that variants in cholesterol 7alpha-hydroxylase (*CYP7A1*) (Pullinger et al. 2002), *ABCG8* (Kajinami et al. 2004) and HMG-CoA reductase (*HMGCR*) (Chasman et al. 2004) are important determinants of the lipid response to statin therapy.

Pravastatin, a hydrophilic HMG-CoA reductase inhibitor, is taken up efficiently from the circulation into the liver by an active transport carrier system, but is not metabolized by CYP enzymes. Human organic anion-transporting polypeptide 1B1 (OATP1B1), transporter of pravastatin, is expressed on the basolateral membrane in the hepatocytes responsible for the hepatocellular uptake of pravastatin (Hsiang et al. 1999). The major site of cholesterol synthesis, the liver, is the main target organ of statins. Recently, Niemi et al. (2005) have shown that the *SLCO1B1**17 allele (containing -11187G>A, 388A>G and 521T>C) is associated with the decreased acute effect of pravastatin on cholesterol synthesis; however, the impact of *SLCO1B1* genotypes on the lipid-lowering response to pravastatin during long-term treatment has not been well investigated.

The aim of this study was to describe the influence of *SLCO1B1* genotypes on the lipid-lowering response to pravastatin in Japanese hypercholesterolemic patients. Furthermore, we evaluated the contribution of genetic variants in other candidate genes (*APOE*, *CYP7A1*, *ABCG8* and *HMGCR*) to the variability in pravastatin efficacy.

Materials and methods

Study design

We studied 33 patients (14 males and 19 females; mean age 62.3 years; age range 34–83 years) with hypercholesterolemia treated in Tottori University Hospital. All subjects were initially prescribed pravastatin (mean dose range 9.4 mg/day) between January 1997 and October 2004. We used the electronic medical database available in the hospital to obtain precise information on patients' backgrounds, laboratory tests, prescribed drugs and adverse events. We collected these data retrospectively for each patient for at least 1 year from the day pravastatin was administered. Patients with serious or uncontrolled renal or liver disease, no drug compliance, other hypolipidemic treatment or uncontrolled diabetes were excluded. The average body mass index (BMI), total cholesterol (TC) and LDL-C values in this study patients were 23.9 kg/m^2 (range 17.3–30.9 kg/m^2), 259.6 mg/dl (range 225.8–315.0 mg/dl) and 167.4 mg/dl (range 112.0–240.7 mg/dl), respectively. This study was approved by the Tottori University Ethics Committee, and informed consent was obtained from all individuals.

Genotyping

All subjects were genotyped for variants in the candidate genes involved in the pharmacokinetics and pharmacodynamics of pravastatin. Details of the genotyping and haplotyping of *SLCO1B1**1b (388A>G), *5 (521T>C) and *15 (388A>G and 521T>C) were described previously (Nishizato et al. 2003). The promoter variant (-11187G>A) in the *SLCO1B1* gene was determined with PCR–SSCP analysis. The *SLCO1B1* -11187G>A variant was observed as heterozygosity (0.212) in this patient group suggesting it was tightly linked to the *SLCO1B1**15 allele. The genotypes in *CYP7A1* (-204A>C) (Hubacek et al. 2003), *APOE* (ϵ2, ϵ3 and ϵ4) (Hixon and Vernier 1990) and *ABCG8* (55G>C) (Kajinami et al. 2004) were examined by previously described methods using PCR restriction fragment length polymorphism analysis. Genetic variants (SNP12 and 29) in the *HMGCR* gene were found as functional variants for variable response to statin therapy in the previous study (Chasman et al. 2004) as determined with PCR–SSCP analysis.

Statistical analysis

Comparisons between two groups were performed using a Student *t*-test and between more than two groups using ANOVA (with Tukey–Kramer multiple comparison test). A 5% level of probability was considered to be significant.

Results and discussion

The mean percent reductions from the baseline in TC and LDL-C values at 8 weeks post-treatment with pravastain were significantly smaller in heterozygous carriers of the *SLCO1B1**15 allele than in homozygous carriers of the *1a and *1b alleles (Fig. 1a, $P<0.05$). Also, the mean percent reduction from the baseline in TC values at 8 weeks post-treatment was significantly smaller in *SLCO1B1**15 carriers than in non-carriers (–9.8 vs –20.9%; $P<0.05$; Fig. 1b). A similar trend was observed in the LDL-C level (–14.1 vs –28.9%, $P<0.05$; Fig. 1b) even though the pravastatin daily dose (mean±SD; non-carriers: 9.4±2.9 mg, carriers:

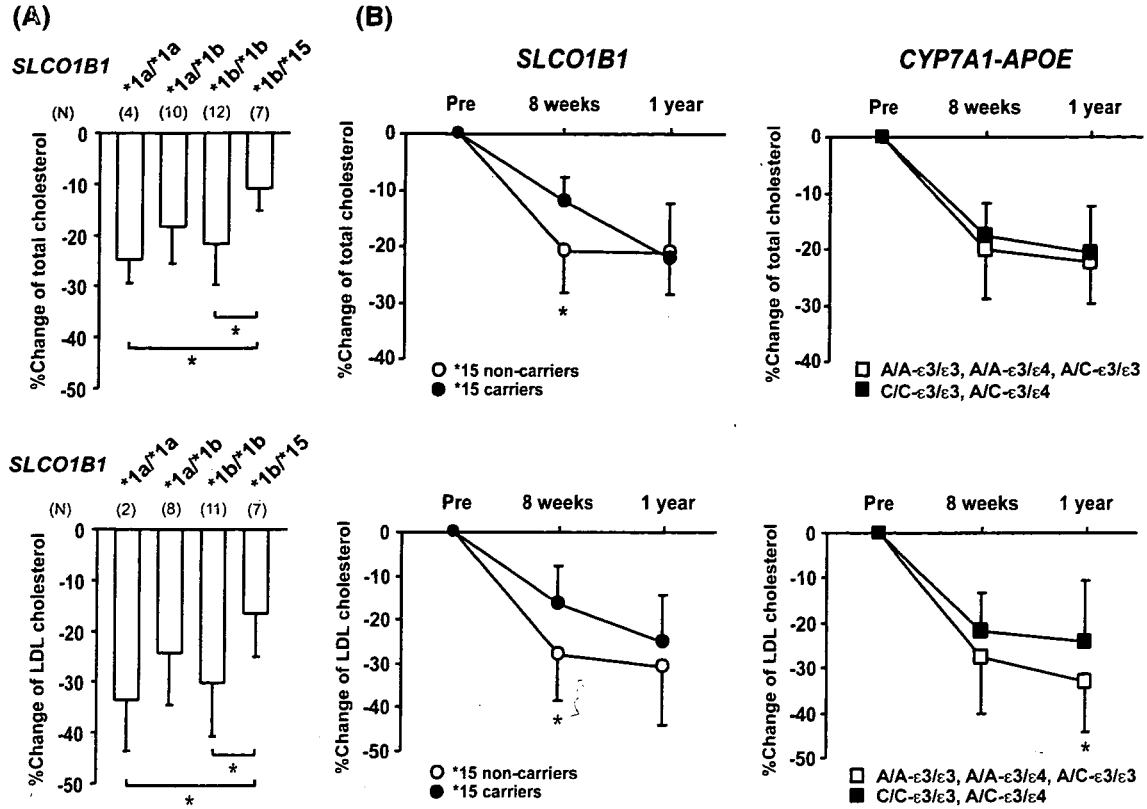

Fig. 1 **a** Influence of the *SLCO1B1* genotypes on percent reduction from baseline in TC and LDL-C values at 8 weeks after pravastatin treatment. *$P<0.05$ when compared between the two groups using Tukey–Kramer multiple comparison test. **b** Influence of the *SLCO1B1*, *CYP7A1* and *APOE* genotypes on time course of percent reduction from baseline in TC and LDL-C value after pravastatin treatment. *$P<0.05$ when compared between the two genotypes was analyzed with Student's *t*-test. Each value is the mean±SD

9.3±2.0 mg,) and BMI (non-carriers: 24.1±3.5 kg/m^2, carriers: 23.5±2.7 kg/m^2) were not significantly different between the two groups. In contrast, at 1 year post-treatment, there were no significant differences in the reduction of TC and LDL-C values between the two groups (Fig. 1b; Table 1).

In an in vitro experiment, Iwai et al. (2004) demonstrated that the transport activity of *SLCO1B1**15 allele is significantly decreased compared with that of the *SLCO1B1**1a or *1b allele using cDNA-transfected HEK293 cells. Previously, we found *SLCO1B1**15 allele was associated with higher plasma concentration of pravastatin, and the non-renal clearance of pravastatin in subjects with *SLCO1B1**1b/*15 and *15/*15 was reduced to 55 and 14% of *1b/*1b subjects, respectively (Nishizato et al. 2003). Thus, it is suggested that the *SLCO1B1**15 allele leads to an increase in plasma pravastatin concentrations but a reduction in the hepatocellular uptake of pravastatin, resulting in a decreased effect of pravastatin. However, interestingly, the genotype-dependent difference in this lowering effect disappeared after long-term treatment. Although its mechanism remains to be elucidated, one possible reason is that all of our patients with the *SLCO1B1**15 allele were heterozygotes for functionally active *1a or *1b alleles (Iwai et al. 2004). Thus, the lipid-lowering profiles in homozygotes for the *15 allele are of interest.

Multidrug resistance-associated protein 2 (MRP2/ABCC2) on the bile canalicular membrane is mainly involved in the biliary excretion of pravastatin (Matsushima et al. 2005). With regard to liver concentration of pravastatin, genetic polymorphisms of *MRP2* might affect response to pravastatin. However, *MRP2* variants have been observed at low frequency in Japanese (Itoda et al. 2002), and functional significance of these variants is not established. Therefore, association of *MRP2* genotypes should be analyzed by further studies.

We also examined the influence of the *CYP7A1* promoter (-204A/C) and *APOE* (ϵ2, ϵ3 and ϵ4) variants on the clinical outcome of pravastatin therapy. As shown in Fig. 1b and Table 1, the reduction from the baseline in LDL-C value at 1 year post-treatment was

Table 1 Association of *SLCO1B1*, *CYP7A1* and *APOE* genotypes with lipid changes

Gene	Genotype	Lipid concentrations (mg/dl)					
		N	Baseline	*N*	8 weeks	*N*	1 year
Total cholesterol							
*SLCO1B1**15	Non-carriers	26	260.9±24.4	26	205.8±22.2	20	201.9±18.5
	Carriers	7	254.8±10.6	7	227.9±19.6	6	204.0±16.5
	P value		NS		<0.05		NS
CYP7A1-APOE	A/A-ε3/ε3, A/A-ε3/ε4, A/C-ε3/ε3	19	261.9±23.9	19	210.3±27.9	14	198.9±12.7
	C/C-ε3/ε3, A/C-ε3/ε4	14	256.4±20.1	14	210.7±16.0	12	206.0±22.3
	P value		NS		NS		NS
LDL cholesterol							
*SLCO1B1**15	Non-carriers	22	170.7±27.4	22	124.0±20.7	17	115.1±23.9
	Carriers	7	157.0±29.3	7	132.0±32.7	6	110.5±10.9
	P value		NS		NS		NS
CYP7A1-APOE	A/A-ε3/ε3, A/A-ε3/ε4, A/C-ε3/ε3	19	168.6±34.4	19	124.0±29.9	12	106.3±20.6
	C/C-ε3/ε3, A/C-ε3/ε4	12	165.7±16.3	12	128.7±12.5	10	123.8±12.5
	P value		NS		NS		<0.05

Values are mean±SD

Statistical significance between the two genotypes was analyzed with Student's *t*-test

NS No significant difference

significantly decreased in carriers of A/A-ε3/ε3, A/A-ε3/ε4 or A/C-ε3/ε3 in *CYP7A1* and *APOE* genes compared with C/C-ε3/ε3 or A/C-ε3/ε4 carriers. There was no significant effect of genotypes (A/A-ε3/ε3, A/A-ε3/ε4 or A/C-ε3/ε3 vs C/C-ε3/ε3 or A/C-ε3/ε4) in the *CYP7A1* and *APOE* genes on pravastatin dose (10.0±2.9 vs 8.8±2.9 mg) and BMI (23.8±3.6 vs 24.5±3.0 kg/m^2). Only one patient was a heterozygous carrier of SNP12 in the *HMGCR* gene. However, no remarkable difference in the lipid-lowering effects was observed in this patient. Also, SNP29 in *HMGCR* and 55G>C in *ABCG8* were not detected.

In contrast to *SLCO1B1* gene, part of the interpatient variability in the efficacy of pravastatin after long-term treatment may be attributable to genetic variation, and combined genotyping of *CYP7A1* and *APOE* genes is useful for describing the lowering effects. Since the basal cholesterol synthesis rate is a key determinant for statin response, loss of CYP7A1 activity, which is involved in bile acid synthesis from cholesterol in the liver, may result in a poor response to statin treatment (Pullinger et al. 2002). A previous study has shown that the nucleotide sequence around position -204 negatively regulates CYP7A1 promoter activity (Cooper et al. 1997). Among the known variants, the *CYP7A1* -204A>C variant is expected to decrease promoter activity (Kajinami et al. 2005). Apolipoprotein E is known as one of the major determinants in lipoprotein metabolism. Previous studies (Ojala et al. 1991; Ordovas et al. 1995) demonstrated that the ε4 allele in primary hypercholesterolemia is associated with lower response to statin, when compared to ε2 and ε3 alleles, because the binding activity of ε4 allele to receptor is relatively higher than that of other alleles. These results suggest that decreased cholesterol 7alpha-hydroxylase activity and increased binding affinity of apolipoprotein E to LDL receptor enhance the intracellular cholesterol content in hepatocytes, resulting in lower HMG-CoA reductase activity, which may also lead to tolerance to statin treatment (Kajinami et al. 2005).

In conclusion, our results suggest that the *SLCO1B1**15 allele is associated with a slow response to pravastatin. Instead of *SLCO1B1**15, combined genotyping of *CYP7A1* -204A>C and *APOE* ε4 variants may be useful for describing the long-term clinical outcomes of pravastatin. Further study is necessary to confirm the influence of genetic variants in these candidate genes on the lipid-lowering efficacy of pravastatin as well as other statins in a large sample size.

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Expert Opinion

Genetic polymorphisms of drug transporters: pharmacokinetic and pharmacodynamic consequences in pharmacotherapy

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There has been increasing appreciation of the role of drug transporters in pharmacokinetic and pharmacodynamic consequences in pharmacotherapy. The clinical relevance of drug transporters depends on the localisation in human tissues (i.e., vectorial movement), the therapeutic index of the substrates and inherent interindividual variability. With regard to variability, polymorphisms of drug transporter genes have recently been reported to be associated with alterations in the pharmacokinetics and pharmacodynamics of clinically useful drugs. A growing number of preclinical and clinical studies have demonstrated that the application of genetic information may be useful in individualised pharmacotherapy for numerous diseases. However, the reported effects of variants in certain drug transporter genes have been inconsistent and, in some cases, conflicting among studies. Furthermore, the incidence of almost all known variants in transporter genes tends to be racially dependent. These observations suggest the necessity of considering interethnic variability before extrapolating pharmacokinetic data obtained in one ethic group to another, especially in the early phase of drug development. This review focuses on the impact of genetic variations in the function of drug transporters (ABC, organic anion and cation transporters) and the implications of these variations for pharmacotherapy from pharmacokinetic and pharmacodynamic viewpoints.

Keywords: drug transporter, genetic polymorphism, pharmacodynamics, pharmacokinetics

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1. Introduction

Many types of drug transporters are expressed in various human tissues, such as the intestine, liver, kidney, skin and the brain, and play roles in drug absorption, distribution and excretion. Accordingly, it is reasonable to hypothesise that factors influencing transport capability could lead to important consequences for interindividual differences in disposition kinetics and interaction profiles of clinically useful drugs, susceptibility to side effects, and treatment efficacy. Among these factors, genetic polymorphism is highly important. The identification of allelic variations and their functional confirmations (i.e., genotype–phenotype relationship) is a necessary step towards the use of genetic information for individualised pharmacotherapy. These backgrounds have led to the study of single nucleotide polymorphisms (SNPs), which has progressed rapidly and generated remarkable findings, and some SNPs have been shown to alter both the expression and function of their gene products. This review highlights recent studies by the groups of Ieiri and others on the role of drug transporter gene polymorphisms in pharmacokinetic and pharmacodynamic consequences in pharmacotherapy. The scope of this review is strictly limited to

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Table 1. General features of drug transporters (localisation in human tissues, substrates and inhibitors)

Name (gene nomenclature)	Chromosome localisation	Main localisation (tissue or subcellular)	Substrates (clinically useful drugs)	Inhibitors (clinically useful drugs)
MDR1 or P-gp (*ABCB1*)	7q21.1	Canalicular membrane (hepatocytes) Brush-border membrane of proximal tubular cells (kidney) Brush-border membrane (enterocytes) Capillary endothelial cells (brain and testis) Placental trophoblast	Anticancers (docetaxel, etoposide, paclitaxel, topotecan, vinblastine) Antihypertensives (diltiazem, losartan) Antiarrhythmics (digoxin, verapamil) Antivirals (indinavir, nelfinavir) Antibiotics (erythromycin, sparfloxacin) Immunosuppressants (ciclosporin, tacrolimus) Others (cimetidine, fexofenadine, loperamide, phenytoin, morphine, ondansetron)	Amiodarone, amitriptyline, diltiazem, dipyridamole, phenothiazines, propafenone, propranolol, quinidine, spironolactone, tamoxifen
MRP2 (*ABCC2*)	10q24	Canalicular membrane (hepatocytes) Brush-border membrane of proximal tubular cells (kidney)	Bilirubin, diglucuronide, sulfates, glutathione conjugates, benzbromarone, indomethacin, vinblastine, telmisartan	Ciclosporin, glibenclamide
BCRP (*ABCG2*)	4q22	Canalicular membrane (hepatocytes) Apical membrane of syncytiotrophoblast cells (placenta, membrane facing maternal blood) Luminal membranes of villous epithelial cells (small intestine and colon)	Epirubicin, topotecan, doxorubicin, daunorubicin, etoposide, SN-38, reserpine	
OATP1A2 or OATP-A (*SLCO1A2*)	12p12	Cerebral endothelial cells luminal membrane (intestinal enterocytes)	Thyroid hormones (T4 and T3), prostaglandin E2, fexofenadine, quinidine	Dexamethasone, erythromycin, quinidine, verapamil
OATP1B1 or OATP-C (*SLCO1B1*)		Basolateral (sinusoidal) Plasma membrane (hepatocytes)	Thyroid hormones (T4 and T3), methotrexate, pravastatin, rifampicin, prostaglandin E2	
OATP1B3 or OATP8 (*SLCO1B3*)		Basolateral (sinusoidal) Plasma membrane (hepatocytes)	Thyroid hormones (T4 and T3), leukotriene C4, digoxin, methothrexate, rifampicin	
OATP2B1 or OATP-B (*SLCO2B1*)	11q13	Basolateral (sinusoidal) Plasma membrane (hepatocytes) Apical membrane (enterocytes)	Narrow substrate specificity (pH dependent?)	
OCT1 (*SLC22A1*)	6q26	Basolateral (sinusoidal) Plasma membrane (hepatocytes)	Acylclovir, ganciclovir, metformin	Acebutolol, amantadine, cimetidine, disopyramide, midazolam, prazosin, quinidine, verapamil
OCT2 (*SLC22A2*)		Basolateral membrane of proximal tubular cells (kidney) Apical side of the distal tubule (kidney)?	Amantadine, metformin, neurotransmitters, monoamine	Desipramine, procainamide
OCT3 (*SLC22A3*)	6q26 – 27	Placenta	Cimetidine, tyramine, neurotransmitters, monoamine	Clonidine, desipramine, imipramine, prazosin, procainamide

BCRP: Breast cancer-resistance protein; OAT: Organic anion transporter; OATP: Organic anion-transporting polypeptide; OCT: Organic cation transporter; MDR: Multi-drug resistance; MRP: Multi-drug resistance-associated protein; P-gp: P-glycoprotein.

Table 1. General features of drug transporters (localisation in human tissues, substrates and inhibitors) (continued)

Name (gene nomenclature)	Chromosome localisation	Main localisation (tissue or subcellular)	Substrates (clinically useful drugs)	Inhibitors (clinically useful drugs)
OAT1 (*SLC22A6*)	11q12.3	Basolateral membrane of proximal tubular cells (kidney)	Methotrexate	β-Lactam antibiotics, diuretics, NSAIDs, probenecid
OAT2 (*SLC22A7*)	6q21.1 – 2	Basolateral (sinusoidal) Plasma membrane (hepatocytes)	Methotrexate, prostaglandin E2	
OAT3 (*SLC22A8*)	11q12.3	Basolateral membrane of proximal tubular cells (kidney) Brush-border membrane of choroid plexus cells and in capillary endothelial cells (brain)	Cimetidine, methotrexate, salicylate, prostaglandin E2	

BCRP: Breast cancer-resistance protein; OAT: Organic anion transporter; OATP: Organic anion-transporting polypeptide; OCT: Organic cation transporter; MDR: Multi-drug resistance; MRP: Multi-drug resistance-associated protein; P-gp: P-glycoprotein.

observations from human (healthy volunteers and patients) studies. This review focuses on the following transporters: ABC transporters (P-glycoprotein [P-gp]/multi-drug resistance 1 [MDR1/ABCB1], multi-drug resistance-associated protein 2 [MRP2/ABCC2] and breast cancer-resistance protein [BCRP/ABCG2]), organic anion-transporting polypeptide family (OATP1A2 [OATP-A]/SLCO1A2, OATP1B1 [OATP-C]/SLCO1B1, OATP1B3 [OATP8]/SLCO1B3 and OATP2B1 [OATP-B]/SLCO2B1), organic anion transporter family (OAT1/SLC22A6, OAT2/SLC22A7 and OAT3/SLC22A8) and organic cation transporter family (OCT1/SLC22A1 and OCT2/SLC22A2).

2. General features

2.1 Localisation in human tissues and basic function

P-gp/MDR1 (ABCB1) is expressed in the small and large intestines, adrenal gland, placental trophoblasts, kidney, liver, pancreas (pancreatic ductile cell) and capillary endothelial cells of the brain and testes (**Table 1**) [1-4]. Evidence including findings in knockout mice support that P-gp excretes substrate drugs via the canalicular membrane of hepatocytes into the bile, via the brush-border membrane of enterocytes into the gut lumen and via the brush-border membrane of proximal tubules into the urine [5,6]. P-gp in trophoblasts and endothelial cells of the blood–brain barrier (BBB) contribute to the function of blocking the transfer of xenobiotics across the human placenta and preventing the entry of substrates into the CNS [7-9].

Although at least 13 structurally and functionally related family members have been identified in MRPs (ABCC proteins), their localisation, expression levels and substrate specificity are different [10,11]. MRP2 (ABCC2 protein) is expressed at the apical membrane in liver hepatocytes, renal proximal tubule cells and enterocytes of the intestine [12-15], and plays roles in the biliary excretion, intestinal excretion and urinary excretion of the substrates [10,11].

Similar to P-gp and MRP2, BCRP (ABCG2 protein) is expressed at the apical membrane in the placenta (trophoblast cells), liver (bile canalicular membrane of hepatocytes), kidney and intestine (enterocytes) [16-19]. The tissue distribution of BCRP suggests that its major physiological role may be the regulation of intestinal absorption and biliary secretion of substrates, and protection of the fetus and brain from toxic xenobiotics. Unlike most other ABC transporters (e.g., P-gp and MRPs), which are characterised by 2 nucleotide-binding domains (NBD) and 12 transmembrane domains (TMD), BCRP has a single NBD at the amino terminus followed by 6 TMDs. Thus, BCRP is a so-called half-transporter and may form a homodimer, although heterodimeric forms are possible [20-24].

OATP1A2 (OATP-A) was first isolated from human liver; however, subsequent studies have identified its expression in the brain, lung, kidney and testes [25,26]. Recently, OATP1A2 has been reported to be expressed on the luminal membrane of human intestinal enterocytes, and to play a possible role in fexofenadine absorption from the intestine [26,27].

Both OATP1B1 (OATP-C) and -3 (OATP8; 80% amino acid identity to OATP-C) have liver-specific tissue distribution [28-31]. Because the uptake of substrates from the blood into hepatocytes, mediated by uptake transporters in the basolateral membrane, is the first step in the hepatocellular elimination process in the human body, the role of these transporters in the liver is of special interest. So far, the functional characterisation of OATP1B1 in the human body has been elucidated progressively among the OATP family due to its liver-specific expression.

Similar to OATP1B1 and -3, OATP2B1 (OATP-B) is predominantly found in the liver, but is also expressed in various tissues, including the brain, lung, kidney, placenta, heart, intestine and testis [32,33]. OATP2B1 is found on the basolateral membrane of hepatocytes, suggesting that this transporter functions in an uptake capacity to remove substrates from the portal circulation [33].

OAT1 and -3 are substantially expressed in the kidney, and localised on the basolateral membrane of the proximal tubules [34,35]. They uptake substrates from the blood side into the proximal tubule cell [36]. Because of key molecules in

renal excretion, OAT1 and -3 have been reported to be responsible for antibiotic- or antiviral-related nephrotoxicity [37-40]. In general, OAT family members are mainly expressed in the kidney; however, OAT2 is abundantly expressed on the basolateral membrane of the liver and, to a lesser extent, in the kidney [41,42]. In the brain, OAT3 is localised on the brush-border membrane of choroids plexus cells, suggesting it functions as the blood–cerebrospinal fluid barrier [43,44].

OCT1 is primarily expressed in the basolateral membrane of hepatocytes and is thought to play a fundamental role in the uptake of substrates into the liver [45-48]. In contrast, OCT2 is detected predominantly in the kidney and is likely to be the major transporter for the uptake of many cations from the blood sides into renal epithelial cells [48]. OCT3 has much more widespread tissue distribution at the mRNA level: aorta, skeletal muscle, prostate, salivary gland, adrenal gland and placenta [49]. Among these tissues, the placental expression level is relatively high.

2.2 Substrate drugs

P-gp accepts a broad spectrum of structurally and functionally unrelated drugs (Table 1). P-gp substrates, inducers and inhibitors are listed in detail elsewhere [50-52]. Interestingly, there is a strong overlap in substrate specificity and tissue distribution between P-gp and CYP3A4/5 [53,54].

MRP2 also has broad substrate specificity covering anticancer drugs [55,56] and organic anions derived from phase I and II metabolism of xenobiotics [57-59].

BCRP recognises various compounds such as negative or positive charge, organic anions and sulfate conjugates [60,61]; however, there is considerable, but not complete, overlap in substrates, especially for anticancer drugs among P-gp, MRP2 and BCRP [62,63].

In general, the substrate specificity of most OATPs is extremely broad and shows substantial overlap between different members of the superfamily. Substrates of OATP1A2 include various endogenous compounds such as bile acids, steroid hormones and thyroid hormones [25,64-66]. In contrast, information on the substrate specificity of OATP2B1 is limited at present [33]. OATP1B1 is involved in the hepatic uptake of a broad array of endogenous compounds such as leukotriene C4, prostaglandin E2, bilirubin and its glucuronides conjugates [29,67]. Furthermore, a variety of drugs, including 3-hydroxy-3-methylgluatryl coenzyme A reductase (HMG-CoA-reductase) inhibitors (e.g., pravastatin and pitavastatin), have been identified as OATP1B1 substrates [30,68,69]. Although OATP1B3 shares substrates with OATP1B1, OATP1B3 is the only OATP member known to transport digoxin [31,33,70].

Substrates of OAT1 and -3 include relatively small and hydrophilic organic anions, such as methotrexate, antiviral agents, β-lactam antibiotics and NSAIDs [40,71,72]. OAT2 also transports small and hydrophilic organic anions including salicylate and indometacin [73].

OCT1, -2 and -3 all transport a broad range of structurally diverse organic cations with extensively overlapping substrate specificities [45]. Clinically useful drugs for which transport has been demonstrated include antiparkinsonians (amantadine), antidiabetics (biguanide metformin) and the H_2-receptor agonist cimetidine [45].

3. Sites of polymorphisms and allelic frequency in different ethnic populations

ABCB1, the MDR1 gene, is located on chromosome 7 at q21, with 28 exons encoding a protein of 1280 amino acids [74]. Recently, Bodor *et al.* [75] used several different human cell lines as well as lymphocytes and liver samples to investigate eventual differences between tissues and/or subjects regarding the *ABCB1* gene locus, and confirmed the length of the *ABCB1* gene is most likely 209 kb, as indicated in the database (accession number NT007933). The first evidence of the presence of naturally occurring polymorphisms in human *ABCB1* was reported by Mickley *et al.* [76] who found two SNPs in exon 21 (2677G→T) and 24 (2995G→A) (**Figures 1** and 2). Subsequently, screening of the entire *ABCB1* gene has been undertaken by various laboratories and, so far, numerous SNPs have been identified [77-82]. Some SNPs are nonsynonymous; for example, G→T (2677G→T) and G→A (2677G→A) transversions at position 2677 in exon 21, located on the intracellular side of P-gp after transmembrane region 10, result in an amino acid change from Ala at codon 893 to Ser and Thr, respectively. In contrast, 1236C→T (exon 12) and 3435C→T (exon 26) are synonymous. Interestingly, some SNPs, such as 1236C→T, 2677G→T/A and 3435C→T are closely linked; thus, haplotype-oriented assignment has been taken into consideration in recent genotype–phenotype studies [78,83-86].

The allelic frequency distributions of SNPs in *ABCB1* have been reported in various racial populations (Table 2). The incidence of the most known SNPs, but also haplotypes, is highly racially dependent. The above-mentioned three variants are found at 45 – 55% frequency in Caucasians and 35 – 50% in Japanese, but only at 5 – 10% frequency in African-Americans. Interethnic differences in the distribution of the variants are a possible cause of interethnic differences in the pharmacokinetics of P-gp substrate drugs. Differences in the oral bioavailability of ciclosporin and tacrolimus and the incidence of resistance and more aggressive tumours are illustrated as samples [87-90].

ABCC2 (MRP2 gene) is composed of 32 exons encoded by an ~ 45-kb gene located on chromosome 10q24 [91,92]. Similar to the *ABCB1* gene, numerous variations have been identified in the *ABCC2* gene. Genetic analysis of *ABCC2* is well documented in patients with Dubin–Johnson syndrome (DJS), an autosomal recessive disorder characterised by conjugated hyperbilirubinaemia. At present, at least 16 variants have been identified in DJS patients, and a wide variety of genetic mechanisms, including missense mutation, nonsense mutation, splice site mutation and deletion mutation, are responsible for DJS [93]. In healthy Japanese volunteers

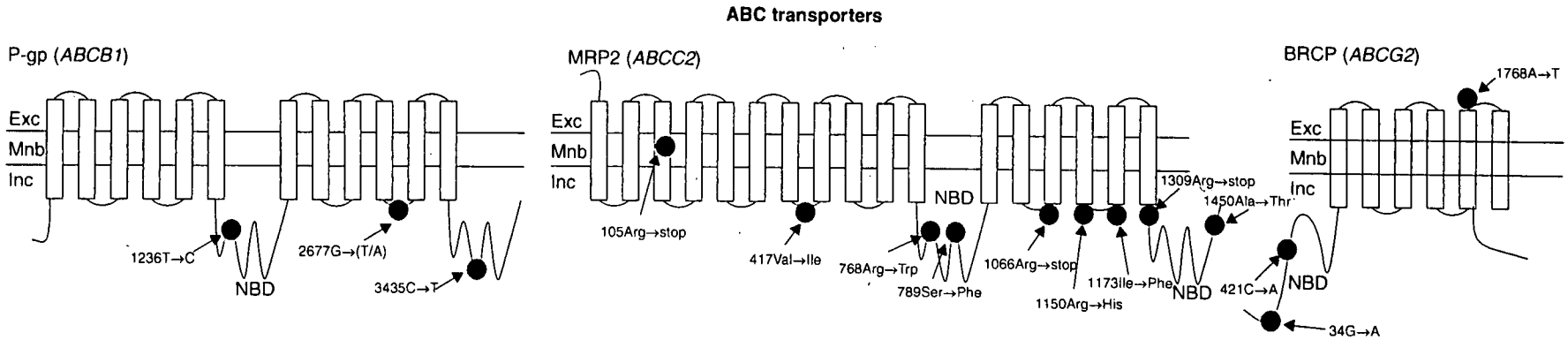

Figure 1. Schematic representation of secondary structures in drug transporters, with some nucleotide substitutions.

BCRP: Breast cancer-resistance protein; Exc: Extracellular; Inc: Intracellular; Mnb: Membrane; MRP: Multi-drug resistance-associated protein; NBD: Nucleotide-binding domain; P-gp: P-glycoprotein.

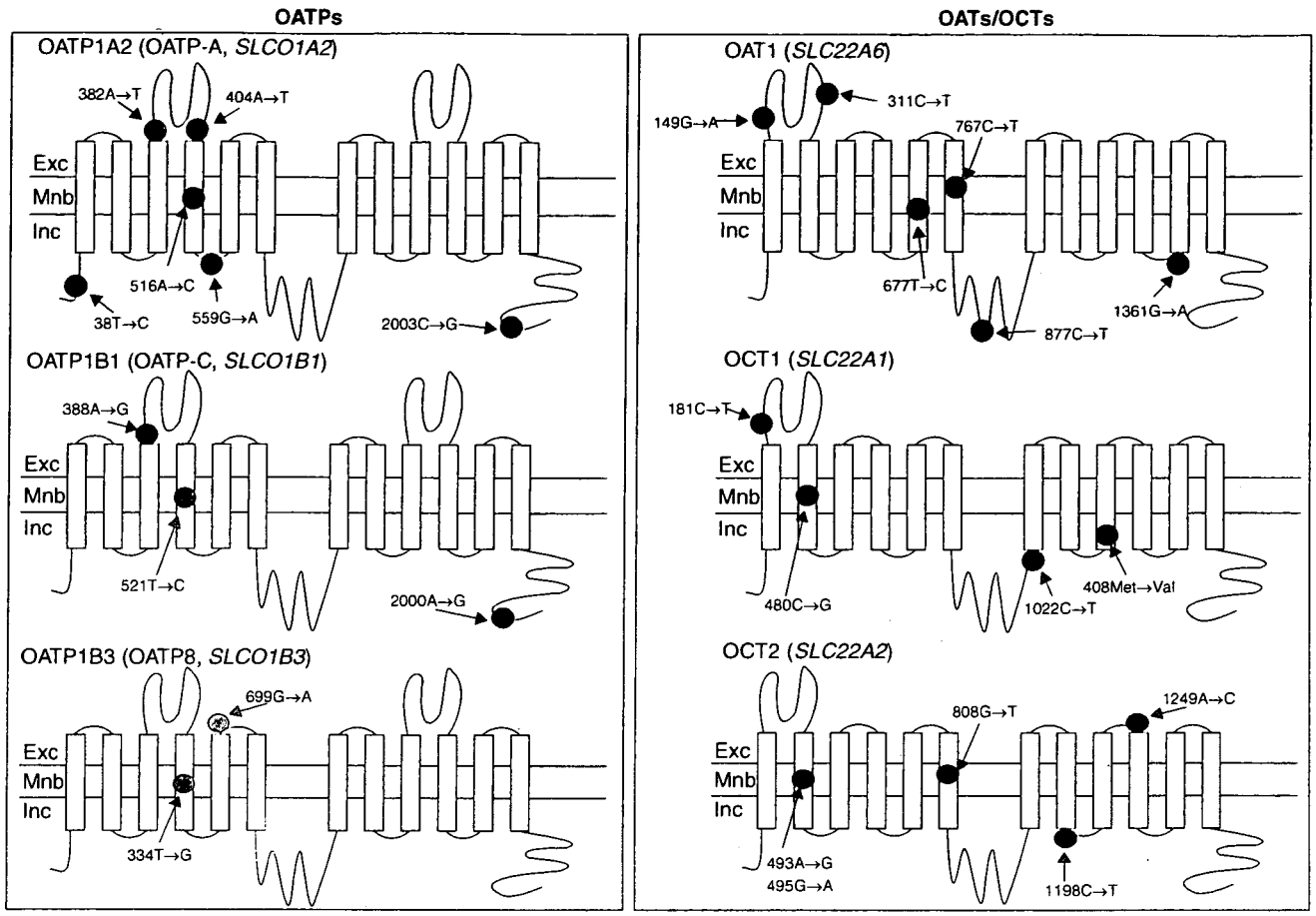

Figure 2. Schematic representation of secondary structures in drug transporters, with some nucleotide substitutions.
Exc: Extracellular; Inc: Intracellular; Mnb: Membrane; OAT: Organic anion transporter; OATP: Organic anion-transporting polypeptide; OCT: Organic cation transporter.

Table 2. Summary of racial genetic data of naturally occurring variations of human drug transporters

Gene	Mutation	Location	Effect	Allelic frequency (n)		
				Japanese (48 – 220)	Caucasians (37 – 461)	African-Americans (23 – 200)
ABCB1 (MDR1)	-129T→C	Exon 1b	Noncoding	0.94/0.06	0.94 – 0.97/ 0.03 – 0.06	
	1236C→T	Exon 12	Synonymous	0.35/0.65	0.54 – 0.66/ 0.34 – 0.46	0.79 – 0.85/ 0.15 – 0.21
	2677G→(T/A)	Exon 21	893Ala→(Ser/Thr)	0.36 – 0.44/ 0.36 – 0.42/ 0.20 – 0.22	0.50 – 0.56/ 0.38 – 0.46/ 0.02 – 0.10	0.85 – 0.89/ 0.10 – 0.15/ 0.00 – 0.01
	3435C→T	Exon 26	Synonymous	0.51 – 0.62/ 0.38 – 0.49	0.43 – 0.54/ 0.46 – 0.57	0.74 – 0.84/ 0.16 – 0.26
ABCC2 (MRP2)	-24C→T	Promoter	Noncoding	0.81/0.19		
	1249G→A	Exon 10	417Val→Ile	0.88/0.13		
	2302C→T	Exon 18	768Arg→Trp	0.99/0.01		
	2366C→T	Exon 18	789Ser→Phe	0.99/0.01		
	4348G→A	Exon 31	1450Ala→Thr	0.99/0.01		
ABCG2 (BCRP)	34G→A	Exon 2	12Val→Met	0.81 – 0.83/ 0.17 – 0.19	0.90 – 0.96/ 0.04 – 0.10	0.94/0.06
	376C→T	Exon 4	126Gln→ (stop codon)	0.98 – 0.99/ 0.01 – 0.02	1.00/0.00	1.00/0.00
	421C>→A	Exon 5	141Gln→Lys	0.67 – 0.73/ 0.27 – 0.33	0.86 – 0.89/ 0.11 – 0.14	0.95 – 0.97/ 0.02 – 0.05
	1515C (deletion)	Exon 13	509Met→ (stop codon)	0.995/0.005		
SLCO1A2 (OATP-A)	38T→C	Exon 1	13Ile→Thr		0.89 – 0.94/ 0.06 – 0.11	0.98/0.02
	382A→T	Exon 4	128Asn→Tyr		1.00/0.00	0.99/0.01
	516A→C	Exon 5	172Glu→Asp		0.95 – 0.98/ 0.02 – 0.05	0.98/0.02
	559G→A	Exon 5	187Ala→Thr		0.99/0.01	1.00/0.00
	2003C→G	Exon 14	668Thr→Ser		0.99/0.01	0.96/0.04
SLCO1B1 (OATP-C)	-11187G→A	Promoter	Noncoding		0.93/0.07	
	388A→G	Exon 4	130Asn→Asp	0.37/0.63	0.53 – 0.69/ 0.31 – 0.47	0.25/0.75
	521T→C	Exon 5	174Val→Ala	0.84 – 0.89/ 0.11 – 0.16	0.82 – 0.88/ 0.12 – 0.18	0.98/0.02
SLCO1B3 (OATP8)	334T→G	Exon 3	112Ser→Ala	0.70/0.30	0.76/0.24	0.49/0.51
	699G→A	Exon 6	233Met→Ile	0.70/0.30	0.76/0.24	0.49/0.51
SLCO2B1 (OATP-B)	9-bp deletion	Exon 2	Frame shift	0.93/0.07		
	1457C→T	Exon 10	486Ser→Phe	0.69/0.31	0.86/0.14	0.63/0.37
SLC22A6 (OAT1)	149G→A	Exon 1	50Arg→His		1.00/0.00	0.97/0.03
	311C→T	Exon 1	104Pro→Leu		1.00/0.00	0.99/0.01

BCRP: Breast cancer-resistance protein; MDR: Multi-drug resistance; MRP: Multi-drug resistance-associated protein; OAT: Organic anion transporter; OATP: Organic anion-transporting polypeptide; OCT: Organic cation transporter.

Table 2. Summary of racial genetic data of naturally occurring variations of human drug transporters (continued)

Gene	Mutation	Location	Effect	Allelic frequency (n)		
				Japanese (48 – 220)	Caucasians (37 – 461)	African-Americans (23 – 200)
	677T→C	Exon 4	226Ile→Thr		0.99/0.01	1.00/0.00
	767C→T	Exon 4	256Ala→Val		1.00/0.00	0.99/0.01
	877C→T	Exon 5	293Arg→Trp		1.00/0.00	0.98/0.02
	1361G→A	Exon 8	454Arg→Gln		1.00/0.01	0.99/0.01
SLC22A8 (OAT3)	1166C→T	Exon 8	389Ala→Val	0.99/0.01		
SLC22A1 (OCT1)	181C→T	Exon 1	61Arg→Cys	1.00/0.00	0.91 – 0.93/ 0.07 – 0.09	1.00/0.00
	262T→C	Exon 1	88Cys→Arg	1.00/0.00	0.99/0.01	1.00/0.00
	480C→G	Exon 2	160Phe→Leu	0.89/0.11	0.78 – 0.93/ 0.07 – 0.22	0.99/0.01
	1022C→T	Exon 6	341Pro→Leu	0.84/0.16	1.00/0.00	0.92/0.08
	17857G→A	Exon 7	401Gly→Ser	1.00/0.00	0.97 – 0.99/ 0.01 – 0.03	0.99/0.01
	17878A→G	Exon 7	408Met→Val	0.17/0.83	0.40/0.60	0.26/0.74
	17914(ATG) deletion	Exon 7	420Met deletion	1.00/0.00	0.81 – 0.84/ 0.16 – 0.19	0.97/0.03
	32870G→A	Exon 9	465Gly→Arg	1.00/0.00	0.96 – 0.99/ 0.01 – 0.04	1.00/0.00
SLC22A2 (OCT2)	495G→A	Exon 2	165Met→Ile	1.00/0.00	1.00/0.00	0.99/0.01
	601C→T	Exon 3	200Thr→Met	0.99/0.01	1.00/0.00	1.00/0.00
	808G→T	Exon 4	270Ala→Ser	0.83 – 0.87/ 0.13 – 0.17	0.84/0.16	0.89/0.11
	1198C→T	Exon 7	400Arg→Cys	1.00/0.00	1.00/0.00	0.98/0.02
	1294A→C	Exon 8	432Lys→Gln	1.00/0.00	1.00/0.00	0.99/0.01
SLC22A3 (OCT3)	1270A→T	Exon 7	424Thr→Ser	0.99/0.01		

BCRP: Breast cancer-resistance protein; MDR: Multi-drug resistance; MRP: Multi-drug resistance-associated protein; OAT: Organic anion transporter; OATP: Organic anion-transporting polypeptide; OCT: Organic cation transporter.

(n = 48), Ito *et al.* [79] analysed the entire *ABCC2* gene and found six SNPs. Among them, 1249G→A in exon 10, a non-synonymous mutation (417Val→Ile) was frequently observed with an allelic frequency of 12.5%. Only one heterozygote (allelic frequency is 1%) was observed out of 48 volunteers for 2302C→T (768Arg→Trp in exon 18), 2366C→T (789Ser→Phe in exon 18) and 4348G→A (1450Ala→Thr in exon 31).

The *ABCG2* gene is located at 4q22 and encodes a 72-kDa membrane protein composed of 655 amino acids [22,94]. So far, systematic mutation analysis of the *ABCG2* gene has been performed in various ethnic populations, and > 40 SNPs have been identified [95-98]. The two most frequent non-synonymous mutations identified in humans are 34G→A (12Val→Met in exon 2) and 421C→A (141Gln→Lys in exon 5). When comparing the frequencies of the three major variants (i.e., 34G→A, 376C→T and 421C →A) among three ethnic populations (Japanese, Caucasian and African-American), Japanese subjects had significantly higher frequencies of 34G→A and 421C→A than the other two ethnic groups. Interestingly, these three variants occurred simultaneously, and the following four haplotypes were identified: G-C-C, G-C-A, A-C-C and G-T-C with their corresponding allelic frequencies of 46, 35, 18 and 1%, respectively [97]. Thus, similar to other transporter genes, the genetic frequency of *ABCG2* appears to be dependent on ethnicity.

Two recent studies have been conducted to identify SNPs in the *SLCO1A2* gene using genomic DNA samples from various ethnic populations [99,100]. Iida *et al.* [99] screened 27-kb wide for *SLCO1A2* in a Japanese population (n = 48). They did not detect SNPs in the exonic regions, but identified several variations in the 5′-flanking region. Among them, three variations (-916G→A, -526T→C and -189A/ins) are of interest because they are located within important transcriptional regulatory

regions (e.g., hepatic nuclear factor 1α). In contrast, Lee *et al.* [100] screened all 14 exons of *SLCO1A2* and identified 6 nonsynonymous SNPs with an allelic frequency in the range of 1.0 – 11.1%. They also demonstrated that allelic frequencies of six identified SNPs are dependent on ethnicity using ethnically defined DNA (European, African, Chinese and Hispanic-Americans).

So far, at least 15 nonsynonymous *SLCO1B1* SNPs have been identified in various ethnic populations. Among them, two commonly occurring nonsynonymous SNPs, 388A→G (130Asn→Asp in exon 4) and 521T→C (174Val→Ala in exon 5), are of special interest, due to not only their marked consequences in transport capability, but also interethnicity in allelic frequency. In addition to SNP-based analysis, haplotype-oriented assessment has also been well documented [101-104]. At least 17 haplotypes have been recognised so far. Major haplotypes in humans are as follows: *SLCO1B1*1a* (130Asn174Val), *-*1b* (130Asp174Val), *-*5* (130Asn174Ala), *-*15* (130Asp174Ala) and *-*17* (-11187G→A130Asp174Ala). Although the frequency of *SLCO1B1*5* is extremely low in Asian and black populations, the frequency in Caucasians is ~ 15%. In contrast, *SLCO1B1*15* is more common in Asian populations [69,101,103]. Interestingly, although the allelic frequency of 521T→C is similar between Asians and Caucasians (~ 15%), their haplotype patterns are different. In Asian populations, the 521T→C polymorphism is combined with the 388A→G variant. In recent Japanese data, the -11187G→A variant is also tightly linked (~ 100%) to the *SLCO1B1*15* allele [105].

A recent report has described the identification of SNPs in *SLCO1B3* in a population of Japanese individuals [99]. Based on this study and the authors' unpublished data, at least two nonsynonymous SNPs, 334T→G (112Ser→Ala in exon 3) and 699G→A (233Met→Ile in exon 6), exist with an allelic frequency ranging 0.24 – 0.51. Because the frequencies of these two SNPs were identical in all ethnic populations studied, these SNPs may occur simultaneously (being haplotyped).

Interestingly, collective evidence indicates that the frequency of nonsynonymous SNPs in OAT family genes (*SLC22A6*, *SLC22A7* and *SLC22A8*) appears extremely lower (< 1%) [99,103,106,107], suggesting these genes are relatively intolerant of nonsynonymous changes. Fujita *et al.* [106] focused on *SLC22A6* (OAT1 gene) and identified 6 nonsynonymous SNPs using 267 DNA samples from an ethnically diverse population. Only two SNPs, 149G→A (50Arg→His in exon 1) and 877C→T (293Arg→Trp in exon 5), were present at ≥ 1% in at least one ethnic population. They also identified 17 distinct haplotypes. Xu *et al.* [107] resequenced the coding regions of four OAT member genes from an ethnically diverse, healthy population (n = 192), and identified two nonsynonymous SNPs in *SLC22A6*, three in *SLC22A7*, one in *SLC22A8* and eight in *SLC22A9*, with an allelic frequency in the range of 0.01 – 0.03.

Some groups have screened for genetic variants of *SLC22A1* (encoding OCT1) in various ethnic populations [108-110]. Kerb *et al.* [110] identified 4 nonsynonymous SNPs (61Arg→Cys, 88Cy→Arg, 160Phe→Leu, 401Gly→Ser) and 1 deletion (420Met→del) in 57 Caucasian samples, with respective allelic frequencies of 9.1, 0.6, 22.0, 3.2 and 16.0%. Subsequently, Shu *et al.* [109] also detected numerous variations from five different ethnic groups. Some known SNPs (e.g., 41Phe→Leu and 117Pro→Leu) were observed in at least one ethnic population, suggesting ethnic diversity in *SLC22A1* polymorphism.

Similar to *SLC22A1*, several genetic variants in the coding region of *SLC22A2* (OCT2 gene) have been identified [111,112]. *SLC22A2* polymorphism was recently investigated comprehensively by screening all 11 exons as well as intronic sequence using 247 ethnically diverse DNA samples [112]. Among eight nonsynonymous SNPs, four (165Met→Ile, 270Ala→Ser, 400Arg→Cys and 432Lys→Gln) were polymorphic, with ethnic-specific allelic frequencies ≥ 1%. Novel variations, including SNPs and deletion, have also been reported in recent Japanese studies [111,113].

4. Impact of polymorphisms on pharmacotherapy

4.1 Pharmacokinetic consequences

In the pharmacogenomics of the *ABCB1* gene, Hoffmeyer *et al.* [77] first reported that a synonymous SNP, 3435C→T, was associated with significantly reduced intestinal P-gp content in subjects with the T/T genotype in comparison with subjects homozygous for the C allele (C/C), leading to higher steady-state plasma concentrations after the oral administration of digoxin in T/T subjects. After this report, a remarkably large number of clinical studies have been conducted around the world on the association of the *ABCB1* genotype and pharmacokinetic phenotypes. Most studies have focused on SNPs in the following two exons, 21 (2677G→T/A) and 26 (3435C→T); however, as summarised in recent reviews [50,82,114-116] and **Table 3**, the published observations conflict even when using the same probe drug and even among the same racial group. For example, Sakaeda *et al.* [117] conducted an ABCB1 genotype–phenotype study using digoxin as a probe and found that the AUC of digoxin in the absorption phase was significantly lower in subjects with 3435T/T genotype than in 3435C/C subjects. These observations are in line with a finding by Kim *et al.* [81], but are in contrast to the findings of Hoffmeyer *et al.* [77] and Kurata *et al.* [118]. In order to overcome these disagreements, some researchers have incorporated haplotype-oriented analysis into the genotype–phenotype study [83-86,119]. Recent studies have demonstrated that haplotype assessment represents more precise prediction of the pharmacokinetics of certain drugs such as digoxin [119] and ciclosporin [83].

Wang *et al.* [120] introduced new approach for the evaluation of the 3435C→T variant. The level of mRNA expression can be regulated in a *cis* or *trans* fashion, and the *cis*-acting polymorphism changes the expression of the gene transcript

Table 3. Impact of *ABCB1* gene variants on PK of drug substrates

Polymorphism	Population	Drug	Functional effect of the variant allele	Ref.
3435C→T	Caucasian HV	Digoxin	Increased AUC after single dose for T/T	[77]
	Caucasian HV	Digoxin	Higher AUC and C_{max} under steady state for T/T*	[119]
	Japanese HV	Digoxin	Higher BA after single dose for T/T*	[118]
	Caucasian and African HV	Digoxin	Higher AUC after single dose for T/T	[191]
	Caucasian HV	Digoxin	No difference in PK data after single dose	[192]
	Japanese HV	Digoxin	Decreased AUC after single dose for T/T	[117]
	Korean HV	Fexofenadine	Higher AUC and C_{max} after single dose for T/T*	[193]
	Caucasian HV	Fexofenadine	No difference in PK data after single dose	[194]
	Caucasian and African HV	Fexofenadine	Decreased AUC after single dose for T/T	[81]
	Asian HT patients	Ciclosporin	Higher AUC under steady state for T/T*	[84]
	Caucasian RT patients	Ciclosporin	No difference in C_{min} under steady state	[195]
	Caucasian and African HV	Ciclosporin	No difference in AUC after single dose	[196]
	Caucasian RT patients	Ciclosporin	Decreased AUC under steady state for C/T and T/T	[197]
	LT patients	Ciclosporin	Higher plasma (or serum) level/dose ratio under steady state for T/T	[198]
	RT patients	Tacrolimus	Higher C_{min} under steady state for T/T	[199]
	HT paediatric patients	Tacrolimus	Higher C_{min} under steady state for C/T and T/T	[200]
	RT patients	Tacrolimus	No difference in C_{min} under steady state	[201]
	Caucasian HV	Talinolol	No difference in AUC*	[202]
	Chinese HV	Talinolol	No difference in AUC after single dose*	[203]
	Caucasian HIV-1 patients	Nelfinavir, efavirenz	Lower C_{min} under steady state for T/T	[143]
	HIV patients	Atazanavir	Lower drug level under steady state for T/T	[204]
	Caucasian HV	Loperamide	No difference in PK data after single dose	[205]
	HV	Dicloxacillin	No difference in C_{max} after single dose	[206]
	Turkish HV	Phenytoin	Higher drug level under steady state for T/T	[207]
	Japanese schizophrenic patients	Risperidone	No difference in C_{min} under steady state	[208]
	ALL paediatric patients	Vincristine	No difference in PK data*	[160]
2677G→(T/A)	Japanese HV	Digoxin	Higher BA after single dose for T/T*	[118]
	Caucasian and African HV	Digoxin	Higher AUC after single dose for T/T	[191]
	Caucasian HV	Digoxin	No difference in PK data after single dose	[192]
	Japanese HV	Digoxin	Lower AUC after single dose for T/T	[209]
	Caucasian and African HV	Fexofenadine	Decreased AUC after single dose for T/T	[81]
	Korean HV	Fexofenadine	Decreased AUC after single dose for A/A*	[193]
	Asian HT patients	Ciclosporin	Higher AUC under steady state for T/T*	[84]
	HT paediatric patients	Tacrolimus	Higher C_{min} under steady state for G/T and T/T	[200]

*Including haplotype assessments.

ALL: Acute lymphoblastic leukaemia; BA: Bioavailability; HT: Heart transplant; HV: Healthy volunteers; LT: Liver transplant; PK: Pharmacokinetics; RT: Renal transplant.

Table 3. Impact of *ABCB1* gene variants on PK of drug substrates (continued)

Polymorphism	Population	Drug	Functional effect of the variant allele	Ref.
	RT patients	Tacrolimus	Higher drug level under steady state for T/T*	[210]
	Caucasian HV	Talinolol	Slightly higher in AUC for T/A and T/T*	[202]
	Chinese HV	Talinolol	No difference in AUC after single dose*	[203]
	ALL paediatric patients	Vincristine	No difference in PK data*	[160]

*Including haplotype assessments.

ALL: Acute lymphoblastic leukaemia; BA: Bioavailability; HT: Heart transplant; HV: Healthy volunteers; LT: Liver transplant; PK: Pharmacokinetics; RT: Renal transplant.

from the allele carrying the polymorphism, leading to the allelic expression imbalance. In order to test for the presence of *cis*-acting polymorphisms in human *ABCB1* that might be responsible for altered mRNA expression of the 3435T allele, they measured differences in allelic mRNA expression between the 3435T and 3435C allele using liver samples from heterozygous individuals carrying the 3435C→T SNP. They indicated that mRNA expression of the 3435C allele was significantly higher than that of the 3435T allele (3435C/3435T ratios in the range of 1.06 – 1.16). Based on the experiments including *in vitro* transfection of mixtures of *ABCB1* variants carrying all possible combinations of 1236C→T, 2677G→T and 3435C→T, they concluded that 3435C→T is the main functional polymorphism affecting mRNA levels, by altering mRNA stability. Interestingly, allelic expression imbalance has been observed in other pharmacokinetic genes such as *ABCG2*, *CYP3A5* and *CYP3A4* [97,121,122].

The question arises as to why the contribution of SNPs to the pharmacokinetics of some probes (e.g., digoxin and fexofenadine) differs among reports. The reasons for this discrepancy remain to be addressed; however, multiple tissue expression of P-gp with various vectorial movements and no suitable specific probe drug for P-gp function may contribute. Recently, Brunner *et al.* [123] measured the brain distribution of a model P-gp substrate, the calcium-channel inhibitor verapamil [124], using positron emission tomography in two groups of healthy volunteers. To these authors' knowledge, this is the first evaluation of P-pg function, as a 'gatekeeper' (i.e., regulating drug uptake to highly sensitive tissue brain), in the BBB directly. They indicate no difference in the brain distribution of [^{11}C]verapamil between the TTT haplotype (1236T, 2677T and 3435T) and the wild-type CGC haplotype (1236C, 2677G and 3435C). Because positron emission tomography has sensitivity in the lower picomolar range for tissue concentrations of drug molecules to be measured, and because P-gp-triggered active efflux may be an unyielding barrier in the brain penetration of substrate drugs, their findings that failed to show an effect of *ABCB1* gene polymorphisms on P-gp functions in the BBB are notable.

Sparreboom *et al.* [125] first studied the effects of naturally occurring, common variant *ABCG2* 421C→A on the pharmacokinetics of diflomotecan, a synthetic derivative of camptothecin, in 22 adult white patients with cancer. They found that plasma levels of diflomotecan after intravenous administration were significantly higher (~ 300%) in patients with 421C/A genotype than in 421C/C patients. However, despite expectations of significant genotype-dependent regulation in intestinal absorption due to its enriched localisation, the pharmacokinetics of diflomotecan did not differ between the two genotype groups after oral administration. Although further investigation is required to resolve this issue, these observations partially agree with some *in vitro* studies, indicating that the *ABCG2* 421C→A allele is associated with low BCRP expression levels [97,126,127]. These *in vitro* observations suggest that carriers of the 421C→A allele may have decreased clearance (increased plasma levels) and/or increased bioavailability. In a preliminary fashion, Sparreboom *et al.* [128] also reported that the heterozygous 421C/A allele observed in 2 patients was associated with a 1.34-fold increased oral bioavailability of topotecan compared with that in 10 patients with the 421C/C genotype. In contrast, de Jong *et al.* [129] reported no difference in the pharmacokinetic parameters of irinotecan and SN-38 between patients with and without the *ABCG2* 421C→A allele. They noted that other processes involved in irinotecan metabolism and elimination that exhibit great interindividual variation might be overshadowing any effect of this *ABCG2* polymorphism.

As described previously, because MRP2 (ABCC2 protein) is responsible for the export of conjugated drug metabolites from hepatocytes to bile, and because many *ABCC2* variants are known to be associated with DJS, naturally occurring *ABCC2* variants are expected to be involved in large interindividual differences in pharmacokinetic and pharmacodynamic consequences of substrate drugs; however, no sufficient human data have been reported so far. To the authors' knowledge, at least two studies have been carried out to assess possible associations of genetic variants in *ABCC2* with phenotypes (i.e., cellular exposure of nelfinavir [130] and pharmacokinetics of pravastatin [102]). However, there were no significant associations between phenotype indices and SNPs and/or haplotypes at *ABCC2*.

Facilitative hepatic uptake from the portal circulation by OATP1B1 is thought to contribute to tissue selectivity and therapeutic response to HMG-CoA reductase inhibitors (statins). Nishizato *et al.* [103] screened genetic polymorphism in the *SLCO1B1* using DNA from 120 Japanese healthy

volunteers and conducted a clinical study to examine whether variants alter transport activity with pravastatin as a selective probe drug. Subjects with the *SLCO1B1*15* allele (130Asp174Ala) had reduced total and nonrenal clearance, as compared with those with the *SLCO1B1*1b* allele (130Asp174Val), and the difference between **1b/*1b* and **1b/*15* subjects was significant. In their study, only one subject harboured the **15/*15* genotype, with nonrenal clearance about a tenth of that in the **1b/*1b* genotype. They first demonstrated that commonly occurring SNPs in the *SLCO1B1* gene are likely to be associated with altered pharmacokinetics of substrate drugs in humans. Niemi *et al.* [102] also evaluated the relationship between *SLCO1B1* variants and the pharmacokinetics of pravastatin. In heterozygous carriers of **15B* (130Asp174Ala), the mean pravastatin AUC_{0-12} was 93% higher compared with noncarriers and, in heterozygous carriers of **17* (-11187G→A and 130Asp174Ala), it was 130% higher compared with noncarriers. They also reported no significant associations between *SLCO2B1*, *ABCC2* or *ABCB1* polymorphisms and the pharmacokinetics of pravastatin. Based on the healthy volunteers study, Mwinyi *et al.* [104] found that **5* allele (130Asn174Ala) delayed the hepatocellular uptake of pravastatin, whereas **1b* allele seemed to accelerate OATP1B1-dependent uptake of the drug. Chung *et al.* [69] characterised the effects of *SLCO1B1* alleles, **1a*, **1b* and **15* on the pharmacokinetics of pitavastatin. Despite small sample sise, the dose-normalised AUC and C_{max} of pitavastatin were 1.4- and 1.8-fold higher, respectively, in subjects heterozygous for the **15* allele versus subjects not varying this allele. Similar to pravastatin, the **15* allele is suggested to be associated with decreased pitavastatin uptake from blood into hepatocytes. Systematic exposure to rosuvastatin had been observed to be ~ 2-fold higher in Japanese subjects living in Japan compared with white subjects in Western Europe or the US [131-133]. Because OATP1B1 contributes to the hepatic uptake of rosuvastatin [134], in order to determine whether polymorphisms in the *SLCO1B1* gene contribute to any pharmacokinetic differences, Lee *et al.* [135] conducted a pharmacokinetic study including four racial populations. They found that *SLCO1B1* 521T→C did not account for the clear population differences in rosuvastatin exposure among white subjects and Asian groups. Although no 521C/C homozygote in Asian subjects seems to be the most likely reason for failed to show up the differences, they concluded that the pharmacogenetics of other rosuvastatin disposition pathways may better explain the ethnic differences in pharmacokinetics [136].

The H_1-receptor antagonist fexofenadine is a P-gp substrate [65]; however, association between the pharmacokinetics of fexofenadine and polymorphism of the *SLCO1B1* gene has recently been reported [137]. The mean total AUC of fexofenadine in the -11187G/G521C/C subjects was 76% higher in subjects with the 521T/C genotype and 127% higher in subjects with the 521T/T genotype. These results suggest that OATP1B1 is involved in fexofenadine exposure, and may partly explain the conflicting observations between fexofenadine pharmacokinetics and *ABCB1* polymorphism.

Niemi *et al.* [138] investigated possible associations between the pharmacokinetics of repaglinide, a meglitinide analogue antidiabetic drug, and SNPs in genes encoding for OATP1B1, P-gp, CYP2C8 and CYP3A5 in 56 healthy subjects. Multiple regression analysis indicated that the *SLCO1B1* 521T→C and *CYP2C8*3* allele were independent predictors of the $AUC_{0-\infty}$ and C_{max} of repaglinide; the $AUC_{0-\infty}$ in the subjects with 521C/C genotype was 107 and 188% higher, respectively, than in subjects with the 521C/T or 521T/T genotype; however, surprisingly, only *SLCO1B1* -11187G→A was significantly associated with an enhanced effect of repaglinide on blood glucose, even though SNPs at positions -11187 and 521 are haplotyped.

Very recently, the authors studied the effects of polymorphism of *SLCO1B1*, particularly the **1b* allele, on the pharmacokinetics of three anionic drugs, pravastatin, valsartan and temocapril in a three-way crossover manner in 23 healthy Japanese volunteers [139]. The authors found that AUC of pravastatin in **1b/*1b* carriers was 65% of that in **1a/*1a* carriers, and AUC of valsartan and temocapril in each subject was significantly correlated with that of pravastatin. These results suggest that: i) *SLCO1B1*1b* allele enhances the hepatic uptake activity of pravastatin; and ii) OATP1B1 is one of the determinant factors governing interindividual variability in the pharmacokinetics of these three drugs. Reduction of pravastatin AUC in the **1b* subjects was well consistent with the above-mentioned study conducted by Mwinyi *et al.* [104].

Two studies have examined the effects of *SLCO1B1*5* and **15* on the functional properties of OATP1B1 using cDNA transfected cells. Kameyama *et al.* [140] evaluated transport capability by transient expression system of HEK293 and HeLa cells using endogenous conjugates, estradiol-17β-D-glucuronide and estrone-3-sulfate, and statins as substrates. Kinetic analysis of pravastatin and atorvastatin showed that K_m values were not altered, but V_{max} values decreased significantly in cells expressing the variants. Immunocytochemical study showed that the variant-typed proteins were localised not only at the plasma membrane, but also in the intracellular space. In contrast, Iwai *et al.* [141] indicated that all SNP variants expressed in HEK293 cells were predominantly located on the cell surface without changes in K_m values for the transport of 17β-estradiol 17β-D-glucuronide. However, the normalised V_{max} value (by the protein expression level estimated from western blotting) for *SLCO1B1*15* was drastically decreased to < 30% compared with **1a*. Although the observation of lower V_{max} values in the *SLCO1B1*15* cells was similar between the studies, the expression manner was clearly controversial. There is no good reason for this discrepancy; however, we need to be careful in expecting *in vivo* cellular localisation from the results of *in vitro* expression system.

Although many SNPs have been identified in the OCT2 gene, no pharmacogenomic human study has yet been

Table 4. Impact of the *ABCB1* (MDR1) genetic variant on PD of drug substrates and their consequences on disease states

Population (disease)	Polymorphism	Drug	Outcome marker	Effect	Ref.
Caucasian RT patients	3435C→T	Ciclosporin	Acute rejection	No significant difference	[195]
Caucasian patients (depression)	3435C→T	Nortriptyline	Nortriptyline-induced postural hypotension	Higher in T/T	[211]
Caucasian patients (HIV infection)	3435C→T	Nelfinavir, efavirenz	CD4 recovery with treatment	Higher in T/T	[143]
HIV patients	3435C→T		Virological suppression	Higher in T/T	[212]
Caucasian patients (HIV infection)	3435C→T		CD4 recovery with treatment	No significant difference	[145]
HIV patients	3435C→T	Efavirenz	Drug-induced HDL-cholesterol level	Higher in C/C	[213]
Paediatric HT patients	3435C→T 2677G→T	Corticosteroids	Steroid weaning 1 year after HT	Higher in T/T	[214]
AML patients	1236C→T 2677G→(T/A) 3435C→T	Menu of SHG-AML-96	OS and PR	Higher OS and low PR in T/T	[155]
AML patients	2677G→T		OS and PR	No significant difference	[156]
ALL patients	3435C→T		OS	No significant difference	[157]
Colon cancer patients	3435C→T		Susceptibility to colon cancer	Higher in T/T	[179]
Japanese LT patients	2677G→(T/A)	Tacrolimus	Tacrolimus-induced neurotoxicity	Higher in T/T	[161]
RT patients	3435C→T	Ciclosporin	Ciclosporin-induced tremor	No significant difference	[215]
RT patients	2677G→(T/A) 3435C→T	Ciclosporin	Ciclosporin-induced nephrotoxicity	Higher in T/T (donor side)	[162]
Lung transplant patients	2677G→T 3435C→T	Tacrolimus + predonisolone + azathiopurine	Acute persistent rejection	Higher in patients with the C allele	[216]
RT patients	3435C→T	Ciclosporin	Gingival overgrowth	No significant difference	[163]
Caucasian patients (renal epithelial cell cancer)	3435C→T		Susceptibility to renal tumour	Higher in T/T	[181]
Caucasian patients (ulcerative colitis)	3435C→T		Susceptibility to ulcerative colitis	Higher in T/T	[168]
Caucasian patients (Crohn's disease)	3435C→T		Susceptibility to Crohn's disease	No significant difference	[168]
White Spanish patients	2677G→(T/A) 3435C→T		Susceptibility to Crohn's disease	Higher in 2677T/3435C	[175]
White Scottish patients	2677G→T 3435C→T		Susceptibility to ulcerative colitis	Higher in 3435T/T	[174]
Caucasian patients (Parkinson's disease)	3435C→T		Susceptibility to Parkinson's disease	Early onset in T/T (trend)	[164]

5-HT: 5-Hydroxytryptamine; ALL: Acute lymphoblastic leukaemia; AML: Acute myeloid leukaemia; HDL: High-density lipoprotein; HT: Heart transplant; LT: Liver transplant; OS: Overall survival; PD: Pharmacodynamics; PR: Probability of relapse; RT: Renal transplant.

Table 4. Impact of the *ABCB1* (MDR1) genetic variant on PD of drug substrates and their consequences on disease states (continued).

Population (disease)	Polymorphism	Drug	Outcome marker	Effect	Ref.
Parkinson's disease patients	3435C→T		Susceptibility to pesticide-induced Parkinson's disease	Higher in C/T	[165]
Breast cancer patients	3435C→T		Response to preoperative chemotherapy	Decreased resistance in T/T	[217]
Epileptic patients	3435C→T		Response to antiepileptics	Higher in T/T	[152]
Epileptic patients	3435C→T		Response to antiepileptics	No significant difference	[154]
Cancer patients	3435C→T	5-HT_3 receptor antagonists	Antiemetic response (granisetron)	Higher in T/T (first 24 h)	[218]
Japanese patients (schizophrenia)	2677G→(T/A) 3435C→T	Bromperidol	Response to bromperidol		[219]

5-HT: 5-Hydroxytryptamine; ALL. Acute lymphoblastic leukaemia; AML: Acute myeloid leukaemia; HDL: High-density lipoprotein; HT: Heart transplant; LT: Liver transplant; OS: Overall survival; PD: Pharmacodynamics; PR: Probability of relapse; RT: Renal transplant.

reported. A recent study of monozygotic twin pairs showed that genetic factors contribute substantially to the renal clearance of metformin [142]. Genetic variation in the OCT2 is expected to explain the large interindividual variability in the pharmacokinetics of metformin.

Fujita *et al.* [106] conducted a small family based clinical study to determine the renal elimination of a model OAT1 substrate, adefovir, with regard to a nonfunctional variant, OAT1 1361G→A. They did not observe significant differences in renal clearance or renal secretory clearance in family members heterozygous for 1361G→A allele, in comparison with family members who did not carry this variant.

In OAT3 pharmacogenomics, one study reported no remarkable differences in both the mean renal and renal secretory clearances of pravastatin among the genotypic groups evaluated (reference, 723T→A and 1166C→T) [103].

4.2 Pharmacodynamic consequences

An association of *ABCB1* gene polymorphisms with pharmacodynamic consequences was reported for HIV therapy, antiepileptic pharmacotherapy, chemotherapy, adverse effects of P-gp substrates such as immunosuppressants and tricyclic antidepressants, and others (Table 4).

Fellay *et al.* [143] have studied the association between the response to antiretroviral treatment and the *ABCB1* genotype in 123 HIV-1-infected patients treated with efavirenz or nelfinavir. They found that patients with 3435T allele have a better response to the drugs after treatment for 6 months, as determined by an increased $CD4^+$ count. Haas *et al.* [144] also found that the 3435T/T genotype was associated with a decreased likelihood of virological failure and decreased emergence of efavirenz-resistant virus, but not with plasma efavirenz exposure after long-term follow up lasting up to 3 years. In contrast, Nasi *et al.* [145] and Winzer *et al.* [146] failed to find an association between the *ABCB1* genotype and virological and immunological responses to antiretroviral therapy.

P-gp can transport antiepileptic drugs [147], and the association of the multiple-drug resistance phenotype in epilepsy with increased lesional P-gp expression levels in resected brain tissues has been speculated [148-151]. Based on these backgrounds, Siddiqui *et al.* [152] genotyped 3435C→T in 315 patients with epilepsy, and demonstrated that patients with drug-resistant epilepsy were more likely to have the C/C genotype than T/T, when compared with patients with drug-responsive epilepsy. However, similar to antiretroviral therapy, controversial observations have been reported by at least two independent laboratories [153,154].

Multi-drug resistance is one of the most serious problems in the failure of chemotherapy, and some clinical studies with regard to *ABCB1* polymorphism and clinical outcomes have been conducted; however, as shown in Table 4, contribution of the *ABCB1* gene variants on outcome markers (e.g., overall survival and probability of relapse) in acute lymphoblastic leukaemia or acute myeloid leukaemia patients is controversial among the studies [155-160].

Numerous adverse reactions including neurotoxicity, nephrotoxicity and gingival hyperplasia are sometimes observed in patients treated with immunosuppressants. Yamauchi *et al.* [161] studied the correlation of the *ABCB1* polymorphism with tacrolimus-induced neurotoxicity (e.g., convulsion and tremor) in patients after living-related donor liver transplantation, and found that the 2677T allele might be a positive predictor of toxicity. Similarly, although daily dose, trough levels, and the concentration per dose ratio were not different between case and control groups, the donor's *ABCB1* 3435T/T genotype was reported to be a predictor of ciclosporin-induced nephrotoxicity [162]. Drug transporters in salivary glands have not yet been characterised; however,

Table 5. Impact of gene variants of the drug transporters PK and PD of substrates and their effect on disease states.

Gene	Polymorphism	Population	Drug/substrate	PK	PD	Functional effect of the variant allele	Ref.
ABCG2 (BCRP)	421C→T (141Gly→Lys)	White cancer patients (n = 22)	Diflomotecan	Yes		Higher AUC for C/T	[125]
	421C→T (141Gly→Lys)	European Caucasian cancer patients (n = 84)	Irinotecan (SN-38 and SN-38G)	Yes		No significant change in PK	[129]
	421C→T (141Gly→Lys)	2 cancer patients	Topotecan	Yes		Higher BA for C/T	[128]
SLCO1B1 (OATP1B1)	**15*	Japanese HV	Pravastatin	Yes		Higher AUC for **15/*15*	[103]
	521T→C	Japanese hyperlipidaemia (n = 66)	HMG-CoA reductase inhibitors		Response to cholesterol-lowering effect	Lower in C/T	[188]
	**15*	Japanese patients	Pravastatin, atorvastatin		Susceptibility to statin-induced myopathy	Higher in **15* patients	[220]
	521 T→C -11187G→A	Caucasian HV (n = 20)	Fexofenadine	Yes		Higher AUC in C/C	[137]
	**17*	Caucasian HV (n = 41)	Pravastatin	Yes	Response to cholesterol synthesis inhibition	Higher AUC and smaller response in **17* subjects	[189]
	521 T→C -11187G→A	HV (n = 56)	Repaglinide	Yes	Response to glucose-lowering effect	Higher AUC in C/C Increased response in G/A	[138]
	388A→G 521T→C	4 racial populations (HV)	Rosuvastatin	Yes		Higher AUC for C/T and C/C	[135]
	**1a, *1b, *15*	Korean HV (n = 24)	Pitavastatin	Yes		Higher AUC for **15* subjects	[69]
	**1a, *1b, *5*	Caucasian HV (n = 30)	Pravastatin	Yes		Higher AUC for **5* subjects Lower AUC for **1b* subjects	[104]
	**15B, *17*	Caucasian HV (n = 41)	Pravastatin	Yes		Higher AUC for **15B* and **17* subjects	[102]
SLC22A6 (OAT1)	1361G→A (454Arg→Gln)	1 African-American family	Adefovir	Yes		No change in renal clearance	[106]
SLC22A8 (OAT3)	1166C→T (389Ala→Val)	1 heterozygote (Japanese HV)	Pravastatin	Yes		No change in renal clearance	[103]

BA: Bioavailability; BCRP: Breast cancer-resistance protein; HMG-CoA: 3-Hydroxy-3-methylgluatryl coenzyme A; HV: Healthy volunteers; OAT: Organic anion transporter; OATP: Organic anion-transporting polypeptide; PD: Pharmacodynamics; PK: Pharmacokinetics.

Drozdzik *et al.* [163] reported no association between *ABCB1* polymorphism and gingival overgrowth in kidney transplant patients with ciclosporin treatment.

Although the physiological role of P-gp is not fully elucidated, it is conceivable that P-gp acts as a cellular barrier at numerous levels in the human body. Therefore, genotype-dependent P-gp function may contribute to disease susceptibility. So far, a number of studies have been reported on the association of *ABCB1* polymorphism with the following diseases: Parkinson's disease [164-167], inflammatory bowel diseases (ulcerative colitis and Crohn's disease) [168-175], cancers (leukaemia [155,176,177], colon cancers [178-180], renal epithelial tumours [181] and glioma [182]), primary biliary cirrhosis [183], rheumatoid arthritis [184] and hypertension [185].

Previous human studies agree that among numerous SNPs in the *SLCO1B1* gene, 521T→C (174Val→Ala) plays an important role in the transport capability, reducing hepatic uptake of pravastatin (Table 5) [103,104]. Because the target tissue of pravastatin is hepatocytes [186,187], subjects with this allele may exhibit reduced cholesterol-lowering effect of pravastatin due to lower pravastatin concentration in the hepatocytes, despite high plasma levels and AUC of pravastatin. At least two studies have been conducted to clarify this hypothesis. Tachibana-Iimori *et al.* [188] conducted a retrospective study on 66 patients who underwent treatment for hyperlipidaemia with HMG-CoA reductase inhibitors. They found that patients with the 521C allele showed an attenuated total-cholesterol-lowering effect compared with those homozygous for the 521T allele. Niemi *et al.* [189] investigated the association between polymorphism in the *SLCO1B1* and plasma concentrations of lathosterol and cholesterol up to 12 h after the intake of a single dose of pravastatin 40 mg in 41 healthy Caucasian subjects, and found that the plasma lathosterol level and lathosterol to cholesterol level ratio, markers of the rate of cholesterol synthesis *in vivo*, were significantly lower among the three heterozygous carriers of the *SLCO1B1*17* haplotype as compared with noncarriers. Both studies suggest that the 521T→C polymorphism modulates the lipid-lowering efficacy of HMG-CoA reductase inhibitors.

5. Conclusion

The polymorphism of genes encoding drug transporters is a useful marker to interpret large interindividual differences in the pharmacokinetics and response (pharmacodynamics) of clinically important drugs, and a great deal of effort is now being directed at assessing genotype–phenotype relationships not only in the clinical setting, but also at all stages of drug development. Numerous drug transporters, except the transporters described here, may also play an important role in the human body. Gene-knockout animals and expression cell systems are now available for the characterisation of basic traits such as substrate specificity, localisation and vectorial movement. Thus, in order to elucidate their *in vivo* functions more precisely, it seems appropriate to integrate the results from *in vitro* experiments/animal studies into the human study. Further refining of this integration will provide more precise and useful observations, allowing for truly genome-based scientific pharmacotherapy.

6. Expert opinion

Genetic polymorphisms have been identified in most known drug transporters. Some of these variants were shown to have an impact on pharmacokinetic and pharmacodynamic consequences in pharmacotherapy, but unfortunately, functional confirmation remains to be elucidated for most of these variants. We are now beginning to elucidate and understand the consequences of these variants in the human body. So far, except for a few cases (e.g., the *SLCO1B1* genotype and statins pharmacokinetics/pharmacodynamics), there are still discrepancies in the results of functional confirmation (i.e., phenotype and genotype relationship), thus necessitating some concerns for further investigations.

Controversial and confused observations relating to the *in vivo* pharmacokinetic relevance of the polymorphisms of some drug transporter genes (e.g., *ABCB1* and *ABCG2*) may have arisen from the nonspecific substrate drugs used in the various studies. For example, in the *ABCB1* polymorphism, although digoxin and fexofenadine have been used as probed drugs for P-gp function, these are also known to be substrates, at least for polymorphic *SLCO1B3* and *SLCO1B1*, respectively.

Despite considerable effort, it is difficult to find specific substrates to corresponding specific transporters because the substrate specificity of most transporters is extremely broad and shows substantial overlap between different members of the superfamily. For this perspective, multiple gene analysis of the network of genes involved in drug metabolism, transport, and response (e.g., receptors), is preferable. For example, previous *in vitro* experiments reported that at least two transporters, but no cytochrome P450s, are involved in the pharmacokinetics of pitavastatin; OATP1B1 for uptake into hepatocytes and BCRP for efflux into the bile and gut lumen [190]. A pharmacogenomic human study of pitavastatin conducted with polymorphisms in *SLCO1B1* and *ABCG2* is of interest. Again, in order to establish a pharmacokinetic gene network, the integration of *in vitro* and animal experiments into the human study is essential.

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