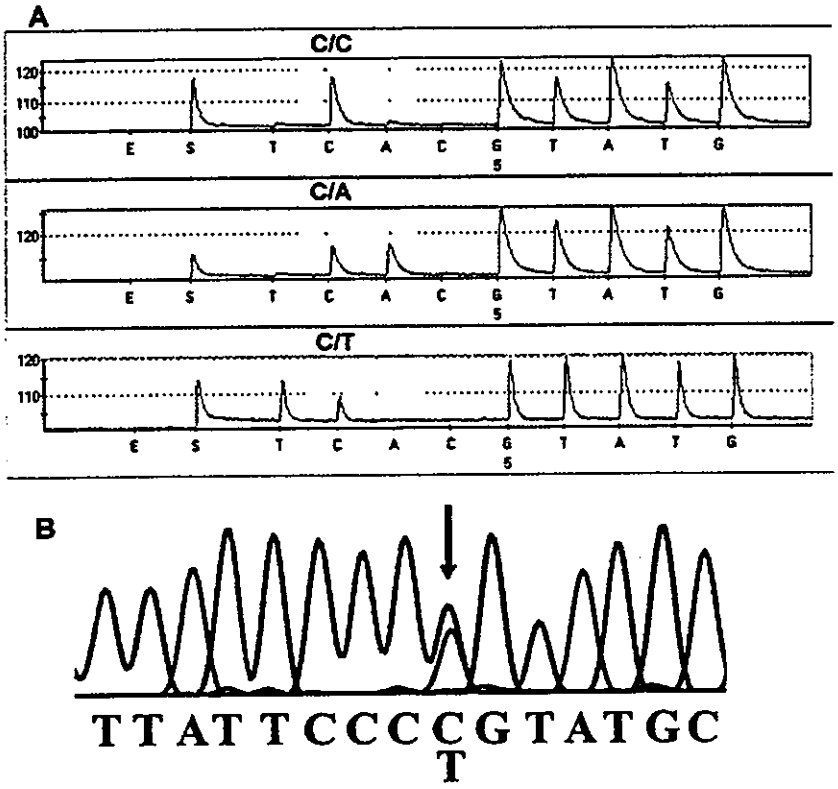

FIG. 1. Pyrosequencing and dideoxy sequencing methods for position 686. A, pyrograms for UGT1A1 686C/C, 686C/A, and 686C/T. The sequence to be analyzed is underlined in GACGTGGTTTATTCCCCGTATG. The first T and the fourth C, nucleotides unrelated to 686C>A, were added to estimate the background value. Top, a pyrogram obtained from a Japanese individual carrying homozygous 686C; middle, a pyrogram obtained from a Japanese individual with 686C/A; bottom, a pyrogram obtained from an African-American with 686C/T. B, the nucleotide sequence around the 686th nucleotide in exon 1 is shown (the A of the translational start codon of the cDNA is designated as 1). The sequence of the sense strand obtained from the same individual as in the bottom of A is shown. The arrow indicates the nucleotide substitution position.

2042C>G in African-Americans and Caucasians. The frequencies of 1813C>T and 2042C>G in the Japanese were not determined in this study, because these alleles were reported to be in complete association with 1941C>G in a Japanese population without exception (Sai et al., 2004; unpublished data). Therefore, allele frequencies of 1813C>T and 2042C>G in the Japanese were assumed to be equal to that of 1941C>G. On the other hand, we found that 1813C>T and 2042C>G were not always in association with 1941C>G in African-Americans and Caucasians, as shown in Table 1. The allele frequencies of the three SNPs were highest in African-Americans and lowest in the Japanese. Significant differences in the allele frequencies were detected ($p < 0.0001$, $p = 0.0074$, and $p < 0.0001$ for 1813C>T, 1941C>G, and 2042C>G, respectively, by the χ^2 test).

Differences in Haplotype Frequencies. Sai et al. (2004) previously reported that *UGT1A1* could be divided into two blocks, with the transcription-regulating and promoter regions and exon 1 in block 1, and exons 2 to 5 in block 2, according to linkage disequilibrium analysis. Therefore, haplotype/diplotype analysis was performed using four marker variations in block 1 (−3279T>G, 211G>A, and 686C>A, and the TATA box), and three marker variations in block 2 (1813C>T, 1941C>G, and 2042C>G). The diplotype configurations (combinations of haplotypes) were estimated for each subject by PHASE software (Stephens et al., 2001; Stephens et al., 2003).

Concerning block 1, the diplotype configurations were inferred with greater than 0.99 certainties for 145 Japanese, 144 Caucasians, and 147 African-Americans. The haplotypes identified are summarized in Table 2 along with their frequencies for each population, where the 14 subjects with ambiguous diplotypes were excluded. We followed the haplotype nomenclature previously reported (Sai et al., 2004). One Japanese subject carrying heterozygous 686C>A also carried homozygous TA_7. Thus, the association of variation 686C>A with TA_7 was also confirmed in this study, as previously reported by Huang et al. (2000) and Sai et al. (2004). A new haplotype, designated **6d*, having variations in both positions −3279 and 211, was identified that was not found in the previous study (Sai et al., 2004). We could not determine whether the novel variation 686T and the **28* allele were on the same chromosome, because the subject with 686T carried heterozygous TA repeats, TA_6/TA_7.

In block 1, haplotype **1a* was predominant in the Japanese population (0.61 in frequency). In contrast, its frequencies in Caucasians and African-Americans were 0.45 and 0.15, respectively. Major haplotypes in African-Americans were **28b* and **60a*. Haplotype frequencies of **1a* and **28b* in Caucasians were comparable. Thus, the haplotype distribution patterns in block 1 for the individual populations were significantly different ($p < 0.0001$ by the χ^2 test).

In block 2, the diplotype configurations using the three SNPs were inferred with certainties greater than 0.98 for all Caucasians and African-Americans (150 each). The haplotypes and their frequencies are summarized in Table 3. Four novel haplotypes, **1C*, **1D*, **1E*, and **1F*, were identified in the Caucasian and African-American populations, although **1E* was not found in the latter. These haplotypes were not found in the Japanese, as shown in a previous study (Sai et al., 2004). Although the three SNPS were reported to be completely associated with each other in the Japanese (Sai et al., 2004), they were not always linked with each other in the rest of the populations. However, it is noteworthy that 1941G allele was always associated with 1813T and 2042G alleles except for the haplotype **1E*, found only in two Caucasians. Haplotype **1A* was predominant for all ethnic groups. The second major haplotype was **1B* in both the Japanese and Caucasians. However, the frequencies of **1B* and **1C* were similar in African-Americans (0.183 and 0.163, respectively). The haplotype **1C* was also found in Caucasians (0.06). Thus, the haplotype distribution patterns in block 2 for the three populations were also significantly different ($p < 0.0001$ by the χ^2 test).

Expression and SN-38 Glucuronidation of a Novel Variant P229L Compared with Wild-Type UGT1A1. The relative expres-

TABLE 2

Haplotypes in block 1 (the enhancer/promoter regions and exon 1) of UGT1A1 for three ethnic groups

Haplotype distribution patterns in block 1 were statistically different among the three ethnic groups ($p < 0.0001$ by the χ^2 test). Fourteen subjects with ambiguous diplotypes were excluded from the analysis.

Position	-3279		TA box				211		686		Haplotype frequency		
Nucleotide	T	G	5	6	7	8	G	A	C	A	African-American	Caucasian	Japanese
**1a*	■			■			■		■		0.150	0.451	0.610
**6a*	■			■				■	■		0.000	0.000	0.141
**6d*		■		■				■	■		0.000	0.000	0.003
**28b*		■			■		■		■		0.446	0.389	0.097
**28c*		■			■		■			■	0.000	0.000	0.003
**36b*		■	■				■		■		0.044	0.017	0.000
**37b*		■				■	■		■		0.065	0.007	0.000
**60a*		■		■			■		■		0.296	0.135	0.145

TABLE 3

Haplotypes in block 2 (exons 2–5) of UGT1A1 for three ethnic groups

Haplotype distribution patterns in block 2 were statistically different among the three ethnic groups ($p < 0.0001$ by the χ^2 test).

Position	1813		1941		2042		Frequency		
Nucleotide	C	T	C	G	C	G	African-American	Caucasian	Japanese[a]
**IA*	■		■		■		0.617	0.757	0.903
**IB*		■		■		■	0.183	0.157	0.097
**IC*		■	■			■	0.163	0.060	0.000
**ID*	■		■			■	0.033	0.017	0.000
**IE*	■			■		■	0.000	0.007	0.000
**IF*		■	■		■		0.003	0.003	0.000

[a] Haplotypes in Japanese were determined according to the genotype of 1941C > G, because it was previously reported that all three markers were completely associated in a Japanese population.

sion level of the novel variant UGT1A1/P229L in the membrane fraction of COS-1 cells was determined by Western blotting using a polyclonal anti-human UGT1A antibody (Fig. 2). Because two bands, one at the same position as the wild-type and the other at a higher molecular weight, were detected for P229L, the total chemiluminescence of the two bands was used to calculate the protein expression level for P229L. The protein expression level of P229L was approximately 60% of the wild type, and this difference was statistically significant by the *t* test ($p = 0.044$). The *UGT1A1* mRNA expression level was then measured by real-time reverse transcription-PCR (RT-PCR) using SYBR Green. As shown in Fig. 3, however, no significant difference in the mRNA level was detected among the COS-1 cells transfected with the expression plasmids carrying wild-type and P229L *UGT1A1* cDNAs.

The glucuronidation activity of SN-38 by P229L expressed in COS-1 cells was compared with that of the wild-type under 11 substrate concentrations ranging from 2.5 to 150 μM. The representative curves of the Michaelis-Menten kinetics are shown in Fig. 4, and the estimated apparent kinetic parameters (K_m, V_{max}, and V_{max}/K_m) are summarized in Table 4. V_{max} values normalized

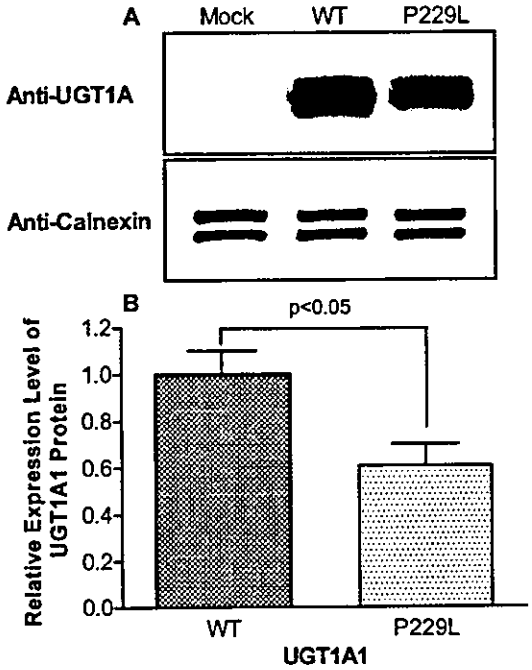

FIG. 2. Expression of wild-type and variant P229L UGT1A1 in COS-1 cells. A, an aliquot (20 μg) of the pooled membrane fractions from three independent preparations was subjected to SDS-polyacrylamide gel electrophoresis, electrophoretically transferred to a polyvinylidene difluoride membrane, and immunochemically detected with a rabbit anti-human UGT1A antiserum. The membrane was subsequently stripped and reprobed with a rabbit anti-calnexin antiserum to confirm that the samples were evenly loaded. B, each Western blot from three independent preparations was quantified densitometrically, and the expression level of UGT1A1 proteins was normalized to that of the wild type. The results are expressed as the mean ± S.E.M. from three independent preparations. The expression level of UGT1A1 proteins of P229L was significantly lower than that of the wild type (p = 0.044).

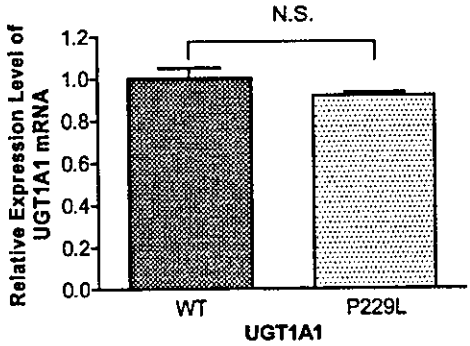

FIG. 3. Quantification of UGT1A1 mRNA by real-time SYBR Green RT-PCR in COS-1 cells transfected with wild-type and variant P229L UGT1A1. UGT1A1 mRNA from the cellular total RNA samples was quantified by SYBR Green RT-PCR. Each sample was normalized on the basis of the β-actin content and expressed as a percentage of the wild type. The results indicate the mean ± S.E.M. from three independent preparations. No significant difference in mRNA level was observed between the wild type and P229L (p = 0.180).

to the expression levels are also shown. Wild-type UGT1A1 catalyzed SN-38 glucuronidation with an average apparent K_m value of 8.67 μM, whereas P229L catalyzed SN-38 glucuronidation with a K_m value of 37.6 μM. The average V_{max} values were 71.2 and 5.27 pmol/min/mg membrane protein for the wild-type and P229L, respectively. The average intrinsic clearances of SN-38 by glucuronidation (V_{max}/K_m) normalized to expressed UGT1A1 protein levels were 8.26 and 0.24 μl/min/mg protein for the wild-type and P229L, respectively.

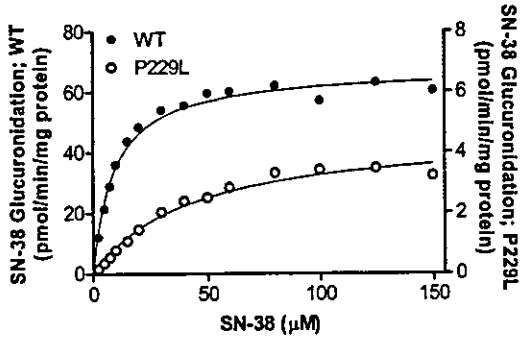

FIG. 4. Representative Michaelis-Menten kinetics of SN-38 glucuronidation by expressed wild-type (●) and variant P229L (○) UGT1A1 in COS-1 cells. SN-38 glucuronidation by expressed UGT1A1 was assayed in the presence of the membrane fractions (100 μg) at substrate concentrations ranging from 2.5 to 150 μM. The solid line indicates predicted glucuronidation using Michaelis-Menten kinetic parameters estimated by nonlinear regression analysis.

Discussion

There have been previous reports of the allele frequencies of variants in the TATA box in various ethnic groups (Beutler et al., 1998; Fertrin et al., 2002; Innocenti et al., 2002; Sugatani et al., 2002; Ki et al., 2003; Premawardhena et al., 2003; Sai et al., 2004). According to these studies, allele frequencies of the wild-type TA_6 were highest in Polynesian people (0.97–0.99), followed by Asians, including Japanese, Koreans, and Taiwanese (0.81–0.92), and Caucasians (0.61–0.73). Allele frequencies were lowest in Africans (0.44–0.52). The allele frequencies of TA_5 and TA_8 in Africans were reported as approximately 0.05, but were very rare in Caucasians and were not detected in Asians. The allele frequencies of the four genotypes in the TATA box in African-American, Caucasian, and Japanese populations observed in this study were in the ranges previously reported. Allele frequencies of other *UGT1A1* variations have not been as extensively studied as those in the TATA box. The allele frequencies for the variant −3279T>G were 0.17 to 0.27 in Japanese and Koreans (Sugatani et al., 2002; Ki et al., 2003; Sai et al., 2004), 0.47 in Caucasians (Innocenti et al., 2002), and 0.85 in African-Americans (Innocenti et al., 2002). Thus, our data (0.257 for Japanese, 0.550 for Caucasians, and 0.847 for African-Americans) are comparable with previously reported frequencies of this allele. The allele frequency of the variant 211G>A observed in this study was comparable to previously reported values for Asian people (0.09–0.21) (Huang et al., 2000; Sugatani et al., 2002; Ki et al., 2003; Sai et al., 2004). The variant is considered to be characteristic for Asians. We found only two individuals with this allele out of 150 Caucasians, and no African-Americans had this allele.

Innocenti et al. (2002) reported on the haplotype frequencies of *UGT1A1* in African-Americans and Caucasians using −3279T>G, the TA repeat, and other SNPs in the transcription-regulating region. Although they did not use the marker SNPs 211G>A and 686C>A (likely due to their rarity in both populations), frequencies of the **28*, **36*, **37*, and **60* haplotype groups in African-Americans can be calculated according to their data as 0.35, 0.04, 0.12, and 0.33, and the calculated frequencies in Caucasian as 0.36, 0.01, 0.01, and 0.09. These frequencies were also comparable to our data. We found two individuals carrying 211G>A in the Caucasian group; however, we could not infer haplotypes for these individuals with high certainty. The observed haplotype **28* group frequency in our study of 0.100 (**28b* plus **28c*) was slightly lower than the reported values of 0.131 in Japanese patients (Sai et al., 2004) and 0.127 in Koreans (Ki et al., 2003). However, the previously reported values are included in the 95% confidence interval of our data. The observed haplotype **6* group

TABLE 4

Kinetic parameters of SN-38 glucuronidation by wild-type and P229L UGT1A1

Parameter	K_m	V_{max}	V_{max}/K_m	Normalized V_{max}	Normalized V_{max}/K_m
UGT1A1	*μM*	*pmol/min/mg protein*	*ml/min/mg protein*	*pmol/min/mg protein*	*ml/min/mg protein*
Wild-type	8.67 ± 0.18	71.2 ± 4.73	8.23 ± 0.65	71.7 ± 2.46	8.26 ± 0.15
P229L	37.6 ± 2.34	5.27 ± 0.32	0.14 ± 0.02	8.93 ± 0.98	0.24 ± 0.03

frequency (**6a* plus **6b*) was also comparable to the previously reported data in Japanese and Koreans. A strong association of TA_7 with −3279G was suggested for Japanese cancer patients (Sai et al., 2004). This association was also observed in all populations measured in this study. Other variations, TA_5 and TA_8 in the TATA box, also had linkage with −3279G.

Recent studies indicate that 3′-untranslated regions may regulate mRNA stability (Day and Tuite, 1998; Conne et al., 2000). Exon 1 is unique for each member of the *UGT1A* subfamily, whereas exons 2 to 5 are common to all members of the subfamily. The variants in the 3′-untranslated region of *UGT1A1* in exon 5, therefore, could have various effects on all enzymes of the *UGT1A* subfamily. However, until recently, their physiological effects have remained unknown. Acuna et al. (2003) reported a protective effect of the variant 1941C>G on liver transaminase levels caused by tolcapone, for which glucuronidation (mainly by UGT1A9) is one metabolic pathway (Acuna et al., 2003). The SNP 1941C>G is a marker for haplotype **1B* in block 2 as shown in Table 3. Sai et al. (2004) reported the **1B*-dependent decreasing trend of the AUC ratio (SN-38G/SN-38) and an increase in serum total bilirubin levels. Highly differential haplotype distributions in both blocks 1 and 2 observed in different ethnic groups may cause different toxicity profiles during therapy using drugs, such as CPT-11, that are metabolized by UGT1A1.

A novel SNP 686C>T was found in an African-American sample in this study, leading to an amino acid change, P229L, which is different from the known SNP 686C>A (P229Q). Studies using a larger sample size may be necessary to elucidate an accurate frequency. The known SNP 686C>A (P229Q) was reported to be related to Gilbert's syndrome and to have very low bilirubin glucuronidation activity (Koiwai et al., 1995). However, Jinno et al. (2003a,b) reported that protein expression and mRNA levels of P229Q expressed in COS-1 cells were comparable to those of the wild type, and the decrease in its SN-38 glucuronidation was marginal. Since the association of P229Q with TA_7 has been suggested previously (Huang et al., 2000; Sai et al., 2004) and confirmed in this study, hyperbilirubinemia observed in Japanese and Taiwanese patients carrying the P229Q variant can be mainly attributed to the TA_7 variation. On the other hand, the novel variant P229L expressed in COS-1 cells was found to have extremely low SN-38 glucuronidation efficacy in this study (less than 3% of that for the wild type). The low glucuronidation activity of P229L was not caused by its low transcription levels, because a significant difference in the mRNA level was not observed between the wild type and variant. The low UGT1A1/P229L expression level in COS-1 cells suggests that this enzyme is unstable compared with the wild-type enzyme. Moreover, a band shifted to a higher molecular weight than the wild-type UGT1A1 was detected by a rabbit anti-human UGT1A antibody in a Western blot. Such additional bands were also observed in Western blots of variant UGT1A1 having extremely low enzyme activities, such as F83L and Y486D in our group (Jinno et al., in press). The band shift to a higher molecular weight in SDS-polyacrylamide gel electrophoresis is considered to be the result of products with different post-translational modifications such as phosphorylation or glycosylation, which may occur due to conformational changes caused by amino acid substitution. This may also contribute to the extremely low catalytic activity of the variant enzyme compared with that of the wild type as shown in Table 4. Although the clinical significance of 229L remains to be confirmed, this novel SNP may be a potential cause of diseases such as hyperbilirubinemia or jaundice and may cause severe adverse effects after administration of CPT-11.

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LEADING ARTICLE

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The *MDR1* (ABCB1) Gene Polymorphism and its Clinical Implications

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Abstract

There has been an increasing appreciation of the role of drug transporters in the pharmacokinetic and pharmacodynamic profiles of certain drugs. Among various drug transporters, P-glycoprotein, the *MDR1* gene product, is one of the best studied and characterised. P-glycoprotein is expressed in normal human tissues such as liver, kidney, intestine and the endothelial cells of the blood-brain barrier. Apical (or luminal) expression of P-glycoprotein in these tissues results in reduced drug absorption from the gastrointestinal tract, enhanced drug elimination into bile and urine, and impeded entry of certain drugs into the central nervous system. The clinical relevance of P-glycoprotein depends on the localisation in human tissues (i.e. vectorial or directional movement), the therapeutic index of the substrate drug and the inherent inter- and intra-individual variability.

With regard to the variability, polymorphisms of the *MDR1* gene have recently been reported to be associated with alterations in disposition kinetics and interaction profiles of clinically useful drugs, including digoxin, fexofenadine, ciclosporin and talinolol. In addition, polymorphism may play a role in patients who do not respond to drug treatment. Moreover, P-glycoprotein is an important prognostic factor in malignant diseases, such as tumours of the gastrointestinal tract.

A growing number of preclinical and clinical studies have demonstrated that polymorphism of the *MDR1* gene may be a factor in the overall outcome of pharmacotherapy for numerous diseases. We believe that further understanding the physiology and biochemistry of P-glycoprotein with respect to its genetic variations will be important to establish individualised pharmacotherapy with various clinically used drugs.

Introduction of the concepts of pharmacogenetics to the clinical setting has had an impact on individualisation of drug treatment, and could therefore contribute significantly to enhanced drug safety and efficacy. Drug-metabolising enzymes are typical cases that have been intensively investigated. For example, the activity of cytochrome P450 (CYP) 2D6 is polymorphically distributed in the population and related to the presence of a number of allelic variants with varying degrees of functional significance. Thus, genotyping is expected to provide a new tool for predicting individual drug-metabolising capabilities before treatment begins. Recently, some naturally occurring polymorphisms of the

MDR1 gene have been reported to be correlated with potential clinical effects,[1-6] with the levels of P-glycoprotein expression in human tissues,[7-10] or with the bioavailability of orally administered drugs.[11] Furthermore, there is some evidence that the extent of P-glycoprotein induction and the interaction profile of P-glycoprotein substrates are dependent on *MDR1* polymorphisms.[9,11,12]

Numerous candidate single nucleotide polymorphisms (SNPs) have been identified and characterised. Notably, the C3435T mutation in exon 26 has been examined in detail. In addition, the G2677T/A mutation is of interest because it is closely linked to C3435T. However, the effects of these SNPs on the expression of P-glycoprotein and the pharmacokinetic and pharmacodynamic outcomes of certain drugs are unclear.[13]

At a time when the potential importance of P-glycoprotein function is receiving much attention, this review highlights recent studies by others and ourselves on the role of the *MDR1* gene polymorphism in expression in various human tissues, its pharmacokinetic and pharmacodynamic impacts, as well as the inter-racial variability of allelic frequencies. In addition, possible explanations for the variable and conflicting results seen among studies are discussed. The scope of this review is strictly limited to observations from human studies.

1. General Features

1.1 Localisation in Human Tissues and Basic Functions

P-glycoprotein, a transmembrane transporter encoded by the *MDR1* gene, acts as an efflux pump in an adenosine triphosphate (ATP)-dependent fashion. It was first identified in human cancer cells as a protein responsible for resistance against many anticancer drugs.[14-17] Subsequently, this efflux transporter has been found in various normal human tissues. Expression is identified in the small and large intestinal epithelium, adrenal gland, placenta (trophoblasts), kidney (the brush border of the renal tubule), liver (the canalicular membrane of the hepatocyte), pancreas (pancreatic ductile cell), and capillary endothelial cells of brain and testes.[18-22] In these tissues, P-glycoprotein is located on the apical or luminal surface of the epithelial cells. P-glycoprotein has also been found in peripheral blood lymphocytes.[23,24] On the basis of its tissue distribution and findings in knockout mice, P-glycoprotein is speculated to play an important role in the excretion of foreign xenobiotics and endogenous substrates 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.[25,26] P-glycoprotein in trophoblasts contributes to the function of blocking the transfer of hydrophobic xenobiotics across the human placenta.[27] In the endothelial cells of the blood-brain barrier, P-glycoprotein prevents the entry of substrates into the central nervous system.[28-30] In summary, P-glycoprotein functions as a defence mechanism against xenotoxins.

1.2 Substrate Drugs

P-glycoprotein recognises and transports a variety of drugs, including chemotherapy agents (paclitaxel[31] and irinotecan[32]), antibacterials (erythromycin[33] and levofloxacin[34]), immunosuppressants (ciclosporin and tacrolimus),[35] cardiac drugs (digoxin[36] and quinidine[37]), calcium channel antagonists (diltiazem[38] and verapamil[39]) and HIV protease inhibitors (ritonavir[40] and saquinavir[25]). P-glycoprotein substrates, inducers and inhibitors are listed in detail elsewhere.[41-43] It is well known that there is a strong overlap in substrate specificity and tissue distribution for P-glycoprotein and CYP3A4/5.[44-46] P-glycoprotein accepts a broad spectrum of structurally and functionally unrelated drugs. It is not clear what determines the substrate specificity of P-glycoprotein. In general, P-glycoprotein substrates are hydrophobic, and/or organic cations at physiological pH, containing one or more aromatic rings, with a molecular weight >400.[47]

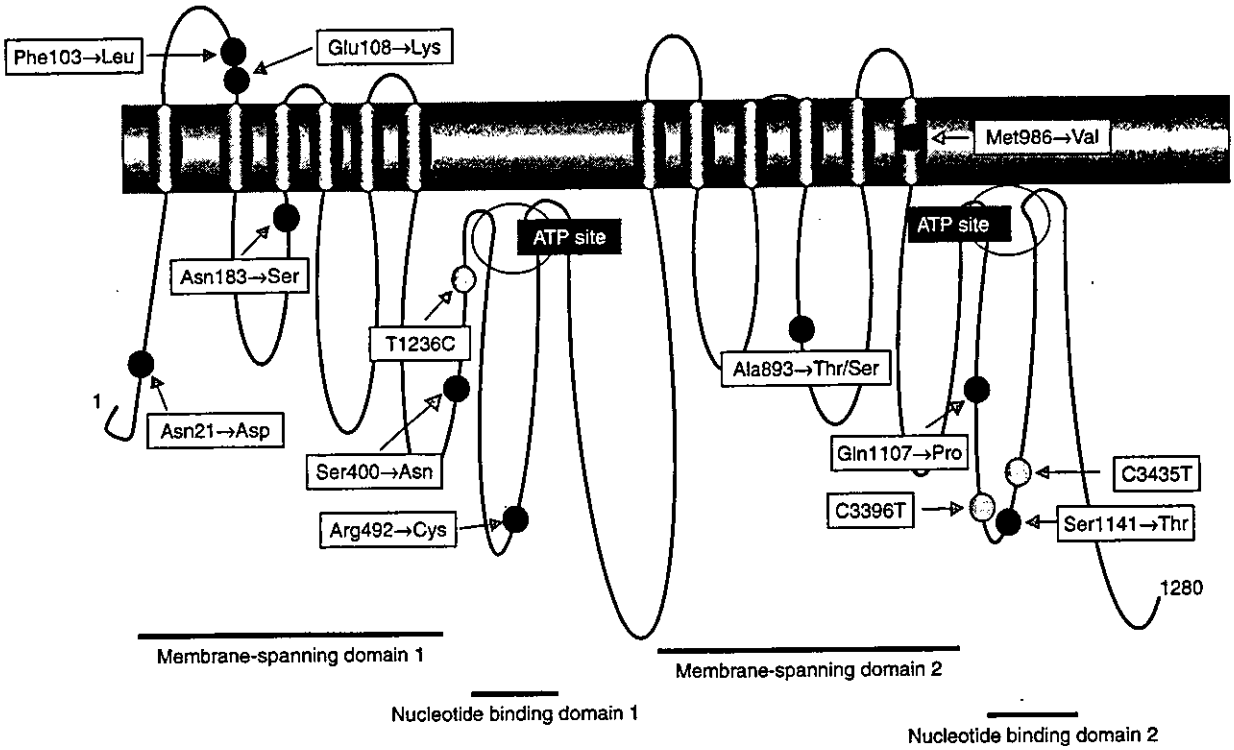

Fig. 1. Schematic representation of P-glycoprotein secondary structure, with known synonymous and non-synonymous nucleotide substitutions. Non-synonymous substitutions are indicated by the corresponding amino acid changes. **ATP** = adenosine triphosphate.

1.3 Gene Structure and Sites of Polymorphism

The *MDR1* gene is located on chromosome 7 at q^{21},[48] with 28 exons encoding a protein of 1280 amino acids. The presence of a highly conserved ATP-binding site in two homologous halves as well as the linker region makes this protein a member of the so-called ATP-Binding Cassette (ABC) transporter superfamily.

Mickley et al.[49] reported the first evidence of the presence of naturally-occurring polymorphisms in the human *MDR1* gene. They found two SNPs in exon 21 (G2677T) and 24 (G2995A). Subsequently, screening of the entire *MDR1* gene has been undertaken by some laboratories[9,10,50-52] and, to date, numerous SNPs have been identified (figure 1 and tables I, II and III). Systematic analysis of the entire *MDR1* gene including the promoter region indicated that at least one SNP existed in all DNA samples obtained from healthy Japanese and Caucasian individuals.[9,10] All of the mutations reported for *MDR1* are SNPs, and some of these are associated with amino acid substitutions. Whole gene deletion, single nucleotide deletion or changes for aberrant transcription have not been reported. Among non-synonymous mutations, the G→T (G2677T) and G→A (G2677A) transversions at position 2677 in exon 21, which is located on the intracellular side of P-glycoprotein after transmembrane region 10, are associated with an amino acid change from Ala at codon 893 (Ala893) to Ser and Thr, respectively. In contrast, the C→T transversion at 3435 in exon 26 (C3435T) does not change the amino acid sequence. Interestingly, G2677T/A and C3435T are closely linked; >90% of Japanese,[10] 62% of European American[52] and 80% of Caucasian German[53] individuals have these two SNPs simultaneously. The association was also observed in paediatric heart transplant patients.[5] As regards the linkage disequilibrium, Tang et al.[54] recently performed a haplotype analysis of the *MDR1* gene in three ethnic Asian groups (Chinese, Malays and Indians) by examining ten intragenic SNPs. Interestingly, three SNPs, located on exons 12 (C1236T), 21 (G2677T/

Table I. Allelic frequencies of multidrug resistance 1 gene (*MDR1*) variants among different ethnic populations and patient groups. Locations of the intronic single nucleotide polymorphisms (SNPs) are as follows: G–35C (intron 4); G–25T (intron 4); C+139T (intron 6); C+145T (intron 6); C+44T (intron 12); T–76A (intron 16); A+137G (intron 17). Amino acid substitutions are indicated with the single-letter code. [Note: Tables II and III are a continuation of table I]

Ethnic background	Subject	A–41aG	C–145G	T–129C	C–4T	G–1A	A61G (21N→D)	G–35C	G–25T	T307C (103F→L)
		A/G	C/G	T/C	C/T	G/A	A/G	G/C	G/T	T/C
Asian/Oceanian										
Japanese (n = 100)[10]	Placental cDNA	0.91/0.09	0.97/0.03	0.94/0.06	1.00/0	1.00/0	1.00/0			1.00/0
Japanese (n = 114)[55]	Volunteers									
Chinese (n = 92–104)[54]	Neonates (umbilical cords)			0.98/0.02		1.00/0	1.00/0			
Chinese (n = 96–132)[56,57]	Volunteers/blood donors									
Filipino (n = 60)[56]	Volunteers/blood donors									
Indian (n = 61–68)[54]	Neonates (umbilical cords)			0.98/0.02		1.00/0	1.00/0			
Indian (n = 87)[57]	Volunteers									
Malay (n = 80–93)[54]	Neonates (umbilical cords)			0.96/0.04		1.00/0	1.00/0			
Malay (n = 92)[57]	Volunteers									
Southwest (n = 89)[58]	Volunteers/blood donors									
Middle East										
Saudi (n = 96)[56]	Volunteers/blood donors									
African										
Ghanaian (n = 172–206)[56,58]	Volunteers/blood donors									
Kenyan (n = 80)[56]	Volunteers/blood donors									
Sudanese (n = 51)[56]	Volunteers/blood donors									
African American										
African American (n = 23–88)[52,56]	Volunteers/blood donors				0.96/0.04	1.00/0	1.00/0			
Caucasian										
Caucasian German (n = 461)[51]	Volunteers					0.91/0.09	0.89/0.11			
Caucasian UK (n = 190)[56]	Volunteers/blood donors									
Portuguese (n = 100)[56]	Volunteers/blood donors									
Italian (n = 106)[59]	Control for Parkinson's disease			0.97/0.03						
Caucasian (n = 537)[3]	Control for renal tumours									

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Table I. Contd

Ethnic background	Subject	A–41aG	C–145G	T–129C	C–4T	G–1A	A61G (21N→D)	G–35C	G–25T	T307C (103F→L)
		A/G	C/G	T/C	C/T	G/A	A/G	G/C	G/T	T/C
Caucasian (n = 37–188)[9,52]	Volunteers			0.94/0.06	1.00/0	(0.94–0.96)/ (0.04–0.06)	(0.90–0.91)/ (0.09–0.10)	0.99/0.01	0.84/0.16	0.99/0.01
Specific patient groups										
Japanese (n = 17)[6]	LDLT recipients	0.79/0.21	0.94/0.06	0.79/0.21	1.00/0	1.00/0	1.00/0			1.00/0
Japanese (n = 68–69)[7]	LDLT recipients					1.00/0	1.00/0			
Italian (n = 25)[59]	Early onset Parkinson's disease			0.98/0.02						
Italian (n = 70)[59]	Late onset Parkinson's disease			0.99/0.01						
Caucasian New Zealand (n = 160)[11]	Depressed patients									
Caucasian (n = 124)[60]	Renal transplant recipients									
Caucasian (n = 212)[3]	Renal epithelial tumours									

LDLT = living donor liver transplantation.

A) and 26 (C3435T), could account for most of the haplotypes, similar to those reported in European- and African-Americans[52] and Japanese[10] populations. They also found inter-ethnic differences in the *MDR1* haplotypes.

On the basis of the assumption that one SNP occurs every 1000 base pairs in a DNA sequence, further novel polymorphisms may be expected. Indeed, we recently observed a novel non-synonymous mutation (Glu108Lys) in Japanese subjects.[61]

2. Allelic Frequencies of MDR1 Variants in Different Ethnic/Racial Populations and Specific Patient Groups

2.1 Ethnic/Racial Distribution

The allelic frequency distributions of SNPs in the *MDR1* gene have been reported in various racial populations. Tables I, II and III show the allelic frequencies for *MDR1* variants reported from different ethnic populations and specific patient groups. Among distinct *MDR1* variants, C3435T has been detected in all ethnic populations studied so far (i.e. Caucasians, African Americans and Asians), albeit with considerable ethnic variation in frequencies: the frequency of the C3435 allele has been reported as 43–54% in Caucasians, 34–63% in Asians and 73–90% in Africans. The incidence of C/T and C/C3435 genotypes in the African is much higher than those in other racial populations. At SNP exon 21 G2677T/A, Caucasians (57%) and Japanese (43%) share a similar frequency of the G2677 allele; however, there is a trend toward a lower frequency in the Indian population (34%). An A61G variant is observed only in Caucasians, suggesting it may be ethnic-specific.

Ethnic differences in P-glycoprotein activity have not been widely studied. However, inter-ethnic differences in the distribution of the *MDR1* variants are a possible cause of the inter-ethnic differences in the pharmacokinetics of P-glycoprotein substrate drugs illustrated in the following examples. The oral bioavailability of ciclosporin was significantly lower in Blacks (mean 30.9%) than Whites (39.6%) or Hispanics (42.1%), with no differences in clearance

Table II. [Note: Tables II and III are a continuation of table I]

Ethnic background	Subject	C+139T C/T	C+145T C/T	A548G (183N→S) A/G	G1199A (400S→N) G/A	T1236C T/C	C+44T C/T	C1474T (492R→C) C/T	T–76A T/A	A+137G A/G
Asian/Oceanian										
Japanese (n = 100)[10]	Placental cDNA			1.00/0	1.00/0	0.65/0.35		1.00/0		
Japanese (n = 114)[55]	Volunteers									
Chinese (n = 92–104)[54]	Neonates (umbilical cords)					0.69/0.31				
Chinese (n = 96–132)[56,57]	Volunteers/blood donors					0.72/0.28				
Filipino (n = 60)[56]	Volunteers/blood donors									
Indian (n = 61–68)[54]	Neonates (umbilical cords)					0.61/0.39				
Indian (n = 87)[57]	Volunteers					0.67/0.33				
Malay (n = 80–93)[54]	Neonates (umbilical cords)					0.69/0.31				
Malay (n = 92)[57]	Volunteers					0.66/0.34				
Southwest (n = 89)[56]	Volunteers/blood donors									
Middle East										
Saudi (n = 96)[56]	Volunteers/blood donors									
African										
Ghanaian (n = 172–206)[56,58]	Volunteers/blood donors									
Kenyan (n = 80)[56]	Volunteers/blood donors									
Sudanese (n = 51)[56]	Volunteers/blood donors									
African American										
African American (n = 23–88)[52,56]	Volunteers/blood donors			1.00/0	1.00/0			1.00/0		
Caucasian										
Caucasian German (n = 461)[51]	Volunteers	0.63/0.37			0.94/0.06	0.41/0.59	0.95/0.05		0.54/0.46	
Caucasian UK (n = 190)[56]	Volunteers/blood donors									
Portuguese (n = 100)[56]	Volunteers/blood donors									
Italian (n = 106)[59]	Control for Parkinson's disease									
Caucasian (n = 537)[3]	Control for renal tumours									
Caucasian (n = 37–188)[9,52]	Volunteers	0.59/0.41	0.99/0.01	0.99/0.01	(0.94–0.95)/ (0.05–0.06)	0.62/0.38	0.94/0.06	0.99/0.01	0.55/0.45	0.99/0.01

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Table II. Contd

Ethnic background	Subject	C+139T	C+145T	A548G (183N→S)	G1199A (400S→N)	T1236C	C+44T	C1474T (492R→C)	T−76A	A+137G
		C/T	C/T	A/G	G/A	T/C	C/T	C/T	T/A	A/G
Specific patient groups										
Japanese (n = 17)[6]	LDLT recipients			1.00/0	1.00/0	0.62/0.38		1.00/0		1.00/0
Japanese (n = 68–69)[7]	LDLT recipients	0.37/0.63			1.00/0	0.65/0.35	1.00/0		0.68/0.32	
Italian (n = 25)[59]	Early onset Parkinson's disease									
Italian (n = 70)[59]	Late onset Parkinson's disease									
Caucasian New Zealand (n = 160)[11]	Depressed patients									
Caucasian (n = 124)[60]	Renal transplant recipients									
Caucasian (n = 212)[3]	Renal epithelial tumours									

LDLT = living donor liver transplantation.

or the volume of distribution at steady state.[62] Since intestinal P-glycoprotein activity is the major determinant of the bioavailability of ciclosporin, patients with a higher level of intestinal P-glycoprotein may have lower bioavailability and whole blood concentrations, and vice versa.[63] Inter-ethnic differences in bioavailability and interaction profiles of tacrolimus have also been reported.[64-66] As described above, the frequency of the C/C3435 genotype is higher in Africans than in other racial populations. Recently, Schaeffeler and colleagues[58] speculated that the reason for the lower bioavailability of ciclosporin in Africans than in Caucasians is an increased frequency of the C/C genotype in Africans, based on their experimental findings that individuals homozygous for the T3435 allele have on average substantially lower intestinal P-glycoprotein levels than those homozygous for the C3435 allele.[9] The high frequency of the C3435 allele in Africans may also explain the high incidence of resistance and more aggressive tumours, such as breast cancer, in individuals of African origin.[56,67] These data highlight the need to consider inter-ethnic variability before extrapolating pharmacokinetic data, including drug interaction profiles, obtained in one ethnic group to another.

2.2 Specific Patient Groups

Since P-glycoprotein may have a role as a protective barrier against a wide variety of substrates as well as the environment, the allelic frequency of *MDR1* variants is expected to differ among patients with various diseases, as has been seen among different racial populations.

Based on the functional role of P-glycoprotein as a neuroprotective barrier, altered P-glycoprotein expression or function in brain capillaries, partially due to polymorphism of the *MDR1* gene, could affect the uptake of neurotoxic xenobiotics, thereby modulating interindividual susceptibility to neurological disorders such as Parkinson's disease. To test this hypothesis, Furuno et al.[59] compared allelic frequencies of three mutations (C3435T, G2677T/A, and T−129C) between 95 Italian patients with Parkinson's disease and 106 non-Parkin-

Table III. [Note: Table III is a continuation of tables I and II]

Ethnic background	Subject	C2650T C/T	G2677T/A (893A→S,T) G/T/A	A2956G (986M→V) A/G	A3320C (1107Q→P) A/C	C3396T C/T	T3421A (1141S→T) T/A	C3435T C/T	G4030C (3'end) G/C	A4036G (3'end) A/G
Asian/Oceanian										
Japanese (n = 100)[10]	Placental cDNA		0.43/0.39/0.18	0.99/0.01	1.00/0	1.00/0	1.00/0	0.58/0.42	0.99/0.01	0.69/0.31
Japanese (n = 114)[55]	Volunteers							0.61/0.39		
Chinese (n = 92–104)[54]	Neonates (umbilical cords)		0.51/0.44/0.06					0.60/0.40		
Chinese (n = 96–132)[56,57]	Volunteers/blood donors		0.38/0.50/0.13					(0.47–0.53)/(0.47–0.53)		
Filipino (n = 60)[56]	Volunteers/blood donors							0.59/0.41		
Indian (n = 61–68)[54]	Neonates (umbilical cords)		0.34/0.62/0.04					0.40/0.60		
Indian (n = 87)[57]	Volunteers		0.33/0.60/0.07					0.37/0.63		
Malay (n = 80–93)[54]	Neonates (umbilical cords)		0.57/0.36/0.07					0.63/0.37		
Malay (n = 92)[57]	Volunteers		0.53/0.44/0.03					0.49/0.51		
Southwest (n = 89)[56]	Volunteers/blood donors							0.34/0.66		
Middle East										
Saudi (n = 96)[56]	Volunteers/blood donors							0.55/0.45		
African										
Ghanaian (n = 172–206)[56,58]	Volunteers/blood donors							(0.83–0.90)/(0.10–0.17)		
Kenyan (n = 80)[56]	Volunteers/blood donors							0.83/0.17		
Sudanese (n = 51)[56]	Volunteers/blood donors							0.73/0.27		
African American										
African American (n = 23–88)[52,56]	Volunteers/blood donors	1.00/0					0.96/0.04	0.84/0.16		
Caucasian										
Caucasian German (n = 461)[51]	Volunteers		0.57/0.42/0.02		0.99/0.01			0.46/0.54		
Caucasian UK (n = 190)[56]	Volunteers/blood donors							0.48/0.52		
Portuguese (n = 100)[56]	Volunteers/blood donors							0.43/0.57		
Italian (n = 106)[59]	Control for Parkinson's disease		0.56/0.41/0.03					0.54/0.46		
Caucasian (n = 537)[3]	Control for renal tumours							0.50/0.50		

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Table III. Contd

Ethnic background	Subject	C2650T (C/T)	G2677T/A (893A→S,T) (G/T/A)	A2956G (986M→V) (A/G)	A3320C (1107Q→P) (A/C)	C3396T (C/T)	T3421A (1141S→T) (T/A)	C3435T (C/T)	G4030C (3'end) (G/C)	A4036G (3'end) (A/G)
Caucasian (n = 37–188)[9,52]	Volunteers	0.97/0.03				0.99/0.01	1.00/0	0.52/0.48		
Specific patient groups										
Japanese (n = 17)[6]	LDLT recipients	1.00/0	0.59/0.32/0.09	1.00/0	1.00/0	1.00/0	1.00/0	0.53/0.47	1.00/0	0.62/0.38
Japanese (n = 68–69)[7]	LDLT recipients		0.45/0.44/0.12					0.56/0.44		
Italian (n = 25)[59]	Early onset Parkinson's disease		0.50/0.48/0.02					0.42/0.58		
Italian (n = 70)[59]	Late onset Parkinson's disease		0.53/0.44/0.04					0.49/0.51		
Caucasian New Zealand (n = 160)[11]	Depressed patients							0.53/0.47		
Caucasian (n = 124)[60]	Renal transplant recipients							0.46/0.55		
Caucasian (n = 212)[3]	Renal epithelial tumours							0.42/0.58		

LDLT = living donor liver transplantation.

son's disease, non-medicated controls. They divided the 95 patients into two groups according to the age at onset of the disease; an early onset group (n = 25, onset age ≤45 years) and a late onset group (n = 70, onset age >45 years). Although the differences did not reach statistical significance, the frequencies of T3435 and T2677 were highest in the early onset Parkinson's disease group, second highest in the late onset group, and lowest in the control group. Siegsmund et al.[3] also compared the frequencies of allelotypes and genotypes of C3435T between patients with renal epithelial tumours and healthy control subjects. They observed a significant disequilibrium with respect to a higher T3435 prevalence in patients.

Ulcerative colitis in humans has histological features resembling a form of colitis developed in mdr1 knockout mice.[68,69] Since ulcerative colitis in mdr1a$^{-/-}$ mice can be prevented by antibiotics and since the mdr1a$^{-/-}$ mice are immunologically normal, functional defects of the intestinal epithelial barrier due to the lack of P-glycoprotein expression are possible reasons for the pathogenesis of colitis.[68] Schwab et al.[70] tested whether C3435T polymorphism, which is associated with a lower intestinal P-glycoprotein expression,[9] predisposes one to the development of ulcerative colitis by comparing allele frequencies and genotype distribution of C3435T between 149 patients with ulcerative colitis and sex-matched healthy controls. They found significantly increased frequencies of the T3435 allele and homozygotes for the T allele in patients with ulcerative colitis compared with controls, and concluded that impairment of barrier function in T3435 subjects could render this genotype more susceptible to the development of ulcerative colitis.

Some SNPs have been identified in the 5′-untranslated region (5′-UTR) of the *MDR1* gene. Interestingly, these mutations are frequently observed in patients with haematological malignancies[71] or osteosarcomas.[72] Several studies have shown that certain specific nucleotide sequences, including SP1 and AP1 binding sites, are important in controlling the level of *MDR1* expression.[73-75] Rund et al.[71] have identified a T→C transversion at position +8

(relative to the transcription start site) localising near (one base pair downstream) the initiator sequence (–6 to +11) required for proper transcription initiation. They also found that this mutation was present in patients with acute myeloid leukaemia (AML) and chronic lymphocytic leukaemia at three times the frequency that it was found in normal subjects. A T→C transversion at position +8 is identical to the T–129C mutation.

3. Polymorphisms and Expression of P-Glycoprotein in Various Human Tissues

P-glycoprotein levels are known to show significant interindividual variability, with 2- to 8-fold variations found in intestinal biopsies from patients and healthy volunteers.[63] Among various mutations in the *MDR1* gene, certain variants have recently been reported to be associated with a change in expression levels of P-glycoprotein in human tissues. Table IV summarises the effect of different *MDR1* genotypes on the expression level of P-glycoprotein in various human tissues, together with *in vivo* pharmacokinetic and pharmacodynamic changes of P-glycoprotein substrates.

Using an immunochemical approach to quantify P-glycoprotein content, Hoffmeyer et al.[9] first reported that C3435T was associated with a significantly reduced intestinal P-glycoprotein content in subjects with the T/T genotype (i.e. the homozygote for the mutant T allele) in comparison with subjects homozygous for the C allele. In the human placenta, Tanabe et al.[10] found that individuals having the C–129 allele showed significantly lower P-glycoprotein levels than those having the T–129 allele, while such a significant correlation was not observed for the C3435T allele, as measured by Western blot analysis. T–129C is located seven basepairs downstream from the transcription initiation site (*A*$^{+1}$TTCGAG*T*AG). In their study, G2677T/A also correlated with the level of P-glycoprotein expression, but not significantly. In the human heart (left ventricular samples), a reduced expression, but not statistically significant, in samples containing the T/T genotype of the C3435T mutation was reported.[84]

P-glycoprotein and *MDR1* mRNA are also expressed in various leucocyte lineages with the highest level of expression in CD56+ natural killer cells, followed by CD4+, CD15+, CD19+ and CD14+ cells.[85,86] Hitzl et al.[82] studied *MDR1* mRNA expression in leucocytes of healthy individuals with different genotypes at position 3435 of the human *MDR1* gene and found that the *MDR1* mRNA level was lowest in the T/T population, intermediate in heterozygous subjects and highest in the C/C group. Reduced P-glycoprotein activity was also found in natural killer cells from healthy individuals having the T/T genotype at position 3435 in comparison with subjects homozygous for the C allele.[79,82] Fellay et al.[2] have quantified by real-time PCR *MDR1* transcripts in peripheral blood mononuclear cells from 59 HIV-1-infected patients, and showed an association between the C3435T T/T genotype and a lower level of *MDR1* expression (arbitrary unit, median 1.87) compared with the C/T genotype (2.36) and C/C genotype (2.79). They also confirmed this association by fluorescence-activated cell-sorter analysis of P-glycoprotein expression in peripheral blood mononuclear cells; the correlation coefficient between transcript and protein expression was $r = 0.58$ ($p < 0.0006$). Siegsmund et al.[3] have also indicated an association of the T3435 allele and lower P-glycoprotein expression in non-cancerous renal tissues.

In contrast to these observations, Nakamura et al.[8] have indicated that the T3435 allele was associated with increased *MDR1* mRNA expression in human duodenal samples. Similarly, Illmer et al.[4] showed a lower *MDR1* expression in blast samples obtained from patients with AML whose *MDR1* genotypes were homozygous for the wild-type allele at all three gene loci investigated (i.e. exons 12, 21, and 26). However, Goto et al.[7] and Siegmund et al.[53] have recently reported that intestinal or duodenal *MDR1* mRNA levels were not influenced by C3435T polymorphism. The latter authors have also determined immunoreactive duodenal P-glycoprotein expression and observed the same results for

Table IV. Effects of genetic polymorphism of *MDR1* on the expression of P-glycoprotein in human tissues and *in vivo* pharmacokinetics and pharmacodynamics of P-glycoprotein substrates

Study	Polymorphism	Substrate	Subject/material	Pharmacodynamic-pharmacokinetic outcome	*In vitro* efflux	Expression level
Hoffmeyer et al.[9]	C3435T	Digoxin	HV/human duodenum	T/T > C/C (C_{max})		C/C > C/T > T/T (protein concentration)
Kerb et al.[76]	C3435T	Phenytoin	HV	T/T > C/C (plasma concentration)		
Kurata et al.[11]	C3435T, G2677T	Digoxin	HV	M/M > W/M > W/W in both loci (F). W/W > W/M > M/M in both loci (CL_R, CL_{sec})		
Min and Ellingrod[77]	C3435T	Ciclosporin	HV	(T/T + C/T) > C/C (C_{max} and AUC, but not significantly different)		
Johne et al.[78]	C3435T, G2677T	Digoxin	HV	TT > TC > CC (C3435T, AUC and C_{max}). Haplotype 12 > haplotype 11 (AUC, C_{max})		
von Ahsen et al.[60]	C3435T	Ciclosporin	RTR	C/C = C/T = T/T (dose-adjusted C_{min} and rejection incidence)		
Chowbay et al.[57]	C1236T, G2677T, C3435T	Ciclosporin	HTR	TT-TT-TT > CT-GT-CT > CC-GG-CC. T-T-T > C-G-C haplotype. (AUC, C_{max}, C_{min})		
Drescher et al.[79]	C3435T	Fexofenadine, rhodamine 123	HV/CD56+ cell	C/C = T/T (AUC)	C/C > C/T > T/T	

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Table IV. Contd

Study	Polymorphism	Substrate	Subject/material	Pharmacodynamic-pharmacokinetic outcome	*In vitro* efflux	Expression level
Goto et al.[7]	C+139T, C1236T, T–76A, G2677T/A, C3435T	Tacrolimus	LDLTR/human intestine	No significant effect of SNPs on tacrolimus concentration/dose ratio		No effect (mRNA level)
Siegmund et al.[53]	C3435T, G2677T/A	Talinolol	HV/human duodenum	W/W = W/M = M/M in both loci (AUC)		No significant effect (protein and mRNA levels)
Goh et al.[80]	C3435T	Docetaxel	Cancer patients	C/C = C/T = T/T (CL)		
Kim et al.[52]	G2677T *2 allele	Fexofenadine, digoxin	HV/NIH-3T3 GP+E86 cells	*1/*1 > *1/*2 > *2/*2 (AUC)	*MDR1*-Ser893 > *MDR1*-Ala893	
Sakaeda et al.[55]	C3435T	Digoxin	HV	C/C > subjects with the T allele (i.e. C/T and T/T) [AUC_4]		
Roberts et al.[1]	C3435T	Nortriptyline	Depressed patients	T/T > C/T > C/C (frequency of drug-induced postural hypotension)		
Fellay et al.[2]	C3435T	Nelfinavir, efavirenz	HIV-1-infected patients/PBMC	T/T > C/T > C/C (CD4+ cell count and recovery of naive CD4+ cells). C/C > C/T > T/T (C_{min})		C/C > C/T > T/T (protein and mRNA levels)
Zheng et al.[5]	C3435T, G2677T	Corticosteroids	Pediatric heart transplant recipients	W/W > W/M > M/M (duration of corticosteroid therapy)		
Illmer et al.[4]	C1236T, G2677T, C3435T		AML patients/blast samples	W/M > M/M > W/M in all 3 loci (overall survival). W/W > M/M > W/M in all 3 loci (probability of relapse)		C/T > T/T > C/C (1236), G/T > T/T > G/G (2677), C/T > T/T > C/C (3435) [mRNA level]

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Table IV. Contd

Study	Polymorphism	Substrate	Subject/material	Pharmacodynamic-pharmacokinetic outcome	*In vitro* efflux	Expression level
Potocnik et al.[81]	T-129C, IVS1-81delG		Tumour samples (colorectal adenocarcinoma)	Association with lymphoid infiltration		W > M (protein level)
Yamauchi et al.[6]	G2677T/A	Tacrolimus	LDLTR	Positive predictor of drug-induced neurotoxicity		
Siegsmund et al.[3]	C3435T		Healthy control and non-CCRCC patients/ non-cancerous renal tissues	T allele as a risk factor		C/C > T/T (protein level)
Schwab et al.[70]	C3435T		Inflammatory bowel disease (Crohn's disease and ulcerative colitis) patients	T > C allele, T/T genotype > other types (allelic frequency in patients with ulcerative colitis)		
Hitzl et al.[82]	C3435T	Rhodamine 123	CD56+ cell		C/C > C/T > T/T	C/C > C/T > T/T (mRNA level)
Calado et al.[83]	T–129C, G2677T, C3435T	Rhodamine 123	CD34+ cells		W/W = W/M = M/M	
Tanabe et al.[10]	T–129C, G2677T/A, C3435T		Human placenta			T/T > T/C (–129), W/W > W/M > M/M (2677), C/C = C/T = T/T (3435) [protein level]
Nakamura et al.[8]	C3435T		Human duodenum			T/T > C/T > C/C (mRNA level)
Meissner et al.[84]	C3435T		Human heart			Reduced in T/T samples (protein and mRNA levels)

AML = acute myeloid leukaemia; **AUC** = area under the plasma concentration-time curve; **CCRCC** = clear cell renal cell carcinoma; **CL** = systemic clearance; $\mathbf{CL_R}$ = renal clearance; $\mathbf{CL_{sec}}$ = renal secretory clearance; $\mathbf{C_{max}}$ = peak plasma concentration; $\mathbf{C_{min}}$ = trough plasma concentration; **F** = bioavailability; **HTR** = heart transplant recipients; **HV** = healthy volunteers; **LDLTR** = living donor liver transplantation recipients; **M** = mutant allele; **mRNA** = messenger RNA; **PBMC** = peripheral blood mononuclear cells; **RTR** = renal transplant recipients; **SNP** = single nucleotide polymorphism; **W** = wild-type allele.

mRNA levels.[53] Thus, the collected evidence indicates that the contribution of the *MDR1* variants to expression (both at protein and mRNA levels) is still controversial.

4. Impact of Polymorphisms on Pharmacotherapy

4.1 Pharmacokinetic Consequences

To date, polymorphisms of the *MDR1* gene that alter *in vivo* transport activity have been focused on: the silent mutation in exon 26 (C3435T) and the non-synonymous mutation in exon 21 (G2677T/A).

Subjects with the T/T genotype at position 3435 had higher steady-state plasma concentrations after oral administration of digoxin in comparison with the C/C subjects.[9,78] Similar results were observed by Kurata et al.,[11] who showed that the mean absolute bioavailability (estimated from oral and intravenous administrations) of digoxin was significantly higher in 2677TT/3435TT subjects (homozygotes for thymine at both positions 2677 and 3435) than 2677GG/3435CC subjects in a gene dose-dependent manner, in that maximum bioavailability was observed in homozygotes for the mutant allele (mean, 87.1%) > heterozygotes (80.9%) > homozygotes for the wild-type allele (67.6%). They also indicated that the renal clearance of digoxin was almost 32% lower in 2677TT/3435TT subjects than in 2677GG/3435CC subjects, with 2677GT/3435CT subjects having an intermediate value. These results suggest that reductions in the intestinal secretion of digoxin into the gut lumen and renal excretion into the urine occur simultaneously in subjects with SNPs.

The histamine H_1 receptor antagonist fexofenadine, which is used for the treatment of seasonal allergic rhinitis and chronic idiopathic urticaria, is also a P-glycoprotein substrate. Kim et al.[52] demonstrated that the *2 allele was associated with differences in fexofenadine concentrations, with the area under the plasma concentration-time curve (AUC) being almost 40% greater in *1/*1 subjects compared with *2/*2 subjects, with *1/*2 heterozygotes having an intermediate value, suggesting enhanced *in vivo* P-glycoprotein activity among subjects with the *MDR1* *2 allele. In their study, the *2 allele was defined as a haplotype in which three SNPs at different polymorphic sites (T1236, T2677 and T3435) occurred simultaneously. However, they reported that fexofenadine is also a good substrate for organic anion transporting polypeptide (OATP).[87,88] In contrast, Drescher et al.[79] did not find any significant differences in fexofenadine disposition between subjects homozygous for the C allele and T allele at position 3435. Siegmund et al.[53] also did not find a significant influence of three *MDR1* variants (C3435T and G2677T/A) on talinolol disposition.

Because of a lack of metabolic biotransformation, digoxin is often used as a probe drug for pharmacogenetic testing (i.e. *in vivo* phenotype-genotype relationship studies) of the *MDR1* gene polymorphism. Sakaeda et al.[55] studied the relationship between the *MDR1* genotype and the pharmacokinetics of digoxin after a single oral administration in healthy subjects. They found that the AUC_4 of digoxin was significantly lower in subjects with the T/T3435 genotype than in C/C3435 subjects. Their observations are in line with a finding by Kim et al.,[52] but in contrast to the findings of Hoffmeyer et al.[9] and Kurata et al.[11]

One study has investigated the relationship between *MDR1* polymorphisms and the pharmacokinetics of oral ciclosporin in healthy subjects. Although the peak concentration and AUC of ciclosporin in the C/T3435 and T/T3435 subjects were 15% and 22% larger than those in C/C3435 subjects, differences in these values did not reach statistical significance.[77]

Functional consequences of *MDR1* polymorphism have also been investigated in two *in vitro* studies. In *in vitro* experiments conducted by Kim et al.[52] with cultured cells expressing MDR1-Ala893 (G2677) and MDR1-Ser893 (T2677) revealed that the Ser893 variant transporter resulted in a 47% lower intracellular digoxin concentration than did the Ala893 variant. Based on these results, they concluded that Ser893 variant-containing cells exhibit enhanced efflux characteristics compared with

those cells in which Ala893 was expressed. Kimchi-Sarfaty et al.[89] also investigated functional consequences of *MDR1* polymorphisms (Asn21Asp, Phe103Leu, Ser400Asn, Ala893Ser, and Ala998Thr) using a vaccinia virus-based transient expression system by two approaches; cell surface localisation and transport function. In contrast to the findings by Kim et al.,[52] they found that cell surface expression and transport capabilities were not substantially affected by any of the polymorphisms tested.

Taking all these findings into consideration, published observations, even when made using the same probe drug and even among the same racial group, are conflicting. The question arises as to why the contribution of C3435T and/or G2677T/A mutations to the pharmacokinetics of digoxin and fexofenadine differs among reports. Discussing possible reasons for this discrepancy will be useful for future studies of the involvement of polymorphisms of *MDR1*, as well as other drug transporters, in *in vivo* transport activity.

Both digoxin and fexofenadine are transported across cells by the OATPs, which are also expressed in various human tissues such as liver, intestine, and kidney. Although the intestinal transport mechanisms responsible for fexofenadine uptake have not yet been defined, fexofenadine has been shown to be a substrate of human OATP-A.[87,88] Similarly, digoxin was reported to be a substrate of liver-specific OATP8, another member of the OATP family.[90] In addition to the *MDR1* gene, the *OATP-C* and *OATP8* genes exhibit genetic variability.[91,92] Although the effects of *OATP8* variants have not yet been elucidated, certain mutations in the *OATP-C* gene could alter the *in vivo* pharmacokinetics of a clinically used drug.[93] Thus, it is possible that other transport mechanisms apart from those involving P-glycoprotein contribute to the variations in digoxin and fexofenadine pharmacokinetics in humans.

Grapefruit is known to inhibit the intestinal metabolism of numerous drugs, including terfenadine, saquinavir, ciclosporin, triazolam and nisoldipine, by inhibiting CYP3A enzymes, resulting in elevated drug bioavailability and then serum concentrations.[94] Recently, a new mechanism for the drug-grapefruit juice interaction has been reported; the bioavailability and serum concentrations of fexofenadine were reduced when grapefruit juice was taken.[95] In the intestine, P-glycoprotein and OATPs are located on the luminal membrane of the enterocyte, but they have opposite vectors for efflux back into the bowel and for uptake into the portal circulation, respectively.[95] Although the specific OATP member(s) responsible for the fexofenadine-grapefruit juice interaction has not been elucidated, OATP-B was recently identified as an OATP member localised at the apical membrane of intestinal epithelial cells in humans.[96] In addition to OATP-B, OATP-D and OATP-E are reported to be expressed in the human small intestine.[97] Since grapefruit juice is a more potent *in vitro* inhibitor of OATP than of P-glycoprotein activities,[95] the entry of fexofenadine from the intestinal lumen to blood may be inhibited by grapefruit juice, resulting in a reduced bioavailability. As grapefruit is able to inhibit P-glycoprotein-mediated drug efflux when present in sufficient concentrations,[95,98,99] the net bioavailability of fexofenadine will depend on the relative contribution of both efflux and uptake mechanisms. Indeed, in an interaction study with grapefruit juice, non-significant but moderate changes in digoxin pharmacokinetics were observed.[99] These findings clearly indicate that the intestinal transport of fexofenadine is determined by at least two drug transporters (P-glycoprotein and OATPs). Thus, multi-transporter-mediated drug transport with genetic variability needs to be considered when evaluating transport activities in the human body. It is clear that the identification of specific probe substrates and inhibitors for P-glycoprotein is required to elucidate the *in vivo* effect of *MDR1* polymorphisms on pharmacotherapy.

The possibility of the existence of functional unobserved SNPs cannot be excluded. As described in section 2, three SNPs, C1236T, G2677T/A and C3435T, have been haplotyped.[10,13,52,54] Recently, Tang et al.[54] demonstrated linkage disequilibrium between the different pairs of these SNPs and speculated on unobserved causal SNP(s) near position