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ORIGINAL ARTICLE

Human organic cation transporter (*OCT1* and *OCT2*) gene polymorphisms and therapeutic effects of metformin

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Abstract Organic cation transporters (OCTs) are responsible for the hepatic and renal transport of metformin. In this study we analyzed variants of *OCT1* and *OCT2* genes in 33 patients (24 responders and nine non-responders) based on the hypothesis that polymorphisms in both genes contribute to large inter-patient variability in the clinical efficacy of metformin. The sequences of the 5′-flanking and coding regions of the two genes of interest were screened by single-strand conformation polymorphism (SSCP) analysis. To compare the causative factors between responders and non-responders, we performed stepwise discriminant functional analysis. Age, body mass index (BMI) and treatment with lipid-lowering agents were demonstrated as positive predictors, and two mutations in the *OCT1* gene, –43T > G in intron 1 and 408Met > Val (1222A > G) in exon 7, were negative and positive predictors, respectively, for the efficacy of metformin; the predictive accuracy was 55.5% ($P < 0.05$). Subsequent study indicated that OCT1 mRNA levels tended to be lower in human livers with the 408Met (1222A) variant, though the differences did not reach the level of significance. In this study it is suggested that *OCT1* and *OCT2* gene polymorphisms have little contribution to the clinical efficacy of metformin.

Keywords Metformin · OCT1 · OCT2 · Polymorphisms · Pharmacokinetics · Pharmacodynamics

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Introduction

Metformin is one of the most commonly used drugs for the treatment of type 2 diabetes, but we sometimes encounter patients who do not respond sufficiently, even under approved dosage conditions (e.g., 500–750 mg/day in Japan). Although the effects of metformin on glycemic control and lipids have been reported to be dose dependent, recent pharmacogenomic studies indicate that genetic polymorphisms of drug-metabolizing enzymes and transporters should be taken into consideration when large inter-patient variability in the intensity and duration of both drug effects and side effects is observed. Among various pharmacokinetic-related genes, since renal secretion, not hepatic metabolism, is the major route of elimination of metformin, the contribution of genetic variations in drug transporters is of interest.

Human organic cation transporters (OCTs; OCT1–3) are poly-specific transporters of small and hydrophilic

organic cations, including toxic substances, endogenous compounds (e.g., dopamine and serotonin), and clinically used drugs (e.g., procainamide and amantadine) (Jonker and Schinkel 2004). Among the OCT family, OCT1 is expressed predominantly in the basolateral membrane of hepatocytes, and mouse Oct1, which is homologically and functionally similar to OCT1, is responsible for the hepatic uptake of metformin (Wang et al. 2002, 2003). Although the precise mechanism of the action of metformin remains unclear, it is believed that hepatic uptake is an essential step in reducing hepatic glucose production as well as the occurrence of life-threatening side effects such as lactic acidosis (Hundal et al. 2000; Stumvoll et al. 1995; Wang et al. 2002). Recently, a number of single nucleotide polymorphisms (SNPs) has been identified in the *OCT1* gene. Some of these SNPs have been found to be associated with altered in vitro transport activity (Hundal et al. 2000; Sakata et al. 2003; Shu et al. 2003; Takeuchi et al. 2003).

In the kidney, OCT2, another subfamily of the OCT family, is expressed on the basolateral membrane of the proximal tubule epithelium and is involved in the uptake of many xenobiotics from the bloodstream into renal epithelial cells (Jonker and Schinkel 2004). Kimura et al. (2005) demonstrated that metformin is a good substrate for OCT2, using HEK293 cells expressing OCT2. Similar to those in the *OCT1* gene, functionally different variants have been identified in the *OCT2* gene (Leabman et al. 2002).

We hypothesized that large inter-patient variability in the clinical efficacy of metformin may occur as a result of variations in *OCT1* and/or *OCT2*. In this report we evaluated the functional significance of genetic polymorphisms of *OCT1* and *OCT2* genes with regard to the efficacy of metformin in patients with type 2 diabetes. To date, no study has addressed the genotype–phenotype relationship in light of *OCT* in humans.

Materials and methods

Study subjects

Thirty-three patients (nine men and 24 women; mean age 60 years, range 29–73 years) treated with metformin for at least 1 month were enrolled. We excluded patients who discontinued metformin because of adverse effects (e.g., diarrhea and headache). There are no generally accepted criteria in the clinical cut-off point to divide patients into responders and non-responders. Thus, we selected the criteria empirically, based on our clinical experiences and a previous report (Takei et al. 2001) as follows: (1) responders [n = 24; mean age 62 years, range 29–73 years; mean body mass index (BMI) 25.4 kg/m^2, range 20.4–34.5 kg/m^2], i.e., those whose HbA_{1c} levels had decreased by more than 0.5% from the baseline within 3 months of metformin therapy and had remained low for more than 3 months; and (2) non-responders (n = 9; mean age 56 years, range 34–69 years; mean BMI 25.1 kg/m^2, range 17.8–30.6 kg/m^2), i.e., those for whom either metformin therapy had been discontinued within 3 months and/or after another hypoglycemic drug (e.g., sulfonylurea) had been added to the therapy because of insufficient improvement in HbA_{1c} levels. Eighteen of the responders and six of the non-responders were treated with the maximum approved daily dose in Japan (i.e., 750 mg/day). Eight of the responders and four of the non-responders received metformin monotherapy, and others were co-medicated with sulfonylurea, α-glycosidase inhibitor or insulin. This study was approved by the Ethics Review Board of the Faculty of Medicine, Tottori University, and all subjects gave informed consent before participating.

Identification of variants in *OCT1* and *OCT2* genes

Genomic DNA was extracted from peripheral blood. The primer design was based on the sequence of the 5'-flanking region and the intron/exon junction of *OCT1* and *OCT2* genes (GenBank accession number AL353625 for *OCT1*, AL162582 for *OCT2*). Primers were designed to divide all 11 exons of each gene into fragments of approximately 350 bp so that mutations could be screened by subsequent single-strand conformation polymorphism (SSCP) analysis. Polymerase chain reaction (PCR) products were sequenced either directly or after subcloning on an ABI 3100 automatic sequencer (Applied Biosystems, Foster City, VA, USA).

Quantitative real-time PCR

Total RNA was extracted with an RNAeasy kit (Qiagen, Hilden, Germany) from 58 human liver samples (33 Caucasian and 25 Japanese non-diabetic donors), and reverse transcribed into cDNA using oligo dT primers and reverse transcriptase. OCT1 mRNA was quantified by real-time PCR using an ABI PRISM 7700 sequence detector (Applied Biosystems) with SYBR-green detection of reaction products. Primers for OCT1 mRNA were directed at a sequence that spans the junction of exons 9 and 10, corresponding to open reading frame 1437–1509; 5'-CAC

CCCCTTCATAGTCTTCAG-3′ (forward) and 5′-GCC CAACACCGCAAACAAAAT-3′ (reverse). The copy number of the transcript was measured against the copy-number standard curve of cloned target templates consisting of serial tenfold dilution points. β_2-microglobin mRNA was used as the reference gene for OCT1 mRNA.

Statistical analysis

The significance of differences in allelic frequency was calculated by χ^2 analysis using 2×2 contingency tables. Statistical differences among the data for each group were determined by analysis of variance (ANOVA), followed by the Fisher least significant difference test. To compare the causative factors between responders and non-responders, we performed stepwise discriminant functional analysis. At each step, improvement in the χ^2 and the P values was used to check whether the variable entered at that step significantly improved the discrimination. The independent variables were as follows: polymorphisms, gender, age, duration of disease, types and numbers of co-medicated anti-hyperglycemic drugs, daily dose of metformin, BMI, aspartate aminotransferase, alanine aminotransferase, total cholesterol, high-density lipoprotein (HDL) and treatment with lipid-lowering agents (statins and fibrates). Data are shown as means ± SDs. A P value <0.05 was considered to be significant.

Results

Although the time course of change in the mean daily dose of metformin (milligrams per kilogram per day) and the initial level of HbA_{1c} did not differ between the two groups, the mean HbA_{1c} level was significantly lower in the responder group than in the non-responder group during metformin therapy (Fig. 1).

To identify polymorphisms, we performed PCR–SSCP analysis of all 11 exons of the two genes of interest (*OCT1* and *OCT2*), using DNA obtained from all patients, and the allelic frequency was compared between the responder and non-responder groups. In the *OCT1* gene, 11 polymorphisms were detected by SSCP analysis and identified by subsequent sequencing; none were novel polymorphisms (Table 1). Of these, five SNPs resulted in the following amino acid substitutions: 123C > G (41Phe > Leu), 350C > T (117Pro > Leu), 480C > G (160Phe > Leu), 1022C > T (341Pro > Leu), and 1222A > G (408Met > Val). Although 480C > G, 1022C > T, and 1222A > G variants had a relatively

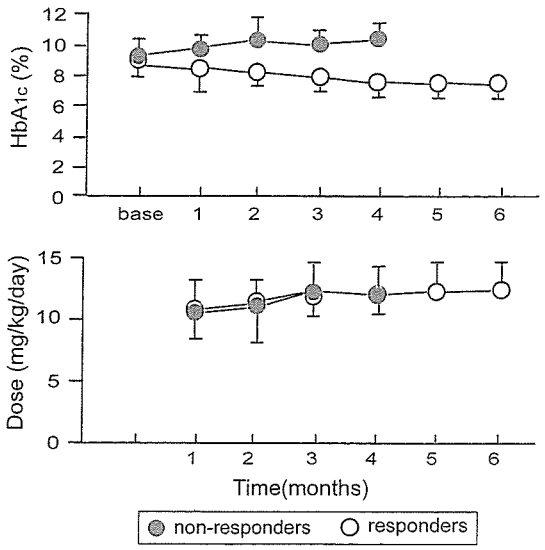

Fig. 1 Time course of changes in HbA_{1c} and metformin daily dose during the observation period in responders and non-responders

high incidence, 123C > G and 350C > T were observed in one patient as heterozygosity. In the *OCT2* gene, two non-synonymous variants were observed: 602C > T (201Thr > Met) and 808G > T (270Ala > Ser). Altogether, there were no remarkable differences in the prevalence of any mutation between responders and non-responders.

The result of discriminant functional analysis is shown in Table 2. Variables selected by the discriminant process were age, BMI, treatment with lipid-lowering agents and two mutations in the *OCT1* gene (–43T > G and 1222A > G). Other variables, such as duration of disease, daily dose of metformin, and types of co-medicated anti-hyperglycemic drugs, had no significant effect on the discrimination. Although age, BMI and treatment with lipid-lowering agents were demonstrated as positive predictors, –43T > G and 1222A > G (408Met > Val) were negative and positive predictors, respectively, for the efficacy of metformin. Total predictive accuracy using these factors was 55.5% ($\chi^2 = 5.59$, $P < 0.05$).

As shown in Table 1, since the frequency of the 408Met allele tended to be higher in non-responders than in responders (0.28 vs 0.19), and since the non-synonymous 408Met > Val variant was selected as a positive predictor, we next examined the association of the 408Met > Val (1222A > G) variant with the expression of OCT1 mRNA in the human liver samples (Fig. 2). Of 58 samples, we analyzed 31 that were homozygotes for the –43T variant (–43T/T). The mean (± SD) hepatic expression level of OCT1 in homozygotes for 408Met (1222A/1222A), heterozygotes for

Table 1 Summary of *OCT1* and *OCT2* gene polymorphisms

Gene	Location	Position[a]	Allele[a]	Nucleotide sequence	Amino acid substitution	Allelic frequency (95% CI)	
						Responders (n = 24)	Non-responders (n = 9)
OCT1	Exon 1	123	C	tcttCctgg	41Phe > Leu	0.98 (0.94–1.02)	1.000
			G	tcttGctgg		0.02 (–0.02–0.06)	0.000
		156	T	agagTcctg	Ser52	0.58 (0.44–0.72)	0.44 (0.21–0.67)
			C	agagCcctg		0.42 (0.28–0.56)	0.56 (0.33–0.79)
		243	C	cgggCgagg	Gly81	1.000	0.94 (0.84–1.05)
			T	cgggTgagg		0.000	0.06 (–0.05–0.16)
		350	C	ctgcCgctg	117Pro > Leu	1.000	0.94 (0.84–1.05)
			T	ctgcTgctg		0.000	0.06 (–0.05–0.16)
	Intron 1	–43	T	atggTtctg	–	0.42 (0.28–0.56)	0.33 (0.12–0.55)
			G	atggGtctg		0.58 (0.44–0.72)	0.67 (0.45–0.89)
	Exon 2	480	C	tcttCtttg	160Phe > Leu	0.88 (0.78–0.97)	0.83 (0.66–1.01)
			G	tcttGtttg		0.13 (0.03–0.22)	0.17 (–0.01–0.34)
	Exon 6	1022	C	acgcCgcgc	341 Pro > Leu	0.81 (0.70–0.92)	0.89 (0.74–1.03)
			T	acgcTgcgc		0.19 (0.08–0.30)	0.11 (–0.03–0.26)
	Exon 7	1222	A	ggccAtgtc	408Met > Val	0.19 (0.08–0.30)	0.28 (0.07–0.49)
			G	ggccGtgtc		0.81 (0.70–0.92)	0.72 (0.52–0.93)
	Inron 7	+8	Deletion	(ggtaagtt)0		0.81 (0.70–0.92)	0.72 (0.52–0.93)
				(ggtaagtt)1		0.19 (0.08–0.30)	0.28 (0.07–0.49)
	Intron 10	+26	C	actcCgagg		0.98 (0.94–1.02)	1.000
			T	actcTgagg		0.02 (–0.02–0.06)	0.000
		–21	C	ccaaCttt		0.46 (0.32–0.60)	0.39 (0.16–0.61)
			T	ccaaTttt		0.54 (0.40–0.68)	0.61 (0.39–0.84)
OCT2	Exon 3	602	C	tataCgtgg	201Thr > Met	0.98 (0.94–1.02)	0.94 (0.84–1.05)
			T	tataTgtgg		0.02 (–0.02–0.06)	0.06 (–0.05–0.16)
	Exon 4	808	G	agttGctct	270Ala > Ser	0.92 (0.88–0.96)	0.94 (0.84–1.05)
			T	agttTctct		0.08 (0.04–0.12)	0.06 (–0.05–0.16)

[a] Position is relative to the ATG start site, and the reference allele for each gene was obtained from the GenBank accession numbers AL353625 for OCT1 and AL162582 for OCT2

408Met > Val (1222A/1222G), and homozygotes for 408Val (1222G/1222G) was 0.69 ± 0.43, 0.92 ± 0.53, and 1.01 ± 0.66, respectively. Although the hepatic expression of OCT1 tended to be lower in livers with the 408Met (1222A) variant, the differences did not reach the level of significance. In the –43T > G variant, the mean OCT1 expression level in –43T/T (n = 18), –43T/G (n = 8), and –43G/G (n = 10) samples (all harbored the 1222G/1222G allele) was 1.01 ± 0.70, 1.04 ± 0.34, and 1.46 ± 0.53, respectively.

Table 2 Stepwise discriminant functional analysis of the efficacy of metformin

Variable	Coefficient	χ^2 value	P
Age	0.09	5.59	0.05
BMI	0.23		
Treatment with lipid-lowering agents	2.25		
–43T > G (intron 1)	–2.35		
408Met > Val (exon 7)	2.51		

Predictive accuracy = 55.5%

Discussion

In this study we first analyzed mutations in *OCT1* and *OCT2* and then examined the association between polymorphisms in these two genes and the efficacy of metformin, because in vitro studies have indicated that OCT1 and OCT2 are responsible, respectively, for the hepatic and renal transport of metformin (Kimura et al. 2005; Wang et al. 2002, 2003). In contrast to studies in vitro and with animals, there are no data from human studies on the contribution of these polymorphisms to the phenotypes of metformin.

In the *OCT1* gene, all non-synonymous variants except 41Phe > Leu and 117Pro > Leu have already been identified in some racial populations, with a frequency of 0.005–0.81 (Kerb et al. 2002; Shu et al. 2003). The 41Phe > Leu and 117Pro > Leu allele frequencies were relatively low (0.004), and they have already been reported in a Japanese population (Itoda et al. 2004). Recent expression studies have indicated that 341Pro > Leu had decreased ability to transport test compounds, while 160Phe > Leu and 408Met > Val were unchanged (Kerb et al. 2002; Sakata et al. 2003;

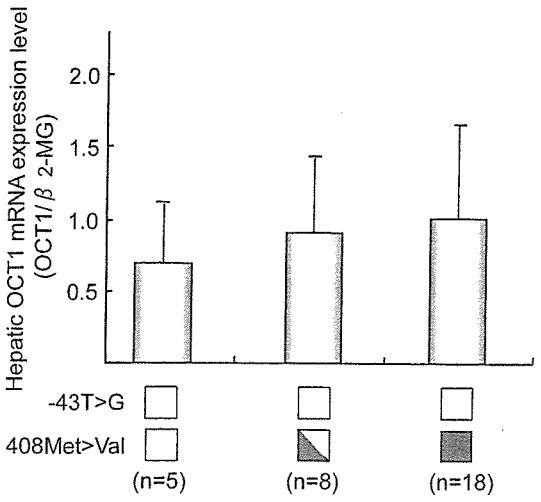

Fig. 2 Hepatic OCT1 mRNA expression levels with regard to the 408Met > Val (1222A > G) variant. Among 58 samples, 31, which were homozygotes for the –43T variant (–43T/T), were analyzed. *Open squares*, *partially filled squares* and *closed squares* correspond to patients homozygous for the 408Met (1222A) allele and heterozygous and homozygous for the 408Val (1222G) allele

Shu et al. 2003). Interestingly, the 341Pro > Leu variant was observed in Asian and African American populations but not in Caucasians (Shu et al. 2003); however, there was no difference in the allele frequency of 341Pro > Leu between responders and non-responders to metformin therapy in this study.

In contrast to those in the *OCT1* gene, it appears that the number of non-synonymous variants in the *OCT2* gene and their allelic frequencies were lower than in other known drug transporter genes such as *MDR1*, *MRP1*, *MRP2*, and *OATP-C* (Nishizato et al. 2003). These observations are consistent with the finding of a lower frequency of non-synonymous variants in ethnically diverse genomic DNA samples (Leabman et al. 2002). Recent population-genetic analysis has demonstrated that selection has acted against amino acid changes in OCT2 (Leabman et al. 2002), suggesting that OCT2 is relatively intolerant of non-synonymous changes. In general, the less frequent non-synonymous variants resulted in more significant and deleterious functional changes. However, the 270Ala > Ser variant was reported to exhibit subtle functional differences from the reference form of OCT2 (Leabman et al. 2002).

Although there were no remarkable differences in the prevalence of any mutation sites between responders and non-responders, we next carried out discriminant functional analysis including not only genetic polymorphisms but also the patients' background. As shown in Table 2, age, BMI and treatment with lipid-lowering agents were demonstrated as positive predictors of metformin efficacy. These observations are partially in agreement with the findings by Knowler et al. (2002), that metformin was less effective in subjects with lower BMI or a lower fasting plasma glucose concentration. BMI > 25 kg/m^2 is defined as obesity in Japan; 66.7% of responders and 44.4% of non-responders were obese in this study. Although the precise mechanism is unknown, these data suggest that metformin is more effective in the case of obesity-induced insulin resistance that is higher fasting plasma glucose. The contribution of lipid-lowering agents was somewhat unexpected, because metformin therapy has been reported to improve both glycemic control and lipid concentrations (i.e., plasma total and low-density lipoprotein cholesterol and triglyceride) in patients with non-insulin-dependent diabetes mellitus (DeFronzo and Goodman 1995). However, in our study, 12 responders and two non-responders were treated with lipid-lowering agents, and most of these patients (11/12 responders and 1/2 non-responders) used HMG-CoA reductase inhibitors (statins). Several studies have shown that low-density lipoprotein (LDL) size rather than plasma LDL level is more correlated with insulin resistance and eventual progression of coronary heart disease (Rizzo and Berneis 2006). Although the efficacy of modifying LDL size is different among agents (fluvastatin and atorvastatin seem to be much more effective agents than pravastatin and simvastatin), statins moderately lower all LDL subclasses, and, somehow, this process seems to make metformin more effective.

Since –43T > G and 408Met > Val (1222A > G) variants were identified as negative and positive predictors, respectively, for the clinical effectiveness of metformin, we evaluated the functional significance of the latter non-synonymous variant in the expression of OCT1 mRNA, using human liver samples. Our findings indicate that samples with the 408Met (1222A) allele tended to be associated with a reduced expression level, as compared with those without the 408Met allele; however, the difference did not reach significance. A recent study using site-directed mutagenesis has indicated that point mutations in the predicted ninth transmembrane domain such as 1222A > G (408Met > Val) do not lead to functional changes (Kerb et al. 2002). We also measured OCT1 mRNA expression with regard to the non-coding –43T > G variant; however, no significant effect was observed. In the present study, the predicted accuracy is still insufficient for its clinical application (i.e., 55.5%). Thus, if these observations are taken into consideration, the contribution of polymorphisms in

OCT1 and *OCT2* genes to metformin efficacy may not be as significant as our expectations had led us to believe. However, since a non-synonymous variant 408Met > Val is often observed simultaneously with other non-synonymous variants (Shu et al. 2003), further in vitro and in vivo studies with regard to the haplotypic consideration, including the non-coding region, are needed to elucidate the functional properties of the variants identified in this study.

While data from only 24 responders and nine non-responders were used, this preliminary investigation is the first study addressing the genotype–phenotype relationship of OCTs in the efficacy of metformin. However, obviously, the small number of patients is a drawback in our study. For example, co-medication of other anti-hyperglycemic drugs in both groups made it difficult for us to judge whether the decreases in HbA_{1c} levels in the responders are attributable to the metformin effect. Clearly, definition of the clinical cut-off point is also essential to divide patients into the two groups correctly. In order to overcome these problems, it is clear that the results in this study should be confirmed in a population study involving large numbers of patients. Nevertheless, this report provides for the possibility of OCTs' functions in humans.

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SHORT COMMUNICATION

Pharmacogenetic determinants of variability in lipid-lowering response to pravastatin therapy

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Abstract Pravastatin is mainly taken up from the circulation into the liver via organic anion-transporting polypeptide 1B1 (*SLCO1B1* gene product). We examined the contribution of genetic variants in the *SLCO1B1* gene and other candidate genes to the variability of pravastatin efficacy in 33 hypercholesterolemic patients. In the initial phase of pravastatin treatment (8 weeks), heterozygous carriers of the *SLCO1B1**15 allele had poor low-density lipoprotein cholesterol (LDL-C) reduction relative to non-carriers (percent reduction: –14.1 vs –28.9%); however, the genotype-dependent difference in the cholesterol-lowering effect disappeared after 1 year of treatment. Cholesterol 7α-hydroxylase (*CYP7A1*) and apolipoprotein E (*APOE*) are known to contribute to lipid metabolism. Homozygous carriers of the *CYP7A1* -204C allele or heterozygotes for both *CYP7A1* -204C and *APOE* ε4 alleles showed significantly poorer LDL-C reduction compared to that in other genotypic groups after 1 year of treatment (–24.3 vs –33.1%). These results suggest that the *SLCO1B1**15 allele is associated with a slow response to pravastatin therapy, and the combined genotyping of *CYP7A1* and *APOE* genes is a useful index of the lipid-lowering effect of pravastatin.

Keywords *SLCO1B1* · *CYP7A1* · *APOE* · Pravastatin · Cholesterol

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Introduction

Coronary heart disease is the leading cause of death worldwide. Several risk factors for cardiovascular disease are well known, especially increased low-density lipoprotein cholesterol (LDL-C) and decreased high-density lipoprotein cholesterol (HDL-C). Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a rate-limiting enzyme in cholesterol biosynthesis. Lipid-lowering therapy by statins has the potential to improve outcomes in patients at risk for cardiovascular disease. Despite these large effects, interindividual variability in the response to statins has been observed in clinical situations (Pazzucconi et al. 1995). Previous studies have demonstrated that the mechanisms responsible for variability in the statin response are due, at least in part, to genetic factors. Most studies have focused on the association between variants (ε2, ε3 and ε4) in apolipoprotein E (*APOE*) gene, which is a primary ligand for the LDL receptor found on the liver, and the response to statins (Ojala et al. 1991; Ordovas et al. 1995). In addition, recent studies have demonstrated

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:

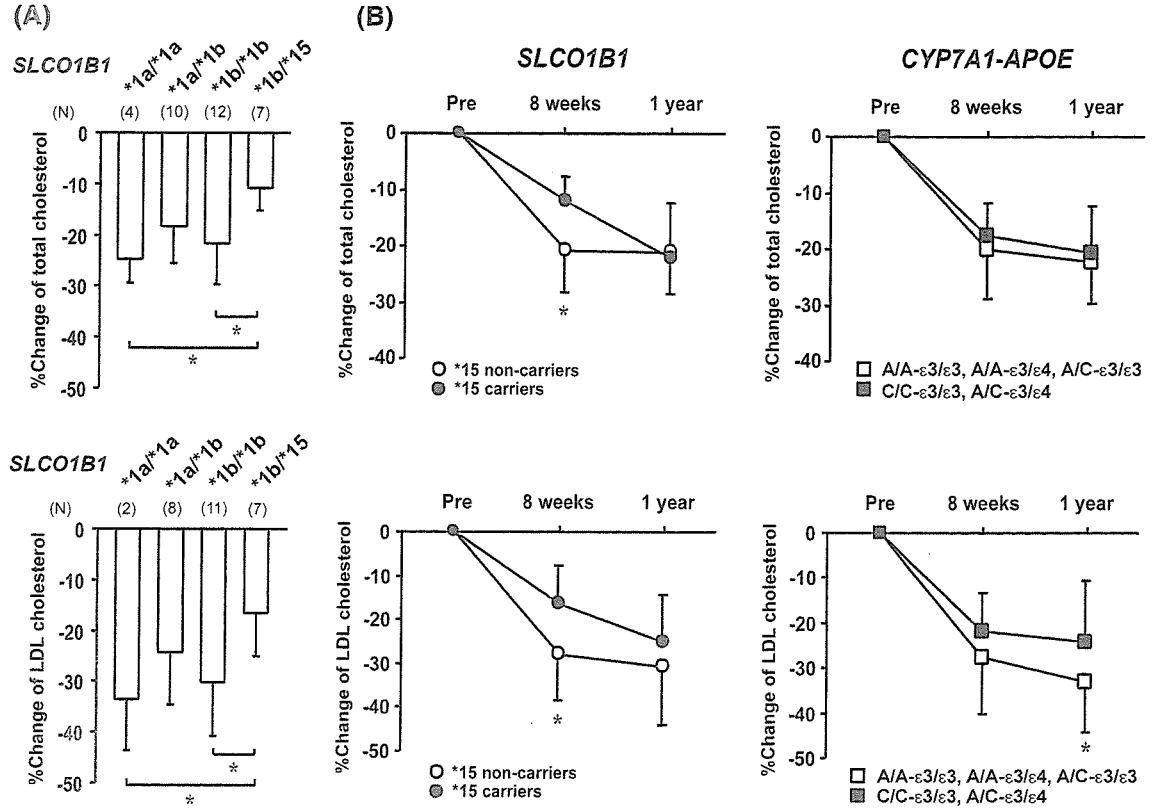

Fig. 1 **a** Influence of the *SLCO1B1* genotypes on percent reduction from baseline in TC and LDL-C values at 8 weeks after pravastatin treatment. *$P<0.05$ when compared between the two groups using Tukey–Kramer multiple comparison test. **b** Influence of the *SLCO1B1*, *CYP7A1* and *APOE* genotypes 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

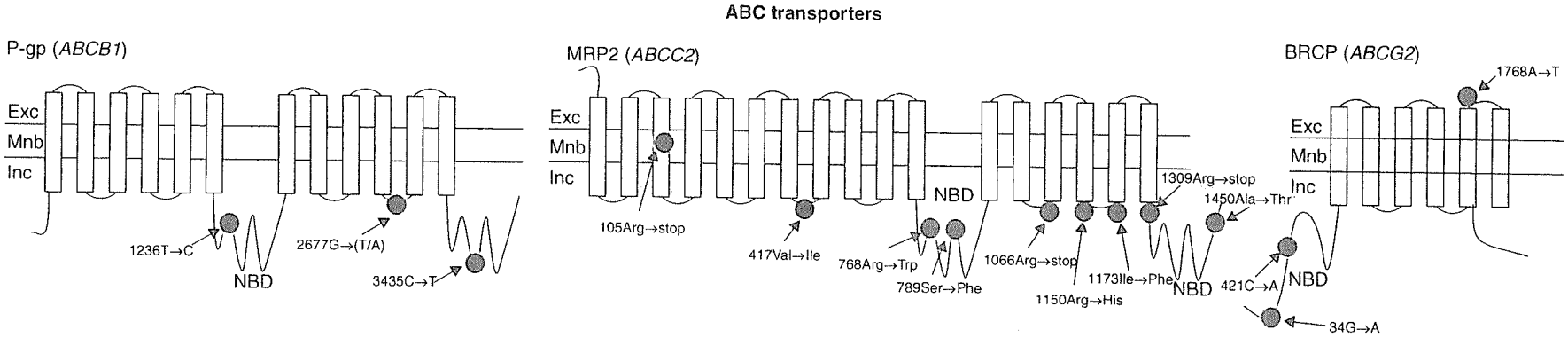

Figure 1. Schematic representation of secondary structures in drug transporters, with some nucleotide substitutions.

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.

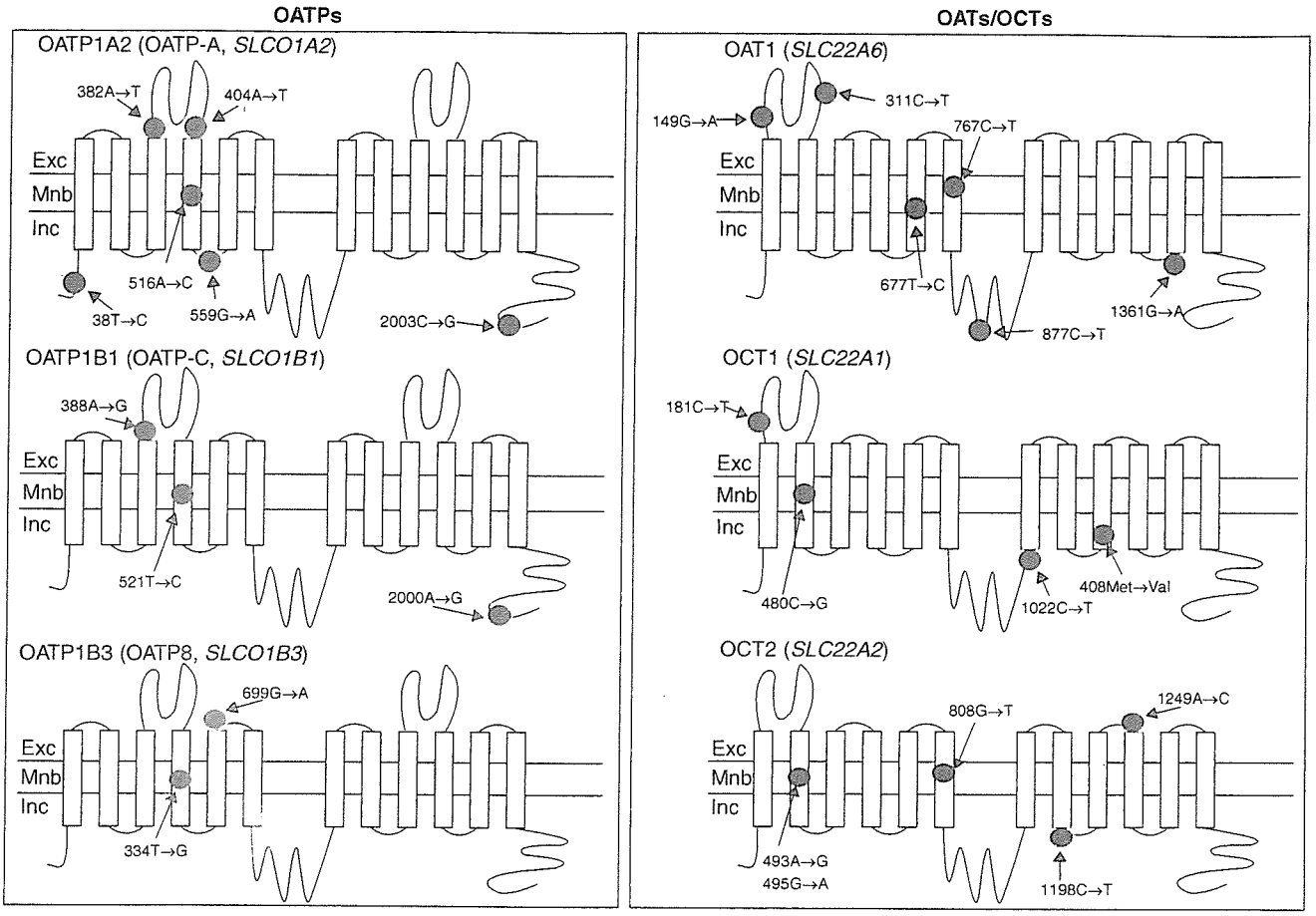

Figure 2. Schematic representation of secondary structures in drug transporters, with some nucleotide substitutions.
Exc: Extracellular; Inc: Intracellular; Mnb: Membrane; OAT: Organic anion transporter; OATP: Organic anion-transporting polypeptide; OCT: Organic cation transporter.