The G−113A polymorphism in *CYP1A2* affects the caffeine metabolic ratio in a Chinese population

Objective: **This study was designed to better understand genetic variation in the cytochrome P450 (CYP) gene *CYP1A2* and its impact on CYP1A2 activity in Chinese subjects.**

Methods: ***CYP1A2* genetic polymorphisms were screened by direct sequencing in 27 selected Chinese subjects. Plasma 1,7-dimethylxanthine/caffeine ratios 5 hours after a 100-mg caffeine administration, used as an index of CYP1A2 in vivo activity, were determined in 422 healthy subjects. Five single-nucleotide polymorphism markers, including G−860A (*CYP1A2*1C*), T−3594G, G−3113A, A−163C (*CYP1A2*1F*), and C5347T (*CYP1A2*1B*), were selected and genotyped by either polymerase chain reaction–restriction fragment length polymorphism or direct sequencing.**

Results: **Thirteen polymorphisms and 2 linkage disequilibrium blocks with a boundary around −2467 were identified at this locus. The allele frequency for −3860A, −3594G, −3113A, −163C, and 5347T was 0.21, 0.15, 0.10, 0.36, and 0.14, respectively, in the CYP1A2-phenotyped cohort. A significant difference in CYP1A2 activity was observed among genotypes of polymorphism G−3113A (P = .038), and CYP1A2 activity in subjects carrying the AA genotype was lower than that in those carrying the GA (P = .096) and GG genotypes (P = .036): −0.45 ± 0.05 (mean ± SD), −0.32 ± 0.16, and −0.29 ± 0.16, respectively. Further analysis based on haplotype pairs found a 1.92-fold variation (95% confidence interval, 1.13-2.71) in mean CYP1A2 activity between haplotype pairs 13 and 15, and the difference was significant (−0.19 ± 0.15 versus −0.45 ± 0.05, P = .016). As compared with haplotype pair 10, haplotype pairs 9 and 15 and most haplotype pairs heterozygous for the haplotype with an A allele at −3113, including pairs 5, 8, and 12, also showed significantly lower CYP1A2 activity (P = .015, .048, .008, .024, and .014 for pairs 5, 8, 9, 12, and 15, respectively). In addition, haplotype pairs 5, 9, and 12 also showed significantly lower CYP1A2 activity than pair 13 (P = .034, .020, and .037 for pairs 5, 9, and 12, respectively).**

Conclusions: **The G−3113A polymorphism is associated with decreased CYP1A2 activity, haplotype pairs 10 and 13 are responsible for high CYP1A2 activity, and haplotype pairs 5, 8, 9, 12, and 15 are responsible for low CYP1A2 activity in Chinese subjects. (Clin Pharmacol Ther 2005;78:249-59.)**

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CYP1A2 is a smoking-inducible isozyme of cytochrome P450 (CYP), a superfamily that plays important roles in the metabolism of numerous structurally diverse endogenous and exogenous molecules. Among all members of CYP identified, CYP1A2 is the second-most abundant CYP in the adult human liver.[1] In addition to endobiotics such as estradiol and uroporphyrinogen, CYP1A2 has been found to metabolize several commonly used drugs, especially antipsychotics such as clozapine,[2] olanzapine,[3] imipramine,[4] and tizani-

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dine.[5] CYP1A2 is also involved in the activation of various toxic compounds and carcinogens such as food-derived aflatoxin B1[6] and heterocyclic or aromatic amines found in tobacco smoke.[7]

Considerable interindividual differences in CYP1A2 expression, exceeding 40-fold in messenger ribonucleic acid levels, have been observed both in vivo and in vitro.[8-12] Interethnic differences in CYP1A2 activity have also been reported, with Chinese women and black subjects demonstrating lower CYP1A2 activity than white subjects.[13,14] These remarkable interindividual and interethnic differences in constitutive or inducible CYP1A2 activity contribute greatly to variation in drug responses, as well as an individual's predisposition to common diseases such as colon cancer,[15] bladder cancer,[16] testicular cancer,[17] and primary hepatocellular carcinoma.[6]

Although environmental exposures such as cigarette smoking, caffeine intake, and cruciferous vegetable consumption and several therapeutic drugs affect CYP1A2 activity to some degree,[7,12] the results of a twins study in white subjects indicate that CYP1A2 in vivo activity is determined mainly by genetic factors, with a heritability estimate of 0.725.[18] The *CYP1A2* gene is located on 15q22 and spans about 7.8 kilobases (kb). Several studies have been devoted to the identification of the functional polymorphisms in *CYP1A2*.[10,19-22] Three single-nucleotide polymorphisms (SNPs), namely, −3860A (*CYP1A2*1C*),[19] C−163A (*CYP1A2*1F*),[20] and C−729T,[10] seem to be associated with CYP1A2 activity and inducibility. However, these polymorphisms could not completely explain the large interindividual variation in CYP1A2 activity. The overall nucleotide diversity pattern in *CYP1A2* and the major genetic basis for the variation of CYP1A2 expression remain largely unexplored.

To better understand the nucleotide diversity pattern at the *CYP1A2* locus and its impact on CYP1A2 activity, we first performed SNP screening of all exons, relevant exon-intron boundaries, and approximately a 3-kb promoter region of *CYP1A2* by polymerase chain reaction (PCR) direct sequencing in 27 selected Chinese individuals. Then, by using caffeine as the probe drug, we evaluated the associations of those common genetic polymorphisms with CYP1A2 activity in vivo in this study.

METHODS

Subjects. A total of 422 healthy unrelated Chinese volunteers, comprising 238 men and 184 women aged 18 to 33 years (mean age, 20 ± 2 years), participated in the study. Each subject was in good health on the basis of medical history, physical examination, and laboratory evaluation. All subjects were students from Central South University, Changsha, China, and had similar foodstuffs provided by an eatery at the university 1 week before and during phenotyping. In addition, all subjects were self-stated nonsmokers and were asked to abstain from coffee, tea, cola, chocolate, or any caffeine-containing drinks within 1 week before and during phenotyping. None of the subjects was taking any medications including oral contraceptives 1 week before and during phenotyping. This study was approved by the Ethics Committee of the School of Pharmaceutical Science, Central South University, and all subjects gave written informed consent before commencing the study.

SNP discovery. SNP screening of all exons, exon-intron boundaries, and approximately 3-kb promoter region of *CYP1A2* (referred to as GenBank[22a] accession no. NT_010194.16) was performed by PCR direct sequencing. The screening panel included 27 unrelated individuals selected randomly from the 422 volunteers. The sample size yielded 95% probability of detecting alleles with a minimal frequency of 5.4%.[23] In brief, the primers for the target regions were designed by use of Web-based software (Primer3.0).[24] Deoxyribonucleic acid (DNA) samples from the 27 Chinese individuals were amplified and purified. The PCR products were then sequenced by use of the ABI PRISM Dye Terminator Sequencing Kit with Amplitaq DNA polymerase and loaded onto an ABI 3700 sequencer (Applied Biosystems, Foster City, Calif). SNP candidates were identified by the PolyPhred program[25] and inspected by 2 observers. SNP positions and individual genotypes were confirmed by reamplifying and resequencing the SNP site from the opposite strand.

Caffeine phenotyping. The caffeine plasma metabolic ratio (MR) indicative of CYP1A2 in vivo activity was determined according to the method of Carrillo et al.[26] In brief, a 100-mg oral dose of a caffeine capsule (Hunan Pharmaceutical, Changsha, China) was given at 7 to 8 AM after overnight fasting. Five-milliliter venous blood samples were drawn into ethylenediaminetetraacetic acid–containing tubes at 0 hours and 5 hours after caffeine administration. Plasma and the peripheral leukocytes were isolated immediately and stored separately at −20°C until analysis. Molar concentrations of caffeine (137X) and its metabolite 1,7-dimethylxanthine (paraxanthine) (17X) were determined by HPLC as described elsewhere.[9]

SNP genotyping. DNA samples were extracted from peripheral leukocytes by standard phenol/chloroform protocols. DNA samples were diluted to 8 ng/μL and

distributed to 96-well plates; each 96-well plate contained 94 samples and 2 DNA-free water controls. Five SNPs (ie, *CYP1A2*1C*, T−3594G, A−3113G, *CYP1A2*1F*, and *CYP1A2*1B*) were genotyped in the phenotyped population. The polymorphisms *CYP1A2*1C*, T−3594G, and *CYP1A2*1F* were genotyped by direct sequencing. PCR amplification of the target fragments consisted of the following 2 sets of primers (forward/reverse), with the forward primers in each set being the sequencing primers: 5′-GAACACAACGGGACTTCTTG-3′/5′-GGATTACGCTCCTTCTCCTT-3′ for *CYP1A2*1C* and T−3594G and 5′-TCTCAAGCACCTGCCTCTAC-3′/5′-AGGGAACAGACTGGGACAAT-3′ for *CYP1A2*1F*. A−3113G and *CYP1A2*1B* were genotyped by PCR–restriction fragment length polymorphism. The A−3113G polymorphism results in the formation of an *Hpy*CHIV restriction site, and the *CYP1A2*1B* polymorphism abolishes an *Eco*57I restriction site. For G−3113A, a 441–base pair (bp) fragment was amplified by use of primers 5′-AAGGAGAAGGAGCGTAATCC-3′/5′-GTTCCAGGACCCATTGGA-3′ (forward/reverse). Five microliters of the PCR products was digested overnight at 37°C in a 10-μL reaction mixture with 1 U of *Hpy*CHIV (New England Biolabs, Beverly, Mass). For *CYP1A2*1B*, a 438-bp fragment was amplified with primers 5′-AGGTCCCATCTCCTCTGTTC-3′ (forward) and 5′-GCACTTGGCTAAAGCTGCTA-3′ (reverse). Five microliters of the PCR products was digested overnight at 37°C in a 10-μL reaction mixture with 1 U of *Eco*57I (MBI Fermentas, St Leon-Rot, Germany). The digested products were subsequently separated on 3% agarose. For A−3113G polymorphism, *Hpy*CHIV digestion gave 2 bands of 398 bp and 43 bp for the GG genotype, 1 band of 443 bp for the AA genotype, and all 3 bands for the AG genotype. In contrast, for *CYP1A2*1B* polymorphism, *Eco*57I digestion gave 2 bands of 269 bp and 169 bp for the *CYP1A2*1A/CYP1A2*1A* genotype, 1 band of 438 bp for the *CYP1A2*1B* genotype, and all 3 bands for the *CYP1A2*1A/CYP1A2*1B* genotype. An additional DNA panel including 94 subjects was also genotyped by direct sequencing for the functional SNP C−729T as has been reported previously in Africans.[10]

Data analysis. Allele frequencies for each SNP were determined by gene counting. Haplotypes were constructed by the Phase 2.0 program.[27] Haplotype tag SNPs were selected by SNPtagger.[28] Tests of fitness to Hardy-Weinberg equilibrium of each polymorphic site and linkage disequilibrium (LD) analysis between SNP pairs were performed with LDA software,[29] and Lewontin's D′ was applied to evaluate the pairwise LD between polymorphisms.

SPSS 10.0 for Windows was applied for statistical analysis of the data (SPSS, Chicago, Ill). Frequency distribution and probit plot were used in analysis of phenotype distribution. Normal distribution of CYP1A2 activity was assessed by the Kolmogorov-Smirnov test. Comparison of CYP1A2 activity (expressed as mean ± SD) between genders was carried out by the Mann-Whitney *U* test. Comparisons of allele frequencies between populations were carried out by chi-square test or Fisher exact test. Comparisons of CYP1A2 activity (indicated by the log transformation of 17X/137X ratios) among genotypes and haplotype pairs were analyzed by ANOVA. Statistical significance was regarded as $P < .05$.

RESULTS

SNP discovery. Resequencing of 7262-bp genomic DNA in the 27 Chinese subjects identified 13 biallelic polymorphisms, yielding a mean density of 1 polymorphism per 605 bp (Table I and Fig 1). Twelve of these polymorphisms were common SNPs with a minor allele frequency (MAF) of approximately 10% or higher. MAF was 50.0% for −2467 delT; 29.6% for A−163C (*CYP1A2*1F*); 22.2% for G−3860A (*CYP1A2*1C*); 20.4% for T−3594G, G2159A, and C5347T (*CYP1A2*1B*); 9.3% for G−3598T, G−3113A, T−2847C, T−1708C, T−739G (*CYP1A2*1E*), and T3613C; and 1.9% for C63G (*CYP1A2*2*). Among these polymorphisms, 2 coding SNPs were identified. *CYP1A2*2* was a singleton that resulted in an amino acid change from phenylalanine to leucine, and *CYP1A2*1B* was a synonymous mutation. In comparison with those in white subjects,[15] allele frequencies for *CYP1A2*1C* (0.9% in white subjects), −739G (0.4% in white subjects), and *CYP1A2*1B* (61.8% in white subjects) varied significantly between populations ($P < .001$) (Table I). The C−729T polymorphism was not observed either in the SNP screening panel or in the additional 94 subjects.

Haplotype structure and LD pattern at CYP1A2 locus. Construction of haplotypes was based on genotype data of all SNPs identified. Eleven haplotypes were identified, 5 of which were common haplotypes with a frequency greater than 5% (Fig 1, *A*). All haplotypes except haplotype 1 could be inferred unambiguously. Among the haplotypes inferred, haplotype 1 was most distinctive at the nucleotide level and showed unique differences at 6 sites from all other haplotypes.

Lewontin's D′ was calculated to evaluate LD extension in this region. The absolute value of D′ ranged

Table I. Single-nucleotide polymorphisms identified in *CYP1A2* in Chinese subjects and comparison with white subjects

Polymorphism	*Location*	*Minor allele*	*Minor allele frequency (%)*		*rs ID‡*	*CYP nomenclature*
			Chinese subjects	*White subjects†*		
G−3860A§	5′-Flanking	A	22.2‖	0.9	2069514	*CYP1A2*1C*
G−3598T	5′-Flanking	T	9.3	—	2069519	—
T−3594G	5′-Flanking	G	20.4	—	2069520	—
G−3113A	5′-Flanking	A	9.3	—	2069521	—
T−2847C	5′-Flanking	C	9.3	—	2069522	—
−2467 delT	5′-Flanking	T	50.0‖	4.82	—	*CYP1A2*1D*
T−1708C	5′-Flanking	C	9.3	—	2069525	—
T−739G	Intron 1	G	9.3‖	0.4	2069526	*CYP1A2*1E*
A−163C	Intron 1	C	29.6	33.3	762551	*CYP1A2*1F*
C63G	Exon 2	G	1.9	0	—	*CYP1A2*2*
G2159A	Intron 4	A	20.4	—	2472304	—
T3613C	Intron 6	C	9.3	—	4646427	—
C5347T	Exon 7	T	20.4‖	61.8	2470890	*CYP1A2*1B*

†Reference data reported by Sachse et al[15] in 114 white subjects.
‡rs (reference SNP) ID in dbSNP database. Dashes indicate polymorphisms not studied in the reference or not deposited in the dbSNP database[29a] or CYP allele nomenclature Web site.[29b]
§The numbers indicate the SNP locations relative to the start codon ATG according to National Center for Biotechnology Information[29a] genomic contig NT_010194.16.
‖$P < .001$, significantly different from those in white subjects.

from 0.20 to 1.0, with a mean value of 0.96. Two LD blocks with a threshold of absolute value of D′ equal to 0.8 were identified (Fig 1, *B*). The boundary between the 2 blocks existed around −2467. The 5′ upstream block (block 1) and 3′ downstream block (block 2) spanned roughly 1.6 kb and 5.7 kb, respectively. Among the SNPs identified, G−3598T, A−3113G, C−2847T, C−1708T, G−739T (*CYP1A2*1E*), and T3613C were in absolute LD; G2159A and *CYP1A2*1B* were also in absolute LD. A−3113G, a polymorphism located in a region containing a potential binding site for positive regulatory transcription factor,[30] the synonymous mutation *CYP1A2*1B*, and other 3 haplotype tag SNPs (including *CYP1A2*1C*, T−3594G, and *CYP1A2*1F*) were selected for further phenotype-genotype association study (Fig 1, *A*). These 5 substitutions described more than 90% of the haplotype diversity in the sequences.

Phenotyping with caffeine. No endogenous interfering peaks were found in any of the 0-hour plasma samples. Caffeine MR was not available for 3 samples (2 for women and 1 for men) because of a lapse during plasma sample treatment. Caffeine MR ranged from 0.18 to 1.51 (log-transformed, −0.74 to 0.18), with a mean value of 0.54 ± 0.21 (log-transformed, −0.29 ± 0.16 [mean ± SD]). In men CYP1A2 activity was slightly higher than in women ($P = .028$)—0.56 ± 0.21 (n = 236) and 0.52 ± 0.21 (n = 183), respectively. Normal distribution was indicated in the overall population ($P = .075$) by the Kolmogorov-Smirnov test. Neither the frequency distribution nor the probit plot of caffeine MRs showed any obvious antimode (Fig 2). After stratification by gender, normal distribution was detected in men ($P = .200$) but not in women ($P = .049$).

Association of individual SNPs and haplotype pairs with caffeine MR. All SNPs were found to be in conformance with Hardy-Weinberg equilibrium in the CYP1A2-phenotyped cohort. A significant difference in CYP1A2 activity was shown among genotypes at the G−3113A polymorphic site ($P = .038$). Individuals carrying the AA genotype at −3113 showed significantly lower CYP1A2 activity (−0.45 ± 0.05) as compared with those carrying either the GG (−0.29 ± 0.16) or AG (−0.32 ± 0.16) genotype ($P = .036$ for AA versus GG and $P = .096$ for AA versus AG) (Table II). Individuals carrying the *CYP1A2*1C/*1C* genotype also showed marginally lower CYP1A2 activity as compared with both *CYP1A2*1A/*1A* and *CYP1A2*1A/*1C* genotypes ($P = .064$ and .078, respectively) (Table II). In addition, *CYP1A2*1B* homozygotes showed marginally significantly higher CYP1A2 activity as compared with *CYP1A2*1A/*1A* homozygotes (−0.19 ± 0.15 versus −0.30 ± 0.16, $P = .052$) (Table II). No differ-

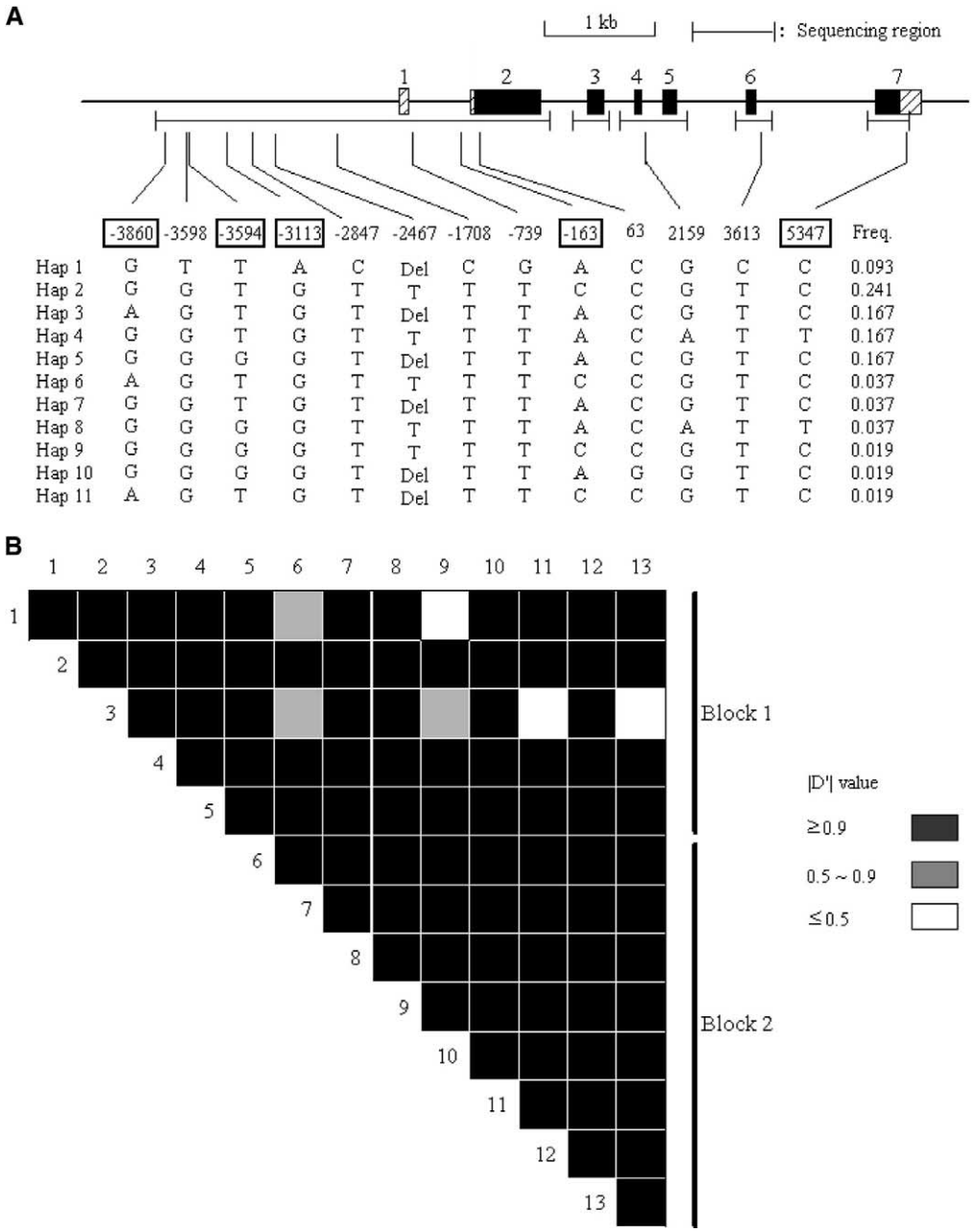

Fig 1. Gene structure, regions resequenced, polymorphisms discovered, and haplotype (Hap) and linkage disequilibrium (LD) pattern at *CYP1A2* locus. **A**, Gene structure, regions resequenced, and locations of all 13 polymorphisms identified at *CYP1A2* locus. Coding exons are marked with *solid boxes*, and 5′- and 3′-untranslated regions are marked by *hatched boxes*. The locations of each polymorphism (relative to the first nucleotide of the open reading frame) are shown under the exon-intron structure of *CYP1A2* (accession number of reference sequence NT_010194.16). Haplotypes and their frequencies in 27 unrelated Chinese individuals can be inferred. Locations of 5 single-nucleotide polymorphisms (SNPs) selected for further phenotype-genotype association study were boxed. bp, Base pairs. **B**, Pairwise LD between polymorphisms identified at *CYP1A2* locus. The absolute values of D′ for each pair of polymorphisms are indicated. Two LD blocks (block 1 and block 2) with low haplotype diversities are also shown.

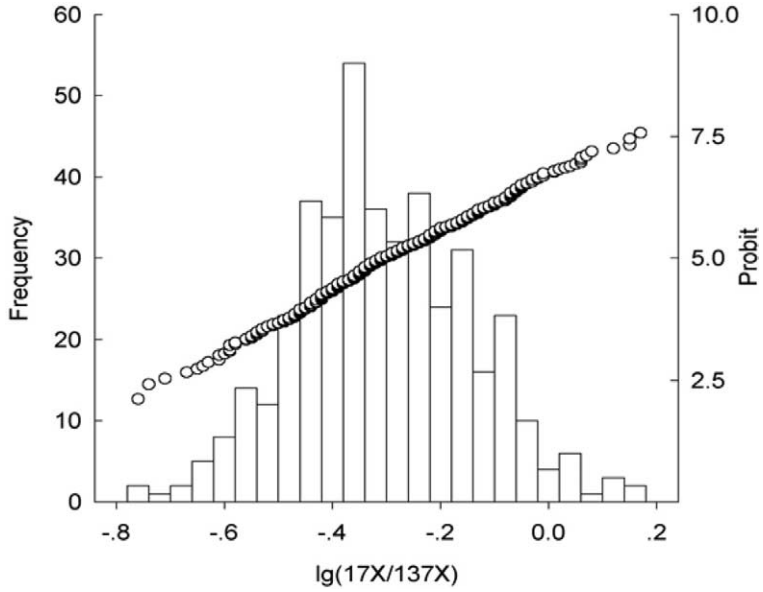

Fig 2. Frequency and probit distribution of CYP1A2 activity in Chinese population as indicated by plasma log-transformed 1,7-dimethylxanthine/caffeine [lg(17X/137X)] ratios (n = 419).

Table II. Association of *CYP1A2*1C*, T−3592G, G−3113A, *CYP1A2*1F*, and *CYP1A2*1B* polymorphisms with plasma caffeine metabolic ratio in Chinese subjects

SNP locus	*Genotype*	*No.†*	*Log-transformed 17X/137X ratio*	*Total P value*
G−3860A	**1A/*1A*	n = 256‡	−0.29 ± 0.16	.176
	**1C/*1A*	n = 140§	−0.29 ± 0.15	
	**1C/*1C*	n = 20	−0.36 ± 0.16	
T−3594G	TT	n = 299	−0.30 ± 0.17	.341
	TG	n = 106	−0.28 ± 0.15	
	GG	n = 9	−0.35 ± 0.08	
G−3113A	GG	n = 332	−0.29 ± 0.16‖	.038
	GA	n = 79	−0.32 ± 0.16¶	
	AA	n = 3	−0.45 ± 0.05	
A−163C	**1A/*1A*	n = 173	−0.30 ± 0.14	.636
	**1F/*1A*	n = 175	−0.30 ± 0.17	
	**1F/*1F*	n = 59	−0.28 ± 0.17	
C5347T	**1A/*1A*	n = 302	−0.30 ± 0.16	.068
	**1B/*1A*	n = 104	−0.28 ± 0.15#	
	**1B/*1B*	n = 8	−0.19 ± 0.15	

SNP, Single-nucleotide polymorphism; 17X/137X, 1,7-dimethylxanthine/caffeine ratio.
†Because of polymerase chain reaction failure, the total number of individuals was less than 422 for all of the polymorphisms.
‡P = .064 and §P = .078, compared with corresponding **1C/*1C* genotype. ‖P = .036 and ¶P = .096, compared with corresponding AA genotype. #P = .052, compared with corresponding **1A/*1A* genotype.

ences in CYP1A2 activity among genotypes of T−3594G or *CYP1A2*1F* polymorphisms were observed (Table II).

Haplotypes were constructed for 403 individuals whose genotype data were complete. A total of 9 haplotypes and 27 haplotype pairs (data not shown) were observed. Differences in CYP1A2 activity among haplotype pairs were also compared. In addition, for the purposes of analysis, the difference in CYP1A2 activity was compared only among homozygous haplotype pairs and heterozygous pairs with frequency higher than 3%. Fifteen pairs representing 92% of the cohort

Table III. Major haplotype pairs at *CYP1A2* locus in Chinese subjects and their association with CYP1A2 activity

Haplotype pair	*Chromosome A haplotype**	*Chromosome B haplotype**	*No. (%)*	*Log-transformed 17X/137X ratio*
1	GTGCC	ATGAC	58 (14.4)	−0.29 ± 0.18
2	GTGCC	GTGCC	55 (13.6)	−0.27 ± 0.17
3	GTGCC	GTGAT	40 (9.9)	−0.27 ± 0.16
4	GTGCC	GGGAC	40 (9.9)	−0.30 ± 0.16
5	GTAAC	GTGCC	27 (6.7)	−0.33 ± 0.19‡§
6	ATGAC	GGGAC	26 (6.4)	−0.30 ± 0.15
7	ATGAC	GTGAT	23 (5.7)	−0.29 ± 0.14
8	GTAAC	ATGAC	22 (5.4)	−0.31 ± 0.15‖
9	ATGAC	ATGAC	18 (4.7)	−0.35 ± 0.16¶#
10	GTGAT	GGGAC	17 (4.2)	−0.21 ± 0.16
11	GTAAC	GGGAC	13 (3.2)	−0.29 ± 0.13
12	GTAAC	GTGAT	12 (3.0)	−0.34 ± 0.15**††
13	GTGAT	GTGAT	8 (2.0)	−0.19 ± 0.15
14	GGGAC	GGGAC	7 (1.7)	−0.35 ± 0.08
15	GTAAC	GTAAC	3 (0.7)	−0.45 ± 0.05‡‡§§
Others†			34 (8.4)	−0.31 ± 0.14‖‖

*Nucleotide positions are in the order of −3860, −3594, −3113, −163, and 5347; chromosomes A and B are arbitrarily assigned.
†Including all 12 other haplotype pairs identified: ‡P = .015, ‖P = .048, ¶P = .008, **P = .024, ‡‡P = .014, and ‖‖P = .029, compared with haplotype pair 10; §P = .034, #P = .020, ††P = .037, and §§P = .016, compared with haplotype pair 13.

met this criterion (Table III). Individuals with the haplotype pair 15 (homozygous for −3860G/−3594T/−3113A/−163A/5347C, n = 3) and the haplotype pair 13 (homozygous for −3860G/−3594T/−3113G/−163A/5347T, n = 8) demonstrated the lowest and highest mean caffeine MRs, respectively, in the population (Table III). To delineate which pairs differ from one another with regard to CYP1A2 activity, comparisons were made for pair 13 and pair 15 versus the other haplotype pairs. The results showed a significant difference in caffeine log-transformed MR between pairs 13 and 15 (−0.45 ± 0.05 versus −0.19 ± 0.15, P = .016) (Table III). A 1.92-fold variation (95% confidence interval, 1.29-2.71) in mean plasma 17X/137X ratio was observed between pairs 13 and 15. A significant difference in CYP1A2 activity was also shown between pair 5 (log-transformed 17X/137X ratio, −0.33 ± 0.19; n = 27) and pair 13 (P = .034), pair 9 (homozygous for −3860A/−3594T/−3113G/−163A/5347C; log-transformed 17X/137X ratio, −0.35 ± 0.16; n = 18) and pair 13 (P = .020), and pair 12 (log-transformed 17X/137X ratio, −0.34 ± 0.15; n = 12) and pair 13 (P = .037). When pairwise tests were performed on the remaining pairs, significant differences in CYP1A2 activity were also observed between pairs 9 and 10 (−0.35 ± 0.16 versus −0.21 ± 0.16, P = .008) and pairs 15 and 10 (−0.45 ± 0.05 versus −0.21 ± 0.16, P = .014). In addition, most pairs heterozygous for the haplotype −3860G/−3594T/−3113A/−163A/5347C (including pairs 5, 8, and 12) also showed decreased CYP1A2 activity as compared with pair 10 (P = .015 for pair 5 versus pair 10, P = .048 for pair 8 versus pair 10, and P = .024 for pair 12 versus pair 10) (Table III).

DISCUSSION

In this study we began with the investigation on nucleotide diversity pattern at the *CYP1A2* locus in Chinese subjects. Twelve of the polymorphisms were common with MAF ranging from 9.3% to 50.0%. As compared with the allele frequencies observed in white subjects,[15] some polymorphisms showed remarkable ethnic difference. No common nonsynonymous polymorphism (with MAF >5%) was identified in our study. The only rare nonsynonymous SNP identified in our study, *CYP1A2*2*, has been reported to be nonfunctional.[31] Most of the polymorphisms are located in the 5′-flanking regulatory region and introns. A similar set of polymorphisms across the *CYP1A2* locus was also observed recently in Japanese subjects.[32] This pattern of polymorphism distribution at the *CYP1A2* locus suggests that the remarkable interindividual variation in CYP1A2 activity in Chinese subjects is not a result of a structural or functional difference in CYP1A2 itself, whereas SNPs located in noncoding regions may play a role. A functional polymorphism located in intron 1, C−729T, has been reported to decrease both CYP1A2 activity and its inducibility in Ethiopians.[10] However,

this SNP was not observed in 121 unrelated Chinese DNA samples. Several other rare coding SNPs reported recently in Japanese subjects also have not been identified in our study.[22]

We found significantly lower CYP1A2 activity in subjects with the AA genotype at the −3113 polymorphic site in this Chinese population. As has been reported previously, G−3113A is located in a 32-bp region, −3115 to −3084 bp, containing a *cis* element for *CYP1A2* transcription.[30] An in vitro study with transfection systems has suggested that the entire 32 bp of this region is necessary for *CYP1A2* promoter activity.[33] However, because this SNP is in complete LD with 5 other SNPs (ie, G−3598T, C−2847T, C1708T, G−739T, and T3613C) observed in *CYP1A2*, it is difficult, in this study, to determine separately the functional relevance of A−3113G. Previous studies using either transient transfection systems or electrophoretic mobility shift assay analysis have excluded the possibility of G−3598T[34] and G−739T (*CYP1A2*1E*)[10] to be functional.

Much attention has also been focused on the functional significance of other SNPs, especially for *CYP1A2*1C* and *CYP1A2*1F*, in recent years. Both *CYP1A2*1C* and *CYP1A2*1F* were initially found to be associated with CYP1A2 inducibility in smokers in white and Japanese subjects, respectively.[19,20] However, contradictory results have been obtained in some other clinical investigations.[35-39] In our study we found marginally lower CYP1A2 activity in *CYP1A2*1C* homozygotes, and this is in accordance with the previous finding that *CYP1A2*1C* is associated with lower theophylline clearance in Japanese patients with asthma.[35] In agreement with other studies performed in schizophrenic patients in Japanese and white populations,[36-39] we did not find any association between *CYP1A2*1F* polymorphism and caffeine MRs in this Chinese population. The lack of association between the T−3594G mutation and CYP1A2 activity observed in our study further supports the result of an in vitro study using transient transfection systems.[34] As for C5347A (*CYP1A2*1B*), a negative result has also been obtained in white subjects.[15] Because of the lack of cigarette smokers recruited in the study, whether these SNPs affect CYP1A2 inducibility deserves further investigation.

The relevance of combinations of multiple SNPs or haplotypes in *CYP1A2* for predicting CYP1A2 activity was also assessed in our study. About a 2-fold difference in mean CYP1A2 activity was demonstrated between individuals carrying haplotype pair 13 (homozygotes of the haplotype −3860G/−3594T/−3113G/−163A/5347T) and pair 15 (homozygotes of the haplotype −3860G/−3594T/−3113A/−163A/5347C), the haplotype pairs that demonstrated the highest and lowest CYP1A2 activity in the overall population. In addition, most haplotype pairs heterozygous for the haplotype −3860G/−3594T/−3113A/−163A/5347C are associated with significantly lower CYP1A2 activity as compared with haplotype pair 10. Because the A allele at −3113 is included within the haplotype −3860G/−3594T/−3113A/−163A/5347C, these results further support the finding that G−3113A is associated with decreased CYP1A2 in vivo activity. Because the G−3113A polymorphism is common, with an MAF of about 10%, in Chinese subjects, this polymorphism may play a role in the interindividual variations in drug response in this population. Because CYP1A2 also plays important roles in the metabolism of environmental carcinogens such as aflatoxin B1,[7] the functional relevance of the G−3113A polymorphism observed in our study makes this SNP an important marker for association studies for relevant diseases such as the aflatoxin B1–associated hepatocellular carcinoma,[6] a malignancy prevalent in China.

The genotype-phenotype association based on haplotype pairs also suggests that the constitutive expression of *CYP1A2* in Chinese subjects is regulated by several SNPs simultaneously and that the contribution of individual SNPs is limited. Similar genotype-phenotype associations based on haplotype pairs have also been reported in other drug response genes such as the β_2-adrenergic receptor gene.[40] According to mean caffeine MRs, haplotype pairs 10 and 13, with a frequency that adds up to 6.2% in the overall population, account for high CYP1A2 activity, whereas haplotype pairs 5, 8, 9, 12, and 15, with a total frequency of 20.3%, account for low CYP1A2 activity in this Chinese population. Most other individuals (73.5%) with inherited haplotypes show intermediate CYP1A2 activity in this population. In addition, it is obvious that polymorphisms including *CYP1A2*1C*, G−3113A, and *CYP1A2*1B* are necessary to discriminate individuals with either low or high CYP1A2 activity in the Chinese population. Because both of the haplotype pairs associated with either low or high CYP1A2 activity are common in Chinese subjects, they are suggested to be relevant to pharmacogenetics and xenobiotic metabolism in this population.

Of note, phenotype-genotype association studies based on haplotypes in *CYP1A2* have also been carried out in other populations. In a study in Ethiopians, subjects with haplotype *CYP1A2*1K* (−739G/−729T/−163A) in intron 1 showed significantly decreased

CYP1A2 in vivo activity.[10] However, this study observed haplotypes limited to intron 1 rather than the entire gene region. In addition, the *CYP1A2*1K* haplotype was not observed either in our study or in a Japanese population as reported recently.[32] Another study has also suggested that there is no haplotype responsible for interindividual variation in CYP1A2 activity in white subjects.[15] Because both frequency spectrums of SNPs and haplotype structures at *CYP1A2* are different between Asian and white subjects,[41] this result is not necessarily contradictory to ours. Because the nucleotide diversity pattern in *CYP1A2* is similar in Chinese and Africans or other Asian populations,[41] our observations would be convincing if they can be replicated in those populations.

With regard to phenotype distribution, polymorphic distribution in CYP1A2 activity is not indicated in the overall population we studied, and this is in disagreement with the former findings of trimodal or bimodal distribution observed in Chinese and African subjects.[9,10,42] When stratified by gender, polymorphic distribution is observed in female, but not male, subjects, and a reduction in population size in the subgroups may account for this difference. Differences in population size may also explain the discordance between our results and those of other reports.[9,41] Other confounding factors such as cigarette smoking and environmental exposure to inhibitory or inducible agents to CYP1A2 may also contribute to this difference.

In conclusion, our study indicates that A−3113G is associated with decreased CYP1A2 activity in vivo in Chinese subjects. Comparing CYP1A2 activity among genotypes that are based on haplotype pairs, we find that haplotype pairs 10 and 13 are responsible for high CYP1A2 activity whereas haplotype pairs 5, 8, 9, 12, and 15 are responsible for low CYP1A2 activity in Chinese subjects. Our observation provides some explanations for interindividual variation in CYP1A2 activity, although other genetic and environmental factors such as passive smoking may also play a role. When drugs that are substrates of CYP1A2 are used, dosages should be adjusted in individuals with inherited genotypes or haplotype pairs that are associated with both high and low CYP1A2 activity.

None of the authors has a conflict of interest.

References

1. Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. 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 1994;270:414-23.
2. Bertilsson L, Carrillo JA, Dahl ML, Llerena A, Alm C, Bondesson U, et al. Clozapine disposition covaries with CYP1A2 activity determined by a caffeine test. Br J Clin Pharmacol 1994;38:471-3.
3. Carrillo JA, Herraiz AG, Ramos SI, Gervasini G, Vizcaino S, Benitez J. Role of the smoking-induced cytochrome P450 (CYP)1A2 and polymorphic CYP2D6 in steady-state concentration of olanzapine. J Clin Psychopharmacol 2003;23:119-27.
4. Lemoine A, Gautier JC, Azoulay D, Kiffel L, Belloc C, Guengerich FP, et al. Major pathway of imipramine metabolism is catalyzed by cytochromes P-450 1A2 and P-450 3A4 in human liver. Mol Pharmacol 1993;43:827-32.
5. Granfors MT, Backman JT, Laitila J, Neuvonen PJ. Tizanidine is mainly metabolized by cytochrome p450 1A2 in vitro. Br J Clin Pharmacol 2004;57:349-53.
6. Eaton DL, Bammler TK, Kelly EJ. Interindividual differences in response to chemoprotection against aflatoxin-induced hepatocarcinogenesis: implications for human biotransformation enzyme polymorphisms. Adv Exp Med Biol 2001;500:559-76.
7. Landi MT, Sinha R, Lang NP, Kadlubar FF. Human cytochrome P4501A2. IARC Sci Publ 1999;148:173-95.
8. Schweikl H, Taylor JA, Kitareewan S, Linko P, Nagorney D, Goldstein JA. Expression of CYP1A1 and CYP1A2 genes in human liver. Pharmacogenetics 1993; 3:239-49.
9. Ou-Yang DS, Huang SL, Wang W, Xie HG, Xu ZH, Shu Y, et al. Phenotypic polymorphism and gender-related differences of CYP1A2 activity in a Chinese population. Br J Clin Pharmacol 2000;49:145-51.
10. Aklillu E, Carrillo JA, Makonnen E, Hellman K, Pitarque M, Bertilsson L, et al. Genetic polymorphism of CYP1A2 in Ethiopians affecting induction and expression: characterization of novel haplotypes with single-nucleotide polymorphisms in intron 1. Mol Pharmacol 2003;64:659-69.
11. Schrenk D, Brockmeier D, Morike K, Bock KW, Eichelbaum M. A distribution study of CYP1A2 phenotypes among smokers and non-smokers in a cohort of healthy Caucasian volunteers. Eur J Clin Pharmacol 1998;53: 361-7.
12. Tantcheva-Poor I, Zaigler M, Rietbrock S, Fuhr U. Estimation of cytochrome P-450 CYP1A2 activity in 863 healthy Caucasians using a saliva-based caffeine test. Pharmacogenetics 1999;9:131-44.
13. Relling MV, Lin JS, Ayers GD, Evans WE. Racial and gender differences in N-acetyltransferase, xanthine oxidase, and CYP1A2 activities. Clin Pharmacol Ther 1992; 52:643-58.

14. Bartoli A, Xiaodong S, Gatti G, Cipolla G, Marchiselli R, Perucca E. The influence of ethnic factors and gender on CYP1A2-mediated drug disposition: a comparative study in Caucasian and Chinese subjects using phenacetin as a marker substrate. Ther Drug Monit 1996;18:586-91.
15. Sachse C, Bhambra U, Smith G, Lightfoot TJ, Barrett JH, Scollay J, et al. Polymorphisms in the cytochrome P450 CYP1A2 gene (CYP1A2) in colorectal cancer patients and controls: allele frequencies, linkage disequilibrium and influence on caffeine metabolism. Br J Clin Pharmacol 2003;55:68-76.
16. Gago-Dominguez M, Bell DA, Watson MA, Yuan JM, Castelao JE, Hein DW, et al. Permanent hair dyes and bladder cancer: risk modification by cytochrome P4501A2 and N-acetyltransferases 1 and 2. Carcinogenesis 2003;24:483-9.
17. Vistisen K, Loft S, Olsen JH, Vallentin S, Ottesen S, Hirsch FR, et al. Low CYP1A2 activity associated with testicular cancer. Carcinogenesis 2004;25:923-9.
18. Rasmussen BB, Brix TH, Kyvik KO, Brosen K. The interindividual differences in the 3-demethylation of caffeine alias CYP1A2 is determined by both genetic and environmental factors. Pharmacogenetics 2002;12:473-8.
19. Nakajima M, Yokoi T, Mizutani M, Kinoshita M, Funayama M, Kamataki T. Genetic polymorphism in the 5′-flanking region of human CYP1A2 gene: effect on the CYP 1A2 inducibility in humans. J Biochem 1999;125: 803-8.
20. Sachse C, Brockmoller J, Bauer S, Roots I. Functional significance of a C−>A polymorphism in intron 1 of the cytochrome P450 CYP1A2 gene tested with caffeine. Br J Clin Pharmacol 1999;47:445-9.
21. Allorge D, Chevalier D, Lo-Guidice JM, Cauffiez C, Suard F, Baumann P, et al. Identification of a novel splice-site mutation in the CYP1A2 gene. Br J Clin Pharmacol 2003;56:341-4.
22. Murayama N, Soyama A, Saito Y, Nakajima Y, Komamura K, Ueno K, et al. Six novel nonsynonymous CYP1A2 gene polymorphisms: catalytic activities of the naturally occurring variant enzymes. J Pharmacol Exp Ther 2004;308:300-6.
22a. National Center for Biotechnology Information. GenBank overview. Available from: URL:http://www.ncbi.nlm.nih.gov/GenBank/. Accessed July 13, 2005.
23. Kruglyak L, Nickerson DA. Variation is the spice of life. Nat Genet 2001;27:234-6.
24. Primer3. Available from: URL:http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi. Accessed July 14, 2005.
25. PolyPhred. Available from: URL:http://droog.mbt.washington.edu/PolyPhred.html. Accessed July 14, 2005.
26. Carrillo JA, Christensen M, Ramos SI, Alm C, Dahl ML, Benitez J, et al. Evaluation of caffeine as an in vivo probe for CYP1A2 using measurements in plasma, saliva, and urine. Ther Drug Monit 2000;22:409-17.
27. Phase 2.0 program. Available from: URL:http://www.stat.washington.edu/stephens/software.html. Accessed Aug 5, 2005.
28. SNPtagger. Available from: URL:http://www.well.ox.ac.uk/~xiayi/haplotype/. Accessed July 14, 2005.
29. Linkage Disequilibrium Analyzer (1.0). Available from: URL:http://www.chgb.org.cn/lda/lda.htm. Accessed July 14, 2005.
29a. National Center for Biotechnology Information. Single nucleotide polymorphism. Available from: URL:http://www.ncbi.nlm.nih.gov/projects/SNP/. Accessed July 13, 2005.
29b. CYP allele nomenclature. Available from: URL:http://www.imm.ki.se/CYPalleles/. Accessed Aug 19, 2005.
30. Chung I, Bresnick E. Regulation of the constitutive expression of the human CYP1A2 gene: cis elements and their interactions with proteins. Mol Pharmacol 1995;47: 677-85.
31. Huang JD, Guo WC, Lai MD, Guo YL, Lambert GH. Detection of a novel cytochrome P-450 1A2 polymorphism (F21L) in Chinese. Drug Metab Dispos 1999;27: 98-101.
32. Soyama A, Saito Y, Hanioka N, Maekawa K, Komamura K, Kamakura S, et al. Single nucleotide polymorphisms and haplotypes of CYP1A2 in a Japanese population. Drug Metab Pharmacokinet 2005;20:24-33.
33. Chung I, Bresnick E. Identification of positive and negative regulatory elements of the human cytochrome P4501A2 (CYP1A2) gene. Arch Biochem Biophys 1997; 338:220-6.
34. Aitchison KJ, Gonzalez FJ, Quattrochi LC, Sapone A, Zhao JH, Zaher H, et al. Identification of novel polymorphisms in the 5′ flanking region of CYP1A2, characterization of interethnic variability, and investigation of their functional significance. Pharmacogenetics 2000;10: 695-704.
35. Obase Y, Shimoda T, Kawano T, Saeki S, Tomari SY, Mitsuta-Izaki K, et al. Polymorphisms in the CYP1A2 gene and theophylline metabolism in patients with asthma. Clin Pharmacol Ther 2003;73:468-74.
36. Shimoda K, Someya T, Morita S, Hirokane G, Yokono A, Takahashi S, et al. Lack of impact of CYP1A2 genetic polymorphism (C/A polymorphism at position 734 in intron 1 and G/A polymorphism at position −2964 in the 5′-flanking region of CYP1A2) on the plasma concentration of haloperidol in smoking male Japanese with schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2002;26:261-5.
37. Mihara K, Kondo T, Suzuki A, Yasui-Furukori N, Ono S, Otani K, et al. Effects of genetic polymorphism of CYP1A2 inducibility on the steady-state plasma concentrations of trazodone and its active metabolite m-chlorophenylpiperazine in depressed Japanese patients. Pharmacol Toxicol 2001;88:267-70.
38. van der Weide J, Steijns LS, van Weelden MJ. The effect

of smoking and cytochrome P450 CYP1A2 genetic polymorphism on clozapine clearance and dose requirement. Pharmacogenetics 2003;13:169-72.
39. Schulze TG, Schumacher J, Muller DJ, Krauss H, Alfter D, Maroldt A, et al. Lack of association between a functional polymorphism of the cytochrome P450 1A2 (CYP1A2) gene and tardive dyskinesia in schizophrenia. Am J Med Genet 2001;105:498-501.
40. Drysdale CM, McGraw DW, Stack CB, Stephens JC, Judson RS, Nandabalan K, et al. Complex promoter and coding region beta 2-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. Proc Natl Acad Sci U S A 2000;97:10483-8.
41. Wooding SP, Watkins WS, Bamshad MJ, Dunn DM, Weiss RB, Jorde LB. DNA sequence variation in a 3.7-kb noncoding sequence 5′ of the CYP1A2 gene: implications for human population history and natural selection. Am J Hum Genet 2002;71:528-42.
42. Han XM, Ou-Yang DS, Lu PX, Jiang CH, Shu Y, Chen XP, et al. Plasma caffeine metabolite ratio (17X/137X) in vivo associated with G-2964A and C734A polymorphisms of human CYP1A2. Pharmacogenetics 2001;11:429-35.