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Organic Anion Transporting Polypeptide (OATP) transporter expression, localization and function in the human intestine

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ABSTRACT

Intestinal transporter proteins, along with drug metabolizing enzymes, play a major role in the disposition of orally administered drugs, nutrients and xenobiotics. In this regard, efflux transporters such as ABCB1 and ABCG2 can limit oral bioavailability, while uptake carriers such as PEPT1 have the potential to facilitate intestinal absorption. In contrast to the extensive information on intestinal efflux pumps, detailed knowledge about the respective uptake carriers is rather limited. During the last decade, organic anion transporting polypeptide (OATP) transporters have been frequently discussed as clinically relevant uptake transporters for endogenous compounds as well as drugs. Many drug-drug interactions or food-drug interactions that have been observed have been attributed to inhibition of intestinal OATPs. Moreover, an OATP-mediated drug delivery approach assumes OATPs to be target for improving oral drug absorption. However, recent data from several research groups question the established paradigm of intestinal OATPs as uptake carriers. This review aims to provide a comprehensive up-to-date overview regarding the expression, localization and function of OATP transporters in the human intestine.

Contents

1. Introduction

Despite the fact that the oral pharmacotherapy is the most commonly used route of administration, intestinal absorption of many drugs is characterized by a high inter-subject variability and poorly predictable drug-drug (DDI) or food-drug interactions (Fisher & Labissiere, 2007;Peters, Jones, Ungell, & Hatley, 2016). This is most likely due to the involvement of several complex physiological processes, such as intestinal motility, drug solubility in the intestinal lumen (dependent on the availability of luminal water), as well as intestinal metabolism and transport. Intestinal drug transport has been shown to play a substantial role in determining the oral bioavailability of many drugs, and in turn, to influence their efficacy and safety in a significant manner (Estudante,

Abbreviations: ABC, ATP-binding cassette; ASBT, Apical sodium–dependent bile acid transporter; AUC, Area under the concentration-time curve; b.i.d., Twice daily; CAR, Constitutive androstane receptor; CYP, Cytochrome P450; DDI, Drug-drug interaction; HEK293, Human embryonic kidney cells 293; IC50, Half maximal inhibitory concentration; Km, Michaelis–Menten constant; MCT1, Monocarboxylate transporter 1; MD, Multiple doses; MDCK, Madin-Darby canine kidney; OATP, Organic anion transporting polypeptide; PEPT1, Peptide transporter 1; PXR, Pregnane X receptor; S.D., Standard deviation; SGLT, Sodium-dependent glucose transporter; SD, Single dose; SLC, Solute carrier; SNP, Single nucleotide polymorphism; t.i.d., Three times a day.

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Morais, Soveral, & Benet, 2013;Muller, Keiser, Drozdzik, & Oswald, 2017;Shugarts & Benet, 2009;Giacomini et al., 2010;Hillgren et al., 2013). This becomes especially apparent in the case of unwanted DDIs, whereby the concomitant oral administration of a victim drug with a potent inducer or inhibitor of the transporter results in increased or decreased systemic exposure of the victim drug (Konig, Muller, & Fromm, 2013;Nakanishi & Tamai, 2015;Yoshida, Maeda, & Sugiyama, 2013).

In this regard, intestinal ATP-binding cassette (ABC) transporters such as ABCB1 (P-glycoprotein) or ABCG2 (breast cancer resistance protein, BCRP) that are localized at the apical (luminal) membrane of the enterocytes are known to limit the intestinal absorption of many drugs by effluxing the compound back into gut lumen. While the expression, regulation and function of the aforementioned ABC transporters, as well as their relevance in drug absorption and DDIs, has been well established, comparable information is rather limited for intestinal uptake solute carrier (SLC) transporters. In addition to apical uptake carriers of endogenous substrates, such as the apical sodium-dependent bile acid transporter (ASBT; bile acids), sodium/glucose cotransporter 2 (SGLT2; glucose) and peptide transporter 1 (PEPT1; di- and tripeptides), members of the organic anion transporting polypeptide (OATP) transporter family are expressed in the intestinal epithelia and have been implicated in the intestinal absorption of drugs, as well as several clinically relevant DDIs (Hediger, Clemencon, Burrier, & Bruford, 2013;Tamai, 2012;Tamai & Nakanishi, 2013). However, as the available data is contradictory, this review article aims to summarize the current knowledge regarding intestinal OATP transporters and to estimate their relevance for intestinal drug absorption.

2. Overview of OATP transporters

OATP transporters belong to the SLC superfamily of transporters, facilitating the influx of polar compounds into the cellular compartment in a sodium-independent manner (Hagenbuch & Stieger, 2013; Hediger et al., 2013;Shitara et al., 2013). OATPs have a broad substrate specificity, mediating the uptake of several endogenous compounds, metabolites and waste products, including bile acids, steroid and thyroid hormones, prostaglandins, leukotrienes and bilirubin glucuronides, playing an important role in maintaining homeostasis (Table 1). Moreover, several OATPs have been shown to affect the absorption, elimination and tissue distribution of drugs which are high affinity substrates (Giacomini et al., 2010;Hillgren et al., 2013;Shitara et al., 2013). Therefore, OATP transporters can significantly affect the serum and tissue exposure of a drug and, in turn, drug efficacy and/or safety. For example, inhibition of hepatic OATP1B1/1B3, which are localized in the basolateral (sinusoidal) membrane of the hepatocytes, results in elevated serum level of statins, such as simvastatin or pravastatin, which is associated with an increased risk for rhabdomyolysis (Konig et al., 2013;Nakanishi & Tamai, 2015). In addition, single nucleotide polymorphisms (SNPs) of several OATPs (OATP1B1/1B3, OATP1A2) have been shown to affect the function of the transporter, thereby contributing to the variability in pharmacokinetics, efficacy and side effects of certain drugs (Gong & Kim, 2013;Kalliokoski & Niemi, 2009;Nakanishi & Tamai, 2012). Considering their physiological and pharmacological relevance, it is not surprising that OATPs are widely expressed in nearly all tissues of the human body, although distinct tissue specificity has been described for some members, for example, OATP1B1/1B3 are assumed to be liver-specific (Hagenbuch & Stieger, 2013;Shitara et al., 2013). In general, high expression of OATPs is observed in organs with excretory function, such as the liver.

OATPs are encoded by the *SLC21/SLCO* genes, and so far 12 OATPs have been identified (Table 1). Of these, OATP1B1, OATP1B3, OATP2B1 and OATP1A2 have been identified as important drug transporters and have been studied extensively *in vitro* and *in vivo* (Hagenbuch & Stieger, 2013;Konig et al., 2013;Shitara et al., 2013;Yoshida et al., 2013;Giacomini et al., 2010;Hillgren et al., 2013). In contrast, for the

Table 1
Summarized overview about human OATP transporters including gene name, protein name, tissue distribution and some established substrates and inhibitors.

Gene symbol	Protein name (alias)	Tissue distribution	Localization	Substrates	Inhibitors	References
SLCO1A2	OATP1A2 (OATP-A)	Brain, liver, kidney, testis	Apical	Aliskiren, fexofenadine, imatinib, levofloxacin, MTX, talinolol, thyroxine, trospium	Apple/orange/grapefruit juice, naringin, rifampicin, verapamil	(Bailey, 2010;Estudante et al., 2013; Giacomini et al., 2010;Gong & Kim, 2013; Hagenbuch & Stieger, 2013;Kalliokoski & Niemi, 2009;Konig et al., 2013;Nakanishi & Tamai, 2012;Nakanishi & Tamai, 2015; Nakanishi et al., 2017;Shitara et al., 2013;Tamai, 2012;Yu et al., 2017)
SLCO1B1	OATP1B1 (OATP-C/- 2, LST1)	Liver	Basolateral	Bosentan, fexofenadine, thyroxine, E3S, DHEAS, BSP, E17G, statins, sartans, HIV PI, MTX	Clarithromycin, cyclosporine, gemfibrozil, paclitaxel, rifampicin	
SLCO1B3	OATP1B3 (OATP-8, LST2)	Liver	Basolateral	Bosentan, fexofenadine, thyroxine, E3S, DHEAS, BSP, E17G, statins, sartans, HIV-PI, MTX, digoxin	Clarithromycin, cyclosporine, erythromycin, rifampicin	
SLCO1B7	OATP1B7 (LST3)	Unknown	Unknown	-	-	
SLCO1C1	OATP1C1 (OATP-F)	Brain, testis	Unknown	Thyroid hormones, BSP	-	
SLCO2A1	OATP2A1 (PGT)	Ubiquitous	Unknown	Prostaglandins	Suramin	
SLCO2B1	OATP2B1 (OATP-B)	Ubiquitous including Intestine	Mostly basolateral	E3S, DHEAS, BSP, statins, glibenclamide, bosentan, fexofenadine, talinolol	Cyclosporine, gemfibrozil, grapefruit juice, naringin, rifampicin	
SLCO3A1	OATP3A1 (OATP-D)	Ubiquitous including intestine	Unknown	Benzylpenicillin, prostaglandins	-	
SLCO4A1	OATP4A1 (OATP-E)	Ubiquitous including intestine	Unknown	Benzylpenicillin, prostaglandins, E3S	-	
SLCO4C1	OATP4C1 (OATP-H)	Kidney	Unknown	Digoxin, ouabain, MTX, thyroxine	-	
SLCO5A1	OATP5A1 (OATP-J)	Unknown	Unknown	-	-	
SLCO6A1	OATP6A1 (GST)	Testis	Unknown	-	-	

BSP, bromosulfophthalein; DHEAS, dehydroepiandrosterone sulfate; E17G, estradiol-17ß-D-glucuronide; E3S, estrone-3-sulfate; HIV-PI, HIV protease inhibitors: saquinavir, lopinavir, darunavir, nevirapine, efavirenz; MTX, methotrexate; statins: atorvastatin, fluvastatin, pitavastatin, pravastatin, rosuvastatin; sartans: olmesartan, telmisartan, valsartan

remaining OATPs, information on their tissue distribution, substrates and inhibitors is limited.

3. Intestinal transporters and intestinal OATPs

The human intestinal brush border consists of enterocytes that are very tightly connected to each other by so-called tight junction proteins, thereby forming an effective absorption barrier that does not allow free paracellular transport. In order to maintain intestinal absorption of essential nutrients such as vitamins, peptides, sugars and fatty acids, or important endogenous compounds like bile acids, the enterocytes are equipped with a complex network of several uptake and efflux transporters expressed at the apical (luminal) and basolateral membranes (Fig. 1). This transporter-mediated uptake machinery enables, on one hand, the selective transcellular transport of physiologically important molecules across the enterocytes that cannot diffuse into the intestinal epithelia due to their polarity. On the other hand, intestinal SLC transporters localized at the apical membrane can also facilitate the intestinal absorption of xenobiotics and drugs (Thwaites & Anderson, 2007). Well-studied examples of this include PEPT1-facilitated intestinal uptake of peptidomimetic drugs, including beta-lactam antibiotics, angiotensin-converting enzyme (ACE) inhibitors and antiviral drugs, such as ampicillin, ramipril and acyclovir, respectively (Daniel, 2004). This strategy has also been used by pharmaceutical companies to increase the intestinal absorption of poorly absorbed compounds by attaching cleavable moieties that are recognized by PEPT1 to drugs, for example cefuroxime-axetil and valacyclovir, which are prodrugs of cefuroxime and acyclovir, respectively (Kramer, 2011). Another example of SLC transporters mediating drug absorption is the monocarboxylate transporter 1 (MCT1), which has been implicated in the intestinal uptake of non-steroidal anti-inflammatory drugs (Choi, Jin, & Han, 2005;Thwaites & Anderson, 2007).

In contrast to the uptake transporters, efflux transporters expressed at the apical membrane of enterocytes can limit the intestinal absorption of drugs by removing them from the enterocytes back to the intestinal lumen (Estudante et al., 2013;Giacomini et al., 2010;Muller et al., 2017;Shugarts & Benet, 2009). This excretory function of intestinal ABC transporters has been extensively studied for ABCB1, ABCC2 (multidrug resistance associated protein 2, MPR2) and ABCG2. Inhibition or induction of these efflux pumps can markedly affect intestinal drug absorption and result in clinically relevant DDIs (Konig et al., 2013; Nakanishi & Tamai, 2015). In contrast, the physiological and pharmacological roles of SLC and ABC transporters expressed at the basolateral membrane of the intestinal epithelia have yet to be elucidated. Although several functions have been hypothesized, including basolateral efflux of anionic drugs or phase II metabolites via ABCC1/3 (Ming, Knight, & Thakker, 2011), experimental evidence has not been conclusive. The reader is directed to several reviews for further insight into the area of intestinal transport proteins, which is beyond the scope of this review (Estudante et al., 2013;Muller et al., 2017;Shugarts & Benet, 2009).

The physiological consequence of intestinal OATP involvement in the apical and basolateral uptake of endogenous compounds and drugs is still not clear (Muller et al., 2017;Tamai, 2012;Tamai & Nakanishi, 2013). Although there are excellent *in vitro* data and convincing evidence on the intestinal expression of OATPs available, final conclusions regarding the *in vivo* contribution are difficult to derive. There have been several recent reviews regarding intestinal OATPs which summarize the clinical evidence provided by clinical DDI or food-drug interaction studies, as well as from pharmacogenetic studies (An, Mukker, Derendorf, & Frye, 2015;Tamai & Nakanishi, 2013;Yoshida et al., 2013;Yu, Zhou, Tay-Sontheimer, Levy, & Ragueneau-Majlessi, 2017). However, these data provide only indirect evidence because an observed interaction can be due to many different reasons apart from intestinal transporters (intestinal pH, intestinal motility, ion pair formation and interference with tight junction proteins), or result from non-specific inhibition of more than one uptake or efflux transporter, as well as drug metabolizing enzymes. Surprisingly, the recent review on intestinal OATP transporters published by Yu et al. from the University of Washington Drug Interaction Database did not consider studies that question the significance of intestinal OATPs (Yu et al., 2017). Consequently, a balanced evaluation of intestinal OATPs was not possible. Moreover, the authors included DDIs or food-drug interactions of drugs that also undergo substantial drug metabolism, which makes it challenging to conclude on the function of one specific intestinal transporter (e.g. saquinavir, rosuvastatin and sildenafil). Finally, the authors did not consider fundamental aspects of OATP transporters which must be taken into consideration to conclude on their functional relevance in the human intestine, namely intestinal gene expression,

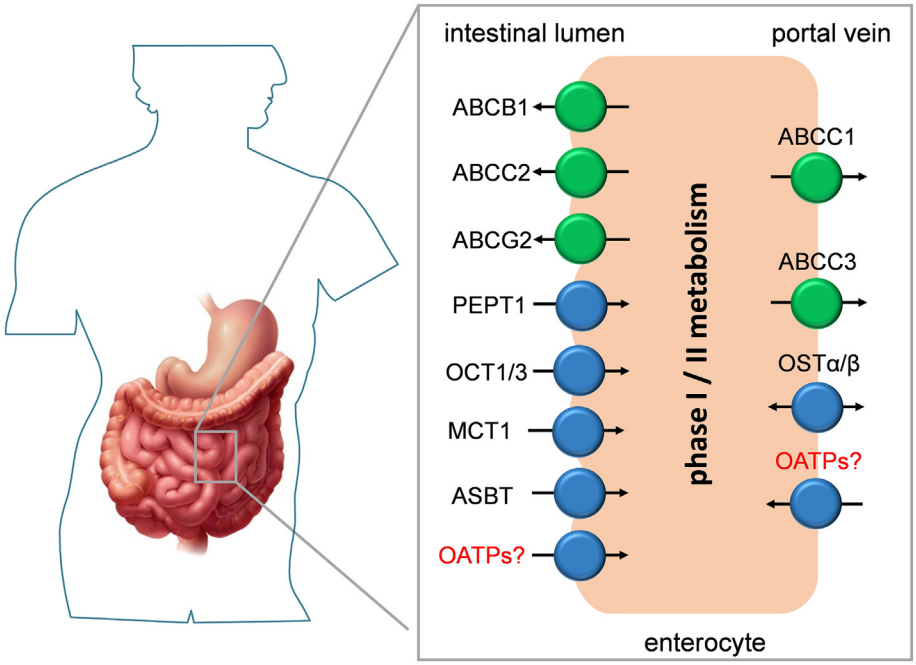

Fig. 1. Schematic overview of human intestinal transporter proteins localized at the apical (intestinal lumen) or the basolateral (facing the portal vein) membrane of enterocytes.

protein abundance, as well as cellular localization. These aspects will be addressed and discussed in the following sections, using clinical findings to conclusively define the clinical relevance of intestinal OATPs.

3.1. Evidence from expression studies

Of the 12 human OATP transporters, only OATP1A2 and OATP2B1 have been proposed to be expressed and of functional relevance in the human intestine (Estudante et al., 2013;Konig et al., 2013;Muller et al., 2017;Tamai, 2012;Tamai & Nakanishi, 2013;Yu et al., 2017). With the exception of OATP1B1 and OATP1B3, which are assumed to be liver-specific transporters, data for the other OATPs are limited (Shitara et al., 2013). We have recently analyzed the gene expression pattern of OATP1A2, OATP1B1, OATP1B3, OATP2B1, OATP3A1, OATP4A1 and OATP4C1 along the entire length of the human intestine from six organ donors (Drozdzik et al., 2014) (Fig. 2). Here, we showed that OATP2B1 exhibits the highest expression, followed by OATP4A1, OATP3A1 and OATP4C1, while only trace amounts of mRNA could be detected for OATP1A2, OATP1B1 and OATP1B3, with gene expression approximately three orders of magnitude lower than that of OATP2B1. A similar gene expression pattern (OATP2B1 > OATP4A1 > OATP3A1, and only trace amounts for OATP1A2) has been shown by other authors (Hilgendorf et al., 2007;Meier et al., 2007;Nishimura & Naito, 2005;Sai et al., 2006).

It is worth noting that the level of mRNA of nearly any gene of interest can be detected in nearly any tissue dependent on the number of PCR cycles used, because even a single mRNA transcript can be amplified to reach a substantial amount of corresponding cDNA molecules after enough PCR cycles (Livak & Schmittgen, 2001). Thus, it is not surprising that some studies were able to detect transcripts of OATP1A2 or OATP1B1/1B3 in the human intestine because there is no classical lower limit of detection, unlike the determination of protein amount, where negative controls can be utilized (Glaeser et al., 2007;Kullak-Ublick et al., 2016). However, compared to genuinely expressed intestinal transporters such OATP2B1 or ABCB1, gene expression levels of OATP1A2 and OATP1B1/1B3 were negligible or not detectable in most studies (Drozdzik et al., 2014;Hilgendorf et al., 2007;Meier et al., 2007;Nishimura & Naito, 2005). Similar to the trace amounts of transcript detected in intestinal tissue, protein expression for these OATPs has not be convincingly demonstrated (Table 2).

Few studies have investigated the abundance of intestinal OATP proteins (Drozdzik et al., 2014;Miyauchi et al., 2016;Nakamura et al., 2016;Vaessen et al., 2017;Drozdzik et al., 2018). Fig. 3 shows data from two independent studies which characterized the protein abundance of several ABC and SLC transporters, including several OATPs, in the plasma membrane of jejunal tissue from 28 obese subjects (Fig. 3a) and in the mucosal tissue lysate taken from 9 healthy organ donors (Fig. 3b). While OATP1A2 and OATP1B1/1B3 protein could not be detected in either study, robust abundance could be demonstrated for several other OATPs (OATP2B1 ≈ OATP4A1 > OATP3A1 > OATP2A1). However, compared to clinically relevant transporters of the intestinal absorption barrier, such as ABCB1, ABCG2 or PEPT1, the protein amount of all detected OATPs was rather low (Fig. 3). Similar findings have been recently observed in human and porcine intestine (Vaessen et al., 2017).

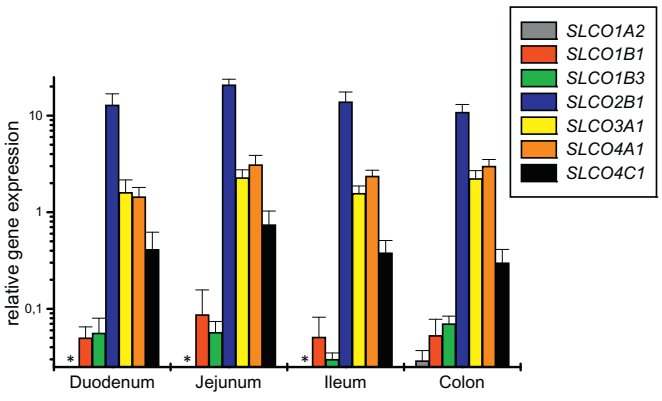

Fig. 2. Relative gene (mRNA) expression of different *SLCO* transporter genes expressed in the mucosa of the human duodenum, jejunum, ileum and colon from six organ donors (intra-subject data measured in duplicate relative to 18S rRNA, mean ± S.D.). Data were taken from Drozdzik et al., 2014 (*, not detectable).

Although many clinical investigations are based on the concept that OATP1A2 and/or OATP2B1 contribute in a significant manner to the intestinal absorption of drugs (see the following section), there is surprisingly little, and partly controversial, data on their intestinal gene expression and protein abundance (Table 2). A review of the current literature clearly indicates stable gene expression and protein abundance of OATP2B1 in the intestinal epithelia, which has also been shown to be homogeneously distributed along the entire length of the human intestine (Drozdzik et al., 2014;Kobayashi et al., 2003;Kullak-Ublick et al., 2001;Miyauchi et al., 2016;Vaessen et al., 2017). In contrast, only one immunohistochemistry-based analysis indicated the presence of OATP1A2 in the apical membrane of the brush border membrane (Glaeser et al., 2007). However, this investigation could not be reproduced by different research groups, either at the gene or protein level (Drozdzik et al., 2014;Hilgendorf et al., 2007;Miyauchi et al., 2016;Vaessen et al., 2017). Even the sensitive and specific method of targeted proteomics did not show any intestinal OATP1A2 using several different protein-specific peptides by different groups (Drozdzik et al., 2014;Drozdzik et al., 2018;Miyauchi et al., 2016;Vaessen et al., 2017). In each of these studies, the presence of OATP2B1 could be verified in the same human intestinal tissue samples. Taken together, it appears reasonable to conclude that in the human intestine only OATP2B1, OATP4A1, OATP3A1 and OATP2A1 are found on the level of protein. However, due to the limited knowledge about the functional consequences of OATP2A1, OATP3A1 and OATP4A1, the remainder of this review will mainly focus on OATP2B1 and the controversial presence of OATP1A2.

3.2. Evidence from clinical drug- drug or food-drug interaction studies

Most clinical evidence for the existence and functional relevance of intestinal OATPs has been provided by DDI or food-drug interaction studies. In nearly all cases, these studies represent inhibition studies in which the assumed inhibition of intestinal OATPs by perpetrators results in diminished intestinal drug absorption, and consequently markedly reduced systemic exposure to the respective OATP substrate (victim drug). Unfortunately, there are no interaction studies available that are based on induction of intestinal OATPs as there have been for efflux pumps like ABCB1, which could thereby strengthen the hypothesis of their *in vivo* relevance. This is most likely due to the differing regulation of OATPs via nuclear receptors apart from PXR, the activation of which is the reason for the striking interaction caused by induction of intestinal ABCB1 and/or CYP3A4 (Chai, Zeng, & Xie, 2013;Urquhart, Tirona, & Kim, 2007).

The first clinical study indicating functional relevance of intestinal OATP transporters in humans was provided by Dresser et al., who studied the effect of different fruit juices on the oral absorption of the antihistamine drug fexofenadine in 10 healthy volunteers (Dresser et al., 2002). They found that compared to the intake of 1200 mL water, the same ingested volume of grapefruit, orange and apple juice reduced the systemic drug exposure by about 67-77%. In additional *in vitro* experiments performed in OATP1A2-transfected HeLa cells, the authors demonstrated inhibitory effects on the cellular uptake of fexofenadine only for grapefruit and orange juice, but not for apple juice, which produced by far the most pronounced reduction in oral drug absorption *in vivo*. Despite the discrepancy between *in vivo* and *in vitro* effects, the inhibitory effect of juices was hypothesized to be related to different content of flavonoids or flavanones such as naringin (grapefruit),

Table 2
Overview of so far available data on mRNA expression and protein abundance of OATP transporters in the human intestine (+, gene/protein expression was shown; n.d., not detectable; -, not investigated). Data are ranked in chronological order (publication date).

Reference	OATP1A2		OATP2A1		OATP3A1		OATP4A1		OATP2B1	
	Gene	Protein (method)	Gene	Protein (method)	Gene	Protein (method)	Gene	Protein (method)	Gene	Protein (method)
(Kullak-Ublick et al., 2001)	-	-	-	-	-	-	-	-	+	-
(Kobayashi et al., 2003)	-	-	-	-	-	-	-	-	-	+ (Immunohisto-chemistry)
(Nishimura & Naito, 2005)	+	-	-	-	+	-	+	-	+	-
(Sai et al., 2006)	-	-	-	-	+	-	+	-	+	-
(Glaeser et al., 2007)	+	+ (Immunohisto-chemistry)	-	-	-	-	-	-	+	-
(Hilgendorf et al., 2007)	n.d.	-	-	-	+	-	+	-	+	-
(Meier et al., 2007)	n.d.	-	-	-	-	-	+	-	+	-
(Mandery, Bujok, Schmidt, Wex, et al., 2010)	-	-	+	+ (Immunohisto-chemistry)	-	-	-	-	-	-
(Drozdzik et al., 2014)	n.d.	n.d. (Targeted proteomics)	-	-	+	-	+	-	+	+ (Targeted proteomics)
(Nakamura et al., 2016)	-	n.d. (Global proteomics)	-	n.d. (Global proteomics)	-	n.d. (Global proteomics)	-	n.d. (Global proteomics)	-	n.d. (Global proteomics)
(Miyauchi et al., 2016)	-	n.d. (Targeted proteomics)	-	+ (Targeted proteomics)	-	+ (Targeted proteomics)	-	+ (Targeted proteomics)	-	+ (Targeted proteomics)
(Kullak-Ublick et al., 2016)	+	-	-	-	-	-	-	-	-	-
(Mooij et al., 2016)	-	-	-	-	-	-	-	-	-	+ (Immunohisto-chemistry)
(Vaessen et al., 2017)	-	n.d. (Targeted proteomics)	-	-	-	-	-	+ (Targeted proteomics)	-	+ (Targeted proteomics)
(Drozdzik et al., 2018)	n.d.	n.d. (Targeted proteomics)	-	-	-	-	-	-	+	+ (Targeted proteomics)

hesperidin (orange) as well as quercetin and phloridzin (apple) as these compounds are present in sufficient concentrations in the aforementioned juices.

Later, the same authors demonstrated that this interaction was also observable during concomitant administration of 300 mL grapefruit juice, although the continuous ingestion of 1200 mL led to a more distinct reduction of fexofenadine absorption, which, according to the authors was most likely due to dose-dependent and prolonged inhibition of intestinal OATP1A2 (Dresser, Kim, & Bailey, 2005). Finally, the same group demonstrated that an aqueous solution of naringin at the same concentration that is present in grapefruit juice (~1.2 mM) reduced oral fexofenadine absorption by about one quarter, which supported their initial hypothesis of this flavonoid being the main ingredient responsible for the observed interaction (Bailey, Dresser, Leake, & Kim, 2007). In line with their hypothesis, the authors published the aforementioned data suggesting intestinal gene expression and protein abundance of OATP1A2 at the human brush border membrane around the same time (Glaeser et al., 2007).

According to some *in vitro* studies, intestinal OATP2B1 may be excluded as an intestinal uptake carrier of fexofenadine, and therefore

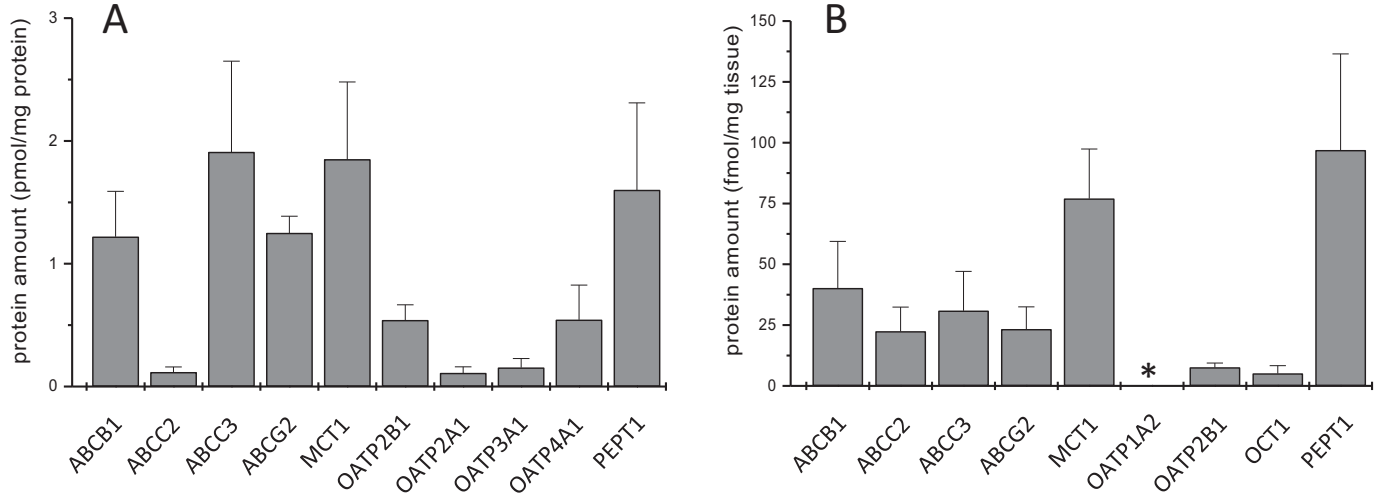

Fig. 3. Comparative protein abundance of intestinal ABC and SLC transporters in the human small intestine. A, Data were generated by targeted proteomics analysis of the plasma membrane of jejunal tissue from 28 obese subjects (Miyauchi et al., 2016). B, Data were generated by targeted proteomics analysis of mucosal tissue lysates (jejunum) from 9 healthy organ donors (Drozdzik et al., 2018). In both studies, samples were measured once each using validated LC-MS/MS methods. The individual amount of peptide was determined as an average of two to four mass transitions. Data are given as mean ± S.D. (*, below the lower limit of quantification).

cannot be involved in the described interactions with different fruit juices (Glaeser et al., 2007;Shimizu et al., 2005). The mechanism of this inhibition was assumed to be competitive, as the intake of grapefruit juice four hours before the administration of fexofenadine did not cause any change in the oral absorption of the drug (Glaeser et al., 2007). This is in contrast to the known mechanism-based, and irreversible, inhibition of CYP3A4 by grapefruit juice (Greenblatt et al., 2003).

Interestingly, another interaction study found that regular-strength grapefruit juice and modified grapefruit juice (containing nearly no furanocoumarins or polymethoxyflavones, but almost exclusively flavanones, such as naringin) both reduced fexofenadine AUC by about 25% (Won et al., 2013). This is nearly half of the effect that was seen in the aforementioned studies. In this study, in vitro experiments demonstrated very weak to no effect of grapefruit juice on the cellular uptake of fexofenadine into OATP1A2-transfected COS-1 or HEK293 cells.

Another extensively studied drug in terms of interactions with fruit juices is the beta-blocker talinolol, an *in vitro* substrate of OATP1A2 and OATP2B1 (Shirasaka et al., 2009;Shirasaka et al., 2010). Comparable to what had been observed for fexofenadine, Schwarz et al. assessed that a single ingestion of 300 mL of grapefruit juice reduced the talinolol serum exposure after oral administration by about 44% (Schwarz et al., 2005). This was an unexpected finding for the authors as talinolol is also a well-established substrate for ABCB1, the inhibition of which would be expected to result in increased instead of reduced oral bioavailability, as seen before in a comparable study in rats (Spahn-Langguth & Langguth, 2001).

Shirasaka et al. speculated that the discrepancy between rat and human data is most likely based on the different inhibitory potencies of the grapefruit juice flavonoid naringin on rat and human Oatp/OATP and Abcb1/ABCB1 (Shirasaka, Kuraoka, et al., 2010). In this regard, they showed that the IC_{50} value for the naringin-mediated inhibition of the cellular uptake of talinolol was much lower for rat Oatp1a5 than that for human OATP1A2 (12.7 μmol/L vs. 343 μmol/L). As the functional counterpart Abcb1/ABCB1, which is expected to pump substrates out of enterocytes back into gut lumen, was also shown to be more susceptible to fexofenadine efflux inhibition by grapefruit juice in rats (IC_{50}: ~600 μmol/L) than in humans (IC_{50}: ~2000 μmol/L), it was postulated that intestinal naringin concentrations of >600 μmol/L (the typical concentration in grapefruit juice being 750-1000 μmol/L) will result in inhibition of both Oatp1a5 and Abcb1 in rats (i.e. increased absorption), but only inhibition of human OATP1A2 (i.e. decreased intestinal absorption) (Shirasaka et al., 2009;Shirasaka, Kuraoka, et al., 2010). Interestingly, the authors did not find any effect of grapefruit juice on the OATP2B1-mediated uptake of talinolol *in vitro*.

However, this elegant hypothesis was substantially shaken by a later interaction study in which high-dose short term administration of naringin (1050 mg) did not cause any effect on the oral availability of talinolol (Nguyen, Staubach, Tamai, & Langguth, 2015). This finding was further confounded by another study from the same group showing significantly reduced serum AUC values of talinolol in healthy subjects after acute co-administration with 1500 mg of the flavonoid quercetin (Nguyen, Staubach, Wolffram, & Langguth, 2014), which is also known as a potent inhibitor of both presumed intestinal OATP transporters, OATP1A2 and OATP2B1, as well as ABCB1 (Mandery et al., 2010).

In addition to talinolol, several other beta-blockers including celiprolol, atenolol and nadolol showed negative food effects with juices, i.e. their oral absorption was markedly diminished when co-administered with juice (Table 3) (Lilja, Backman, Laitila, Luurila, & Neuvonen, 2003;Lilja, Juntti-Patinen, & Neuvonen, 2004;Lilja, Raaska, & Neuvonen, 2005;Itohda et al., 2012). One of the most stinking interactions attributed to inhibition of intestinal OATP transporters was demonstrated for nadolol and green tea (Misaka et al., 2014). Here, the systemic AUC was reduced by about 85% when a single dose of the drug was administered with 350 ml green tea (b.i.d. for 14 days before drug intake). As the authors also presented *in vitro* experiments showing that nadolol was taken up into OATP1A2-overexpressing HEK293 cells, which could then be inhibited in a concentration dependent-manner by green tea and (-)-epigallocatechin gallate, it was suggested that this substantial interaction was due to the inhibition of intestinal OATP1A2 (Abe et al., 2018;Misaka et al., 2014).

Comparable interaction studies have been performed for the renin inhibitor aliskiren. As this compound is similarly not metabolized by CYP3A4, it appears to be a suitable probe compound to study transporter-related interactions *in vivo*. Aliskiren was shown to be a substrate of ABCB1; consequently, co-medication with strong ABCB1 inhibitors such as cyclosporine, verapamil and ketoconazole markedly increased its serum exposure (Rebello et al., 2011;Rebello, Leon, Hariry, Dahlke, & Jarugula, 2011). In the same manner, the combination of aliskiren with grapefruit juice, which is also an inhibitor of ABCB1, would thereby be expected to increase oral drug absorption. However, Tapaninen et al. could show that grapefruit juice reduced the serum exposure, compared to intake with water, by more than 60% (Tapaninen, Neuvonen, & Niemi, 2010). The same authors also performed similar aliskiren interaction studies with orange and apple juice which resulted in nearly the same extent of decreased intestinal drug absorption (Tapaninen, Neuvonen, & Niemi, 2011). Although the authors did not provide further *in vitro* evidence for their findings, they attributed this phenomenon to inhibition of intestinal OATP2B1. The interaction between aliskiren – grapefruit juice was later on also replicated by another group (Rebello et al., 2012). Although the inhibitory effect on drug absorption, i.e. the reduction of serum AUC of aliskiren, was much weaker than reported by Tapaninen et al., 2010 (37% vs. 61%), this study also provided *in vitro* data demonstrating the cellular uptake of aliskiren into OATP1A2- but not into OATP2B1-trasfected HEK293 cells, which could be inhibited in a concentration-dependent manner by naringin (IC_{50}: 75.5 μmol/L).

Interestingly, there are few clinical studies available for compounds that are very frequently used *in vitro* to characterize the function of OATP2B1, such as sulfasalazine or pravastatin, which could confirm the functional importance of OATP-mediated drug uptake in the human intestine (Grube et al., 2006;Satoh et al., 2005;Shirasaka, Suzuki, Nakanishi, & Tamai, 2010). In this regard, only one study demonstrated a significantly decreased oral absorption of the anti-inflammatory drug sulfasalazine by the co-administration of grapefruit juice (Kashihara et al., 2017). All available studies with pravastatin did not provide functional evidence for intestinal OATPs (Deng et al., 2009;Koitabashi et al., 2006;Lilja, Kivisto, & Neuvonen, 1999;Wu et al., 2012)(see next paragraph, Table 4).

In addition, there are several studies available using other well-established substrates of OATP1A2 and OATP2B1 which also undergo substantial intestinal and/or hepatic metabolism, including atorvastatin, pitavastatin, rosuvastatin, imatinib, glibenclamide, montelukast and saquinavir (Ando et al., 2005;Eechoute et al., 2011;Johnson et al., 2017;Kashihara et al., 2017;Kupferschmidt, Fattinger, Ha, Follath, & Krahenbuhl, 1998;Lilja, Niemi, Fredrikson, & Neuvonen, 2007;Mougey, Lang, Wen, & Lima, 2011;Yu et al., 2017). Considering the complex interplay of metabolism and transport, these DDI studies are very challenging to interpret as established perpetrators, such as grapefruit juice, inhibit both CYP3A4-mediated metabolism as well as OATP-mediated transport. Consequently, these studies have been excluded from further analysis.

3.3. Contradictory findings from clinical DDI studies

The critical review of clinical trials investigating the interaction between substrates and inhibitors of OATP1A2 and OATP2B1 published thus far revealed several contradictory findings that are not aligned with the findings summarized in the previous section (Table 3), and do not support the contribution of OATPs to intestinal drug absorption. In this regard, the first inconsistencies were provided by the first, and previously mentioned, study in this field (Dresser et al., 2002). In this study, apple juice produced the most pronounced *in vivo* effect,

Table 3
Overview of clinically relevant drug-drug or food-drug interactions that have been explained by inhibition of intestinal OATP transporters.

Substrate (victim drug)	Perpetrator (inhibitor)	PK change compared to control	Assumed reason/hypothesis	Reference
Fexofenadine (SD, 120 mg)	Grapefruit juice (MD, 1200 mL) orange juice (MD, 1200 mL) apple juice (MD, 1200 mL)	$AUC_{0\text{-}8h}$↓ 67% $AUC_{0\text{-}8h}$↓ 72% $AUC_{0\text{-}8h}$↓ 77%	Inhibition of intestinal OATP1A2; OATP1A2-mediated uptake demonstrated in vitro, only strongly inhibited by orange/grapefruit juice	Dresser et al., 2002
Fexofenadine (SD, 120 mg)	Grapefruit juice (SD, 300 mL) grapefruit juice (MD, 1200 mL)	$AUC_{0\text{-}8h}$↓ 42% $AUC_{0\text{-}8h}$↓ 64%	Dose-dependent and prolonged inhibition of intestinal OATP1A2	Dresser et al., 2005
Fexofenadine (SD, 120 mg)	Grapefruit juice (SD, 300 mL) naringin solution (SD, 300 mL)	$AUC_{0\text{-}8h}$↓ 45% $AUC_{0\text{-}8h}$↓ 25%	Inhibition of intestinal OATP1A2; concentration-dependent inhibition of OATP1A2 was shown for naringin in vitro (IC_{50}: 3.6 μM)	Bailey et al., 2007
Fexofenadine (SD, 120 mg)	Grapefruit juice (SD, 300 mL) grapefruit juice (- 2h, SD, 300 mL) grapefruit juice (- 4h, SD, 300 mL)	$AUC_{0\text{-}8h}$↓ 52% $AUC_{0\text{-}8h}$↓ 38% $AUC_{0\text{-}8h}$ unchanged	Inhibition of intestinal OATP1A2; in vitro uptake shown for human OATP1A2 (but not OATP2B1) and rat Oatp1a1/1a4/1a5/1b2	Glaeser et al., 2007
Fexofenadine (SD, 120 mg)	Grapefruit juice (SD, 240 mL) mod. grapefruit juice (SD, 240 mL)	$AUC_{0\text{-}\infty}$ ↓ 24% $AUC_{0\text{-}\infty}$ ↓ 25%	Inhibition of intestinal OATPs	Won et al., 2013
Talinolol (SD, 50 mg)	Grapefruit juice (SD, 300 mL)	AUC↓ 44%	Inhibition of intestinal OATPs	Schwarz et al., 2005
Talinolol (SD, 100 mg)	Quercetin (SD, 1500 mg)	$AUC_{0\text{-}48h}$↓23%	Inhibition of intestinal OATP1A2	Nguyen et al., 2014
Celiprolol (SD, 100 mg)	Grapefruit juice (MD, *t.i.d.* 200 ml)	$AUC_{0\text{-}33h}$↓87%	Inhibition of intestinal OATPs and/or increased ionization and thereby lower absorption	Lilja et al., 2003
Celiprolol (SD, 100 mg)	Orange juice (MD, *t.i.d.* 200 ml)	$AUC_{0\text{-}33h}$↓83%	Inhibition of intestinal OATPs and/or increased ionization and thereby lower absorption	Lilja et al., 2004
Celiprolol (SD, 200 mg)	Apple juice (SD, 500 ml)	$AUC_{0\text{-}24h}$↓84%	Inhibition of intestinal OATPs (comment: only data of 5 from 15 volunteers presented)	Itohda et al., 2012
Atenolol (SD, 50 mg)	Orange juice (MD, *t.i.d.* 200 ml)	$AUC_{0\text{-}33h}$↓40%	Inhibition of intestinal OATPs and/or increased ionization and thereby lower absorption	Lilja et al., 2005
Atenolol (SD, 50 mg)	Apple juice (MD, 600 mL) apple juice (MD, 1200 mL)	$AUC_{0\text{-}48h}$↓58% $AUC_{0\text{-}48h}$↓82%	Inhibition of intestinal OATPs	Jeon et al., 2013
Nadolol (SD, 30 mg)	Green tea (MD, *b.i.d.* 350 ml)	$AUC_{0\text{-}33h}$↓85%	Inhibition of intestinal OATP1A2; OATP1A2-(not OATP2B1) mediated uptake and its concentration-dependent inhibition shown in vitro (IC_{50}: 1.4% tea)	Misaka et al., 2014
Aliskiren (SD, 150 mg)	Grapefruit juice (MD, *t.i.d.* 200 ml)	$AUC_{0\text{-}72h}$↓61%	Inhibition of intestinal OATP2B1	Tapaninen et al., 2010
Aliskiren (SD, 300 mg)	Grapefruit juice (SD, 300 ml)	$AUC_{0\text{-}96h}$↓37%	Inhibition of intestinal OATP1A2; OATP1A2-(not OATP2B1) mediated uptake and its concentration-dependent inhibition shown in vitro (IC_{50}: 75.5 μM)	Rebello et al., 2012
Aliskiren (SD, 150 mg)	Orange juice (MD, *t.i.d.* 200 ml) apple juice (MD, *t.i.d.* 200 ml)	$AUC_{0\text{-}72h}$↓62% $AUC_{0\text{-}72h}$↓63%	Inhibition of intestinal OATP2B1	Tapaninen et al., 2011
Sulfasalazine (SD, 300 μg)	Grapefruit juice (MD, *t.i.d.* 200 ml)	$AUC_{0\text{-}24h}$↓34%	Inhibition of intestinal OATP2B1	Kashihara et al., 2017

AUC, area under the concentration-time curve; b.i.d., twice a day; MD, multiple doses; SD, single dose; t.i.d., three times a day

reducing the AUC of fexofenadine by about 80%, while the corresponding *in vitro* experiments could not show any significant inhibition of OATP1A2 by apple juice, though inhibition could be demonstrated for orange and grapefruit juice *in vitro*. Moreover, although both fexofenadine and talinolol were shown to be *in vitro* substrates of OATP1A2, and naringin was capable of inhibiting the cellular accumulation of both compounds in OATP1A2-transfected cells, a significant clinical interaction with oral naringin could be only demonstrated for fexofenadine, not talinolol (Bailey et al., 2007;Nguyen et al., 2015). Conversely, the OATP1A2/2B1 inhibiting flavonoid quercetin that is present in several fruit juices at sufficiently high concentrations only produced a significant interaction with talinolol, but not fexofenadine *in vivo* (Kim, Park, & Park, 2009;Mandery, Bujok, Schmidt, Keiser, et al., 2010;Nguyen et al., 2014). Similarly and in contrast to expectations, no interaction was observed for the combination of grapefruit juice and the OATP1A2 substrate nadolol, which showed the striking interaction with green tea (Misaka et al., 2013;Misaka et al., 2014).

Interestingly, all available *in vivo* interaction studies involving the frequently used *in vitro* OATP2B1 substrate pravastatin also contradicted the relevance of intestinal OATPs. The concomitant administration of the potent OATP2B1 inhibitors grapefruit juice, orange juice and quercetin significantly increased the systemic drug exposure of the statin instead of decreasing it, as expected and as seen for other OATP substrates (summarized in Table 3) (Koitabashi et al., 2006;Lilja et al., 1999;Wu et al., 2012). These rather surprising findings cannot be explained by inhibition of intestinal ABCB1, for which grapefruit juice and quercetin are known inhibitors, because pravastatin is not a substrate of ABCB1 (Holtzman, Wiggins, & Spinler, 2006). One may argue that inhibition of hepatic OATP2B1 by the aforementioned perpetrators may contribute to the unexpected finding, as blockade of hepatic OATPs is known to increase serum levels of the respective substrates (Konig et al., 2013;Nakanishi & Tamai, 2015). Nevertheless, inhibition of the initial step of intestinal drug uptake should result in reduced oral absorption, and in turn, to reduced serum exposure of the drug. Moreover, it is uncertain whether the respective flavonoids that inhibit OATPs can reach sufficient concentrations in the portal vein (Bailey, 2010).

Although the OATP2B1-related discrepancies have been explained by the presence of at least two different binding sites, this model does not convincingly explain all the contrary findings from the aforementioned *in vitro* and clinical DDI studies (Table 4, Fig. 4) (Shirasaka, Shichiri, Mori, Nakanishi, & Tamai, 2013;Shirasaka, Mori, Murata, Nakanishi, & Tamai, 2014;Tamai & Nakanishi, 2013). Moreover, even the identification of whether or not a drug is a substrate of OATP1A2/OATP2B1 *in vitro* remains controversial. For example, Glaeser et al. showed that fexofenadine is not a substrate of OATP2B1, but of OATP1A2, in transfected HeLa cells, while Shirasaka et al. demonstrated uptake of fexofenadine into *X. laevis* oocytes expressing OATP2B1 (Glaeser et al., 2007;Shirasaka et al., 2013).

Table 4
Overview of drug-drug or food-drug interactions which do not support the hypothesis of intestinal OATP transporters.

Substrate (victim drug)	Perpetrator (inhibitor)	PK change compared to control	Contradictory finding	Reference
Fexofenadine (SD, 60 mg)	Rifampicin (SD, 600 mg)	AUC↑ 73-76%	Increased oral absorption in the presence of rifampicin (potent inhibitor of OATP1A2/2B1, Vavricka et al., 2002)	Kusuhara et al., 2013
Fexofenadine (SD, 60 mg)	Quercetin (MD, 500 mg)	AUC↑ 56%	Increased oral absorption in the presence of quercetin (potent inhibitor of OATP1A2/2B1, Mandery et al., 2010)	Kim et al., 2009
Fexofenadine (SD, 120 mg)	Vitamin D3 (MD, 0.5 μg)	$AUC_{0\text{-}24h}$ unchanged	Unchanged oral absorption although induction of OATP1A2 was shown in Caco-2 cells after a 3-day treatment with vitamin D3 (Eloranta et al., 2012)	Kullak-Ublick et al., 2016
Talinolol (SD, 100 mg)	Naringin (MD, *t.i.d.* 350 mg, 7 d)	$AUC_{0\text{-}48h}$ unchanged	Unchanged oral absorption despite the affinity of talinolol to OATP1A2/2B1 and their strong inhibition by grapefruit juice (Shirasaka, Kuraoka, et al., 2010)	Nguyen et al., 2015
Nadolol (SD, 30 mg)	Grapefruit juice (SD, 300 mL)	$AUC_{0\text{-}48h}$ unchanged	Unchanged oral absorption compared to the strong interaction with green tea (Misaka et al., 2014)	Misaka et al., 2013
Pravastatin (SD, 40 mg)	Grapefruit juice (MD, *t.i.d.* 200 ml)	$AUC_{0\text{-}72h}$ unchanged	Unchanged oral absorption despite the affinity of pravastatin to OATP2B1 and its strong inhibition by grapefruit juice (Shirasaka, Suzuki, et al., 2010)	Lilja et al., 1999
Pravastatin (SD, 10 mg)	Orange juice (MD, 800 mL)	$AUC_{0\text{-}4h}$↑ 52%	Increased oral absorption despite the affinity of pravastatin to OATP2B1 and its strong inhibition by orange juice (Shirasaka, Shichiri, Mori, et al., 2013)	Koitabashi et al., 2006
Pravastatin (SD, 40 mg)	Quercetin (MD, 500 mg)	$AUC_{0\text{-}10h}$↑ 24%	Increased oral absorption despite the affinity of pravastatin to OATP2B1 and its strong inhibition by quercetin (Shirasaka, Shichiri, Mori, et al., 2013)	Wu et al., 2012
Pravastatin (SD, 20 mg)	Rifampicin (SD, 600 mg)	$AUC_{0\text{-}12h}$↑ 234%	Increased oral absorption despite the affinity of pravastatin to OATP2B1 and its strong inhibition by SD rifampicin (Vavricka et al., 2002)	Deng et al., 2009

AUC, area under the concentration-time curve; b.i.d., twice a day; MD, multiple doses; SD, single dose; t.i.d., three times a day

As already stated, functional evidence from DDI studies caused by transporter induction would strengthen the hypothesis of functionally relevant intestinal OATPs. However, there is only one study available in which this approach was attempted. In this study, Kullak-Ublick and colleagues intended to induce intestinal OATP1A2 by pretreatment with vitamin D3 (1,25-dihydroxyvitamin D3), as *in vitro* data from Caco-2 cells suggested a strong OATP1A2 inducing effect (9-fold on mRNA) (Eloranta, Hiller, Juttner, & Kullak-Ublick, 2012). To investigate the function of intestinal OATP1A2, the pharmacokinetic effects of vitamin D3 (0.5 μg administered for 10 days) on the orally administered OATP1A2 substrate fexofenadine was studied (Kullak-Ublick et al., 2016). However, the authors failed to detect any induction of OATP1A2 at the gene level or any pharmacokinetic changes in the probe drug. Additionally, it was convincingly demonstrated that human OATP1A2 and OATP2B1 are regulated by the nuclear receptor PXR (Meyer zu Schwabedissen, Tirona, Yip, Ho, & Kim, 2008; Moscovitz et al., 2018), meaning that chronic pretreatment with prototypical inducers such as rifampicin or St. John's wort would be expected to up-regulate the intestinal expression and function of both OATP transporters. In contrast, we and others were not able to detect any rifampicin-mediated induction of intestinal OATPs (Meyer zu Schwabedissen et al., 2012;Oscarson et al., 2007;Oswald et al., 2012).

In addition, a single oral dose of rifampicin is expected to result in clinically relevant interactions with orally administered substrates of OATP1A2 and OATP2B1, assuming their intestinal expression, as rifampicin is a potent inhibitor of these transporters (Vavricka, Van, Ha, PJ, & Fattinger, 2002). Of note, interaction studies including pretreatment with rifampicin for several days are not suitable for this evaluation, as pretreatment with the PXR ligand for more than five days results in dramatic up-regulation in the expression and function of several intestinal and hepatic enzymes and transporters, including CYP3A4, CYP2C9/19, UGT1A1, ABCB1 and ABCC2, which would be confounding (Oscarson et al., 2007;Oswald et al., 2006). However, coadministration of either pravastatin or fexofenadine (single oral dose) with single dose rifampin did not result in reduced oral absorption; in fact, the serum exposure to both drugs was significantly increased by 73-234% (Deng et al., 2009;Kusuhara et al., 2013). Although these findings may be confounded by inhibition or saturation of hepatic OATPs (OATP1B1/1B3), which are known to recognize fexofenadine and pravastatin as substrates and are also sensitive to inhibition by rifampicin, inhibition of an intestinal uptake transporter essential for oral drug absorption should nevertheless dominate this interaction, resulting in decreased instead of increased serum levels of the respective substrates.

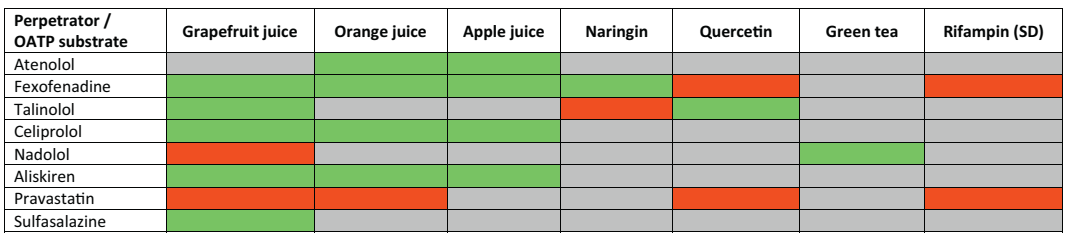

Fig. 4. Heatmap for intestinal OATP-mediated drug-drug and food-drug interactions in humans indicating the heterogeneity of so far available data and the existing gaps in knowledge (Tables 3 & 4). Green fields indicate inhibition of intestinal OATPs as suggested by significantly decreased AUC of OATP substrates in the presence of an inhibitor, while red fields indicate no pharmacokinetic changes or even opposite effects, i.e. increased AUC of OATP substrates in the presence of an inhibitor. Grey fields indicate that this interaction has not been studied yet.

A general limitation which has to be taken into consideration when interpreting clinical DDI studies is that nearly all studies are challenged by the fact that the investigated probe drugs are substrates of multiple transporters and/or enzymes, and specific inhibitors for each transporter are not available. For example, fexofenadine has been reported to be a substrate of OATP1A2, OATP2B1, OATP1B1, ABCB1, ABCC2 and ABCC3 whereas grapefruit juice is known to inhibit OATP1A2, OATP2B1, ABCB1 and CYP3A4/5. Moreover, other important aspects of intestinal drug absorption, such as intestinal motility, luminal availability of water, intestinal pH, osmolality and passive diffusion, may contribute to the observed DDIs and food-drug interactions and were, in most cases, not further investigated. Finally, the involvement of other transporters that may contribute to observed DDI or food-drug interactions cannot be ruled out (Mimura, Yasujima, Ohta, Inoue, & Yuasa, 2017).

In summary, while some of the aforementioned studies clearly provide evidence for the existence and clinical relevance of intestinal OATP1A2 and OATP2B1, other studies raise serious question as to whether the transporters are genuinely expressed in the human intestine (i.e. OATP1A2) or of any functional meaning for intestinal drug absorption. Fig. 4 illustrates the existing discrepancies as a heatmap.

3.4. Evidence from pharmacogenetic studies

Additional evidence for the functional relevance of intestinal OATPs has been provided by some pharmacogenetics studies. Compared to the extensively studied polymorphisms of the hepatic carriers OATP1B1 and OATP1B3, there are only a relatively small number of pharmacogenetic studies considering genetic polymorphisms of OATP1A2 and OATP2B1 (Gong & Kim, 2013;Kalliokoski & Niemi, 2009;Nakanishi & Tamai, 2012). For the other intestinal OATPs, namely OATP2A1, OATP3A1 and OATP4A1, pharmacogenetic studies are even more sparse.

For our evaluation, we have exclusively considered confirmed substrates of OATP1A2 and OATP2B1, such as aliskiren and fexofenadine (Table 5). In theory, functionally relevant non-synonymous loss-of-function single-nucleotide polymorphisms (SNPs) would be expected to result in decreased intestinal OATP-mediated drug absorption, and in hence to reduced systemic exposure of the respective OATP substrates. For OATP2B1, the non-synonymous SNP *SLCO2B1* c.935G > A (rs12422149) was frequently investigated, as *in vitro* data suggested diminished uptake (Mougey, Feng, Castro, Irvin, & Lima, 2009). For this polymorphism, which occurs with an allelic frequency of about 8-14% in Caucasians and 13% in African-Americans (Gong & Kim, 2013), no pharmacokinetic differences were observed for aliskiren or celiprolol in heterozygous (*SLCO2B1* c.935GA) or homozygous (*SLCO2B1* c.935AA) carriers, compared to the wild-type (*SLCO2B1* c.935GG) (Ieiri et al., 2012;Tapaninen, Karonen, Backman, Neuvonen, & Niemi, 2013). Data for montelukast is controversial regarding this OATP2B1 polymorphism (Kim, Lee, Joo, IB, & Park, 2013;Mougey et al., 2011; Tapaninen et al., 2013). However, as this drug undergoes extensive metabolism, partly by polymorphic enzymes (e.g. CYP3A4, CYP2C8, CYP2C9), the interpretation of these studies is challenging as the data may be confounded by the metabolic component, therefore this substrate was not considered in our analysis. For the same reason, glibenclamide, mycophenolic acid and tacrolimus have been excluded from our analysis.

The other frequently studied *SLCO2B1* SNP is the non-synonymous *SLCO2B1* c.1457C > T (*SLCO2B1**3) SNP (rs230618), the allele frequency of which was reported to be only 3% in Caucasians, but over 30% in Asians (Gong & Kim, 2013;Nakanishi & Tamai, 2012). Although this polymorphism was shown to decrease the expression and function of OATP2B1 in *in vitro* systems (Nozawa et al., 2002;Tamai et al., 2000), it's *in vivo* effects remains controversial. While the exposure of orally administered atenolol was not significantly affected in carriers of the genetic variant, the serum AUC of celiprolol was nearly halved in homozygous SNP carriers (*SLCO2B1* c.1457TT) compared to the reference genotype (Ieiri et al., 2012;Jeon et al., 2013). Moreover, the mean AUC of fexofenadine after a single 60 mg oral dose was decreased by 38% and 36% in heterozygous and homozygous carriers of the variant allele, respectively (Imanaga et al., 2011). However, the completely opposite effect was reported by Akamine et al. for the (R)- and (S)-enantiomers of fexofenadine (Akamine et al., 2010). Here, the authors observed significantly increased serum AUC values for (S)-fexofenadine (+51%, $p = 0.031$), but not for (R)-fexofenadine (+20%, $p = 0.212$) in carriers of the SLCO2B1 c.1457C > T allele. In addition, a very recent study could not detect any effect of the *SLCO2B1**3 polymorphism on the pharmacokinetics of sulfasalazine or celiprolol after small oral doses of either drug (Kashihara et al., 2017). In the same study, similar results were observed for rosuvastatin and glibenclamide; however, as already mentioned above, due to the considerable metabolism of these compounds, they were not considered in our analysis. Other *SLCO2B1* polymorphisms, including c.601G>A, c.1175C>T and g.-282G>A, have been reported but their clinical impact is unknown and needs to be further assessed in humans.

With respect to OATP1A2, there are, to the best of our knowledge, only pharmacogenetic data on the anticancer drug imatinib available. While the results of the potential clinical impact of *SLCO1A2* polymorphisms are controversial (Angelini et al., 2013;Eechoute et al., 2011; Yamakawa et al., 2011), we do not consider this drug a good probe substrate to conclude on the *in vivo* relevance of OATP1A2 polymorphisms, as imatinib is transported by multiple transporters (e.g. ABCB1, ABCG2, OATP1A2 and OATP1B1) and is furthermore subjected to substantial metabolism.

Table 5
Overview of pharmacogenetics studies for OATP2B1. Only drugs with a negligible metabolism were included.

Substrate (dosage)	Genotype	Ethnicity/number of subjects	Significant PK change/AUC change compared to reference genotype	Reference
Aliskiren (150 mg, SD)	*SLCO2B1* c.935GA	Finnish/12	No/AUC↓ 2%	Tapaninen et al., 2013
	SLCO2B1 c.935AA	Finnish/5	No/AUC↑ 24%	
Atenolol (50 mg, SD)	*SLCO2B1* c.1457TT	Korean/6	No/AUC↓ 7%	Jeon et al., 2013
Celiprolol (100 mg, SD)	*SLCO2B1* c.935GA	Japanese/6	No/AUC↓ 23%	Ieiri et al., 2012
	SLCO2B1 c.935AA	Japanese/5	No/AUC↓ 12%	
	SLCO2B1 c.1457CT	Japanese/6	No/AUC↓ 29%	
	SLCO2B1 c.1457TT	Japanese/4	Yes/AUC↓ 50%	
Celiprolol (1200 μg, SD)	*SLCO2B1* c.1457TT	Japanese/7	No/unchanged AUC	Kashihara et al., 2017
Fexofenadine (60 mg, SD)	*SLCO2B1* c.1457CT	Japanese/5	No/AUC↓ 38%	Imanaga et al., 2011
	SLCO2B1 c.1457TT	Japanese/4	Yes/AUC↓ 36%	
(R)-Fexofenadine (60 mg, SD)	*SLCO2B1* c.1457CT/TT	Japanese/10	No/AUC↑ 20%	Akamine et al., 2010
(S)-Fexofenadine (60 mg, SD)	*SLCO2B1* c.1457CT/TT	Japanese/10	Yes/AUC↑ 51%	
Sulfasalazine (300 μg, SD)	*SLCO2B1* c.1457TT	Japanese/7	No/unchanged AUC	Kashihara et al., 2017

As a general limitation, all available pharmacogenetics studies on intestinal OATPs suffer from a very limited number of subjects enrolled in each genotype group, ranging from 4-12 (Table 5). Considering the substantial inter-subject pharmacokinetic variability known for the probe drugs used (e.g. fexofenadine, coefficient of variation for serum AUC: 31-46% (Glaeser et al., 2007;Imanaga et al., 2011)) and the maximal observed pharmacokinetic difference observed in different groups of SNP carriers (a 50% increase/decrease of the serum AUC), the statistical power of all available studies can be estimated to be low, for example it was 65% in the Imanaga et al. study for the comparison of *SLCO2B1* c.1457CC vs. *SLCO2B1* c.1457TT. Thus, future studies may address this issue by including more volunteers/patients and by using appropriate probe drugs that do not undergo substantial metabolism. Due to the controversial outcome of the pharmacogenetics studies involving OATP1A2 and OATP2B1, clear and reliable conclusions on the expression or function of intestinal OATP transporters cannot be derived.

4. Localization of intestinal OATP2B1: back to square one?

While we and others did not observe the presence of intestinal OATP1A2 protein, OATP2B1 has undoubtedly been shown to be expressed in the intestinal epithelia along the entire length of the human intestine (see Section 3.1). However, an indisputable prerequisite for OATP2B1 to be involved in the intestinal absorption of orally administered drugs is its localization at the apical membrane of the enterocytes. Despite this fundamental premise, there are very few studies that directly addressed this question. In this regard, Kobayashi et al. and Sai et al. showed localization of OATP2B1 to the apical membrane of human enterocytes, as well as the adenocarcinoma cell line Caco-2, using immunohistochemistry (Kobayashi et al., 2003;Sai et al., 2006). In contrast, Mooij et al. reported basolateral localization of OATP2B1, using immunohistochemical analysis of paraffin-embedded small intestinal samples, which was indirectly verified by co-localization of ABCB1, ABCC2 and PEPT1, which were all detected, as expected, in the apical membrane (Mooij et al., 2016).

Similarly, our group verified the basolateral localization of OATP2B1 in the human jejunum and Caco-2 cells by separating the basolateral and apical membrane fraction and using the targeted proteomics approach for specific and sensitive protein detection in each membrane fraction (Fig. 5) (Keiser et al., 2017). In the same work, we were not able to detect OATP2B1 in the basolateral or apical membrane of human jejununal enterocytes in a reliable manner using immunofluorescence staining, which again demonstrated the uncertainty of this method. Moreover, in functional experiments studying the vectorial transport of the OATP2B1 substrates pravastatin and sulfasalazine across human and porcine jejunum, as well as Caco-2 cell monolayers, we could confirm the basolateral localization of the protein, as the transport of both probe substrates was substantially higher from the basolateral to the apical compartment than in the opposite direction and could be modulated in the presence of OATP2B1 inhibitors (Keiser et al., 2017). In line with our findings, OATP2B1 is also located in the basolateral membrane of other human tissues such as the liver or the placenta, as well as in transfected MDCKII-OATP2B1 cells (Giacomini et al., 2010;Grube et al., 2007). Thus, it remains questionable whether the DDI studies discussed previously, which had assumed apical localization, can be explained by inhibition OATP2B1, as an uptake transporter that is located in the basolateral membrane of the enterocytes cannot contribute directly to intestinal absorption of drugs.

Interestingly, OATP2B1 was demonstrated to exhibit pH-dependent transport which is increased at acidic pH, suggesting that luminal localization would be expected to promote intestinal uptake transport function, considering the slightly acidic pH in the upper small intestine (Nozawa, Imai, Nezu, Tsuji, & Tamai, 2004). However, although this concept may be plausible it does not fit to our data on the intestinal localization and function of OATP2B1. Therefore, further studies from independent groups are needed to clarify this important uncertainty.

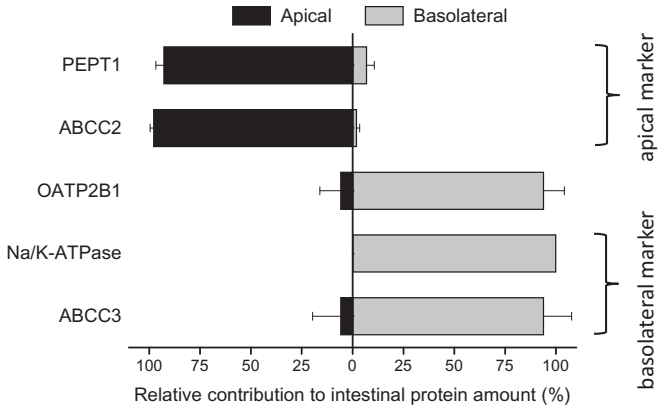

Fig. 5. Relative contribution of different intestinal transporter proteins to the apical or basolateral membrane fraction in the human jejunum taken from six organ donors. PEPT1 and ABCC2 were used as established apical reference proteins, while Na/K-ATPase and ABCC3 were used as basolateral marker proteins. Intestinal fractions were measured once each using a validated LC-MS/MS method. The individual amount of peptide was determined as an average of two to four mass transitions. Data are given as mean ± S.D. and were taken from Keiser et al., 2017.

5. Limitations of available research models for intestinal OATPs

Despite the assumed importance of OATPs for the intestinal uptake of drugs and the vast number of DDI studies which have been explained by the inhibition of intestinal OATP-mediated drug transport, most available *in vitro* data were generated using non-biorelevant models that do not mimic the complex setting found in human enterocytes, i.e. the complexity of uptake and efflux transporters localized in the apical and basolateral membrane and the interplay of metabolizing enzymes and transporter proteins (Fig. 1). In this regard, the available *in vitro* studies on the functional characterization of OATP2B1 were mostly performed in transfected and OATP2B1-overexpressing HEK293 cells or oocytes from *Xenopus laevis* (Oswald et al., 2008;Sai et al., 2006;Shirasaka, Suzuki, et al., 2010;Shirasaka, Suzuki, Nakanishi, & Tamai, 2011;Shirasaka, Shichiri, Mori, et al., 2013). Although these *in vitro* models are appropriate for the estimation of important kinetic parameters such as Km and Vmax, they do not allow any conclusions on the transport function *in vivo*, such as the localization or the vectorial transport properties of OATP2B1, because neither HEK293 cells nor *Xenopus* oocytes are polarized cells and do not form confluent monolayers, which would allow bidirectional transport studies to be performed. In contrast, MDCKII-OATP2B1 cells are able to form confluent monolayers of polarized cells, but are not suitable for bidirectional transport studies, as many established OATP2B1 *in vitro* probe substrates, such as estrone-3-sulfate, require an additional cellular efflux transporter, such as ABCG2, due to their high polarity (Grube et al., 2007;Kopplow, Letschert, Konig, Walter, & Keppler, 2005). Because of this limitation, these cells have predominately been used to study OATP2B1-dependent cellular uptake of compounds (Grube et al., 2006;Kock et al., 2010;Letschert, Faulstich, Keller, & Keppler, 2006).

To overcome this limitation, Caco-2 cells are frequently used as a rather unspecific *in vitro* model to conclude on the transport properties of OATP2B1 (Keiser et al., 2017;Ming et al., 2011;Mougey et al., 2009). Despite the established limitation that these cells were originally derived from a colorectal adenocarcinoma while the indisputable site of intestinal drug absorption is the jejunum, they form a tight cellular monolayer and express many clinically relevant uptake and efflux transporters, including ABCB1, ABCG2, PEPT1 and OATP2B1 (Bruck, Strohmeier, Busch, Drozdzik, & Oswald, 2017;Hilgendorf et al., 2007; Olander, Wisniewski, Matsson, Lundquist, & Artursson, 2016;Seithel, Karlsson, Hilgendorf, Bjorquist, & Ungell, 2006;Taipalensuu et al., 2001;Uchida et al., 2015). However, it has also been shown that gene expression and protein abundance can differ considerably between

Caco-2 cells and the human small intestine. Accordingly, especially the protein abundance of OATP2B1 was shown to be up to 16-fold higher in Caco-2 cells than in the human jejunum (Bruck et al., 2017;Olander et al., 2016;Vaessen et al., 2017). Thus, data on drug transport generated by using Caco-2 cells may bear the risk to overestimate the functional relevance of OATP2B1 (Ming et al., 2011;Sai et al., 2006). Considering, moreover, the tremendous inter-laboratory variability in the expression and function of transporter proteins that has been observed in Caco-2 cells, this *in vitro* model may not be an appropriate system to study the function of intestinal OATP2B1 (Bentz et al., 2013;Hayeshi et al., 2008). Although it was shown that Caco-2 cells express OATP2A1, OATP3A1 and OATP4A1 in addition to OATP2B1 (Kasai et al., 2016;Sai et al., 2006), the suitability of Caco-2 cells to mimic their respective *in vivo* transport properties cannot be estimated and needs further research

Unfortunately, rodent and human OATP transporters show substantial sequence differences. Thus, native animal models, such as rats and mice, are not useful to predict the *in vivo* function of human intestinal OATPs (Cao et al., 2006;Glaeser & Fromm, 2008;Tamai, 2012).

Even though there are many efforts nowadays to develop primary human enterocytes or complex organ-on-a-chip models as advanced and biorelevant intestinal *in vitro* systems, they are far off from being established and need to prove their suitability to simulate the complex processing of drugs and xenobiotics taking place in the human intestinal mucosa (Bein et al., 2018;Ho, Ring, Amaral, Doshi, & Li, 2017). From today's perspective, the most biorelevant and best established *in vitro*/*ex vivo* experiments so far may be bidirectional transport studies across fresh human or porcine intestinal mucosa using the Ussing chamber. This setting enables the simultaneously study of metabolism and transport of drugs in a biorelevant manner, as the amount and localization of the respective transport proteins can be assumed to be preserved (Kisser et al., 2017;Sjoberg et al., 2013;Westerhout et al., 2014).

6. Summary and concluding remarks on the physiological and pharmacological role of intestinal OATPs

Given the aforementioned uncertainties in the expression, localization and function of intestinal OATP transporters, it is challenging to conclude on their *in vivo* relevance in terms of translocating physiologically or pharmacologically relevant compounds. As mentioned previously, there is cumulative evidence only for the presence of OATP2A1, OATP3A1, OATP4A1 and OATP2B1 in the human intestine (Section 3.1). With respect to the frequently discussed OATP1A2, there is only one highly cited study that described its expression in the human small intestinal epithelia, whereas several other studies from different laboratories were not able to confirm this finding.

Although numerous clinically relevant interactions with juices and other perpetrators have been attributed to the inhibition of intestinal OATP1A2 and OATP2B1 (Section 3.2), there are several controversial *in vitro* and *in vivo* findings that do not support this hypothesis (Section 3.3). The same is true for the very weak and contradictory evidence provided by pharmacogenetic studies (Section 3.4). By far the most data is available for OATP2B1. Because recent data indicate basolateral localization of this protein in the intestinal epithelia (Keiser et al., 2017), there are serious doubts about the role of this transporter as an intestinal uptake transporter for drugs (Section 4). However, basolateral localization of OATP2B1 does not necessarily mean that this protein is irrelevant for the oral bioavailability of drugs. In contrast to the current understanding, basolateral OATP2B1 is expected to limit the oral absorption of its substrates by taking them up from the portal blood flow into the enterocytes to support their subsequent excretion by apical ABC transporters such as ABCB1, ABCC2 or ABCG2 (Estudante et al., 2013;Keiser et al., 2017;Muller et al., 2017). In this regard, there is substantial overlap in the substrate spectra of OATP2B1 and ABCG2, which supports this hypothesis (Giacomini et al., 2010; Kalliokoski & Niemi, 2009;Lee et al., 2015;Yu et al., 2017).

An alternative hypothesis, considering the bidirectional transport properties that have been described for some OATPs including OATP1B1/1B3 and OATP2A1, might be that basolateral OATP2B1 may also function as a basolateral gatekeeper, mediating the transport of intestinally absorbed compounds from the intracellular space of human enterocytes to the portal blood, comparable to the function of the bile acid carriers OSTalpha/beta (Mahagita, Grassl, Piyachaturawat, & Ballatori, 2007;Nakanishi & Tamai, 2017). However, this hypothesis is rather speculative and is not supported by our own transport data from Ussing chamber experiments (Keiser et al., 2017).

Finally, also from the physiological perspective, apical localization of OATP2B1 in the intestinal mucosa is questionable. In this regard, OATP2B1, which accepts several phase II metabolites such as estrone-3-sulfate and DHEAS (Tamai et al., 2001), would be expected to mediate the intestinal reabsorption of biliary secreted waste products, which is in contrast to the elimination function of the human intestine. Moreover, to the best of our knowledge, there are no pharmacological agents nor physiologically relevant compounds or nutrients known which require OATP2B1 for their intestinal absorption. This is the case, however, for several other uptake transporters that are known to be expressed at the luminal membrane of the human enterocytes, such as PEPT1 (di-/tripeptides), LAT1/2 (amino acids), MCT1 (monocarboxylates), ABCG5/G8 (cholesterol/phytosterols), ASBT (bile acids) and SGLT1/2 (glucose) (Daniel, 2004;Estudante et al., 2013;Muller et al., 2017; Thwaites & Anderson, 2007;Hediger et al., 2013).

With respect to intestinal OATP2A1, OATP3A1 and OATP4A1, the available data is very limited. OATP2A1 is known to mediate the cellular uptake of prostaglandins such as PGE_2, $PGF2\alpha$, PGD_2 and TxB_2, thus may playing a crucial role in their intracellular catabolism (Nakanishi & Tamai, 2017). While the physiological role OATP2A1 has been well-characterized in the lung, kidney, reproductive tissues, eyes and the central nervous system, rather little is known regarding its relevance in the human intestine (Nakanishi & Tamai, 2017). Because OATP2A1 gene and protein expression could be detected in human stomach and duodenum and some drugs showed substantial inhibitory or even stimulatory effects on the transport of the mucosal protective PGE_2, it was hypothesized that this may contribute to the different gastrointestinal side effects of nonsteroidal anti-inflammatory drugs (Mandery et al., 2010). OATP2A1 was also shown to control the PGE_2 release from human colorectal cancer cells *in vitro* and to promote tumorgenesis by PGE_2 uptake into the endothelial cells of a murine model, suggesting that inhibition of OATP2A1 might be a promising pharmacologic approach to treat colon cancer (Kasai et al., 2016;Nakanishi et al., 2017). Moreover, genetic polymorphisms of the *SLCO2A1* gene were reported to contribute to chronic non-specific ulcers of the small intestine (Umeno et al., 2015).

Although the limited data on intestinal gene expression and protein abundance of OATP3A1 and OATP4A1 suggest that they are more highly expressed than OATP2A1, the knowledge about their pharmacological or (patho-) physiological relevance is even more incomplete (Miyauchi et al., 2016;Nishimura & Naito, 2005;Sai et al., 2006). In this regard, in tissue from colorectal cancer patients, a significant reduction of OATP3A1 mRNA was detected, most likely due to DNA hypermethylation in the OATP3A1 promoter region, however a significant increase in OATP4A1 mRNA levels was also detected, most likely due to promoter region hypomethylation, as compared to healthy control tissue (Rawluszko-Wieczorek et al., 2015). Very recently, this induction of OATP4A1 was verified at the protein level by immunohistochemistry analysis of paraffin-embedded samples obtained from patients with early-stage colorectal cancer (Buxhofer-Ausch et al., 2018). Considering that OATP3A1 and OATP4A1 are also able to transport prostaglandins (Hagenbuch & Stieger, 2013;Shitara et al., 2013), the function of these OATPs may affect the progression of colorectal cancer and/or its response to drug therapy. A significant increase in the gene expression of OATP4A1 was also reported by Wojtal et al. in tissue samples from patients suffering from inflammatory bowel disease, which implies that

any kind of intestinal inflammation may lead to increased expression levels of OATP4A1 (Wojtal et al., 2009).

Very recently, OATP3A1 was also reported to be a cellular uptake carrier of simvastatin acid (Atilano-Roque & Joy, 2017). However, whether this might be of relevance for the intestinal absorption of the drug needs to be clarified. Similarly, OATP3A1 and OATP4A1 were reported to be transporters of triiodothyronine (T_3) and thyroxine (T_4) (Hagenbuch, 2007). Thus, these intestinal carriers are plausible uptake transporters for orally administered thyroid hormones, especially since the very recently suggested idea of OATP2B1 being an uptake carrier for T_4 may be challenged by the aforementioned basolateral localization of OATP2B1 in the intestinal epithelia (Meyer zu Schwabedissen et al., 2018).

In addition, OATP4C1 and OATP1C1 may also contribute to the intestinal absorption of T_3 and T_4 because they are high affinity transporters for these compounds and have been found to be expressed in the human small and large intestine (Hagenbuch, 2007;Hilgendorf et al., 2007;Nishimura & Naito, 2005). Moreover, digoxin and sitagliptin are also potential candidates for intestinal OATP-mediated uptake because they are established substrates of OATP4C1 (Chu et al., 2007; Yamaguchi et al., 2010).

However, this hypothesis is solely based on the available gene expression data of OATP4C1 and OATP1C1 and has yet to be verified on the protein level, which would allow reliable conclusions on their potential function in the human intestine. Moreover, the summarized evidence for OATP2A1, OATP3A1, OATP4A1, and probably also for OATP4C1 and OATP1C1, assume their apical localization in the brush border membrane of the human intestine, which also needs to be confirmed by respective analysis.

In conclusion, even after more than a decade of research on intestinal transporter proteins, the understanding of the functional relevance of intestinal OATP transporters is still very limited. In contrast to hepatic OATP transporters and intestinal efflux transporters, which have been extensively studied *in vivo* and *in vitro* and for which functional contribution can even be predicted in a quantitative manner by sophisticated PBPK algorithms (Fan, Chen, Chow, & Pang, 2010), there are many gaps regarding very basic data on the expression, localization, regulation and function of intestinal OATP transporters. This might be due to the lack of appropriate *in vitro* and *ex vivo* models of the human intestine (Section 5). In addition, the frequently used DDI and food-drug interaction studies are much too complex to allow reliable conclusions on the function of a specific intestinal OATP transporter.

Finally, there is so far a very limited interest by the research community and even the regulatory authorities to comprehensively understand the process of intestinal drug absorption; instead, preclinical and clinical programs focus almost exclusively on the intestinal efflux transport by ABCB1 or ABCG2, the hepatic by OATPs or renal uptake by OATs and OCTs. However, one must consider that the majority of all drugs on the market are administered via the oral route. Considering, the high inter-subject variability in the oral bioavailability of many drugs and the frequently occurring but poorly predictable DDI due to inhibition or induction of intestinal transporters or metabolizing enzymes, which altogether may threaten the therapeutic efficacy and safety of drugs, we should perhaps pay more attention to the molecular mechanisms of intestinal drug uptake by OATPs and other transporters such as OCTs, PMAT, SLC22A18 and HPT1 to try to fill the existing gaps in knowledge which have been described in this review.

Conflict of interest statement

The author declares to have no conflict of interest.

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