Pharmaceutical Research, Vol. 22, No. 10, October 2005 (© 2005)
DOI: 10.1007/s11095-005-6810-2

Expert Review

Apical/Basolateral Surface Expression of Drug Transporters and its Role in Vectorial Drug Transport

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Received February 1, 2005; accepted June 21, 2005

Abstract. It is well known that transporter proteins play a key role in governing drug absorption, distribution, and elimination in the body, and, accordingly, they are now considered as causes of drug–drug interactions and interindividual differences in pharmacokinetic profiles. Polarized tissues directly involved in drug disposition (intestine, kidney, and liver) and restricted distribution to naive sanctuaries (blood–tissue barriers) asymmetrically express a variety of drug transporters on the apical and basolateral sides, resulting in vectorial drug transport. For example, the organic anion transporting polypeptide (OATP) family on the sinusoidal (basolateral) membrane and multidrug resistance-associated protein 2 (MRP2/ABCC2) on the apical bile canalicular membrane of hepatocytes take up and excrete organic anionic compounds from blood to bile. Such vectorial transcellular transport is fundamentally attributable to the asymmetrical distribution of transporter molecules in polarized cells. Besides the apical/basolateral sorting direction, distribution of the transporter protein between the membrane surface (active site) and the intracellular fraction (inactive site) is of practical importance for the quantitative evaluation of drug transport processes. The most characterized drug transporter associated with this issue is MRP2 on the hepatocyte canalicular (apical) membrane, and it is linked to a genetic disease. Dubin–Johnson syndrome is sometimes caused by impaired canalicular surface expression of MRP2 by a single amino acid substitution. Moreover, single nucleotide polymorphisms in OATP-C/SLC21A6 (SLCO1B1) also affect membrane surface expression, and actually lead to the altered pharmacokinetic profile of pravastatin in healthy subjects. In this review article, the asymmetrical transporter distribution and altered surface expression in polarized tissues are discussed.

KEY WORDS: epithelial cells; sorting; transporter; vectorial transport.

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ABBREVIATIONS: ABC, ATP-binding cassette; AP, apical; BBB, blood–brain barrier; BCRP, breast cancer resistance protein; BCSFB, blood–cerebrospinal fluid barrier; BL, basolateral; BPB, blood–placenta barrier; BSEP, bile salt export pump; BSP, bromosulfophthalein; BTB, blood–testis barrier; CLAMP, C-terminal linking and modulating protein; ER, endoplasmic reticulum; $E_2$17βG, estradiol-17β-D-glucuronide; F-actin, filamentous actin; HAX-1, HS1-associated protein X-1; ISBT, ileal Na^+-dependent bile salt transporter; MDR, multidrug resistance protein; MRP, multidrug resistance-associated protein; NTCP, Na^+/taurocholate cotransporting polypeptide; OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; PEPT, peptide transporter; PFICII, progressive familial intrahepatic cholestasis type II; SNP, single nucleotide polymorphism; UDC, ursodeoxycholate.

DISTRIBUTION OF DRUG TRANSPORTERS IN THE BODY

In drug absorption and distribution/excretion processes, permeability through polarized cells can lead to problems. The polarized cell surface is composed of an apical and basolateral membrane (further subdivided into a basal and lateral membrane) separated by a tight junction (Fig. 1). The membrane on which the drug transporter is located, i.e., apical or basolateral, is critical in determining the net transcellular transport and, ultimately, governing the pharmacokinetic profiles of drug substrates in the body. Although most of the transporters are specifically expressed on the apical or basolateral side in different tissues, some exceptions have been reported. For example, rat organic anion transporting polypeptide 1 (Oatp1) is expressed on the basolateral membrane of hepatocytes to take up anionic substrates from blood, whereas it is expressed on the apical membrane of renal epithelia to reabsorb substrates from the urine. If Oatp1 were located on the basolateral side of renal epithelia,

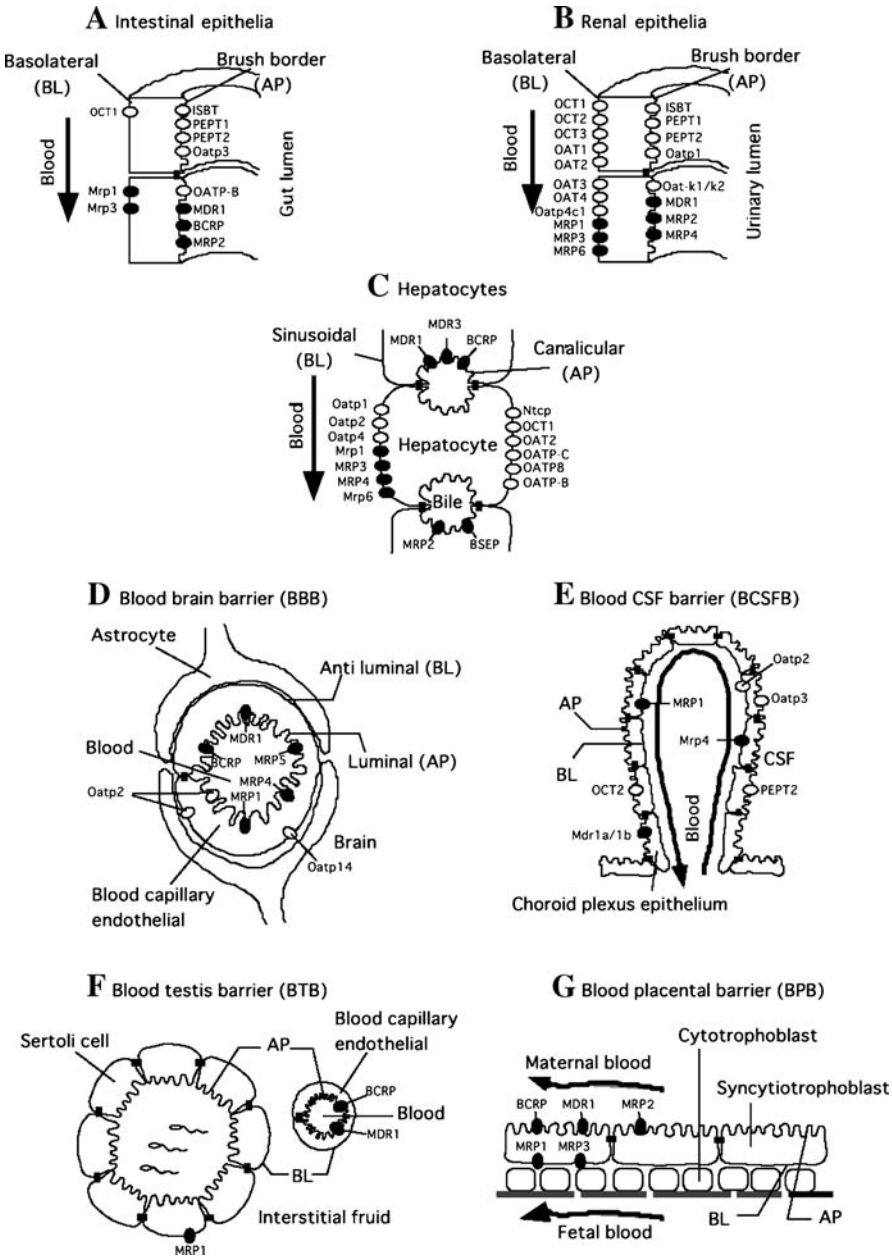

Fig. 1. Distribution and cellular localization of drug transporters in polarized tissues. Intestinal epithelia (A), renal epithelia (B), hepatocytes (C), blood–brain barrier (D), blood–CSF barrier (E), blood–testis barrier (F) and blood–placental barrier (G) are shown. These tissues are composed of polarized epithelial or endothelial cells with a tight junction structure to limit passive diffusion. Selective expression of particular transporter molecules on the apical or basolateral side determine the net transport of compounds across these monolayers. ABC transporters are indicated by closed circles. Detailed information is given in Table I.

it would act as a transporter for renal secretion rather than reabsorption. Although the precise molecular mechanism of the heterologous sorting direction is not fully understood, it is plausible that putative sorting signals in the transporter molecules are differentially decoded by respective host cells. It has been reported that apical/basolateral sorting is determined by the ambiguous nature of the sorting signals (tyrosine motif and di-leucine motif as the basolateral sorting signal and glycosylphosphatidylinositol anchor, *N*- and *O*-linked oligosaccharides and other sequences scattered over peptides as the apical sorting signal) and the irregular hierarchy of these signals (1). Although precise molecular information is not available at present, it is still worthwhile to examine the apical/basolateral surface localization of drug transporters in the body to understand their cooperative roles in drug distribution and elimination.

In epithelial cells of the mucosa of the small intestine, kidney urinary tubules, and the choroid plexus forming the blood–cerebrospinal fluid barrier (BCSFB), the blood side corresponds to the basolateral side and the lumen or cerebro-

Table I. Cellular Localization of Drug Transporters *in Vivo* Tissues and *in Vitro* Polarized Cell Lines

Name	Symbol	Species	Expression	Vivo		Cell		PDZ motif (S/T-X-Φ)
				Apical	Basolateral	Apical	Basolateral	
NTCP	SLC10A1	Human	Liv				MDCK(131)	–
Ntcp	Slc10a1	Rat	Liv(>>Kid, Int)		Liv(132)		MDCK(133)	–
Ntcp	Slc10a1	Mouse	Liv					–
ISBT	SLC10A2	Human	Int(>>Kid)			Caco2(131)		–
Isbt	Slc10a2	Rat	Int	Kid(8, 134), Int(7, 8), BD(8, 135, 136)	Kid, Int, BD (Splicing variant)(8)	MDCK(133)		–
Isbt	Slc10a2	Mouse						–
PEPT1	SLC15A1	Human	Liv, Kid	Int(2, 3)				–
Pept1	Slc15a1	Rat	Kid, Int, Tes	Kid(4, 137, 138), Int(4)		LLC(139)		+
Pept1	Slc15a1	Mouse						+
PEPT2	SLC15A2	Human	Kid					+
Pept2	Slc15a2	Rat	BCSFB, Kid(>>Int)	Kid(4, 137), Int(4), BCSFB(63)				+
Pept2	Slc15a2	Mouse		BCSFB(63)				+
OCT1	SLC22A1	Human	Liv, Int					+/–
Oct1	Slc22a1	Rat	Kid, Liv, Int		Liv(140), Kid(33–36), Int(141)		MDCK(33)	+/–
Oct1	Slc22a1	Mouse	Kid		Liv			–
OCT2	SLC22A2	Human	Int, Liv		Kid(28)			–
Oct2	Slc22a2	Rat	Kid	BCSFB(142)	Kid(35, 36)		MDCK(33, 143), LLC(143)	+/–
Oct2	Slc22a2	Mouse	Kid					–
OCT3	SLC22A3	Human	Liv, Int		Kid(37)			+
Oct3	Slc22a3	Rat	Int, BCSFB, Liv, Kid, Tes		Kid(36)			+
Oct3	Slc22a3	Mouse	Ret					+
OCTN1	SLC22A4	Human	Ubiquitous					+
OctN1	Slc22a4	Rat	Kid, Int, BCSFB, Hea, Liv					+
OctN1	Slc22a4	Mouse	Kid, Hea, Liv					+
OCTN2	SLC22A5	Human	Ubiquitous					+
OctN2	Slc22a5	Rat	Kid, Int, BCSFB, Hea, Liv, Pla					+
OctN2	Slc22a5	Mouse	Kid, Hea, Liv					+
OAT1	SLC22A6	Human	Kid		Kid(27, 28)			–
Oat1	Slc22a6	Rat	Kid		Kid(29, 30)			–
Oat1	Slc22a6	Mouse	Kid					–
OAT2	SLC22A7	Human	Liv, Kid		Kid(144)			–
Oat2	Slc22a7	Rat	Liv, BCSFB, Kid, Tes	Kid(29)	Liv(82, 83)			–
Oat2	Slc22a7	Mouse	Liv, Kid					–
OAT3	SLC22A8	Human	BCSFB		Kid(28, 31)			–
Oat3	Slc22a8	Rat	Kid, BCSFB, Liv		Kid(29, 32), BCSFB(64)		LLC(145)	+/–
Oat3	Slc22a8	Mouse	BBB, Eye, BCSFB, Kid		Kid			–

Table 1. Cellular Localization of Drug Transporters *in Vivo* Tissues and *in Vitro* Polarized Cell Lines (Continued)

Name	Symbol	Species	Expression	Vivo		Cell		PDZ motif (S/T-X-Φ)
				Apical	Basolateral	Apical	Basolateral	
OAT4 (NM_080866)	SLC22A9	Human	Liv					+
OctN3	Slc22a9	Mouse	Kid, Tes					−
OAT5	SLC22A10	Human	Liv					
OAT4 (NM_018484)	SLC22A11	Human	Liv	Kid(146, 147)				+
Oatp1	Slc21a1	Rat	Kid, Liv	Kid(41)	Liv(41, 148–151)			+
Oatp1	Slc21a1	Mouse	Kid					+
OATP-A	SLC21A3 (SLCO1A2)	Human	Liv, Kid, Bra, Pro					+
Oat-K1/K2	Slc21a4	Rat	Kid	Kid(42)		MDCK(87)	LLC(88)	+
Oatp2	Slc21a5	Rat	Liv, BCSFB, Tes	BBB(56)	Liv(149, 152), BBB(56), BCSFB(56)			+
Oatp2	Slc21a5	Mouse	Kid					+
OATP2/ OATP-C/ LST-1	SLC21A6 (SLCO1B1)	Human	Liv		Liv(153, 154)		MDCKII(114, 155)	+/−
Oatp3	Slc21a7	Rat	BCSFB, Tes	Int(156), BCSFB		MDCK(156)		+
Oatp3	Slc21a7	Mouse						+
OATP8/LST-2	SLC21A8 (SLCO1B3)	Human			Liv(154, 157–159)		MDCKII(160)	−
OATP-9/OATP-B	SLC21A9	Human	Kid, Ova, Tes, Spl	Int(9)	Liv(81)			+
moat1	Slc21a9	Rat	Kid, Int					−
	Slco2b1	Mouse						−
Oatp-4/Lst-1	Slc21a10	Rat	Liv		Liv(153, 157, 161)		MDCKII(162)	+
	Slc21a10	Mouse	Liv					+
OATP-D	SLC21A11	Human	Ubiquitous					+
	Slc21a11	Rat	Ubiquitous					+
	Slc21a11	Mouse						+
OATP-E	SLC21A12	Human	Ubiquitous					+
	Slco4a1	Rat						+
	Slco4a1	Mouse						+
Oatp-5	Slc21a13	Rat	Kid					+
	Slc21a13	Mouse	Kid					+
OATP-F	SLC21A14	Human	Bra					+
Oatp14	Slc21a14	Rat	Bra					+
Oatp14	Slc21a14 (Slco1c1)	Mouse	Bra		BBB(163)			+
OATP4C1	SLCO4C1	Human	Kid					−
Oatp4c1	Slco4c1	Rat	Kid		Kid(164)		MDCK	−
Oatp4c1	Slco4c1	Mouse						−
MDR1	ABCB1	Human	Ubiquitous	Liv(11), Kid(11), Int(11, 165), BBB, BD(11, 166), Pla(74), BTB(Endothelial)(71)		MDCK(167), LLC(168, 169), Caco2(170)		−

Mdr1a/1b	Abcb1a/1b	Rat	Ubiquitous	Liv(12, 51), Kid, Int(12), BBB, BCSFB(66), BD(171)				−
Mdr1a/1b	Abcb1a/1b	Mouse	Ubiquitous	Liv, Kid, Int, BBB		LLC(172, 173)		−
MDR2/3	ABCB4	Human	Liv	Liv		LLC(169)		−
Mdr2	Abcb4	Rat	Liv(>>Int)	Liv				−
Mdr2	Abcb4	Mouse	Liv	Liv				−
BSEP	ABCB11	Human	Liv	Liv(174)		MDCK(128)		+
Bsep	Abcb11	Rat	Liv	Liv(51, 175)				−
Bsep	Abcb11	Mouse	Liv	Liv(176)				−
MRP1	ABCC1	Human	Ubiquitous	BBB(177)	BCSFB(66), Pla(74–76), BTB(Sertoli)(71)		MDCK(178, 179), LLC(180)	−
Mrp1	Abcc1	Rat	BCSFB, Kid, Tes, Int		Liv(50, 181), Kid(50), BCSFB(66)			−
Mrp1	Abcc1	Mouse	Ubiquitous		Kid(21, 70), Int(21), BTB(Sertoli)(70), BCSFB(182)			−
MRP2	ABCC2	Human	Liv, Kid, Int	Liv(183, 184), Kid(47), Int(13), pla(76)		MDCK(160, 185), MDCKII(155), LLC(186), Caco2(170)		+
Mrp2	Abcc2	Rat	Liv, Kid, Int	Int(14, 15), Liv(184, 187), Kid(46)		MDCKII(162)		+
Mrp2	Abcc2	Mouse	Liv, Kid, Int	Liv				+
MRP3	ABCC3	Human	Liv, Adr, Pan, Kid, Int		Liv(188, 189), Kid(189), BD(189), Pla(76)		MDCKII(190), Caco2	−
Mrp3	Abcc3	Rat	Int, Kid		Liv(191), Int(22), BD(191)			
Mrp3	Abcc3	Mouse						−
MRP4	ABCC4	Human	Ubiquitous	Kid(45), BBB(177)	Liv(192)			+
Mrp4	Abcc4	Rat	BCSFB, Kid(>>Int), Tes	Kid(45)	Liv(192)			+
Mrp4	Abcc4	Mouse	Liv	BBB(68)	Liv(192), BCSFB(68)			−
MRP5	ABCC5	Human	Ubiquitous	BBB(177)			MDCKII(193)	−
Mrp5	Abcc5	Rat	BCSFB, Kid, Tes					−
Mrp5	Abcc5	Mouse	Kid					−
MRP6	ABCC6	Human	Liv, Kid, Int	Liv	Kid(194)		MDCKII(195)	−
Mrp6	Abcc6	Rat	Liv, Kid, Int, BCSFB	Liv(196)	Liv(196)			−
Mrp6	Abcc6	Mouse	Liv, Int		Liv			−
BCRP/MXR	ABCG2	Human	Pla, Liv, Int, Ova, Kid, HEa	Liv(16), Int(16), BBB(57), BD(197), Pla(16), BTB (Endothelial)(71)		MDCKII(77), LLC(198)		−
Bcrp	Abcg2	Rat	Kid, Int, Liv, Pla	BBB(199)				−
Bcrp	Abcg2	Mouse	Kid, Liv, Int, Pla, BBB	Liv, Kid, Int(17)				−

Liv, liver; Kid, kidney; Int, intestine; Pla, placenta; Pro, prostate; Hea, heart; Ova, ovary; Spl, spleen; Bra, brain; Ret, retina; Pan, pancreas; Adr, adrenal; BD, bile duct epithelium; BCSFB, blood cerebrospinal fluid barrier, BBB, blood brain barrier; BTB, blood testis barrier; BPB, blood placental barrier. Carboxy terminal three amino acids are considered for possible PDZ protein binding motif(+, consensus PDZ motif; −, not consensus; +/−, partially consensus but last amino acid is not hydrophobic nature). Information without references are cited from Transporter Database (http://www.ilab.rise.waseda.ac.jp/transdb/). RNA expression profiles are summarized from refs 6, 16, 25, 38, 48, 68, 67, 200–203.

spinal fluid side corresponds to the apical side (Fig. 1). However, especially in the case of the blood–brain barrier (BBB), the blood (luminal) side corresponds to the apical side and the brain (antiluminal) side corresponds to the basolateral side (Fig. 1). Table I summarizes the polarized distribution of drug transporters in the body. The tissue distribution and intracellular localization of the transporters are reviewed first. Although the nomenclature of the transporter gene family [i.e., solute carrier (SLC) and ATP-binding cassette (ABC) superfamily] has been approved and unified by the HUGO Gene Nomenclature Committee (HGNC), earlier gene symbols such as OATP and multidrug resistance-associated protein (MRP) are used throughout the text because most of the readers are still familiar with the earlier transporter names. (Please refer to Table I to see how the official gene symbols correspond to their earlier transporter names.) In some cases, human transporters are given entirely in capital letters (e.g., OATP1), whereas only the initial letters are in capitals in the case of rat/mouse genes (e.g., Oatp1) to distinguish between human and rodent transporters.

Intestine

In the epithelium of the small intestine, secondary active uptake transporters, such as peptide transporter (PEPT) 1 for small peptides (di- or tripeptide) (2–5) and ileal Na^+-dependent bile salt transporter (ISBT) (6–8) for bile salts, are expressed on the apical membrane. Epithelial cells avidly take up these essential substrates and then secrete them into the circulating blood. Among the drug transporter families, OATP-B and rat Oatp3 are reported to be expressed on the apical membrane (9). OATP-B accepts bile salts as well as pravastatin and sulfate conjugates of steroid hormones at a low pH, as demonstrated in transfected HEK293 cells, although the contribution of this to absorption is not yet known (10). Primary active efflux transporters (ABC transporters), including multidrug resistance protein (MDR)1 (11,12), MRP2 (13–15), and breast cancer resistance protein (BCRP) (16,17), are also expressed on the apical membrane for the purpose of extruding xenobiotics (18,19). These efflux transporters contribute to the elimination of drugs from the systemic circulation. After an intravenous administration of 1-chloro-2,4-dinitrobenzene (a precursor of the Mrp2 substrate, dinitrophenol glutathione, and its *N*-acetylated form) to rats, secretion of these substrates into the intestinal lumen was observed (20). This type of secretion was significantly reduced in Mrp2-deficient rats. Mrp1 (21) and Mrp3 (22) are expressed on the basolateral side, although their physiological significance remains to be clarified. The role of basolateral transporters is not yet fully understood in the small intestine compared with the apical transporters mentioned above. Several organic anions, including bile acids, are transported in an ATP-dependent manner into isolated basolateral membrane vesicles from rat jejunum, ileum, and colon (23). These are mediated, at least partly, by Mrp3 on the basolateral membrane as the transport kinetics and inhibitor sensitivity are similar to those determined in the rat Mrp3 expression system, and there is a positive correlation of the transport activity and protein expression profile along the intestine (23). Organic cation transporter (Oct1) is also functionally expressed on the basolateral membrane to facilitate the elimination of its substrates from the circulating blood into the intestinal lumen as demonstrated in Oct1 knockout mice (24). The expression of MRP2, MRP3, MDR1, and BCRP is highest at the top of the villus, where absorption of the compounds takes place. However, MRP1 expression is highest in the crypt region where extensive cell division occurs (21).

Kidney

In the kidney epithelium, many of the uptake transporters are localized on the basolateral side and the efflux transporters are on the apical side (25,26). As a result, vectorial transport from the blood side to the urinary lumen is achieved. Moreover, small peptides, sugars, and other essential nutrients are reabsorbed from the urinary lumen via secondary active uptake transporters, including the PEPT and sodium glucose transporters on the apical membrane. OAT1 (27–30) and OAT3 (28,29,31,32) are expressed on the basolateral membrane of the renal proximal epithelium in both humans and rats. However, localization of OAT2/Oat2 in the renal epithelium is different between rats and humans. Human OAT2 is localized on the basolateral membrane of the renal proximal tubule epithelium, whereas rat Oat2 is localized on the apical surface of the tubules in the medullary thick ascending limb of Henle's loop and cortical and medullary collecting ducts (29). OCT families (OCT1–3) are all localized on the basolateral membrane of the renal epithelium (28,33–37) to take up organic cations in a voltage-dependent manner. Among these three OCT families, OCT2 expression seems the highest and most restricted in the human kidney (28). However, OCT1 expression is quite low in the kidney, but highly expressed in the human liver (28). Possibly, OCT2 is the primary transporter for renal uptake and OCT1 is the primary transporter for hepatic uptake of organic cationic compounds. OCT3 expression is not restricted to kidney or liver, but found widely throughout the body. OCTN family members, including OCTN1, OCTN2, and OCTN3, have all been detected in the kidney. All these OCTN family members can transport carnitine and exhibit different transport properties, i.e., Na^+-dependence or pH-dependence (see (38) for details). OctN2-deficient juvenile mice with visceral steatosis exhibit lower blood carnitine levels due to the impaired renal reabsorption of carnitine (39). More importantly, the high-affinity carnitine transporter OCTN2 can transport cationic drug molecules other than carnitine (40). Although the subcellular localization of OCTN members is unknown at present, it is likely that OCTN2 is localized on the brush border membrane and is involved in the reabsoption of carnitine following exchange with cationic compounds (40). Oatp1 (41) and Oat-K1 (42) are expressed on the apical side in the rat kidney. As Oatp1 (43) and Oat-K1 (44) have been demonstrated to be bidirectional transporters, they probably act as both reabsorption and secretory pathways for the substrates. To date, no human counterparts of Oatp1 and Oat-K1 have been reported. Localization of efflux transporters on the apical side of the renal epithelium has been reported for MRP2/Mrp2 and MRP4/Mrp4 in both humans and rats (45–47). Moreover, MDR1 is also located on the apical side of the renal epithelium (11). These ABC transporters may contribute to the unidirectional secretion of their substrates (25).

Liver

Hepatocytes are highly polarized cells responsible for the vectorial efflux of water. For most drugs, uptake carriers such as Na^+-taurocholate cotransporting polypeptide (NTCP), OAT2, several OATP families, and OCT1 are all located on the basolateral membrane, whereas efflux transporters are expressed on the apical canalicular membrane under normal conditions (48). In particular, active and unidirectional efflux of organic anions into the bile via MRP2 and the bile salt export pump (BSEP) produce a high osmotic gradient across the tight junctions to drag water from the blood into the bile—the so-called bile flow. However, under obstructive cholestasis (49,50) or inflammatory stress (51), these canalicular transporters are downregulated. Alternatively, MRP3, which have a similar or overlapping substrate specificity with MRP2 and BSEP, is induced on the basolateral membrane to extrude these compounds into the circulating blood and, finally, excrete them into urine (52). This switching of the transport direction without changing the overall substrate specificity is thought to be one of the defense mechanisms of hepatocytes used to protect them from damage due to toxic material (53).

BBB/BCSFB

As far as many of the transporters in the BBB are concerned, their localization is not yet fully understood. The general consensus is that MDR1 is located on the apical (blood side) membrane of the brain capillary endothelial cells. This acts as an efflux transporter for lipophilic compounds as demonstrated in knockout mice (54). Moreover, in rat BBB, Oatp2, a bidirectional transporter for organic anions (55), is found on both the apical and basolateral sides (56). (BCRP) is found on the apical side of the BBB in humans (57). Its substrates include neutral and negatively charged molecules, including cytotoxic compounds (mitoxantrone, topotecan, flavopiridol, methotrexate), sulfated conjugates of therapeutic drugs, and hormones (estrogen sulfate) (58,59). This efflux transporter possibly restricts the entry of these substrates into the brain, although contradictory results have also been obtained when Bcrp knockout mice were used (60). Lee *et al.* (60) demonstrated that the brain uptake of [^{3}H]dehydroepiandrosterone sulfate and [^{3}H]mitoxantrone, both established Bcrp substrates, did not differ between wild-type and knockout mice. They concluded that the contribution of Bcrp is minor in the BBB, at least in limiting these two compounds entering the brain. OATP-F/Oatp14, a bidirectional transporter for the thyroid hormones T3 and T4, is expressed in the brain (61,62), and was recently shown to be located on the basolateral side of the BBB. In BCSFB, Mdr1, PepT2 (63), Oat3 (64) and, possibly, Oatp3, but not Oatp1 (56,65), are expressed on the apical (brain) side, whereas Mrp1 (66) and Oatp2 (56) are located on the basolateral (blood) side in rat BCSFB. The messenger RNA expression profile has been investigated in detail in the rat choroid plexus in comparison with the liver, kidney, and ileum using a branched DNA signal amplification technique (67). In addition to the transporters mentioned above, other family members, including Oat2, Oct3, Oatp9, Mrp4, and Mrp5, have been detected (67). Interestingly, it was recently reported that the subcellular localization of Mrp4 in the BCSFB and BBB was basolateral and apical, respectively, in mice (68). This is the first transporter exhibiting a subcellular localization that differs between the BBB and BCSFB. These heterologous localizations (both facing the blood side) are consistent with the protective role of Mrp4 in the brain as far as toxic compounds are concerned. Accordingly, topotecan (Mrp4 substrate) accumulation in the brain was increased in Mrp4 knockout mice and resulted in higher sensitivity to this anticancer reagent (68).

Blood–Testis and –Placental Barrier

In the testis, mRNA expression of many other transporters has been confirmed by RT-PCR in Sertoli cells in rats (69). Sertoli cells and testicular blood endothelial cells independently form blood–testis barriers to protect developing germ cells from xenobiotics and immunological effects (Fig. 1). The Sertoli cell layer expresses MRP1 on the basolateral side (70,71), and endothelial cells express MDR1 and BCRP on the luminal side (71). These transport directions are preferable for limiting the free entrance of toxic compounds from the systemic circulation (72,73). In fact, increased etoposide-induced damage to the mucosa of the seminiferous tubules of the testis has been demonstrated in Mrp1 knockout mice (70).

Expression of MDR1 and MRP family has been confirmed in the placenta (74–76). MDR1, MRP2 and BCRP are found on the apical membrane of placental syncytiotrophoblasts, whereas MRP1 and MRP3 are found on the basolateral membrane of these cells. Although MDR1, MRP2, and BCRP seem to protect the fetus from toxic materials in the maternal circulation (72), the exact role of MRP1 and MRP3 has not yet been determined. In mdr1a/1b double knockout mice, the distribution of topotecan into the fetal compartment was increased twofold in the presence of the BCRP inhibitor GF120918, suggesting the functional significance of BCRP in the fetal–maternal barrier (77).

APICAL/BASAL SORTING OF TRANSPORTER DEPENDS ON HOST CELLS

We can predict the intracellular sorting of proteins for specific cytosolic organelles (78), such as the nucleus (79), endoplasmic reticulum (ER) (80), mitochondria, and lysosomes. However, it is too complicated to predict the sorting direction of membrane transporters, apical or basolateral, in heterologous tissues or in *in vitro* cell lines from their primary sequences. Actually, some of the transporters are sorted to the opposite direction in different tissue or cell lines. For example, rat Oatp2 is sorted to both the apical and basolateral sides of the BBB, although it is localized on the basolateral side of choroid plexus epithelia (56). As mentioned above, Mrp4 is localized on the apical and basolateral side of BBB and BCSFB, respectively, in mice (68). Human OATP-B is localized on the apical and basolateral membranes of intestinal epithelia (9) and hepatocytes (81), respectively. Rat Oat2 is located on the apical and basolateral membranes of the renal epithelium (29) and hepatocytes (82,83), respectively. Rat Oatp1 is located on the

basolateral membrane of hepatocytes (41), whereas it is located on the apical side of the renal epithelium to reabsorb compounds from the lumenal side (41). There is a marked difference in the molecular weight of the hepatic and renal forms of Oatp1 (83 kDa in liver and 33–37 kDa in kidney (84–86)), although there is no further evidence supporting the significance of this size difference as far as intracellular sorting is concerned. Another typical example is rat Oat-K1. It is expressed on the apical side of the kidney proximal epithelia (42) and MDCK cells (87), whereas it is expressed on the basolateral membrane in LLC-PK1 cells (88). Inverse sorting of membrane proteins in MDCK and LLC-PK1 cells has also been reported for other nontransporter proteins (89,90). The H^+/K^+ ATPase β-subunit, LDL receptors, and transferrin receptors are localized on the basolateral membrane of MDCK cells, whereas they are localized on the basolateral membrane of LLC-PK1. In some, but not most, cases, this can be explained by the lack of important intracellular adapter protein μ1B, a component of clathrin adapter complex AP-1 involved in the basolateral sorting in LLC-PK1 cells. In such cases, basolateral sorting is restored to that in MDCK cells by introducing μ1B into LLC-PK1 cells (90). Although none of the transporters mentioned above has been examined in terms of an interaction with these adapter proteins, cell type specific sorting machinery is likely to be involved in the complex sorting heterogeneity. As we have little information about the sorting motifs and the interacting machinery for the sorting of drug transporters, this remains an unexplored field that will be investigated in the future.

MRP2 AND BSEP AROUND THE PERICANALICULAR MEMBRANE

Besides sorting of newly synthesized proteins from the ER to the plasma membrane, insertion and retrieval from the plasma membrane also affects the steady-state level of protein expression. Extensively studied examples include the biliary transporters located on the bile canalicular membrane as reviewed by Crocenzi *et al.* (91). Mrp2 is internalized from and reinserted into the bile canalicular membrane under cholestatic conditions (92), following drug treatment (93), cytokine stimulation (51,94), or an osmotic effect (94–96). Similar phenomena have also been reported for Bsep (51,95,97,98), and these local changes rapidly affect the transport activity and bile flow rate. In clinical situations, genipin, a Kanpo medicine widely used for jaundice, stimulates Mrp2 localization on the canalicular microvilli and induces GSH-dependent choleresis (Fig. 2) (99). The bile flow was rapidly increased after an intravenous infusion of genipin (1 μmol/min/100 g body weight), whereas this effect was absent in Eisai hyperbilirubinemic rats (EHBR) lacking Mrp2 (Fig. 2B). On the other hand, the bile flow increased significantly after choleretic ursodeoxycholate (UDC) treatment both in normal Sprague–Dawley (SD) rats and EHBR (Fig. 2B). These results indicate the specific relocalization of Mrp2 by genipin, which has a choleretic mechanism different from UDC. The canalicular surface expression of Mrp2 was increased by about twofold within 30 min of genipin administration (Fig. 2A).

Although the precise molecular mechanism is not fully decoded, several interacting proteins have been reported for biliary transporters. The PDZ motif is found in MRP2/Mrp2 at the carboxy terminal end (Table I) and is reported to be able to interact with PDZK1 as well as other PDZ proteins *in vitro* (100,101). The PDZ protein is a member of the family of cytoplasmic proteins containing several consensus PDZ motifs conserved between PSD95, Dlg, and ZO-1. These motifs can bind to carboxy terminal sequences such as the T/S-*X*-*Φ* of target protein, where *X* is any amino acid and *Φ* is a lipophilic amino acid. Importantly, many of the drug transporters have this consensus motif (Table I), suggesting a possible interaction with some PDZ proteins. In general, plural target proteins are linked to each other via the PDZ protein, so they can cross-talk in close cooperation or be stabilized under such conditions (102,103). The role of the PDZ binding motif in MRP2 localization or activity is still controversial. Although the carboxy terminal PDZ binding motif is important for the expression of MRP2 on the apical membrane in MDCK (104), it is not necessary in other cases (105–107). Recently, PDZK1 knockout mice have been produced (108). However, subcellular localization of known interacting proteins (MRP2, type IIa Na/Pi cotransporter) is normal in the kidney. Moreover, the serum bilirubin concentration is not affected in knockout mice, suggesting normal Mrp2 localization on the canalicular membrane of hepatocytes. The only observed difference is an increase in serum cholesterol. This may be because of the role of PDZK1 as a regulator of the cholesterol clearance system. PDZK1 corresponds to rat C-terminal linking and modulating protein (CLAMP), which modulates the surface expression and/or function of cholesteryl ester acceptor protein in the hepatocyte basolateral membrane (109). However, it is still possible that other PDZ family proteins compensate for the function of PDZK1, as has been discussed elsewhere (108).

Linking to the cytoskeleton structure also seems to be important for Mrp2. Radixin knockout mice exhibit conjugated hyperbilirubinemia (Fig. 3B) (110). Radixin is a member of the ERM family (ERM is an acronym for the cytosolic proteins Ezrin, Radixin, and Moesin sharing 70% amino acid identity) (102). They localize at the back of the apical membrane and act as a connector of integral membrane proteins and the filamentous actin (F-actin) that extends toward the apical microvilli. F-actin also binds to the myosin at the base of the microvilli and is involved in the contraction of the bile canaliculi and bile flow formation. Mrp2 on the bile canalicular membrane is reduced to 20% of the value in control mice, whereas that in whole-cell lysate is only reduced to 60% (Fig. 3A). Although localization of Mdr1 (P-GPs) and CD26 on the canalicular membrane was also decreased to some extent, similar reductions were also observed in whole-cell lysate for these proteins (Fig. 3A). The effect of radixin deficiency seems specifically to alter Mrp2 localization, which can be explained by direct interaction between radixin and Mrp2. Interaction of the amino-terminal half of radixin and the carboxy region of human MRP2 [1232–1545] has been demonstrated in a pull-down assay and also in hepatocytes and MDCK cells expressing MRP2 (110). Mrp2 may be linked to cytoskeleton filaments via radixin, and so Mrp2 may be removed from the membrane surface without radixin in knockout mice. Such a concept has been examined using the liver from

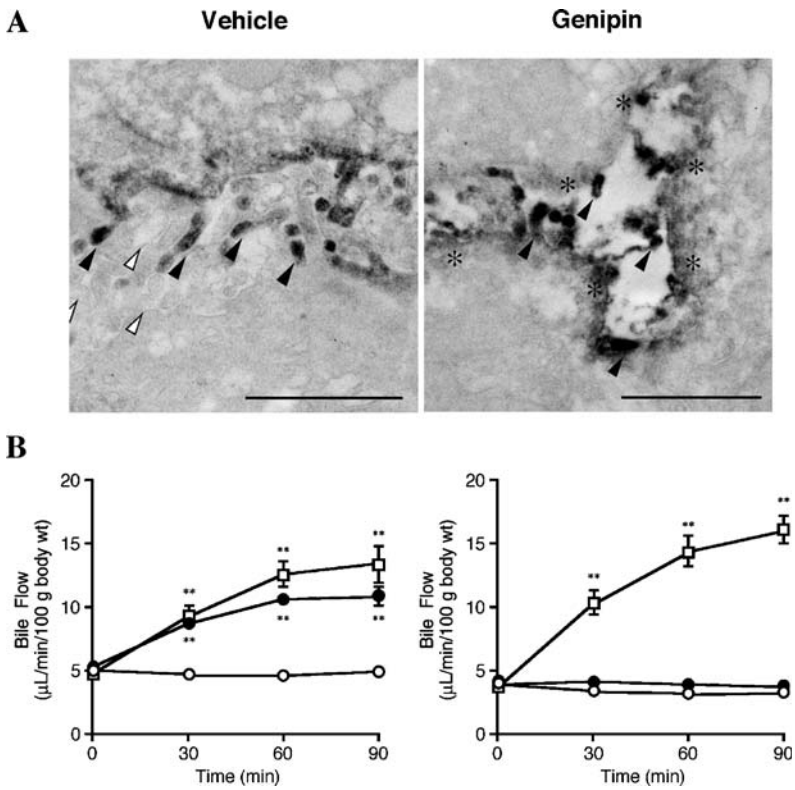

Fig. 2. Genipin specifically enhances Mrp2 localization and induces GSH-dependent choleresis (99). (A) Immunohistochemical analysis of Mrp2 after Genipin treatment in rats (electron microscopy). Liver tissue sections were prepared from SD rat livers 30 min after intravenous administration of vehicle or genipin. The Mrp2 protein was localized mostly in canalicular microvilli and canalicular membrane in genipin-treated livers. The proportion of Mrp2-containing microvilli (▲) to total microvilli [including Mrp2-negative microvilli (□)] was greater in genipin-treated than in vehicle-treated liver tissue sections and the membrane density of Mrp2 protein around the base of microvilli in genipin-treated liver tissue sections (*) was markedly increased in genipin-treated tissue sections. Bars = 1.0 μm. (B) Ursodeoxycholate (UDC) (□), genipin (●), or control vehicle (○) was intravenously infused (1 μmol/min/100 g) in normal SD rats (left panel) and Mrp2-deficient EHBR (right panel). Bile flow was increased by genipin and UDC treatment (left panel). Stimulation of GSH excretion via Mrp2 may be the mechanism of genipin-induced choleresis. UDC-induced choleresis seems to be independent of Mrp2 but affects Bsep function (right panel).

patients with primary biliary cirrhosis (PBC) (111). The redistribution of MRP2 protein in the hepatocytes of PBC stage III patients has been observed (111). The areas of irregular MRP2 immunostaining showed largely reduced radixin immunostaining implying the importance of radixin for proper MRP2 expression, whereas normal hepatocytes exhibit precise colocalization of MRP2 and radixin on the canalicular membrane. The role of radixin in the regulation of Mrp2 surface expression should be further addressed under other cholestatic conditions where Mrp2 downregulation has been considered (94,96).

MDR1, MDR2, and BSEP do not contain obvious PDZ interacting motifs (Table I), although these proteins are localized on the canalicular membrane. Ortiz *et al.* (112) recently found that these biliary ABC transporters interact with HAX-1 (HS1-associated protein X-1) via their linker region, as initially found in two hybrid screening systems in yeast; this was also confirmed by coimmunoprecipitation assay using rat hepatocytes. Most of the HAX-1 was colocalized with Mdr1a/1b, Mdr2, and Bsep in the canalicular membrane fraction. Moreover, specific reduction of the endogenous HAX-1 by 70% in MDCK cells using an RNA interference technique increased the apical surface expression of exogenously transfected rat Bsep by 71%, perhaps because of retarded internalization of Bsep (112). Similarly, inhibition of endogenous cortactin, which links HAX-1 and actin filaments, also increased the apical surface expression of Bsep in MDCK cells. These data suggest the cooperative role of HAX-1 and cortactin in the internalization of Bsep from the canalicular membrane of hepatocytes, although the physiological significance of these interactions needs to be explored *in vivo* using hepatocytes. The role of HAX-1 on the membrane localization of Mdr1a/1b and Mdr2 has not yet been elucidated.

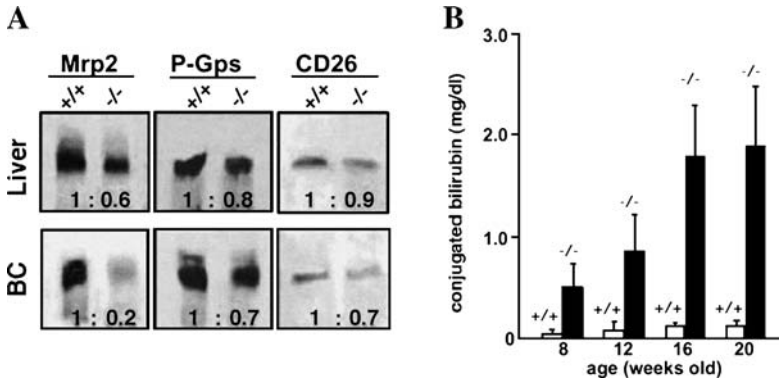

Fig. 3. Mrp2 surface expression is impaired in Radixin knockout mice and results in jaundice (110). (A) Western blot analysis of canalicular membrane proteins [Mrp2, Mdr (P-Gps), and CD26] in normal (+/+) and radixin knockout mice (−/−). The relative expression of these proteins in total cell homogenate (liver) and bile canalicular membrane (BC) is indicated. The efficiency of Mrp2 expression on BC is relatively low compared with other canalicular proteins. (B) The serum bilirubin concentration is significantly higher in Radixin knockout mice (closed columns) than that in normal mice (open columns) presumably because of impaired Mrp2 expression on the canalicular membrane surface.

Collectively, heterogeneous factors including the PDZ family, ERM family, and other unknown proteins interact with MRP2 and, possibly, BSEP to sort and/or anchor them to the canalicular membrane. As PDZ binding motifs have also been observed in the carboxy terminal of many drug transporters (Table I), the existence of a spatial and/or functional regulation mechanism will be an interesting field of investigation.

SINGLE NUCLEOTIDE POLYMORPHISMS AND LOCALIZATION OF DRUG TRANSPORTERS

Although information about single nucleotide polymorphisms (SNPs) is steadily increasing, little is known about the functional effect of SNPs *in vitro*, not to mention *in vivo*. In addition to the total amount of protein in the cell lysate and intrinsic transport activity of a single molecule, the effect on the sorting efficiency is also important for the evaluation of interindividual differences in drug disposition and distribution. Although a limited number of studies have been focused on relevant cells, such as human hepatocytes for hepatic transporter studies as shown below, it is still informative to examine the effect of SNPs on the surface expression of transporters in heterologous cell lines including MDCK cells and other nonpolarized cells.

OATP-C

One of the few examples of an investigation of the effect of SNP on intracellular sorting and the impact on the pharmacokinetic profile involves OATP-C (also referred to as OATP2 and LST-1) SNPs. OATP-C participates in the hepatic uptake of a broad range of organic anions, including bile salts, hormones, peptides, and other organic anions and cations (65). Single nucleotide polymorphisms have been found and some of them are involved in the efficiency of membrane sorting (Fig. 4) (113–118). In some SNPs, reduced surface expression of the transporter was observed without any change in total cell lysate (113). In a study of 81 Caucasian volunteers, OATP-C protein expression was substantially reduced in the liver of one subject (114). The SNP (L193R), located in the fourth transmembrane domain, resulted in accumulation inside the ER and no surface expression of the product in MDCK cells. Reduced surface expression was also observed in the V174A mutant in HeLa cells, where V174 is also located in the fourth transmembrane domain (Fig. 4) (113). However, the surface expression was not affected in V174A when it was introduced into HEK293 cells, suggesting that the sorting efficiency was not the same in HeLa and HEK293 cells. The pharmacological significance of OATP-C SNPs is slowly becoming better understood in human volunteers. Indeed, the nonrenal clearance of pravastatin may be mediated by OATP-C in the liver, and a study on Japanese subjects has shown that it is significantly reduced in healthy volunteers with OATP-C*15/*15 (DI30 A174 homozygote) compared with groups with OATP-C*1b/*15 (DI30V174/D130 A174 heterozygote) alone (Fig. 5) (115). Decreased surface expression of OATP-C*15 is possible in human hepatocytes because OATP-C*15 exhibits reduced surface expression when introduced into HEK293 cells, although N130D and V174A alone do not affect the surface expression (116,117). Altered pravastatin pharmacokinetics in Caucasian volunteers have also been reported in subjects with N130D/V174A SNPs (118). In heterozygous carriers of OATP-C*15 (N130D/V174A), the mean pravastatin AUC_{0-12} was 93% ($p = 0.024$), higher than that for noncarriers. Moreover, in heterozygous carriers of *17 (containing the −11187G>A in the promoter region and N130D/V174A), it was 130% ($p = 0.0053$), higher than that for noncarriers. Again, these results support the importance of OATP-C*15, whose surface expression and/or function has also been identified in a Caucasian population.

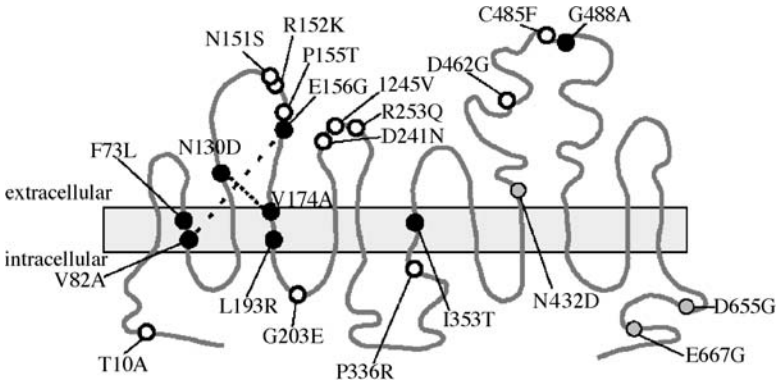

Fig. 4. OATP-C SNPs affecting surface expression. OATP-C alleles reducing its surface expression *in vitro* are indicated by closed circles: F73L (OATP-C*2), V82A/E156G (OATP-C*3), V174A (OATP-C*5), I353T (OATP-C*6), G488A (OATP-C*9) (113), N130D/V174A (OATP-C*15) (115,116), and L193R (113). Three of these SNPs (N130D, V174A, and L193R) have actually been shown to affect its surface expression and/or function *in vivo* in humans (Fig. 5 and see text). Note that the surface expression and transport activity of OATP-C were not affected in N130D (OATP-C*1b) and V174A (OATP-C*5) alone, but were significantly reduced in the N130D/V174A simultaneous variant (OATP-C*15) in HEK293 cells (116,117), although reduced surface expression of OATP-C*5 (V174A) in HeLa cells was also reported (113). V82A and E156G were not examined alone but only a combination of these SNPs (V82A/E156G: OATP-C*3) reduced the surface expression in HeLa cells (113). SNPs affecting transport K_m or V_{max} are shown in hatched circles, although D655G was examined only in combination with F73L (as OATP-C*12) and E667G was examined only in combination with V82A/E156G (as OATP-C*13) (113). Other SNPs without any apparent effect (113) or only appearing in the Entrez SNP database (http://www.ncbi.nlm.nih.gov/entrez/) without any functional information are indicated by open circles.

BCRP

Breast cancer resistance protein is a member of the half-size ABC transporter family on the apical membrane of hepatocytes, the small intestinal epithelium, placenta, prostate, and BBB. Recently, some of the nonsynonymous SNPs of BCRP have been reported to cause impaired apical expression in LLC-PK1 cells (119,120). Mizuarai *et al.* (120) reported that the V12M SNP mutant exhibited severely impaired apical expression in LLC-PK1 cells. The allele frequency of V12M reached as high as 10.3%, and actually 27 and 2 out of the 150 normal healthy Caucasians were hetero- and homozygotes of this SNP, respectively. Kondo *et al.* (119) also examined the cellular localization of a total of seven SNP variants of BCRP (V12M, Q141K, A149P, R163K, Q166E, P269S, and S441N) in LLC-PK1. As a result, reduced protein expression levels of Q141K and S441N were observed compared with the wild-type BCRP. In contrast to the report from Mizuarai *et al.*, V12M was found to be normally localized on the apical membrane of LLC-PK1 cells (119). The reason why such a contradictory result was observed while using the same SNP variant of BCRP (V12M) in the same host cell (LLC-PK1) is currently unknown, but the different cell culture conditions in laboratories might be one possible cause. The intestinal absorption, biliary excretion, and brain penetration profile of BCRP substrates are possibly affected in these SNP subjects, although this has not yet been tested in humans. It is also possible that the incidence of acquired drug resistance of BCRP is related to this SNP, because its substrates include several chemotherapeutic agents, such as mitoxantrone, SN38, doxorubicin, and daunorubicin (58).

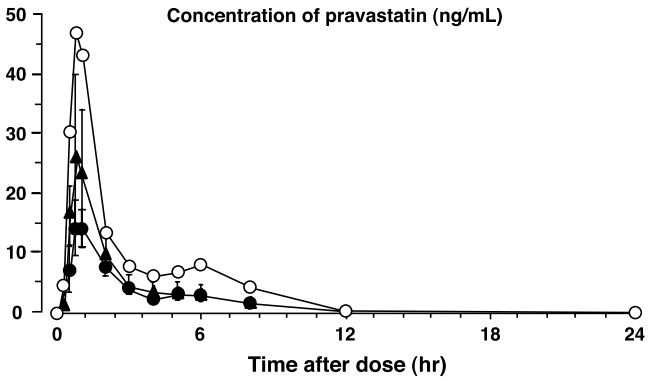

Fig. 5. OATP-C SNPs affect the pravastatin elimination time profile in humans. Three genotypic groups with OATP-C *1b/*1b (D130V174 homozygote, ●), *1b/*15 (D130V174/D130A174 heterozygote, ▲), and *15/*15 (D130A174 homozygote, ○) were orally administered with pravastatin (10 mg). The nonrenal clearance of pravastatin mainly depends on OATP-C function and this was significantly reduced in *1b/*15 compared with *1b/*1b (115). Reduced surface expression of OATP-C is a possible cause because OATP-C*15 exhibited reduced surface expression when introduced into HEK293 cells (116,117).

MRP2 and BSEP

MRP2 and BSEP on the bile canalicular membrane are involved in the unidirectional excretion of organic anions and bile acids, respectively (121,122). A hereditary defect in MRP2 and BSEP activity causes conjugated hyperbilirubinemia (Dubin–Johnson syndrome) and progressive familial intrahepatic cholestasis type II (PFICII), respectively, and some conditions are caused by a sorting problem (123–128). Figure 6 shows all the reported SNPs with amino acid substitutions and also the mutations linking them to Dubin–Johnson syndrome (MRP2) and PFICII (BSEP). All the mutant forms of MRP2 found in patients with Dubin–Johnson syndrome can be classified as due to miss-folding in the ER or loss of transport function (Fig. 6) (123–127). Among eight amino acid mutations observed in Dubin–Johnson syndrome patients, three of them are nonsense mutations (R105*, R1066*, and R1309*). At least three of the other mutations (R768W, I1173F, and deletion 1392–1394) are related to sorting problems from the ER to Golgi, as these mutant MRP2 accumulated within the ER in core-glycosylated forms. Q1382R MRP2 was mainly localized on the apical membrane of transfected LLC-PK1 cells as wild-type MRP2. However, efflux of glutathione monochlorobimane and ATP-dependent leukotriene C_4 uptake into plasma membrane vesicles from cells expressing Q1382R MRP2 were markedly reduced, suggesting that the Q1382R MRP2 on the apical membrane was nonfunctional (125). Similarly, R1150H was also found as the mature glycosylated form on the membrane surface of transfected HEK293 cells but was not functional (123).

Seven amino acid substitutions in BSEP, linked to PFICII (G238V, E297G, C336S, D482G, G982R, R1153C, R1268Q), have been reported and have been examined using rat Bsep expressed in MDCK (128). Five of these mutations resulted in disappearance from the apical surface in MDCK cells (G238V, E297G, G982R, R1153C, R1268R) (128). These substitutions simply affect the folding and stability in MDCK cells rather than the specific apical sorting motif, as the transport activities of these five mutants also disappeared in nonpolarized Sf9 cell membrane vesicles. C336S affected neither Bsep transport activity nor the apical trafficking of rat Bsep, suggesting that this mutation alone may not cause this disease. Recently, the intracellular sorting and transport function of the D482G mutant was further examined in detail using mouse Bsep expressed in HepG2 cells and Sf21 insect cells (129). A considerable amount of D482G mutant mBsep protein was still detected in the cytoplasm as well as the bile canalicular space. Such reduced sorting efficiency was attributable to the unstable and temperature-sensitive nature of the

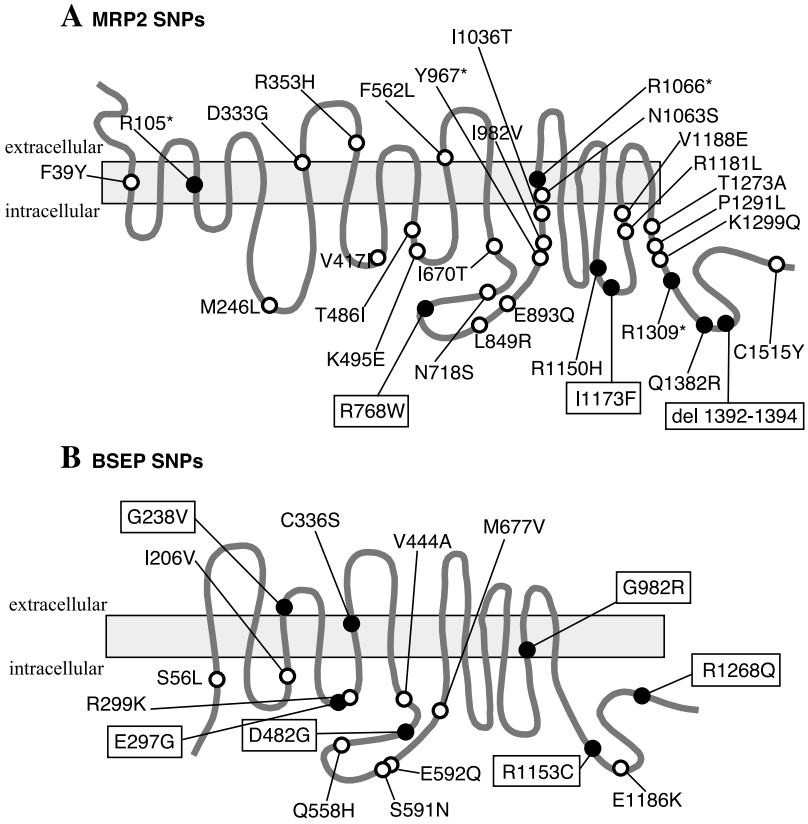

Fig. 6. Mutations in MRP2 and BSEP lead to impaired surface expression and genetic diseases. Closed circles are mutations associated with Dubin–Johnson syndrome (MRP2) and PFICII (BSEP). Some of them (indicated in the boxes) lead to impaired surface expression. Open circles are SNPs not related to these diseases. Asterisks indicate the nonsense mutations producing immature stop codons. Data are obtained from the Entrez SNP database (http://www.ncbi.nlm.nih.gov/entrez/) and the literature [MRP2 (123–127) and BSEP (128,129)].

D482G mutant without loss of transport function. D482G expressed in the nonpolarized Sf21 insect cell membrane was functionally active. Moreover, culturing the D482G expressing HepG2 cells at a lower temperature (30°C) resulted in increased expression of the fully glycosylated form of mouse Bsep (12.6-fold) compared with that at 37°C. These observations offer hope of therapy using putative chemostabilizers, which can stabilize unstable mutant proteins inside cells and promote surface expression of transport-competent transporters. Such a concept has already been accepted as a new form of therapy for cystic fibrosis with the delta508 genotype, which is the most frequently observed mutation in CFTR patients (130).

CONCLUSION

Localization of the transporters is well conserved among species, resulting in the vectorial transport of endo- and xenobiotic compounds. In some cases, substitution of critical amino acids for folding inside the ER leads to protein aggregation and degradation before reaching the cell surface. It might be also possible that specific apical/basal sorting is disrupted by genetic polymorphism, although clear experimental evidence is not yet available. If typical endogenous compounds are the substrates of the affected drug transporters, the phenotype will be apparent as shown in patients with Dubin–Johnson syndrome. Moreover, as drug transporters with nonsynonymous SNPs are susceptible to altered surface expression and pharmacokinetic profiles as demonstrated in OATP-C, other transporter SNPs and their effects on intracellular sorting and stability are also important issues to be addressed.

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