Discovery of a novel allelic variant of *CYP2C8, CYP2C8*11*, in Asian populations and its clinical effect on the rosiglitazone disposition *in vivo*

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P450, cytochrome P450; LC-MS/MS, liquid chromatography–tandem mass spectrometry; C_{max}, peak plasma concentration; AUC_{0-24} , Area under the concentration- time curve from time 0 to 24 hours; AUC_{inf}, Area under the concentration- time curve from time 0 to infinity; CL/F, oral clearance; Vd_{ss}/F, apparent volume of distribution; AUMC, the area under the first moment of the concentration-time curve; k_e is the elimination rate constant

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 seventeen *CYP2C8* variants including a novel coding variant (E274Stop) were identified. The novel CYP2C8 E274Stop variant was assigned as *CYP2C8*11* by the Cytochrome P450 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), Caucasians (n=100), Han-Chinese (n=348), Vietnamese (n=100), and African-American (n=93) was performed by a newly developed pyrosequencing method. The frequency of *CYP2C8*11* was 0.3% in Koreans, 1% in Vietnamese, and 0.14% in Chinese. However, none of the Caucasians and African-Americans contained the *CYP2C8*11* allele. Subjects with the *CYP2C8*1/*11* exhibited a higher plasma concentration-time profiles of rosiglitazone compared to those of 9 control subjects carrying *CYP2C8*1/*1*. The AUC and C_{max} of rosiglitazone in individuals carrying *CYP2C8*1/*11* (n=5) were 54% and 34% higher compared to the mean values observed in the control subjects carrying *CYP2C8*1/*1*, respectively (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 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 (CYP2C8) is one of the major hepatic cytochrome P450s, constituting 3-7% of the total P450 content in the human liver (Rendic and Di Carlo, 1997; Totah and Rettie, 2005). CYP2C8 involves in the metabolism of various endogenous compounds such as retinoic acid and arachidonic acid (AA) (Totah and Rettie, 2005). CYP2C8-mediated AA metabolism has an important physiological role that affects hepatic glycogenolysis, platelet aggregation, vascular smooth muscle tone, and water reabsorption in 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-Jarvinen, 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 the P450 Nomenclature Website (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; Rodriguez-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 the *CYP2C8*11*, a novel null allele, on the disposition of rosiglitazone was evaluated in the individuals carrying *CYP2C8*11* allele.

Materials and Methods

Subjects. Genomic DNAs from 50 Koreans for direct DNA sequencing and an extended set of DNA for genotyping which includes 400 Koreans, 100 Viet Kinh Vietnamese, 348 Han Chinese, 93 African–Americans, and 100 Caucasians 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; Lee et al., 2007; Lee et al., 2010b). The approval and the research protocol for the usage 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 (IRB) 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 fifty normal healthy Korean subjects who participated in the study. For this, genomic DNA was isolated from whole blood cells using the Qiagen DNA Extraction Kit (QIAGEN GmbH, Germany) according to the manufacturer's protocol. Specific primers which were slightly modified in addition to the previous study (Soyama et al., 2002) were used to amplify nine exons of *CYP2C8*, from up to 3 kb of upstream region of 5'-UTR to the 722 bp of 3'-UTR (Table S1, which is provided as Supplemental information). PCR was performed in a 20 µL reaction volume containing 150 ng of genomic DNA, 1X PCR buffer, 0.2 mM dNTPs, 0.2 M of each primer, 1.5 mM MgCl$_2$, and 1 U Taq polymerase (Roche Pharmaceutical Co., Basel, Switzerland). Sequencing was performed using the Applied Biosystems Model 3700XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Identification of SNP with single-nucleotide substitutions in heterozygous or homozygous mutations was performed by using a software package, PC Gene (Oxford Molecular, Campbell, CA, USA).

Genotyping. To determine the allelic frequency of a novel null variant, CYP2C8 E274Stop, a pyrosequencing method was developed. Briefly, the 488 bp PCR fragment, containing 23452G>T change coding for E274Stop, was amplified with a biotinylated forward primer (5′-

CTTCTGCTTTTATTTCTGGG-3') and a non-biotinylated reverse primer (5′-CAAGGTGGAGGATACTGGC -3′). After an initial denaturation at 94℃ for 5 min, 35 cycles of the PCR reaction composed of 30 s at 94℃, 30 s at 58℃, and 30 s at 72℃ were carried out and terminated after a final elongation at 72℃ for 5 min. Following PCR, the biotinylated PCR product was bound to streptavidin-coated Sepharose beads (Amersham Biosciences, Piscataway, NJ) 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, denaturation solution (0.2 mol/L 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'-TGACTTTTGGTTGTCCT-3'. The sequencing primer/bead mixture was heated at 90℃ for 2 min and then cooled to room temperature. The genotyping analysis was performed with an automated PSQ96MA and data were analyzed with automated PSQ96MA SNP software (Biotage AB, Uppsala, Sweden). 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 number 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 (LD), haplotype analysis, and Tag SNP selection. Allele frequency, Hardy–Weinberg equilibrium, haplotype, and LD were analyzed by SNPAlyze software (version 4.1; Dynacom Co., Ltd., Yokohama, Japan) as described previously (Lee et al., 2009; Lee et al., 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/). Detailed method for tagging SNP selection has been 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). Four mg rosiglitazone with 240 mL water were orally administered 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℃ until assayed.

Measurement of rosiglitazone and its metabolites in human plasma.
Plasma concentrations of rosiglitazone, hydroxyrosiglitazone, and N-desmethyl rosiglitazone were assayed by liquid chromatography–tandem mass spectrometry (LC-MS/MS). In brief, 20 µL of the internal standard (chlorpropamide, 1 µg/mL) was added to 0.1 mL of plasma and followed by protein precipitation for 5 min with 0.4 mL of acetonitrile. After centrifugation, the 20 µL of organic phase was injected for analysis. An API 3000 LC-MS/MS system (Sciex Division of MDS, Toronto, Ontario, Canada) equipped with an Agilent 1100 series HPLC system (Agilent, Wilmington, DE, USA) was used. The compound was separated chromatographically on a Luna C18 column (2.0×30 mm, 3 µm; Phenomenex, Torrance, CA, USA), 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 by the usage of an electrospray in positive ionization mode. For rosiglitazone, hydroxyrosiglitazone, N-desmethyrosiglitazone, and chlorpropamide, the precursor-to-product ion reactions monitored yielded mass-to-charge

ratios of 358→135, 374→151, 344→121, and 277→175, respectively. The retention times for rosiglitazone, hydroxyrosiglitazone, N-desmethyrosiglitazone, and chlorpropamide were 0.67, 0.79, 0.70, and 1.67 min, respectively. The detection limit for rosiglitazone, hydroxyrosiglitazone, and N-desmethyrosiglitazone was 2 ng/mL. The inter-assay precision for all analysis was less than 14.8%.

Pharmacokinetic evaluations. The pharmacokinetic parameters of rosiglitazone and its metabolites were estimated by noncompartmental methods by use of WinNonlin Professional software (version 5.1; Pharsight, Mountain View, CA). The peak plasma concentration (C_{max}) values and reaching times 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 hours (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: $t_{1/2} = \ln2/k_e$. The oral clearance (CL/F) of rosiglitazone was calculated as follows: CL/F = Dose/$AUC_{0-\infty}$. The apparent volume of distribution at steady state (Vd_{ss}/F)

was calculated as follows: $Vd_{ss}/F = Dose \cdot AUMC/(AUC_{0-\infty})^2$, 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 SNP

analyzer (Dynacom Co., Yokohama, Japan). Pharmacokinetics analysis

for rosiglitazone and its metabolites were performed using WinNonlin

Professional software (version 5.1; Pharsight, Mountain View, CA). All data

for pharmacokinetic evaluation for rosiglitazone are presented as mean ±

S.D. The values of pharmacokinetic parameter between two different

genotypes, *CYP2C8*1/*1* and *CYP2C8*1/*11*, were compared using

Wilcoxon's Rank sum test. All statistical analyses were performed using

SAS software (version 9.1.3, SAS Institute, NC, USA). P value less than

0.05 was considered to be statistically significant.

Results

From the direct sequencing of the *CYP2C8* gene, 16 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 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 CYP nomenclature committee (http://www.cypalleles.ki.se/). The *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 *CYP2C8 gene* in the present study were summarized in Table 1. Allelic frequency of *CYP2C8*11* was evaluated in the different ethnic populations, which was observed 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 Caucasian subjects (Table 2). None of the Korean subjects contained *CYP2C8*7* and *8* (n=450), although they were reported to occur at low frequency (one individual each out 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 over 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 4mg rosiglitazone. The five subjects with *CYP2C8*1/*11* showed higher plasma concentrations of rosiglitazone (Fig. 2A) and tendency of lower plasma concentrations of hydroxyrosiglitazone (Fig. 2B) compared to those of 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 5 subjects with *CYP2C8*1/*11* (2754.04 ± 404.26 ng·h/mL, and 442.47 ± 60.59 ng/mL) were significantly higher than those in subjects with *CYP2C8*1/*1* (1788.83 ± 391.80 ng·h/mL and 329.61 ± 49.82 ng/mL; *P*=0.0150 and *P*=0.0250, respectively). The AUC of rosiglitazone in 5 subjects with *CYP2C8*1/*11* was 54% higher, and the C_{max} was 34% higher than those in subjects with *CYP2C8*1/*1*, respectively. The oral clearance of rosiglitazone in 5 subjects with *CYP2C8*1/*11* (21.86 ± 2.05

mL/h/kg) was about 31% lower than the mean value found in *CYP2C8*1/*1* subjects (31.87 ± 5.68 mL/h/kg; *P*=0.0116). Finally, the plasma AUC_{0-24} ratio of hydroxyrosiglitazone / rosiglitazone in 5 subjects with *CYP2C8*1/*11* was about 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 fifteen variants of *CYP2C8* have been reported on the Human Cytochrome P450 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, thus this was expected to cause the loss of CYP2C8 enzyme activity. From the extensive scoring of the *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 Caucasians. 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 Malaysian.

As 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 higher plasma concentrations of rosiglitazone than those subjects with *CYP2C8*1/*1,* respectively and demonstrated the higher AUC_{0-24} and C_{max} of rosiglitazone as well as a longer terminal half-life than those with wild-type. Oral clearance of rosiglitazone in subjects with heterozygous *CYP2C8*1/*11* decreased by about 31% compared to that of the wild-type subjects. The plasma concentrations of the hydroxy-metabolite, produced mainly by *CYP2C8*, were observed to be lower in *CYP2C8*1/*11* subjects than those with wild type. The AUC_{0-24} ratios of hydroxyrosiglitazone/rosiglitazone in 5 subjects heterozygous for *CYP2C8*1/*11* were almost half compared to the mean value in 9 wild type subjects. These results suggest that the *CYP2C8*11* genotype causes a decreased rosiglitazone hydroxylation *in vivo*. Since a part of N-desmethylrosiglitazone formation is catalyzed by CYP2C9 (Baldwin et al.), 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 these subjects were identified to

possess 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, since 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 increase 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 *CYP2C8*11* allele, statistical significance of this allele could not be analyzed in this study. However, our results appeared to be clear that individuals having *CYP2C8*1/11* genotype changed the disposition of the parent compound rosiglitazone compared to 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 Caucasians. 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 due to 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, Rhee, Shin, Lee (Lee, SS), Shon

Conducted experiments: Yeo, Lee (Lee, SS), Bae, Shon, Kim

Performed data analysis: Yeo, Shin, Lee (Lee, SJ), Bae, Kim

Wrote or contributed to the writing of the manuscript: Yeo, Lee (Lee, SJ),

Shin, Rhee

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Footnotes

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Legends for figures

Fig. 1. Linkage disequilibrium (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.

Fig. 2. Plasma concentration-time profiles for rosiglitazone (a), hydroxyrosiglitazone (b), and N-desmethylrosiglitazone (c) according to *CYP2C8* genotypes. Open circles, *CYP2C8*1/*1* (n=9) and closed circles, *CYP2C8*1/*11*(n=5) after a single oral administration of 4 mg rosiglitazone. Values are described as mean ± S.D.

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

The reference sequence used was GenBank accession No. NC_000010.9.

[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

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

Ethnic group	Subject (n)	No. of allele	Frequency (%)	95% CI
Korean	447	3	0.3	0.3 (0.00-0.81)
Han-Chinese	348	1	0.1	0.1 (0.00-0.54)
Vietnamese	100	2	1	0.5 (0.00-1.15)
Caucasian	100	0	0	-
African-American	93	0	0	-

95%CI, 95% confidence interval.

Table 3 Pharmacokinetic parameters of rosiglitazone in individuals having a novel variant of *CYP2C8*11*

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

C_{max}, peak plasma concentration; T_{max}, time to maximum plasma concentration; $t_{1/2}$, half-life;

AUC_t, area under the concentration time curve from zero to last observation; CL/F, total oral clearance;

Vd_{ss}/F, apparent volume of distribution. Data are presented as mean ± S.D.

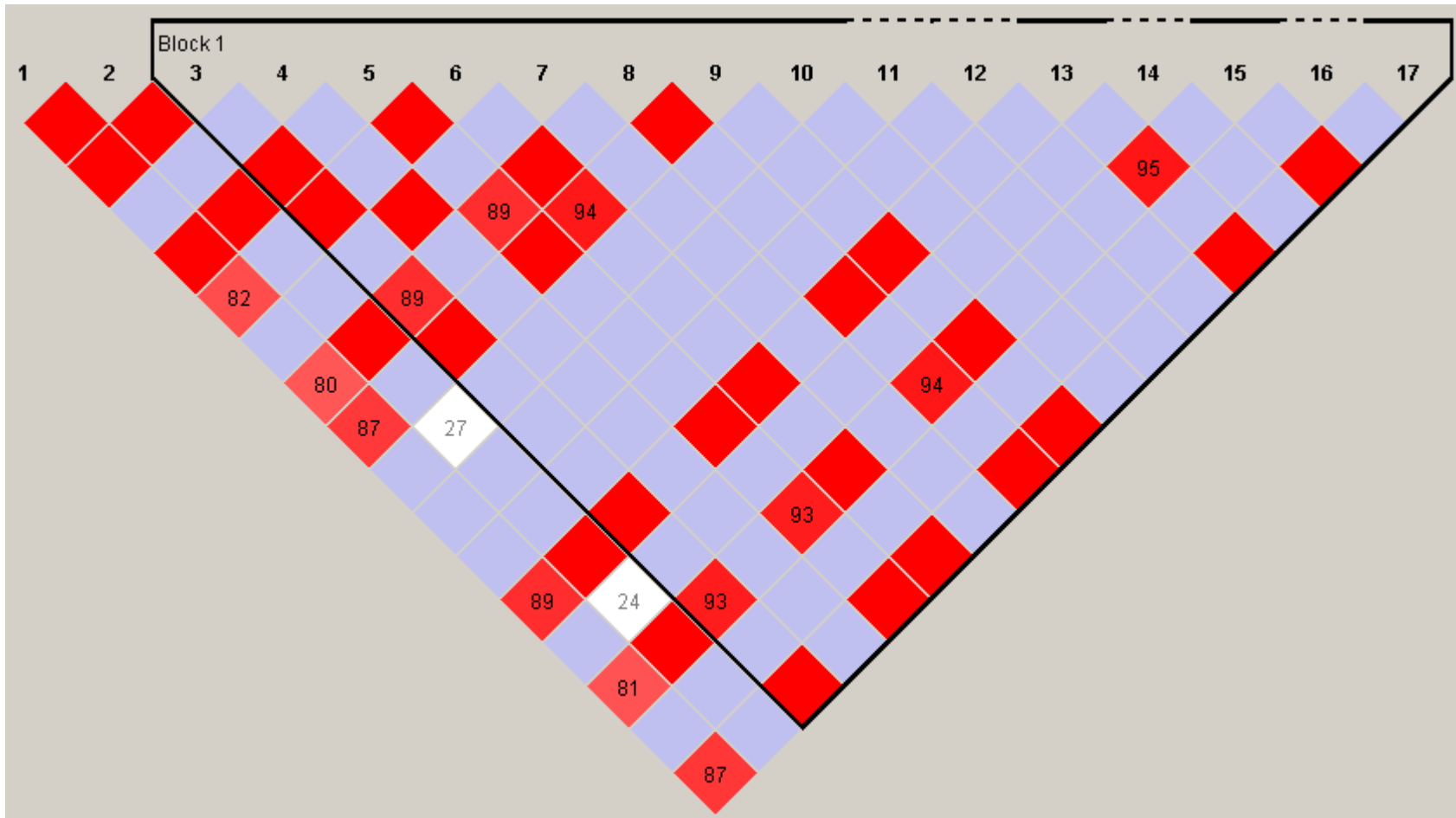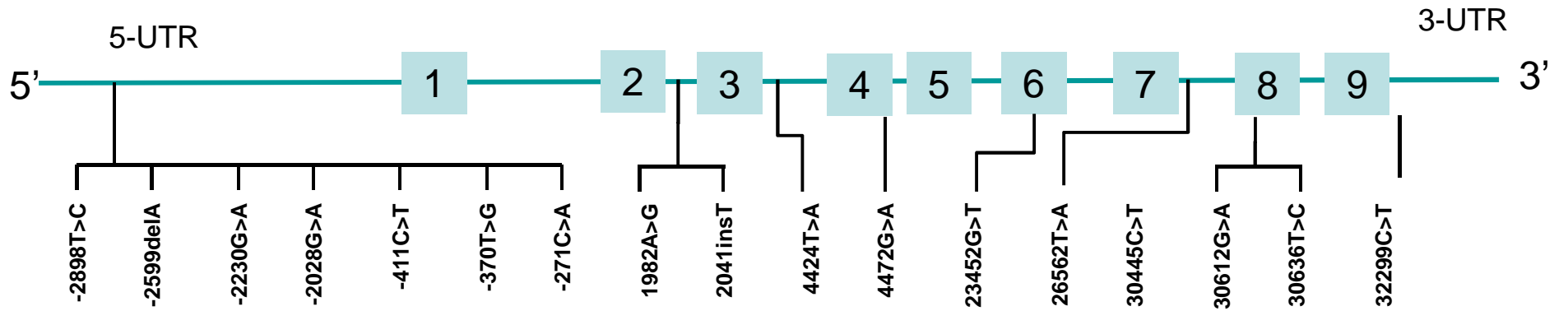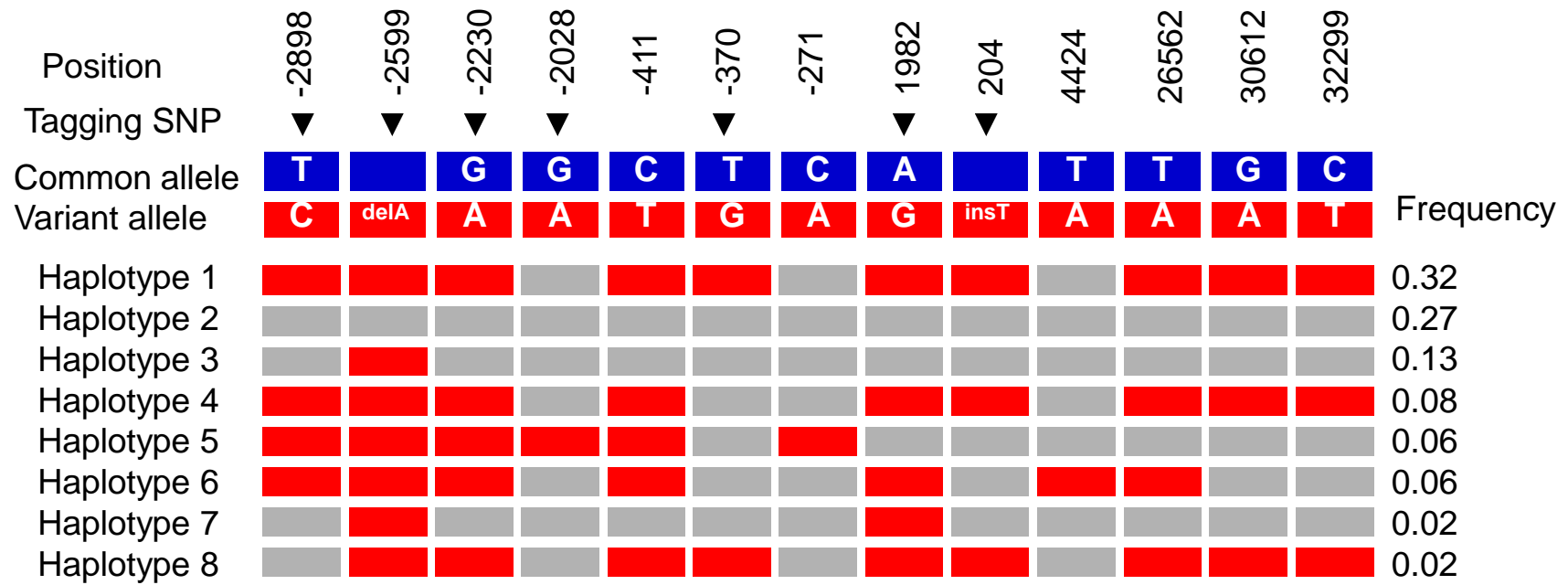* P<0.05, Wilcoxon's Rank sum test for *CYP2C8*1/*1* versus *CYP2C8*1/*11*.
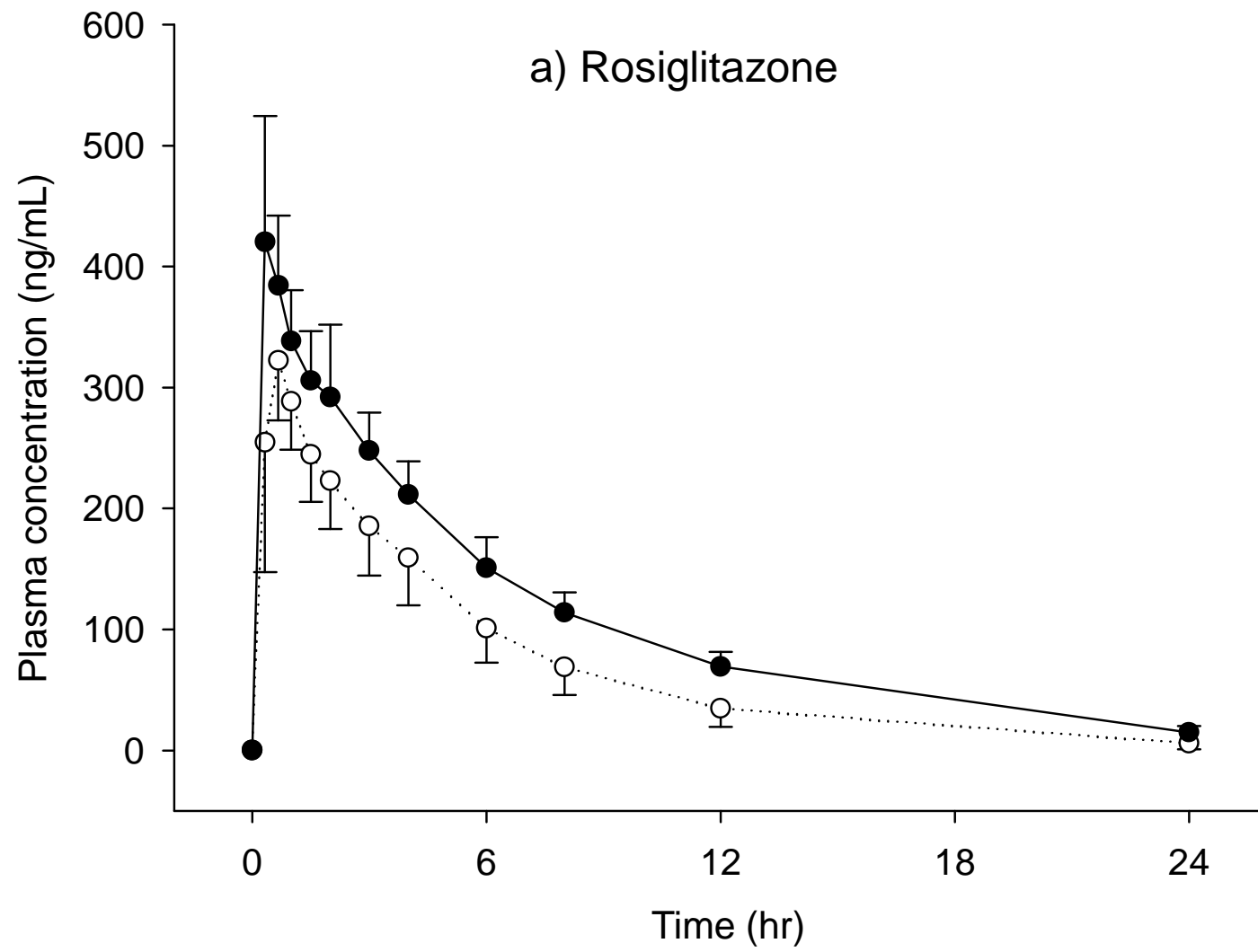
Fig.1A

Fig.1B

Fig.2A

a) Rosiglitazone

Fig.2B

b) Hydroxyrosiglitazone

Fig.2C

c) N-desmethylrosiglitazone