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Editors

Drug Transporters

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Preface

In the past 15 years, there has been a dramatic increase in the extent of our knowledge regarding the importance of transporter proteins that govern drug disposition and response. For example, the human multidrug and toxin extrusion protein 1, which is important for the elimination of organic cations and drugs, was cloned and functionally characterized in 2005. Regarding their function, transporters can be classified as uptake or efflux transporters and mediate the uptake of endogenous compounds and drugs into or out of the cells, respectively. This book focuses on transporters of the solute carrier family (SLCs; e.g., organic anion transporting polypeptides, OATPs; organic anion transporters, OATs; organic cation transporters, OCTs; multidrug and toxin extrusion proteins, MATEs; apical sodium-dependent bile acid cotransporter, ASBT; sodium taurocholate transporting polypeptide, NTCP) and the ATP-binding cassette (ABC) transporters (e.g., bile salt export pump, BSEP; P-glycoprotein; multidrug resistance proteins, MRPs; breast cancer resistance protein, BCRP). Each tissue has a distinct pattern of expression for uptake and efflux transporters.

The main focus of this book is transporters expressed in the intestine, liver, and kidney of relevance to drug response and toxicity. Increasing intensity of research in this area has resulted in a much better understanding of the localization of transporters in cell membranes and their role for polarized drug transport. For example, drugs are delivered via the portal venous blood to the basolateral membrane of hepatocytes and taken up by distinct transporters localized in the basolateral membrane into hepatocytes with subsequent intracellular phase I and II metabolism and excretion of parent compounds or of metabolites via other transporters localized to the canalicular membrane into bile. Individual chapters in this book will also address the role of transporters located in tissues other than intestine, liver, and kidney to the local accumulation and effect of drugs at the site of action (e.g., CNS accumulation of HIV protease inhibitors and P-glycoprotein in the blood–brain barrier).

As highlighted in this book, transporters are also important to our understanding of (patho-)physiological processes as well as drug disposition and effects. For

example, the chapter on intestinal bile acid transporters highlights not only our current understanding of the absorption of bile acids from the intestinal lumen, but also shows how this knowledge is currently used for development of new hypocholesterolemic or hepatoprotective drugs.

Interindividual variability in drug response is a major problem for optimal drug therapy. The transporter field has contributed substantially to a better understanding of the determinants that account for intersubject differences in drug disposition and effects. Genetic polymorphisms in transporters can cause certain diseases, for example the Dubin-Johnson syndrome, in patients with certain mutations in the *ABCC2* gene encoding MRP2. Moreover, genetic polymorphisms in genes encoding uptake and efflux transporters have been identified as determinants of drug disposition. The knowledge summarized in this book on substrate specificity of individual transporters as well as the potential of drugs for inhibiting specific transporters has helped improve our understanding of mechanisms for drug–drug interactions. For example, increased plasma concentrations and toxicity of the cardiac glycoside digoxin with coadministration of multiple drugs (e.g., quinidine, verapamil) have been observed dating back to the 1970s, but the mechanism underlying these drug–drug interactions remained unclear for a long time. This changed when digoxin was identified as substrate of the efflux pump P-glycoprotein and when comedications such as quinidine and verapamil were identified as potent inhibitors of P-glycoprotein function.

For these reasons, regulatory agencies are increasingly asking pharmaceutical companies for detailed information on whether transporters are involved in disposition of a new drug entity and whether the new drug entity itself might cause undesired drug–drug interactions due to inhibition of specific drug transporters. This process is supported by the International Transporter Consortium, which recently published recommendations for investigations on Membrane Transporters in Drug Development (International Transporter Consortium 2010). However, it should be noted that our knowledge of the role of transporters in the disposition and effects of older, marketed drugs is far from complete and clearly requires further investigation.

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Reference

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Uptake Transporters of the Human OATP Family

Molecular Characteristics, Substrates, Their Role in Drug–Drug Interactions, and Functional Consequences of Polymorphisms

Jörg König

Contents

Abstract Organic anion transporting polypeptides (OATPs, gene family: *SLC21/SLCO*) mediate the uptake of a broad range of substrates including several widely prescribed drugs into cells. Drug substrates for members of the human OATP family include HMG-CoA-reductase inhibitors (statins), antibiotics, anticancer agents, and cardiac glycosides. OATPs are expressed in a variety of different tissues including brain, intestine, liver, and kidney, suggesting that these uptake transporters are important for drug absorption, distribution, and excretion. Because of their wide tissue distribution and broad substrate spectrum, altered transport kinetics, for example, due to drug–drug interactions or due to the functional consequences of genetic variations (polymorphisms), can contribute to the interindividual variability of drug effects. Therefore, the molecular characteristics of human OATP family members, the role of human OATPs in drug–drug interactions, and the in vitro analysis of the functional consequences of genetic variations in *SLCO* genes encoding OATP proteins are the focus of this chapter.

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Abbreviations

BSP	Bromosulfophthalein
K_m	Kinetic constant (Michaelis–Menten constant)
OATP	Organic anion transporting polypeptide
OCT	Organic cation transporter
SLCO	Gene of the SLCO family encoding OATP uptake transporters
V_{max}	Maximal transport velocity

1 Introduction

Transport proteins located in plasma membranes of cells are important for the absorption, distribution, and excretion of endogenous compounds and drugs. Principally, they can be subdivided into two major groups: uptake transporters mediating the uptake of substances from the extracellular space into cells, and export pumps actively secreting substances out of cells. Most of these export pumps belong to the superfamily of ABC (ATP-binding cassette) transporters (Fromm 2003; Keppler et al. 2001; König et al. 1999; Kruh and Belinsky 2003) energized by the hydrolysis of ATP and secreting substances (e.g., drugs or drug conjugates) against a concentration gradient [see respective chapters about P-glycoprotein, multidrug resistance proteins (MRPs), or the breast cancer resistance protein (BCRP)]. Uptake transporters mostly belong to the superfamily of solute carriers [SLC superfamily (Hediger et al. 2004)]. Currently, this superfamily is comprised of 48 SLC families (SLC1–SLC48) with more than 360 identified members (see: www.bioparadigms.org/slc/intro). Genes encoding the organic anion transporting polypeptide (OATP in humans and Oatp in rodents) are classified as the SLCO family (formerly termed SLC21 family). Today, the human OATP family consists of 11 members (Hagenbuch and Meier 2003; Mikkaichi et al. 2004b) including 10 OATPs and the prostaglandin transporter OATP2A1 [formerly termed PGT (Lu et al. 1996)]. Because trivial names for individual proteins do not correspond to the continuous numbering based on the chronology of protein identification and because some rodent Oatp proteins have no direct human orthologue, Hagenbuch and Meier (2004) introduced a new nomenclature for the entire OATP family. In this chapter I will follow this new nomenclature and designate all human proteins in capitals (e.g., OATP1B1), all rodent proteins as, for example, Oatp1a1, and all

genes encoding OATP proteins according to the SLCO gene nomenclature in italics (e.g., *SLCO1B1* encoding the human hepatocellular uptake transporter OATP1B1).

OATPs are a group of membrane carriers with a wide spectrum of amphipathic transport substrates (König et al. 2006; Kullak-Ublick et al. 2000, 2001; Meier et al. 1997). Although some members in rodents and humans are predominantly, if not exclusively expressed in liver (Hsiang et al. 1999; König et al. 2000a, b), most OATP/Oatp family members are expressed in multiple tissues including brain (Kusuhara and Sugiyama 2005), kidney (van Montfoort et al. 2003), and intestine (Kunta and Sinko 2004; Zair et al. 2008). As characterized so far, OATPs have a wide substrate spectrum transporting several endogenously synthesized compounds (e.g., bile salts, hormones steroid conjugates) as well as several xenobiotics and widely prescribed drugs like HMG-CoA-reductase inhibitors (statins), anticancer agents, and antibiotics [see reviews (Hagenbuch and Gui 2008; König et al. 2006; Kullak-Ublick et al. 2001; Niemi 2007)].

Because of their wide substrate spectrum and their expression in several tissues important for the absorption, distribution, and excretion of drugs, altered transport kinetics of these uptake transporters may contribute to the interindividual variability in drug response. Two major molecular mechanisms may account for altered uptake transporter kinetics. First, the functional consequences of genetic variations (so-called polymorphisms) in transporter genes resulting in amino acid exchanges in the transport protein can influence the expression, localization or transport kinetics of the uptake transporter. If, for example, a mutation in the hepatocyte-specific uptake transporter OATP1B1 leads to reduced hepatic uptake of drug substrates, the plasma concentration of these drugs increases due to decreased transport of the drug from blood into hepatocytes compared with individuals carrying the wild-type protein. A second possibility for altered uptake kinetics of one drug is the coadministration of a second drug, which is also a substrate of the drug transporter. This so-called transporter-mediated drug–drug interaction is another reason for adverse drug reactions as the inhibition or stimulation of drug uptake into cells may, as described already in detail for the inhibition of metabolizing enzymes, alter the pharmacokinetics of one or both drug substrates.

Therefore, members of the human OATP family of uptake transporters, their molecular features, their role in transporter-mediated drug–drug interactions, and the in vitro analysis of the functional consequences of polymorphisms in transporter genes encoding mutated uptake transporter proteins are the focus of this chapter.

2 The Human OATP Family

2.1 Molecular Characteristics of Human OATP Family Members

Rat Oatp1a1 (formerly termed oatp or Oatp1) was the first member of the OATP/Oatp family identified in 1994 by expression cloning (Jacquemin et al. 1994). It was demonstrated that this transport protein mediates the uptake of several organic

anions including conjugated and unconjugated bile salts (Kullak-Ublick et al. 1994). Based on this sequence information several Oatp1a1-related transport proteins could be identified and characterized in the following years, and today more than 40 different OATP/Oatp family members from rat, mouse, and human are known (Hagenbuch and Meier 2003; Mikkaichi et al. 2004b). The phylogenetic classification of human and rodent (mouse and rat) members of the OATP/Oatp family demonstrates that most of the human OATP family members have direct orthologous proteins in both rodent species as shown by related rat and mouse sequences (Fig. 1). Interestingly, the founding member of the human OATP family, OATP1A2, and both highly-in-hepatocytes-expressed family members OATP1B1 and OATP1B3, have no direct rodent orthologues. For OATP1A2 several rat and mouse Oatps could be identified as closely related proteins, and OATP1B1 and OATP1B3 have the highest sequence homology to each other, with mouse or rat Oatp1b2 being a more remote relative (Fig. 1). Orthologous proteins in rat and/or mouse showing the same tissue expression pattern and a comparable substrate spectrum to the respective human family member are the prerequisite for using animals as model systems for the functional characterization of the human proteins. Because both hepatocyte-specific OATPs OATP1B1 and OATP1B3 have no

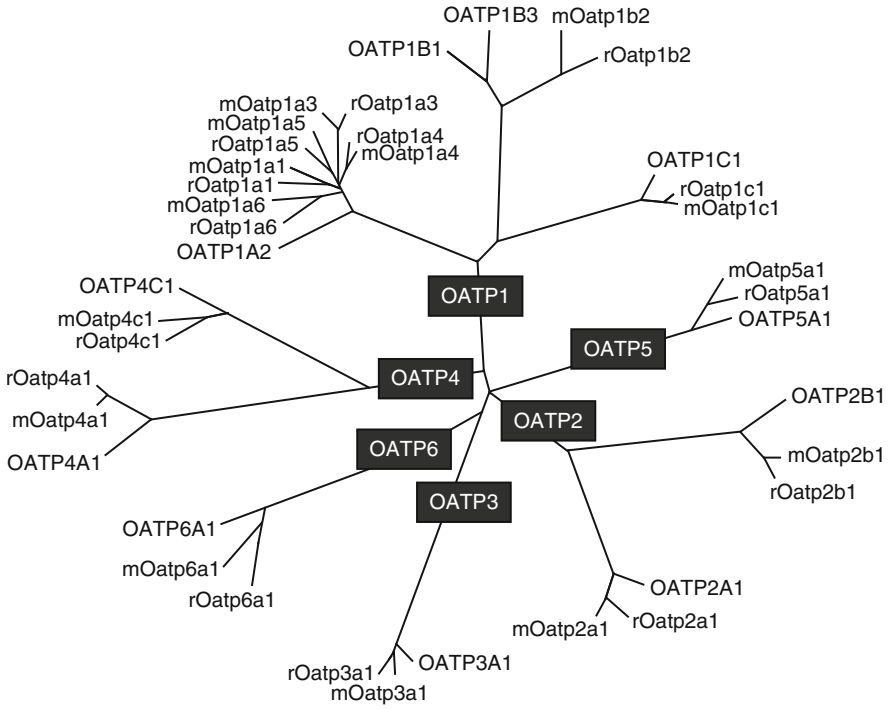


Fig. 1 Phylogenetic tree of human and rodent OATP/Oatp family members. The OATP subfamilies (OATP1–OATP6) are depicted in boxes. rOatp stands for the rat family member, mOatp for the respective mouse Oatp family member

orthologous proteins in other species, it has become evident that the transferability of animal data (e.g., analyzing the hepatobiliary elimination of endogenous substances or drugs) to the human situation at least regarding the function of the hepatic OATP proteins is limited. Therefore, the availability of genetically engineered cell models stably expressing the respective human OATP family members is important for the functional analysis of these uptake transporters.

Interestingly, the four human OATPs of the OATP1 subfamily (OATP1A1, OATP1B1, OATP1B3, and OATP1C1) are all localized on the short arm of chromosome 12 (chromosome 12p12), whereas the genes of all other members of the OATP family are distributed over the whole genome (Table 1). The proteins have a medial length of 710 amino acids with OATP2A1 being the shortest family member (643 amino acids) and OATP5A1 the longest (848 amino acids).

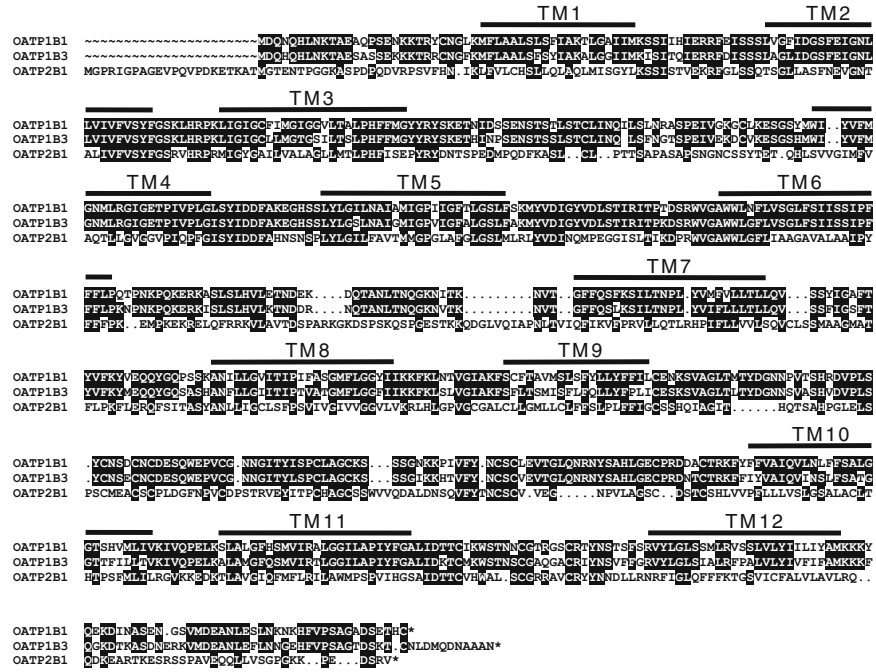
All OATP/Oatp family members share a very similar topology (Fig. 2). Based on computational analyses they consist of 12 transmembrane domains [so-called TMs; (Fig. 2)] and a large fifth extracellular loop between TMs 9 and 10. This loop contains many conserved cysteine residues. N-glycosylation sites are in the extracelluar loops two and five and the OATP family signature D-X-RW-(I,V)-GAWW-X-G-(F,L)-L is located at the border between extracellular loop 3 and TM 6 (Hagenbuch and Meier 2003). Based on expressed sequence tags (EST) and genomic database entries OATP-related proteins were identified in many other species including nematodes (*Caenorhabditis elegans*), zebrafish (*Danion rerio*), the frog *Xenopus laevis*, chicken (*Gallus gallus*), fruitfly (*Drosophila melanogaster*), pig (*Sus scrofa*), and cow (*Bos taurus*). Interestingly, although studied intensively, no sequence similarities were found in the genomes of bacteria and yeast.

Although most of the human OATP family members are expressed in multiple tissues, OATP1B1 and OATP1B3 are predominantly if not exclusively expressed in liver (Hsiang et al. 1999; König et al. 2000a, b). Despite the fact that some studies identified *SLCO1B1* (expressing OATP1B1) and *SLCO1B3* (expressing OATP1B3)

Table 1 Characteristics of human OATP family members

Protein name	Gene symbol	Sequence accession ID	Chromosomal localization	Amino acids	Tissue distribution
OATP1A2	*SLCO1A2*	NM_021094	12p12	670	Brain, Kidney
OATP1B1	*SLCO1B1*	NM_006446	12p	691	Liver
OATP1B3	*SLCO1B3*	NM_019844	12p12	702	Liver
OATP1C1	*SLCO1C1*	NM_017435	12p12.2	712	Brain, Testis
OATP2A1	*SLCO2A1*	NM_005630	3q21	643	Ubiquitous
OATP2B1	SLCO2B1	NM_007256	11q13	709	Ubiquitous
OATP3A1	*SLCO3A1*	NM_013272	15q26	710	Ubiquitous
OATP4A1	*SLCO4A1*	NM_016354	20q13.33	722	Ubiquitous
OATP4C1	*SLCO4C1*	NM_180991	5q21.2	724	Kidney
OATP5A1	*SLCO5A1*	NM_030958	8q13.3	848	?
OATP6A1	*SLCO6A1*	NM_173488	5q21.1	719	Testis(?)

Data compiled from NCBI (www.ncbi.nml.nih.gov) and SLC tables (www.bioparadigms.org/slc) databases



	OATP1A2	OATP1B1	OATP1B3	OATP1C1	OATP2B1	OATP3A1	OATP4A1	OATP4C1	OATP5A1	OATP6A1
OATP1A2	100	43,8	42,2	47,5	33,9	35,9	32,4	32,5	34,5	24,6
OATP1B1		100	43,8	46,6	34,7	37,3	31,4	34,4	35,9	27,5
OATP1B3			100	48,3	35,6	36,0	33,6	34,9	36,9	26,1
OATP1C1				100	34,1	37,3	33,8	32,9	33,8	25,4
OATP2B1					100	36,0	33,8	34,8	36,2	27,1
OATP3A1						100	38,3	39,0	38,8	28,6
OATP4A1							100	46,1	41,5	35,3
OATP4C1								100	37,4	44,8
OATP5A1									100	28,9
OATP6A1										100

Fig. 2 Box alignment of the hepatic OATP family members OATP1B1, OATP1B3, and OATP2B1 and amino acid identities of the human OATP family members. Predicted transmembrane domains are shown above the alignment (TM1–TM12)

mRNA expression in the intestine (Glaeser et al. 2007) or (in the case of OATP1B3) in colorectal carcinoma (Abe et al. 2001; Lee et al. 2008) no protein data have been published so far demonstrating the occurrence of both proteins in other tissues than human liver. Although identified and cloned from human liver (Kullak-Ublick et al. 1995), OATP1A2 shows a very low expression there, but it is highly expressed in brain and testis (Kullak-Ublick et al. 1995). OATP2B1, OATP3A1, and OATP4A1 seem to be ubiquitously expressed in all tissues investigated so far, whereas

OATP4C1 is highly expressed in kidney (Mikkaichi et al. 2004a). No protein expression data have been published from human OATP5A1 and OATP6A1, in rats Oatp6a1 is expressed at the blood–testis barrier (Augustine et al. 2005). Almost all OATP family members are localized to the basolateral membrane of polarized cells. OATP1B1, OATP1B3, and OATP2B1 are localized to the basolateral membrane of human hepatocytes (Hsiang et al. 1999; König et al. 2000a, b), whereas OATP1A2 has been localized to the basolateral membrane of brain epithelial cells (Lee et al. 2005) and to the basolateral membrane of proximal kidney tubule cells (Kullak-Ublick et al. 1995). Interestingly, in addition to the basolateral localization of OATP1A2 and OATP2B1 both proteins have been detected in the apical membrane of enterocytes (Glaeser et al. 2007; Kobayashi et al. 2003). OATP4A1 is predominantly localized at the apical surface of the syncytiotrophoblast in placenta (Sato et al. 2003) and, together with OATP3A1 and OATP1C1 to the basolateral membrane of the nonpigmented human ciliary body epithelium (Gao et al. 2005). OATP4C1 has been found to be highly expressed at mRNA level in human kidney, and so far only rat Oatp4c1 has been localized to the basolateral membrane of kidney proximal tubular cells (Mikkaichi et al. 2004a). The expression and subcellular localization of human OATP5A1 and OATP6A1 remains to be analyzed.

Despite the fact that all human OATP proteins share a similar membrane topology the knowledge on protein regions or structures involved in the transport process is limited. Some indirect evidence identifying amino acid residues important for substrate recognition or transport resulted from the characterization of the functional consequences of polymorphisms. Analyzing the effect of mutations in the second extracellular loop of the OATP1B1 protein showed that some residues located there are important for substrate recognition (Michalski et al. 2002), whereas several in vitro and in vivo studies have demonstrated that the exchange OATP1B1p.V174A (allelic nomenclature: OATP1B1*5) is critical for transport kinetics [for review see: (König et al. 2006; Seithel et al. 2008)]. Only a few studies directly used side-directed mutagenesis to identify structural domains and/or residues important for substrate selectivity or transport. One study identified three key residues located in the transmembrane domain 10 (TM10) of the OATP1B3 protein (Gui and Hagenbuch 2008), being important for the transport of the OATP1B3 substrate choleocystokinin-8 (CCK-8). In detail, mutations of the residues Tyr537, Ser545, and Thr550 alone or in combination alter the kinetic constant (K_m value) as well as the maximal transport velocity (V_{max} value) of the OATP1B3 protein, suggesting that TM10 is important for the transport process. A second study published by Miyagawa et al. (2009) demonstrated that alterations in the eighth and ninth transmembrane domain of the OATP1B1 protein resulted in a change of the transport kinetics of the mutated protein. A detailed study analyzing conserved lysine and arginine in transmembrane helices demonstrated that the amino acid residues Lys41 (in TM1) and Arg580 (in TM11) are pivotal for the transport activity of the OATP1B3 protein (Glaeser et al. 2009). These few studies demonstrated that several regions in the protein should be important for substrate recognition and transport, and it would be important to have data based on the crystal

structure of an OATP/Oatp protein to gain more insight into the molecular nature of the translocation of substrates from one site of the membrane to the other.

2.2 Substrate Spectrum of Human OATP Family Members

OATP1A2 (formerly termed OATP or OATP-A) was cloned based on its homology to rat Oatp1a1 [rat Oatp1 (Jacquemin et al. 1994)] as a sodium-independent uptake transporter for the organic anion bromosulfophthalein and bile salts in human liver (Kullak-Ublick et al. 1995). Using *Xenopus laevis* oocytes it was shown that OATP1A2 transports the bile acids cholate, taurocholate, glycocholate, taurochenodeoxycholate, and tauroursodeoxycholate (Kullak-Ublick et al. 1995). Further studies investigating the substrate spectrum of OATP1A2 in more detail demonstrated the capability of OATP1A2 to transport a wide range of amphipathic organic anions including bile salts, steroid hormones, and their conjugates (Bossuyt et al. 1996), thyroid hormones (Friesema et al. 1999) as well as some organic cations like *N*-methyl-quinidine (van Montfoort et al. 1999).

Interestingly, studies determining the substrate spectrum of other human OATP family members demonstrated that most of them have a similarly broad substrate spectrum at least partially overlapping with the substrate spectrum of OATP1A2. Best-characterized members of the human OATP family with respect to the substrate spectrum are the hepatocyte-specific OATPs OATP1B1 and OATP1B3. For these uptake transporters several endogenously synthesized substances have been identified as substrates including bile salts like taurocholate (Abe et al. 1999, 2001; Cui et al. 2001; Hsiang et al. 1999; Letschert et al. 2004), conjugated steroids like estradiol-17ß-glucuronide (König et al. 2000a, b), or estrone-3-sulfate (Nozawa et al. 2004c; Tamai et al. 2000) and hormones like tyroxine (Abe et al. 1999; Kullak-Ublick et al. 2001; Omote et al. 2006). The so-called model substrate for these OATPs and for OATP1A2 is the organic anion bromosulfophthalein (BSP), which is transported with very low K_m values of 20 µM, 0.1 µM, and 3.3 µM for OATP1A2, OATP1B1, and OATP1B3, respectively (Cui et al. 2001; König et al. 2000a; Kullak-Ublick et al. 2001). Interestingly, for other human OATPs the substrate spectrum has not been characterized in that detail, and only a few substances have been identified as substrates for OATP2B1, OATP1C1, OATP3A1, OATP4A1, and OATP4C1, whereas to this day no substrates have been identified for OATP5A1 and OATP6A1. A substrate common for all OATPs (except OATP4C1) is estrone-3-sulfate, whereas BSP is transported in addition by OATP1C1 and OATP2B1 (Kullak-Ublick et al. 2001; Pizzagalli et al. 2002).

Beside endogenously synthesized substances, several drugs have been identified as substrates for human OATP family members (Table 2). Especially the hepatocyte-specific family member OATP1B1 seems to be important in drug transport mediating the uptake of a variety of different drug substrates from blood into hepatocytes. Drug substrates of OATP1B1 include HMG-CoA-reductase inhibitors (statins) like

Table 2 Selected drug substrates for human OATP family members

Substrate	K_m value (μM)	References
OATP1A2		
BQ123		Kullak-Ublick et al. (2001)
Chloambuciltaurocholate		Kullak-Ublick et al. (1997)
Bamet-R2	24	Briz et al. (2002)
Bamet-UD2	14	Briz et al. (2002)
Deltorphin II	330	Gao et al. (2000)
DPDPE	202	Gao et al. (2000)
Fexofenadine	6	Cvetkovic et al. (1999)
GD-B20790	92	Pascolo et al. (1999)
N-Methyl-quinidine	26	van Montfoort et al. (1999)
Methotrexate	457	Badagnani et al. (2006)
Ouabain	5.5	Bossuyt et al. (1996)
Rocuronium		van Montfoort et al. (1999)
Tauroursodeoxycholate	19	Kullak-Ublick et al. (1995)
OATP1B1		
Atorvastatin	12	Kameyama et al. (2005)
Atrasentan		Katz et al. (2006)
Benzylpenicillin		Tamai et al. (2000)
Bamet-R2	10	Briz et al. (2002)
Bamet-UD2	10	Briz et al. (2002)
Bosentan	44	Treiber et al. (2007)
BQ-123		Kullak-Ublick et al. (2001)
Caspofungin		Sandhu et al. (2005)
Cerivastatin		Shitara et al. (2004b)
DADLE		Nozawa et al. (2003)
DPDPE		Kullak-Ublick et al. (2001)
Enalapril	260	Liu et al. (2006)
Fluvastatin	3.5	Kopplow et al. (2005)
Glycoursodeoxycholate	5	Maeda et al. (2006b)
Methotrexate		Abe et al. (2001)
Olmesartan	13	Yamada et al. (2007)
Pitavastatin	3	Hirano et al. (2004)
Pravastatin	35	Hsiang et al. (1999)
Rifampin	2	Tirona et al. (2003)
Rosuvastatin	8	Schneck et al. (2004)
SN-38		Nozawa et al. (2005)
Tauroursodeoxycholate	8	Maeda et al. (2006b)
Temocaprilat		Maeda et al. (2006a)
Troglitazone sulphate		Nozawa et al. (2004b)
TR-14035	7.5	Tsuda-Tsukimoto et al. (2006)
Valsartan	1.4	Yamashiro et al. (2006)
OATP1B3		
Atrasentan		Katz et al. (2006)
Bosentan	141	Treiber et al. (2007)
BQ-123		Kullak-Ublick et al. (2001)
Deltorphin II		Kullak-Ublick et al. (2001)
Digoxin		Kullak-Ublick et al. (2001)
Docetaxel		Smith et al. (2005)
DPDPE		Abe et al. (2001)
Enalapril		Liu et al. (2006)
Fexofenadine	108	Shimizu et al. (2005)

(*continued*)

Table 2 (continued)

Substrate	K_m value (μM)	References
Fluvastatin		Kopplow et al. (2005)
Glycoursodeoxycholate	25	Maeda et al. (2006b)
Methotrexate	25	Abe et al. (2001)
Olmesartan	44	Yamada et al. (2007)
Ouabain		Kullak-Ublick et al. (2001)
Paclitaxel	7	Smith et al. (2005)
Pitavastatin	3	Hirano et al. (2004)
Pravastatin		Seithel et al. (2007)
Rifampin	2	Vavricka et al. (2002)
Tauroursodeoxycholate	16	Maeda et al. (2006b)
Telmisartan	0.8	Ishiguro et al. (2006)
TR-14035	5.3	Tsuda-Tsukimoto et al. (2006)
Valsartan	18	Yamashiro et al. (2006)
OATP2B1		
Atorvastatin	0.2	Grube et al. (2006)
Benzylpenicillin		Tamai et al. (2000)
Bosentan	202	Treiber et al. (2007)
CP-671.305	4	Kalgutkar et al. (2007)
Fexofenadine		Nozawa et al. (2004a)
Fluvastatin	0.8	Kopplow et al. (2005)
Glibenclamide	6.3	Satoh et al. (2005)
M17055	4.5	Nishimura et al. (2007)
OATP3A1		
Benzylpenicillin		Tamai et al. (2000)
OATP4A1		
Benzylpenicillin		Tamai et al. (2000)
OATP4C1		
Digoxin	8	Mikkaichi et al. (2004a)
Methotrexate		Mikkaichi et al. (2004a)
Ouabain	0.4	Mikkaichi et al. (2004a)
OATP5A1 and OATP6A1		
Neither endogenous substrates nor drugs as substrates identified yet		

Bamet-R2: [*cis*-diammine-chloro-cholylglycinate-platinum(II)], Bamet-UD2: [*cis*-diammine-bisursodeoxycholate-platinum(II)], DADLE: [D-Ala(2), D-Leu(5)]enkephalin, DPDPE: [D-penicillamine-2,5]enkephalin, M17055: 7-chloro-2,3-dihydro-1-(2-methylbenzoyl)-4(1H)-quinolinone 4-oxime-O-sulfonic acid potassium salt, TR-14035: *N*-(2,6-dichlorobenzoyl)-4-(2′,6′-bismethoyphenyl)phenylamine

pravastatin (Hsiang et al. 1999), rosuvastatin (Schneck et al. 2004), atorvastatin (Kameyama et al. 2005), and pitavastatin (Hirano et al. 2004). Other drugs transported by OATP1B1 include antibiotics [benzylpenicillin, rifampicin (Tamai et al. 2000; Tirona et al. 2003)], antineoplastic agents like methotrexate (Abe et al. 1999), the endothelin receptor antagonist bosentan (Treiber et al. 2007), and the angiotensin II receptor antagonist valsartan (Yamashiro et al. 2006). Interestingly, for some drugs it has been shown by in vivo studies that genetic variations in the *SLCO1B1* gene encoding OATP1B1 are associated with altered pharmacokinetics of this drug, but direct evidence that this drug is also a transport substrate for OATP1B1 is lacking. So it has been demonstrated that the genetic variation *SLCO1B1c.521T>C*

(resulting in the protein OATP1B1p.Val174Ala; OATP1B1*5) is associated with an increase in the area under the concentration time curve (AUC) for the oral antidiabetic drug repaglinide (Niemi et al. 2005), clearly showing that this genetic variation is a major determinant in the interindividual variability of repaglinide pharmacokinetics. In vitro studies using stably transfected HEK293 cells confirmed these results showing that repaglinide inhibits OATP1B1-mediated BSP and pravastatin uptake (Bachmakov et al. 2008), but studies demonstrating that repaglinide is an OATP1B1 substrate have not been published to date. On the other hand it has been demonstrated that this genetic variation did not affect the pharmacokinetic of rosiglitazone, a second oral antidiabetic drug (Kalliokoski et al. 2007), whereas in vitro studies have demonstrated that rosiglitazone affects OATP1B1-mediated uptake (Bachmakov et al. 2008). Like shown for the organic cation transporters (OCTs, gene family *SLC22*) it seems that substances may interact with OATPs by influencing transport kinetics without being transported themselves.

Because OATP1B1 and OATP1B3 share a nearly identical substrate spectrum, most of the drugs identified as substrates for OATP1B1 are also transported by OATP1B3 (Table 2). These OATP1B3 drug substrates include statins [pravastatin, fluvastatin, pitavastatin (Hirano et al. 2004; Kopplow et al. 2005; Seithel et al. 2007)], as well as antibiotics [rifampicin (Vavricka et al. 2002)] and antineoplastic agents [methotrexate (Abe et al. 2001)]. In addition, the antihistaminic drug fexofenadine (Shimizu et al. 2005) and the chemotherapeutic agent paclitaxel (Smith et al. 2005) have been identified as substrates for OATP1B3. Fexofenadine has also been shown to be a substrate for OATP1A2 (Cvetkovic et al. 1999), which in addition also transports the muscle relaxant rocuronium (van Montfoort et al. 1999) and the cardiac glycoside ouabain (Bossuyt et al. 1996). The knowledge on drugs as substrates for other human OATP family members is limited. Some drugs have been identified as substrates for OATP2B1 including statins [atorvastatin, fluvastatin (Grube et al. 2006; Kopplow et al. 2005), fexofenadine (Shimizu et al. 2005), and the antidiabetic drug glibenclamide (Satoh et al. 2005)]. Selected drug substrates for human OATP family members are presented in Table 2.

2.3 *Hepatic OATPs and Drug–Drug Interactions*

Besides the impact of polymorphisms in *SLCO*/*SLC21* genes encoding human OATPs on the pharmacokinetics of drug substrates, a second possibility influencing drug plasma concentrations are transporter-mediated drug–drug interactions. Because several widely prescribed drugs (e.g., statins) are substrates of hepatic OATP proteins, concomitantly administered drugs, which are also substrates for these uptake transporters, may lead to elevated plasma levels of statins due to their reduced hepatic uptake. Statins are used as inhibitors of the de novo synthesis of cholesterol in the liver and are widely used to treat dyslipidaemia. OATP-mediated uptake of statins into hepatocytes is a prerequisite for the subsequent intracellular inhibition of the HMG-CoA-reductase, and reduced hepatic uptake of statins due to

coadministered OATP drug substrates may cause severe side effects such as myopathy and rhabdomyolysis (Bruno-Joyce et al. 2001; East et al. 1988).

Macrolides are known to cause severe drug–drug interactions and it has been demonstrated that some of these interactions are due to the inhibition of metabolizing enzymes. Some macrolides have been identified as potent inhibitors of the phase I enzyme CYP3A4, and this macrolides-induced inhibition may increase the plasma concentrations of coadministered drugs that are also CYP3A4 substrates (Ito et al. 2003; Polasek and Miners 2006). Clarithromycin, for example, increases the plasma concentrations of the concomitantly administered statins atorvastatin and simvastatin that are both metabolized by CYP3A4. Interestingly, clarithromycin also increases the plasma concentration of coadministered pravastatin, which is not metabolized by cytochromes and excreted almost unchanged into bile (Jacobson 2004), and the observed interaction cannot be due to the inhibition of metabolizing enzymes. Therefore, Seithel et al. analyzed whether macrolides inhibit OATP1B1- and OATP1B3-mediated uptake of pravastatin in stably transfected HEK cells recombinantly expressing these uptake transporters (Seithel et al. 2007). They found that not only clarithromycin but also erythromycin and roxithromycin inhibited OATP1B1- and OATP1B3-mediated pravastatin uptake (Fig. 3). Interestingly, this uptake inhibition by clarithromycin and roxithromycin was significant at the tested low macrolides concentration of 10 μM, which is below the calculated macrolides concentration in the portal venous blood after oral administration. These in vitro studies demonstrated that besides the inhibition of metabolizing enzymes by macrolides the inhibition of uptake transport proteins could be responsible for macrolides-induced alterations in the plasma concentrations of concomitantly administered drugs.

In addition to oral antidiabetic drugs (metformin, glitazones, or repaglinide), most patients with type 2 diabetes are concomitantly treated with cardiovascular drugs (e.g., statins or ACE inhibitors). Niemi and coworkers have demonstrated that the genetic variation *SLCO1B1c.521T>C* encoding the OATP1B1*5 variant is associated with altered plasma concentrations of repaglinide (Niemi et al. 2005), whereas glitazones are known substrates for OATP1B1 (Nozawa et al. 2004b). Interestingly, although the OATP1B1*5 variant leads to altered plasma concentrations of repaglinide it has obviously no effect on the pharmacokinetics of rosiglitazone (Kalliokoski et al. 2007). Based on these data Bachmakov and coworkers investigated whether the oral antidiabetic drugs metformin, repaglinide, and rosiglitazone affect OATP1B1- and OATP1B3-mediated pravastatin uptake (Bachmakov et al. 2008). Using HEK293 cells stably expressing human OATP1B1 or OATP1B3 they demonstrated that repaglinide inhibited OATP1B1- and OATP1B3-mediated pravastatin uptake at a low concentration of 10 μM. Interestingly, rosiglitazone at the same low concentration stimulated pravastatin uptake into OATP1B1- and OATP1B3-expressing cells to 170% and 400%, respectively, and inhibited pravastatin uptake only at a high concentration of 100 μM (Bachmakov et al. 2008). These results suggest that not only inhibition of uptake transporter function but also the stimulation of uptake rates have to be considered as possible molecular mechanisms for altering drug plasma concentrations.

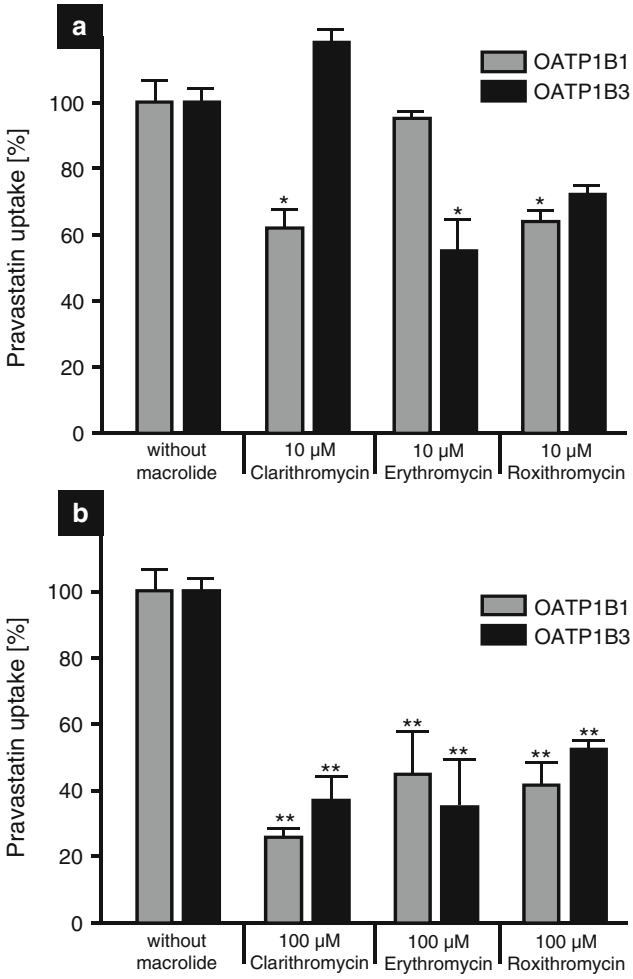


Fig. 3 OATP1B1- and OATP1B3-mediated drug–drug interactions using pravastatin as drug substrate and macrolides antibiotics as potential uptake inhibitors. Uptake of pravastatin was measured in HEK293 cells stably expressing the respective uptake transporter. (**a**) Macrolides (clarithromycin, erythromycin, and roxithromycin) were added in a concentration of 10 μM into the uptake solution. (**b**) Macrolides were added in a concentration of 100 μM. Pravastatin uptake without added macrolides was set to 100%

Interactions of statins with coadministered drugs have been reported earlier with cerivastatin given together with the immunosuppressant drug cyclosporin A (Shitara et al. 2003) or the fibrate gemfibrozil (Shitara et al. 2004b). Kidney transplant recipients treated simultaneously with cerivastatin and cyclosporin A showed increased plasma concentrations of cerivastatin (Muck et al. 1999). This interaction was studied using human liver microsomes and it was demonstrated that

cyclosporin A inhibits both the metabolism and the hepatic uptake of cerivastatin mediated by OATP1B1 (Shitara et al. 2004a). Interestingly, uptake inhibition was observed at lower cyclosporin A concentrations than the inhibition of metabolizing enzymes, suggesting that uptake inhibition is also an important mechanism in vivo. In the case of gemfibrozil it has been reported that coadministration together with cerivastatin leads to a sixfold increase in cerivastatin AUC (Backman et al. 2002). Several studies described potential molecular mechanisms of this interaction. Possible molecular targets could be the phase I enzyme CYP2C8 and the phase II enzymes UGT1A1 and UGT1A3 (Prueksaritanont et al. 2002). Both enzymes are important for the metabolism of cerivastatin and both could be inhibited by gemfibrozil. Furthermore, it has been reported using MDCK (Madin-Darby canine kidney cells) recombinantly expressing human OATP1B1 that gemfibrozil and its metabolite gemfibrozil 1-O-ß-glucuronide inhibit OATP1B1-mediated cerivastatin uptake (Shitara et al. 2004b). Therefore, this uptake inhibition may lead to elevated cerivastatin plasma levels with an increased risk for statin-induced myopathy. All three observed interactions may contribute to these severe drug–drug interactions, and consequently cerivastatin was withdrawn from the market.

Another example for transporter-mediated drug–drug interactions has been observed with the endothelin receptor antagonist bosentan. Bosentan is metabolized in hepatocytes mainly by CYP2C9 and CYP3A4. Several studies have demonstrated that simultaneously administered drugs can increase the plasma concentration of bosentan. It has been reported, for example, for coadministered ketoconazol, cyclosporin A, rifampicin, and sildenafil that all of these drugs increased the plasma concentration of bosentan (Treiber et al. 2007; van Giersbergen et al. 2002). Interestingly, cyclosporin A led to a 30-fold increase of bosentan concentrations, whereas ketoconazol, a potent CYP3A4 inhibitor, led only to a twofold increase in bosentan plasma concentrations suggesting that other molecular mechanisms besides the inhibition of metabolizing enzymes occur. Recently, it has been demonstrated that bosentan is a substrate for OATP1B1 and OATP1B3 (Treiber et al. 2007). Using CHO (Chinese hamster ovary) cells stably expressing OATP1B1 or OATP1B3 it has been shown that bosentan uptake is inhibited by cyclosporine A, rifampicin, and sildenafil. Furthermore, also the transporter-mediated uptake of the bosentan metabolite Ro 48-5033 is inhibited. Kinetic analysis of the inhibitory effect revealed that rifampicin inhibited bosentan uptake with IC_{50} values of 0.3 μM and 0.8 μM for OATP1B1 and OATP1B3, respectively, whereas rifampicin inhibited OATP1B1 with an IC_{50} value of 3.2 μM and OATP1B3 with an IC_{50} value of 1.6 μM (Treiber et al. 2007). Because cyclosporin A could reach plasma concentrations of 1.3 μM and rifampicin of 15 μM a combined effect of uptake inhibition and of the inhibition of metabolizing enzymes may be responsible for the in vivo observed increase in bosentan plasma concentrations in the presence of both drugs. For sildenafil IC_{50} values of 1.5 μM (for OATP1B1) and 0.8 μM (for OATP1B3) were determined whereas plasma concentrations of 1.2 μM could be reached. Because sildenafil is not an inhibitor of CYP3A4 or CYP2C9, inhibition of uptake transporters may be the major determinant of this drug–drug interaction.

Taken together, the presented in vitro studies demonstrated that inhibition of uptake transporter-mediated drug transport by concomitantly administered drugs could be an additional important mechanism underlying previously observed drug–drug interactions.

2.4 *Functional Consequences of Genetic Variations in Transporter Genes*

2.4.1 Pharmacogenomics of OATP1B1

Besides transporter-mediated drug–drug interactions the functional consequences of frequent genetic variations, so-called polymorphisms, in transporter genes play an important role in the interindividual variability of drug disposition and drug response. Considerable effort has been made in recent years to identify single nucleotide polymorphisms (SNPs) or haplotypes to determine their frequency in different ethnic populations and to establish the functional consequences on protein expression, localization, or transport function. A summary of reported sequence variations in the *SLCO1B1* gene, encoding human OATP1B1, and of variations in the genes *SLCO1A2*, *SLCO1B3*, and *SLCO2B1*, encoding human OATP1A2, OATP1B3, and OATP2B1, is depicted in Fig. 4. Functional relevant polymorphisms in other human OATP family members have not been identified so far. A recently published study investigating thyroid hormone transport mediated by human OATP1C1 described two frequent polymorphisms in the *SLCO1C1* gene that both had no effect on the function of the mutated OATP1C1 protein (van der Deure et al. 2008).

Tirona and coworkers have published the first detailed in vitro analysis of genetic variations in the *SLCO1B1* gene encoding the hepatocellular uptake transporter OATP1B1 (Tirona et al. 2001). They investigated 14 nonsynonymous polymorphisms identified in a population of European and African Americans and found that some polymorphisms of haplotypes affect protein localization or transport function of the mutated OATP1B1 proteins. In general, they found that the genotypic frequencies were dependent on race and that amino acid exchanges located within the transmembrane-spanning domains and in the extracellular fifth loop were associated with alteration in transport kinetics. In detail, the variant OATP1B1*2 (*SLCO1B1c.217T>C*) alone or together with the exchange *SLCO1B1c.1964A>G* (OATP1B1*12) increased the K_m value for estrone-3-sulfate from 0.54 to 5.9 μM and 8.1 μM, respectively. A significant increase in the K_m value (2.2 μM) could also be observed analyzing the haplotype OATP1B1*13 (*SLCO1B1c.245T>C/c.467A>G/c.2000A>G*). Both variants also reduced transport compared to transport mediated by the OATP1B1*1a allele. Reduced transport of the substrates estrone-3-sulfate and estradiol-17ß-glucuronide was also observed for the variants OATP1B1*3 (*SLCO1B1c.245T>C/c.467A>G*, OATP1B1p.V82A/p.Q156G), OATP1B1*5 (*SLCO1B1c.521T>C*, OATP1B1p.V174A), OATP1B1*6

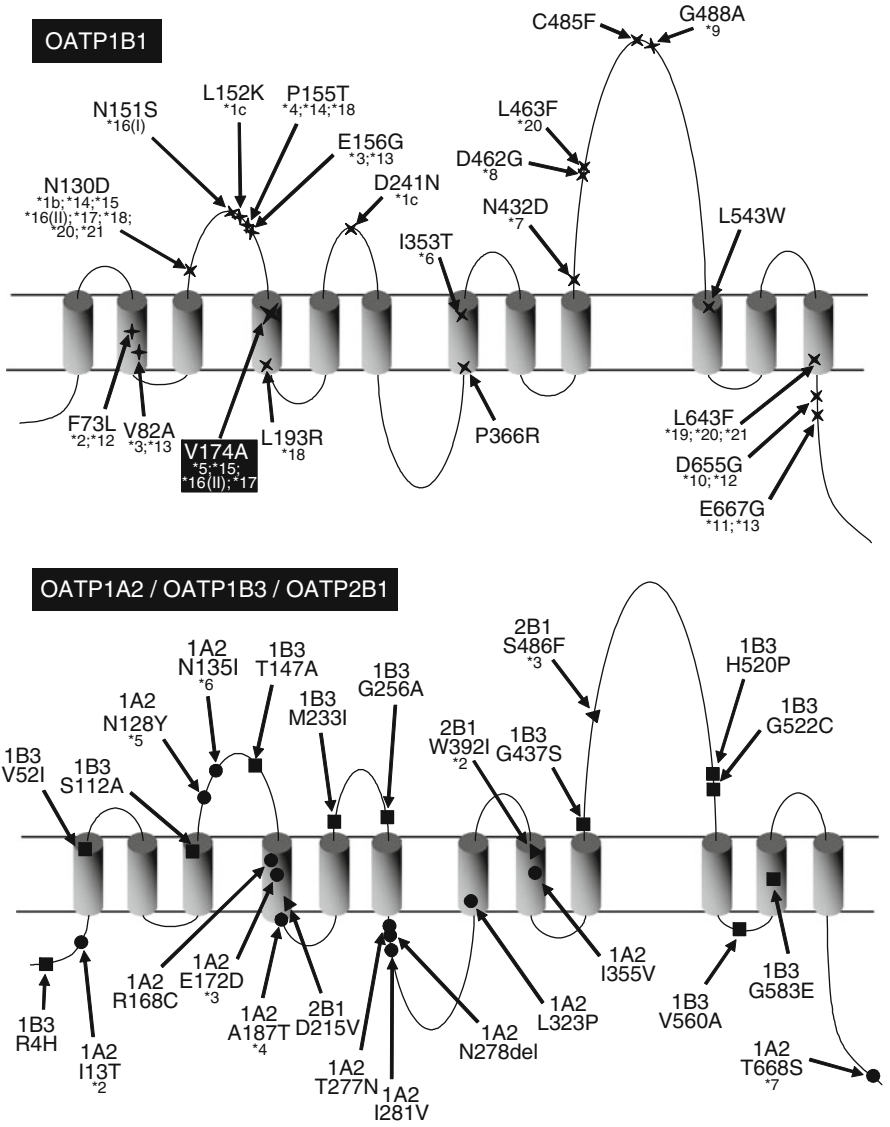


Fig. 4 Schematic two-dimensional model of human OATP1B1 (*above*) and OATP1A2/OATP1B3/OATP2B1 (*below*). For OATP1B1 the localization of genetic variations (polymorphisms) after translation is indicated as *stars*, for OATP1A2 as *circles*, for OATP1B3 as *boxes*, and for OATP2B1 as *triangles*. If valid, the respective allele and haplotype nomenclature of the variation is written (*1–*21). For OATP1B1, the important allele OATP1B1p.V174A (contained in the haplotypes *5, *15, *16(II), and *17) is highlighted

(*SLCO1B1c.1058T>C*, OATP1B1p.I353T), and OATP1B1*9 (*SLCO1B1c.1463G>C*, OATP1B1p.G488A). As mentioned previously, most of these variations are located within transmembrane-spanning regions or in the second or fifth extracellular loop (Fig. 4) suggesting that these protein regions are important for substrate recognition and/or transport (Tirona et al. 2001).

Michalski et al. (2002) have published the first naturally occurring mutation within the *SLCO1B1* gene together with the detailed analysis of the functional consequences of two frequent polymorphisms. They analyzed 81 human liver samples originating from Caucasians and identified one sample showing reduced OATP1B1 protein amount compared to the protein amount in all other liver samples. Analyzing the sequence of the *SLCO1B1* gene from this sample identified one haplotype containing two synonymous and three nonsynonymous base pair exchanges. Two of them corresponded to the recently before identified frequent polymorphisms OATP1B1*1b and OATP1B1*4 (OATP1B1p.P155T), whereas the third nonsynonymous exchange (OATP1B1*18, OATP1B1p.L193R) could be analyzed as the first naturally occurring mutation in the *SLCO1B1* gene with a frequency below 0.3%. For the analysis of the functional consequences of these variations, the authors established stably transfected MDCKII cells recombinantly expressing single-mutated OATP1B1 proteins for the analysis of each polymorphism and one MDCKII cell line expressing the mutated OATP1B1 protein encoded by the haplotype gene. Analyzing the protein localization in these OATP1B1-expressing cell lines it was demonstrated that both frequent variants were localized like the OATP1B1*1a protein in the lateral membrane of MDCKII cells, whereas a pronounced change in localization was observed for the OATP1B1p.L193R protein, which was hardly detectable in the lateral plasma membrane. Using bromosulfophthalein (BSP), estradiol-17ß-glucuronide ($E_2$17ßG), and taurocholate as substrates they found that BSP was transported by the OATP1B1*1b and by the *4 variant according to transport rates determined for the OATP1B1*1a allele; transport of $E_2$17ßG was significantly reduced by the OATP1B1*4 variant. Using taurocholate as substrate, the transport by the OATP1B1*1b variant was reduced and totally abolished by the OATP1B1*4 protein. None of the tested substrates was transported by the OATP1B1 protein carrying the single mutation L193R or by the protein encoded by the haplotype gene (Michalski et al. 2002). These analyses demonstrated that alterations in the second extracellular loop, where both frequent analyzed variations were located, could influence the substrate spectrum of the OATP1B1 protein, whereas the mutation OATP1B1p.L193R totally abolishes the transport function and influences in addition the localization of the protein.

Nozawa et al. (2002) have published a detailed investigation of polymorphisms in the Japanese population. They found that the previously identified OATP1B1*1c allele could not be detected in 267 Japanese subjects, whereas the OATP1B1*1b and *5 alleles were present with 54% and 0.7% in the Japanese population, respectively. Furthermore, they identified the novel haplotype OATP1B1*15, containing the two mutations *SLCO1B1c.388A>G* and *SLCO1B1c.521T>C*, which has an allelic frequency of 3.0% in the investigated population. Using transfected HEK293 cells expressing the different mutant OATP1B1 proteins they investigated

the consequences of the variations on OATP1B1-mediated transport. They found no significant changes in V_{max} or K_m values for estrone-3-sulfate uptake. Using estradiol-17ß-glucuronide as substrate, these results were confirmed in a second study analyzing the same genetic variations (Iwai et al. 2004). In this study the authors found no changes in K_m values, whereas the V_{max} value was slightly decreased for the haplotype OATP1B1*15. In a second study analyzing the functional consequences of the OATP1B1*15 haplotype Nozawa and coworkers demonstrated that the *15 allele shows decreased transport activities for the HMG-CoA-reductase inhibitor pravastatin and for SN-38, the active metabolite of the topoisomerase inhibitor irinotecan, suggesting that this haplotype contributes to the interindividual variability in drugs that are substrates for this hepatic uptake transporter (Nozawa et al. 2005). Kameyama and colleagues systematically investigated the effect of the OATP1B1*1a, *1b, *5, and *15 alleles alone and in combination with the polymorphism *SLCO1B1c.1007C>G* on OATP1B1-mediated transport of several statins using stably transfected HEK293 cells recombinantly expressing the different OATP1B1 proteins (Kameyama et al. 2005). For atorvastatin and pravastatin, the maximum transport velocities (V_{max} values) and the intrinsic clearance (V_{max}/K_m) were significantly reduced for the alleles OATP1B1*5, *15, and *15 + 1007C>G compared to the transport mediated by the wild-type protein (*1a allele). No differences in transport could be observed in HEK cells expressing the OATP1B1*1b allele. Interestingly, all alleles affecting transport have the polymorphism *SLCO1B1c.521T>C* (OATP1B1*5) in common, demonstrating that this polymorphism is responsible for the reduced transport activity of the OATP1B1 protein.

The functional consequences of the polymorphisms OATP1B1*1b and OATP1B1*5 on the transport of ezetimibe have been investigated using stably transfected HEK293 cells expressing the mutated OATP1B1 proteins (Oswald et al. 2008). In this study, the authors compared in vivo data obtained by studies with healthy participants with in vitro transport data and found that the ezetimibe metabolite ezetimibe glucuronide inhibited OATP1B1-, OATP1B3- and OATP2B1-mediated bromosulfophthalein (BSP) uptake with very low IC_{50} values of 0.15 μM, 0.26 μM, and 0.14 μM, respectively. Analyzing the functional consequences of the genetic variations they showed that uptake of ezetimibe glucuronide was significantly reduced for the OATP1B1*1b and OATP1B1*5 variant. Together with their in vivo data, these results demonstrated that the pharmacokinetics of ezetimibe is influenced by OATP1B1 polymorphisms (Oswald et al. 2008). Recently, the OATP1B1*5 variant was found to be associated with higher lopinavir plasma concentrations (Hartkoorn et al. 2010), also suggesting decreased hepatic uptake due to reduced OATP1B1-mediated transport function. In summary, it can be stated that several polymorphisms in the *SLCO1B1* gene leading to amino acid exchanges in the OATP1B1 protein have been characterized with respect to their impact of drug transport. The variant *SLCO1B1c.512T>C* [OATP1B1*5, also present in the haplotypes *15, *16(II), and *17 (see Fig. 4)], is associated with reduced transport activity of the OATP1B1 protein leading in vivo to altered pharmacokinetics of drugs that are substrates of this uptake transporter. The impact

of this polymorphism has been demonstrated recently in a genomewide association study demonstrating that this variant is strongly associated with an increased risk of statin-induced myopathy (Link et al. 2008).

2.4.2 Pharmacogenomics of Other Human OATP Family Members

In contrast to polymorphisms in the *SLCO1B1* gene investigated in detail, genetic variations in other *SLCO* genes have not been investigated with such detail. The following chapter will briefly summarize reported findings on the functional consequences of genetic variations in the *SLCO1B3*, *SLCO2B1,* and *SLCO1A2* genes.

Like OATP1B1, OATP1B3 (gene symbol *SLCO1B3*) is localized in the basolateral membrane of human hepatocytes (König et al. 2000a). It has 80% amino acid identity to OATP1B1 (Fig. 2), and both have an overlapping, nearly identical substrate spectrum with differences in the transport kinetics of the substrates. Like OATP1B1, OATP1B3 transports endogenous substances as well as several highly prescribed drugs like statins and antibiotics (see Table 2). Interestingly, literature analyzing functional consequences in the *SLCO1B3* gene is relatively sparse. Iida et al. have described genetic variations in the *SLCO1B3* gene (Iida et al. 2001) without the analysis of the functional consequences of the identified variations. Letschert et al. (2004) identified additional mutations in the Caucasian population and investigated their functional impact on protein localization and transport activity of the mutated OATP1B3 proteins. In their study they identified the common variants *SLCO1B3c.334T>G* (OATP1B3p.S112A) and *c.699G>A* (p.M233I) with allelic frequencies of 74% and 71%, respectively. These frequencies were confirmed in a study by Smith et al. (2007) where they also demonstrated that these two variants exist in complete linkage disequilibrium. Furthermore, using stably transfected HEK293 and MDCKII cells expressing the mutated proteins, Letschert and coworkers analyzed the impact of the polymorphisms *SLCO1B3c.334T>G* (OATP1B3p.S112A), *c.699A>G* (p.M233I) and *c.1564G>T* (p.G522C) and of the artificial mutation *c.1748G>A* (p.G583E) on the cellular localization and the transport function. These results demonstrated that effects of the mutations on the cellular localization and on the transport function were substrate and cell line dependent (Letschert et al. 2004). The frequent polymorphisms *SLCO1B3c.334T>G* and c.*699G>A* did not lead to significant changes in protein expression or transport activity of the OATP1B3 substrates bromosulfophthalein (BSP), taurocholate, or estrone-3-sulfate. For these two polymorphisms, the results were confirmed in *Xenopus laevis* oocytes using the chemotherapeutic drug paclitaxel as substrate for OATP1B3 (Smith et al. 2007). Interestingly, in vivo both variations seem to have an impact on the pharmacokinetics of mycophenolate mofetil (MPA) and its glucuronide [MPAG (Picard et al. 2010)] when studied in 70 renal transplant patients receiving combination treatment of MMF with either tacrolimus or sirolimus. In contrast to the data for the frequent variations, the infrequent variant *SLCO1B3c.1564G>T* (allelic frequency in the

Caucasian population = 1.9%) revealed substrate and cell line-dependent changes in transport activity. For BSP and taurocholate this variant showed significantly reduced transport activity in both cell lines, whereas for estradiol-17ß-glucuronide the transport was almost totally abolished in the HEK293 cells compared with the transport measured in the MDCKII cells expressing this mutant. The transport of cholecystokinin-8 was significantly reduced in both cell lines, whereas the transport of estrone-3-sulfate was totally repressed in HEK293 cells but not in MDCKII cells. Furthermore, increased intracellular retention and a decreased amount of protein could be detected in both cell lines, suggesting that for the analysis of the functional consequences of polymorphisms several prototypic substrates of one transport protein have to be tested because mutations may alter the substrate specificity instead of changing the transport kinetics of one given substrate.

OATP2B1 (gene symbol *SLCO2B1*) is the third hepatic OATP family member but in contrast to the more liver-specific OATPs OATP1B1 and OATP1B3, this uptake transporter is expressed in addition in several other tissues (Grube et al. 2006, 2007). To date, only three variants were characterized with respect to their functional impact on OATP2B1-mediated transport. Tamai et al. (2000) identified two variations and subsequently the allelic frequencies and the functional consequences of these polymorphisms were investigated in detail (Nozawa et al. 2002). The most common variant *SLCO2B1c.1457C>T* (OATP2B1p.S486F; OATP2B1*3) occurred with an allelic frequency of 30.9% in the Japanese population and showed a reduced maximal transport velocity (V_{max}) for estrone-3-sulfate compared to the transport mediated by the wild-type protein. The variant *SLCO2B1c.1175C>T* (OATP2B1p.W392I; OATP2B1*2) was not found in the investigated Japanese population and the occurrence of this polymorphism needs to be verified in other ethnicities. Mougey et al. (2009) analyzed the impact of a third variant (*SLCO2B1c.935G>A*; OATP2B1p.Arg312Gln) and found that this variant is associated with reduced plasma concentrations of the leukotriene receptor antagonist Montelukast.

OATP1A2 (formerly termed OATP-A or OATP, gene symbol *SLCO1A2*) was the first human OATP family member to be cloned (Kullak-Ublick et al. 1995) and functionally characterized. *SLCO1A2* mRNA expression has been detected in various tissues with a high expression in brain (Gao et al. 2000). There, the protein has been localized to the capillary endothelium suggesting that OATP1A2 plays a role in the constitution of the blood–brain barrier. Given its tissue distribution and its ability to transport drug substrates it is reasonable to assume that polymorphisms in the *SLCO1A2* gene may also have an impact on drug disposition. Several studies have identified genetic variations within this gene, and some of them studied the functional consequences of the resulting mutated OATP1A2 protein. Iida and colleagues identified several polymorphisms within the 5′-regulatory region of the *SLCO1A2* gene without further analyzing their consequences on gene regulation (Iida et al. 2001). A detailed analysis published by Lee et al. identified six variations in exonic regions from people of a mixed ethnic background with three polymorphisms having functional consequences (Lee et al. 2005). When analyzed in transfected HeLa cells the variants *SLCO1A2c.404A>T* (OATP1A2p.N135I,