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Genetic Polymorphisms and Haplotypes of Major Drug Metabolizing Enzymes in East Asians and Their Comparison with Other Ethnic Populations

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Abstract: Remarkable ethnic differences in drug response are well known, and many of these can be attributed to differences in genetic backgrounds. Accumulating evidence has shown that genetic polymorphisms can cause the alteration or even loss of activity in drug metabolizing enzymes, transporters and receptors. Thus, genetic polymorphisms may be important in understanding these ethnic differences in drug response. Furthermore, haplotypes, linked combinations of genetic polymorphisms on a chromosome, have the advantage of providing more useful information on phenotype–genotype links than individual polymorphisms. In the past 6 years, mostly as a Japanese national project, we resequenced the exons and enhancer/promoter regions of more than 30 drug metabolizing enzymes, transporters and receptors using genomic DNA from 100 to 500 Japanese subjects, analyzed linkage-disequilibrium (LD), and estimated haplotype structures. Regarding *CYP2C9* and *2C19*, we found linkages between *CYP2C19*2* or **3* and *CYP2C9*1*, and between *CYP2C9*3* and *CYP2C19*1* haplotypes. Haplotype structures of *CYP2D6* are complicated by gene duplication or recombination. In contrast, the haplotype structure of *CYP3A4* was simple, but close linkages were observed with other *CYP3As*. As for *UGT1As*, the 8 first exons encoding active isoforms and common exons 2-5 were divided into 5 blocks by LD analysis, and intra- and inter-block haplotypes were estimated. Several linkages of haplotypes with functional importance were revealed, such as *UGT1A7*3 - UGT1A6*2 - UGT1A1*28* or **6*. In this review, we summarize polymorphisms and haplotype structures of these clinically important drug metabolizing enzymes in East Asians, mainly from our Japanese data, and compare them with those of other ethnicities.

INTRODUCTION

Remarkable ethnic differences in drug response are well known, and thus optimal drug dosages for prescription vary among or even within countries [Tate and Goldstein, 2004 for review]. For example, reduction of diastolic blood pressure by propranolol is more evident in Caucasians than in Africans [Cubeddu *et al.*, 1986]. Daily maintenance doses of warfarin, an anti-coagulant, are known to be different among Caucasians, Asians and Afro-Caribbeans [Blann *et al.*, 1999]. Many of the differences in drug response now can be attributed to genetic background. Development of DNA sequencing/genotyping technology and world-wide human genome projects has prompted the identification of clinically important genetic polymorphisms for diverse ethnic populations (see Grant 2005 for overview of genotyping technologies). As a result, accumulating data has shown that genetic polymorphisms specific for different ethnicities cause the alteration or even loss of activities in drug metabolizing enzymes, transporters and receptors [Evans and Relling, 1999, Chowbay *et al.*, 2005]. Thus, genetic polymorphisms are important in understanding ethnic differences in drug response. Furthermore, haplotypes, linked combinations of genetic polymorphisms on a chromosome, sometimes have the advantage of providing more useful information on phenotype–genotype links than individual polymorphisms [Judson *et al.*, 2000]. In addition, long-range haplotypes covering the gene clusters such as human Cytochrome P450 (CYP) *2Cs*, *CYP3As* and uridinediphosphoglucuronate glucuronosyltransferase (UGT) *1As* could help to elucidate the pharmacokinetics and pharmacodynamics of drugs with complicated metabolic pathways.

For the past 6 years, mostly as a Japanese national project to elucidate the genetic contribution to drug response in Japanese, we performed pharmacogenetic studies for more than 10 clinically important drugs. In these approaches, more than 30 genes encoding drug metabolizing enzymes, transporters and receptors were resequenced from genomic DNA from 100 to 500 Japanese subjects. Our studies cannot fully explain the interindividual or ethnic differences in drug response; however, identification of novel and/or known defective polymorphisms and haplotypes in Japanese suggests their involvement in such differences and highlights the necessity of ethnic-specific pharmacogenetic data.

In this review, we focus on four clinically important drug metabolizing enzyme groups: 1) CYP2C9 and CYP2C19, 2) CYP2D6, 3) CYP3A4, and 4) UGT1A1 and other UGT1As, and summarize the genetic polymorphisms and haplotype structures of these enzymes in East Asians, mainly from our Japanese data, and compare them with data from other ethnicities. Note that our sequence analysis to identify genetic polymorphisms focused on enhancer/promoter regions, exons and surrounding introns; thus, many intronic variations that were far from the exon-intron boundaries were excluded in the haplotype estimations. Haplotypes in this review are shown as a number plus alphabetical letters. The numbers are based on assignments by the Human Cytochrome P450 Allele Nomenclature Committee Home Page (http://www.cypalleles.ki.se/, as of July 11-15, 2006) or the UDP-

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Glucuronosyltransferase (UGT) Alleles Nomenclature Home Page (http://galien.pha.ulaval.ca/alleles/alleles.html, as of July 14, 2006). Capital alphabetical letters were used when the corresponding haplotypes were already shown in the web sites listed above. The other inferred haplotypes in our study are provisionally shown with small alphabetical letters, basically depending on the frequencies, as shown in our original reports cited in each section.

CYP2C9 AND CYP2C19

Human CYP2C subfamily accounts for 20% of the total P450 content in human liver microsomes [Shimada *et al.*, 1994]. The four known human *CYP2C* genes are located in a cluster spanning 500 kb in chromosome 10q24 in the following order: *CYP2C18*, *CYP2C19*, *CYP2C9* and *CYP2C8*, each of which is estimated to be at least 50 kb. These genes have similar structures consisting of nine exons with conserved exon/intron boundaries and greater than 82% deduced amino acid homology [Goldstein and de Morais, 1994]. In this section, we focus on the genetic polymorphisms and haplotypes of *CYP2C9* and *CYP2C19*.

CYP2C9 Polymorphisms

More than 100 currently used drugs have been identified as substrates of CYP2C9, corresponding to about 10 to 20% of commonly prescribed drugs [Evans and Relling, 1999]. These include the narrow therapeutic index agents warfarin and phenytoin and other routinely prescribed compounds such as angiotensin II receptor blockers (losartan and irbesartan), sulfonylureas (tolbutamide, glibenclamide, glipizide and glimepiride), the diuretic torsemide, and various nonsteroidal anti-inflammatory agents (ibuprofen, diclofenac, piroxicam, flurbiprofen, and celecoxib) [Rettie and Jones, 2005 for review].

The possible genetic regulation of tolbutamide metabolism was first reported in 1979 [Scott and Poffenbarger, 1979]. *CYP2C9* polymorphisms had been recognized since multiple cDNA clones were isolated in the late 1980s and early 1990s. To date, more than 50 single nucleotide polymorphisms (SNPs) of *CYP2C9* including regulatory and coding SNPs have been identified (http://www.cypalleles.ki.se/cyp2c9.htm, as of July 15, 2006). Some SNPs have been reported to exhibit reduced catalytic activities compared with the wild-type by both *in vitro* functional studies and clinical pharmacokinetic/pharmacodynamic studies (Table 1).

A number of population genotyping studies also demonstrated that these SNPs were distributed with different frequencies among various ethnic populations. Two nonsynonymous SNPs, *CYP2C9*2* (430C>T, R144C) and *CYP2C9*3* (1075A>C, I359L), are found at allele frequencies of 10-15% and 5-10%, respectively, in Caucasians (American, European, Scandinavian, and Russian), Turkish, and Hispanic populations (Table 1) [Scordo *et al.*, 2001, Bravo-Villalta *et al.*, 2005, Garcia-Martin *et al.*, 2006]. In contrast, these SNPs are less prevalent in African and Asian populations. African-Americans and Ethiopians exhibit 2-4% and 1-2% allele frequencies for *CYP2C9*2* and *CYP2C9*3*, respectively [Scordo *et al.*, 2001; Bravo-Villalta *et al.*, 2005; Garcia-Martin *et al.*, 2006]. In East Asians, *CYP2C9*3* is found at 1-4% allele frequencies, while *CYP2C9*2* is hardly detected [Wang *et al.*, 1995; Nasu *et al.*, 1997; Yoon *et al.*, 2001]. In most *in vitro* studies, *CYP2C9*2* exhibited a small decrease in *V*max (0-35%) and little or no change in the *K*m for catalysis of various substrates [Lee *et al.*, 2002 for review]. The recombinant *CYP2C9*3* enzyme shows a greater *K*m and/or lower *V*max compared to wild-type for most CYP2C9 substrates although the magnitude of alterations in metabolic activity varies significantly among substrates [Takanashi *et al.*, 2000]. Both alleles, *CYP2C9*2* and *CYP2C9*3*, affect pharmacokinetics and/or the dose requirements of a number of substrates such as warfarin, phenytoin, losartan, and glimepiride [Kirchheiner and Brockmoller, 2005 for review].

Other reported alleles (*CYP2C9*4* to **24*) are mostly ethnic specific and/or relatively rare (Table 1). Due to the low frequencies of these alleles, *in vivo* elucidation of their functional significance is generally difficult. As for the defective alleles revealed by *in vitro* studies, *CYP2C9*5* (D360E) [Dickmann *et al.*, 2001; Tracy *et al.*, 2002; Yasar *et al.*, 2002a; Allabi *et al.*, 2004 and 2005; Takahashi *et al.*, 2006] and *CYP2C9*6* (K273RfsX34) with a null-activity mutation [Kidd *et al.*, 2001; Allabi *et al.*, 2004 and 2005; Takahashi *et al.*, 2006] were found only in Africans at allele frequencies around 0.017 and 0.006, respectively. *CYP2C9*11* (R335W) is present both in Africans and in Caucasians at allele frequencies around 0.01 [Higashi *et al.*, 2002; Blaisdell *et al.*, 2004; King *et al.*, 2004; Tai *et al.*, 2005; Veenstra *et al.*, 2005; Takahashi *et al.*, 2006], but is absent in Asians. Caucasians also carry two other rare defective alleles, *CYP2C9*12* (P489S) [Blaisdell *et al.*, 2004; Veenstra *et al.*, 2005] and *CYP2C9*14* (R125H) [Veenstra *et al.* 2005]. In Asians, 10 defective alleles have been identified: *CYP2C9*4* (I359T) [Ieiri *et al.*, 2000; Imai *et al.*, 2000], *CYP2C9*13* (L90P) [Si *et al.*, 2004], *CYP2C9*14* (R125H), *CYP2C9*15* (S162X), *CYP2C9*16* (T299A), *CYP2C9*18* (D397A+I359L) [Zhao *et al.*, 2004; Delozier *et al.*, 2005], *CYP2C9*25* (K118RfsX9), *CYP2C9*26* (T130R), *CYP2C9*28* (Q214L), and *CYP2C9*30* (A477T) [Maekawa *et al.*, 2006]. Especially, *CYP2C9*13* (L90P), an allele detected in a Chinese poor metabolizer (PM) of lornoxicam, has been found independently both in Chinese and Japanese at allele frequencies of 0.01 and 0.002, respectively [Si *et al.*, 2004; Maekawa *et al.*, 2006]. Guo *et al.* [2005a and 2005b] have revealed that the L90P substitution markedly decreased the intrinsic clearance of lornoxicam, tolbutamide and diclofenac *in vitro* and/or *in vivo*. Although further clinical investigation is required for these rare alleles, not only *CYP2C9*3* but also many other defective alleles described above would be at least partially responsible for highly variable interindividual and ethnic differences in the metabolism of CYP2C9 substrate drugs in Asians.

CYP2C9 Haplotypes

Recently, several groups reported comprehensive haplotype structures with high-density SNPs in *CYP2C9*, which will provide more useful information than single SNP genotyping in investigating interindividual or ethnic differences in the *in vivo* metabolic activity of CYP2C9. Veenstra *et al.* [2005] reported whole-gene high-resolution haplotype structures of *CYP2C9* in 192 European American patients administered warfarin. They determined 23 haplotypes, only 8 of

Table 1. Summary of *CYP2C9* Alleles

Allele	Nucleotide Change	Amino Acid Change	Allele Frequency			Functional Effect	Reference
			African	Caucasian	Asian		
*CYP2C9*2*	430C>T	R144C	0.02 - 0.04	0.10 - 0.15	ND	Decreased activity (*in vitro* and *in vivo*)	Lee *et al.* 2002, Schwarz 2003, Bravo-Villalta *et al.* 2005, Kirchheiner and Brockmoller 2005, Garcia-Martin *et al.* 2006
*CYP2C9*3*	1075A>C	I359L	0.01 - 0.02	0.05 - 0.10	0.01 - 0.04	Decreased activity (*in vitro* and *in vivo*)	Lee *et al.* 2002, Schwarz 2003, Bravo-Villalta *et al.* 2005, Kirchheiner and Brockmoller 2005, Garcia-Martin *et al.* 2006
*CYP2C9*4*	1076T>C	I359T	ND	ND	0.004 (1/264)	Decreased activity (*in vitro*)	Ieiri *et al.* 2000, Imai *et al.* 2000
*CYP2C9*5*	1080C>G	D360E	0.017	ND	ND	Decreased activity (*in vitro* and *in vivo*)	Dickmann *et al.* 2001, Yasar *et al.* 2002a, Tracy *et al.* 2002, Allabi *et al.* 2004 and 2005, Takahashi *et al.* 2006
*CYP2C9*6*	818delA	K273R fsX34	0.006	ND	ND	Decreased activity (*in vivo*)	Kidd *et al.* 2001, Allabi *et al.* 2004 and 2005, Takahashi *et al.* 2006
*CYP2C9*7*	55C>A	L19I	0.056 (1/18)	ND	ND	Unaltered activity (*in vitro*)	Blaisdell *et al.* 2004
*CYP2C9*8*	449G>A	R150H	0.036 (1/28)	ND	ND	Increased activity (*in vitro*)	Blaisdell *et al.* 2004, Allabi *et al.* 2004 and 2005
*CYP2C9*9*	752A>G	H251R	0.133 (4/30)	0.003	ND	Unaltered activity (*in vitro*)	Blaisdell *et al.* 2004, Allabi *et al.* 2005, Veenstra *et al.* 2005,
*CYP2C9*10*	815A>G	E272G				Unaltered activity (*in vitro*)	Blaisdell *et al.* 2004
*CYP2C9*11*	1003C>T	R335W	0.056 (1/18)	0.01	ND	Decreased activity (*in vitro* and *in vivo*)	Higashi *et al.* 2002, Blaisdell *et al.* 2004, King *et al.* 2004, Allabi *et al.* 2004 and 2005, Tai *et al.* 2005, Veenstra *et al.* 2005, Takahashi *et al.* 2006
*CYP2C9*12*	1465C>T	P489S		0.003		Decreased activity (*in vitro*)	Blaisdell *et al.* 2004, Veenstra *et al.* 2005
*CYP2C9*13*	269T>C	L90P	ND	ND	0.01	Decreased activity (*in vitro* and *in vivo*)	Si *et al.* 2004, Guo *et al.* 2005a and 2005b
*CYP2C9*14*	374G>A	R125H	ND	0.003	0.019	Decreased activity (*in vitro*)	Zhao *et al.* 2004, Veenstra *et al.* 2005, DeLozier *et al.* 2005
*CYP2C9*15*	485C>A	S162X	ND	ND	0.019	No holoprotein expression (*in vitro*)	Zhao *et al.* 2004, DeLozier *et al.* 2005
*CYP2C9*16*	895A>G	T299A	ND	ND	0.008	Decreased activity (*in vitro*)	Zhao *et al.* 2004, DeLozier *et al.* 2005
*CYP2C9*17*	1144C>T	P382S	ND	ND	0.008	Unaltered activity (*in vitro*)	Zhao *et al.* 2004, DeLozier *et al.* 2005
*CYP2C9*18*	1190A>C (+1075A>C)	D397A (+I359L)	ND	ND	0.019	No protein expression (D397A alone, *in vitro*)	Zhao *et al.* 2004, DeLozier *et al.* 2005
*CYP2C9*19*	1362G>C	Q454H	ND	ND	0.008	Unaltered activity (*in vitro*)	Zhao *et al.* 2004, DeLozier *et al.* 2005

(Table 1. Contd....)

Allele	Nucleotide Change	Amino Acid Change	Allele Frequency			Functional Effect	Reference
			African	Caucasian	Asian		
*CYP2C9*20*	208G>C	G70R	ND	ND	0.014		Zhao *et al.* 2004
*CYP2C9*21*	89C>T	P30L	ND	0.005	ND		Veenstra *et al.* 2005
*CYP2C9*22*	121A>G	N41D	ND	0.003	ND		Veenstra *et al.* 2005
*CYP2C9*23*	226G>A	V76M	ND	0.005	ND		Veenstra *et al.* 2005
*CYP2C9*24*	1060G>A	E354K	ND	0.002 (1/408)	ND		Herman *et al.* 2006
*CYP2C9*25*	353_362delAG AAATGGAA	K118R fsX9	ND	ND	0.002	No protein expression (*in vitro*)	Maekawa *et al.* 2006
*CYP2C9*26*	389C>G	T130R	ND	ND	0.002	Decreased activity (*in vitro*)	Maekawa *et al.* 2006
*CYP2C9*27*	449G>T	R150L	ND	ND	0.004	Unaltered activity (*in vitro*)	Maekawa *et al.* 2006
*CYP2C9*28*	641A>T	Q214L	ND	ND	0.002	Decreased activity (*in vitro*)	Maekawa *et al.* 2006
*CYP2C9*29*	835C>A	P279T	ND	ND	0.002	Unaltered activity (*in vitro*)	Maekawa *et al.* 2006
*CYP2C9*30*	1429G>A	A477T	ND	ND	0.002	Decreased activity (*in vitro*)	Maekawa *et al.* 2006

ND: not detected.

which occurred at frequencies greater than 5%, indicating that the overall haplotype structure of *CYP2C9* was not complex. In another study, 21 haplotypes were inferred from 92 individuals in three racial groups (Africans, Caucasians, and Asians) [Blaisdell *et al.*, 2004]. In our study, 46 haplotypes were assigned from 263 Japanese subjects, of which only 5 haplotypes with frequencies of >2% accounted for most (>87%) of the inferred haplotypes [Maekawa *et al.*, 2006], indicating that the haplotype structure of *CYP2C9* in Japanese is also simple. We determined 6 haplotype-tagging SNPs (htSNP), IVS8-109A>T (intronic variations are designated by "IVS" (intervening sequence), the intron number, and then positive numbers starting from the end of the preceding exon or negative numbers from the beginning of the proceeding exon), IVS8+147C>T, -1565C>T, IVS7+38C>T, IVS6+95A>G, and 1075A>C (I359L), which can distinguish the major haplotypes *CYP2C9*1A*, *CYP2C9*1B*, *CYP2C9*1e*, *CYP2C9*1f*, *CYP2C9*1h*, and *CYP2C9*3B*, respectively. Allele frequencies of these htSNPs exhibit interethnic differences between Japanese and other ethnicities publicized by the International HapMap Project (http://www.hapmap.org/index.html.ja, as of July 15, 2006) (Table **2**).

Because HapMap data revealed substantial interethnic differences in the allele frequencies of htSNPs (Table **2**), we then compared the precise haplotype frequency distribution in Japanese [Maekawa *et al.*, 2006] with those in other ethnic populations from previous reports in Caucasians [Veenstra *et al.*, 2005] and Africans [Blaisdell *et al.*, 2004]. The frequency of the wild-type haplotype *CYP2C9*1A* was higher in Japanese (haplotype frequency = 0.489; this frequency differs slightly from the allele frequency of htSNP shown in Table **2**) than in Caucasians (0.281) as reported by Veenstra *et al.* [2005]. The haplotype *CYP2C9*1B*, first assigned by King *et al.* [2004], contained 6 linked noncoding SNPs, -3089G>A, -2665_-2664delTG, -1188T>C, IVS3+239C>T, IVS8+147C>T, and IVS8-109A>T, was found at comparable frequencies between Japanese (0.222) and Caucasians (0.175). Several studies on Caucasians and Asians showed that there was no association of the haplotype *CYP2C9*1B* or its promoter SNPs (-2665_-2664delTG and -1188T>C) with warfarin sensitivity [King *et al.*, 2004; Zhao *et al.*, 2004; Veenstra *et al.*, 2005] or acenocoumarol pharmacodynamics [Morin *et al.*, 2004]. The third dominant haplotype in Japanese, *CYP2C9*1e* (0.118) harboring the htSNP -1565C>T, was found at a frequency of 0.043 in Asians and at a frequency of 0.133 in African-Americans [Blaisdell *et al.*, 2004], but was absent in Caucasians [Veenstra *et al.*, 2005]. The fourth dominant haplotype in Japanese *CYP2C9*1f* (0.023), tagged by IVS7+38C>T, might be Asian-specific (0.022) [Blaisdell *et al.*, 2004]. These differences in the haplotype (*CYP2C9*1e* and *CYP2C9*1f*) between the various ethnicities might contribute to variance in CYP2C9 activity across populations. For example, East Asians require a lower maintenance dose of warfarin than Caucasians and Indians [Takahashi *et al.*, 2003; Zhao *et al.*, 2004]. In fact, Chern *et al.* [2006] reported that IVS3-65G>C, the *CYP2C9*1e*-tagging SNP linked perfectly with -1565C>T, is associated with an elevated warfarin sensitivity in Taiwan Chinese, leading to a lowered warfarin dose for patients who were heterozygous or homozygous carriers of this allele.

Table 2. Ethnic Differences in Allelic Frequencies of Haplotype-Tagging SNPs of *CYP2C9*

Haplotype-Tagging SNP in *CYP2C9*	dbSNP ID (NCBI)	Haplotype[a]	Our Study	HapMap*			
			Japanese (263 Subjects)	CEU (60 Subjects)	YRI (60 Subjects)	CHB (45 Subjects)	Japanese (45 Subjects)
IVS8-109A[b]	rs1934969	*CYP2C9*1A*	0.544		0.297¶	0.648	0.611
IVS8+147C>T	rs2298037	*CYP2C9*1B*	0.287	0.167	ND¶	0.267	0.330
-1565C>T	rs9332096	*CYP2C9*1e*	0.125	ND¶	0.183	0.033	0.044
IVS7+38C>T	rs17847029	*CYP2C9*1f*	0.034				
IVS6+95A>G[c]	rs9332174	*CYP2C9*1h*	0.011	0.225¶	0.267¶	0.023	ND
430C>T (R144C)	rs1799853	*CYP2C9*2*	ND	ND	ND	ND	ND
1075A>C (I359L)	rs1057910	*CYP2C9*3B*	0.030	0.058	ND		
IVS6-32T>C	rs9332197	-	ND	0.067¶	ND	ND	ND

ND: not detected.

[a]*CYP2C9* haplotypes in a Japanese population are defined by Maekawa *et al.* [2006].

*http://www.hapmap.org/index.html.ja (as of July 15, 2006). CEU, YRI and CHB are U.S. (residents with ancestry from Northern and Western Europe), Nigeria (Yoruba) and Chinese populations, respectively.

¶Significant differences ($P<0.01$, chi-square test) in allele frequencies between our Japanese population and each ethnic population. The multiple comparison was corrected by Bonferroni's method.

[b]The major allele, IVS8-109A, tags *CYP2C9*1A* (minor allele is IVS8-109T).

[c]In the previous papar [Maekawa *et al.*, 2006], we chose IVS2+73T>C as a htSNP of *CYP2C9*1h*, which was perfectly linked with IVS6+95A>G.

Further clinical studies are needed to evaluate the functional relevance of these Asian- (and/or African-) specific haplotypes, *CYP2C9*1e* and *CYP2C9*1f*, to the metabolism of CYP2C9 substrates. The minor Japanese haplotype, *CYP2C9*1h* (0.008) tagged by IVS6+95A>G, seems more frequent in Caucasians (0.205) and African-Americans (0.100) than in Asians (0.043) as reported by Blaisdell *et al.* [2004]. The frequency of the haplotype *CYP2C9*3B* harboring I359L in Japanese (0.027) was comparable to that in Asians (0.022) [Blaisdell *et al.* 2004], but was slightly lower than those in Caucasians (0.057-0.081) [King *et al.*, 2004; Morin *et al.*, 2004; Veenstra *et al.*, 2005].

A previous study in a Japanese population demonstrated that haplotypes harboring the promoter SNPs of *CYP2C9* (-1911T>C, -1885C>G, -1537G>A and -981G>A) resulted in a reduction of promoter activity [Shintani *et al.*, 2001]. However, the majority of the promoter SNPs are shown to be closely linked with *CYP2C9*2* (-1096A>G, -620G>T, -485T>A, -484C>A and R144C) and *CYP2C9*3* (-1911T>C, -1885C>G, -1537G>A, -981G>A and I359L) [Blaisdell *et al.*, 2004; King *et al.*, 2004; Veenstra *et al.*, 2005; Maekawa *et al.*, 2006]. It remains unclear whether these promoter SNPs contribute to the impaired activities of *CYP2C9*2* and *CYP2C9*3*.

CYP2C19 Polymorphisms

Another member of the human CYP2C subfamily, CYP2C19, accounts for only 1% of the total P450 in human liver microsomes [Inoue *et al.*, 1997]. However, it is responsible for the metabolism of clinically important drugs such as the anticonvulsant mephenytoin, proton-pump inhibitors (omeprazole, lansoprazole, rabeprazole and pantoprazole), the antimalarial proguanil and the anxiolytic diazepam [Desta *et al.*, 2002 for review]. CYP2C19 substrates are either neutral or weakly basic compounds, while CYP2C9 substrates are relatively acidic. In addition, CYP2C19 and CYP2C9 share a number of common substrates, but display different substrate stereospecificity and regioselectivity [Bajpai *et al.*, 1996; Lewis *et al.*, 1998].

Interindividual differences in the activity of CYP2C19 were first characterized by 4'-hydroxylation of *S*-mephenytoin [Andersson *et al.*, 1990]. The phenotypes of this enzyme are classified into two groups, extensive metabolizers (EMs) and PMs. The two genetic defects, *CYP2C19*2* (681G>A, splice defect) and *CYP2C19*3* (636G>A, W212X) are primarily responsible for the PM phenotype of mephenytoin [De Morais *et al.*, 1994a, 1994b]. The pharmacokinetics and/or pharmacodynamics of other CYP2C19 substrate drugs such as proton-pump inhibitors [Furuta *et al.*, 2005], diazepam [Inomata *et al.*, 2005] and antidepressants [Kirchheiner *et al.*, 2004] are also affected by *CYP2C19* genotypes. As shown in Table **3**, the allele frequencies of *CYP2C19*2* (21-45%) and *CYP2C19*3* (5-13%) in Asian populations were higher than European-American populations (*CYP2C19*2*, 13-19%; *CYP2C19*3*, 0-0.3%) and Africans (*CYP2C19*2*, 11-25%; *CYP2C19*3*, 0-1.8%) [Bravo-Villalta *et al.*, 2005], resulting in significant interethnic differences in PM frequencies.

Both *CYP2C19*2* and **3* account for >99% of PM alleles in Asians and ~87% of Caucasian PM alleles [De Morais *et al.*, 1994a and 1994b]. Unequal distributions of these alleles among various ethnic groups are the primary cause of different population pharmacokinetics of CYP2C19 substrate

Table 3. Summary of *CYP2C19* Alleles

Allele	Nucleotide Change	Amino Acid Change or Effect	Allele Frequency			Functional Effect	Reference
			African	Caucasian	Asian		
*CYP2C19*2*	681G>A	Splice defect	0.11 - 0.25	0.13 - 0.19	0.21 - 0.45	No activity (*in vitro* and *in vivo*)	De Morais *et al.*, 1994a and 1994b, Desta *et al.* 2002, Kirchheiner *et al.* 2004, Furuta *et al.* 2005, Inomata *et al.* 2005
*CYP2C19*3*	636G>A	W212X	0 - 0.018	0 - 0.003	0.05 - 0.13	No activity (*in vitro* and *in vivo*)	De Morais *et al*, 1994a and 1994b, Desta *et al.* 2002, Kirchheiner *et al.* 2004, Furuta *et al.* 2005, Inomata *et al.* 2005
*CYP2C19*4*	1A>G	No translation	ND	0.006	0.004	No activity (*in vitro*)	Ferguson *et al.* 1998, Garcia-Barcelo *et al.* 1999
*CYP2C19*5*	1297C>T	R433W	ND	ND	0.0025		Xiao *et al.* 1997
*CYP2C19*6*	395G>A	R132Q	ND	0.003 (1/346)	ND	No activity (*in vitro*)	Ibeanu *et al.* 1998
*CYP2C19*7*	IVS5+2T>A	Splice defect	ND	0.002 (1/650)	ND		Ibeanu *et al.* 1999
*CYP2C19*8*	358T>C	W120R	ND	0.003	ND	Decreased activity (*in vitro*)	Ibeanu *et al.* 1999
*CYP2C19*9*	431G>A	R144H	0.17	ND	ND	Decreased activity (*in vitro*)	Blaisdell *et al.* 2002
*CYP2C19*10*	680C>T	P227L	0.03	ND	ND	Decreased activity (*in vitro*)	Blaisdell *et al.* 2002
*CYP2C19*11*	449G>A	R150H	ND	0.03	ND	Unaltered activity (*in vitro*)	Blaisdell *et al.* 2002
*CYP2C19*12*	1473A>C	X491C	0.03	ND	ND	No holoprotein (*in vitro*)	Blaisdell *et al.* 2002
*CYP2C19*13*	1228C>T	R410C	0.06	ND	ND	Unaltered activity (*in vitro*)	Blaisdell *et al.* 2002
*CYP2C19*14*	50T>C	L17P	0.06	ND	ND		Blaisdell *et al.* 2002
*CYP2C19*15*	55A>C	I19L	0.06	ND	ND		Blaisdell *et al.* 2002
*CYP2C19*16*	1324C>T	R442C	ND	ND	rare		Morita *et al.* 2004
*CYP2C19*17*	-3402C>T, -806C>T	Increased transcription	0.18	0.18	0.04	Increased activity (*in vitro* and *in vivo*)	Sim *et al.* 2006
*CYP2C19*18*	986G>A	R329H	ND	ND	0.002		Fukushima-Uesaka *et al.* 2005
*CYP2C19*19*	151A>G	S51G	ND	ND	0.002		Fukushima-Uesaka *et al.* 2005

ND: not detected.

drugs [Desta *et al.*, 2002 for review]. As summarized in Table 3, however, subsequent studies have revealed additional defective *CYP2C19* alleles. A null allele, *CYP2C19*4* (1A>G), was found in Caucasians and Chinese with 0.6% and 0.4% frequencies, respectively [Ferguson *et al.* 1998, Garcia-Barcelo *et al.*, 1999]. *CYP2C19*5* (1297C>T, R433W), located in the conserved heme-binding region, was found in one Chinese Bai subject who was a PM of mephenytoin

[Xiao *et al.*, 1997]. *CYP2C19*6* to **15* were found in Caucasians or Africans, but not in Asians [Ibeanu *et al.*, 1998 and 1999; Blaisdell *et al.*, 2002]. *CYP2C19*16* (1324C>T, R442C) located near the heme-binding region, was found in a Japanese subject with impaired mephobarbital 4'-hydroxylation activity [Morita *et al.*, 2004]. *CYP2C19*17* harboring -806C>T and -3402C>T in the 5'-flanking region was identified with frequencies of 0.18 in both Swedes and Ethiopians and 0.04 in Chinese [Sim *et al.*, 2006]. The **17* carriers had increased *in vivo* omeprazole metabolism, probably due to the mutated -806T site, which consistently increased the transcription of *CYP2C19* by luciferase reporter transfection experiments *in vivo* in mice. Recently, we identified 2 novel alleles, *CYP2C19*18* (986G>A, R329H) and *CYP2C19*19* (151A>G, S51G) in a Japanese population [Fukushima-Uesaka *et al.*, 2005], and their functional analysis is ongoing.

CYP2C19 Haplotypes

Although *CYP2C19*2* and *CYP2C19*3* polymorphisms were extensively studied in relation to the pharmacokinetics/pharmacodynamics of CYP2C19 substrate drugs, pharmacogenetic studies using haplotypes of *CYP2C19* in various ethnic groups are currently lacking. Recently, we performed a comprehensive haplotype analysis using 48 genetic variations obtained from 253 Japanese subjects, and inferred 31 haplotypes in *CYP2C19*, of which only 5 haplotypes (haplotype frequency in parentheses) had frequencies of >2%: *CYP2C19*1d* (0.492), *CYP2C19*2c* (0.241), *CYP2C19*3b* (0.115), *CYP2C9*1e* (0.043), and *CYP2C19*1f* (0.022) accounted for most (>91%) of the observed haplotypes [Fukushima-Uesaka *et al.* 2005]. The htSNPs that resolved the 6 common haplotypes were IVS7-106T>C (*CYP2C19*1d*), 681G>A (*CYP2C19*2c*), 636G>A (*CYP2C19*3b*), 991A>G (*CYP2C9*1e*), IVS7-201G>A (*CYP2C19*1f*) and -806 C>T (*CYP2C19*17a*, originally designated *CYP2C19*1j* in Fukushima-Uesaka *et al.* [2005]). We compared the allele frequencies of these 6 htSNPs in Japanese with those of the International HapMap Project (http://www.hapmap.org/index.html.ja, as of July 15, 2006) (Table 4) although caution should be taken that Nigerian (Yoruba) may not necessarily represent Africans. The allele frequency of IVS7-106T>C tagging haplotype *CYP2C19*1d* in Japanese (0.530) was comparable to that of Caucasians (0.508), but was quite higher than that of Nigerians (0.183). 681G>A (splicing defect), the htSNP of *CYP2C19*2c* in Japanese, was found at an allele frequency of 0.267, which was comparable to that in Chinese (0.256) in the HapMap Project, but was slightly higher than those in Caucasians (0.150) and Nigerians (0.167). In agreement with previous reports [Bravo-Villalta *et al.*, 2005], 636G>A (W212X) tagging *CYP2C19*3b* was not found in Caucasians and Nigerians. The allele frequency of 991A>G (I331V), the htSNP of *CYP2C19*1e*, was comparable between Japanese and Caucasians. Marked differences in allele frequencies of -806C>T tagging the *CYP2C19*17a* haplotype were observed among East Asians, Nigerians, and Caucasians. Its frequency was about twenty times higher in Caucasians (0.217) and in Nigerians (0.275) than in Japanese (0.008). As described above, Sim *et al.* [2006] reported that -806C>T together with -3402C>T (*CYP2C19*17*) showed interracial differences in allelic frequency among Swedes, Ethiopians and Chinese, and was associated with the ultra-EM phenotype for omeprazole due to augmented expression of CYP2C19. They predicted that the omeprazole AUC (area under the plasma concentration-time curve) in subjects homozygous for *CYP2C19*17* would be 60% of that of subjects homozygous for *CYP2C19*1*. Thus, it is possible that *CYP2C19*17* (-806C>T, -3402C>T) and its representative haplotype *CYP2C19*17a* in Japanese cause therapeutic failures in treatment with proton-pump inhibitors and antidepressants. Further studies on comprehensive haplotype structures in *CYP2C19* of major ethnic groups and their associations with the metabolism of CYP2C19 substrate drugs are necessary.

Table 4. Ethnic Differences in Allelic Frequencies of Haplotype-tagging SNPs of *CYP2C19*

Haplotype-Tagging SNP in *CYP2C19*	dbSNP ID (NCBI)	Haplotype[a]	Our Study	HapMap*			
			Japanese (253 Subjects)	CEU (60 Subjects)	YRI (60 Subjects)	CHB (45 Subjects)	Japanese (45 Subjects)
IVS7-106T>C	rs4917623	*CYP2C19*1d*	0.530	0.508	0.183¶	0.602	0.593
681G>A (splicing defect)	rs4244285	*CYP2C19*2c*	0.267	0.150†	0.167	0.256	0.284
636G>A (W212X)	rs4986893	*CYP2C19*3b*	0.128	ND¶	ND¶	0.033	0.045
991A (I331)[b]	rs3758581	*CYP2C19*1e*	0.045	0.058	ND	0.056	0.091
IVS7-201G>A	rs17882222	*CYP2C19*1f*	0.024				
-806C>T	rs12248560	*CYP2C19*17a*	0.008	0.217¶	0.275¶	0.022	ND

ND: not detected.

[a]*CYP2C19* haplotypes in a Japanese population are defined by Fukushima-Uesaka *et al* [2005].

*http://www.hapmap.org/index.html.ja (as of July 15, 2006). CEU, YRI and CHB are U.S. (residents with ancestry from Northern and Western Europe), Nigeria (Yoruba) and Chinese populations, respectively. Significant differences (†$P<0.05$, ¶$P<0.01$, chi-square test) in allele frequencies between our Japanese population and each ethnic population. The multiple comparison was corrected by Bonferroni's method.

[b]The minor allele, 991A (I331), tags *CYP2C19*1e* [(major allele is 991G (V331)].

LDs and Haplotype Structures of the CYP2C Cluster

It has recently become evident that alleles or haplotypes in the *CYP2C* subfamily gene (*CYP2C18*, *CYP2C19*, *CYP2C9* and *CYP2C8*) are closely linked with each other. By genotyping 1468 subjects in Stockholm, Yasar *et al.* [2002b] showed a strong linkage of *CYP2C9*2* with *CYP2C8*3* harboring two SNPs, 416G>A (R139K) and 1196A>G (K399R). In their study, approximately 96% of the subjects with the *CYP2C8*3* alleles also carried *CYP2C9*2*, and 85% of the subjects that had *CYP2C9*2* also carried *CYP2C8*3*. A similar linkage has been reported between *CYP2C18* and *CYP2C19* variations [Mamiya *et al.* 1998]. A coding region polymorphism in *CYP2C18*, which generates a premature stop codon (204T>A, Y68X), was completely linked to the *CYP2C19*3* allele in a Japanese population, suggesting that individuals who lack CYP2C19 activity also lack CYP2C18 activity. In addition, an upstream *CYP2C18* polymorphism (-478T>C) was in complete linkage with the *CYP2C19*2* allele although the effect of this upstream polymorphism on gene expression is currently unknown.

The LD profiles of SNPs in the polygenic *CYP2C* region from two population samples (European and Japanese) indicated that the four *CYP2C* genes are possibly divided into two LD blocks (clusters): *CYP2C18* and *CYP2C19* in cluster 1 and *CYP2C9* and *CYP2C8* in cluster 2 [Ahmadi *et al.*, 2005]. Analysis using HapMap data from Europeans, Yoruba, Chinese, and Japanese suggested that a more extensive LD block is observed in *CYP2C* across populations: *CYP2C* cluster 1 spans *CYP2C18* and *CYP2C19* and also includes the exonic part of *CYP2C9*, and *CYP2C* cluster 2 includes *CYP2C8* and a small part of the *CYP2C9* 3'-flanking region [Walton *et al.*, 2005]. We analyzed LD patterns for 253 Japanese subjects and revealed the associations of haplotypes between *CYP2C9* and *CYP2C19*. As shown in Fig. (**1**), of all 1225 pairwise |D'| values between 50 common SNPs consisting of 24 in *CYP2C19* [Fukushima-Uesaka *et al.*, 2005] and 26 in *CYP2C9* (> 0.01 in their allele frequencies) [Maekawa *et al.*, 2006], 988 pairs (81%) had |D'|>0.90, indicating an extended LD block covering both *CYP2C19* and *CYP2C9*. The long-range haplotypes spanning *CYP2C19* and *CYP2C9* were inferred using 12 htSNPs (Fig. **2**). The most dominant haplotype, *H1* (0.524 frequency), is the combination of the wild-type haplotypes of both *CYP2C19* (*CYP2C19*1d*) and *CYP2C9* (*CYP2C9*1A*) in Japanese that are associated with extensive metabolic phenotypes. The defective allele of *CYP2C19*, *CYP2C19*2* (681G>A, splicing defect), was assigned to either *H2* or *H4* with a frequency of

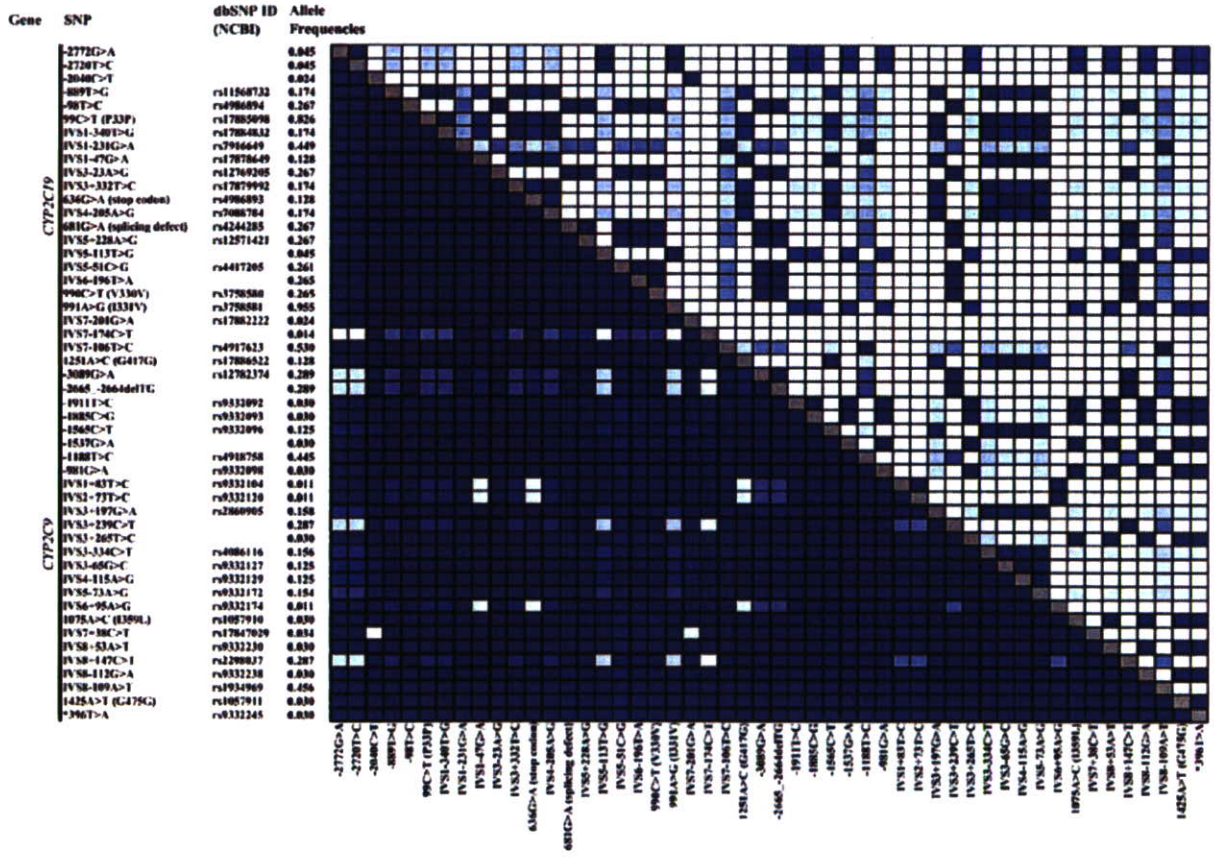

Fig. (1). Linkage disequilibrium (LD) analysis of *CYP2C19* and *CYP2C9* in a Japanese population (253 subjects). Pairwise LD between 50 common variations is expressed as /D'| (lower left) and r^2 (upper right) by 10-graded blue colors. The denser color indicates higher linkage. Allele frequencies of variations in Japanese are also shown.

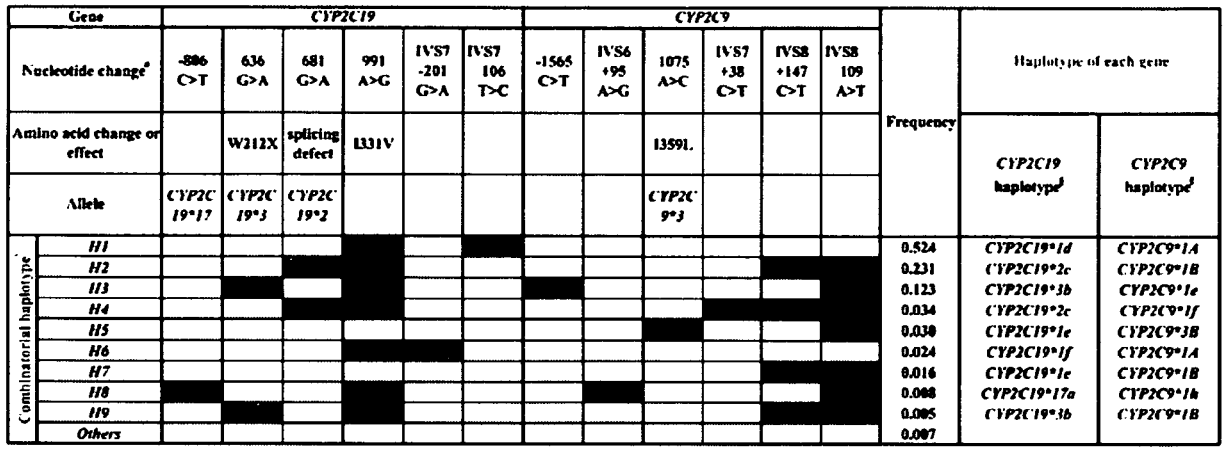

Fig. (2). Long-range haplotypes spanning *CYP2C19* and *CYP2C9* in a Japanese population (253 subjects). #A of the translational start codon of *CYP2C19* or *CYP2C9* is numbered +1. NT_030059.12 was used as the reference sequence. *Major allele, white; minor allele, gray. §Refer to Fukushima-Uesaka *et al.* [2005] for detailed *CYP2C19* haplotypes. ¶Refer to Maekawa *et al.* [2006] for detailed *CYP2C9* haplotypes.

0.231 and 0.034, respectively. Two haplotypes, *H3* and *H9* with frequencies of 0.123 and 0.005, respectively, contained another defective allele of *CYP2C19*, *CYP2C19*3* (636G>A, W212X). *CYP2C9*3* (1075A>C, I359L) was assigned to *H5* with a frequency of 0.030. There is no linkage among *CYP2C19*2*, *CYP2C19*3*, and *CYP2C9*3*, suggesting that statistically, Japanese individuals are unlikely to show PM phenotypes simultaneously for both *CYP2C19* and *CYP2C9*. However, the diplotype configurations showed that about 67% of Japanese individuals bear one or two copies of haplotypes harboring either *CYP2C19*2*, *CYP2C19*3* or *CYP2C9*3* (*H2*, *H3*, *H4*, *H5*, *H9*) (data not shown). *CYP2C19*17* associated with the increased transcriptional activity [Sim *et al.*, 2006] and *CYP2C9*1h* were linked mutually and formed *H8* with frequencies of 0.008. This linkage might be conserved across populations because allele frequencies of both -806C>T in *CYP2C19* and IVS6+95A>G in *CYP2C9*, tagging *CYP2C19*17a* and *CYP2C9*1h*, respectively, was significantly different between Japanese (probably Asians) and the other ethnicities described above, but parallel within a population (Tables **2** and **4**). Some CYP2C9 substrate drugs are also metabolized by CYP2C19 (phenytoin, tolbutamide, and chlorpropamide) or by CYP2C8 (troglitazone, pioglitazone, and rosiglitazone). The evaluation of LD profiles and long-range haplotype structures in the *CYP2C* gene region including *CYP2C18*, *CYP2C19*, *CYP2C9*, and *CYP2C8* will facilitate pharmacogenetic studies aimed at detecting phenotypic differences of drugs with dual (complicated) metabolic pathways mediated by at least two enzymes.

CYP2D6

Cytochrome P450 (CYP) 2D6 metabolizes a number of clinically important drugs such as anti-arrythmics, psychiatrics, anti-histamines, and anti-depressants as well as endogenous substances [Ingelman-Sundberg, 2005 for review]. As for the major defective alleles **4* (1846G>A, splicing defect) and **5* (gene deletion), the frequency of **4* is relatively high in Caucasians but very low in the Chinese and Japanese [Ingelman-Sundberg, 2005; Bradford *et al.*, 2002]. Instead, the **10* allele, which confers a partially reduced enzymatic activity, is found at much higher allele frequencies in Japanese (38 to 43%) [Dahl *et al.*, 1995; Tateishi *et al.*, 1999; Nishida *et al.*, 2000; Kubota *et al.*, 2000], Chinese (40 to 50%) [Wang *et al.*, 1993; Johansson *et al.*, 1994; Dahl *et al.*, 1995; Droll *et al.*, 1998] and Koreans (35 to 50%) [Dahl *et al.*, 1995; Roh *et al.*, 1996; Yoon *et al.*, 2000] than in Caucasians (1 to 3%) [Sachse *et al.*, 1997; Droll *et al.*, 1998; Griese *et al.*, 1998].

A number of other *CYP2D6* variant alleles have been reported (http://www.cypalleles.ki.se/cyp2d6.htm, as of July 15, 2006, SNP positions were shown following this web site). Among them, relatively frequent alleles found in Caucasians and/or Africans are **2*, **3*, **6*, **9*, **17* [See Bradford 2002 for ethnic distributions], **29* and **41*. The **2* allele (R296C and S486T) is thought to be the second wild-type but may have slightly altered substrate specificity [Tsuzuki *et al.*, 2001; Marcicci *et al.*, 2002]. The **3* allele (2549delA, frame-shift) [Kagimoto *et al.*, 1990] found in Caucasians is rare in Africans. The **6* (1707delT, frame-shift) [Saxena *et al.*, 1994] is found in Caucasians and American Indians. The **9* allele (K281del) [Tyndale *et al.*, 1991; Broly and Meyer, 1993] is found in Caucasians and Malays [Teh *et al.*, 2001]. The **17* allele (T107I, R296C, and S486T), which has changed substrate specificity [Masimirembwa *et al.*, 1996; Wennerholm *et al.*, 2002], and **29* (V136M, R296C, V338M and S486T) [Marez *et al.*, 1997; Wennerholm *et al.*, 2001] are commonly found in black Africans. Except for **2* and **41*, these alleles were hardly found in East Asians.

Genetic Polymorphisms of CYP2D6 Found in East Asians

In addition to **2* and **10*, **41* is relatively frequently found in Japanese [Ikenaga *et al.*, 2005; our unpublished data] and Koreans [Lee *et al.*, 2006a] at allele frequencies around 0.02. This allele is a low-activity **2* variant with -1584C and intronic 2988G>A [Raimundo *et al.*, 2004; Toscano *et al.*, 2006], conferring the intermediate metabolizer phenotype to Caucasians [Raimundo *et al.*, 2000; Zanger *et al.*, 2001] and Mexicans [Luo *et al.*, 2005]. In

Japanese, -1584C and 2988A are perfectly linked to each other [our unpublished data].

The **2*-group minor alleles **14* (G169R, R296C and S486T) (Ji *et al.*, 2002a) and **21* (2573_2574insC, frameshift) [Chida *et al.*, 1999a; Yamazaki *et al.*, 2003] are found at frequencis of 0.001 to 0.02 [Nishida *et al.*, 2000; Soyama *et al.*, 2004; Ikenaga *et al.*, 2005; Ebisawa *et al.*, 2005; Ji *et al.*, 2002b; Lee *et al.*, 2006a]. The **1*-group **18* allele (468_470dupVPT) [Yokoi *et al.*, 1996] identified in a Japanese poor metabolizer has been found in Japanese at frequencies of 0.002 to 0.007 [Yokoi *et al.*, 1996; Chida *et al.*, 1999b; Soyama *et al.*, 2004] but not in Chinese [Garcia-Barcelo *et al.*, 2000a] or Koreans [Lee *et al.*, 2006a]. As described above, the **4* allele is rare (at allele frequencies of 0.002 to 0.008), and **3* is hardly found in East Asians [Wang *et al.*, 1993; Pang *et al.*, 1998; Garcia-Barcelo *et al.*, 2000a; Kubota *et al.*, 2000; Nishida *et al.*, 2000; Soyama *et al.*, 2004; Ebisawa *et al.*, 2005; Lee *et al.*, 2006a].

Our comprehensive resequencing of the gene in 263 Japanese subjects [Soyama *et al.*, 2002; Soyama *et al.*, 2004; our unpublished data] detected *CYP2D6*1A*, **2A*, and **10B*, which are known to exist with high frequencies in the Japanese, their known (**14*, **18*, **21*, **41*, and **44*) and novel (**47* to **51*) variant alleles and a number of intronic variations [Soyama *et al.*, 2002; Soyama *et al.*, 2004]. Ebisawa *et al.* [2005] have reported additional novel alleles **53* to **55* as well as **27* and **39* from a study with 286 Japanese subjects. Lee *et al.* [2006a] also resequenced the *CYP2D6* gene in 400 Koreans and detected the minor alleles **14*, **21*, **27*, **35*, **39*, and **47*.

Prevalence of *36-*10B in Japanese

The **10* allele was first reported as a single nucleotide polymorphism 100C>T (P34S) in exon 1 in a Japanese population [Yokota *et al.*, 1993]. Johansson *et al.* [1994] found two low-activity *CYP2D6* genes, *CYP2D6Ch*$_1$ (**10B*) and *CYP2D6Ch*$_2$ (**36*), in Chinese subjects who were intermediate metabolizers. These genes were tandemly organized downstream of *CYP2D7P* in the following order: *CYP2D8P-CYP2D7P-CYP2D6Ch*$_2$ (**36*)-*CYP2D6Ch*$_1$ (**10B*). This genomic organization confers the *Xba*I 44-kb haplotype. In addition, a single-type (*Xba*I 29-kb) **10B*, *CYP2D8P-CYP2D7P-CYP2D6Ch*$_1$ (**10B*), was also found. *CYP2D6*Ch*$_2$, originally designated **10C* and renamed **36*, is thought to be generated by recombination with the pseudogene *CYP2D7P* at a site upstream of exon 9, resulting in 13 nucleotide changes with six amino acid substitutions.

Although the tandem form of **36-*10B* was assumed to be a major form [Johansson *et al.*, 1994; Garcia-Barcelo *et al.*, 2000b; Nishida *et al.*, 2000], no detailed information has been published for its intervening and flanking regions. We first confirmed the presence of the tandem-type **36-*10B* utilizing long-range PCR with an intron 6-specific forward primer and an intron 2-specific reverse primer and then resequenced both genes. Our sequence data have shown that most (83%) of the **10*-positive haplotypes harbor the upstream **36* gene [Soyama *et al.*, 2006a]. Frequencies of the single-type **10B* and **36-*10B* were 0.055 and 0.278 [our unpublished data], respectively.

Since the regions between *CYP2D7P* and **36* and between **36* and **10B* have not been sequenced yet, the complete sequence of the entire **36-*10B* region was also obtained [GenBank DQ211353]. Our sequence data indicated the structure of *CYP2D6*36*-REP7-*CYP2D6*10*, and the downstream **10* was confirmed to be **10B* (or its variants). Moreover, the single-type **10B* was shown to have the structure of *CYP2D7P*-REP7-*CYP2D6*10B*-REP6, and the distance between the 3'-end of **10* and CYP-REP6 was 1.6-kb shorter than that between the 3'-end of **36* and CYP-REP7 [Soyama *et al.*, 2006a].

Gene Duplication in East Asians

The other type found in the Chinese by Johansson *et al.* [1994] was the *Xba*I 42-kb duplicated genes, which had the structure of *CYP2D8P-CYP2D7P-CYP2D6L2-CYP2D6L1* (*CYP2D6*2X2*). Several research groups have investigated duplicated *CYP2D6* genes in Asians and have found *CYP2D6*1X2*, *CYP2D6*10X2* [Roh *et al.*, 1996; Garcia-Barcelo *et al.*, 2000b; Nishida *et al.*, 2000; Ishiguro *et al.*, 2003; Mitsunaga *et al.*, 2002; Soyama *et al.*, 2006a; Lee *et al.*, 2006a], and *CYP2D6*36X2* [Chida *et al.*, 2002; Gaedigk *et al.*, 2006]. The allele frequencies were low (mostly around 0.005), and their detailed structures and functional relevance in Asian populations remains mostly unclear. Ishiguro et al. [2004a] have reported that **1X2/*1* and **2X2/*1* subjects show an ultrarapid metabolizer phenotype for dextromethorphan *O*-demethylation, but that **10X2* does not show a gene-dose effect.

Novel CYP2D6 Haplotypes Containing Chimeric REP7/6

Recently, a novel **10*-related haplotype, named *CYP2D6*10D* (**10D*) [Ishiguro *et al.*, 2004b], was found with a frequency of approximately 0.003 in Japanese [Fukuda *et al.*, 2005]. The **10D* haplotype harbors a downstream *CYP2D7*-derived region and a chimeric repetitive sequence, CYP-REP7/6 (REP7/6). REP7/6 structures are also present in the deletion haplotype **5* [Steen *et al.*, 1995] and have been often utilized for **5*-typing [Hersberger *et al.*, 2000]. Thus, for Japanese and probably Chinese and Koreans, the typing of REP7/6 might have caused misplacement of **10D* as **5* [Ishiguro *et al.*, 2004b, Lee *et al.*, 2006a]. In addition to the single-type **10D*, we found an additional **10D*-bearing haplotype, **36-*10D*, at a frequency of 0.004. Moreover, a novel defective structure consisting of *CYP2D6*36* followed by 3'-flanking REP7/6 (single-type **36*-REP7/6) was also found in a Japanese population at a frequency of 0.004 [Soyama *et al.*, 2006b]. Gaedigk *et al.* [2006] have also found a single-type **36* in an Asian subject as well as in 9 African-Americans. The haplotype structures that we have found in Japanese are shown in Fig. (**3**).

Then, the REP7/6 sequences in **5*, **10D*, **36-*10D*, and **36*-REP7/6 were determined and classified into 5 types: types A to D for **5*, type E for **10D* and **36-*10D*, and type F for **36* [Soyama *et al.*, 2006b]. Comparisons of the sequences revealed that types A, C, and D were derived from the **1* sequence, and type B from the **2* sequence, and type E from the **10* sequence. These findings could be useful for accurate determination of the **5* and REP7/6-harboring aberrant *CYP2D6* haplotypes in Asian populations.

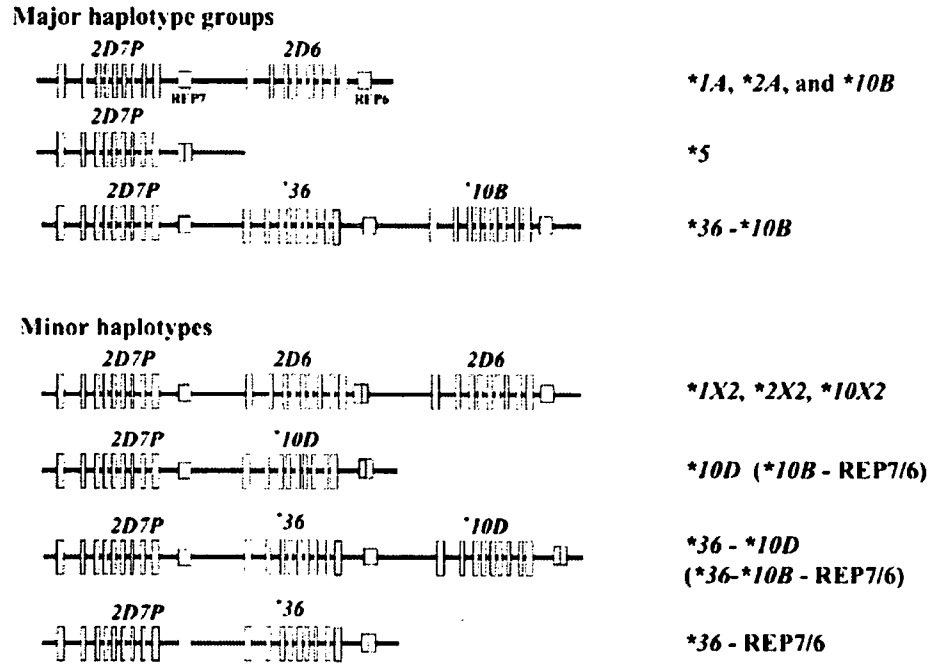

Fig. (3). Structures of *CYP2D6* haplotypes in a Japanese population. Exon and repetitive sequences derived from *CYP2D7P* are shown by dotted boxes.

Enzymatic activity of **10D* is considered almost the same as that of **10B* because sequences in the coding and proximal promoter regions of **10D* are identical to that of **10B* [Ishiguro *et al.*, 2004b; Soyama *et al.*, 2006b; our unpublished data]. Since CYP2D6.36 shows very low activities towards several drugs [Johansson *et al.*, 1994; Fukuda *et al.*, 2000; Hanioka *et al.*, 2006], **36-*10D* activity is considered similar to that of **10B* and **10D*. On the other hand, single-type **36* (*CYP2D6*36*-REP7/6) would be defective.

CYP3A4

The human cytochrome P450 (CYP) 3A subfamily has been estimated to be involved in the metabolism of 50% of the currently used therapeutic drugs [Wrighton *et al.*, 1996, Thummel and Wilkinson, 1998; Guengerich, 1999]. The *CYP3A5*, *CYP3A7*, *CYP3A4*, and *CYP3A43* genes consist of a cluster spanning 231 kb on chromosome 7 in the order listed above [Gellner *et al.*, 2001]. Overall, the CYP3A subfamily is the predominant P450 isoforms in human adult liver (approximately 30% of the total P450 content) [Shimada *et al.*, 1994]. The expression of CYP3A enzymes is differentially regulated in the developmental process: CYP3A7 levels are high in fetal liver, and CYP3A4 is abundant in adult liver. CYP3A5 is present in both fetal and adult livers, but its expression is known to be highly polymorphic. Since CYP3A43 is expressed at very low levels in several tissues including liver, it is believed not to play a substantial role in drug metabolism. In this review, we focus on the genetic polymorphisms of *CYP3A4*.

Among the subfamily members, CYP3A4 is the most predominant form in the adult human liver. This enzyme metabolizes a wide variety of substrates without structural similarity including steroids, fatty acids and xenobiotics (drugs, pesticides and carcinogens) [Wrighton *et al.*, 1996; Thummel and Wilkinson, 1998; Guengerich, 1999]. Up to 90-fold interindividual variations in CYP3A4 expression levels have been observed in Caucasian liver microsomes [Hustert *et al.*, 2001]. Furthermore, there are 40-60 fold variations in the metabolism of CYP3A substrates *in vivo* [Shimada *et al.*, 1994; Thummel and Wilkinson, 1998]. These interindividual differences are likely to influence pharmacokinetics, drug-drug interactions, efficacy, and adverse effects of drugs. Thus, it is clinically important to predict CYP3A4 activity in the liver or other tissues, such as the intestine.

CYP3A4 Polymorphisms

It has been suggested that approximately 85% of the interindividual variability in hepatic CYP3A4 activity is due to genetic factors [Ozdemir *et al.*, 2000]. Thus, several research groups have focused on the identification of *CYP3A4* variations (Lamba *et al.*, 2002). To date, 40 *CYP3A4* alleles (or haplotypes), including 20 subtypes, have been published on the Human Cytochrome P450 Allele Nomenclature Committee homepage (http://www.cypalleles.ki.se/cyp3a4.htm, as of July 11, 2006) [Lee and Goldstein, 2005; Krishna and Shekar, 2005 for review]. The distribution of *CYP3A4* alleles among different ethnic populations is summarized in Table **5** and Table **6**.

An A to G mutation at -392 in the 5'-flanking region is designated as *CYP3A4*1B*. This allele is found at 0.53 to 0.87 frequencies in Africans, 0.04 to 0.10 in Caucasians, 0.06 to 0.09 in Hispanics, and 0.09 in Saudi, but is absent in other Asians (Table **5**). The functional significance of this allele has been controversial. It has been reported that *CYP3A4*1B* caused a reduction in nuclear protein binding to

Table 5. Allelic Frequencies of *CYP3A4*1B* (-392A>G) in Different Ethnic Populations

Population	Allele Frequency	Number of Subjects	Reference
Caucasians			
Caucasian-American[¶]	0.096 0.036 0.090	94 273 132	Rebbeck *et al.* 1998 Ball *et al.* 1999 Walker *et al.* 1998
Finnish[¶]	0.042	59	Sata *et al.* 2000
Scottish[¶]	0.054	101	Tayeb *et al.* 2000
Dutch[¶]	0.053	199	van Schaik *et al.* 2000
Portuguese[¶]	0.040	100	Cavaco *et al.* 2003
Africans			
African-American[¶]	0.546 0.530 0.667	186 70 75	Ball *et al.* 1999 Walker *et al.* 1998 Sata *et al.* 2000
Ghanaian[¶]	0.690	100	Tayeb *et al.* 2000
Senegalese[¶]	0.780	178	Zeigler-Johnson *et al.* 2002
Nigerian[¶]	0.866	82	Kittles *et al.* 2002
Asians			
Japanese	ND ND	150 416	Naoe *et al.* 2000 Fukushima-Uesaka *et al.* 2004
Japanese-American	ND	77	Ball *et al.* 1999
Chinese-American	ND	78	Ball *et al.* 1999
Chinese	ND	96	Chowbay *et al.* 2003
Taiwanese	ND ND	130 59	Walker *et al.* 1998 Sata *et al.* 2000
Malay	ND	92	Chowbay *et al.* 2003
Indian	ND	87	Chowbay *et al.* 2003
Saudi[¶]	0.089	101	Tayeb *et al.* 2000
Hispanics			
Hispanic-American[¶]	0.093	188	Ball *et al.* 1999
Mexican[¶]	0.058	69	Reyes-Hernandez *et al.* 2004

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.

Table 6. Distribution of Nonsynonymous *CYP3A4* Alleles among Different Populations

Allele	Nucleotide Change	Amino Acid Change	Population	Allele Frequency	Number of Subjects	Functional Effect	Reference
**2*	664T>C	S222P	Finnish	0.027	55	Altered activity depending on the substrates (*in vitro*)	Sata *et al.* 2000
			Portuguese	0.045	100		Cavaco *et al.* 2003

(Table 6. Contd....)

Allele	Nucleotide Change	Amino Acid Change	Population	Allele Frequency	Number of Subjects	Functional Effect	Reference
*3	1334T>C	M445T	Caucasian	0.005	213	No apparent change in activity (*in vitro*)	Eiselt *et al.* 2001
			Caucasian	0.042	24	No apparent change in activity (*in vitro*)	Dai *et al.* 2001
						No change in activity (*in vitro*)	Lee *et al.* 2005
			European	0.021	94		Garsa *et al.* 2005
*4	352A>G	I118V	Chinese	0.015	102	Reduced activity (*4:n=3, *in vivo*)	Hsieh *et al.* 2001
			Chinese	0.033	211	Reduced activity (*4:n=8, *in vivo*)	Wang *et al.* 2005
			Chinese	0.004	387		Wen *et al.* 2004
			Chinese	0.024	451		Liu *et al.* 2005
*5	653C>G	P218R	Chinese	0.010	102	Reduced activity (*5:n=2, *in vivo*)	Hsieh *et al.* 2001
			Chinese	0.007	387		Wen *et al.* 2004
			Chinese	0.007	451		Liu *et al.* 2005
*6	830_831insA	frame-shift	Chinese	0.005	102	Reduced activity (*6:n=1, *in vivo*)	Hsieh *et al.* 2001
			Chinese	0.005	387		Wen *et al.* 2004
			Malay	0.005	104		Chowbay *et al.* 2003
			Indian	0.005	101		Chowbay *et al.* 2003
			Japanese	0.001	416		Fukushima-Uesaka *et al.* 2004
*7	167G>A	G56D	Caucasian	0.014	213	No apparent change in activity (*in vitro*)	Eiselt *et al.* 2001
*8	389G>A	R130Q	Caucasian	0.003	150	No holoprotein (*in vitro*)	Eiselt *et al.* 2001
*9	508G>A	V170I	Caucasian	0.002	212	No apparent change in activity (*in vitro*)	Eiselt *et al.* 2001
*10	520G>C	D174H	Caucasian	0.002	212	Reduced activity? (*in vitro*)	Eiselt *et al.* 2001
			Caucasian	0.020	53		Lamba *et al.* 2002
			African-American	0.020	21		Lamba *et al.* 2002
			Mexican	0.050	10		Lamba *et al.* 2002
*11	1088C>T	T363M	Caucasian	0.003	149	Reduced holoprotein level (*in vitro*)	Eiselt *et al.* 2001
			Japanese	0.002	416		Fukushima-Uesaka *et al.* 2004
						Reduced activity (*in vitro*)	Murayama *et al.* 2002
*12	1117C>T	L373F	Caucasian	0.003	149	Altered activity depending on the substrates (*in vitro*)	Eiselt *et al.* 2001

(Table 6. Contd....)

Allele	Nucleotide Change	Amino Acid Change	Population	Allele Frequency	Number of Subjects	Functional Effect	Reference
**13*	1247C>T	P416L	Caucasian	0.003	149	No holoprotein (*in vitro*)	Eiselt *et al.* 2001
**14*	44T>C	L15P	unknown	0.060	8	Unknown	Lamba *et al.* 2002
**15*	485G>A	R162Q	African	0.042	24	Unknown	Dai *et al.* 2001
**16*	554C>G	T185S	Mexican	0.050	10		Lamba *et al.* 2002
			Japanese	0.050	10		Lamba *et al.* 2002
			Japanese	0.014	416		Fukushima-Uesaka *et al.* 2004
						Reduced activity (*in vitro*)	Murayama *et al.* 2002
			Japanese			Reduced activity (**16*:n=9, *in vivo*)	Nakajima *et al.* 2006
**17*	566T>C	F189S	Caucasian	0.021	24	Reduced activity (*in vitro*)	Dai *et al.* 2001
			Caucasian	ND	100	Markedly reduced activity (*in vitro*)	Lee *et al.* 2005
**18*	878T>C	L293P	Asian	0.021	24	Increased activity (*in vitro*)	Dai *et al.* 2001
			Japanese	0.028	416		Fukushima-Uesaka *et al.* 2004
						Increased activity (*in vitro*)	Murayama *et al.* 2002
						No change in activity (*in vitro*)	Lee *et al.* 2005
			Chinese	0.008	387		Wen *et al.* 2004
**19*	1399C>T	P467S	Asian	0.021	24	No apparent change in activity (*in vitro*)	Dai *et al.* 2001
						No change in activity (*in vitro*)	Lee *et al.* 2005
**20*	1461_1462insA	frame-shift	Brazilian	unknown	unknown	No holoprotein (*in vitro*)	Westlind-Johnsson *et al.* 2006

ND: not detected.

the promoter region, resulting in reduced enzymatic activity [Rodriguez-Antona *et al.*, 2005], and lowered systemic clearance of midazolam [Wandel *et al.*, 2000]. Other studies, however, suggested no changes (or rather an increase) in enzyme activity both *in vivo* [Ball *et al.*, 1999; Hesselink *et al.*, 2004] and *in vitro* [Westlind *et al.*, 1999]. A recent report also showed its association with even higher expressions of mRNA and protein and enzymatic activity [Schirmer *et al.*, 2006]. At least part of this discrepancy *in vivo* might be explained by the linkage between *CYP3A4*1B* and *CYP3A5*1* (wild-type) as discussed below.

In contrast to the **1B* allele, nonsynonymous polymorphisms were relatively rare in all ethnic groups (Table 6). The allele **2* with a S222P change showed a higher *K*m and lower *V*max for nifedipine, resulting in a 6 to 9-fold reduction of intrinsic clearance *in vitro* [Sata *et al.*, 2000]. However, no significant change was observed in testosterone 6β-hydroxylase activity. This allele was found at frequencies of 0.027 in Finnish [Sata *et al.*, 2000] and 0.045 in Portuguese [Cavaco *et al.*, 2003]. *CYP3A4*3* (M445T) had no functional changes for chlorpyrifos [Dai *et al.*, 2001], testosterone and progesterone [Eiselt *et al.*, 2001], and nifedipine [Lee *et al.*, 2005] when the variant enzyme was expressed in *E. coli*. *CYP3A4*4* to **6* were found only in Asians. *CYP3A4*4* (I118V) has been detected at 0.01 to 0.03 frequencies only in Chinese, but has not been detected in 416 Japanese subjects [Fukushima-Uesaka *et al.*, 2004]. The subjects with the **4* allele showed a reduced ratio of urine 6β-hydroxycortisol to free cortisol, suggesting reduced enzyme activity [Hsieh *et al.*, 2001; Wang *et al.*, 2005]. Furthermore, heterozygous **4* patients showed significantly increased lipid-lowering effects of simvastatin compared with homozygous **1* patients [Wang *et al.*, 2005]. Two Chinese subjects with heterozygous **5* (P218R) also showed a reduced ratio of urine 6β-hydroxycortisol to free cortisol [Hsieh *et al.*, 2001]. *CYP3A4*5* was detected at ~1% frequency only in Chinese, but not in 416 Japanese [Fukushima-Uesaka *et al.*, 2004]. *CYP3A4*6* allele, a rare allele detected in Chinese, Japanese,

Malay and Indian, has an insertion of adenine between 830 and 831, resulting in a frame-shift from E277 and an immature stop codon at 285 [Hsieh *et al.*, 2001]. Thus, the variant enzyme is most likely to be non-functional. A patient heterozygous for this allele showed a reduced urine 6β-hydroxycortisol/free cortisol ratio [Hsieh *et al.*, 2001]. *CYP3A4*7* (G56D), **8* (R130Q), and **9* (V170I) were detected only in Caucasians [Eiselt *et al.*, 2001]. The **8* variant exhibited no holoprotein formation in *E. coli*, although its apoprotein was slightly expressed. *CYP3A4*10* (D174H) was found in Caucasians, African-Americans, and Mexicans but not in Japanese. The variant protein exhibited a slightly reduced testosterone and progesterone hydroxylase activity at a low substrate concentration (25 μM) [Eiselt *et al.*, 2001]. *CYP3A4*11* (T363M) is a rare allele detected in Caucasians and Japanese, and its recombinant protein had reduced holoprotein levels [Eiselt *et al.*, 2001]. When expressed in a human liver cell line, HepG2, the variant enzyme had ~40% decrease in intrinsic clearance (*V*max/*K*m) for the testosterone 6β-hydroxylation reaction [Murayama *et al.*, 2002]. Both *CYP3A4*12* (L373F) and **13* (P416L) with reduced expression levels were detected only in Caucasians with very low frequencies [Eiselt *et al.*, 2001]. Functional significance of *CYP3A4*14* (L15P) and **15* (R162Q) has not been reported until now. *CYP3A4*16* (T185S) was found in Japanese and Mexicans with 0.01 to 0.05 frequencies. The CYP3A4.16 proteins expressed in HepG2 cells exhibited about 50% reduction in the intrinsic clearance (*V*max/*K*m) in testosterone 6β-hydroxylation activity with about 60% decrease in *V*max [Murayama *et al.*, 2002]. Very recently, we have shown for the first time that heterozygous **16* patients administered paclitaxel show significantly reduced 3'-*p*-hydroxypaclitaxel/paclitaxel AUC ratio and increased 6α-hydroxypaclitaxel/paclitaxel AUC ratio, suggesting that *CYP3A4*16* is indeed a low-activity allele [Nakajima *et al.*, 2006]. *CYP3A4*17* (F189S) detected in Caucasians had a low activity to testosterone and almost negligible activities for chlorpyrifos and nifedipine *in vitro* [Dai *et al.*, 2001; Lee *et al.*, 2005]. *CYP3A4*18* allele (L293P) was found in Asians at 0.01 to 0.03 frequencies. The CYP3A4.18 protein exhibited increased activities for testosterone and chlorpyrifos, but was unchanged for nifedipine compared with wild-type CYP3A4 enzymes [Dai *et al.*, 2001; Lee *et al.*, 2005]. Our *in vitro* analysis revealed that increased activity for testosterone was attributed to an increased *V*max [Murayama *et al.*, 2002]. However, the *in vivo* activity did not alter in Japanese heterozygotes [Nakajima *et al.*, 2006]. *CYP3A4*19* (P467S) in Asians catalyzes testosterone, chlorpyrifos and nifedipine similar to that of the wild-type enzymes [Dai *et al.*, 2001; Lee *et al.*, 2005]. The most recently identified **20* allele, found in a Brazilian patient, is defective, but was not detected in 413 Caucasian, 195 African and 230 Chinese samples [Westlind-Johnsson *et al.*, 2006]. Overall, functionally important and relatively frequent (≥1%) alleles are **4*, **16* and **18* (and probably **5*) for Asians (at least for East Asians) and **2* for Caucasians.

Since nonsynonymous polymorphisms are relatively rare, the transcriptional regulatory regions have also been analyzed. It has been shown that CYP3A4 induction is mediated by pregnane/steroid X receptor (PXR/SXR), constitutive androstane receptor and the vitamin D receptor (VDR) through binding to the distal xenobiotic-responsive enhancer module (XREM) (-7.7 kb and -7.3 kb upstream of the transcriptional start site), and to the proximal promoter region, especially to the proximal PXR/SXR response element (-169 to -152 from the transcriptional start site) [Goodwin *et al.*, 1999, 2002; Drocourt *et al.*, 2002]. Hepatocyte nuclear factor-4α also binds to the region immediately upstream of XREM and increases basal and the above transcriptional factor-mediated reporter gene expression [Tirona *et al.*, 2003]. However, no functional polymorphism has been found in these transcriptional factor-binding elements in Japanese [Fukushima-Uesaka *et al.*, 2004]. Recently, Matsumura *et al.* [2004] reported that a TGT insertion between -11,129 and -11,128 (from the transcriptional start site) resulted in the disruption of transcriptional factor USF1 binding and a 36% reduction of enhancer activity. This polymorphism was detected at a 0.031 frequency in 511 French subjects, but not in 131 Japanese subjects, suggesting that this polymorphism is an important factor in regulating CYP3A4 activity in Caucasians.

LDs and Haplotype Structures of the CYP3A Cluster

The *CYP3A5*, *CYP3A7*, *CYP3A4*, and *CYP3A43* genes are located on chromosome 7 (7q21.1) in this order. Extreme interethnic variability exists in CYP3A5 expression, and this variation has been shown to be mostly due to IVS3-237A>G (6986A>G) in intron 3, designated *CYP3A5*3*. This polymorphism generates an incorrectly spliced mRNA and a non-functional protein [Kuehl *et al.*, 2001]. This allele has reported in the frequencies of 0.06-0.84 in Africans, 0.85-0.95 in Caucasians, 0.59-0.82 in Asians, 0.71-0.85 in Hispanics, and 0.79-0.82 in Pacific Islanders (summarized in Table 7). Thus, there is extreme variation in allele frequencies in Africans. This allele was known to be associated with reduced midazolam hydroxylation [Kuehl *et al.*, 2001] and clearance [Wong *et al.*, 2004], increased AUC of alprazolam [Park *et al.*, 2006], and reduced oral clearance of sirolimus [Le Meur *et al.*, 2006]. We analyzed haplotype structures of *CYP3A4* in 416 Japanese subjects [Fukushima-Uesaka *et al.*, 2004] and *CYP3A5* in 187 Japanese subjects [Saeki *et al.*, 2003]. Overall, 25 haplotypes were inferred in *CYP3A4*. Then, the association between the *CYP3A4* and *CYP3A5* haplotypes was analyzed [Fukushima-Uesaka *et al.*, 2004]. In Japanese, LD analysis of *CYP3A4* and *CYP3A5* showed strong linkages of polymorphisms between both genes. The *CYP3A4* haplotypes containing the IVS10+12G allele (such as **1A*) are very closely linked to *CYP3A5*3*. Inversely, most of the *CYP3A4* haplotypes with IVS10+12A (such as **1G*) are linked to *CYP3A5*1*. Thus, these results suggested that, in a Japanese population, genotyping the IVS10+12 position in *CYP3A4* can predict whether the subject has *CYP3A5*3* although the IVS10+12G>A polymorphism itself lacks functional significance [Nakajima *et al.*, 2006]. In addition, the low-activity haplotype *CYP3A4*16B* (with T185S and IVS10+12G>A) is perfectly linked with *CYP3A5*1E* (with IVS9+77G>T), but not with **3*. We also found that a *CYP3A7* SNP -425G>C (A of the translational start site of *CYP3A7* is numbered +1) was perfectly linked with a *CYP3A5* haplotype containing IVS2-102C>T and IVS11+177C>T [Fukushima-Uesaka *et al.*, 2004]. These results suggested that *CYP3A4*, *CYP3A5*, and *CYP3A7* (in the order

Table 7. Allelic Frequencies of *CYP3A5*3* (IVS3-237A>G) in Different Ethnic Populations

Population	Allele Frequency	Number of Subjects	Reference
Caucasians			
Caucasian-American*	0.85	27	Kuehl *et al.* 2001
Dutch¶	0.92	500	van Schaik *et al.* 2002
Caucasian-Canadian¶	0.93	77	Roy *et al.* 2005
French*	0.91	29	Thompson *et al.* 2004
Russian*	0.92	25	Thompson *et al.* 2004
Italian (Sardinian)*	0.95	28	Thompson *et al.* 2004
Africans			
African-American¶	0.55 0.27	20 45-50	Kuehl *et al.* 2001 Hustert *et al.* 2001
Nigerian (Yoruba)*	0.06	25	Thompson *et al.* 2004
Senegalese (Mandenka)*	0.31	24	Thompson *et al.* 2004
Algerian (Mozabite)*	0.84	29	Thompson *et al.* 2004
Zimbabwean	0.78	100	Roy *et al.* 2005
Asians			
Japanese	0.71 0.77 0.74 0.76	45-50 200 196 187	Hustert *et al.* 2001 Fukuen *et al.* 2002 Hiratsuka *et al.* 2002 Saeki *et al.* 2003
Chinese	0.73 0.76	45-50 108	Hustert *et al.* 2001 Balram *et al.* 2003
Korean*	0.70	45-50	Hustert *et al.* 2001
Malay¶	0.61	98	Balram *et al.* 2003
Indian¶	0.59	90	Balram *et al.* 2003
Cambodian*	0.73	11	Thompson *et al.* 2004
Pakistani (Kalash)*	0.76	25	Thompson *et al.* 2004
Palestinian	0.82	51	Thompson *et al.* 2004
Hispanics			
Mexican (Maya)*	0.71	24	Thompson *et al.* 2004
Brazilian (Karitiana)*	0.77	24	Thompson *et al.* 2004
Colombian*	0.85	13	Thompson *et al.* 2004
Pacific islanders			
Papua New Guinean*	0.79	17	Thompson *et al.* 2004
Melanesian (Bougainville)*	0.82	22	Thompson *et al.* 2004

¶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 due to the small number of subjects (<50 subjects).

3A5-3A7-3A4 on chromosome 7) are in the same LD block in Japanese.

In Caucasians, a close linkage between *CYP3A4*1A* and *CYP3A5*3* (inversely, *CYP3A4*1B* and *CYP3A5*1*) was

also observed [Plummer *et al.*, 2003; Zeigler-Johnson *et al.*, 2004]. A weaker linkage was also observed in African-Americans. The *CYP3A4*1B* and **1G* alleles were very closely linked in Caucasians [Schirmer *et al.* 2006], and no **1B* allele was detected in Japanese. Therefore, the linkage patterns between *CYP3A4* and *CYP3A5* are similar between Caucasians and Japanese. Similar LD patterns between *CYP3A4* and *CYP3A5* were also reported by a different group [Thompson *et al.*, 2004], where haplotype structures were shown to be different between African-Americans and non-African-Americans (Europeans and Han Chinese). The same group extended the analysis to all four genes in the *CYP3A* cluster using the 224 detected polymorphisms for 3 ethnic groups [Thompson *et al.*, 2006]. In Han Chinese and Europeans, strong LDs were observed among *CYP3A5*, *CYP3A7* and *CYP3A4*, but only between *CYP3A5* and *CYP3A7* in African-Americans. In all populations, LD decays substantially between *CYP3A4* and *CYP3A43* [Thompson *et al.*, 2006]. Thus, in the two non-African populations, the LD profiles of the *CYP3A* locus are relatively similar. Another group reported the LD patterns and haplotype structures of the *CYP3A* locus for 5 different ethnic groups (African-Americans, African Sans, European Caucasians, Chinese, and Japanese) [Schirmer *et al.*, 2006]. Strong LDs were also observed for the *CYP3A5-CYP3A7-CYP3A4* region in European Caucasians and Japanese, and their most frequent haplotype was similar. The tendency observed in Chinese was similar, but the LD between *CYP3A7* and *CYP3A4* was far weaker than in European Caucasians and Japanese. In African-Americans and African Sans, strong linkages were not detected between the *CYP3A7* and *CYP3A4* regions. The *CYP3A43* region forms discrete LD blocks in African-Americans and African Sans. The 5'-part of *CYP3A4* was in LD with *CYP3A43* in European Caucasians. In Chinese and Japanese, strong linkages were not observed in this region. This paper also described haplotype structures of the entire *CYP3A* cluster region and revealed that the most common haplotype was the same among Caucasians, Japanese and Chinese although the linkages downstream of the *CYP3A43* gene were different.

The clinical importance of these linkages among the *CYP3A* genes has not been investigated. However, the linkage between *CYP3A4*1B* and *CYP3A5*1* is probably important if a substrate drug is metabolized by both enzymes. In Japanese, the low-activity haplotype *CYP3A4*16B* was perfectly linked with *CYP3A5*1*, but not **3*, suggesting that the resulting expression of CYP3A5 can compensate for decreased CYP3A4 activity.

UGT1A1 AND OTHER UGT1AS

Glucuronidation, catalyzed by UGTs, is one of the critical steps in the detoxification and elimination of various endogenous and exogenous compounds [Radominska-Pandya *et al.*, 1999, Tukey and Strassburg, 2000]. Glucuronidation accounts for about 35% of phase II drug metabolism [Evans and Relling, 1999]. As for the genes encoding UGTs, the four subfamilies, *UGT1*, *UGT2*, *UGT3* and *UGT8*, have been identified in humans [Mackenzie *et al.*, 2005]. As the *UGT1* subfamily, the *UGT1A* gene complex, located on chromosome 2q37, spans approximately 200 kb and consists of 9 active and 4 inactive first exons (in the following order: *UGT1A12P*, *1A11P*, *1A8*, *1A10*, *1A13P*, *1A9*, *1A7*, *1A6*, *1A5*, *1A4*, *1A3*, *1A2P* and *1A1*) and common exons 2 to 5. One of the 9 active first exons (namely, *1A1* and *1A3* to *1A10*) can be used in conjunction with the common exons [Tukey and Strassburg, 2000]. The UGT1A *N*-terminal domains (encoded by the first exons) determine the substrate-binding specificity, and the *C*-terminal domain (encoded by exons 2 to 5) is important for binding to UDP-glucuronic acid [Radominska-Pandya *et al.*, 1999]. The first exons, encoding substrate-binding domains, confer the substrate specificity of UGT1A isoforms, and the 5'-flanking region of each exon 1 is presumed to independently regulate the expression of each isoform. UGT1A1, 1A3, 1A4, 1A6, and 1A9 are expressed in liver as well as extrahepatic tissues such as colon [Tukey and Strassburg, 2000]. Recently, UGT1A5 was reported to be also expressed in liver and intestine at low levels and shown as catalytically active [Finel *et al.*, 2005]. In contrast, UGT1A7, 1A8, and 1A10 are expressed only in extrahepatic tissues including esophagus, stomach, small intestine and colon [Tukey and Strassburg, 2000; Basu *et al.*, 2004]. Substantial interindividual differences were detected in mRNA, protein and activity levels of UGT1A isoforms [Ritter *et al.*, 1999; Congiu *et al.*, 2002].

UGT1A1 Polymorphisms and Segmental Haplotypes

A number of genetic polymorphisms including SNPs in *UGT1As* have been identified and publicized in the UDP-Glucuronosyltransferase (UGT) Alleles Nomenclature Home Page (http://galien.pha.ulaval.ca/alleles/alleles.html, as of July 14, 2006), and some of them are known to affect glucuronidation rates [Guillemette, 2003 for review]. UGT1A1 is known to be the principal isoform for the glucuronidation of bilirubin and SN-38, an active metabolite of the anticancer drug irinotecan [Ando and Hasegawa, 2005]. To date, 64 alleles were reported in this isoform, most of which are rare and related to two severe familial forms of unconjugated hyperbilirubinemia syndromes (Crigler-Najjar types I and II). However, several genetic polymorphisms are relatively common and involved in altered drug metabolism. $A(TA)_nTAA$ number polymorphisms in the TATA box region (-54 to -39 from the translational start codon) include four variant alleles (n=5, *UGT1A1*36*; n=6, wild-type; n=7, *UGT1A1*28*; n=8, *UGT1A1*37*). *In vitro* and *in vivo* studies showed that increasing the TA repeat number leads to a decrease in the transcriptional activity of *UGT1A1*. Given the transcriptional activity of n=6 was defined as 100%, those of n=5, 7, and 8 were approximately 130%, 65% and 50%, respectively [Beutler *et al.*, 1998]. The frequencies of these repeat polymorphisms in various populations are summarized in Table 8. *UGT1A1*28* was distributed at 0.35-0.56 frequencies in Africans, 0.26-0.39 in Caucasians, and 0.07-0.19 in East and South-East Asians, and 0.25-0.49 in South and Middle East Asians. While the frequencies in South and Middle East Asians were comparable to Caucasians, there were no remarkable differences between East and South-East Asians. *UGT1A1*36* and **37* were detected at 0.01-0.12 frequencies in Africans and 0-0.02 in Caucasians, but not found in Asians. *UGT1A1*28* is known to be associated with an increased risk of SN-38 (an active irinotecan metabolite)-induced toxicity [Ando *et al.* 2000] as well as a mild type of inherited unconjugated hyperbilirubinemia syndrome

Table 8. Frequencies of TATA Box Polymorphism of *UGT1A1* in Different Ethnic Populations

Population	Allele Frequency				Number of Subjects	Reference
	**36*	Wild-Type	**28*	**37*		
	A(TA)$_5$TAA	A(TA)$_6$TAA	A(TA)$_7$TAA	A(TA)$_8$TAA		
Caucasians						
Sardinian[#]	ND	0.743	0.257	ND	70	Hall *et al.* 1999
European[#]	ND	0.613	0.387	ND	71	Beutler *et al.* 1998
Caucasian[¶]	0.005	0.698	0.295	0.002	101	Lampe *et al.* 1999
	0.009	0.616	0.366	0.009	56	Innocenti *et al.* 2002
	0.017	0.588	0.388	0.007	147	Kaniwa *et al.* 2005
	0.004	0.659	0.337	ND	132	Innocenti *et al.* 2005
Caucasian-Brazilian[¶]	0.007	0.662	0.324	0.007	71	Fertrin *et al.* 2002
Africans						
African-American[¶]	0.035	0.470	0.426	0.069	101	Beutler *et al.* 1998
	0.080	0.520	0.380	0.020	200	Guillemette *et al.* 2000a
	0.038	0.500	0.346	0.115	56	Innocenti *et al.* 2002
	0.044	0.446	0.446	0.064	149	Kaniwa *et al.* 2005
African-Brazilian[¶]	0.065	0.519	0.407	0.009	54	Fertrin *et al.* 2002
Pygmy Mbenzele (Cameroon)[*]	0.036	0.333	0.560	0.071	42	Hall *et al.* 1999
Kenyan[¶]	0.100	0.444	0.444	0.013	80	Premawardhena *et al.* 2003
at Ivory coast[¶]	0.061	0.466	0.358	0.115	74	Premawardhena *et al.* 2003
Asians						
Japanese	ND	0.903	0.097	ND	150	Kaniwa *et al.* 2005
	ND	0.914	0.086	ND	116	Kanai *et al.* 2005
	ND	0.870	0.130	ND	301	Saeki *et al.* 2006
Koreans	ND	0.873	0.127	ND	324	Ki *et al.* 2003
	ND	0.932	0.068	ND	81	Han *et al.* 2006
Chinese	ND	0.840	0.160	ND	89	Balram *et al.* 2002
Taiwanese	ND	0.876	0.124	ND	218	Huang *et al.* 2002
Thai	ND	0.844	0.156	ND	96	Boyd *et al.* 2006
Vietnamese	ND	0.916	0.084	ND	83	Premawardhena *et al.* 2003
Malay	ND	0.920	0.080	ND	50	Yusoff *et al.* 2006
	ND	0.812	0.188	ND	93	Balram *et al.* 2002
Indonesian	ND	0.808	0.192	ND	60	Premawardhena *et al.* 2003
Indian[¶]	ND	0.649	0.351	ND	84	Balram *et al.* 2002
	ND	0.592	0.408	ND	119	Premawardhena *et al.* 2003
Sri lankan[¶]	ND	0.506	0.493	ND	229	Premawardhena *et al.* 2003
Yemenite[¶]	ND	0.746	0.254	ND	61	Premawardhena *et al.* 2003

(Table 8. Contd....)

Population	Allele Frequency				Number of Subjects	Reference
	36	Wild-Type	*28*	*37*		
	A(TA)$_5$TAA	A(TA)$_6$TAA	A(TA)$_7$TAA	A(TA)$_8$TAA		
Lebanese*	ND	0.643	0.357	ND	42	Premawardhena *et al.* 2003
Hispanics*	0.011	0.614	0.375	ND	44	Hall *et al.* 1999
Parakana Indian*	ND	0.672	0.328	ND	32	Fertrin *et al.* 2002

ND: not detected.

"Significant differences (P<0.01, chi-square test) in allele frequencies between the Japanese population and each ethnic population. Between Japanese and Caucasian or African populations, all four alleles were compared. Between Japanese and other Asian populations, only A(TA)$_6$TAA and A(TA)$_7$TAA were compared. 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 due to the small number of subjects (<50 subjects).

[c]Not applicable for chi-square test.

(Gilbert's syndrome) [Bosma *et al.*, 1995; Monaghan *et al.*, 1996]. Another *1A1* polymorphism 211G>A (G71R, **6* allele) in exon 1 is also a causative factor for Gilbert's syndrome [Aono *et al.*, 1995], reduced metabolic activity to SN-38 [Gagne *et al.*, 2002; Jinno *et al.*, 2003a], and lower tumor response and higher incidence of grade 4 neutropenia in Koreans [Han *et al.*, 2006]. This allele is found at intermediate frequencies (0.13-0.24) in East Asians, and at low (0.01-0.10) levels in South-East Asians, but hardly found in Caucasians and Africans (Table **9**). In addition, *1A1*60* allele (-3279T>G) is located in the distal enhancer region (so called phenobarbital–responsive enhancer module) and shows reduced transcriptional activity [Sugatani *et al.*, 2002]. The frequencies of this allele are very high (0.85) in Africans, high (0.35-0.55) in Caucasians, and moderate (0.17-0.34) in Asians (Table **10**). A minor allele **27* (686C>A, P229Q) was reported to be associated with Gilbert's syndrome in Asians [Aono *et al.*, 1995], but its effect on enzymatic activity is marginal *in vitro* [Jinno *et al.*, 2003a]. Using these alleles, haplotypes were estimated for *UGT1A1* exon 1 in Japanese, Caucasians, and African-Americans (Fig. 4) [Sai *et al.*, 2004; Kaniwa *et al.*, 2005 for detail]. The **28* (A(TA)$_7$ TAA), **36* (A(TA)$_5$TAA), and **37* (A(TA)$_8$TAA) alleles were found to be linked with the **60* allele (-3279T>G) in most cases forming **28b* (and **28c*), **36b*, and **37b* haplotypes, respectively. The **27* allele (686C>A, P229Q), detected only in Asians, was exclusively harbored by the **28b* haplotype (forming **28c* haplotype), suggesting that its association with Gilbert's syndrome may be due to its linkages with A(TA)$_7$TAA and -3279T>G. The **6* (211G>A, G71R) and **28* (A(TA)$_7$TAA) alleles are mutually exclusive. The wild-type haplotype **1a* is less frequent in African-Americans (0.15), but about half of the Caucasians or Asians has this active haplotype (Table **11**). The frequencies of **28* haplotypes were more than 0.34 in Caucasians and African-Americans, but less than 0.14 in Asians. The **60a* haplotype was frequent in African-Americans (0.30-0.33) but less frequent in Asians (0.14-0.23) and Caucasians (0.09-0.14). The **6* haplotypes were found only in Asians with 0.13-0.24 frequencies.

In addition to the *UGT1A1* exon 1, segmental haplotypes for *UGT1A8, 1A10, 1A9, 1A7, 1A6, 1A4, 1A3*, and common exons 2-5 were estimated in Japanese [Sai *et al.*, 2004; Saeki *et al.*, 2005; 2006]. Thomas *et al.* also sequenced the *UGT1A* complex including *1A5* for 92 Caucasians and 46 Asians, and estimated segmental haplotypes of these populations separately [Thomas *et al.*, 2006]. The haplotype configurations for all segments of the complex were significantly different between Caucasians and Asians.

LDs and Haplotype Structures of UGT1A Gene Complex

Co-occurrence of the segmental haplotypes with functional changes in the *UGT1A* complex could lead to a cooperative alteration in glucuronidation activity. Using the genetic variations obtained from 196 Japanese subjects, linkage disequilibrium analysis was performed for the *UGT1A* gene complex [Saeki *et al.*, 2006 for detail]. Strong linkages were observed between *1A8* and *1A10*, among *1A9, 1A7* and *1A6*, and between *1A3* and *1A1*. Thus, the region from *1A8* to common exons was divided into five LD blocks: Block 8/10 (*1A8* and *1A10*), Block 9/6 (*1A9, 1A7* and *1A6*), Block 4 (*1A4*), Block 3/1 (*1A3* and *1A1*), and Block C (common exons 2-5). This block partitioning was similar to that of Thomas *et al.* [2006] in Asians except that they further divided the blocks at region *1A7*. LD profiles were considerably different between Caucasians and Asians: close linkage was observed among *UGT1A6, 1A5, 1A4, 1A3* and *1A1*, forming one LD block in Caucasians [Thomas *et al.*, 2006]. Furthermore, a recent report showed that the LD profile across *UGT1A1, 1A6* and *1A9* in African-Americans was clearly different from those of Caucasians and Asians [Maitland *et al.*, 2006].

Block haplotyping was only reported in Japanese [see Saeki *et al.*, 2006 for detail]. As for Block 8/10 consisting of two segments *1A8* and *1A10*, 14 haplotypes were inferred, and the 4 haplotypes with frequencies ≥5% accounted for 93.8% of the total haplotypes. It is noteworthy that the low-activity *1A10* haplotype **3* (containing 605C>T, T202I, now renamed to *UGT1A10*6*) [Jinno *et al.*, 2003b] was completely linked with the *1A8*1* haplotype (wild-type). Regarding Block 9/6 (*1A9-1A7-1A6*), 22 haplotypes were inferred, and the 3 haplotypes with frequencies ≥5% accounted for 85.2% of the total haplotypes. Notably, most of the high-activity segment haplotype *1A9*22* (with -126_-118 T_9>T_{10}, now renamed to be **1b* allele) [Yamanaka *et al.* 2004] was linked with *1A7*1* (wild-type) and *1A6*1* (wild-type). The