

Discovery of a Novel Allelic Variant of *CYP2C8*, *CYP2C8*11*, in Asian Populations and Its Clinical Effect on the Rosiglitazone Disposition In Vivo^[S]

Chang-Woo Yeo, Su-Jun Lee, Sang Seop Lee, Soo Kyung Bae,¹ Eun-Young Kim, Ji-Hong Shon, Byoung Doo Rhee, and Jae-Gook Shin

Departments of Pharmacology and Pharmacogenomics Research Center (C.-W.Y., S.-J.L., S.S.L., S.K.B., J.-H.S., J.-G.S.), Clinical Pharmacology (C.-W.Y., E.-Y.K., J.-H.S., J.-G.S.), and Internal Medicine (B.D.R.), Inje University College of Medicine, Inje University Busan Paik Hospital, Busan, Korea

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ABSTRACT:

The objectives of this study were to identify the genetic variants of *CYP2C8*, analyze *CYP2C8* single nucleotide polymorphisms (SNPs), and characterize their functional consequences in the *CYP2C8* substrate drug rosiglitazone in humans. The direct full sequencing of *CYP2C8* genomic DNA was performed in a Korean population ($n = 50$). A total of 17 *CYP2C8* variants including a novel coding variant (E274Stop) were identified. The novel *CYP2C8* E274Stop variant was assigned as *CYP2C8*11* by the Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee. Seventeen SNPs were used to characterize linkage disequilibrium, haplotype structures, and haplotype tagging SNPs. Genotyping for *CYP2C8*11* in an extended set of Koreans ($n = 400$), whites ($n = 100$), Han Chinese ($n = 348$), Vietnamese ($n = 100$), and African Americans ($n = 93$) was performed by a newly developed pyrosequencing method. The frequency of *CYP2C8*11* was 0.3% in Ko-

reans, 1% in Vietnamese, and 0.14% in Chinese. However, none of the whites or African Americans contained the *CYP2C8*11* allele. Subjects with *CYP2C8*11* exhibited higher plasma concentration-time profiles of rosiglitazone than those of nine control subjects carrying *CYP2C8*1*. The area under the concentration-time curve and peak plasma concentration of rosiglitazone in individuals carrying *CYP2C8*11* ($n = 5$) were 54 and 34% higher than the mean values observed in the control subjects carrying *CYP2C8*1* ($P = 0.015$ and $P = 0.025$, respectively). In summary, this is the first report to characterize the allele frequency and haplotype distribution of *CYP2C8* in a Korean population, and it provides functional analysis of a new variant *CYP2C8*11*. Our findings suggest that individuals carrying *CYP2C8*11*, a null allele found in Asians only, may have lower activity for metabolizing *CYP2C8* substrate drugs.

Introduction

Cytochrome P450 2C8 is one of the major hepatic cytochrome P450s, constituting 3 to 7% of the total cytochrome P450 content in the human liver (Rendic and Di Carlo, 1997; Totah and Rettie, 2005). *CYP2C8* is involved in the metabolism of various endogenous compounds such as retinoic acid and arachidonic acid (Totah and Rettie, 2005). *CYP2C8*-mediated arachidonic acid metabolism has an important physiological role that affects hepatic glycogenolysis, platelet aggregation, vascular smooth muscle tone, and water reabsorption in

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¹ Current affiliation: College of Pharmacy, The Catholic University of Korea, Bucheon, Korea.

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renal tubules (Moreno et al., 2001; Roman, 2002; Pomposiello et al., 2003; Sacerdoti et al., 2003; Yasar et al., 2003). *CYP2C8* also contributes to the metabolism of therapeutic agents, including amiodarone, cerivastatin, paclitaxel, repaglinide, and rosiglitazone (Rendic and Di Carlo, 1997; Totah and Rettie, 2005).

Rosiglitazone is an oral thiazolidinedione compound that acts as an insulin sensitizer to improve glycemic control and has been developed for the treatment of type 2 diabetes mellitus (Yki-Järvinen, 2004). The major elimination route of rosiglitazone is extensive hepatic metabolism, *para*-hydroxylation of the pyridine ring, and N-demethylation, followed by conjugation with sulfate and glucuronic acid (Baldwin et al., 1999; Klose et al., 1999). In vitro data have shown that this hydroxylation is catalyzed primarily by *CYP2C8* (Baldwin et al., 1999; Klose et al., 1999; Jaakkola et al., 2006). Many researchers have adopted rosiglitazone in pharmacokinetic studies to quantify the functional activity of *CYP2C8* in relation to its genetic polymorphism. Thus, it is widely accepted that rosiglitazone is an effective *CYP2C8* probe drug in vivo and in vitro (Baldwin et al., 1999; Cox et al., 2000).

The *CYP2C8* gene locus is highly polymorphic and various mutations have been reported, resulting in more than 16 different alleles in

ABBREVIATIONS: SNP, single nucleotide polymorphism; UTR, untranslated region; PCR, polymerase chain reaction; LD, linkage disequilibrium; MS/MS, tandem mass spectrometry; AUC, area under the concentration-time curve.

the Cytochrome P450 Nomenclature Web site (<http://www.cypalleles.ki.se/cyp2c8.htm>). These *CYP2C8* genetic polymorphisms have been implicated in the variability of *CYP2C8* activity with different phenotypes (Daily and Aquilante, 2009). These different phenotypes are distributed unequally in major ethnic populations. Ethnic differences in the *CYP2C8* genotype frequencies have been observed in a number of populations (Dai et al., 2001; Saito et al., 2007; Rodríguez-Antona et al., 2008; Daily and Aquilante, 2009). However, no screening studies for finding *CYP2C8* variants have been performed in Korean populations.

To determine the distribution of *CYP2C8* variants and haplotypes, we conducted direct DNA sequencing of the *CYP2C8* gene in a Korean population for the first time. The frequencies of functional *CYP2C8* variants were further analyzed in an extended DNA set of ethnically different populations. In addition, the in vivo clinical effect of *CYP2C8*11*, a novel null allele, on the disposition of rosiglitazone was evaluated in the individuals carrying the *CYP2C8*11* allele.

Materials and Methods

Subjects. Genomic DNAs from 50 Koreans for direct DNA sequencing and an extended set of DNA for genotyping that included 400 Koreans, 100 Viet Kinh Vietnamese, 348 Han Chinese, 93 African Americans, and 100 whites were obtained from the DNA repository bank at INJE Pharmacogenomics Research Center (Inje University College of Medicine, Busan, Korea) as reported previously (Lee et al., 2005a, 2007, 2010b). The approval and the research protocol for the use of human DNA were obtained according to the institutional guideline. Nine healthy volunteers with the *CYP2C8*1/*1* genotype and five individuals having *CYP2C8*1/*11* participated in the phenotyping study. Three individuals having *CYP2C8*11* were found and recruited from the genotyping procedure in an extended set of the DNA repository bank, and two individuals carrying *CYP2C8*11* and nine control subjects were from the local communities through the advertisement put up by the Inje University Pharmacogenomics Research Center. All participants gave written informed consent, which was approved by the institutional review board of Busan Paik Hospital (Busan, Korea). The subjects ranged in age from 21 to 34 years and in body weight from 59 to 90 kg. All the participants were healthy, according to their medical history, physical examination, and laboratory tests.

Identification of *CYP2C8* SNPs in a Korean Population. The *CYP2C8* SNP profiles were analyzed by the direct full sequencing of the *CYP2C8* gene from 50 normal healthy Korean subjects who participated in the study. For this, genomic DNA was isolated from whole blood cells using a DNA Extraction Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's protocol. Specific primers, which were slightly modified in addition to those in the previous study (Soyama et al., 2002), were used to amplify nine exons of *CYP2C8*, from up to 3 kilobases of the upstream region of the 5'-UTR to the 722 base pairs of 3'-UTR (Supplemental Table S1). Polymerase chain reaction (PCR) was performed in a 20- μ l reaction volume containing 150 ng of genomic DNA, 1 \times PCR buffer, 0.2 mM dNTPs, 0.2 M concentrations of each primer, 1.5 mM MgCl₂, and 1 U of Taq polymerase (Roche Pharmaceutical Co., Basel, Switzerland). Sequencing was performed using a model 3700XL Genetic Analyzer (Applied Biosystems, Foster City, CA). SNPs with single nucleotide substitutions in heterozygous or homozygous mutations were identified by using the software package PC Gene (Oxford Molecular, Campbell, CA).

Genotyping. To determine the allelic frequency of a novel null variant, *CYP2C8* E274Stop, a pyrosequencing method, was developed. In brief, the 488-base pair PCR fragment, containing 23452G>T change coding for E274Stop, was amplified with a biotinylated forward primer (5'-CTTCT-GCTTTTATTCTGGG-3') and a nonbiotinylated reverse primer (5'-CAAGGTGGAGGATACTGGC-3'). After an initial denaturation at 94°C for 5 min, 35 cycles of the PCR composed of 30 s at 94°C, 30 s at 58°C, and 30 s at 72°C were performed and terminated after a final elongation at 72°C for 5 min. After PCR, the biotinylated PCR product was bound to streptavidin-coated Sepharose beads (GE Healthcare, Little Chalfont, Buckinghamshire, UK) in binding buffer. After a 10-min incubation period vortex at 1400 rpm, the beads were isolated using a vacuum filtration system (Vacuum Prep Tool; Biotage AB, Uppsala, Sweden) and treated with 70% ethanol for 5 s, dena-

uration solution (0.2 M NaOH) for 5 s, and washing buffer for 10 s. The beads were released into a PSQ 96-well plate containing an annealing buffer and a sequencing primer, 5'-TGACTTTTGGTTGCTCT-3'. The sequencing primer/bead mixture was heated at 90°C for 2 min and then cooled to room temperature. The genotyping analysis was performed with automated PSQ96MA, and data were analyzed with automated PSQ96MA SNP software (Biotage AB). In addition, genotyping for *CYP2C8*7* and **8* was performed by TaqMan assays in 400 Koreans according to the manufacturer's instructions (Applied Biosystems protocol PN 4332856D). Genotyping for the presence of *CYP2C9*3*, **13*, and **14* in all phenotyped subjects was performed as described previously (Lee et al., 2005b; Kim et al., 2009).

Linkage Disequilibrium, Haplotype Analysis, and Tag SNP Selection. Allele frequency, Hardy-Weinberg equilibrium, haplotype, and LD were analyzed by SNP Alyze software (version 4.1; Dynacom Co., Ltd., Yokohama, Japan) as described previously (Lee et al., 2009, 2010a). Thirteen SNPs having >5% frequency were applied to select haplotype tagging SNPs. Seven representative tagging SNPs were selected on the basis of the exclusion of redundant SNPs displaying high levels of linkage disequilibrium by the Tagger program, which combines the simplicity of pairwise methods with the potential efficiency of multimarker approaches (<http://www.broad.mit.edu/mpg/tagger/>). A detailed method for tagging SNP selection was described previously (Lee et al., 2010a).

In Vivo Study Design. The study was done in an open, parallel design with single-dose rosiglitazone (Avandia; GlaxoSmithKline, Uxbridge, Middlesex, UK). Rosiglitazone (4 mg with 240 ml of water) was administered orally to all subjects after overnight fasting. The subjects were asked to remain in a seated position for 3 h after taking rosiglitazone, after which they were then allowed to perform their usual daily activities in the clinical trial center; however, strenuous activity and exercise were prohibited. Standardized meals were provided at 4 and 10 h after the administration of rosiglitazone. Blood samples were drawn immediately before and at 0.33, 0.66, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after rosiglitazone administration, as described previously (Niemi et al., 2004; Park et al., 2004; Kim et al., 2005). The blood samples were centrifuged, and the separated plasma samples were stored at -80°C until assayed.

Measurement of Rosiglitazone and Its Metabolites in Human Plasma. Plasma concentrations of rosiglitazone, hydroxyrosiglitazone, and *N*-desmethylrosiglitazone were assayed by liquid chromatography-tandem mass spectrometry (MS/MS). In brief, 20 μ l of the internal standard (1 μ g/ml chlorpropamide) was added to 0.1 ml of plasma, followed by protein precipitation for 5 min with 0.4 ml of acetonitrile. After centrifugation, 20 μ l of the organic phase was injected for analysis. An API 3000 liquid chromatography-MS/MS system (MDS Sciex, Toronto, ON, Canada) equipped with an 1100 series high-performance liquid chromatography system (Agilent, Wilmington, DE) was used. The compound was separated chromatographically on a Luna C18 column (2.0 \times 30 mm, 3 μ m; Phenomenex, Torrance, CA), with a mobile phase consisting of water and acetonitrile (5:5, v/v) containing 0.1% formic acid, delivered at a flow rate of 0.2 ml/min. The MS/MS system was operated with use of an electrospray in positive ionization mode. For rosiglitazone, hydroxyrosiglitazone, *N*-desmethylrosiglitazone, and chlorpropamide, the precursor-to-product ion reactions monitored yielded *m/z* of 358 \rightarrow 135, 374 \rightarrow 151, 344 \rightarrow 121, and 277 \rightarrow 175, respectively. The retention times for rosiglitazone, hydroxyrosiglitazone, *N*-desmethylrosiglitazone, and chlorpropamide were 0.67, 0.79, 0.70, and 1.67 min, respectively. The detection limit for rosiglitazone, hydroxyrosiglitazone, and *N*-desmethylrosiglitazone was 2 ng/ml. The interassay precision for all analyses was less than 14.8%.

Pharmacokinetic Evaluations. The pharmacokinetic parameters of rosiglitazone and its metabolites were estimated by noncompartmental methods with use of WinNonlin Professional (version 5.1; Pharsight, Mountain View, CA). The peak plasma concentration (C_{max}) values and times to reach C_{max} (T_{max}) were estimated directly from the observed plasma concentration versus time curves. The area under the plasma concentration versus time curve from 0 to 24 h (AUC_{0-24}) was calculated by using the linear trapezoidal rule. The AUC from 0 to infinity ($AUC_{0-\infty}$) was calculated as follows: $AUC_{0-\infty} = AUC_{0-24} + C_{24}/k_e$, where C_{24} is the last plasma concentration measured and k_e is the elimination rate constant, which was determined by linear regression analysis of the logarithm-linear part of the plasma concentration versus time curve. The half-life ($t_{1/2}$) of rosiglitazone was calculated as follows: $t_{1/2} = \ln 2/k_e$. The oral clearance (CL/F) of rosiglitazone was calculated as follows: $CL/F = \text{dose}/$

TABLE 1
Allelic frequencies of CYP2C8 genetic variants in a Korean population

n = 50.

SNP ^a	Allele	Location	Effect	Subjects (n)			95% CI	Frequency %
				Wt/Wt	Wt/Mt	Mt/Mt		
-2898T>C		5-UTR		9	27	14	41.21-68.78	55
-2599delA		5-UTR		9	27	14	41.21-68.78	55
-2230G>A		5-UTR		8	27	15	43.27-70.72	57
-2028G>A		5-UTR		43	7	0	0-14.07	7
-411C>T		5-UTR		8	27	15	43.27-70.72	57
-370T>G	*1C	5-UTR		21	23	6	21.77-48.22	35
-271C>A	*1B	5-UTR		42	8	0	0.48-15.51	8
1982A>G		Intron 2		10	29	11	37.14-64.85	51
2041insT		Intron 2		24	26	0	13.84-38.15	26
4424T>A		Intron 3		45	4	1	0-12.58	6
4472G>A	*6	Exon 4	G171S	49	1	0	0-3.75	1
23452G>T ^b	*11	Exon 6	E274Stop	49	1	0	0-3.75	1
26562T>A		Intron 7		10	29	11	37.14-64.85	51
30445C>T ^b		Exon 8	G400G	48	2	0	0-5.88	2
30612G>A		Intron 8		4	28	8	30.24-57.75	44
30636T>C		Intron 8		49	1	0	0-3.75	1
32299C>T		3-UTR		15	27	8	29.27-56.72	43

Wt, wild type; Mt, mutant.

^a Position is indicated in relation to the start codon ATG of the CYP2C8 gene; the A in ATG is +1.

^b New variants found in the present study.

AUC_{0-∞}. The apparent volume of distribution at steady state (Vd_{ss}/F) was calculated as follows: Vd_{ss}/F = dose · AUMC/(AUC_{0-∞})², where AUMC is the area under the first moment of the concentration-time curve.

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 an SNP Alyze (Dynacom Co.). Pharmacokinetic analysis for rosiglitazone and its metabolites was performed using WinNonlin Professional (version 5.1; Pharsight). All data for pharmacokinetic evaluation for rosiglitazone are presented as means \pm S.D. The values for pharmacokinetic parameters between two different genotypes, CYP2C8*1/*1 and CYP2C8*1/*11, were compared using the Wilcoxon rank-sum test. All statistical analyses were performed using SAS software (version 9.1.3; SAS Institute, Cary, NC). *P* values less than 0.05 were considered to be statistically significant.

Results

From the direct sequencing of the CYP2C8 gene, 17 genetic variants were identified from 50 healthy Korean subjects. Three variants were found in exons, six in introns, seven in 5'-flanking regions, and one in the 3'-UTR region. Of the three coding variants, two SNPs (23452G>T and 30445C>T) were newly identified from the Korean population. The 23452G>T change resulted in a premature termination at position 274, whereas 30445C>T was a silent mutation (G400G). The novel variant 23452G>T (E274Stop) was designated as CYP2C8*11 by the Human Cytochrome P450 (CYP) Allele Nomenclature Committee (<http://www.cypalleles.ki.se/>). CYP2C8*6 (G171S), a previously reported variant, was also found in 50 Koreans with 1% frequency. χ^2 tests were used to compare the observed variants with the expected variants in the study population. No deviation from Hardy-Weinberg equilibrium was observed for the SNPs identified (*P* > 0.05). Frequencies of identified variants in the CYP2C8 gene in the present study are summarized in Table 1. Allelic frequency of CYP2C8*11 was evaluated in the different ethnic populations and was found to be at 0.3% in Koreans (*n* = 450), 0.14% in Chinese (*n* = 348), and 0.5% in Vietnamese (*n* = 100), but no subject with this novel variant was identified from 93 African-American and 100 white subjects (Table 2). None of the Korean subjects had CYP2C8*7 and *8 (*n* = 450), although these variants were reported to occur at low frequency (one individual each of 201 subjects) in

Japanese populations (Hichiya et al., 2005) (Table 2). The haplotype map of the CYP2C8 locus was generated using Haploview (version 4.1) from 17 variants detected in the present studies, which resulted in one LD block (Fig. 1A). Distribution of haplotype patterns was analyzed in the CYP2C8 locus using 13 SNPs having minor allele frequencies >5% (Fig. 1B), of which 6 haplotypes were sufficient to represent more than 90% of the haplotypes in the present study. Seven haplotype tagging SNPs were determined by the Tagger program as described previously (Lee et al., 2010a).

The plasma concentrations versus time profiles of rosiglitazone were compared between the CYP2C8 genotype groups after a single oral dose of 4 mg of rosiglitazone. The five subjects with CYP2C8*1/*11 showed higher plasma concentrations of rosiglitazone (Fig. 2A) and a tendency for lower plasma concentrations of hydroxyrosiglitazone (Fig. 2B) than the nine subjects with CYP2C8*1/*1. Plasma concentrations of *N*-desmethylrosiglitazone tended to be higher in CYP2C8*1/*11 than in CYP2C8*1/*1 wild type (Fig. 2C). The AUC and C_{max} of rosiglitazone in five subjects with CYP2C8*1/*11 (2754.04 \pm 404.26 ng · h/ml and 442.47 \pm 60.59 ng/ml) were significantly higher than those in subjects with CYP2C8*1/*1 (1788.83 \pm 391.80 ng · h/ml and 329.61 \pm 49.82 ng/ml; *P* = 0.0150 and *P* = 0.0250, respectively). The AUC of rosiglitazone in five subjects with CYP2C8*1/*11 was 54% higher and the C_{max} was 34% higher than those in subjects with CYP2C8*1/*1. The oral clearance of rosiglitazone in five subjects with CYP2C8*1/*11 (21.86 \pm 2.05 ml per h/kg) was approximately 31% lower than the mean value found in subjects with CYP2C8*1/*1 (31.87 \pm 5.68 ml per h/kg; *P* =

TABLE 2
Allele frequency of CYP2C8*11 in different ethnic populations

Ethnic Group	Subject (n)	No. Alleles	Frequency %	95% CI
Han Chinese	348	1	0.1	0.1 (0.00-0.54)
Vietnamese	100	2	1	0.5 (0.00-1.15)
White	100	0	0	
African American	93	0	0	

95% CI, 95% confidence interval.

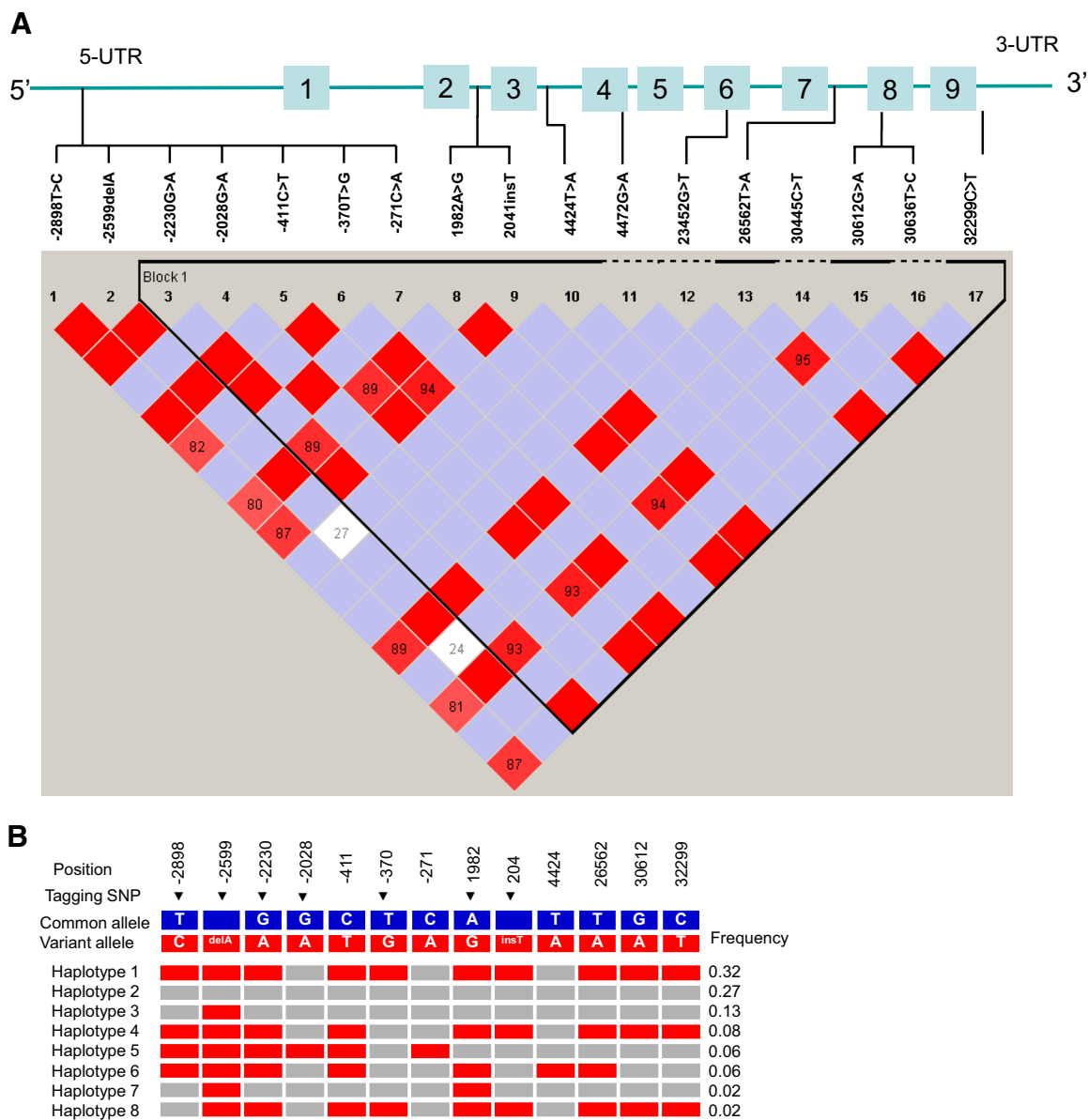


FIG. 1. LD map of *CYP2C8* single nucleotide polymorphisms in a Korean population ($n = 50$). Seventeen *CYP2C8* SNPs identified were included in the LD analysis using the statistics $|D'|$ and r^2 values. A, haploview of *CYP2C8* SNPs along with their locations in *CYP2C8* gene. Red depicts a significant linkage between the pair of SNPs. Numbers inside the square refer to the D' value multiplied by 100. B, *CYP2C8* SNPs and their occurrences in common haplotype structures. Seven tag SNPs are marked by closed triangle symbols. The frequency of each haplotype is shown at the edge.

0.0116). Finally, the plasma AUC_{0-24} ratio of hydroxyrosiglitazone/rosiglitazone in five subjects with *CYP2C8*11/11* was approximately 30% lower than the mean value in those with *CYP2C8*1/1* (Table 3).

Discussion

Genetic polymorphisms in *CYP2C8* may change the function of the enzyme, resulting in altered levels of its endogenous substrate and foreign substrate drugs during systemic exposure in humans. Although *CYP2C8* is an important enzyme in the metabolism of human drugs, no resequencing efforts have yet been made in the people of Korea. Herein, direct DNA sequencing analysis of the *CYP2C8* gene in 50 Koreans revealed a total of 17 variations. To date, more than 15 variants of *CYP2C8* have been reported on the Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee Web site (<http://www.cypalleles.ki.se/cyp2c8.htm>) (Dai et al., 2001; Bahadur et al., 2002; Soyama et al., 2002; Nakajima et al., 2003; Hichiya et al., 2005; Saito et al., 2007).

In the present study, through direct sequencing of the *CYP2C8* gene in 50 Korean subjects, we identified a novel allele, *CYP2C8*11*, in which a mutation causes premature termination at position 274 and encodes a truncated protein; this was expected to cause the loss of *CYP2C8* enzyme activity. From the extensive scoring of *CYP2C8*11* in various ethnic groups, the present study found that this allele was only observed in Koreans (0.3%), Han Chinese (0.14%), and Vietnamese (1%), but not in 93 African Americans and 100 whites. Considering the genetic similarity among Asian populations, the frequency of *CYP2C8*11* should be further determined in other Asian groups, such as Japanese, Thais, and Malaysians.

Because this variant protein lacks the heme-binding domain of the intact *CYP2C8* enzyme, the functional loss was expected. To assess the clinical relevance of this variant, the *in vivo* functionality of *CYP2C8*11* was tested using rosiglitazone, a *CYP2C8* probe drug. Five subjects with the heterozygous *CYP2C8*11* genotype showed

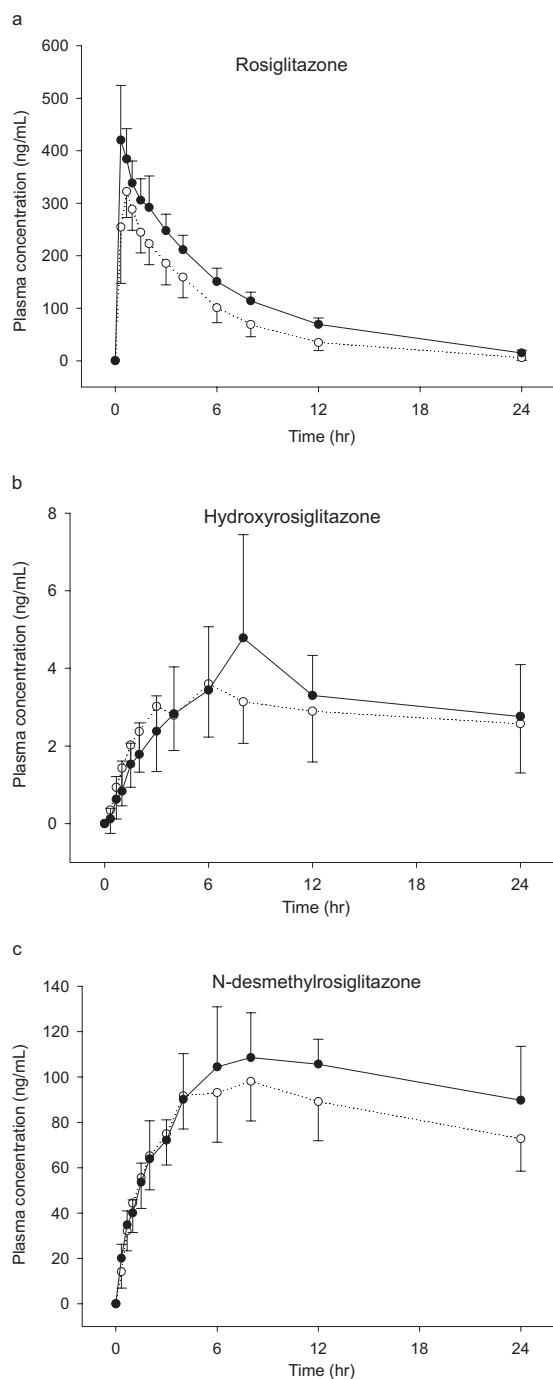


FIG. 2. Plasma concentration-time profiles for rosiglitazone (a), hydroxyrosiglitazone (b), and *N*-desmethylrosiglitazone (c) according to *CYP2C8* genotypes. ○, *CYP2C8*1/*1* ($n = 9$); ●, *CYP2C8*1/*11* ($n = 5$) after a single oral administration of 4 mg of rosiglitazone. Values are described as means \pm S.D.

higher plasma concentrations of rosiglitazone than those subjects with *CYP2C8*1/*1* and demonstrated higher AUC_{0-24} and C_{max} of rosiglitazone as well as a longer terminal half-life than those with the wild type. Oral clearance of rosiglitazone in subjects with heterozygous *CYP2C8*1/*11* decreased by approximately 31% compared with that of the wild-type subjects. The plasma concentrations of the hydroxy metabolite, produced mainly by *CYP2C8*, were observed to be lower in subjects with *CYP2C8*1/*11* than those with the wild type. The AUC_{0-24} ratios of hydroxyrosiglitazone/rosiglitazone in five subjects heterozygous for *CYP2C8*1/*11* were almost half compared with the

TABLE 3

Pharmacokinetic parameters of rosiglitazone in individuals having a novel variant of *CYP2C8*11*

Data are presented as the mean \pm S.D.

	<i>CYP2C8*1/*1</i> ($n = 9$)	<i>CYP2C8*1/*11</i> ($n = 5$)	<i>P</i> Value
RSG			
C_{max} (ng/ml)	329.61 \pm 49.82	442.47 \pm 60.59	0.025*
T_{max} (h)	0.67	0.33	
$t_{1/2}$ (h)	4.24 \pm 0.97	5.41 \pm 0.84	0.085
AUC_{0-24} (ng \cdot h/ml)	1746.91 \pm 354.49	2632.70 \pm 364.63	0.015*
$AUC_{0-\infty}$ (ng \cdot h/ml)	1788.83 \pm 391.80	2754.04 \pm 404.26	0.015*
CL/F (ml per h/kg)	31.87 \pm 5.68	21.86 \pm 2.05	0.012*
Vd_{ss} /F (liters)	12.89 \pm 1.61	10.70 \pm 1.44	0.041*
AUC_{0-24} ratio			
Hydroxy-RSG/RSG	0.04 \pm 0.01	0.03 \pm 0.01	0.252
<i>N</i> -Desmethyl-RSG/RSG	1.14 \pm 0.21	0.86 \pm 0.12	0.032*

RSG, rosiglitazone.

* $P < 0.05$, Wilcoxon rank-sum test for *CYP2C8*1/*1* versus *CYP2C8*1/*11*.

mean value in nine wild-type subjects. These results suggest that the *CYP2C8*11* genotype causes a decreased rosiglitazone hydroxylation in vivo. Because a part of *N*-desmethylrosiglitazone formation is catalyzed by *CYP2C9* (Baldwin et al., 1999), the genotype of *CYP2C9* may influence the disposition of rosiglitazone. In our study, therefore, all phenotyped subjects were genotyped for the presence of *CYP2C9*3*, **13*, and **14*. All of these subjects were identified as possessing a *CYP2C9*1/*1* genotype. It could be difficult to link the role of the *CYP2C8*11* allele to a particular metabolite of rosiglitazone, *N*-desmethylrosiglitazone, because this metabolite could be further metabolized to conjugated products by phase II enzymes, thereby masking the primary effect of *CYP2C8*11* on rosiglitazone metabolism. Genetic polymorphisms of phase II enzymes may define more confounding factors that will further enhance our understanding of the increased metabolite amount of *N*-desmethylrosiglitazone in the subjects with *CYP2C8*1/*11*. Because of the limited number of subjects containing the *CYP2C8*11* allele, the statistical significance of this allele could not be analyzed in this study. However, our results seemed to clearly indicate that individuals having the *CYP2C8*1/11* genotype changed the disposition of the parent compound rosiglitazone compared with the control group (*CYP2C8*1/*1*).

In summary, we resequenced the *CYP2C8* gene for the first time in a Korean population and identified 17 variations. Frequencies, haplotypes, LD structures, and tagging SNPs were determined. The *CYP2C8*11* was first identified in Korean subjects and also observed in Asian ethnic populations including Chinese and Vietnamese but not in African Americans and whites. Although no definitive conclusions regarding the impact of the *CYP2C8*11* on the disposition of rosiglitazone can be made in a quantitative manner at this time because of the limited number of the subjects, clinical influence of the *CYP2C8*11* on the rosiglitazone in the present study indicated a decreased pharmacokinetic parameter for rosiglitazone clearance in the subjects. Thus, the *CYP2C8*11* genotype should be considered in the predictions of drug disposition of *CYP2C8* substrate drugs in Asian populations at least.

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Authorship Contributions

Participated in research design: Yeo, S. S. Lee, Shon, and Rhee.

Conducted experiments: Yeo, S. S. Lee, Bae, Kim, and Shon.

Performed data analysis: Yeo, S.-J. Lee, Bae, Kim, and Shin.

Wrote or contributed to the writing of the manuscript: Yeo, S.-J. Lee, Rhee, and Shin.

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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
