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Discovery of Novel Functional Variants and Extensive Evaluation of *CYP2D6* Genetic Polymorphisms in Koreans

Su-Jun Lee, Sang Seop Lee, Hyun-Ju Jung, Ho-Sook Kim, Soo-Jin Park, Chang-Woo Yeo, and Jae-Gook Shin

Department of Pharmacology and Pharmacogenomics Research Center (S.-J.L., S.S.L., H.-J.J., H.-S.K., S.-J.P., C.-W.Y., J.-G.S.), and Department of Clinical Pharmacology (C.-W.Y., J.-G.S.), Inje University College of Medicine, Inje University Busan Paik Hospital, Busan, Korea

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ABSTRACT:

Our objectives were to identify *CYP2D6* genetic polymorphisms in a Korean population, to compare the allele frequencies with those of other ethnic groups, and to evaluate variant-induced functional variations in dextromethorphan (DM) metabolism in vitro and in vivo. Thirty-eight single nucleotide polymorphisms of *CYP2D6* were identified by direct DNA sequencing in 51 Koreans. An extended set of 707 subjects were screened for the identified variants. A group of 202 healthy subjects was subjected to phenotypic analysis on DM metabolism. *CYP2D6*10* was found to be the most frequent allele (45.6%), followed by *CYP2D6*1* (32.3%), **2* (9.9%), **5* (5.6%), **41* (2.2%), **49* (1.4%), and some other rare alleles (<1%). The newly identified E418K and S183Stop were assigned as *CYP2D6*52* and *CYP2D6*60*, respectively, by the Human P450 (*CYP*) Allele Nomenclature Committee. Individuals having the *CYP2D6*10/*49* genotype ($n = 5$) exhibited a significant decrease in CYP2D6 metabolic activity compared with those with the *CYP2D6*1/*1* genotype ($n = 31$) ($P < 0.019$). Variations in CYP2D6 protein levels in liver tissues ($n = 49$) were observed with *CYP2D6* genotypes, and correlation between the CYP2D6 protein content and the activity was significant ($r^2 = 0.7$). Given the importance of CYP2D6 in drug metabolism, subjects with the *CYP2D6*10/*49* genotype may benefit from genotype analysis to achieve optimal drug therapy.

Polymorphisms of the gene for cytochrome P450 2D6 (*CYP2D6*) influence the rate of elimination of CYP2D6 substrates, which represent approximately 20% of commonly prescribed therapeutic drugs. CYP2D6 substrates include antipsychotic drugs (haloperidol and risperidone), antiarrhythmic agents (flecainide and perphenazine), tricyclic antidepressants (imipramine and amitriptyline), β-blockers (metoprolol and carvedilol), and opioids (codeine and tramadol) (Zanger et al., 2004; Ingelman-Sundberg, 2005). The *CYP2D6* gene locus is highly polymorphic, and various point mutations, nucleotide deletions or insertions, gene rearrangements, and multiplication/deletion of the entire *CYP2D6* gene, resulting in more than 106 different alleles (http://www.cypalleles.ki.se/cyp2d6.htm), have been reported. These genetic polymorphisms have been implicated in the extreme variability of CYP2D6 activity, whereby subjects can be categorized into ultra-rapid, extensive, intermediate, and poor metabolizers (PMs) based on *CYP2D6* genotype (Sachse et al., 1997; Griese et al., 1998; Raimundo et al., 2000). These phenotypes are distributed unequally in major populations (Dahl et al., 1995a; Bertilsson et al., 2002). Ethnic differences in the *CYP2D6* genotype frequencies have been observed in a number of populations after the discovery of different debrisoquine phenotypes (Mahgoub et al., 1977; Ingelman-Sundberg, 1999), and most of the phenotypic variants have been attributed to genetic polymorphisms of *CYP2D6* (Zanger et al., 2004; Eichelbaum et al., 2006). Phenotypic studies have revealed that approximately 5 to 10% of whites are PMs (Marez et al., 1997; Gaedigk et al., 1999; Bradford, 2002). However, the frequency of PMs is less than 1% in Asian populations and 0 to 8% in the African American population (Lou et al., 1987; Lennard et al., 1992; Droll et al., 1998; Bertilsson et al., 2002). The PM phenotype is attributed mainly to the *CYP2D6*3*, **4*, **5*, and **6* alleles, which predict 93 to 98% of PMs in whites; these variant alleles have also been found in an African population, albeit at lower frequency (Droll et al., 1998; Xie et al., 2001). In contrast, certain nonfunctional allelic variants (*CYP2D6*3*, **4*, and **6*) have not been observed in Asians (Heim and Meyer, 1992; Garcia-Barceló et al., 2000; Kubota et al., 2000). The *CYP2D6*10* allele, the most common allele in Asians at frequencies of up to ~60%, is a reduced function allele and contributes to intermediate metabolism in a large number of Asian subjects (Kubota et al., 2000; Bertilsson et al., 2002). *CYP2D6*18* and **44* have been found exclusively in Japanese (Chida et al., 1999a; Yamazaki et al., 2003). Wang et al. (1999) reported an Asian-specific PM allele (*CYP2D6*14*), which has not been detected in whites. *CYP2D6*17* has been well described as the most frequent

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ABBREVIATIONS: PM, poor metabolizer; DM, dextromethorphan; LA, long and accurate; PCR, polymerase chain reaction; UTR, untranslated reaction; SNP, single nucleotide polymorphism; DX, dextrorphan; MR, metabolic ratio; bis-Tris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol.

TABLE 1

Primers used for the amplification and sequencing for the CYP2D6 gene

Primer Name	Orientation	Site	Sequence (5′ to 3′)	Position in Reference Sequence[a]
CYP505F	Forward	Amplification of *CYP2D6* gene	CACTGGCTCCAAGCATGGCAG	13044–13064[b]
3′2D6R	Reverse		ACTGAGCCCTGGGAGGTAGGTA	6350–6371[c]
Sequencing primers				
CYP507	Forward	Promoter	AACGTTCCCA CCAGATTTC	561–579
CYP509	Forward	Promoter	GTAAGT GCCAGTGACA GATAAG	1115–1136
2d6-11	Reverse	Promoter	AGGATCCTTTGTTCAGGATATGTTGC	531–556
2d6-12	Reverse	Promoter	CACCAAGTACCCCACTTCCC	811–820
2d6-1	Forward	Exon 1	CATGTGGACTTCCAGAACACACC	1761–1783
2d6-2	Forward	Intron 1	GGTTCAAACCTTTTGCACTG	2179–2198
2d6-3	Forward	Exon 2	GTCGTGCTCAATGGGCTG	2557–2574
2d6-4	Forward	Intron 2	AAGGTGGATGCACAAAGAGT	3077–3096
2d6-5	Forward	Exon 4	GACCTAGCTCAG GAGGGACT	3579–3596
2d6-6	Forward	Exon 5	AGCTGGATGAGCTGCTAACT	4142–4161
2d6-7	Forward	Intron 6	CCTGACCTCCTCCAACATAG	4686–4705
2d6-8	Forward	Intron 7	CACCTAGTCCTCAATGCCAC	5062–5080
2d6-9	Forward	Intron 8	GAGTCTTGCAGGGGTATCAC	5598–5617

[a] Position in the reference sequence, M33388.
[b] The forward primer is the same as that in the previous report (Lovlie et al., 1996).
[c] The reverse primer for the amplification of *CYP2D6* gene was described previously (Gaedigk et al., 1999).

reduced activity allele in Africans and African Americans (Masimirembwa et al., 1996; Wan et al., 2001; Bradford, 2002).

There has been a lack of comprehensive reports on updated information regarding the influence of *CYP2D6* genotypes on the metabolic activity of CYP2D6 in Korean subjects. Because different ethnic groups exhibit different genetic polymorphisms of *CYP2D6*, the determination of genotype frequencies and identification of new alleles in a large population of Korean subjects would be helpful in ensuring safe drug therapy. Correlation studies between *CYP2D6* genotypes and metabolic activities have been reported in racially different ethnic groups; however, no studies have been carried out in Koreans using dextromethorphan (DM), which is a prototype substrate for CYP2D6. Therefore, this study was undertaken to obtain comparative information on the metabolic index between Korean and other ethnic groups, and this information would be useful in establishing criteria for phenotype/genotype correlation in the future study. The objectives of the present study were to determine the frequencies of *CYP2D6* genotypes and to elucidate the *CYP2D6* genotype and phenotype associations in a large group of subjects.

Materials and Methods

Subjects. Healthy Korean subjects were enrolled under the protocol approved by the institutional review board of Busan Paik Hospital (Busan, Korea). The study protocol was explained in detail to all volunteers, and written informed consent was obtained from all participants. The volunteers were recruited after screening by physical examination, medical history, and routine laboratory tests. None of the participants were alcoholics or taking any medication, including herbal medicines and food supplements. Liver tissues from 49 Korean donors (mean age ± S.D., 57 ± 9.3 years) at Busan Paik Hospital (Busan, Korea) were obtained from the tissue repository bank at Inje Pharmacogenomics Research Center (Inje University College of Medicine, Busan, Korea). The approval and the research protocol for the usage of human liver microsomes were obtained according to institutional guidelines.

Genotyping and DNA Sequencing. Blood samples were collected from 758 volunteers for genotyping analysis. No participants from the previous study (Lee et al., 2006) were included in this study. Genomic DNA was isolated from blood and liver tissue samples using a QIAamp Blood Mini Kit (QIAGEN, Chatsworth, CA) and stored at −20°C until use. From the 758 genomic DNA samples, 51 subjects were randomly selected for complete DNA sequencing analysis. All subjects recruited in this study were genotyped for *CYP2D6*2*, **3*, **4*, **5*, **10*, **14*, **18*, **21*, **41*, **49*, and **52*, and one novel variant (3877G>A, *CYP2D6*60*). Variants exclusively found in Asian populations with clinical consequences were selected for genotyping. However, rare variants in Asia and the variants undetected both in the present study and the previous sequencing report (Lee et al., 2006) in Koreans were excluded from the screening. The presence of a 100T>C change was collectively described as *CYP2D6*10* in the present study. Therefore, the known alleles *CYP2D6*10A/B*, **36x2*, and **36+*10* tandem were not differentiated in the current genotyping method. Detection of *CYP2D6*5* (Gaedigk et al., 1999) and detailed methods and primers used have been described previously (Table 1) (Yokoi et al., 1996; Chida et al., 1999b; Chou et al., 2003; Soyama et al., 2004; Lee et al., 2006). The presence of gene duplication and the allele carrying the duplication were determined according to published methods (Lovlie et al., 1996; Sachse et al., 1997; Soyama et al., 2004). For direct DNA sequencing analysis, the *CYP2D6* gene was amplified by long and accurate (LA) polymerase chain reaction (PCR) (TaKaRa LA-PCR kit; TaKaRa Shuzo, Shiga, Japan) using the previous reported primers (Lovlie et al., 1996; Gaedigk et al., 1999). The 6.6-kilobase LA-PCR product contained all nine exons, the intron/exon boundaries, and the 5′-UTR and 3′-UTR of the *CYP2D6* gene. The genomic DNA (200 ng) was dissolved in LA-PCR buffer that contained 3.0 mM $MgCl_2$, 0.2 μM concentrations of primers, 400 mM concentrations of dNTPs, and 2.5 U of LA-Taq polymerase. After an initial denaturation step at 94°C for 1 min, 30 cycles of 10 s at 98°C, 30 s at 64°C, and 7 min at 72°C were performed, and the reaction was terminated after a final elongation step at 72°C for 10 min. The entire PCR product was directly sequenced using the Applied Biosystems model 377A DNA Sequencer (Table 1). On-line bioinformatics tools (http://www.fruitfly.org/seq_tools/splice.html; http://www.cbrc.jp/research/db/TFSEARCH.html) were used to analyze whether the novel SNPs interfere with RNA splicing or affect transcription factor binding sites.

Phenotyping. The in vivo phenotype study was performed on 202 subjects (mean age ± S.D., 25 ± 3.6 years), who agreed to follow the experimental schedule. They were admitted to overnight housing at the Clinical Trial Center of Busan Paik Hospital and were prohibited from taking alcohol or any medicine including food supplements for 1 week before and throughout the trial period. After subjects empty their bladders, blank urine was collected, and the subjects took a single oral dose of 30 mg of DM (Romilar; Roche Korea Co., Ltd., Ansung, Korea). Food was not allowed for 4 h before and after dosing. All the urine produced within 8 h after the administration of DM was collected. The urine volume was measured, and aliquots were stored at −20°C until assayed. The DM and dextrorphan (DX) levels in the urine were determined by reverse-phase high-performance liquid chromatography with fluorescence detection, as described previously (Ryu et al., 2007). The interassay precision values for all samples were less than 9.3%. The mean values of urinary metabolic ratio (MR) were used as an index to reflect CYP2D6 activity.

Western Blotting. Human liver microsomes were prepared from the randomly chosen liver tissues, the *CYP2D6* genotypes of which were determined as described under *Materials and Methods*. Microsomal proteins (30 μg/lane) were separated by NuPAGE 4 to 12% bis-Tris gel (Invitrogen, Carlsbad, CA) electrophoresis and then transferred to a polyvinylidene difluoride membrane

TABLE 2

SNPs detected in the CYP2D6 gene in 51 Korean subjects[a]

	Location	SNP and Surrounding Sequence (5′ to 3′)	Effect	Subject No. (Frequency %)
−1770G>A	5′-Flanking	GTGGATGATCCC**G/A**TAGAAGTCCAGA		17 (16.7)
−1584C>G	5′-Flanking	CTTGGAAGAACC**C/G**GGTCTCTACAAA		15 (14.6)
−1426C>T	5′-Flanking	CTACTGAAAATA**C/T**AAAAAGCTAGAC		53 (51.0)
−1235A>G	5′-Flanking	AAAAAAAAAAAG**A/G**ATTAGGCTGGGT		73 (70.8)
−1000G>A	5′-Flanking	GTGGAGGAGGAC**G/A**ACCCTCAGGCAG		53 (51.0)
−740C>T	5′-Flanking	GAGAATGTGTGC**C/T**CTAAGTGTCAGT		18 (17.7)
−678G>A	5′-Flanking	GTGATTTTCTGC**G/A**TGTGTAATCGTG		18 (17.7)
−498C>A	5′-Flanking	CCCAGGTAAGTG**C/A**CAGTGACAGATA		7 (7.3)
−138A>G	5′-Flanking	ACAGCAGAGGGC**A/G**AAGGCCATCATC		1 (1.0)
100C>T	Exon 1	GCTGCACGCTAC**C/T**CACCAGGCCCCC	P34S	53 (51.0)
214G>C	Intron 1	GGCGGCAGAGGT**G/C**CTGAGGCTCCCC		18 (17.7)
221C>A	Intron 1	GAGGTGCTGAGG**C/A**TCCCCTACCAGA		18 (17.7)
223C>G	Intron 1	GGTGCTGAGGCT**C/G**CCCTACCAGAAG		18 (17.7)
227T>C	Intron 1	CTGAGGCTCCCC**T/G**ACCAGAAGCAAA		18 (17.7)
232G>C	Intron 1	GCTCCCCTACCA**G/C**AAGCAAACATGG		18 (17.7)
233A>C	Intron 1	CTCCCCTACCAG**A/C**AGCAAACATGGA		18 (17.7)
245A>G	Intron 1	AAGCAAACATGG**A/G**TGGTGGGTGAAA		18 (17.7)
310G>T	Intron 1	GGTTTGGGGGAC**G/T**CCTGGAGAAGGG		71 (68.8)
746C>G	Intron 1	ACCCCCGCCCCA**C/G**ATCAGGAGGCT		18 (17.7)
843T>G	Intron 1	TGTAGTCTGGGG**T/G**GATCCTGGCTTG		70 (67.7)
1039C>T	Exon 2	GATCCTGGGTTT**C/T**GGGCCGCGTTCC		53 (51.0)
1611T>A	Exon 3	CCCCCAGGGGTG**T/A**TCCTGGCGCGCT	F120I	3 (3.1)
1661G>C	Exon 3	GCGCTTCTCCGT**G/C**TCCACCTTGCGC		70 (67.7)
1790A>G	Intron 3	GCACAAAGCGGG**A/G**ACTGGGAAGGCG		2 (2.1)
1887insTA	Exon 4	TTGGACAAAGCCGTG-/**TA**AGCAACGTGATCGCC	S183Stop	1 (1.0)
2097A>G	Intron 4	AGCAGGATTTGC**A/G**TAGATGGGTTTG		52 (50.0)
2303C>T	Intron 4	GGTGGAGGCTGG**C/T**ACTTGGGGAGGG		4 (4.2)
2663G>A	Intron 5	GGTGGGGTTGAGC**G/A**TCCCAGGAGGAA		13 (12.5)
2850C>T	Exon 6	GATGAGAACCTG**C/T**GCATAGTGGTGG	R296C	18 (17.7)
2988G>A	Intron 6	GGGCCGAGGGAG**G/A**AAGGGTACAGGC		2 (2.1)
3384A>C	Intron 7	CTCAGCACCAGC**A/C**CCTGGTGATAGC		71 (68.8)
3582A>G	Intron 7	TTGGAGGACCCA**A/G**CGCCTGCAGGGA		52 (50.0)
3584G>A	Intron 7	GGAGGACCCAAC**G/A**CCTGCAGGGAGA		18 (17.7)
3790C>T	Intron 7	CACCCTGCATCT**C/T**CTGCCCAGGGAA		18 (17.7)
3877G>A	Exon 8	CGCTTCCACCCC**G/A**AACACTTCCTGG	E418K	2 (2.1)
4180G>C	Exon 9	CTTTCCTGGTGA**G/C**CCCATCCCCCTA	S486T	70 (67.7)
4401C>T	3′-Flanking	GAGACAGGGGTA**C/T**GTTGAGGCTGAG		53 (51.0)
4481G>A	3′-Flanking	CTGCCCAGATTG**G/A**TGACAAGGACTA		17 (16.7)
Total deletion				4 (5.9)

[a] The number indicates the position in the *CYP2D6* gene relative to the start codon of translation. The accession number of the *CYP2D6* genomic DNA is M33388. Nucleotide change is indicated by bold and underline.

(GE Healthcare Bio-Sciences, Little Chalfont, Buckinghamshire, UK). Specific anti-CYP2D6 antiserum (Santa Cruz Biotechnology Inc., Santa Cruz, CA) was used at a 1:1000 dilution as a primary antibody. The immunoreactive proteins were detected with the ECL enhanced chemiluminescence system (GE Healthcare Bio-Sciences). Protein bands containing CYP2D6 were visualized by autoradiography on an LAS 3000 (Fuji Film Life Science, Stamford, CT). Band densities for CYP2D6 and β-actin were quantified using Multi Gauge (Fuji Photo Film Core Science Laboratory, Tokyo, Japan). The relative CYP2D6 levels in the Western blots were normalized to β-actin, which was immunoblotted as an internal control. Pure recombinant CYP2D6 protein (BD Gentest; BD Biosciences, San Diego, CA) (130 fmol/lane) was used as a reference standard.

Metabolism and Enzyme Activity. The microsomal enzyme activity was determined using DM as a prototype substrate. The incubation mixtures (0.25 ml) contained 0.25 mg/ml microsomal protein, 0.1 M phosphate buffer (pH 7.4), and 5 μM DM. After a 5-min preincubation at 37°C, the triplicate reactions were initiated by the addition of the NADPH-generating system (3.3 mM glucose 6-phosphate, 1.3 mM β-$NADP^+$, 3.3 mM $MgCl_2$, and 1.0 U/ml glucose-6-phosphate dehydrogenase) and incubated at 37°C for 15 min (Liu et al., 2006). The metabolite formation was within a linear range at 15 min. The reaction was terminated by placing the incubation tubes on ice and adding 100 μl of ice-cold acetonitrile containing 10 μM chlorpropamide as an internal standard. The incubation mixtures were then centrifuged at 20,000*g* for 10 min at 4°C. Aliquots of the supernatants were injected into the liquid chromatography-tandem mass spectrometry system, which comprises an Agilent 1100 series high-performance liquid chromatograph (Agilent Technologies, Santa Clara, CA) and an API 3000 tandem mass spectrometer (Applied Biosystems, Foster City, CA). The liquid chromatography-tandem mass spectrometry methods for quantification of DX was based on previous methods (Kim et al., 2006; Lee et al., 2006).

Statistical Analysis. The allele frequencies observed in the present study were analyzed by the Hardy-Weinberg equation. The 95% confidence intervals for all the genotype data and the χ^2 test were determined by SNP analyzer (Dynacom Co., Yokohama, Japan). All data are presented as mean ± S.D. Normal distribution of log (DM/DX) in phenotype studies was assessed using the Shapiro-Wilk test. The values of log (DM/DX) between two different genotypes were compared using a Wilcoxon rank-sum test. All statistical analyses were performed using SAS software (version 9.1.3; SAS Institute, Cary, NC). The correlation coefficients between the formation rates of DX and CYP2D6 protein content in the different human liver microsomes (n = 49) were calculated by parametric regression analysis using SAS software. $P < 0.05$ was considered to be statistically significant.

Results

Thirty-eight variant alleles were detected in the direct DNA sequencing analysis of the *CYP2D6* gene in 51 Koreans. The frequency of *CYP2D6* genetic variants is summarized in Table 2. Eight SNPs were found within exons, including two new SNPs that cause an amino acid change (E418K) and introduce a stop codon (S183Stop), respectively. The alleles carrying these SNPs were designated as *CYP2D6*52* and *CYP2D6*60*, respectively, by the Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee (http://www.cypalleles.ki.se/cyp2d6.htm). Nine SNPs in the 5′-UTR (sequenced

TABLE 3

Allele frequencies of CYP2D6 (n = 758)

Allele	Present Study		Reference Data[a]: Observed Frequency (95% Confidence Interval)
	Occurrence	Observed Frequency (95% Confidence Interval) (%)	
**1*	489	32.32 (28.67–35.32)	33.25 (29.88–36.63)
**2*[b]	151	9.89 (7.76–12.01)	10.13 (8.13–12.25)
**2*[c]	2	0.99 (0.28–0.16)	N.D.
**5*	85	5.61 (3.97–7.24)	6.13 (4.50–7.88)
**10*	691	45.58 (42.03–49.12)	45.00 (41.63–48.25)
**14*	5	0.33 (0.00–0.73)	0.50 (0.13–1.00)
**18*	4	0.26 (0.00–0.62	N.D.
**21*	5	0.33 (0.00–0.73)	0.25 (0.00–0.63)
**41*	34	2.24 (1.34–3.53)	1.88 (0.00–0.88)
**49*	21	1.39 (0.55–2.22)	N.D.
**52*	5	0.33 (0.00–0.73)	N.D.
**60*	1	0.07 (0.00–0.26)	N.D.
**1xN*	2	0.07 (0.00–0.26)	0.13 (0.00–0.38)
**2xN*	15	0.99 (0.28–0.16)	0.50 (0.13–1.00)
**10xN*	6	0.40 (0.00–0.84)	0.50 (0.13–1.00)

N.D., not detected.
[a] *CYP2D6* polymorphisms in 400 subjects were described previously (Lee et al., 2006).
[b] Alleles carrying −1584G.
[c] Alleles carrying −1584C.

up to −1800 nucleotides), 19 SNPs in introns, and 2 SNPs in the 3′-UTR (500 nucleotides after the stop codon) were identified. None of these SNPs appeared to be involved in alternative splicing or were situated in transcription factor binding sites. All of the 51 individuals containing a 100C>T change were found as *CYP2D6*10B* in the sequencing analysis as reported in the other independent sequencing study (Lee et al., 2006). However, the other 707 subjects were only genotyped for the presence of a 100C>T and were collectively described as *CYP2D6*10*. The frequency of *CYP2D6*10* in the present study represents the combined calculation from the 51 sequenced and the 707 genotyped subjects. Combined allele frequencies from the 51 sequenced and the 707 genotyped subjects are presented in Table 3. The most frequent variant allele in the present study (n = 758) was *CYP2D6*10* (45.6%), followed by *CYP2D6*2* (9.9%), **5* (5.6%), **41* (2.2%), **49* (1.4%), **2xN* (1%), and the remaining rare alleles (<1% frequency). *CYP2D6*18, *49,* and **60* were identified as rare variants in the present study. However, these SNPs were not detected in a previous report (Lee et al., 2006). The observed *CYP2D6* allele frequencies were similar to those reported for other Asian populations, with the exception of *CYP2D6*49*, which was found at a frequency of 0.5% in a Japanese population (Soyama et al., 2004). The 37 different genotypes are listed, together with their respective frequencies in Table 4. *CYP2D6*1/*10* was the most frequent genotype (26.8%), followed by *CYP2D6*10/*10*, **1/*1*, **2/*10*, and **1/*2*, which were found at frequencies of 20.8, 12.4, 9.9, and 5.8%, respectively. The frequencies of the variants observed in the present study were in good agreement within Hardy-Weinberg equilibrium.

The genotype and phenotype relationships of *CYP2D6* were evaluated in 202 subjects after a single oral administration of 30 mg of DM. Both DM and its metabolite DX were quantitated in all of the urine samples with detection limits of 1 and 5 ng/ml, respectively. Individuals with a *CYP2D6*10/*49* genotype had higher urinary metabolic ratios compared with those with a *CYP2D6*1/*1* genotype (P = 0.019) in a Wilcoxon rank-sum test (Fig. 1). No significant difference was observed between individuals with the *CYP2D6*1/*1* and *CYP2D6*1/*49* genotypes (Fig. 1). The mean log MR values increased on the order of *CYP2D6*1/*1*, **1/*49*, **10/*52*, **10/*49*, and **10/*10.* The log MR values for all individuals having the *CYP2D6*41* allele (**1/*41*, **10/*41*, **14/*41*, and **41/*41*) were higher than those for subjects with the *CYP2D6*1/*1* genotype, as shown previously by Cai et al. (2007). The log MR values of individuals with the *CYP2D6*10/*41* genotype were significantly higher than those for subjects with the *CYP2D6*1/*1* genotype (P < 0.003). Two individuals with the *CYP2D6*10/*52* genotype and one with the *CYP2D6*49/*52* genotype showed slightly lower or comparable log

TABLE 4

Frequency of CYP2D6 genotypes in a Korean population (n = 758)

CYP2D6 Genotype	*n*	Frequency (%)	CI
**1/*1*	94	12.40	10.05–14.74
**1/*1xN*	2	0.26	0.00–0.62
**1/*10*	203	26.78	23.62–29.93
**1/*2*	44	5.80	4.13–7.46
**1/*2*[a]	1	0.13	0.00–0.38
**1/*2xN*	7	0.92	0.24–1.59
**1/*5*	27	3.56	2.24–4.87
**1/*21*	1	0.13	0.00–0.38
**1/*41*	8	1.06	0.33–1.79
**1/*49*	8	1.06	0.33–1.79
**2/*2*	9	1.19	0.42–1.96
**2/*5*	8	1.06	0.33–1.79
**2/*5*[a]	1	0.13	0.00–0.38
**2/*10*	75	9.89	7.76–12.01
**2/*14*	1	0.13	0.00–0.38
**2/*18*	1	0.13	0.00–0.38
**2/*21*	1	0.13	0.00–0.38
**2/*41*	1	0.13	0.00–0.38
**2/*52*	2	0.26	0.00–0.62
**2xN/*5*	1	0.13	0.00–0.38
**2xN/*10*	7	0.92	0.24–1.59
**5/*5*	2	0.26	0.00–0.62
**5/*10*	42	5.54	3.91–7.17
**5/*14*	1	0.13	0.00–0.38
**5/*41*	1	0.13	0.00–0.38
**14/*41*	1	0.13	0.00–0.38
**10/*10*	158	20.84	17.95–23.73
**10/*10xN*	6	0.79	0.16–1.42
**10/*14*	2	0.26	0.00–0.62
**10/*18*	3	0.40	0.00–0.85
**10/*21*	3	0.40	0.00–0.85
**10/*41*	19	2.51	1.40–3.62
**10/*49*	12	1.58	0.69–2.47
**10/*52*	2	0.26	0.00–0.62
**10/*60*	1	0.13	0.00–0.38
**41/*41*	2	0.26	0.00–0.62
**49/*52*	1	0.13	0.00–0.38

CI, 95% confidence interval.
[a] *CYP2D6*2* allele carrying −1584C.

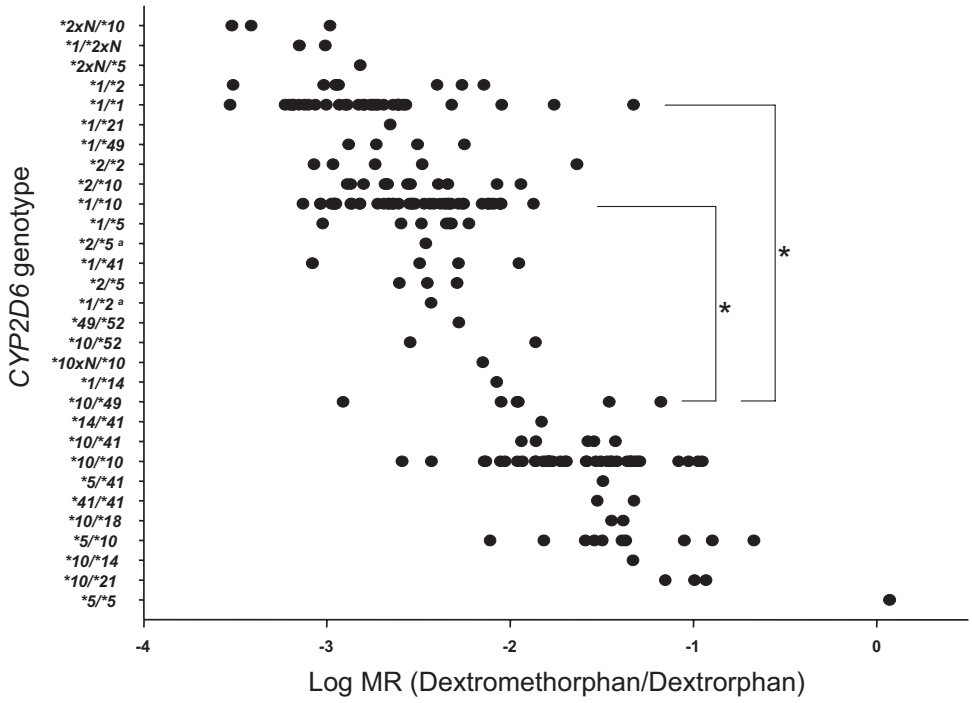

FIG. 1. Relationship between genotype and metabolic ratio of DM to DX in 202 subjects. The subjects received a single oral dose of 30 mg of DM, and all urine up to 8 h was collected after administration. The DM and DX levels in the urine samples were determined by high-performance liquid chromatography, as described under *Materials and Methods*. The *P* value between the *CYP2D6*10/*52* and *CYP2D6*1/*1* genotype groups determined by a Wilcoxon rank-sum test was insignificant ($P = 0.06$). [a]*CYP2D6*2* allele carrying −1584C. *, $P < 0.05$, Wilcoxon rank-sum test for *CYP2D6*10/*49* versus **1/*1* and **1/*10* genotype groups, respectively.

MR values than individuals having the *CYP2D6*10/*10* genotype, suggesting that the *CYP2D6*52* allele may confer decreased activity. Individuals having *CYP2D6*10/*10*, **10/*41*, **10/*49*, **10/*14*, **10/*18*, and **10/*21* showed higher log MR values than subjects with *CYP2D6*1* or **2*. Although the MR values were high for individuals with the *CYP2D6*5/*5*, **41/*41*, **10/*18*, or **10/*21* genotype, the number of subjects in these groups was too low for statistical analysis.

We performed quantitative immunoblotting of CYP2D6 protein content in 49 liver microsomes. There was a 6.8-fold variation in CYP2D6 protein amounts between the lowest and the highest values (Fig. 2A). The highest amounts of CYP2D6 protein were found in microsomes of samples with *CYP2D6*1/*1* ($n = 6$), **1/*2* ($n = 4$), **2/*10* ($n = 4$), and **2xN/*1* ($n = 1$) genotypes, respectively (Fig. 2A). Microsomes prepared from samples genotyped as *CYP2D6*10/*10* ($n = 9$), **5/*10* ($n = 4$), and **10/*41* ($n = 3$) contained less CYP2D6 protein. Lower protein content was observed to exhibit lower activity as assessed with DM (Fig. 2, A and B). Samples with *CYP2D6*1/*1* and *CYP2D6*10/*10* genotypes contained significantly different amounts of protein ($P = 0.017$). However, there were no liver tissues containing the *CYP2D6*49*, **52*, or **60* alleles for the comparisons with the DM phenotype results in vivo.

Discussion

The present study is the first to evaluate the distribution of *CYP2D6* variants and their effects on DM metabolism in a large Korean population. *CYP2D6*10* was found to be the most frequent allele at 45.6%. The *CYP2D6*10* allele has been observed at frequencies of 33 to 50% in Japanese (Dahl et al., 1995b; Sistonen et al., 2007) and 58.4% in Chinese (Cai et al., 2000), which suggests that the frequency of this allele is significantly higher in Asian populations than in white and African populations (Yokoi et al., 1996; Gaedigk et al., 1999). We also found that among the nonfunctional alleles of *CYP2D6*, the *CYP2D6*5* allele was the most frequent at 5.6%, followed by *CYP2D6*14*, **21*, and **18* at frequencies of 0.33, 0.33, and 0.26%, respectively.

Genotype-phenotype relationships have been explored using CYP2D6 probe drugs, and observed discordances have led to the discovery of several variants in Asians, such as *CYP2D6*14*, **18*, **21*, **39*, and **44* (Yokoi et al., 1996; Chida et al., 1999b; Wang et al., 1999; Shimada et al., 2001; Yamazaki et al., 2003). Even though no phenotyping data are available for the *CYP2D6*60* allele, the presence of the S183stop codon clearly suggests that this variant does not encode functional enzyme in humans. The discovery of this allele increases our understanding of the genotype-phenotype relationships in Koreans and possibly other Asian populations as well. We evaluated the genotype and phenotype correlations with the most recent genotype information extensively to predict the metabolic contribution of each variant allele to CYP2D6 activity in Asians. Although our study was conducted in a small number of individuals for certain alleles, making statistical analysis difficult, the MR values indicate a similar pattern of results from the present and previous studies for individuals with the same genotype (Yokoi et al., 1996; Chida et al., 1999b). In particular, the present study offers the first evaluation of the effect of the *CYP2D6*49* allele on DM metabolism in vivo. Individuals with the *CYP2D6*10/*49* genotype (1.6%) exhibited a significant impact on CYP2D6 metabolic activity for DM, compared with individuals with the *CYP2D6*1/*1* ($P = 0.019$) and *CYP2D6*1/*10* ($P = 0.02$) genotypes, respectively. Our data provide evidence that interindividual variation in CYP2D6 activity can be explained by the presence of subjects with a *CYP2D6*10/*49* genotype.

We were unable to further characterize protein encoded by *CYP2D6*49* in microsomal preparations, but our in vivo data imply that it may have minor impact on DM metabolism. More recently, it was reported that CYP2D6*49 protein, in a COS-7 expression system, exhibited decreased activity compared with the wild-type (Sakuyama et al., 2008), suggesting that decreased activity of *CYP2D6*10/*49* in vivo observed in our present study would be attributed to the reduced enzymatic activity of CYP2D6*49.

The location of Phe120 has been identified in the B′-C loop area in a study of the crystal structure of CYP2D6 (Rowland et al., 2006), and Phe120 is suggested to be an important part of the foot arch structure.

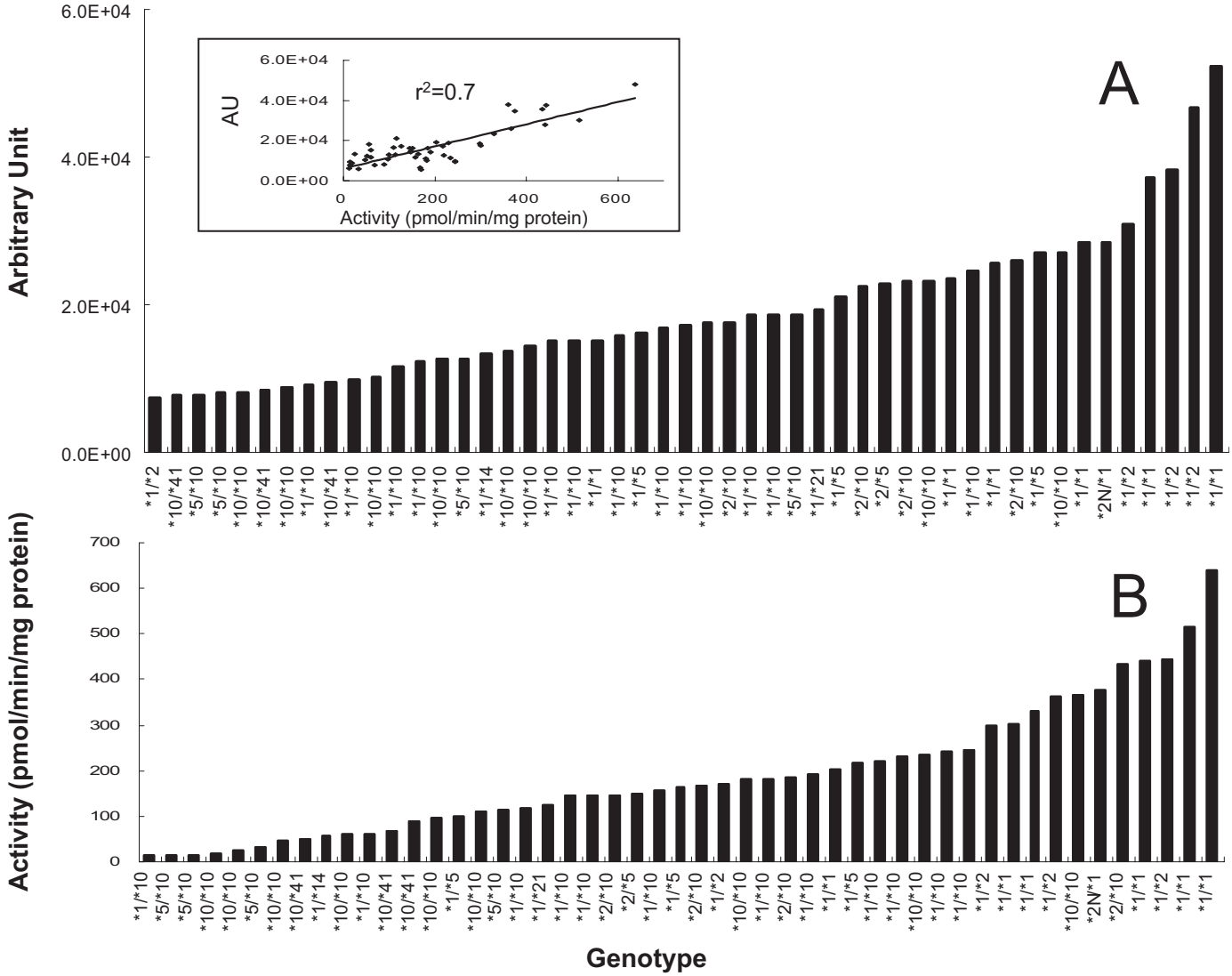

FIG. 2. Relationship between the genotype and hepatic content of CYP2D6 protein (A) and the CYP2D6 activity (B). Liver microsomal proteins (30 μg/lane) were separated by SDS-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes, and developed with an antibody to CYP2D6 using the ECL chemiluminescence kit. AU, arbitrary unit, indicates the relative pixel values of CYP2D6 protein that was normalized to the β-actin level as described under *Materials and Methods*. The plot between the activity and CYP2D6 protein content is in the inset ($r^2 = 0.7$, in linear regression analysis using the Spearman test). CYP2D6 activity was measured using DM as a prototype substrate. Values represent the means ± S.D. of triplicate reactions.

The change from F120I (*CYP2D6*49*) would affect the structural conformation between the B′-C loop and *N* terminus of the I helix, resulting in altered CYP2D6 activity, because this residue is located in the border regions of the B′-C loop and near the *N* terminus of the I helix (Rowland et al., 2006). The newly identified *CYP2D6*52* allele seems to encode a protein of lower activity compared with that encoded by the *CYP2D6*1* wild-type, because two individuals having the *CYP2D6*10/*52* genotype exhibited a higher MR value compared with both *CYP2D6*1/*10* ($P = 0.28$)- and *CYP2D6*1/*1* ($P = 0.06$)-containing subjects. However, further in vitro study using a recombinant system would be helpful to confirm its activity compared with that of the wild-type.

The smallest amounts of CYP2D6 protein in 49 liver tissue samples were found in those genotyped as *CYP2D6*10/*10*, **5/*10*, and **10/*41*, which was consistent with slow metabolizer phenotypes (i.e., higher DM/DX urinary ratios) in subjects of respective genotypes (Figs. 1 and 2). One of four microsomes carrying *CYP2D6*1/*2* exhibited the lowest amount of protein, but the other three showed relatively high expression profiles (Fig. 2A). The correlation between the protein content and the DM activity was significant, as shown in the inset of Fig. 2A ($r^2 = 0.7$). These results suggest that variations in DM metabolism would be largely affected by *CYP2D6* polymorphisms. Because there were no liver tissues containing *CYP2D6*49* and **52*, the correlation between the Western blotting and in vivo DM metabolism for these alleles was not determined.

Unlike other cytochromes P450 such as CYP3A, which exhibits up to 40-fold interindividual variations in protein amounts in liver (Lamba et al., 2002), a panel of liver samples, which did not comprise any with a null/null genotype, exhibited a 6.8-fold variation for CYP2D6 protein content. There were 50-fold variations between the lowest and the highest DM metabolism in the liver microsomes. Our log MR ratio in urine samples exhibited a 52-fold variation between the least and the highest values. Unlike for CYP2C9 and CYP3A4 in humans, there has been a lack of evidence for multiple transcriptional factors for CYP2D6 induction via foreign and endogenous compounds. This may explain the lower interindividual variations in CYP2D6 protein amounts compared with those of the CYP2Cs and CYP3As in humans. Therefore, polymorphic expression of CYP2D6

seems to be the single most important factor explaining variations in drug disposition.

In conclusion, because this is the most extensive genotyping analysis in Koreans to our knowledge, it would cover almost all of current *CYP2D6* genetic variants in Koreans and would be applicable to provide guidance for clinical prediction of CYP2D6 substrates in use. The influence of the *CYP2D6*49* allele on DM metabolism is determined in vivo in various genotypes for the first time. Great care in use of this information would be helpful to optimize drug dosages for individuals with the *CYP2D6*10/*49* genotype. Genotyping *CYP2D6*49* together with other reduced activity alleles would be also helpful in predicting CYP2D6 activity in the clinical setting.

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Address correspondence to: Dr. Jae-Gook Shin, Department of Pharmacology and Pharmacogenomics Research Center, Department of Clinical Pharmacology, Inje University College of Medicine, 633-165 Gaegum-Dong, Jin-Gu, Busan 614-735, Korea. E-mail: phshinjg@inje.ac.kr