Table 9. Allelic Frequencies of *UGT1A1*6* (211G>A, G71R) in Different Ethnic Populations

Population	Allele Frequency	Number of Subjects	Reference
Caucasians			
German¶	ND	50	Akaba *et al.* 1998
Caucasian¶	ND	132	Innocenti *et al.* 2005
	0.007	150	Kaniwa *et al.* 2005
	ND	92	Thomas *et al.* 2006
Africans			
African-American¶	ND	150	Kaniwa *et al.* 2005
Asians			
Japanese	0.130	101	Akaba *et al.* 1998
	0.157	150	Kaniwa *et al.* 2005
	0.177	116	Kanai *et al.* 2005
	0.153	301	Saeki *et al.* 2006
Korean¶	0.230	50	Akaba *et al.* 1998
	0.213	324	Ki *et al.* 2003
	0.241	81	Han *et al.* 2006
Chinese	0.230	50	Akaba *et al.* 1998
Taiwanese	0.156	218	Huang *et al.* 2002
Thai	0.104	96	Boyd *et al.* 2006
Malay¶	0.030	50	Yusoff *et al.* 2006
	0.014	36	Sutomo *et al.* 2004
Indonesian (Javanese)¶	0.015	68	Sutomo *et al.* 2004
Asians (mostly East-Asians)*	0.130	150	Innocenti *et al.* 2005

ND: not detected.

¶Significant differences ($P<0.01$, chi-square test or Fisher's exact test) in allele frequencies between the Japanese population and each ethnic population. When plural studies were undertaken for each ethnic population, combined data were used for comparison. The multiple comparison was corrected by Bonferroni's method.

*Not statistically analyzed because of mixed populations.

Table 10. Allelic Frequencies of *UGT1A1*60* (-3279T>G) in Different Ethnic Populations

Population	Allele Frequency	Number of Subjects	Reference
Caucasians			
Caucasian¶	0.473	55	Innocenti *et al.* 2002
	0.550	150	Kaniwa *et al.* 2005
	0.439	132	Innocenti *et al.* 2005
German	0.351	57	Kanai *et al.* 2005
Africans			
African-American¶	0.851	37	Innocenti *et al.* 2002
	0.847	150	Kaniwa *et al.* 2005
Asians			
Japanese	0.167	27	Sugatani *et al.* 2002
	0.257	150	Kaniwa *et al.* 2005
	0.261	157	Kanai *et al.* 2005
	0.262	301	Saeki *et al.* 2006

(Table 10. Contd....)

Population	Allele Frequency	Number of Subjects	Reference
Korean	0.327 0.267 0.235	55 324 81	Kanai *et al.* 2005 Ki *et al.* 2003 Han *et al.* 2006
Chinese	0.300	50	Kanai *et al.* 2005
Asians (mostly East-Asians)*	0.340	150	Innocenti *et al.* 2005

"Significant differences ($P<0.01$, chi-square test) in allele frequencies between the Japanese population and each ethnic population. When plural studies were undertaken for each ethnic population, combined data were used for comparison. The multiple comparison was corrected by Bonferroni's method.

*Not statistically analyzed because of mixed populations.

1A7 low-activity haplotype **3* (containing –57T>G, 387T> G, 391C>A, 392G>A, and 622T>C, resulting in N129K, R131K, and W208R) [Guillemette *et al.* 2000b, Villeneuve *et al.* 2003] was mostly linked with *1A6* high-activity haplotype **2* (containing 19T>G, 541A>G, and 552A>C, resulting in S7A, T181A, and R184S, respectively) [Krishnaswamy *et al.*, 2005]. The single *UGT1A4* segment is Block 4 [Saeki *et al.*, 2005]. Using 19 genetic polymorphisms, 16 haplotypes were inferred. Regarding Block 3/1 (*1A3-1A1*), 16 haplotypes were inferred, and the 5 haplotypes with frequencies ≥5% accounted for 89.5% of the total haplotypes. It is noteworthy that the high-activity segment haplotype *1A3*2* (containing 31T>C and 140T>C, resulting in W11R and V47A respectively, previous haplotype *1A3*11R47A*) [Iwai *et al.* 2004] was completely linked with the low-activity haplotype *1A1*28*. The low-activity haplotype *1A1*6* was linked with the *1A3*1* haplotype (wild-type) or *1A3*4* haplotype (containing 133C>T, R45W, previous haplotype *1A3*45W*). The *1A3*3* haplotype (containing 31T>C, W11R, previous haplotype *1A3*11R*) was perfectly linked with the low-activity *1A1*60* haplotype. As for common exons 2-5 (Block C), 14 haplotypes were inferred using 13 polymorphisms [Sai *et al.*, 2004].

Then, block-haplotype combinations (whole complex haplotypes) among Block 9/6, Block 4, and Block 3/1 were also estimated for Japanese [see Saeki *et al.*, 2006 for detail]. Block 8/10 and Block C (common exons 2 to 5) were excluded due to a high degree of recombination. We found several functionally important linkages across the blocks. The haplotype *UGT1A9*1-1A7*2-1A6*4* (containing *UGT1A7* N129K and R131K, and *UGT1A6* S7A and R184S, low activity in 1A7) [Guillemette *et al.*, 2000b] and *1A3*2-1A1*28c* (containing *UGT1A3* W11R and V47A, and UGT1A1 –3279T>G, $A(TA)_7TAA$ and P229Q, high activity in 1A3 and low in 1A1) were perfectly linked. Most of the *UGT1A1*6*-containing haplotypes (G71R, low activity) were associated with *UGT1A7*3-1A6*2* (containing *UGT1A7* N129K, R131K and W208R, and *UGT1A6* S7A, T181A and R184S, low activity in 1A7 and high in 1A6). Inversely, most of *UGT1A7*3-1A6*2* haplotypes were associated with the *1A3*2-1A1*28b* (having -3279T>G and $A(TA)_7TAA$, low activity in 1A1) haplotypes (26% of *UGT1A7*3-1A6*2* haplotype) or *UGT1A1*6*-containing haplotypes (67%). The *UGT1A7*2* (low activity), *1A4*3* (containing 142T>G, L48V, activity changes depending on the substrates) [Ehmer *et al.*, 2004, Mori *et al.* 2005] and *1A1*60* (-3279T>G, low

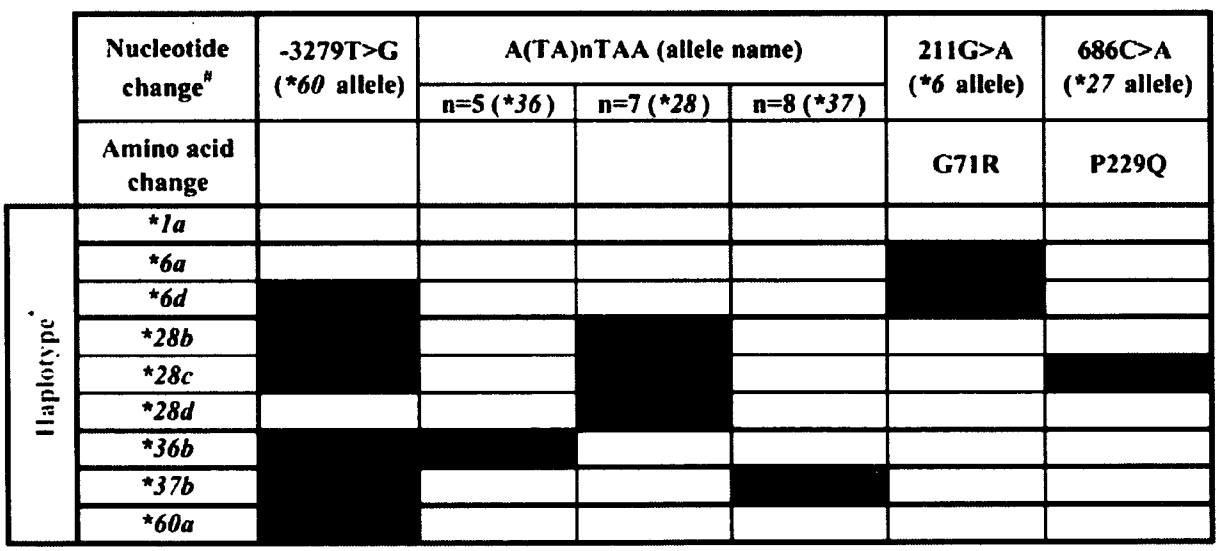

Fig. (4). Haplotype structure of *UGT1A1*. #A of the translational initiation codon is numbered +1 according to the reference sequence AF297093.1. *Major allele, white; minor allele, gray.

Table 11. Haplotype Frequencies of *UGT1A1* in Different Ethnic Populations

Population	*1a	*6		*28			*36b	*37b	*60a	Number of Subjects	Reference
		*6a	*6d	*28b	*28c	*28d					
Caucasian	0.53	-*	-*	0.36	-*	ND	0.01	0.01	0.09	55	Innocenti *et al.* 2002
	0.451	ND	ND	0.389	ND	ND	0.017	0.007	0.135	147	Kaniwa *et al.* 2005
	0.558	ND	ND	0.340	ND	ND	-**	ND	0.102	132	Innocenti *et al.* 2005
African-American	0.15	-*	-*	0.35	-*	ND	0.04	0.12	0.33	37	Innocenti *et al.* 2002
	0.150	ND	ND	0.446	ND	ND	0.044	0.065	0.296	149	Kaniwa *et al.* 2005
Asian	0.526	0.130	ND	0.076	0.034	ND	ND	ND	0.233	150	Innocenti *et al.* 2005
Japanese	0.582	0.151	ND	0.121	0.005	0.005	ND	ND	0.136	195	Sai *et al.* 2004
	0.610	0.141	0.003	0.097	0.003	ND	ND	ND	0.145	150	Kaniwa *et al.* 2005
Korean	0.518	0.235	ND	0.061	-***	0.012	ND	ND	0.172	81	Han *et al.* 2006

ND: Not detected.

*211G>A and 686C>A were not genotyped.

**$A(TA)_5TAA$ was detected in an extra subject but excluded from the haplotype analysis.

***686C>A was not genotyped.

activity) were very closely linked with each other. In addition, we found that *UGT1A10*3* (now **6*, having T202I, low activity) was strongly linked with these *1A7*2*, *1A4*3*, and *1A1*60* (80% of *UGT1A10*3*). These linkages across the segments were also reported in other populations. Kohle *et al.* reported close linkages among *1A1*28* ($A(TA)_7TAA$), *1A6*2* (T181A/R184S) and *1A7*3* (N129K/R131K/W208R) in Caucasians and Egyptians [Kohle *et al.*, 2003]. Linkage between *1A1*6* and *1A7*3* alleles was also suggested in Taiwanese [Huang *et al.*, 2005]. Note that different profiles for the linkage of *1A7*3* with the *1A1* polymorphisms between the Caucasians and East Asians reflect the facts that the frequency of the *1A1*6* haplotype in the East Asian populations was relatively high, and that the *1A1*28* and **6* alleles were mutually exclusive [Sai *et al.*, 2004]. Innocenti *et al.* reported the linkage between *UGT1A9* and *1A1* haplotypes, and the most common three *1A9-1A1* haplotype combinations were *1A9*22* (now **1b*, with -126_-118 $T_9>T_{10}$)-*1A1*1* (frequency: 36.4%), *1A9*1-1A1*28b* (28.0%) and *1A9*1-1A1*1* (18.6%) for Caucasians, and *1A9*22* (**1b*)-*1A1*1* (45.3%), *1A9*1-1A1*60* (22.3%) and *1A9*1-1A1*6* (12.7%) for Asians (mostly from East Asians) [Innocenti *et al.*, 2005]. For Japanese, *1A9*22* (**1b*)-*1A1*1*, *1A9*1-1A1*60*, and *1A9*1-1A1*6* (58.5%, 11.9%, and 13.3%, respectively) were also the three most common combinations, and most of the *1A1*1* haplotype (98%) was linked with *1A9*22* (**1b*), and 87% of *1A1*6*, 100% of *1A1*28c*, and 93% of *1A1*60* were associated with *1A9*1* [Saeki *et al.*, 2006]. A recently published report also showed that *1A9*22* (**1b*)-*1A1*1*, *1A9*1-1A1*60*, and *1A9*1-1A1*6* (48.1%, 16.0%, and 20.4%, respectively) were also the most common three combinations in Koreans [Han *et al.*, 2006]. Collectively, haplotype combinations are suggested to be different between Caucasians and East Asians.

These linkages might be crucial for the metabolism of a certain drug in which two or more UGT1A isoforms are significantly involved. In fact, multiple UGT isoforms contribute to glucuronidation of several compounds. For example, UGT1A1, 1A9 and 1A7 have glucuronidation activity to SN-38 [Ciotti *et al.*, 1999; Gagne *et al.*, 2002]. The *1A1*60*, **28*, and **6* haplotypes are associated with reduced *UGT1A1* activity [Beutler *et al.*, 1998; Sugatani *et al.*, 2002; Jinno *et al.*, 2003a]. The *1A7*3*, but not **2*, haplotype has a reduced (by 60%) glucuronidation activity to SN-38 [Gagne *et al.*, 2002]. As described in the above haplotype analyses, most *1A7*3*-containing haplotypes were estimated to be linked with *1A1*28* in Caucasians or with either *1A1*6* or *1A1*28* in East Asians. Thus, it is often difficult to distinguish the contributions of low-activity *1A1* and *1A7* haplotypes *in vivo*.

Since plural UGT isoforms are often involved in the glucuronidation of "one" compound, co-occurrence of the functionally less active haplotypes in the entire *UGT1A* gene complex needs careful consideration in studies on the association of genetic polymorphisms with pharmacokinetic parameters and both clinical and epidemiological data.

CONCLUDING REMARKS

In this review, we described the influence of genetic polymorphisms/haplotypes on drug metabolism and drug response. However, it should be noted that the genetic polymorphisms/haplotypes are just one of the important factors that contribute to the ethnic and interindividual differences in drug response. For example, the contribution of genetic polymorphisms/haplotypes (mainly *CYP2C9* and *VKORC1* encoding a target enzyme of warfarin) were estimated to be 25 to 44% to the anti-coagulant warfarin dose requirements in 4 different Asian populations [Lee *et al.*, 2006b; Obayashi *et al.*, 2006]. Other non-genetic factors such as age, gender, co-medications, and diagnosis are also important determinants for the dosage. Epigenetic factors may also be important to determine the expression levels of drug metabolizing enzymes. As for CYP3A4, Hirota *et al.* [2004] reported

skewed expression of CYP3A4 mRNA between two alleles, and the allelic expression ratio (less expressed mRNA/more expressed mRNA) varied from 0.3 to 1. This allelic expression ratio correlated well with CYP3A4 mRNA levels as well as testosterone 6β-hydroxylation activity. In addition, DNA methylation was also suggested to influence the CYP3As' expression in HepG2 cells [Dannenberg and Edenberg 2006].

Although depending on the genes, expression of CYPs and UGT1As were also regulated by nuclear receptors such as PXR/SXR and VDR as discussed in the CYP3A4 section [Drocourt *et al.*, 2002; Handschin and Meyer, 2003]. Since many xenobiotics are ligands for PXR/SXR [Handschin and Meyer 2003], co-medications, supplements and/or food ingredients are thought to be the influencing factors for PXR/SXR activation, thereby enhancing target gene expression. Since vitamin D also enhances the expression of CYP3A4 and CYP2C9 through binding to VDR [Drocourt *et al.*, 2002], it is possible that supplements and/or food ingredients could change the expression of these genes. Therefore, these environmental factors may also affect the enzymatic activity and thus the drug response.

However, under certain combinations of enzyme and drug, genetic polymorphisms could explain interindividual or interethnic diversities of pharmacokinetic and/or pharmacodynamic parameters. For example, pharmacogenetic studies in Caucasians have shown close associations of *UGT1A1*28* with reduced glucuronidation of SN-38 and incidence of severe neutropenia [Marsh and McLeod, 2004; Ando and Hasegawa, 2005]. Accordingly, the Food and Drug Administration in the United States has approved an amendment of the label for Camptosar (irinotecan HCl), to which was added a warning to consider a reduction in the starting dose of irinotecan for **28* homozygous patients (NDA 20-571). However, in East Asians, the influence of *UGT1A1*6* on irinotecan toxicities could be also substantial as suggested by *in vitro* and *in vivo* studies [Gagne *et al.*, 2002; Jinno *et al.*, 2003a; Sai *et al.*, 2004; Han *et al.*, 2006]. Thus, ethnic profiles of polymorphisms and haplotypes should be determined prior to clinical applications of genetic polymorphisms. Detailed haplotype data for drug metabolizing enzymes, transporters and receptors would be useful for further pharmacogenetic studies.

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ABBREVIATIONS

AUC = Area under the plasma concentration-time curve

CYP = Cytochrome P450

EM = Extensive metabolizer

htSNP = Haplotype-tagging SNPs

IVS = Intervening sequence

LD = Linkage disequilibrium

PM = Poor metabolizer

PXR/SXR = Pregnane/steroid X receptor

SNP = Single nucleotide polymorphism

UGT = Uridinediphosphoglucuronate glucuronosyltransferase

VDR = Vitamin D receptor

XREM = Xenobiotic-responsive enhancer module

REFERENCES

Ahmadi, K. R.; Weale, M. E.; Xue, Z. Y.; Soranzo, N.; Yarnall, D. P.; Briley, J. D.; Maruyama, Y.; Kobayashi, M.; Wood, N. W.; Spurr, N. K.; Burns, D. K.; Roses, A. D.; Saunders, A. M. and Goldstein, D. B. (2005) A single-nucleotide polymorphism tagging set for human drug metabolism and transport. *Nat. Genet.* **37**, 84-89.

Akaba, K.; Kimuram, T.; Sasaki, A.; Tanabe, S.; Ikegami, T.; Hashimoto, M.; Umeda, H.; Yoshida, H.; Umetsu, K.; Chiba, H.; Yuasa, I. and Hayasaka, K. (1998) Neonatal hyperbilirubinemia and mutation of the bilirubin uridine diphosphate-glucuronosyltransferase gene: a common missense mutation among Japanese, Koreans and Chinese. *Biochem. Mol. Biol. Int.* **46**, 21-26.

Allabi, A. C.; Gala, J. L.; Horsmans, Y.; Babaoglu, M. O.; Bozkurt, A.; Heusterspreute, M. and Yasar, U. (2004) Functional impact of CYP2C9*5, CYP2C9*6, CYP2C9*8, and CYP2C9*11 *in vivo* among black Africans. *Clin. Pharmacol. Ther.* **76**, 113-118.

Allabi, A. C.; Gala, J. L. and Horsmans, Y. (2005) CYP2C9, CYP2C19, ABCB1 (MDR1) genetic polymorphisms and phenytoin metabolism in a Black Beninese population. *Pharmacogenet. Genomics* **15**, 779-786.

Andersson, T.; Regardh, C. G.; Dahl-Puustinen, M. L. and Bertilsson, L. (1990) Slow omeprazole metabolizers are also poor S-mephenytoin hydroxylators. *Ther. Drug Monit.* **12**, 415-416.

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.

Ando, Y. and Hasegawa, Y. (2005) Clinical pharmacogenetics of irinotecan (CPT-11). *Drug Metab. Rev.* **37**, 565-574.

Aono, S.; Adachi, Y.; Uyama, E.; Yamada, Y.; Keino, H.; Nanno, T.; Koiwai, O. and Sato, H. (1995) Analysis of genes for bilirubin UDP-glucuronosyltransferase in Gilbert's syndrome. *Lancet* **345**, 958-959.

Bajpai, M.; Roskos, L. K.; Shen, D. D. and Levy, R. H. (1996) Roles of cytochrome P4502C9 and cytochrome P4502C19 in the stereoselective metabolism of phenytoin to its major metabolite. *Drug Metab. Dispos.* **24**, 1401-1403.

Ball, S. E.; Scatina, J.; Kao, J.; Ferron, G. M.; Fruncillo, R.; Mayer, P.; Weinryb, I.; Guida, M.; Hopkins, P. J.; Warner, N. and Hall, J. (1999) Population distribution and effects on drug metabolism of a genetic variant in the 5' promoter region of CYP3A4. *Clin. Pharmacol. Ther.* **66**, 288-294.

Balram, C.; Sabapathy, K.; Fei, G.; Khoo, K. S. and Lee, E. J. (2002) Genetic polymorphisms of UDP-glucuronosyltransferase in Asians: UGT1A1*28 is a common allele in Indians. *Pharmacogenetics* **12**, 81-83.

Balram, C.; Zhou, Q.; Cheung, Y. B. and Lee, E. J. (2003) CYP3A5*3 and *6 single nucleotide polymorphisms in three distinct Asian populations. *Eur. J. Clin. Pharmacol.* **59**, 123-126.

Basu, N. K.; Ciotti, M.; Hwang, M. S.; Kole, L.; Mitra, P. S.; Cho, J. W. and Owens, I. S. (2004) Differential and special properties of the major human UGT1-encoded gastrointestinal UDP-glucuronosyltransferases enhance potential to control chemical uptake. *J. Biol. Chem.* **279**, 1429-1441.

Beutler, 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.

Blaisdell, J.; Mohrenweiser, H.; Jackson, J.; Ferguson, S.; Coulter, S.; Chanas, B.; Xi, T.; Ghanayem, B. and Goldstein, J. A. (2002) Identification and functional characterization of new potentially defective alleles of human CYP2C19. *Pharmacogenetics* **12**, 703-711.

Blaisdell, J.; Jorge-Nebert, L. F.; Coulter, S.; Ferguson, S. S.; Lee, S. J.; Chanas, B.; Xi, T.; Mohrenweiser, H.; Ghanayem, B. and Goldstein, J. A. (2004) Discovery of new potentially defective alleles of human CYP2C9. *Pharmacogenetics* **14**, 527-537.

Blann, A.; Hewitt, J.; Siddiqui, F. and Bareford, D. (1999) Racial background is a determinant of average warfarin dose required to maintain the INR between 2.0 and 3.0. *Br. J. Haematol.* **107**, 207-209.

Bosma, P. J.; Chowdhury, J. R.; Bakker, C.; Gantla, S.; de Boer, A.; Oostra, B. A.; Lindhout, D.; Tytgat, G. N.; Jansen, P. L. and Oude Elferink, R. P. (1995) The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. *N. Engl. J. Med.* **333**, 1171-1175.

Boyd, M. A.; Srasuebkul, P.; Ruxrungtham, K.; Mackenzie, P. I.; Uchaipichat, V.; Stek, M. Jr.; Lange, J. M.; Phanuphak, P.; Cooper, D. A.; Udomuksorn, W.; Miners, J. O. (2006) Relationship between hyperbilirubinaemia and UDP-glucuronosyltransferase 1A1 (UGT1A1) polymorphism in adult HIV-infected Thai patients treated with indinavir. *Pharmacogenet. Genomics* **16**, 321-329.

Bradford, L. D. (2002) CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics* **3**, 229-243.

Bravo-Villalta, H. V.; Yamamoto, K.; Nakamura, K.; Baya, A.; Okada, Y. and Horiuchi, R. (2005) Genetic polymorphism of CYP2C9 and CYP2C19 in a Bolivian population: an investigative and comparative study. *Eur. J. Clin. Pharmacol.* **61**, 179-184.

Broly, F. and Meyer, U. A. (1993) Debrisoquine oxidation polymorphism: phenotypic consequences of a 3-base-pair deletion in exon 5 of the CYP2D6 gene. *Pharmacogenetics* **3**, 123-130.

Cavaco, I.; Gil, J. P.; Gil-Berglund, E. and Ribeiro, V. (2003) CYP3A4 and MDR1 alleles in a Portuguese population. *Clin. Chem. Lab. Med.* **41**, 1345-1350.

Chern, H. D.; Ueng, T. H.; Fu, Y. P. and Cheng, C. W. (2006) CYP2C9 polymorphism and warfarin sensitivity in Taiwan Chinese. *Clin. Chim. Acta.* **367**, 108-113.

Chida, M.; Yokoi, T.; Nemoto, N.; Inaba, M.; Kinoshita, M. and Kamataki, T. (1999a) A new variant CYP2D6 allele (CYP2D6*21) with a single base insertion in exon 5 in a Japanese population associated with a poor metabolizer phenotype. *Pharmacogenetics* **9**, 287-293.

Chida, M.; Yokoi, T.; Kosaka, Y.; Chiba, K.; Nakamura, H.; Ishizaki, T.; Yokota, J.; Kinoshita, M.; Sato, K.; Inaba, M.; Aoki, Y.; Gonzalez, F. J. and Kamataki, T. (1999b) Genetic polymorphism of CYP2D6 in the Japanese population. *Pharmacogenetics* **9**, 601-605.

Chida, M.; Ariyoshi, N.; Yokoi, T.; Nemoto, N.; Inaba, M.; Kinoshita, M. and Kamataki, T. (2002) New allelic arrangement CYP2D6*36 x 2 found in a Japanese poor metabolizer of debrisoquine. *Pharmacogenetics* **12**, 659-662.

Chowbay, B.; Cumaraswamy, S.; Cheung, Y. B.; Zhou, Q. and Lee, E. J. (2003) Genetic polymorphisms in MDR1 and CYP3A4 genes in Asians and the influence of MDR1 haplotypes on cyclosporin disposition in heart transplant recipients. *Pharmacogenetics* **13**, 89-95.

Chowbay, B.; Zhou, S. and Lee, E. J. (2005) An interethnic comparison of polymorphisms of the genes encoding drug-metabolizing enzymes and drug transporters: experience in Singapore. *Drug Metab. Rev.* **37**, 327-378.

Ciotti, M.; Basu, N.; Brangi, M. and Owens, I. S. (1999) Glucuronidation of 7-ethyl-10-hydroxycamptothecin (SN-38) by the human UDP-glucuronosyltransferases encoded at the UGT1 locus. *Biochem. Biophys. Res. Commun.* **260**, 199-202.

Congiu, M.; Mashford, M. L.; Slavin, J. L. and Desmond, P. V. (2002) UDP glucuronosyltransferase mRNA levels in human liver disease. *Drug Metab. Dispos.* **30**, 129-134.

Cubeddu, L. X.; Aranda, J.; Singh, B.; Klein, M.; Brachfeld, J.; Freis, E.; Roman, J. and Eades, T. A. (1986) comparison of verapamil and propranolol for the initial treatment of hypertension. Racial differences in response. *JAMA* **256**, 2214-2221.

Dahl, M. -L.; Yue, Q. -Y.; Roh, H. -K.; Johansson, I.; Sawe, J.; Sjoqvist, F. and Bertilsson, L. (1995) Genetic analysis of the CYP2D locus in relation to debrisoquine hydroxylation capacity in Korean, Japanese and Chinese subjects. *Pharmacogenetics* **5**, 159-165.

Dai, D.; Tang, J.; Rose, R.; Hodgson, E.; Bienstock, R. J.; Mohrenweiser, H. W. and Goldstein, J. A. (2001) Identification of variants of CYP3A4 and characterization of their abilities to metabolize testosterone and chlorpyrifos. *J. Pharmacol. Exp. Ther.* **299**, 825-831.

Dannenberg, L. O. and Edenberg, H. J. (2006) Epigenetics of gene expression in human hepatoma cells: expression profiling the response to inhibition of DNA methylation and histone deacetylation. *BMC Genomics* **19**, 181.

DeLozier, T. C.; Lee, S. C.; Coulter, S. J.; Goh, B. C. and Goldstein, J. A. (2005) Functional characterization of novel allelic variants of CYP2C9 recently discovered in southeast Asians. *J. Pharmacol. Exp. Ther.* **315**, 1085-1090.

De Morais, S. M.; Wilkinson, G. R.; Blaisdell, J.; Nakamura, K.; Meyer, U. A. and Goldstein, J. A. (1994a) The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. *J. Biol. Chem.* **269**, 15419-15422.

De Morais, S. M.; Wilkinson, G. R.; Blaisdell, J.; Meyer, U. A.; Nakamura, K. and Goldstein, J. A. (1994b) Identification of a new genetic defect responsible for the polymorphism of (S)-mephenytoin metabolism in Japanese. *Mol. Pharmacol.* **46**, 594-598.

Desta, Z.; Zhao, X.; Shin, J. -G. and Flockhart, D. A. (2002) Clinical significance of the cytochrome P450 2C19 genetic polymorphism. *Clin. Pharmacokinet.* **41**, 913-958.

Dickmann, L. J.; Rettie, A. E.; Kneller, M. B.; Kim, R. B.; Wood, A. J.; Stein, C. M.; Wilkinson, G. R. and Schwarz, U. I. (2001) Identification and functional characterization of a new CYP2C9 variant (CYP2C9*5) expressed among African Americans. *Mol. Pharmacol.* **60**, 382-387.

Drocourt, L.; Ourlin, J. C.; Pascussi, J. M.; Maurel, P. and Vilarem, M. J. (2002) Expression of CYP3A4, CYP2B6, and CYP2C9 is regulated by the vitamin D receptor pathway in primary human hepatocytes. *J. Biol. Chem.* **277**, 25125-25132.

Droll, K.; Bruce-Mensah, K.; Otton, S. V.; Gaedigk, A.; Sellers, E. M. and Tyndale, R. F. (1998) Comparison of three CYP2D6 probe substrates and genotype in Ghanaians, Chinese and Caucasians. *Pharmacogenetics* **8**, 325-333.

Ebisawa, A.; Hiratsuka, M.; Sakuyama, K.; Konno, Y.; Sasaki, T. and Mizugaki, M. (2005) Two novel single nucleotide polymorphisms (SNPs) of the CYP2D6 gene in Japanese individuals. *Drug Metab. Pharmacokinet.* **20**, 294-299.

Ehmer, U.; Vogel, A.; Schutte, J. K.; Krone, B.; Manns, M. P. and Strassburg, C. P. (2004) Variation of hepatic glucuronidation: Novel functional polymorphisms of the UDP-glucuronosyltransferase UGT1A4. *Hepatology* **39**, 970-977.

Eiselt, R.; Domanski, T. L.; Zibat, A.; Mueller, R.; Presecan-Siedel, E.; Hustert, E.; Zanger, U. M.; Brockmoller, J.; Klenk, H. P.; Meyer, U. A.; Khan, K. K.; He, Y. A.; Halpert, J. R. and Wojnowski, L. (2001) Identification and functional characterization of eight CYP3A4 protein variants. *Pharmacogenetics* **11**, 447-458.

Evans, W. E. and Relling, M. V. (1999) Pharmacogenomics: translating functional genomics into rational therapeutics. *Science* **286**, 487-491.

Ferguson, R. J.; De Morais, S. M.; Benhamou, S.; Bouchardy, C.; Blaisdell, J.; Ibeanu, G.; Wilkinson, G. R.; Sarich, T. C.; Wright, J. M.; Dayer, P. and Goldstein, J. A. (1998) A new genetic defect in human CYP2C19: mutation of the initiation codon is responsible for poor metabolism of S-mephenytoin. *J. Pharmacol. Exp. Ther.* **284**, 356-361.

Fertrin, K. Y.; Goncalves, M. S.; Saad, S. T. and Costa, F. F. (2002) Frequencies of UDP-glucuronosyltransferase 1 (UGT1A1) gene promoter polymorphisms among distinct ethnic groups from Brazil. *Am. J. Med. Genet.* **108**, 117-119.

Finel, M.; Li, X.; Gardner-Stephen, D.; Bratton, S.; Mackenzie, P. I. and Radominska-Pandya, A. (2005) Human UDP-glucuronosyltransferase 1A5: identification, expression, and activity. *J. Pharmacol. Exp. Ther.* **315**, 1143-1149.

Fukuda, T.; Nishida, Y.; Imaoka, S.; Hiroi, T.; Naohara, M.; Funae, Y. and Azuma, J. (2000) The decreased *in vivo* clearance of CYP2D6 substrates by CYP2D6*10 might be caused not only by the low-expression but also by low affinity of CYP2D6. *Arch. Biochem. Biophys.* **380**, 303-308.

Fukuda, T.; Maune, H.; Ikenaga, Y.; Naohara, M.; Fukuda, K. and Azuma, J. (2005) Novel structure of the CYP2D6 gene that confuses geno-

typing for the CYP2D6*5 allele. *Drug Metab. Pharmacokinet.* **20**, 345-350.

Fukuen, S.; Fukuda, T.; Maune, H.; Ikenaga, Y.; Yamamoto, I.; Inaba, T. and Azuma, J. (2002) Novel detection assay by PCR-RFLP and frequency of the CYP3A5 SNPs, CYP3A5*3 and *6, in a Japanese population. *Pharmacogenetics* **12**, 331-334.

Fukushima-Uesaka, H.; Saito, Y.; Watanabe, H.; Shiseki, K.; Saeki, M.; Nakamura, T.; Kurose, K.; Sai, K.; Komamura, K.; Ueno, K.; Kamakura, S.; Kitakaze, M.; Hanai, S.; Nakajima, T.; Matsumoto, K.; Saito, H.; Goto, Y.; Kimura, H.; Katoh, M.; Sugai, K.; Minami, N.; Shirao, K.; Tamura ,T.; Yamamoto, N.; Minami, H.; Ohtsu, A.; Yoshida, T.; Saijo, N.; Kitamura, Y.; Kamatani, N.; Ozawa, S. and Sawada, J. (2004) Haplotypes of CYP3A4 and their close linkage with CYP3A5 haplotypes in a Japanese population. *Hum. Mutat.* **23**, 100.

Fukushima-Uesaka, H.; Saito, Y.; Maekawa, K.; Ozawa, S.; Hasegawa, R.; Kajio, H.; Kuzuya, N.; Yasuda, K.; Kawamoto, M.; Kamatani, N.; Suzuki, K.; Yanagawa, T.; Tohkin, M. and Sawada, J. (2005) Genetic variations and haplotypes of CYP2C19 in a Japanese population. *Drug Metab. Pharmacokinet.* **20**, 300-307.

Furuta, T.; Shirai, N.; Sugimoto, M.; Nakamura, A.; Hishida, A. and Ishizaki, T. (2005) Influence of CYP2C19 pharmacogenetic polymorphism on proton pump inhibitor-based therapies. *Drug Metab. Pharmacokinet.* **20**, 153-167.

Gaedigk, A.; Bradford, L. D.; Alander, S. W. and Leeder, J. S. (2006) CYP2D6*36 gene arrangements within the CYP2D6 locus: association of CYP2D6*36 with poor metabolizer status. *Drug Metab. Dispos.* **34**, 563-569.

Gagne, J. F.; Montminy, V.; Belanger, P.; Journault, K.; Gaucher, G. and Guillemette, C. (2002) Common human UGT1A polymorphisms and the altered metabolism of irinotecan active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38). *Mol. Pharmacol.* **62**, 608-617.

Garcia-Barcelo, M.; Chow, L. Y.; Kum Chiu, H. F.; Wing, Y. K.; Shing Lee, D. T.; Lam, K. L. and Waye, M. M. (1999) Frequencies of defective CYP2C19 alleles in a Hong Kong Chinese population: detection of the rare allele CYP2C19*4. *Clin. Chem.* **45**, 2273-2274.

Garcia-Barcelo, M.; Chow, L. Y.; Chiu, H. F.; Wing, Y. K.; Lee, D. T.; Lam, K. L. and Waye, M. M. (2000a) Genetic analysis of the CYP2D6 locus in a Hong Kong Chinese population. *Clin. Chem.* **46**, 18-23.

Garcia-Barcelo, M.; Chow, L. Y.; Lam, K. L.; Chiu, H. F.; Wing, Y. K. and Waye, M. M. (2000b) Occurrence of CYP2D6 gene duplication in Hong Kong Chinese. *Clin. Chem.* **46**, 1411-1413.

Garcia-Martin, E.; Martinez, C.; Ladero, J. M. and Agundez, J. A. (2006) Interethnic and intraethnic variability of CYP2C8 and CYP2C9 polymorphisms in healthy individuals. *Mol. Diagn. Ther.* **10**, 29-40.

Garsa, A. A.; McLeod, H. L. and Marsh, S. (2005) CYP3A4 and CYP3A5 genotyping by Pyrosequencing. *BMC Med. Genet.* **6**, 19.

Gellner, K.; Eiselt, R.; Hustert, E.; Arnold, H.; Koch, I.; Haberl, M.; Deglmann, C. J.; Burk, O.; Buntefuss, D.; Escher, S.; Bishop, C.; Koebe, H. G.; Brinkmann, U.; Klenk, H. P.; Kleine, K.; Meyer, U. A. and Wojnowski, L. (2001) Genomic organization of the human CYP3A locus: identification of a new, inducible CYP3A gene. *Pharmacogenetics* **11**, 111-121.

Goldstein, J. A. and de Morais, S. M. (1994) Biochemistry and molecular biology of the human CYP2C subfamily. *Pharmacogenetics* **4**, 285-299.

Goodwin, B.; Hodgson, E. and Liddle, C. (1999) The orphan human pregnane X receptor mediates the transcriptional activation of CYP3A4 by rifampicin through a distal enhancer module. *Mol. Pharmacol.* **56**, 1329-1339.

Goodwin, B.; Hodgson, E.; D'Costa, D. J.; Robertson, G. R. and Liddle, C. (2002) Transcriptional regulation of the human CYP3A4 gene by the constitutive androstane receptor. *Mol. Pharmacol.* **62**, 359-365.

Grant D. M. (2005) Technologies for the analysis of single nucleotide polymorphisms-an overview. In *Pharmacogenomics: Second Edition*; Kalow, W.; Meyer, U. A.; Tyndale, R. F.; Eds.; Taylor and Francis Group: Boca Raton, FL, pp. 305-312.

Griese, E. -U.; Zanger, U. M.; Brudermanns, U; Gaedigk, A.; Mikus, G.; Morike, K.; Stuven, T. and Eichelbaum, M. (1998) Assessment of the predictive power of genotypes for the in-vivo catalytic function of CYP2D6 in a German population. *Pharmacogenetics* **8**, 5-26.

Guengerich, F. P. (1999) Cytochrome P-450 3A4: regulation and role in drug metabolism. *Annu. Rev. Pharmacol. Toxicol.* **39**, 1-17.

Guillemette, C.; Millikan, R. C.; Newman, B. and Housman, D. E. (2000a) Genetic polymorphisms in uridine diphospho-glucuronosyltransferase 1A1 and association with breast cancer among African Americans. *Cancer Res.* **60**, 950-956.

Guillemette, C.; Ritter, J. K.; Auyeung, D. J.; Kessler, F. K. and Housman, D. E. (2000b) Structural heterogeneity at the UDP-glucuronosyltransferase 1 locus: functional consequences of three novel missense mutations in the human UGT1A7 gene. *Pharmacogenetics* **10**, 629-644.

Guillemette, C. (2003) Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. *Pharmacogenomics J.* **3**, 136-158.

Guo, Y.; Zhang, Y.; Wang, Y.; Chen, X.; Si, D.; Zhong, D.; Fawcett, J. P. and Zhou, H. (2005a) Role of CYP2C9 and its variants (CYP2C9*3 and CYP2C9*13) in the metabolism of lornoxicam in humans. *Drug Metab. Dispos.* **33**, 749-753.

Guo, Y.; Wang, Y.; Si, D.; Fawcett, P. J.; Zhong, D. and Zhou, H. (2005b) Catalytic activities of human cytochrome P450 2C9*1, 2C9*3 and 2C9*13. *Xenobiotica* **35**, 853-861.

Hall, D.; Ybazeta, G.; Destro-Bisol, G.; Petzl-Erler, M. L. and Di Rienzo, A. (1999) Variability at the uridine diphosphate glucuronosyltransferase 1A1 promoter in human populations and primates. *Pharmacogenetics* **9**, 591-599.

Han, J. Y.; Lim, H. S.; Shin, E. S.; Yoo, Y. K.; Park, Y. H.; Lee, J. E.; Jang, I. J.; Lee, D. H. and Lee, J. S. (2006) Comprehensive analysis of UGT1A polymorphisms predictive for pharmacokinetics and treatment outcome in patients with non-small-cell lung cancer treated with irinotecan and cisplatin. *J. Clin. Oncol.* **24**, 2237-2244.

Handschin, C. and Meyer, U. A. (2003) Induction of drug metabolism: the role of nuclear receptors. *Pharmacol. Rev.* **55**, 649-673.

Hanioka, N.; Okumura, Y.; Saito, Y.; Hichiya, H.; Soyama, A.; Saito, K.; Ueno, K.; Sawada, J. and Narimatsu, S. (2006). Catalytic roles of CYP2D6.10 and CYP2D6.36 enzymes in mexiletine metabolism: *in vitro* functional analysis of recombinant proteins expressed in *Saccharomyces cerevisiae*. *Biochem. Pharmacol.* **71**, 1386-1395.

Herman, D.; Peternel, P.; Stegnar, M.; Breskvar, K. and Dolzan, V. (2006) A novel sequence variant in exon 7 of CYP2C9 gene (CYP2C9*24) in a patient on warfarin therapy. *Thromb. Haemost.* **95**, 192-194.

Hersberger, M.; Marti-Jaun, J.; Rentsh, K. and Hanseler, E. (2000) Rapid detection of the CYP2D6*3, CYP2D6*4, and CYP2D6*6 alleles by tetra-primer PCR and of the CYP2D6*5 allele by multiplex long PCR. *Clin. Chem.* **46**, 1072-1077.

Hesselink, D. A.; van Gelder, T.; van Schaik, R. H.; Balk, A. H.; van der Heiden, I. P.; van Dam, T.; van der Werf, M.; Weimar, W. and Mathot, R. A. (2004) Population pharmacokinetics of cyclosporine in kidney and heart transplant recipients and the influence of ethnicity and genetic polymorphisms in the MDR-1, CYP3A4, and CYP3A5 genes. *Clin. Pharmacol. Ther.* **76**, 545-556.

Higashi, M. K.; Veenstra, D. L.; Kondo, L. M.; Wittkowsky, A. K.; Srinouanprachanh, S. L.; Farin, F. M. and Rettie, A. E. (2002) Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. *JAMA* **287**, 1690-1698.

Hiratsuka, M.; Takekuma, Y.; Endo, N.; Narahara, K.; Hamdy, S. I.; Kishikawa, Y.; Matsuura, M.; Agatsuma, Y.; Inoue, T. and Mizugaki, M. (2002) Allele and genotype frequencies of CYP2B6 and CYP3A5 in the Japanese population. *Eur. J. Clin. Pharmacol.* **58**, 417-421.

Hirota, T.; Ieiri, I.; Takane, H.; Maegawa, S.; Hosokawa, M.; Kobayashi, K.; Chiba, K.; Nanba, E.; Oshimura, M.; Sato, T.; Higuchi, S. and Otsubo, K. (2004) Allelic expression imbalance of the human CYP3A4 gene and individual phenotypic status. *Hum. Mol. Genet.* **13**, 2959-2969.

Hsieh, K. P.; Lin, Y. Y.; Cheng, C. L.; Lai, M. L.; Lin, M. S.; Siest, J. P. and Huang, J. D. (2001) Novel mutations of CYP3A4 in Chinese. *Drug Metab. Dispos.* **29**, 268-273.

Huang, C. S.; Chang, P. F.; Huang, M. J.; Chen, E. S.; Hung, K. L. and Tsou, K. I. (2002) Relationship between bilirubin UDP-glucuronosyl transferase 1A1 gene and neonatal hyperbilirubinemia. *Pediatr. Res.* **52**, 601-605.

Huang, M. J.; Yang, S. S.; Lin, M. S. and Huang, C. S. (2005) Polymorphisms of uridine-diphosphoglucuronosyltransferase 1A7 gene in Taiwan Chinese. *World J. Gastroenterol.* **11**, 797-802.

Hustert, E.; Haberl, M.; Burk, O.; Wolbold, R.; He, Y. Q.; Klein, K.; Nuessler, A. C.; Neuhaus, P.; Klattig, J.; Eiselt, R.; Koch, I.; Zibat, A.; Brockmoller, J.; Halpert, J. R.; Zanger, U. M. and Wojnowski,

L. (2001) The genetic determinants of the CYP3A5 polymorphism. *Pharmacogenetics* **11**, 773-779.

Ibeanu, G. C.; Goldstein, J. A.; Meyer, U.; Benhamou, S.; Bouchardy, C.; Dayer, P.; Ghanayem, B. I. and Blaisdell, J. (1998) Identification of new human CYP2C19 alleles (CYP2C19*6 and CYP2C19*2B) in a Caucasian poor metabolizer of mephenytoin. *J. Pharmacol. Exp. Ther.* **286**, 1490-1495.

Ibeanu, G. C.; Blaisdell, J.; Ferguson, R. J.; Ghanayem, B. I.; Brosen, K.; Benhamou, S.; Bouchardy, C.; Wilkinson, G. R.; Dayer, P. and Goldstein, J. A. (1999) A novel transversion in the intron 5 donor splice junction of CYP2C19 and a sequence polymorphism in exon 3 contribute to the poor metabolizer phenotype for the anticonvulsant drug S-mephenytoin. *J. Pharmacol. Exp. Ther.* **290**, 635-640.

Ieiri, I.; Tainaka, H.; Morita, T.; Hadama, A.; Mamiya, K.; Hayashibara, M.; Ninomiya, H.; Ohmori, S.; Kitada, M.; Tashiro, N.; Higuchi, S. and Otsubo, K. (2000) Catalytic activity of three variants (Ile, Leu, and Thr) at amino acid residue 359 in human CYP2C9 gene and simultaneous detection using single-strand conformation polymorphism analysis. *Ther. Drug Monit.* **22**, 237-244.

Ikenaga, Y.; Fukuda, T.; Fukuda, K.; Nishida, Y.; Naohara, M.; Maune, H. and Azuma, J. (2005) The frequency of candidate alleles for CYP2D6 genotyping in the Japanese population with an additional respect to the -1584C to G substitution. *Drug Metab. Pharmacokinet.* **20**, 113-116.

Imai, J.; Ieiri, I.; Mamiya, K.; Miyahara, S.; Furuumi, H.; Nanba, E.; Yamane, M.; Fukumaki, Y.; Ninomiya, H.; Tashiro, N.; Otsubo, K. and Higuchi, S. (2000) Polymorphism of the cytochrome P450 (CYP) 2C9 gene in Japanese epileptic patients: genetic analysis of the CYP2C9 locus. *Pharmacogenetics* **10**, 85-89.

Ingelman-Sundberg, M. (2005) Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J.* **5**, 6-13.

Innocenti, F.; Grimsley, C.; Das, S.; Ramirez, J.; Cheng, C.; Kuttab-Boulos, H.; Ratain, M. J. and Di Rienzo, A. (2002) Haplotype structure of the UDP-glucuronosyltransferase 1A1 promoter in different ethnic groups. *Pharmacogenetics* **12**, 725-733.

Innocenti, F.; Liu, W.; Chen, P.; Desai, A. A.; Das, S. and Ratain, M. J. (2005) Haplotypes of variants in the UDP-glucuronosyltransferase1A9 and 1A1 genes. *Pharmacogenet. Genomics* **15**, 295-301.

Inomata, S.; Nagashima, A.; Itagaki, F.; Homma, M.; Nishimura, M.; Osaka, Y.; Okuyama, K.; Tanaka, E.; Nakamura, T.; Kohda, Y.; Naito, S.; Miyabe, M. and Toyooka, H. (2005) CYP2C19 genotype affects diazepam pharmacokinetics and emergence from general anesthesia. *Clin. Pharmacol. Ther.* **78**, 647-655.

Inoue, K.; Yamazaki, H.; Imiya, K.; Akasaka, S.; Guengerich, F. P. and Shimada, T. (1997) Relationship between CYP2C9 and 2C19 genotypes and tolbutamide methyl hydroxylation and S-mephenytoin 4'-hydroxylation activities in livers of Japanese and Caucasian populations. *Pharmacogenetics* **7**, 103-113.

Ishiguro, A.; Kubota, T.; Sasaki, H.; Yamada, Y. and Iga, T. (2003) Common mutant alleles of CYP2D6 causing the defect of CYP2D6 enzyme activity in a Japanese population. *Br. J. Clin. Pharmacol.* **55**, 414-415.

Ishiguro, A.; Kubota, T.; Ishikawa, H. and Iga, T. (2004a) Metabolic activity of dextromethorphan O-demethylation in healthy Japanese volunteers carrying duplicated CYP2D6 genes: duplicated allele of CYP2D6*10 does not increase CYP2D6 metabolic activity. *Clin. Chim. Acta* **344**, 201-204.

Ishiguro, A.; Kubota, T.; Sasaki, H. and Iga, T. (2004b) A long PCR assay to distinguish CYP2D6*5 and a novel CYP2D6 mutant allele associated with an 11-kb EcoRI haplotype. *Clin. Chim. Acta* **347**, 217-221.

Iwai, M.; Maruo, Y.; Ito, M.; Yamamoto, K.; Sato, H. and Takeuchi, Y. (2004) Six novel UDP-glucuronosyltransferase (UGT1A3) polymorphisms with varying activity. *J. Hum. Genet.* **49**, 123-128.

Ji, L.; Pan, S.; Marti-Jaun, J.; Hanseler, E.; Rentsch, K. and Hersberger, M. (2002a) Single-step assays to analyze CYP2D6 gene polymorphisms in Asians: allele frequencies and a novel *14B allele in mainland Chinese. *Clin. Chem.* **48**, 983-988.

Ji, L.; Pan, S.; Wu, J.; Marti-Jaun, J. and Hersberger, M. (2002b) Genetic polymorphisms of CYP2D6 in Chinese mainland. *Chin. Med. J.* **115**, 1780-1784.

Jinno, H.; Tanaka-Kagawa, T.; Hanioka, N.; Saeki, M.; Ishida, S.; Nishimura, T.; Ando, M.; Saito, Y.; Ozawa, S. and Sawada, J. (2003a) 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.

Jinno, H.; Saeki, M.; Tanaka-Kagawa, T.; Hanioka, N.; Saito, Y.; Ozawa, S.; Ando, M.; Shirao, K.; Minami, H.; Ohtsu, A.; Yoshida, T.; Saijo, N. and Sawada, J. (2003b) Functional characterization of wild-type and variant (T202I and M59I) human UDP-glucuronosyltransferase 1A10. *Drug Metab. Dispos.* **31**, 528-532.

Johansson, I.; Oscarson, M.; Yue, Q. Y.; Bertilsson, L.; Sjoqvist, F. and Ingelman-Sundberg, M. (1994) Genetic analysis of the Chinese cytochrome P4502D locus: characterization of variant CYP2D6 genes present in subjects with diminished capacity for debrisoquine hydroxylation. *Mol. Pharmacol.* **46**, 452-459.

Judson, R.; Stephens, J. C. and Windemuth, A. (2000) The predictive power of haplotypes in clinical response. *Pharmacogenomics* **1**, 15-26.

Kagimoto, M.; Heim, M.; Kagimoto, K.; Zeugin, T. and Meyer, U. A. (1990) Multiple mutations of the human cytochrome P450IID6 gene (CYP2D6) in poor metabolizers of debrisoquine. Study of the functional significance of individual mutations by expression of chimeric genes. *J. Biol. Chem.* **265**, 17209-17214.

Kanai, M.; Kijima, K.; Shirahata, E.; Sasaki, A.; Akaba, K.; Umetsu, K.; Tezuka, N.; Kurachi, H.; Aikawa, S. and Hayasaka, K. (2005) Neonatal hyperbilirubinemia and the bilirubin uridine diphosphate-glucuronosyltransferase gene: the common -3263T > G mutation of phenobarbital response enhancer module is not associated with the neonatal hyperbilirubinemia in Japanese. *Pediatr. Int.* **47**, 137-141.

Kaniwa, N.; Kurose, K.; Jinno, H.; Tanaka-Kagawa, T.; Saito, Y.; Saeki, M.; Sawada, J.; Tohkin, M. and Hasegawa, R. (2005) Racial variability in haplotype frequencies of UGT1A1 and glucuronidation activity of a novel single nucleotide polymorphism 686C> T (P229L) found in an African-American. *Drug Metab. Dispos.* **33**, 458-465.

Ki, C. S.; Lee, K. A.; Lee, S. Y.; Kim, H. J.; Cho, S. S.; Park, J. H.; Cho, S.; Sohn, K. M. and Kim, J. W. (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.

Kidd, R. S.; Curry, T. B.; Gallagher, S.; Edeki, T.; Blaisdell, J. and Goldstein, J. A. (2001) Identification of a null allele of CYP2C9 in an African-American exhibiting toxicity to phenytoin. *Pharmacogenetics* **11**, 803-808.

King, B. P.; Khan, T. I.; Aithal, G. P.; Kamali, F. and Daly, A. K. (2004) Upstream and coding region CYP2C9 polymorphisms: correlation with warfarin dose and metabolism. *Pharmacogenetics* **14**, 813-822.

Kirchheiner, J.; Nickchen, K.; Bauer, M.; Wong, M. L.; Licinio, J.; Roots, I. and Brockmoller, J. (2004) Pharmacogenetics of antidepressants and antipsychotics: the contribution of allelic variations to the phenotype of drug response. *Mol. Psychiatry* **9**, 442-473.

Kirchheiner, J. and Brockmoller, J. (2005) Clinical consequences of cytochrome P450 2C9 polymorphisms. *Clin. Pharmacol. Ther.* **77**, 1-16.

Kittles, R. A.; Chen, W.; Panguluri, R. K.; Ahaghotu, C.; Jackson, A.; Adebamowo, C. A.; Griffin, R.; Williams, T.; Ukoli, F.; Adams-Campbell, L.; Kwagyan, J.; Isaacs, W.; Freeman, V. and Dunston, G. M. (2002) CYP3A4-V and prostate cancer in African Americans: causal or confounding association because of population stratification? *Hum. Genet.* **110**, 553-560.

Kohle, C.; Mohrle, B.; Munzel, P. A.; Schwab, M.; Wernet, D.; Badary, O. A. and Bock, K. W. (2003) Frequent co-occurrence of the TATA box mutation associated with Gilbert's syndrome (UGT1A1*28) with other polymorphisms of the UDP-glucuronosyltransferase-1 locus (UGT1A6*2 and UGT1A7*3) in Caucasians and Egyptians. *Biochem. Pharmacol.* **65**, 1521-1527.

Krishna, D. R. and Shekar, M. S. (2005) Cytochrome P450 3A: genetic polymorphisms and inter-ethnic differences. *Methods Find. Exp. Clin. Pharmacol.* **27**, 559-567.

Krishnaswamy, S.; Hao, Q.; Al-Rohaimi, A.; Hesse, L. M.; von Moltke, L. L.; Greenblatt, D. J. and Court, M. H. (2005) UDP glucuronosyltransferase (UGT) 1A6 pharmacogenetics: II. Functional impact of the three most common nonsynonymous UGT1A6 polymorphisms (S7A, T181A, and R184S). *J. Pharmacol. Exp. Ther.* **313**, 1340-1346.

Kubota, T; Yamaura, Y.; Ohkawa, N.; Hara, H. and Chiba, K. (2000) Frequencies of CYP2D6 mutant alleles in a normal Japanese popula-

tion and metabolic activity of dextromethorphan O-demethylation in different CYP2D6 genotypes. *Br. J. Clin. Pharmacol.* **50**, 31-34.

Kuehl, P.; Zhang, J.; Lin, Y.; Lamba, J.; Assem, M.; Schuetz, J.; Watkins, P. B.; Daly, A.; Wrighton, S. A.; Hall, S. D.; Maurel, P.; Relling, M.; Brimer, C.; Yasuda, K.; Venkataramanan, R.; Strom, S.; Thummel, K.; Boguski, M. S. and Schuetz, E. (2001) Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat. Genet.* **27**, 383-391.

Lamba, J. K.; Lin, Y. S.; Thummel, K.; Daly, A.; Watkins, P. B.; Strom, S.; Zhang, J. and Schuetz, E. G. (2002) Common allelic variants of cytochrome P4503A4 and their prevalence in different populations. *Pharmacogenetics* **12**, 121-132.

Lampe, J. W.; Bigler, J.; Horner, N. K. and Potter, J. D. (1999) UDP-glucuronosyltransferase (UGT1A1*28 and UGT1A6*2) polymorphisms in Caucasians and Asians: relationships to serum bilirubin concentrations. *Pharmacogenetics* **9**, 341-349.

Lee, C. R.; Goldstein, J. A. and Pieper, J. A. (2002) Cytochrome P450 2C9 polymorphisms: a comprehensive review of the *in-vitro* and human data. *Pharmacogenetics* **12**, 251-263.

Lee, S. J. and Goldstein, J. A. (2005) Functionally defective or altered CYP3A4 and CYP3A5 single nucleotide polymorphisms and their detection with genotyping tests. *Pharmacogenomics* **6**, 357-371.

Lee, S. J.; Bell, D. A.; Coulter, S. J.; Ghanayem, B. and Goldstein, J. A. (2005) Recombinant CYP3A4*17 is defective in metabolizing the hypertensive drug nifedipine, and the CYP3A4*17 allele may occur on the same chromosome as CYP3A5*3, representing a new putative defective CYP3A haplotype. *J. Pharmacol. Exp. Ther.* **313**, 302-309.

Lee, S. -Y.; Sohn, K. M.; Ryu, J. Y.; Yoon, Y. R.; Shin, J. G. and Kim, J. -W. (2006a) Sequence-based *CYP2D6* genotyping in the Korean population. *Ther. Drug Monit.* **28**, 382-387.

Lee, S. C.; Ng, S. S.; Oldenburg, J.; Chong, P. Y.; Rost, S.; Guo, J. Y.; Yap, H. L.; Rankin, S. C.; Khor, H. B.; Yeo, T. C.; Ng, K. S.; Soong, R. and Goh, B. C. (2006b) Interethnic variability of warfarin maintenance requirement is explained by VKORC1 genotype in an Asian population. *Clin. Pharmacol. Ther.* **79**, 197-205.

Le Meur, Y.; Djebli, N.; Szelag, J. C.; Hoizey, G.; Toupance, O.; Rerolle, J. P. and Marquet, P. (2006) CYP3A5*3 influences sirolimus oral clearance in *de novo* and stable renal transplant recipients. *Clin. Pharmacol. Ther.* **80**, 51-60.

Lewis, D. F.; Dickins, M.; Weaver, R. J.; Eddershaw, P. J.; Goldfarb, P. S. and Tarbit, M. H. (1998) Molecular modelling of human CYP2C subfamily enzymes CYP2C9 and CYP2C19: rationalization of substrate specificity and site-directed mutagenesis experiments in the CYP2C subfamily. *Xenobiotica* **28**, 235-268.

Liu, C. H.; Peck, K.; Huang, J. D.; Lin, M. S.; Wang, C. H.; Hsu, W. P.; Wang, H. W.; Lee, H. L. and Lai, M. L. (2005) Screening CYP3A single nucleotide polymorphisms in a Han Chinese population with a genotyping chip. *Pharmacogenomics* **6**, 731-747.

Luo, H. R.; Gaedigk, A.; Aloumanis, V. and Wan, Y. J. (2005) Identification of CYP2D6 impaired functional alleles in Mexican Americans. *Eur. J. Clin. Pharmacol.* **61**, 797-802.

Mackenzie, P. I.; Walter Bock, K.; Burchell, B.; Guillemette, C.; Ikushiro, S.; Iyanagi, T.; Miners, J. O.; Owens, I. S. and Nebert, D. W. (2005) Nomenclature update for the mammalian UDP glycosyltransferase (UGT) gene superfamily. *Pharmacogenet. Genomics* **15**, 677-685.

Maekawa, K.; Fukushima-Uesaka, H.; Tohkin, M.; Hasegawa, R.; Kajio, H.; Kuzuya, N.; Yasuda, K.; Kawamoto, M.; Kamatani, N.; Suzuki, K.; Yanagawa, T.; Saito, Y. and Sawada, J. (2006) Four novel defective alleles and comprehensive haplotype analysis of CYP2C9 in Japanese. *Pharmacogenet. Genomics* **16**, 497-514.

Maitland, M. L.; Grimsley, C.; Kuttab-Boulos, H.; Witonsky, D.; Kasza, K. E.; Yang, L.; Roe, B. A. and Di Rienzo, A. (2006) Comparative genomics analysis of human sequence variation in the UGT1A gene cluster. *Pharmacogenomics J.* **6**, 52-62.

Mamiya, K.; Ieiri, I.; Miyahara, S.; Imai, J.; Furuumi, H.; Fukumaki, Y.; Ninomiya, H.; Tashiro, N.; Yamada, H. and Higuchi, S. (1998) Association of polymorphisms in the cytochrome P450 (CYP) 2C19 and 2C18 genes in Japanese epileptic patients. *Pharmacogenetics* **8**, 87-90.

Marcucci, K. A.; Pearce, R. E.; Crespi, C.; Steimel, D. T.; Leeder, J. S. and Gaedigk, A. (2002) Characterization of cytochrome P450 2D6.1 (CYP2D6.1), CYP2D6.2, and CYP2D6.17 activities toward model CYP2D6 substrates dextromethorphan, bufuralol, and debrisoquine. *Drug Metab. Dispos.* **30**, 595-601.

Marez, D.; Legrand, M.; Sabbagh, N.; Guidice, J. M.; Spire, C.; Lafitte, J. J.; Meyer, U. A. and Broly, F. (1997) Polymorphism of the cytochrome P450 CYP2D6 gene in a European population: characterization of 48 mutations and 53 alleles, their frequencies and evolution. *Pharmacogenetics* **7**, 193-202.

Marsh, S. and McLeod, H. L. (2004) Pharmacogenetics of irinotecan toxicity. *Pharmacogenomics* **5**, 835-843.

Masimirembwa, C.; Persson, I.; Bertilsson, L.; Hasler, J. and Ingelman-Sundberg, M. (1996) A novel mutant variant of the CYP2D6 gene (CYP2D6*17) common in a black African population: association with diminished debrisoquine hydxylase activity. *Br. J. Clin. Pharmacol.* **42**, 713-719.

Matsumura, K.; Saito, T.; Takahashi, Y.; Ozeki, T.; Kiyotani, K.; Fujieda, M.; Yamazaki, H.; Kunitoh, H. and Kamataki, T. (2004) Identification of a novel polymorphic enhancer of the human CYP3A4 gene. *Mol. Pharmacol.* **65**, 326-334.

Mitsunaga,Y.; Kubota, T.; Ishiguro, A.; Yamada, Y.; Sasaki, H.; Chiba, K. and Iga, T. (2002) Frequent occurrence of CYP2D6*10 duplication allele in a Japanese population. *Mutat. Res.* **505**, 83-85.

Monaghan, G.; Ryan, M.; Seddon, R.; Hume, R. and Burchell, B. (1996) Genetic variation in bilirubin UPD-glucuronosyltransferase gene promoter and Gilbert's syndrome. *Lancet* **347**, 578-581.

Mori, A.; Maruo, Y.; Iwai, M.; Sato, H. and Takeuchi, Y. (2005) UDP-glucuronosyltransferase 1A4 polymorphisms in a Japanese population and kinetics of clozapine glucuronidation. *Drug Metab. Dispos.* **33**, 672-675.

Morin, S.; Bodin, L.; Loriot, M. A.; Thijssen, H. H.; Robert, A.; Strabach, S.; Verstuyft, C.; Tregouet, D. A.; Dubert, L.; Laurent-Puig, P.; Funck-Brentano, C.; Jaillon, P.; Beaune, P. H. and Becquemont, L. (2004) Pharmacogenetics of acenocoumarol pharmacodynamics. *Clin. Pharmacol. Ther.* **75**, 403-414.

Morita, J.; Kobayashi, K.; Wanibuchi, A.; Kimura, M.; Irie, S.; Ishizaki, T. and Chiba, K. (2004) A novel single nucleotide polymorphism (SNP) of the CYP2C19 gene in a Japanese subject with lowered capacity of mephobarbital 4'-hydroxylation. *Drug Metab. Pharmacokinet.* **19**, 236-238.

Murayama, N.; Nakamura, T.; Saeki, M.; Soyama, A.; Saito, Y.; Sai, K.; Ishida, S.; Nakajima, O.; Itoda, M.; Ohno, Y.; Ozawa, S. and Sawada, J. (2002) CYP3A4 gene polymorphisms influence testosterone 6beta-hydroxylation. *Drug Metab. Pharmacokinet.* **17**, 150-156.

Nakajima, Y.; Yoshitani, T.; Fukushima-Uesaka, H.; Saito, Y.; Kaniwa, N.; Kurose, K.; Ozawa, S.; Aoyagi, N.; Kamatani, N.; Yamamoto, N.; Kunitoh, H.; Ohe, Y.; Tamura, T.; Yoshida, T.; Minami, H.; Saijo, N.; Katori, N. and Sawada, J. (2006) Impact of the haplotype CYP3A4*16B harboring the Thr185Ser substitution on paclitaxel metabolism in Japanese patients with cancer. *Clin. Pharmacol. Ther.* **80**, 179-191.

Naoe, T.; Takeyama, K.; Yokozawa, T.; Kiyoi, H.; Seto, M.; Uike, N.; Ino, T.; Utsunomiya, A.; Maruta, A.; Jin-nai, I.; Kamada, N.; Kubota, Y.; Nakamura, H.; Shimazaki, C.; Horiike, S.; Kodera, Y.; Saito, H.; Ueda, R.; Wiemels, J. and Ohno, R. (2000) Analysis of genetic polymorphism in NQO1, GST-M1, GST-T1, and CYP3A4 in 469 Japanese patients with therapy-related leukemia/ myelodysplastic syndrome and *de novo* acute myeloid leukemia. *Clin. Cancer Res.* **6**, 4091-4095.

Nasu, K.; Kubota, T. and Ishizaki, T. (1997) Genetic analysis of CYP2C9 polymorphism in a Japanese population. *Pharmacogenetics* **7**, 405-409.

Nishida, Y.; Fukuda, T.; Yamamoto, I. and Azuma, J. (2000) CYP2D6 genotypes in a Japanese population: low frequencies of CYP2D6 gene duplication but high frequency of CYP2D6*10. *Pharmacogenetics* **10**, 567-570.

Obayashi, K.; Nakamura, K.; Kawana, J.; Ogata, H.; Hanada, K.; Kurabayashi, M.; Hasegawa, A.; Yamamoto, K. and Horiuchi, R. (2006) VKORC1 gene variations are the major contributors of variation in warfarin dose in Japanese patients. *Clin. Pharmacol. Ther.* **80**, 169-178.

Ozdemir, V.; Kalowa, W.; Tang, B. K.; Paterson, A. D.; Walker, S. E.; Endrenyi, L. and Kashuba, A. D. (2000) Evaluation of the genetic component of variability in CYP3A4 activity: a repeated drug administration method. *Pharmacogenetics* **10**, 373-388.

Pang, C. P.; Zhang, J.; Woo, J.; Chan, D.; Law, L. K.; Tong, S. F.; Kwok, T. and Kay, R. (1998) Rarity of debrisoquine hydroxylase gene polymorphism in Chinese patients with Parkinson's disease. *Mov. Disord.* **13**, 529-532.

Park, J. Y.; Kim, K. A.; Park, P. W.; Lee, O. J.; Kang, D. K.; Shon, J. H.; Liu, K. H. and Shin, J. G. (2006) Effect of CYP3A5*3 genotype on the pharmacokinetics and pharmacodynamics of alprazolam in healthy subjects. *Clin. Pharmacol. Ther.* **79**, 590-599.

Plummer, S. J.; Conti, D. V.; Paris, P. L.; Curran, A. P.; Casey, G. and Witte, J. S. (2003) CYP3A4 and CYP3A5 genotypes, haplotypes, and risk of prostate cancer. *Cancer Epidemiol. Biomarkers Prev.* **12**, 928-932.

Premawardhena, A.; Fisher, C. A.; Liu, Y. T.; Verma, I. C.; de Silva, S.; Arambepola, M.; Clegg, J. B. and Weatherall, D. J. (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.

Radominska-Pandya, A.; Czernik, P. J.; Little, J. M.; Battaglia, E. and Mackenzie, P. I. (1999) Structural and functional studies of UDP-glucuronosyltransferases. *Drug Metab. Rev.* **31**, 817-899.

Raimundo, S.; Fischer, J.; Eichelbaum, M.; Griese, E. U.; Schwab, M. and Zanger, U. M. (2000) Elucidation of the genetic basis of the common 'intermediate metabolizer' phenotype for drug oxidation by CYP2D6. *Pharmacogenetics* **10**, 577-581.

Raimundo, S.; Toscano, C.; Klein, K.; Fischer, J.; Griese, E. U.; Eichelbaum, M.; Schwab, M. and Zanger, U. M. (2004) A novel intronic mutation, 2988G>A, with high predictivity for impaired function of cytochrome P450 2D6 in white subjects. *Clin. Pharmacol. Ther.* **76**, 128-138.

Rebbeck, T. R.; Jaffe, J. M.; Walker, A. H.; Wein, A. J. and Malkowicz, S. B. (1998) Modification of clinical presentation of prostate tumors by a novel genetic variant in CYP3A4. *J. Natl. Cancer Inst.* **90**, 1225-1229.

Rettie, A. E. and Jones, J. P. (2005) Clinical and toxicological relevance of CYP2C9: Drug-drug interactions and pharmacogenetics. *Annu. Rev. Pharmacol. Toxicol.* **45**, 477-494.

Reyes-Hernandez, O. D.; Arteaga-Illan, G. and Elizondo, G. (2004) Detection of CYP3A4*1B and CYP3A4*2 polymorphisms by RFLP. Distribution frequencies in a Mexican population. *Clin. Genet.* **66**, 166-168.

Ritter, J. K.; Kessler, F. K.; Thompson, M. T.; Grove, A. D.; Auyeung, D. J. and Fisher, R. A. (1999) Expression and inducibility of the human bilirubin UDP-glucuronosyltransferase UGT1A1 in liver and cultured primary hepatocytes: evidence for both genetic and environmental influences. *Hepatology* **30**, 476-484.

Rodriguez-Antona, C.; Sayi, J. G.; Gustafsson, L. L.; Bertilsson, L. and Ingelman-Sundberg, M. (2005) Phenotype-genotype variability in the human CYP3A locus as assessed by the probe drug quinine and analyses of variant CYP3A4 alleles. *Biochem. Biophys. Res. Commun.* **338**, 299-305.

Roh, H. K.; Dahl, M. L.; Johansson, I.; Ingelman-Sundberg, M.; Cha, Y. N. and Bertilsson, L. (1996) Debrisoquine and S-mephenytoin hydroxylation phenotypes and genotypes in a Korean population. *Pharmacogenetics* **6**, 441-447.

Roy, J. N.; Lajoie, J.; Zijenah, L. S.; Barama, A.; Poirier, C.; Ward, B. J. and Roger, M. (2005) CYP3A5 genetic polymorphisms in different ethnic populations. *Drug Metab. Dispos.* **33**, 884-887.

Sachse, C.; Brockmoller, J.; Bauer, S. and Roots, I. (1997) Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. *Am. J. Hum. Genet.* **60**, 284-295.

Saeki, M.; Saito, Y.; Nakamura, T.; Murayama, N.; Kim, S. R.; Ozawa, S.; Komamura, K.; Ueno, K.; Kamakura, S.; Nakajima, T.; Saito, H.; Kitamura, Y.; Kamatani, N. and Sawada, J. (2003) Single nucleotide polymorphisms and haplotype frequencies of CYP3A5 in a Japanese population. *Hum. Mutat.* **21**, 653.

Saeki, M.; Saito, Y.; Jinno, H.; Sai, K.; Hachisuka, A.; Kaniwa, N.; Ozawa, S.; Kawamoto, M.; Kamatani, N.; Shirao, K.; Minami, H.; Ohtsu, A.; Yoshida, T.; Saijo, N.; Komamura, K.; Kotake, T.; Morishita, H.; Kamakura, S.; Kitakaze, M.; Tomoike, H. and Sawada, J. (2005) Genetic variations and haplotypes of UGT1A4 in a Japanese population. *Drug Metab. Pharmacokinet.* **20**, 144-151.

Saeki, M.; Saito, Y.; Jinno, H.; Sai, K.; Ozawa, S.; Kurose, K.; Kaniwa, N.; Komamura, K.; Kotake, T.; Morishita, H.; Kamakura, S.; Kitakaze, M.; Tomoike, H.; Shirao, K.; Tamura, T.; Yamamoto, N.; Kunitoh, H.; Hamaguchi, T.; Yoshida, T.; Kubota, K.; Ohtsu, A.; Muto, M.; Minami, H.; Saijo, N.; Kamatani, N. and Sawada, J. (2006) Haplotype structures of the UGT1A gene complex in a Japanese population. *Pharmacogenomics J.* **6**, 63-75.

Sai, K.; Saeki, M.; Saito, Y.; Ozawa, S.; Katori, N.; Jinno, H.; Hasegawa, R.; Kaniwa, N.; Sawada, J.; Komamura, K.; Ueno, K.; Kamakura, S.; Kitakaze, M.; Kitamura, Y.; Kamatani, N.; Minami, H.; Ohtsu, A.; Shirao, K.; Yoshida, T. and Saijo, N. (2004) UGT1A1 haplotypes associated with reduced glucuronidation and increased serum bilirubin in irinotecan-administered Japanese patients with cancer. *Clin. Pharmacol. Ther.* **75**, 501-515.

Sata, F.; Sapone, A.; Elizondo, G.; Stocker, P.; Miller, V. P.; Zheng, W.; Raunio, H.; Crespi, C. L. and Gonzalez, F. J. (2000) CYP3A4 allelic variants with amino acid substitutions in exons 7 and 12: evidence for an allelic variant with altered catalytic activity. *Clin. Pharmacol. Ther.* **67**, 48-56.

Saxena, R.; Shaw, G. L.; Relling, M. V.; Frame, J. N.; Moir, D. T.; Evans, W. E.; Caporaso, N. and Weiffenbach, B. (1994) Idetification of a new variant CYP2D6 allele with a single base deletion in exon 3 and its association with the poor metabolizer phenotype. *Hum. Mol. Genet.* **3**, 923-926.

Schirmer, M.; Toliat, M. R.; Haberl, M.; Suk, A.; Kamdem, L. K.; Klein, K.; Brockmoller, J.; Nurnberg, P.; Zanger, U. M. and Wojnowski, L. (2006) Genetic signature consistent with selection against the CYP3A4*1B allele in non-African populations. *Pharmacogenet. Genomics* **16**, 59-71.

Schwarz, U. I. (2003) Clinical relevance of genetic polymorphisms in the human CYP2C9 gene. *Eur. J. Clin. Invest.* **33**, 23-30.

Scordo, M. G.; Aklillu, E.; Yasar, U.; Dahl, M. L.; Spina, E. and Ingelman-Sundberg, M. (2001) Genetic polymorphism of cytochrome P450 2C9 in a Caucasian and a black African population. *Br. J. Clin. Pharmacol.* **52**, 447-450.

Scott, J. and Poffenbarger, P. L. (1979) Pharmacogenetics of tolbutamide metabolism in humans. *Diabetes* **28**, 41-51.

Shimada, T.; Yamazaki, H.; Mimura, M.; Inui, Y. and Guengerich, F. P. (1994) Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J. Pharmacol. Exp. Ther.* **270**, 414-423.

Shintani, M.; Ieiri, I.; Inoue, K.; Mamiya, K.; Ninomiya, H.; Tashiro, N.; Higuchi, S. and Otsubo, K. (2001) Genetic polymorphisms and functional characterization of the 5'-flanking region of the human CYP2C9 gene: *in vitro* and *in vivo* studies. *Clin. Pharmacol. Ther.* **70**, 175-182.

Si, D.; Guo, Y.; Zhang, Y.; Yang, L.; Zhou, H. and Zhong, D. (2004) Identification of a novel variant CYP2C9 allele in Chinese. *Pharmacogenetics* **14**, 465-469.

Sim, S. C.; Risinger, C.; Dahl, M. L.; Aklillu, E.; Christensen, M.; Bertilsson, L. and Ingelman-Sundberg, M. (2006) A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. *Clin. Pharmacol. Ther.* **79**, 103-113.

Soyama, A.; Saito, Y.; Komamura, K.; Ueno, K.; Kamakura, S.; Ozawa, S. and Sawada, J. (2002) Novel single nucleotide polymorphisms in the CYP2D6 gene associated with CYP2D6*2 and/or CYP2D6*10 alleles. *Drug Metab. Pharmacokinet.* **17**, 475-478.

Soyama, A.; Kubo, T.; Miyajima, A.; Saito, Y.; Shiseki, K.; Komamura, K.; Ueno, K.; Kamakura, S.; Kitakaze, M.; Tomoike, H.; Ozawa; S. and Sawada, J. (2004) Novel nonsynonymous single nucleotide polymorphisms in the CYP2D6 gene. *Drug Metab. Pharmacokinet.* **19**, 313-319.

Soyama, A.; Saito, Y.; Kubo, T.; Miyajima, A.; Ohno, Y.; Komamura, K.; Ueno, K.; Kamakura, S.; Kitakaze, M.; Tomoike, H.; Ozawa, S. and Sawada, J. (2006a) Sequence-based analysis of the CYP2D6*36-CYP2D6*10 tandem-type arrangement, a major CYP2D6*10 haplotype in the Japanese population. *Drug Metab. Pharmacokinet.* **21**, 208-216.

Soyama, A.; Saito, Y.; Ohno, Y.; Komamura, K.; Kamakura, S.; Kitakaze, M.; Tomoike, H.; Ozawa, S. and Sawada, J. (2006b) A novel defective CYP2D6 haplotype harboring single-type *36 and chimeric CYP-REP7/6 and CYP-REP7/6 diversity in Japanese. *Drug Metab. Pharmacokinet.* **21**, 395-405.

Steen, V. M.; Molven, A.; Aarskog, N. K. and Gulbrandsen, A. K. (1995) Homologous unequal cross-over involving a 2.8 kb direct repeat as a mechanism for the generation of allelic variants of human cytochrome P450 CYP2D6 gene. *Hum. Mol. Genet.* **4**, 2251-2257.

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.

Sutomo, R.; Talib, N. A.; Yusoff, N. M.; Van Rostenberghe, H.; Sadewa, A. H.; Sunarti Sofro, A. S.; Yokoyama, N.; Lee, M. J.; Matsuo, M. and Nishio, H. (2004) Screening for G71R mutation of the UGT1A1 gene in the Javanese-Indonesian and Malay-Malaysian populations. *Pediatr. Int.* **46**, 565-569.

Tai, G.; Farin, F.; Rieder, M. J.; Dreisbach, A. W.; Veenstra, D. L.; Verlinde, C. L. and Rettie, A. E. (2005) *In-vitro* and *in-vivo* effects of the CYP2C9*11 polymorphism on warfarin metabolism and dose. *Pharmacogenet. Genomics* **15**, 475-481.

Takanashi, K.; Tainaka, H.; Kobayashi, K.; Yasumori, T.; Hosakawa, M. and Chiba, K. (2000) CYP2C9 Ile359 and Leu359 variants: enzyme kinetic study with seven substrates. *Pharmacogenetics* **10**, 95-104.

Takahashi, H.; Wilkinson, G. R.; Caraco, Y.; Muszkat, M.; Kim, R. B.; Kashima, T.; Kimura, S. and Echizen, H. (2003) Population differences in S-warfarin metabolism between CYP2C9 genotype-matched Caucasian and Japanese patients. *Clin. Pharmacol. Ther.* **73**, 253-263.

Takahashi, H.; Wilkinson, G. R.; Nutescu, E. A.; Morita, T.; Ritchie, M. D.; Scordo, M. G.; Pengo, V.; Barban, M.; Padrini, R.; Ieiri, I.; Otsubo, K.; Kashima, T.; Kimura, S.; Kijima, S. and Echizen, H. (2006) Different contributions of polymorphisms in VKORC1 and CYP2C9 to intra- and inter-population differences in maintenance dose of warfarin in Japanese, Caucasians and African-Americans. *Pharmacogenet. Genomics* **16**, 101-110.

Tate, S. K. and Goldstein, D. B. (2004) Will tomorrow's medicines work for everyone? *Nat. Genet.* **36**, S34-42.

Tateishi, T.; Chida, M.; Ariyoshi, N.; Mizorogi, Y.; Kamataki, T. and Kobayashi, S. (1999) Analysis of the CYP2D6 gene in relation to dextromethorphan O-demethylation capacity in a Japanese population. *Clin. Pharmacol. Ther.* **65**, 570-575.

Tayeb, M. T.; Clark, C.; Ameyaw, M. M.; Haites, N. E.; Evans, D. A.; Tariq, M.; Mobarek, A.; Ofori-Adjei, D. and McLeod, H. L. (2000) CYP3A4 promoter variant in Saudi, Ghanaian and Scottish Caucasian populations. *Pharmacogenetics* **10**, 753-756.

Teh, L. K.; Ismail, R.; Yusoff, R.; Hussein, A.; Isa, M. N. and Rahman, A. R. (2001) Heterogeneity of the CYP2D6 gene among Malays in Malaysia. *J. Clin. Pharm. Ther.* **26**, 205-211.

Thomas, S. S.; Li, S. S.; Lampe, J. W.; Potter, J. D. and Bigler, J. (2006) Genetic variability, haplotypes, and htSNPs for exons 1 at the human UGT1A locus. *Hum. Mutat.* **27**, 717.

Thompson, E. E.; Kuttab-Boulos, H.; Witonsky, D.; Yang, L.; Roe, B. A. and Di Rienzo, A. (2004) CYP3A variation and the evolution of salt-sensitivity variants. *Am. J. Hum. Genet.* **75**, 1059-1069.

Thompson, E. E.; Kuttab-Boulos, H.; Yang, L.; Roe, B. A. and Di Rienzo, A. (2006) Sequence diversity and haplotype structure at the human CYP3A cluster. *Pharmacogenomics J.* **6**, 105-114.

Thummel, K. E. and Wilkinson, G. R. (1998) *In vitro* and *in vivo* drug interactions involving human CYP3A. *Annu. Rev. Pharmacol. Toxicol.* **38**, 389-430.

Tirona, R. G.; Lee, W.; Leake, B. F.; Lan, L. B.; Cline, C. B.; Lamba, V.; Parviz, F.; Duncan, S. A.; Inoue, Y.; Gonzalez, F. J.; Schuetz, E. G. and Kim, R. B. (2003) The orphan nuclear receptor HNF4alpha determines PXR- and CAR-mediated xenobiotic induction of CYP3A4. *Nat. Med.* **9**, 220-224.

Toscano, C.; Klein, K.; Blievernicht, J.; Schaeffeler, E.; Saussele, T.; Raimundo, S.; Eichelbaum, M.; Schwab, M. and Zanger, U. M. (2006) Impaired expression of CYP2D6 in intermediate metabolizers carrying the *41 allele caused by the intronic SNP 2988G>A: evidence for modulation of splicing events. *Pharmacogenet. Genomics* **16**, 755-766.

Tracy, T. S.; Hutzler, J. M.; Haining, R. L.; Rettie, A. E.; Hummel, M. A. and Dickmann, L. J. (2002) Polymorphic variants (CYP2C9*3 and CYP2C9*5) and the F114L active site mutation of CYP2C9: effect on atypical kinetic metabolism profiles. *Drug Metab. Dispos.* **30**, 385-390.

Tsuzuki, D.; Takemi, C.; Yamamoto, S.; Tamagake, K.; Imaoka, S.; Funae, Y.; Kataoka, H.; Shinoda, S. and Narimatsu, S. (2001) Functional evaluation of cytochrome P450 2D6 with Gly42Arg substitution expressed in *Saccharomyces cerevisiae*. *Pharmacogenetics* **11**, 709-718.

Tukey, R. H. and Strassburg, C. P. (2000) Human UDP-glucuronosyltransferases: metabolism, expression, and disease. *Annu. Rev. Pharmacol. Toxicol.* **40**, 581-616.

Tyndale, R.; Aoyama, T.; Broly, F.; Matsunaga, T.; Inaba, T.; Kalow, W.; Gelboin, H. V.; Meyer, U. A. and Gonzalez, F. J. (1991) Identification of a new variant CYP2D6 allele lacking the codon encoding Lys-281: possible association with the poor metabolizer phenotype. *Pharmacogenetics* **1**, 26-32.

van Schaik, R. H.; de Wildt, S. N.; van Iperen, N. M.; Uitterlinden, A. G.; van den Anker, J. N. and Lindemans, J. (2000) CYP3A4-V polymorphism detection by PCR-restriction fragment length polymorphism analysis and its allelic frequency among 199 Dutch Caucasians. *Clin. Chem.* **46**, 1834-1836.

van Schaik, R. H.; van der Heiden, I. P.; van den Anker, J. N. and Lindemans, J. (2002) CYP3A5 variant allele frequencies in Dutch Caucasians. *Clin. Chem.* **48**, 1668-1671.

Veenstra, D. L.; Blough, D. K.; Higashi, M. K.; Farin, F. M.; Srinouanprachan, S.; Rieder, M. J. and Rettie, A. E. (2005) CYP2C9 haplotype structure in European American warfarin patients and association with clinical outcomes. *Clin. Pharmacol. Ther.* **77**, 353-364.

Villeneuve, L.; Girard, H.; Fortier, L. C.; Gagne, J. F. and Guillemette, C. (2003) Novel functional polymorphisms in the UGT1A7 and UGT1A9 glucuronidating enzymes in Caucasian and African-American subjects and their impact on the metabolism of 7-ethyl-10-hydroxycamptothecin and flavopiridol anticancer drugs. *J. Pharmacol. Exp. Ther.* **307**, 117-128.

Walker, A. H.; Jaffe, J. M.; Gunasegaram, S.; Cummings, S. A.; Huang, C. S.; Chern, H. D.; Olopade, O. I.; Weber, B. L. and Rebbeck, T. R. (1998) Characterization of an allelic variant in the nifedipine-specific element of CYP3A4: ethnic distribution and implications for prostate cancer risk. *Hum. Mutat.* **12**, 289.

Walton, R.; Kimber, M.; Rockett, K.; Trafford, C.; Kwiatkowski, D. and Sirugo, G. (2005) Haplotype block structure of the cytochrome P450 CYP2C gene cluster on chromosome 10. *Nat. Genet.* **37**, 915-916.

Wandel, C.; Witte, J. S.; Hall, J. M.; Stein, C. M.; Wood, A. J. and Wilkinson, G. R. (2000) CYP3A activity in African American and European American men: population differences and functional effect of the CYP3A4*1B5'-promoter region polymorphism. *Clin. Pharmacol. Ther.* **68**, 82-91.

Wang, S. -L.; Huang, J. -d.; Lai, M. -D.; Liu, B. -H. and Lai, M. -L. (1993) Molecular basis of genetic variation in debrisoquin hydroxylation in Chinese subjects: Polymorphism in RFLP and DNA sequence of CYP2D6. *Clin. Pharmacol. Ther.* **53**, 410-418.

Wang, S. L.; Huang, J.; Lai, M. D. and Tsai, J. J. (1995) Detection of CYP2C9 polymorphism based on the polymerase chain reaction in Chinese. *Pharmacogenetics* **5**, 37-42.

Wang, A.; Yu, B. N.; Luo, C. H.; Tan, Z. R.; Zhou, G.; Wang, L. S.; Zhang, W.; Li, Z.; Liu, J. and Zhou, H. H. (2005) Ile118Val genetic polymorphism of CYP3A4 and its effects on lipid-lowering efficacy of simvastatin in Chinese hyperlipidemic patients. *Eur. J. Clin. Pharmacol.* **60**, 843-848.

Wen, S.; Wang, H.; Ding, Y.; Liang, H. and Wang, S. (2004) Screening of 12 SNPs of CYP3A4 in a Chinese population using oligonucleotide microarray. *Genet. Test* **8**, 411-416.

Wennerholm, A.; Johansson, I.; Hidestrand, M.; Bertilsson, L.; Gustafsson, L. L. and Ingelman-Sundberg, M. (2001) Characterization of the CYP2D6*29 allele commonly present in a black Tanzanian population causing reduced catalytic activity. *Pharmacogenetics* **11**, 417-427.

Wennerholm, A.; Dandara, C.; Sayi, J.; Svenson, J. -O.; Abdi, Y. A.; Ingelman-Sundberg, M.; Bertilsson, L.; Hasler, J. and Gustufsson, L. L. (2002) The African-specific CYP2D6*17 allele encodes an enzyme with changed substrate specificity. *Clin. Pharmacol. Ther.* **71**, 77-88.

Westlind, A.; Lofberg, L.; Tindberg, N.; Andersson, T. B. and Ingelman-Sundberg, M. (1999) Interindividual differences in hepatic expression of CYP3A4: relationship to genetic polymorphism in the 5'-upstream regulatory region. *Biochem. Biophys. Res. Commun.* **259**, 201-205.

Westlind-Johnsson, A.; Hermann, R.; Huennemeyer, A.; Hauns, B.; Lahu, G.; Nassr, N.; Zech, K.; Ingelman-Sundberg, M. and von Richter, O. (2006) Identification and characterization of CYP3A4*20, a

novel rare CYP3A4 allele without functional activity. *Clin. Pharmacol. Ther.* **79**, 339-349.

Wong, M.; Balleine, R. L.; Collins, M.; Liddle, C.; Clarke, C. L. and Gurney, H. (2004) CYP3A5 genotype and midazolam clearance in Australian patients receiving chemotherapy. *Clin. Pharmacol. Ther.* **75**, 529-538.

Wrighton, S. A.; VandenBranden, M. and Ring, B. J. (1996) The human drug metabolizing cytochromes P450. *J. Pharmacokinet. Biopharm.* **24**, 461-473.

Xiao, Z. S.; Goldstein, J. A.; Xie, H. G.; Blaisdell, J.; Wang, W.; Jiang, C. H.; Yan, F. X.; He, N.; Huang, S. L.; Xu, Z. H. and Zhou, H. H. (1997) Differences in the incidence of the CYP2C19 polymorphism affecting the S-mephenytoin phenotype in Chinese Han and Bai populations and identification of a new rare CYP2C19 mutant allele. *J. Pharmacol. Exp. Ther.* **281**, 604-609.

Yamanaka, H.; Nakajima, M.; Katoh, M.; Hara, Y.; Tachibana, O.; Yamashita, J.; McLeod, H. L. and Yokoi, T. (2004) A novel polymorphism in the promoter region of human UGT1A9 gene (UGT1A9*22) and its effects on the transcriptional activity. *Pharmacogenetics* **14**, 329-332.

Yamazaki, H.; Kiyotani, K.; Tsubuko, S.; Matsunaga, M.; Fujieda, M.; Saito, T.; Miura, J.; Kobayashi, S. and Kamataki, T. (2003) Two novel haplotypes of CYP2D6 gene in a Japanese population. *Drug Metab. Pharmacokinet.* **18**, 269-271.

Yasar, U.; Aklillu, E.; Canaparo, R.; Sandberg, M.; Sayi, J.; Roh, H. K. and Wennerholm, A. (2002a) Analysis of CYP2C9*5 in Caucasian, Oriental and black-African populations. *Eur. J. Clin. Pharmacol.* **58**, 555-558.

Yasar, U.; Lundgren, S.; Eliasson, E.; Bennet, A.; Wiman, B.; de Faire, U. and Rane, A. (2002b) Linkage between the CYP2C8 and CYP2C9 genetic polymorphisms. *Biochem. Biophys. Res. Commun.* **299**, 25-28.

Yokoi, T.; Kosaka, Y.; Chida, M.; Chiba, K.; Nakamura, H.; Ishizaki, T.; Kinoshita, M.; Sato, K.; Gonzalez, F. J. and Kamataki, T. (1996) A new CYP2D6 allele with a nine base insertion in exon 9 in a Japanese population associated with poor metabolizer phenotype. *Pharmacogenetics* **6**, 395-401.

Yokota, H.; Tamura, S.; Furuya, H.; Kimura, S.; Watanabe, M.; Kanazawa, I.; Kondo, I. and Gonzalez, F. J. (1993) Evidence for a new variant CYP2D6 allele CYP2D6J in a Japanese population associated with lower *in vivo* rates of sparteine metabolism. *Pharmacogenetics* **3**, 256-263.

Yoon, Y. -R.; Cha, I. -J.; Shon, J. -H.; Kim, K. -A.; Cha, Y. -N.; Jang, I. -J.; Park, C. -W.; Shin, S. -G.; Flockhart, D. A. and Shin, J. -G. (2000) Relationship of paroxetine disposition to metoprorol metabolic ratio and *CYP2D6*19* genotype of Korean subjects. *Clin. Pharmacol. Ther.* **67**, 567-576.

Yoon, Y. R.; Shon, J. H.; Kim, M. K.; Lim, Y. C.; Lee, H. R.; Park, J. Y.; Cha, I. J. and Shin, J. G. (2001) Frequency of cytochrome P450 2C9 mutant alleles in a Korean population. *Br. J. Clin. Pharmacol.* **51**, 277-280.

Yusoff, S.; Van Rostenberghe, H.; Yusoff, N. M.; Talib, N. A.; Ramli, N.; Ismail, N. Z.; Ismail, W. P.; Matsuo, M. and Nishio, H. (2006) Frequencies of A(TA)7TAA, G71R, and G493R mutations of the UGT1A1 gene in the Malaysian population. *Biol. Neonate* **89**, 171-176.

Zanger, U. M.; Fischer, J.; Raimundo, S.; Stuven, T.; Evert, B. O.; Schwab, M. and Eichelbaum, M. (2001) Comprehensive analysis of the genetic factors determining expression and function of hepatic CYP2D6. *Pharmacogenetics* **11**, 573-585.

Zeigler-Johnson, C. M.; Walker, A. H.; Mancke, B.; Spangler, E.; Jalloh, M.; McBride, S.; Deitz, A.; Malkowicz, S. B.; Ofori-Adjei, D.; Gueye, S. M. and Rebbeck, T. R. (2002) Ethnic differences in the frequency of prostate cancer susceptibility alleles at SRD5A2 and CYP3A4. *Hum. Hered.* **54**, 13-21.

Zeigler-Johnson, C.; Friebel, T.; Walker, A. H.; Wang, Y.; Spangler, E.; Panossian, S.; Patacsil, M.; Aplenc, R.; Wein, A. J.; Malkowicz, S. B. and Rebbeck, T. R. (2004) CYP3A4, CYP3A5, and CYP3A43 genotypes and haplotypes in the etiology and severity of prostate cancer. *Cancer Res.* **64**, 8461-8467.

Zhao, F.; Loke, C.; Rankin, S. C.; Guo, J. Y.; Lee, H. S.; Wu, T. S.; Tan, T.; Liu, T. C.; Lu, W. L.; Lim, Y. T.; Zhang, Q.; Goh, B. C. and Lee, S. C. (2004) Novel CYP2C9 genetic variants in Asian subjects and their influence on maintenance warfarin dose. *Clin. Pharmacol. Ther.* **76**, 210-219.

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Functional characterization of two novel CYP2C19 variants (*CYP2C19*18* and *CYP2C19*19*) found in a Japanese population

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Abstract

Cytochrome P450 2C19 (CYP2C19) plays an important role in the metabolism of a wide range of therapeutic drugs and exhibits genetic polymorphism with interindividual differences in metabolic activity. We have previously described two CYP2C19 allelic variants, namely *CYP2C19*18* and *CYP2C19*19* with Arg329His/Ile331Val and Ser51Gly/Ile331Val substitutions, respectively. In order to investigate precisely the effect of amino acid substitutions on CYP2C19 function, CYP2C19 proteins of the wild-type (CYP2C19.1B having Ile331Val) and variants (CYP2C19.18 and CYP2C19.19) were heterologously expressed in yeast cells, and their *S*-mephenytoin 4′-hydroxylation activities were determined. The K_m value of CYP2C19.19 for *S*-mephenytoin 4′-hydroxylation was significantly higher (3.0-fold) than that of CYP2C19.1B. Although no significant differences in V_{max} values on the basis of microsomal and functional CYP protein levels were observed between CYP2C19.1B and CYP2C19.19, the V_{max}/K_m values of CYP2C19.19 were significantly reduced to 29–47% of CYP2C19.1B. By contrast, the K_m, V_{max} or V_{max}/K_m values of CYP2C19.18 were similar to those of CYP2C19.1B. These results suggest that Ser51Gly substitution in CYP2C19.19 decreases the affinity toward *S*-mephenytoin of CYP2C19 enzyme, and imply that the genetic polymorphism of *CYP2C19*19* also causes variations in the clinical response to drugs metabolized by CYP2C19.

Keywords: *CYP2C19, genetic polymorphism, CYP2C19*18, CYP2C19*19, S-mephenytoin 4′-hydroxylation*

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Introduction

Members of the cytochrome P450 (CYP) superfamily of hemoproteins catalyze the oxidative metabolism of exogenous chemicals such as drugs, carcinogens and toxins, as well as endogenous substances such as steroids and fatty acids (Nelson et al. 1996). CYP2Cs are the major subfamily of CYPs that represent approximately 20% of CYP enzymes in human liver and metabolize a similar proportion of clinically used drugs (Goldstein 2001). The CYP2C subfamily consists of four members in humans (CYP2C8, CYP2C9, CYP2C18 and CYP2C19), and their genes are tandemly located on chromosome 10 (10q24.1–q24.3) (Gray et al. 1995).

CYP2C19 plays important roles in the metabolism of a number of therapeutic drugs such as anti-ulcer drugs, omeprazole and lansoprazole, anti-convulsants, *S*-mephenytoin, anti-diabetic drugs, tolbutamide, and anxiolytic drugs, diazepam (Rendic and Di Carlo 1997). The metabolism of these drugs *in vivo* is known to be polymorphic, and individuals can be divided into an extensive metabolizer (EM) group and a poor metabolizer (PM) group. PMs are characterized by a higher area under the concentration-time curve values of the drugs (Katsuki et al. 1997; Furuta et al. 1999; Qin et al. 1999). For example, PMs show a higher cure rate for gastric and duodenal ulcers by omeprazole (Furuta et al. 1998, 1999; Goldstein 2001). It has been also reported that there are ethnic differences in the frequencies of PMs of CYP2C19 enzyme: 2–5% in Caucasian populations, 2–5% in African populations and 13–25% in Asian populations (Wedlund 2000; Goldstein 2001). These differences are known to be attributed to the genetic polymorphism of the *CYP2C19* gene (Goldstein 2001). Various mutations of the *CYP2C19* gene have been identified from ethnically different populations (http://www.imm.ki.se/CYPalleles/cyp2c19.htm). The PM-related *CYP2C19* polymorphism of oriental populations can be explained by the combination of two-point mutations, *CYP2C19*2* (681G > A, splicing defect) of exon 5 and *CYP2C19*3* (636G > A, Trp212Stop) of exon 4 (de Morais et al. 1994a, 1994b). In Caucasian populations, additionally deficient *CYP2C19* alleles have been subsequently found, although only 2–5% of populations show the PM phenotype (Ferguson et al. 1998; Ibeanu et al. 1998a, 1998b, 1999; Blaisdell et al. 2002; Sim et al. 2006).

Recently, Morita et al. (2004) found another minor allele, *CYP2C19*16* (1324C > T, R442C), at 0.6% frequency in Japanese subjects who had received mephobarbital. With respect to other oriental populations, the defective alleles *CYP2C19*4* (1A > G, no protein) and *CYP2C19*5* (1297C > T, Arg433Trp) were found at low frequencies (<0.5%) in a Chinese population (Xiao et al. 1997; Garcia-Barcelo et al. 1999). More recently, *CYP2C19*17* (−806C > T/−3402C > T) has been reported in the Chinese, Swedish and Ethiopian populations at frequencies of 4–19%) (Sim et al. 2006); however, the other *CYP2C19* alleles (*CYP2C19*6–CYP2C19*15*) have not been detected in oriental populations. Additionally, we also identified two alleles (haplotypes) termed *CYP2C19*18* (986G > A/991A > G, Arg329His/Ile331Val) and *CYP2C19*19* (151A > G/991A > G, Ser51Gly/Ile331Val) in a Japanese population at frequencies of 0.2–0.3%, which cause amino acid substitutions in combination with Ile331Val identified in *CYP2C19*1B* or *CYP2C19*1C* (Table I). Wild-type alleles having 991A > G (Ile331Val) were more frequent compared with alleles with no 991A > G mutation (Fukushima-Uesaka et al. 2005).

The purpose of the current study was to examine the influence of *CYP2C19*18* and *CYP2C19*19* polymorphisms on the catalytic activity of the CYP2C19 enzyme. To achieve this, CYP2C19 cDNAs of wild-type (CYP2C19*1C, encoding CYP2C19.1B protein) and variants (CYP2C19*18 and CYP2C19*19) were constructed, and the corresponding CYP2C19 proteins were heterologously expressed in yeast cells. The enzymatic properties

Table I. Characterization of *CYP2C19* alleles examined.

Allele	Protein	Nucleic acid change	Amino acid substitution
*CYP2C19*1C*[a]	CYP2C19.1B[a]	991A > G	Ile331Val
*CYP2C19*18*	CYP2C19.18	99C > T/986G > A/991 A > G/IVS7-106T > C	Arg329His/Ile331Val
*CYP2C19*19*	CYP2C19.19	99C > T/151 A > G/991 A > G/IVS7-106T > C	Ser51Gly/Ile331Val

[a]Wild-type.

of the CYP2C19 proteins were subsequently examined by kinetic analysis using *S*-mephenytoin as a substrate.

Materials and methods

Materials

CYP2C19*1A cDNA cloned into pBluescript-SK(±) vector (pBluescript/CYP2C19*1A) was kindly provided by Dr Joyce A. Goldstein (National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA). KOD-plus DNA polymerase was purchased from Toyobo (Osaka, Japan); *Hin*dIII was from Takara Bio (Ohtsu, Japan); BigDye terminator cycle sequencing reaction kit v3.1 was from Applied Biosystems (Foster City, CA, USA); pcDNA3.1(+) vector was from Invitrogen (Carlsbad, CA, USA); and QuikChange site-directed mutagenesis kit was from Stratagene (La Jolla, CA, USA). The expression vector pGYR1, which has a GAPDH promoter and includes the yeast NADPH-cytochrome P450 reductase gene (Sakaki et al. 1992), was kindly provided by Dr Yoshihiko Funae (Osaka City University, Osaka, Japan). Yeast nitrogen base was purchased from BD Diagnostics (Franklin Lakes, NJ, USA); Zymolyase 100T was from Seikagaku Corporation (Tokyo, Japan); *S*-mephenytoin was from Toronto Research Chemicals (North York, ON, Canada); 4′-hydroxymephenytoin was from Ultrafine Chemicals (Manchester, UK); NADPH, glucose 6-phosphate and glucose 6-phosphate dehydrogenase were from Oriental Yeast (Tokyo, Japan); rabbit anti-human CYP2C19 antibody was from BD Biosciences (San Jose, CA, USA); peroxidase-conjugated goat anti-rabbit immunoglobulin was from Zymed Laboratories (South San Francisco, CA, USA); and enhanced chemiluminescence-plus reagents were from GE Healthcare Bio-Sciences (Little Chalfont, UK). All other chemicals and reagents used were of the highest quality commercially available.

Construction of CYP2C19 plasmids

CYP2C19*1A cDNA was amplified by polymerase chain reaction from pBluescript/CYP2C19*1A as a template using the forward primer 5′-CCCAAGCTTAA*AAAA*ATGGATCCTTTTGTGGTCC-3′ and the reverse primer 5′-GGAAAGCTTAGGAGCAGCCAGACCATCTGT-3′. *Hin*dIII sites (marked with the solid lines) were introduced to the 5′-end of the start codon and the 3′-end of the stop codon to facilitate subcloning into pGYR1. A yeast consensus sequence (Romanos et al. 1992) (marked in italics) was also introduced upstream of the start codon to achieve a high expression of protein in yeast cells. The PCR product was digested with *Hin*dIII and ligated into the same restriction enzyme site of pcDNA3.1(+), resulting in pcDNA3.1/CYP2C19*1A.

The pcDNA3.1/CYP2C19*1A plasmid was sequenced in both forward and reverse directions using a BigDye terminator cycle sequencing reaction kit v3.1 to confirm that there were no PCR errors. The cDNAs of CYP2C19*1C, CYP2C19*18 and CYP2C19*19 were constructed with a QuikChange site-directed mutagenesis kit according to the manufacturer's instructions using the primers listed in Table II. A mutation for CYP2C19*1C (991A > G) was introduced using pcDNA3.1/CYP2C19*1A as a template, resulting in pcDNA3.1/CYP2C19*1C. Mutations for CYP2C19*18 (991A > G and 986G > A) and CYP2C19*19 (991A > G and 151A > G) were successively introduced using pcDNA3.1/CYP2C19*1C as a template. All CYP2C19 plasmids were sequenced to confirm successful mutagenesis (data not shown). The cDNAs of CYP2C19*1C, CYP2C19*18 and CYP2C19*19 were subsequently subcloned into the pGYR1 yeast expression vector.

Expression of CYP2C19 enzymes

The pGYR1 vectors containing CYP2C19 cDNAs were used to transform *Saccharomyces cerevisiae* AH22 by the lithium acetate method, and yeast transformants were cultivated (Wan et al. 1997). Microsomes from yeast cells were prepared as described previously (Hichiya et al. 2002), and stored at −80°C until use. Protein concentrations were determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Assay for CYP2C19 holo- and apoproteins

Microsomal fractions were diluted to a protein concentration of 10 mg/ml with 100 mM potassium phosphate buffer (pH 7.4) containing 20% (v/v) glycerol and 0.4% (w/v) Emulgen 911, and total functional CYP protein levels were spectrophotometrically measured as reduced carbon monoxide (CO) spectra according to the method of Omura and Sato (1964) using $91\,mM^{-1}\,cm^{-1}$ as an absorption coefficient for the 450–490 wavelength couple. Total CYP2C19 protein levels of holo- and apoforms in yeast cell

Table II. Primers used for site-directed mutagenesis.

Mutation	Primer	Sequence	Position
991A > G[a]	I331V-F	5′-GATTGAACGTGTC**G**TTGGCAGA AACCGGAGCC-3′	978–1009
	I331V-R	5′-GGCTCCGGTTTCTGCCAA**C**GAC ACGTTCAATC-3′	
986G > A/ (991A > G)[b]	R329H/(I331V)-F	5′-CCAGGAAGAGATTGAAC**A**TGTC GTTGGCAGAAACCGG-3′	969–1005
	R329H/(I331 V)-R	5′-CCGGTTTCTGCCAACGACA**T**GTTC AATCTCTTCCTGG-3′	
151A > G[c]	S51G-F	5′-CCTACAGATAGATATTAAGGATGTC **G**GCAAATCCTTAACC-3′	126–165
	S51G-R	5′-GGTTAAGGATTTGC**C**GACATCCT TAATATCTATCTGTAGG-3′	

Bold and underlined letters indicate the mutation sites introduced by PCR-based mutagenesis.
[a]Primer for CYP2C19*1C, CYP2C19*18 and CYP2C19*19.
[b]Primer for CYP2C19*18.
[c]Primer for CYP2C19*19.

microsomes were determined by immunoblotting. Microsomal fractions (10 μg protein) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Laemmli 1970) and electrotransferred to a polyvinylidene fluoride sheet as described by Towbin et al. (1979). The sheet was incubated with rabbit anti-human CYP2C19 antibody (diluted at 1:2000) as the primary antibody and then with peroxidase-conjugated goat anti-rabbit immunoglobulin (diluted at 1:5000) as the secondary antibody. Immunoreactive proteins were visualized with chemifluorescence (enhanced chemiluminescence-plus reagents), and the band densities were relatively determined with Scion Image v4.0 (Scion Corporation, Frederick, MD, USA). The anti-human CYP2C19 antibody recognized a single band in human liver microsomes which co-migrated with microsomes from yeast cells expressing wild-type CYP2C19 in a preliminary study.

Assay for S-mephenytoin 4′-hydroxylation

S-Mephenytoin 4′-hydroxylation was determined by high-performance liquid chromatography (HPLC) as described previously with some modifications (Hanioka et al. 2002). The incubation mixture contained *S*-mephenytoin as a substrate (2–500 μM), microsomes from yeast cells (2 mg protein/ml), 1 mM $NADP^+$, 10 mM glucose 6-phosphate, 2.0 unit/ml glucose 6-phosphate dehydrogenase and 10 mM $MgCl_2$ in 50 mM potassium phosphate buffer (pH 7.4) in a final volume of 500 μl. *S*-Mephenytoin was dissolved in methanol-dimethyl sulfoxide (50:50, v/v). The final concentration of organic solvent (methanol and dimethyl sulfoxide) in the incubation mixture was 1%. The reaction was initiated by the addition of microsomes from yeast cells after pre-incubation at 37°C for 1 min. After incubation at 37°C for 20 min, the reaction was terminated by the addition of 4 ml of dichloromethane. The incubation mixture was spiked with 5 nmol phenobarbital as an internal standard and vigorously vortexed for 2 min. After centrifugation at 2000 *g* for 15 min, the organic phase was evaporated to dryness under a gentle stream of nitrogen at 35°C. The residues were dissolved in 200 μl of methanol-water (50:50, v/v) and analyzed by HPLC. The HPLC system consisted of an L-2130 pump (Hitachi, Tokyo, Japan), an L-2300 column oven (Hitachi) and an L-2400 UV detector (Hitachi) equipped with an Inertsil ODS-80A column (4.6 mm i.d. × 150 mm; GL Sciences, Tokyo, Japan). The column was maintained at 40°C. Data acquisition was accomplished using D-2000 v1.1 software (Hitachi). The product (4′-hydroxymephenytoin) was eluted isocratically with 20 mM potassium dihydrogenphosphate/acetonitrile/methanol (77:17:6, v/v/v) at a flow rate of 1.0 ml/min. UV detection absorbance was recorded at 204 nm. Standard samples were prepared in the same manner as incubation samples. Under these conditions, the retention times of 4′-hydroxymephenytoin, phenobarbital and *S*-mephenytoin were 6.2, 12.7 and 22.0 min, respectively. The detection limit for 4′-hydroxymephenytoin was 5 pmol/assay with a signal-to-noise ratio of 3. The 4′-hydroxymephenytoin formation was linear for at least 40 min in microsomes from livers and yeast cells expressing wild-type CYP2C19. The intra- and inter-day variation coefficients did not exceed 10% in any assay.

Data analysis

Kinetic parameters including K_m and V_{max} for *S*-mephenytoin 4′-hydroxylation were estimated by analyzing Michaelis-Menten plots using Prism v4.0 software (GraphPad Software, San Diego, CA, USA). Intrinsic clearance values were determined as the ratio of V_{max}/K_m. All values are expressed as the mean ± SD of three independent

transfection experiments. Statistical comparisons were performed by one-way analysis of variance with Dunnett's *post-hoc* test using Prism v4.0 software. Differences were considered statistically significant when the p value was <0.05.

Results

Expression of wild-type and variant CYP2C19s in yeast cells

The expression levels of CYP2C19 proteins in microsomal fractions obtained from yeast cells transfected with CYP2C19*1C, CYP2C19*18 and CYP2C19*19 cDNAs were examined by reduced CO difference spectral and immunoblot analyses. As shown in Figure 1(a), the reduced CO difference spectra of CYP2C19.1B, CYP2C19.18 and CYP2C19.19 proteins showed a Soret peak at around 450 nm. The expressed CYP level of CYP2C19.1B was 15.6 pmol/mg of microsomal protein. The level of CYP2C19.18 was 2.0-fold higher than that of CYP2C19.1B, whereas the level of CYP2C19.19 was 65% that of CYP2C19.1B (Figure 1b). The expression levels of wild-type and variant CYP2C19 proteins in yeast cell microsomes were also assessed by immunoblotting which recognized

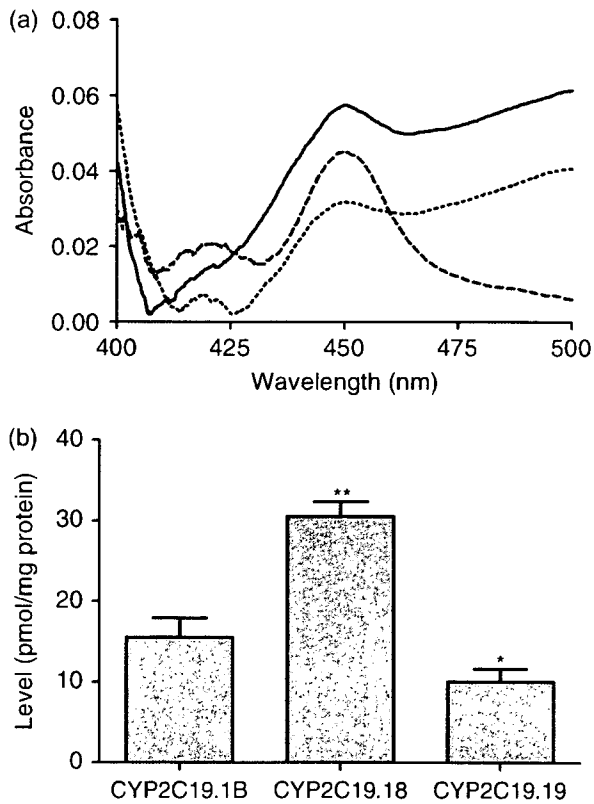

Figure 1. Reduced CO difference spectra of microsomes from yeast cells expressing wild-type and variant CYP2C19s. (a) Representative results of pooled microsomes from three independent preparations. The microsomal protein concentration used was 10 mg/ml. Solid line, CYP2C19.1B; broken line, CYP2C19.18; dotted line, CYP2C19.19. (b) Expression level of CYP2C19 holoprotein. The results are expressed as pmol/mg protein. Each bar represents the mean ± SD of three separate experiments derived from independent preparations. *Significantly different from CYP2C19.1B ($p < 0.05$). **Significantly different from CYP2C19.1B ($p < 0.01$).

both holo- and apoforms. All constructs except the negative control yielded immunodetectable CYP2C19 protein. The stained bands of CYP2C19.18 and CYP2C19.19 were 171% and 57% of CYP2C19.1B, respectively (Figure 2). The profile for the levels of recombinant protein within yeast cells was reproducible in three independent transfection experiments (data not shown).

Enzymatic properties of wild-type and variant CYP2C19s

S-Mephenytoin 4′-hydroxylation activities in microsomes from yeast cells expressing wild-type and variant CYP2C19s were then examined. Figure 3 shows the activities at low (5 μM) and high (200 μM) substrate concentrations on the basis of microsomal and functional CYP protein levels. *S*-Mephenytoin 4′-hydroxylation activities of CYP2C19.1B at substrate concentrations of 5 and 200 μM on the basis of microsomal and functional CYP protein levels were 9.45 and 62.2 pmol/min/mg protein, and 0.60 and 3.94 pmol/min/pmol CYP, respectively. The activities of CYP2C19.19 at 5 μM substrate were significantly lower (34–54%) than those of CYP2C19.1B in both unit terms. By contrast, the activities of CYP2C19.18 at substrate concentrations of 5 and 200 μM were not significantly different from those of CYP2C19.1B in any unit term. The ratio of activities at substrate concentrations of 5 and 200 μM for CYP2C19.18 on the basis of functional CYP protein

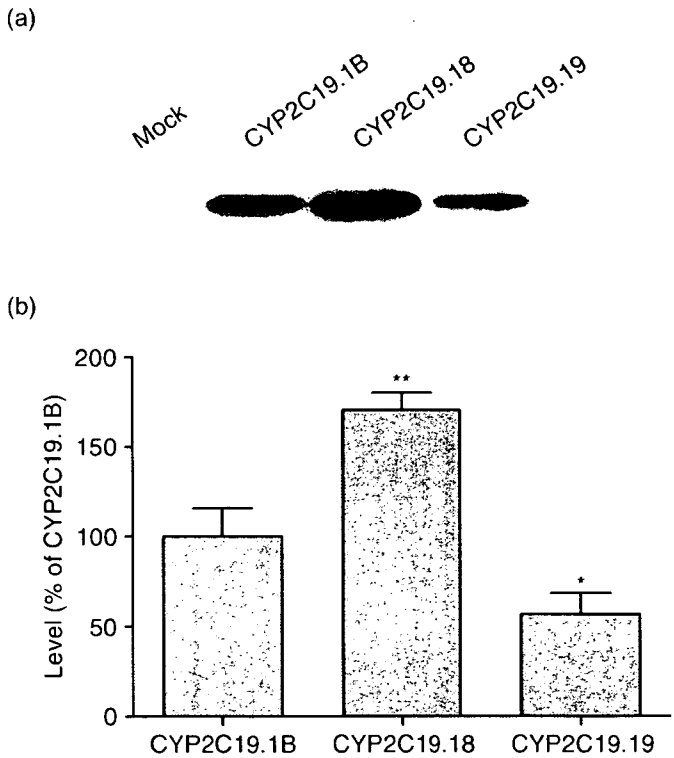

Figure 2. Immunoblotting of microsomes from yeast cells expressing wild-type and variant CYP2C19s. (a) Representative results of pooled microsomes from three independent preparations. The microsomal protein level applied was 10 μg/lane. (b) Expression level of CYP2C19 holo- and apoprotein. The results are expressed as a percentage of the level of CYP2C19.1B. Each bar represents the mean ± SD of three separate experiments derived from independent preparations. *Significantly different from CYP2C19.1B ($p < 0.05$). **Significantly different from CYP2C19.1B ($p < 0.01$).

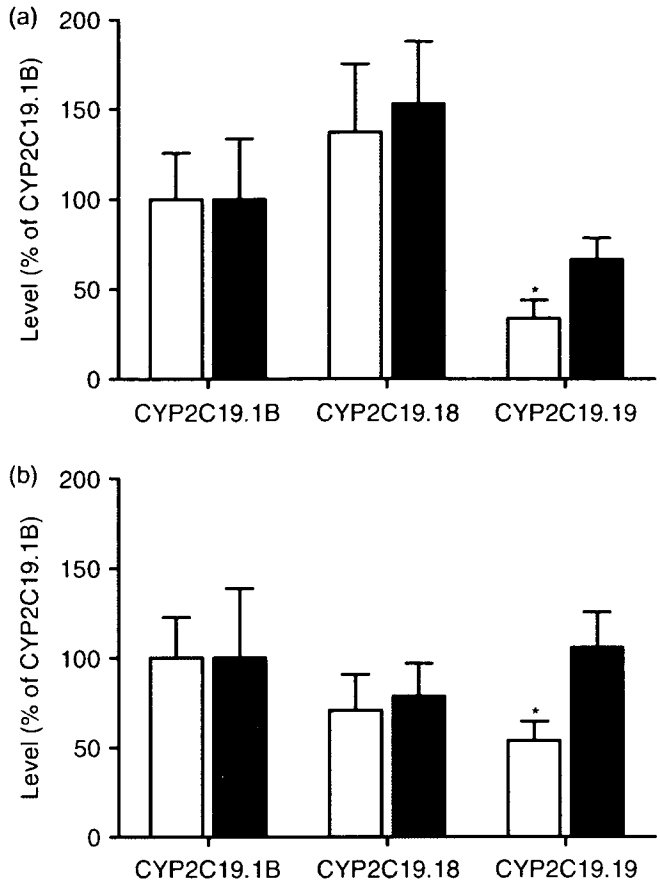

Figure 3. *S*-Mephenytoin 4′-hydroxylation activities in microsomes from yeast cells expressing wild-type and variant CYP2C19s. The results are expressed as a percentage of the activity of CYP2C19.1B. (a) Results on the basis of microsomal protein level. The activities of CYP2C19.1B at 5- and 200 μM substrate concentrations were 9.45 ± 2.43 and 62.2 ± 20.8 pmol/min/mg protein, respectively. (b) Results on the basis of functional CYP protein level. The activities of CYP2C19.1B at 5- and 200 μM substrate concentrations were 0.60 ± 0.14 and 3.94 ± 1.54 pmol/min/pmol CYP, respectively. Each bar represents the mean ± SD of three separate experiments derived from independent preparations. □, 5 μM substrate; ■, 200 μM substrate. *Significantly different from CYP2C19.1B ($p < 0.05$).

level was similar to that for CYP2C19.1B, whereas the relative activity levels of CYP2C19.19 at a substrate concentration of 200 μM was 2.0-fold higher than that at a substrate concentration of 5 μM. No microsomal activity of the negative control was detected at any substrate concentration (data not shown).

To obtain further information on the enzymatic properties of variant CYP2C19s as well as wild-type CYP2C19, kinetic analysis for *S*-mephenytoin 4′-hydroxylation was performed. The nonlinear regression curves of Michaelis-Menten kinetics are shown in Figure 4. The calculated kinetic parameters are summarized in Table III. The K_m value for *S*-mephenytoin 4′-hydroxylation of CYP2C19.1B was 33.5 μM. The K_m value of CYP2C19.19 was significantly higher (3.0-fold) than that of CYP2C19.1B, whereas no significant difference was observed in the K_m values between CYP2C19.1B and CYP2C19.18. The V_{max} and V_{max}/K_m values for *S*-mephenytoin 4′-hydroxylation of CYP2C19.1B on the basis of microsomal protein level were 73.0 pmol/min/mg protein and 2.19 μl/min/mg protein, respectively. When the activities were normalized to CYP holoprotein levels to assess the intrinsic function of wild-type and variant CYP2C19 enzymes, the V_{max} and V_{max}/K_m values

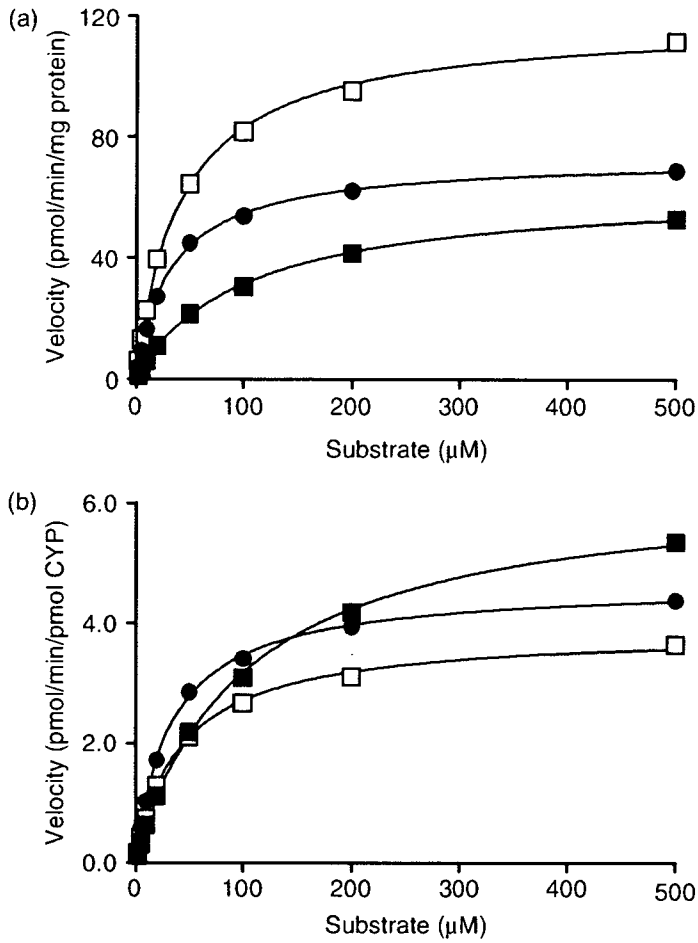

Figure 4. Michaelis-Menten kinetics for *S*-mephenytoin 4′-hydroxylation by microsomes from yeast cells expressing wild-type and variant CYP2C19s. (a) Results on the basis of microsomal protein level. (b) Results on the basis of functional CYP protein level. The substrate concentrations used were 2–500 μM. ●, CYP2C19.1B; □, CYP2C19.18; ■, CYP2C19.19. Each point represents the mean of three separate experiments derived from independent preparations.

of CYP2C19.1B were 4.64 pmol/min/pmol CYP and 138 nl/min/pmol CYP, respectively. The V_{max}/K_m values of CYP2C19.19 on the basis of microsomal and functional CYP protein levels were significantly reduced to 29–47% of CYP2C19.1B, although there were no significant differences in V_{max} values between CYP2C19.1B and CYP2C19.19. By contrast, no significant differences in K_m, V_{max} and V_{max}/K_m values were observed between CYP2C19.1B and CYP2C19.18 in any unit term, although an increasing tendency in the V_{max} value on the basis of microsomal protein level was observed in CYP2C19.18, paralleling the increased holoprotein level (Figure 1).

Discussion

CYP2C19 is a clinically important metabolic enzyme responsible for the metabolism of a number of therapeutic drugs and other xenobiotics (Rendic and Di Carlo 1997). Based on differences in the metabolism of *S*-mephenytoin and other CYP2C19 substrates, individuals