

Figure 1. Single nucleotide polymorphisms (SNPs) in the 5'-flanking region of *CYP2C9* arranged in 9 distinct patterns: the allelic frequencies of the respective SNPs and their association with the coding region polymorphisms in white and Japanese patients. *CYP2C9*1*, *CYP2C9*2*, and *CYP2C9*3* represent Arg114/Ile359, Cys144/Ile359, and Arg144/Leu359 in the coding region, respectively. **P* < .05 and †*P* < .01 between white and Japanese patients; ‡ indicates a subject possessing *CYP2C9*1*11*. Del indicates deletion; Ins, insertion; HNF-1, hepatic nuclear factor-1; CAR, constitutive androstane receptor; PXR, pregnane X receptor; GR, glucocorticoid receptor; DR, direct repeat; and ND, not determined.

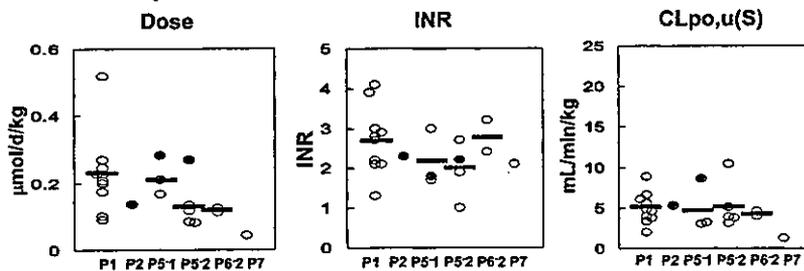
Results and discussion

There was no difference in the mean age of the white and Japanese populations (59 years vs 60 years). While there were significant (*P* < .01) differences in the mean body weight (84 kg vs 57 kg) and oral dosage of warfarin (16.0 μmol/d [5.3 mg/d as warfarin-Na] vs 9.2 μmol/d [3.2 mg/d as warfarin-K]), the mean body weight-normalized daily warfarin dosages (0.18 μmol/d/kg [0.060 mg/d/kg as warfarin-Na] vs 0.16 μmol/d/kg [0.056 mg/d/kg as warfarin-K]) were comparable between the populations. The mean body weight-normalized CL_{po,u} for S-warfarin in Japanese patients was approximately 2-fold greater (*P* < .01) than that in white patients (10.5 mL/min/kg vs 4.8 mL/min/kg). There were significant (*P* < .01) differences in the mean Cu of S-warfarin and international normalized ratio (INR) between white and Japanese patients: 15.6 nM (4.8 ng/mL) versus 6.5 nM (2.0 ng/mL) and 2.4 versus 1.6, respectively. These data are consistent with the recent studies demonstrating that Southeast Asians (Japanese and Chi-

nese) may require lower INR values (eg, 1.5-2.5) for anticoagulation than whites.^{10,11}

We found 13 SNPs, 1 insertion, and 2 deletions within the -2.1-kb 5'-flanking region of *CYP2C9* (Figure 1). Because all white and Japanese participants possessed the sequence -2080T, -2079A, -2078G, Ins T-839, Del T-828, and Del A-820, as compared with the reference sequence (GenBank accession no. L16877), we considered the above sequence to be the "wild-type" in our populations (ie, pattern 1). Because some of the SNPs occurred together, they were classified into 9 different patterns (ie, patterns 1, 2, 3, 4, 5-1, 5-2, 6-1, 6-2, and 7; Figure 1). Patterns 6-1 and 6-2 were in complete linkage disequilibrium either with *CYP2C9*1*3* or *CYP2C9*3*3* in both Japanese and white patients. Similarly, patterns 5-1 and 5-2 were linked either with *CYP2C9*1*2* or *CYP2C9*2*2* in white patients. A white patient possessing *CYP2C9*2*3* had all 8 SNPs for the patterns 5-1 and 6-1, and another such patient with pattern 2 was found to have *CYP2C9*11*. Patterns 5-1, 5-2, and 7 were found only in white patients. Our white patients appeared to have

A White patients



B Japanese patients

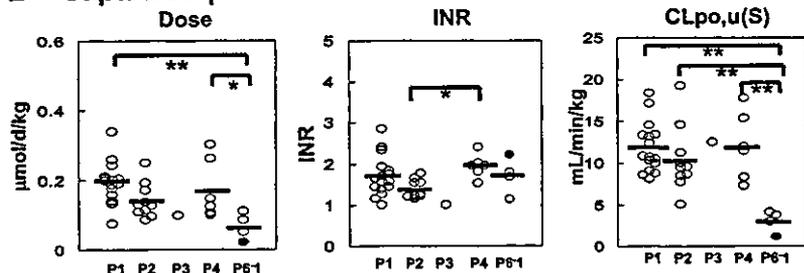


Figure 2. Body weight-normalized warfarin doses, INR, and unbound oral clearance for S-warfarin (CL_{po,u}[S]) in white and Japanese patients with different sequence patterns in the 5'-flanking region of the *CYP2C9* gene. Horizontal bars represent mean values of the respective groups. (A) White patients; (B) Japanese patients. Filled circles represent the patients with SNPs in the coding region of the *CYP2C9* gene: Japanese patients with homozygous *CYP2C9*2* or *CYP2C9*1*11* (see detailed combinations of the promoter and coding region SNPs in Figure 1). **P* < .05 and ***P* < .01 for comparisons between the patients with different sequence patterns in the 5'-flanking region of *CYP2C9* in Japanese patients.

CYP2C9 variants in a greater frequency than those previously reported.⁵ However, further studies with a large number of subjects will be required for comparing population differences in the frequencies of haplotype (combinations of the 5'-flanking and coding region SNPs) of *CYP2C9*.

Previous studies have shown putative binding sites for transcription enhancers in 5'-flanking region of *CYP2C9* (eg, TATA box, CAAT box, HepG2-specific factor-1 [HPF-1], C/EBP, AP-1, glucocorticoid response elements [GREs], Barbie box, hepatic nuclear factor-1 [HNF-1], and DR4 motif).¹²⁻¹⁵ Some of the promoter region SNPs are located at or around these transcription enhancer responsive elements (Figure 1), indicating that they may contribute to within and between population variability in *in vivo* *CYP2C9* activity.

Figure 2 shows doses of warfarin, INR values, and body weight-normalized CL_{po,u} for S-warfarin in the white and Japanese patients possessing the various patterns of SNPs in the *CYP2C9* promoter region. Japanese patients with pattern 6-1 had significantly ($P < .01$) reduced CL_{po,u} for S-warfarin as compared with those with pattern 1; the warfarin doses in these patients were also lower. This finding is in agreement with that of Shintani et al⁶ based on studies in Japanese patients with another *CYP2C9* substrate, phenytoin. However, because pattern 6-1 in the promoter region is in complete linkage disequilibrium with the functionally defective allele *CYP2C9*3*, it cannot be excluded that this observation might instead be attributable to this coding region variant rather than the promoter pattern 6-1. Because the promoter SNP patterns of 5-1 and 5-2 were completely linked with *CYP2C9*2* in the white patients, these promoter SNPs would have only a limited

effect on *in vivo* *CYP2C9* activity as shown for *CYP2C9*2* previously.⁵

When the population differences in the *CYP2C9* activity were compared between those having the wild-type alleles in both promoter and coding regions of *CYP2C9* gene (Figure 2, pattern 1), the Japanese patients had significantly ($P < .01$) greater CL_{po,u} for S-warfarin (11.8 mL/min/kg \pm 3.0 mL/min/kg) than the white patients (5.0 mL/min/kg \pm 1.9 mL/min/kg). These data strongly indicate that the population differences in the *in vivo* *CYP2C9* activity cannot be accounted for either the 13 SNPs in the promoter region or the 6 SNPs in the coding region. However, we cannot exclude the possibility that unidentified SNPs located more upstream in the 5'-flanking region than we currently investigated (eg, constitutive androstane receptor-responsive elements around -2.9 kb)¹⁶ might account for the *in vivo* findings. Further studies are, therefore, required to identify other possible factors (eg, diet and environmental factors) contributing to the population difference in the *CYP2C9* activity.

Acknowledgments

The authors thank Dr Joyce A Goldstein, National Institutes of Environmental Health Sciences, Research Triangle Park, NC, for the generous gift of the genomic DNA for *CYP2C9*6* and Miss Mamiko Shimoyama, Miss Keiko Miyazaki, Miss Masumi Matsumoto, and Miss Eri Yamamoto for their excellent technical assistance.

References

- O'Reilly RA. Studies on the optical enantiomorphs of warfarin in man. *Clin Pharmacol Ther*. 1974;16:348-354.
- Takahashi H, Wilkinson GR, Caraco Y, et al. Population differences in S-warfarin metabolism between *CYP2C9* genotype-matched Caucasian and Japanese patients. *Clin Pharmacol Ther*. 2003;73:253-263.
- Rettie AE, Korzekwa KR, Kunze KL, et al. Hydroxylation of warfarin by human cDNA-expressed cytochrome P-450: a role for P-450C9 in the etiology of (S)-warfarin-drug interactions. *Chem Res Toxicol*. 1992;5:54-59.
- Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee. Available at: <http://www.imm.ki.se/CYPalleles/>. Accessed January 29, 2004.
- Takahashi H, Echizen H. Pharmacogenetics of *CYP2C9* and interindividual variability in anticoagulant response to warfarin. *Pharmacogenomics J*. 2003;3:202-214.
- Shintani M, Ieiri I, Inoue K, et al. Genetic polymorphisms and functional characterization of the 5'-flanking region of the human *CYP2C9* gene: *in vitro* and *in vivo* studies. *Clin Pharmacol Ther*. 2001;70:175-182.
- Takahashi H, Kashima T, Kimura S, et al. Determination of unbound warfarin enantiomers in human plasma and 7-hydroxywarfarin in human urine by chiral stationary-phase liquid chromatography with ultraviolet or fluorescence and on-line circular dichroism detection. *J Chromatogr B*. 1997;701:71-80.
- Takahashi H, Kashima T, Nomizo Y, et al. Metabolism of warfarin enantiomers in Japanese patients with heart disease having different *CYP2C9* and *CYP2C19* genotypes. *Clin Pharmacol Ther*. 1998;63:519-528.
- Takahashi H, Kashima T, Nomoto S, et al. Comparisons between *in vitro* and *in vivo* metabolism of (S)-warfarin: catalytic activities of cDNA-expressed *CYP2C9*, its Leu₃₅₉ variant and their mixture versus unbound clearance in patients with the corresponding *CYP2C9* genotypes. *Pharmacogenetics*. 1998;8:365-373.
- Yamaguchi T. Optimal intensity of warfarin therapy for secondary prevention of stroke in patients with nonvalvular atrial fibrillation: a multicenter, prospective, randomized trial. *Stroke*. 2000;31:817-821.
- Leung A-YH, Chow H-CH, Kwong YL, et al. Genetic polymorphism in exon 4 of cytochrome P450 *CYP2C9* may be associated with warfarin sensitivity in Chinese patients. *Blood*. 2001;98:2584-2587.
- de Morais SMF, Schweikl H, Blaisdell J, Goldstein JA. Gene structure and upstream regulatory regions of human *CYP2C9* and *CYP2C18*. *Biochem Biophys Res Commun*. 1993;194:194-201.
- Ibeanu GC, Goldstein JA. Transcriptional regulation of human *CYP2C* genes: functional comparison of *CYP2C9* and *CYP2C18* promoter regions. *Biochemistry*. 1995;34:8028-8036.
- Gerbal-Chaloin S, Daujat M, Pascussi JM, Pichard-Garcia L, Vilarem MJ, Maurel P. Transcriptional regulation of *CYP2C9* gene: role of glucocorticoid receptor and constitutive androstane receptor. *J Biol Chem*. 2002;277:209-217.
- Pascussi JM, Gerbal-Chaloin S, Drocourt L, Maurel P, Vilarem MJ. The expression of *CYP2B6*, *CYP2C9* and *CYP3A4* genes: a tangle of networks of nuclear and steroid receptors. *Biochim Biophys Acta*. 2003;1619:243-253.
- Ferguson SS, Lecluyse EL, Negishi M, Goldstein JA. Regulation of human *CYP2C9* by the constitutive androstane receptor: discovery of a new distal binding site. *Mol Pharmacol*. 2002;62:737-746.

RACIAL VARIABILITY IN HAPLOTYPE FREQUENCIES OF *UGT1A1* AND GLUCURONIDATION ACTIVITY OF A NOVEL SINGLE NUCLEOTIDE POLYMORPHISM 686C>T (P229L) FOUND IN AN AFRICAN-AMERICAN

Nahoko Kaniwa, Kouichi Kurose, Hideto Jinno, Toshiko Tanaka-Kagawa, Yoshiro Saito, Mayumi Saeki, Jun-ichi Sawada, Masahiro Tohkin, and Ryuichi Hasegawa

Division of Medicinal Safety Science (N.K., K.K., M.T., R.H.), Division of Environmental Chemistry (H.J., T.T-K.), and Project Team for Pharmacogenetics (Y.S., M.S., J.S.), National Institute of Health Sciences, Tokyo, Japan

Received August 10, 2004; accepted November 23, 2004

ABSTRACT:

Ethnic differences in genetic polymorphisms in UDP-glucuronosyltransferase 1A1 (*UGT1A1*) were investigated among African-Americans, Caucasians, and Japanese using samples obtained from 150 individuals for each population. Genotyping of -3279T>G in the phenobarbital-responsive enhancer module, TA repeats in the TATA box, 211G>A (G71R) and 686C>A (P229Q) in exon 1, and three single nucleotide polymorphisms (SNPs) (1813C>T, 1941C>G, and 2042C>G) in the 3'-untranslated region in exon 5 was performed. Eight haplotypes of block 1 (exon 1 and its 5'-flanking region) harboring the first four variations were assigned to each individual. The dominant haplotype for African-Americans was *28b (-3279G;TA₇;211G;686C) (0.446), whereas that for the Japanese was *1a (-3279T;TA₆;211G;686C) (0.610). Frequencies of the two haplotypes *1a and *28b were comparable in Caucasians. Haplotype *6a (-3279T;TA₆;211A;686C) was characteristic of the Japanese, whereas haplotypes

*36b and *37b (-3279T;TA₅ and TA₆;211G;686C) were found mostly in African-Americans. Although the three SNPs in block 2 (exons 2-5) were in complete linkage in the Japanese, they were not completely linked in African-Americans or Caucasians. These differences in haplotype distribution patterns among the three populations suggest the possibility of ethnic differences in toxicity profiles of drugs detoxicated by *UGT1A1*. A novel SNP, 686C>T (P229L), was found in an African-American. The intrinsic clearance of 7-ethyl-10-hydroxycamptothecin (SN-38) by P229L *UGT1A1* expressed in COS-1 cells was about 3% of the wild type. The results of Western blotting and real-time reverse transcription-polymerase chain reaction suggest that the low glucuronidation activity of the variant was partly due to its low stability. The variation 686C>T may cause high toxicity during 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin (CPT-11) therapy or hyperbilirubinemia in patients.

UDP-glucuronosyltransferases catalyze the glucuronidation of various lipophilic endogenous and exogenous substances including drugs and environmental toxicants to produce glucuronides. UDP-glucuronosyltransferase 1A1 (*UGT1A1*) is a member of the *UGT1A* family and is exclusively involved in the metabolism of bilirubin (Tukey and Strassburg, 2000). It is also known to glucuronidate 7-ethyl-10-hydroxycamptothecin (SN-38), the active metabolite of an anticancer drug, irinotecan (CPT-11; 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin), to form inactive 7-ethyl-10-hydroxycamptothecin glucuronide (SN-38G) (Iyer et al., 1998; Hanioka et al., 2001). Since SN-38 is associated with severe diarrhea due to administration of CPT-11 (Araki et al., 1993), detoxication of SN-38 by *UGT1A1* could play a significant role in protecting against the severe side effects caused by CPT-11.

This study was supported in part by the program for the Promotion of Studies in Health Sciences of the Ministry of Health, Labor and Welfare and the program for the Promotion of Fundamental Studies in Health Sciences of the Pharmaceuticals and Medical Devices Agency of Japan.

Article, publication date, and citation information can be found at <http://dmd.aspetjournals.org>.
doi:10.1124/dmd.104.001800.

The wide interindividual variability in SN-38G formation in hepatic tissues is known and has been shown to correlate with *UGT1A1* genetic polymorphisms (Iyer et al., 1999). To date, a wide variety of allelic polymorphisms of human *UGT1A1* have been reported, most of which lead to a partial or complete deficiency of enzyme activity and are associated with diseases, such as the Criegler-Najjar (CN) syndrome types I and II or Gilbert's syndrome, with hyperbilirubinemia and jaundice as main symptoms (<http://som.flinders.edu.au/FUSA/ClinPharm/UGT/1A1alleles.html>; Mackenzie et al., 1997; Tukey and Strassburg, 2000). To understand these syndromes and their possible genetic causes, polymorphisms in the TATA box have been the most extensively studied. An inverse relationship between the number of TA repeats (five to eight) and transcriptional activity has been reported (Beutler et al., 1998). It has been reported that serum bilirubin levels in individuals with seven TA repeats are higher than the levels in individuals without a seventh repeat (Bosma et al., 1995; Rajmakers et al., 2000). Patients suffering from Gilbert's syndrome, CN syndrome, or prolonged neonatal jaundice also often carry seven or eight TA repeats (Iolascon et al., 1999; Burchell et al., 2000). The *UGT1A1* promoter polymorphisms are also considered a determinant of CPT-11 disposition and toxicity (Iyer et al., 1999; Ando et al.,

ABBREVIATIONS: SN-38, 7-ethyl-10-hydroxycamptothecin; CPT-11, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin; SN-38G, 7-ethyl-10-hydroxycamptothecin glucuronide; CN, Criegler-Najjar; SNP, single nucleotide polymorphism; PCR, polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction.

2000; Iyer et al., 2002). Other variations often associated with neonatal hyperbilirubinemia, CN syndromes, Gilbert's disease, or irinotecan toxicity are 211G>A (G71R), 1456T>G (Y486D), and 686C>A (P229Q) (Ando et al., 2000; Kraemer and Klinker, 2002; Yamamoto et al., 2002) (In this report, the A of the translational start codon of the cDNA is designated 1.) Recently, the possibility of a synergistic effect of 3'-untranslated region variations with -3279T>G on the bilirubin level and SN-38 pharmacokinetics was also proposed (Sai et al., 2004). The single nucleotide polymorphism (SNP) -3279T>G is located in the phenobarbital-responsive enhancer module, which is involved in activation of *UGT1A1* transcription by the constitutive androstane receptor (Sugatani et al., 2002).

A recent pharmacogenetic study has suggested that there are advantages in the use of haplotypes rather than individual SNPs to investigate the association between genotypes and phenotypes (Judson et al., 2000). Racial variability in haplotype frequencies of *UGT1A1* may contribute to an interpretation of the racial diversity of pharmacokinetics and toxicity of drugs metabolized by the enzyme. Recently, Sai et al. (2004) revealed the haplotype structure of *UGT1A1* in Japanese cancer patients using -3279T>G, TA repeats in the TATA box, 211G>A, 686C>A, and three SNPs (1813C>T, 1941C>G, and 2042C>G) in the 3'-untranslated region in exon 5 as markers. Therefore, we studied in this report the differences in the haplotype frequencies of *UGT1A1* among African-American, Caucasian, and Japanese populations using genomic DNA obtained from 150 individuals in each population, assessing the genotypes of these markers with dideoxy sequencing and pyrosequencing methods previously reported by Saeki et al. (2003). The function of a novel SNP 686C>T (P229L) that was found in an African-American individual during this study was also investigated by utilizing a heterologous expression system with COS-1 cells.

Materials and Methods

Materials. SN-38 (lot 970507R) and SN-38G (lot 970507R) were kindly supplied by Yakult Honsha Co. Ltd. (Tokyo, Japan). COS-1 cells were obtained from the Health Science Research Resources Bank (Osaka, Japan). Other chemicals used were all reagent grade.

Peripheral Blood Samples. One hundred fifty peripheral blood samples were obtained from healthy Japanese volunteers, 100 of which were kindly provided by Professor Ichiro Ieiri from the Tottori University Hospital with permission of the ethics committees of the Tottori University Faculty of Medicine. Written informed consent was obtained from all participants. Peripheral blood samples of healthy Caucasian and African-American volunteers (150 each) were purchased from the Tennessee Blood Service Corporation (Memphis, TN). The ethics committee of the National Institute of Health Sciences approved this study.

***UGT1A1* Genotyping by Pyrosequencing and Dideoxy Sequencing.** Genotyping of *UGT1A1* using Pyrosequence methods was performed as previously reported (Saeki et al., 2003). Briefly, genomic DNA (10–15 ng) was amplified by Ex-Taq (1 U; Takara Shuzo, Otsu, Japan) with a specific primer pair in which one of the primers was biotinylated. The primers for amplification and sequencing used for detection of polymorphisms -3279T>G, the TA repeat, 211G>A (G71R), and 686C>A/T (P229Q/L) have been shown previously (Saeki et al., 2003). Genotyping of 1941C>G for the Japanese samples was performed using an amplification primer pair (biotin-ATTGAATATG-TATCGTGCC and CATTCAATTCATTTACCTACT) and a sequencing primer (CAGTAGGGGCAGC). Generation of the single-stranded fragment and annealing of the sequencing primers have been described previously (Saeki et al., 2003). Genotypes were determined using the PSQ 96MA (Biostage AB, Uppsala, Sweden) and the PSQ 96 SNP reagent set (Biostage AB). Genotyping of 1813C>T, 1941C>G, and 2042C>G for African-American and Caucasian samples as well as the detection of a genotype 686C>T in the African-American individual were done by dideoxy sequencing as reported previously (Saeki et al., 2002; Sai et al., 2004).

Haplotype Analysis. Genotyping was successfully determined for 150

Japanese, 147 Caucasians, and 148 African-Americans. Diplotypes (combinations of haplotypes) in both blocks 1 and 2 were inferred separately for each population by PHASE version 2.0 (Stephens et al., 2001; Stephens et al., 2003).

Expression and Enzyme Assay of Wild-Type and Variant *UGT1A1*s. Expression of wild-type and variant *UGT1A1*s in COS-1 cells was carried out as described previously (Jinno et al., 2003b) with a minor modification; attB-flanked *UGT1A1* cDNA was amplified by the two-step attB adaptor polymerase chain reaction (PCR) (Jinno et al., 2003a) using pcDNA3.1-*UGT1A1*/WT (Jinno et al., 2003b) as a template and gene-specific primers, 5'-AAAAAGCAGGCTGCAAAGGCGCCATGGCTGT-3' and 5'-AGAAA-GCTGGGTCTCAATGGGTCTTGGATTGTGGG-3'. The resulting PCR fragment was cloned into the pDONR201 vector (Invitrogen, Carlsbad, CA). The 686C>T mutation was introduced into the wild-type *UGT1A1* cDNA clone in pDONR201, using a QuickChange multisite-directed mutagenesis kit (Stratagene, La Jolla, CA) with the 5'-phosphorylated oligonucleotide primer 5'-phospho-GCGACGTGGTTTATTCCCTGTATGCAACCCTTGCCCTC-3' (the nucleotide base changed is underlined). Subcloning of each *UGT1A1* fragment from pDONR201 into pcDNA-DEST40, a mammalian expression vector, was performed by the Gateway LR reaction.

Expression levels of *UGT1A1* mRNA and protein were determined as described previously (Jinno et al., 2003b). SN-38 glucuronidation activity of the wild-type and P229L variant *UGT1A1* was assayed according to the method of Hanioka et al. (2001).

Data Analysis. The χ^2 test in the SAS Preclinical Package (SAS Institute Japan Ltd., Tokyo, Japan) was applied to $s \times r$ contingency tables for comparison of genotype frequencies and analysis of haplotype distribution patterns among the three ethnic groups. In vitro Michaelis-Menten kinetic parameters were estimated by nonlinear regression analysis using Prism version 3.0 (GraphPad Software Inc., San Diego, CA). The average values of the kinetic parameters were calculated using results from three independent preparations. The t test supplied in Prism version 3.0 was applied to the comparison of the average values of protein expression and mRNA levels between wild-type and variant *UGT1A1* at a significance level of 0.05.

Results

Differences in Allele Frequencies among Three Ethnic Groups.

Allele frequencies of polymorphisms in *UGT1A1* tested in this study are summarized in Table 1. The estimated allele frequency of -3279T>G (*UGT1A1**60; for *UGT1A1* allele nomenclature refer to Mackenzie et al., 1997 and <http://som.flinders.edu.au/FUSA/ClinPharm/UGT1A1alleles.htm>) was 0.847, 0.550, and 0.257 in African-Americans, Caucasians, and Japanese, respectively, and they were significantly different ($p < 0.0001$ by the χ^2 test). Regarding the variation in the TATA box, the allele TA₆ was most common in the Japanese population, whereas frequencies of TA₆ and TA₇ (*UGT1A1**28) were equally frequent in African-Americans. The allele frequencies of TA₅ (*UGT1A1**36) or TA₈ (*UGT1A1**37) in African-Americans were about 5%, but no carriers with TA₅ or TA₈ were found in the Japanese. The allele distribution patterns for the wild-type and three variants in the TATA box of the three ethnic groups were significantly different ($p < 0.0001$ by the χ^2 test). The variant 211G>A (G71R) (*UGT1A1**6) was frequently found in the Japanese population, but not in the Caucasians population (only two heterozygous subject), and none were found in African-Americans. The variant 686C>A (P229Q) (*UGT1A1**27) was very rare in all the ethnic groups, and only one heterozygous carrier was found in the Japanese group. A novel variation in the same position as the variant 686C>A, but with a different nucleotide change (686C>T), was found in an African-American. This novel variant led to an amino acid change from proline to leucine (P229L). Pyrograms for 686C/C, 686C/A, and 686 C/T are compared in Fig. 1A and the nucleotide change of the novel variant was confirmed by the dideoxy sequencing method (Fig. 1B). This is the first report detailing allele frequencies of three SNPs in the 3'-untranslated region in exon 5, 1813C>T, 1941C>G and

TABLE 1
Allele frequencies of genetic polymorphisms of UGT1A1 in three ethnic groups

Position	Change	Ethnic group				Allele Frequency (lower limit, upper limit)	Number of Heterozygotes/ Homozygotes ^a Individuals ^b	Allele Frequency (lower limit, upper limit)	Number of Heterozygotes/ Homozygotes ^a Individuals ^b	Allele Frequency (lower limit, upper limit)	p (χ^2 test)
		African-American	Caucasian	Japanese	Japanese						
	Nucleotide	Amino Acid	Number of Heterozygotes/ Homozygotes ^a	Allele Frequency (lower limit, upper limit)	Number of Heterozygotes/ Homozygotes ^a Individuals ^b	Allele Frequency (lower limit, upper limit)	Number of Heterozygotes/ Homozygotes ^a Individuals ^b	Allele Frequency (lower limit, upper limit)	Number of Heterozygotes/ Homozygotes ^a Individuals ^b		
-3279	T > G		36/109 (150)	0.847 (0.806, 0.888)	65/50 (150)	0.550 (0.494, 0.606)	55/11 (150)	0.257 (0.208, 0.306)		<0.0001	
TA box	TA ₃		13/0	0.044 (0.021, 0.067)	5/0	0.017 (0.002, 0.032)	0/0	0.000		}	
	TA ₆		69/32	0.446 (0.390, 0.502)	77/48	0.588 (0.532, 0.644)	23/124	0.903 (0.870, 0.936)			
	TA ₇		65/34	0.446 (0.390, 0.502)	78/18	0.388 (0.332, 0.444)	23/3	0.097 (0.064, 0.130)			
	TA ₈		17/1 (149)	0.064 (0.000, 0.092)	2/0 (147)	0.007 (0.000, 0.017)	0/0 (150)	0.000			
211	G > A	G71R	0/0 (150)	0.000	2/0 (150)	0.007 (0.000, 0.016)	35/6 (150)	0.157 (0.116, 0.198)		<0.0001	
686	C > A	P229Q	0/0	0.000	0/0	0.000	1/0	0.003 (0.000, 0.009)		}	
	C > T	P229L	0/1 (149)	0.003 (0.000, 0.009)	0/0 (150)	0.000	0/0 (150)	0.000			
1813	C > T		61/22 (150)	0.350 (0.296, 0.404)	54/6 (150)	0.220 (0.173, 0.267)		0.097 ^b		<0.0001	
1941	C > G		43/6 (150)	0.183 (0.139, 0.227)	47/1 (150)	0.163 (0.121, 0.205)	25/2 (150)	0.097 (0.064, 0.130)		0.0074	
2042	C > G		68/23 (150)	0.380 (0.325, 0.435)	60/6 (150)	0.240 (0.192, 0.288)		0.097 ^b		<0.0001	

^a Values in parentheses are numbers of individuals determined.

^b Assumed to be equal to the frequency for 1941C > G.

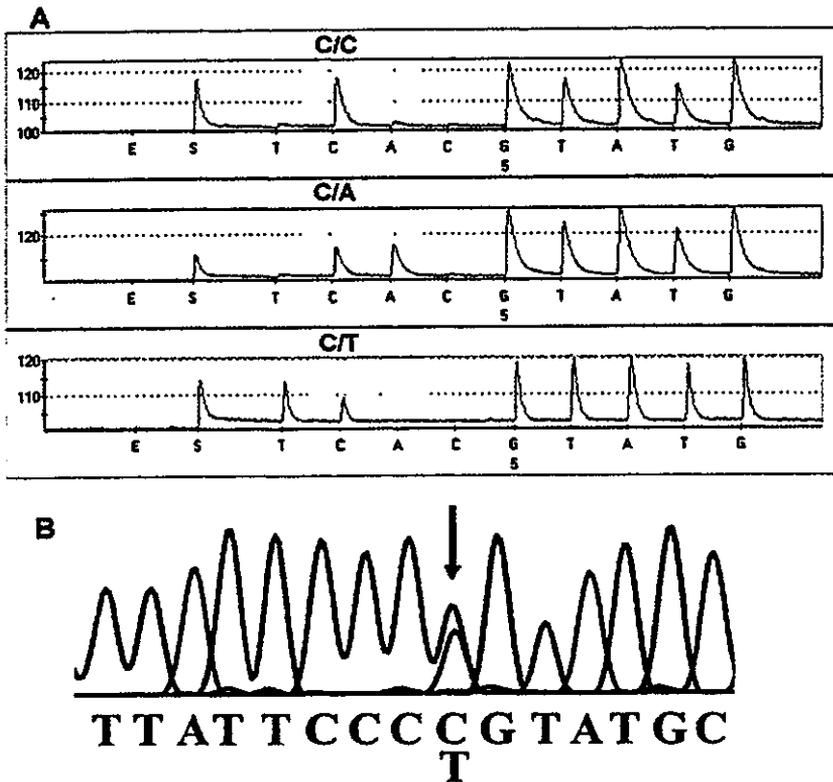


FIG. 1. Pyrosequencing and dideoxy sequencing methods for position 686. A, pyrograms for UGT1A1 686C/C, 686C/A, and 686C/T. The sequence to be analyzed is underlined in GACGTGGTTATTCCCCGTATG. The first T and the fourth C, nucleotides unrelated to 686C>A, were added to estimate the background value. Top, 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 TA₇. Thus, the association of variation 686C>A with TA₇ 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, TA₆/TA₇.

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		
	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		
	C	T	C	G	C	G	African-American	Caucasian	Japanese ^a
*1A	■		■		■		0.617	0.757	0.903
*1B		■		■		■	0.183	0.157	0.097
*1C		■	■			■	0.163	0.060	0.000
*1D	■		■			■	0.033	0.017	0.000
*1E	■			■		■	0.000	0.007	0.000
*1F		■	■		■		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 μ M. 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

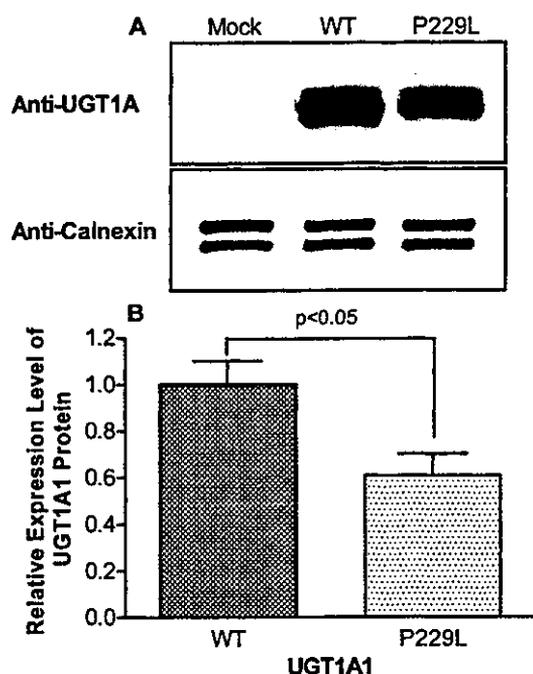


FIG. 2. Expression of wild-type and variant P229L UGT1A1 in COS-1 cells. A, an aliquot (20 μ g) of the pooled membrane fractions from three independent preparations was subjected to SDS-polyacrylamide gel electrophoresis, electrophoretically transferred to a polyvinylidene difluoride membrane, and immunochemically 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 \pm S.E.M. from three independent preparations. The expression level of UGT1A1 proteins of P229L was significantly lower than that of the wild type ($p = 0.044$).

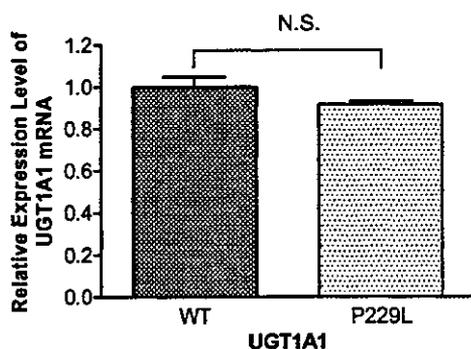


FIG. 3. Quantification of UGT1A1 mRNA by real-time SYBR Green RT-PCR in COS-1 cells transfected with wild-type and variant P229L UGT1A1. UGT1A1 mRNA from the cellular total RNA samples was quantified by SYBR Green RT-PCR. Each sample was normalized on the basis of the β -actin content and expressed as a percentage of the wild type. The results indicate the mean \pm 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 μ M, whereas P229L catalyzed SN-38 glucuronidation with a K_m value of 37.6 μ M. 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 μ l/min/mg protein for the wild-type and P229L, respectively.

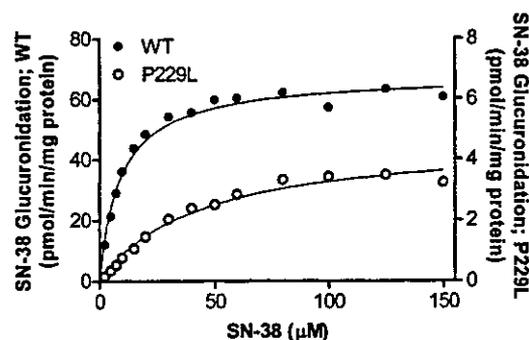


FIG. 4. Representative Michaelis-Menten kinetics of SN-38 glucuronidation by expressed wild-type (\bullet) and variant P229L (\circ) UGT1A1 in COS-1 cells. SN-38 glucuronidation by expressed UGT1A1 was assayed in the presence of the membrane fractions (100 μ g) at substrate concentrations ranging from 2.5 to 150 μ M. 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 TA₇ 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, TA₅ and TA₈ 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 TA₇ 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 TA₇ 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.

Acknowledgments. We thank Professor Ichiro Ieiri from Tottori University Hospital for extracting the genomic DNA. We also thank Yakult Honsha Co. Ltd. for kindly providing the SN-38 and SN-38G.

References

- Acuna G, Foerzler D, Leong D, Rabbia M, Smit R, Dorflinger E, Gasser R, Hoh J, Ott J, Borroni E, et al. (2002) Pharmacogenetic analysis of adverse drug effect reveals genetic variant for susceptibility to liver toxicity. *Pharmacogenomics* 3:327-334.
- Ando Y, Saka H, Ando M, Sawa T, Muro K, Ueoka H, Yokoyama A, Saitoh S, Shimokata K, and Hasegawa Y (2000) Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. *Cancer Res* 60:6921-6926.
- Araki E, Ishikawa M, Iigo M, Koide T, Itabashi M, and Hoshi A (1993) Relationship between development of diarrhea and the concentration of SN-38, an active metabolite of CPT-11, in the intestine and the blood plasma of athymic mice following intraperitoneal administration of CPT-11. *Jpn J Cancer Res* 84:697-702.
- Beuder E, Gelbart T, and Demina A (1998) Racial variability in the UDP-glucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for regulation of bilirubin metabolism? *Proc Natl Acad Sci USA* 95:8170-8174.
- Bosma PJ, Chowdhury JR, Bakker C, Gantla S, de Boer A, Oostra BA, Lindhout D, Tytgat GN, Jansen PL, Oude Elferink RP, et al. (1995) The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. *N Engl J Med* 333:1171-1175.
- Burchell B, Soars M, Monaghan G, Cassidy A, Smith D, and Ethell B (2000) Drug-mediated toxicity caused by genetic deficiency of UDP-glucuronosyltransferases. *Toxicol Lett* 112-113:333-340.
- Conne B, Stutz A, and Vassalli JD (2000) The 3' untranslated region of messenger RNA: a molecular "hotspot" for pathology? *Nat Med* 6:637-641.
- Day DA and Tuite MF (1998) Post-transcriptional gene regulatory mechanisms in eukaryotes: an overview. *J Endocrinol* 157:361-371.
- Fertrin KY, Goncalves MS, Saad ST, and Costa FF (2002) Frequencies of UDP-glucuronosyltransferase 1 (UGT1A1) gene promoter polymorphisms among distinct ethnic groups from Brazil. *Am J Med Genet* 108:117-119.
- Hanioka N, Ozawa S, Jinno H, Ando M, Saito Y, and Sawada J (2001) Human liver UDP-glucuronosyltransferase isoforms involved in the glucuronidation of 7-ethyl-10-hydroxycamptothecin. *Xenobiotica* 31:687-699.
- Huang CS, Luo GA, Huang ML, Yu SC, and Yang SS (2000) Variations of the bilirubin uridine-diphosphoglucuronosyl transferase 1A1 gene in healthy Taiwanese. 10:539-544.
- Innocenti F, Grimsley C, Das S, Ramirez J, Cheng C, Kuttab-Boulos H, Ratain MJ, and Di Rienzo A (2002) Haplotype structure of the UDP-glucuronosyltransferase 1A1 promoter in different ethnic groups. *Pharmacogenetics* 12:725-733.
- Iolascon A, Faienza MF, Centra M, Storelli S, Zelante L, and Savoia A (1999) TA8 allele in the UGT1A1 gene promoter of a Caucasian with Gilbert's syndrome. *Haematologica* 84:106-109.
- Iyer L, Das S, Janisch L, Wen M, Ramirez J, Karrison T, Fleming GF, Vokes EE, Schilsky RL, and Ratain MJ (2002) UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. *Pharmacogenomics* 3:43-47.
- Iyer L, Hall D, Das S, Mortell MA, Ramirez J, Kim S, Di Rienzo A, and Ratain MJ (1999) Phenotype-genotype correlation of in vitro SN-38 (active metabolite of irinotecan) and bilirubin glucuronidation in human liver tissue with UGT1A1 promoter polymorphism. *Clin Pharmacol Ther* 65:576-582.
- Iyer L, King CD, Whittington PF, Green MD, Roy SK, Tephly TR, Coffman BL, and Ratain MJ (1998) Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. *J Clin Invest* 101:847-854.
- Jinno H, Hanioka N, Tanaka-Kagawa T, Saito Y, Ozawa S, and Sawada J (2005) Transfection assays with allele specific constructs: functional analysis of UDP-glucuronosyltransferase variants, in *Methods in Molecular Biology*, Vol. 311: *Pharmacogenomics: Methods and Applications* (Innocenti F ed), Humana Press Inc., Totowa, NJ, in press.
- Jinno H, Saeki M, Saito Y, Tanaka-Kagawa T, Hanioka N, Sai K, Kaniwa N, Ando M, Shiro K, Minami H, et al. (2003a) Functional characterization of human UDP-glucuronosyltransferase 1A9 variant, D256N, found in Japanese cancer patients. *J Pharmacol Exp Ther* 306:688-693.
- Jinno H, Tanaka-Kagawa T, Hanioka N, Saeki M, Ishida S, Nishimura T, Ando M, Saito Y, Ozawa S, and Sawada J (2003b) Glucuronidation of 7-ethyl-10-hydroxycamptothecin (SN-

- 38), an active metabolite of irinotecan (CPT-11), by human UGT1A1 variants, G71R, P229Q, and Y486D. *Drug Metab Dispos* 31:108-113.
- Judson R, Stephens JC, and Windemuth A (2000) The predictive power of haplotypes in clinical response. *Pharmacogenomics* 1:15-26.
- Ki CS, Lee KA, Lee SY, Kim HJ, Cho SS, Park JH, Cho S, Sohn KM, and Kim JW (2003) Haplotype structure of the UDP-glucuronosyltransferase 1A1 (UGT1A1) gene and its relationship to serum total bilirubin concentration in a male Korean population. *Clin Chem* 49:2078-2081.
- Koiwai O, Nishizawa M, Hasada K, Aono S, Adachi Y, Mamiya N, and Sato H (1995) Gilbert's syndrome is caused by a heterozygous missense mutation in the gene for bilirubin UDP-glucuronosyltransferase. *Hum Mol Genet* 4:1183-1186.
- Kraemer D and Klunker H (2002) Crigler-Najjar syndrome type II in a Caucasian patient resulting from two mutations in the bilirubin uridine 5'-diphosphate-glucuronosyltransferase (UGT1A1) gene. *J Hepatol* 36:706-707.
- Mackenzie PI, Owens IS, Burchell B, Bock KW, Bairoch A, Belanger A, Fournel-Gigleux S, Green M, Hum DW, et al. (1997) The UDP glycosyltransferase gene superfamily: recommended nomenclature update based on evolutionary divergence. *Pharmacogenetics* 7:255-269.
- Premawardhena A, Fisher CA, Liu YT, Verma IC, de Silva S, Arambepola M, Clegg JB, and Weatherall DJ (2003) The global distribution of length polymorphisms of the promoters of the glucuronosyltransferase 1 gene (UGT1A1): hematologic and evolutionary implications. *Blood Cells Mol Dis* 31:98-101.
- Rajmakers MT, Jansen PL, Steegers EA, and Peters WH (2000) Association of human liver bilirubin UDP-glucuronosyltransferase activity with a polymorphism in the promoter region of the UGT1A1 gene. *J Hepatol* 33:348-351.
- Saeki M, Ozawa S, Saito Y, Jinno H, Hamaguchi T, Nokihara H, Shimada Y, Kunitoh H, Yamamoto N, Ohe Y, et al. (2002) Three novel single nucleotide polymorphisms in UGT1A10. *Drug Metab Pharmacokinet* 17:488-490.
- Saeki M, Saito Y, Jinno H, Tohkin M, Kurose K, Kaniwa N, Komamura K, Ueno K, Kamakura S, Kitakaze M, et al. (2003) Comprehensive UGT1A1 genotyping in a Japanese population by pyrosequencing. *Clin Chem* 49:1182-1185.
- Sai K, Saeki M, Saito Y, Ozawa S, Katori N, Jinno H, Hasegawa R, Kaniwa N, Sawada J, Komamura K, et al. (2004) UGT1A1 haplotypes associated with reduced glucuronidation and increased serum bilirubin in irinotecan-administered Japanese patients with cancer. *Clin Pharmacol Ther* 75:501-515.
- Stephens M and Donnelly P (2003) A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet* 73:1162-1169.
- Stephens M, Smith NJ, and Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 68:978-989.
- Sugatani J, Yamakawa K, Yoshinari K, Machida T, Takagi H, Mori M, Kakizaki S, Sueyoshi T, Negishi M, and Miwa M (2002) Identification of a defect in the UGT1A1 gene promoter and its association with hyperbilirubinemia. *Biochem Biophys Res Commun* 292:492-497.
- Tukey RH and Strassburg CP (2000) Human UDP-glucuronosyltransferases: metabolism, expression and disease. *Annu Rev Pharmacol Toxicol* 40:581-616.
- Yamamoto A, Nishio H, Waku S, Yokoyama N, Yonetani M, Uetani Y, and Nakamura H (2002) Gly71Arg mutation of the bilirubin UDP-glucuronosyltransferase 1A1 gene is associated with neonatal hyperbilirubinemia in the Japanese population. *Kobe J Med Sci* 48:73-77.

Address correspondence to: Dr. Nahoko Kaniwa, Division of Medicinal Safety Science, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan. E-mail: nkaniwa@nihs.go.jp

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

Ichiro Ieiri, Hiroshi Takane and Kenji Otsubo

Department of Hospital Pharmacy, Faculty of Medicine, Tottori University, Yonago, Japan

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 anti-cancer 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]

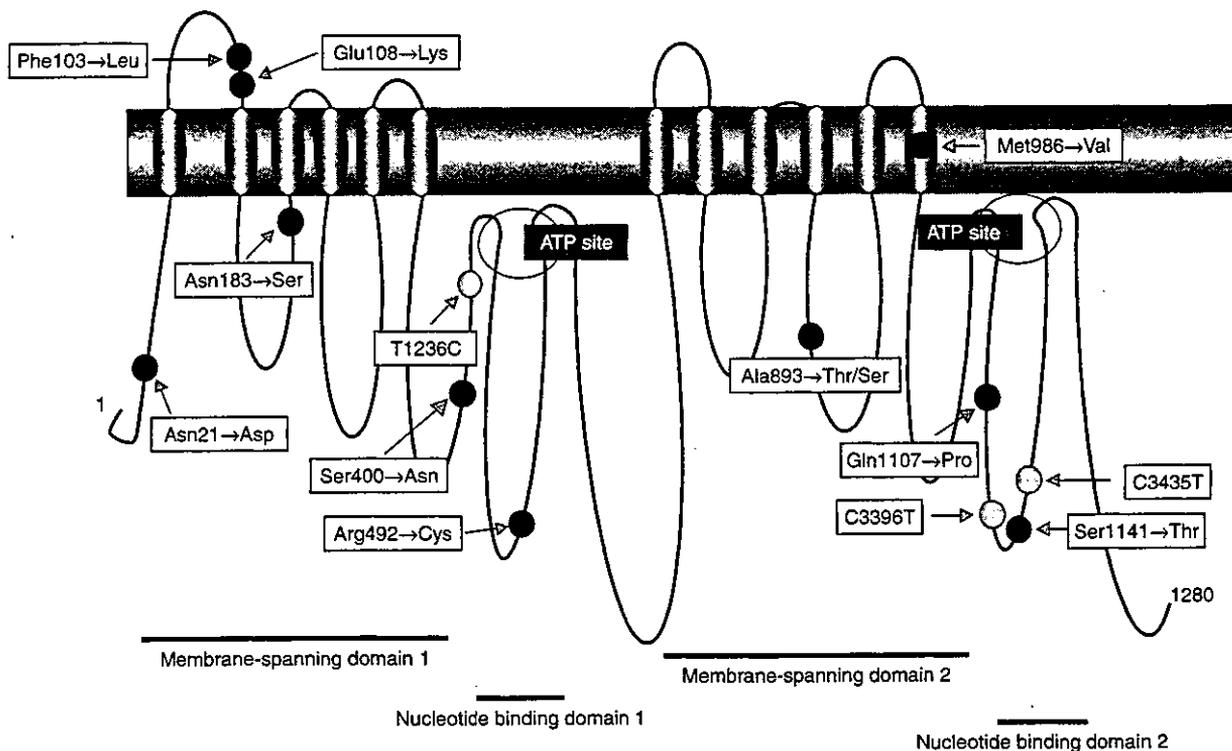


Fig. 1. Schematic representation of P-glycoprotein secondary structure, with known synonymous and non-synonymous nucleotide substitutions. Non-synonymous substitutions are indicated by the corresponding amino acid changes. **ATP** = adenosine triphosphate.

1.3 Gene Structure and Sites of Polymorphism

The *MDR1* gene is located on chromosome 7 at q21,^[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 1. 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 6); T-76A (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) ^[58]	Volunteers/blood donors			0.98/0.02		1.00/0	1.00/0			
Indian (n = 61-68) ^[54]	Neonates (umbilical cords)									
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) ^[58]	Volunteers/blood donors									
African										
Ghanaian (n = 172-206) ^[56,58]	Volunteers/blood donors									
Kenyan (n = 80) ^[58]	Volunteers/blood donors									
Sudanese (n = 51) ^[58]	Volunteers/blood donors									
African American										
African American (n = 23-88) ^[52,58]	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) ^[58]	Volunteers/blood donors									
Portuguese (n = 100) ^[58]	Volunteers/blood donors									
Italian (n = 106) ^[59]	Control for Parkinson's disease			0.97/0.03						
Caucasian (n = 537) ^[3]	Control for renal tumours									

Continued next page

Table 1. Contd

Ethnic background	Subject	A-41aG A/G	C-145G C/G	T-129C T/C	C-4T C/T	G-1A G/A	A61G (21N→D) A/G	G-35C G/C	G-25T G/T	T307C (103F→L) 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) ^[58]	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) ^[1]	Depressed patients									
Caucasian (n = 124) ^[60]	Renal transplant recipients									
Caucasian (n = 212) ^[9]	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+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
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) ^[58]	Volunteers/blood donors									
Middle East										
Saudi (n = 96) ^[59]	Volunteers/blood donors									
African										
Ghanaian (n = 172-206) ^[56,59]	Volunteers/blood donors									
Kenyan (n = 80) ^[58]	Volunteers/blood donors									
Sudanese (n = 51) ^[59]	Volunteers/blood donors									
African American										
African American (n = 23-88) ^[52,59]	Volunteers/blood donors			1.00/0	1.00/0			1.00/0		
Caucasian										
Caucasian German (n = 461) ^[51]	Volunteers	0.63/0.37							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

Continued next page

Table II. Cont'd

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
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) ^[1]	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) (986M→V) G/T/A	A2956G 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 = 95-132) ^(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										
Ghanalan (n = 172-206) ^(58,59)	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) ⁽⁵⁸⁾	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	

Continued next page

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) ^[6,52]	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) ^[1]	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. Siegmund 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⁺TTTCGAGTAG). 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 $r = 0.58$ ($p < 0.0006$). Siegmund 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