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RESEARCH ARTICLE

CYP2D6 allele frequencies in Korean population, comparison with East Asian, Caucasian and African populations, and the comparison of metabolic activity of *CYP2D6* genotypes

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Abstract Cytochrome P450 (CYP) 2D6 is present in less than about 2% of all CYP enzymes in the liver, but it is involved in the metabolism of about 25% of currently used drugs. CYP2D6 is the most polymorphic among the CYP enzymes. We determined alleles and genotypes of *CYP2D6* in 3417 Koreans, compared the frequencies of *CYP2D6* alleles with other populations, and observed the differences in pharmacokinetics of metoprolol, a prototype CYP2D6 substrate, depending on *CYP2D6* genotype. A total of 3417 unrelated healthy subjects were recruited for the genotyping of *CYP2D6* gene. Among them, 42 subjects with different *CYP2D6* genotypes were enrolled in the pharmacokinetic study of metoprolol. The functional allele **1* and **2* were present in frequencies of 34.6 and 11.8%, respectively. In decreased functional alleles, **10* was the most frequent with 46.2% and **41* allele was present in 1.4%. The nonfunctional alleles **5* and **14* were present at 4.5 and 0.5% frequency, respectively. The **X* × N allele was present at a frequency of 1.0%. *CYP2D6*1/*1*, **1/*2* and **2/*2* genotypes with normal enzyme activity were present in 12.1%, 8.6% and 1.4% of the subjects, respectively. *CYP2D6*5/*5*, **5/*14*, and **14/*14* genotypes classified as poor metabolizer were only present in 4, 2, and 1 subjects, respectively. Mutant genotypes with frequencies of more than 1% were *CYP2D6*1/*10* (32.0%), **10/*10* (22.3%), **2/*10* (11.7%), **5/*10* (3.7%), **1/*5* (2.5%), and **10/*41* (1.2%). The relative clearance of metoprolol in *CYP2D6*1/*10*, **1/*5*, **10/*10*, **5/*10*, and **5/*5* genotypes were 69%, 57%, 24%, 14% and 9% of *CYP2D6*wt/*wt* genotype, respectively. These results will be very useful in establishing a strategy for precision medicine related to the genetic polymorphism of *CYP2D6*.

Keywords CYP2D6 · Allele · Genotype · Metoprolol · Ethnic difference · Clearance

Introduction

Cytochrome P450 (CYP) enzymes are classified into 10 classes according to the domain architecture and cellular location of CYP enzymes and redox proteins. According to amino acid sequence homology of CYP enzymes, there are 57 genes in CYP superfamily (Cook et al. 2016). The enzymatic activity of CYP enzymes can vary widely depending on the gene variation, and currently the variant alleles of 31 CYP enzymes are summarized in the Pharmacogene Variation Consortium (http://www.pharmvar.org/genes).

CYP2D6 is present in less than about 2% of all CYP450 enzymes in the liver, but it is involved in the metabolism of about 25% of currently used drugs (Ingelman-Sundberg

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2005; Ingelman-Sundberg et al. 2007). To date, more than 113 different human *CYP2D6* variant and subvariant alleles (*CYP2D6*1B* to **113*) have been identified (http://www.pharmvar.org/gene/CYP2D6). However, at present only nine alleles constitute more than 95% of *CYP2D6* diplotypes. Among them, **1* and **2* are fully functional alleles, **3*, **4*, **5*, and **6* are nonfunctional alleles and **10*, **17*, and **41* are reduced functional alleles. These alleles show significant differences in distribution between race and ethnicity, and the *CYP2D6*2*, **5*, and **10* alleles, along with the *CYP2D6* gene duplication, are the most clinically important and widely distributed polymorphisms in East Asians. However, the distribution of these alleles in East Asians including Koreans, Chinese, and Japanese is somewhat different from one another (Roh et al. 2001; Hosono et al. 2009; Man et al. 2010; Park et al. 2011; Ota et al. 2015; Goh et al. 2017; Zhou et al. 2017), and the information on the enzymatic activity of each *CYP2D6* genotype is insufficient. Accurate information on the distribution of alleles and genotypes of polymorphic drug metabolizing enzyme genes in a population and the enzymatic activity of each genotype is important to establish a strategy for precision medicine. Therefore, in this study, we determined alleles and genotypes of *CYP2D6* in 3417 Koreans, compared the frequencies of *CYP2D6* alleles with other populations, and observed the differences in pharmacokinetics of metoprolol, a prototype CYP2D6 substrate, depending on *CYP2D6* genotype.

Materials and methods

Subjects

A total of 3417 unrelated healthy subjects were recruited for the genotyping of *CYP2D6* gene. After genotyping, 42 of the recruited subjects with different *CYP2D6* genotypes were enrolled in the pharmacokinetic study of metoprolol.

All study procedures were carried out in accordance with the recommendations of the Declaration of Helsinki on biomedical research involving human subjects, and the Institutional Review Board of Sungkyunkwan University, Suwon, Republic of Korea approved the research protocol. Written informed consent was obtained from all subjects.

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes for genotyping of the *CYP2D6* with a commercial blood kit (Wizard® Genomic DNA Purification Kit, Promega, Madison, WI, USA).

Analyses of the *CYP2D6*2*, **4*, **5*, **10*, and **X* × *N* alleles were performed using polymerase chain reaction restriction fragment length polymorphisms (PCR-RFLP) and long PCR analyses, as described previously (Byeon et al. 2015). The samples carrying the 2850C>T mutation were further genotyped for *CYP2D6*14* and **41*.

Genotyping of *CYP2D6*14* was performed by PCR-RFLP method (Wang et al. 1999) with minor modifications. For the PCR amplification of the *CYP2D6*14* allele the forward (5′-GTG GAT GGT GGG GCT AAT GCC TT-3′) and the reverse primer (5′-CAG AGA CTC CTC GGT CTC TCG CT-3′) were used. PCR cycling conditions were as follows: pre-denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s, followed by a final extension at 72 °C for 10 min. The PCR amplification was carried out using the T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA). After amplification, the PCR product was digested with 0.25 µl of the specific restriction enzyme *Msp* I (New England Biolabs, Ipswich, MA, USA) and incubated at 37 °C for 1 h. Digested PCR products were analyzed by gel electrophoresis on 2% agarose gels and stained with ethidium bromide, then directly visualized under the UV light.

Genotyping of *CYP2D6*41* was performed using direct sequencing. Therefore, the genomic DNA was amplified in T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA) using the forward primer 5′-GTA CTT CGA TGT CAC GGG ATG-3′ and the reverse primer 5′-TGA CAG GTG CAG AAT TGG AG-3′. After initial denaturation at 94 °C for 5 min, denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min and extension at 72 °C for 1 min were repeated 40 times, and then terminated with a final extension at 72 °C for 10 min. The amplicons were subsequently purified using LaboPass PCR Purification Kit (CosmoGenetech, Seoul, Korea) and afterwards sequenced on ABI 3730xl DNA Analyzer using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA).

Pharmacokinetic study

Forty-two subjects with different *CYP2D6* genotypes were enrolled. All subjects were in good health as determined by their medical histories, physical examinations, vital signs (blood pressure, pulse rate and body temperature) and routine laboratory tests (blood chemistry, hematology and urine analysis). For the study, subjects were not permitted to ingest any medication, alcohol or caffeine-containing beverages for 10 days prior to the study and during the study. All subjects were given identical meals and then fasted from 10 h before to 4 h after drug administration. Standard meals were served for lunch and dinner 4 and 10 h after drug administration, respectively. A single oral dose of 100 mg metoprolol tablet (Betaloc Tab.,

AstraZeneca Korea, Seoul, Korea) was administered with 240 ml of water to each subject. Blood samples (7 ml) were collected before and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 24 h after dosing, and plasma samples from all blood samples were immediately separated for the determination of the concentrations of metoprolol and stored at − 70 °C until the completion of the analysis.

Determination of metoprolol

Metoprolol in the plasma samples was determined by high-performance liquid chromatography (HPLC) system, which consisted of a Waters 515 HPLC pump, a Waters 717 plus autosampler and a Waters 474 scanning fluorescence detector (Ex = 280, Em = 300 nm) (Waters Corporation, Milford, MA, USA). The separation was performed on a Sunfire C18 column (5 μm, 4.6 i.d. × 250 mm, Waters Corporation, Milford, MA, USA) using 10 mM KH_2PO_4 (pH 3.0) containing 12% acetonitrile and 15% methanol at a flow rate of 1 ml/min. Fifty microliter of 5 μg/ml atenolol (as an internal standard) and 100 μl of 1 M NaOH were added to 0.5 ml of plasma sample. After brief vortex mixing, 6 ml of dichloromethane was added. The mixture was mixed for 30 s and centrifuged at 3500 rpm for 10 min. The organic layer was transferred to a 10 ml tube and evaporated to dryness under nitrogen stream in a 50 °C bath. The residue was dissolved in 300 μl of mobile phase and 70 μl aliquot was injected into the HPLC system.

Statistical analysis

Data were compiled according to the genotype and allele frequencies. The frequencies of each allele are reported with 95% confidence intervals. Hardy–Weinberg equilibrium was evaluated by comparing the genotype frequencies with the expected values using a contingency table χ^2 test. Statistical significance was determined by the χ^2 test. The pharmacokinetic data are expressed as mean ± SD Statistical analysis for AUC_{inf}, C_{max}, and CL/F ratio among genotype groups were performed using one-way analysis of variance (ANOVA) with Bonferroni post hoc test or Kruskal–Wallis one-way ANOVA with Dunn's post hoc test after normality test and equal variance test. All statistical analyses were carried out using the statistical program SigmaPlot 12.5® (Systat Software Inc., San Jose, CA, USA). P value less than 0.05 was considered statistically significant.

Results

In this study, the frequency of *CYP2D6* allele was measured in 3417 Korean subjects who were not related to each other. Then, these results were compared to the previous reports, and the frequency of major alleles of *CYP2D6* measured in Koreans was compared with the results of previous studies that measured the frequencies of major *CYP2D6* alleles in three East Asian populations (Korean, Japanese, and Chinese), Caucasians, and Africans.

CYP2D6 allele frequencies in Korean population

In 3417 Koreans, the functional allele **1* and **2* were present in frequencies of 34.6 and 11.8%, respectively. In the case of two decreased functional alleles, the *CYP2D6*10* was the most frequent allele at 46.2% and the *CYP2D6*41* allele was present in 1.4% of the subjects. The nonfunctional allele **5* and **14* were present at 4.5 and 0.5% frequency, respectively. The **X* × N allele was present at a frequency of 1.0% (Table 1). The results of this study were similar to those of previous studies measured in Koreans (Lee et al. 2006, 2009; Man et al. 2010; Park et al. 2011, 2012).

Comparison of *CYP2D6* allele frequencies among East Asian populations

Comparing the frequencies of major *CYP2D6* alleles between Korean and Japanese populations, *CYP2D6*1* frequency was lower in Koreans (34.6%) than in Japanese (43.6%) ($P < 0.001$), but *CYP2D6*10* frequency was higher in Koreans (46.2%) than in Japanese (37.5%) ($P < 0.001$). *CYP2D6*5* frequency was slightly lower in Koreans (4.5%) than in Japanese (5.5%) ($P < 0.05$). The frequencies of other alleles were not significantly different.

Comparing the frequencies of major *CYP2D6* alleles between Korean and Chinese populations, *CYP2D6*1* frequency was higher in Korean population (34.6% vs. 26.4%, $P < 0.001$), but the frequencies of *CYP2D6*10* (46.2% vs. 52.5%, $P < 0.001$), **14* (0.5% vs. 1.2%, $P < 0.001$), and **41* (1.4% vs. 3.5%, $P < 0.001$) were lower in Korean population. The frequency of *CYP2D6*X* × *N* alleles was slightly lower in Koreans (1.0% vs. 2.3%, $P < 0.001$). The frequencies of other alleles were not significantly different.

The frequencies of *CYP2D6*1* and **10* alleles showed remarkable differences among the three East Asian populations (Table 1).

Table 1 *CYP2D6* allele frequency in Korean population and its comparison to different races and ethnic populations

Populations	n	*CYP2D6* allele frequency (%)									References
		**1*	**2*	**4*	**5*	**10*	**14*	**17*	**41*	**X × N*	
Korean	6834	34.2	12.4	ND	3.8	47.3	0.5	ND	1.1	0.7	Present data
	1516	35.0	10.1	ND	5.6	45.6	0.3	ND	2.2	1.5	Lee et al. (2009)
	800	35.0	10.1	0.3	6.1	45.0	0.5	0.0	1.9	1.1	Lee et al. (2006)
	400	33.9	12.1	0.8	6.2	44.4	0.5	0.0	2.1	ND	Man et al. (2010)
	1530	36.1	11.6	0.1	5.6	42.7	0.4	ND	1.7	1.6	Park et al. (2011)
Sum of Koreans	11,080	34.6 (33.7, 35.5)	11.8 (11.2, 12.4)	0.3 (0.1, 0.5)	4.5 (4.1, 4.9)	46.2 (45.3, 47.1)	0.5 (0.4, 0.6)	0.0	1.4 (1.2, 1.6)	1.0 (0.8, 1.2)	
Japanese	196	43.4	9.2	0.5	6.1	40.8	ND	ND	ND	ND	Tateishi et al. (1999)
	324	40.0	13.0	ND	6.2	38.6	2.2	ND	ND	ND	Kubota et al. (2000)
	412	43.2	12.3	0.2	4.5	38.1	0.7	ND	ND	1.0	Nishida et al. (2000)
	324	39.8	12.7	ND	6.2	38.6	2.2	ND	ND	0.6	Ishiguro et al. (2003)
	910	44.4	12.9	0.4	5.7	35.4	0.3	ND	ND	0.9	Hosono et al. (2009)
	1000	45.3	10.0	0.1	5.1	37.8	0.1	0.0	1.6	ND	Man et al. (2010)
Sum of Japanese	3166	43.6 (41.8, 45.4)***	11.7 (10.6, 12.8)	0.3 (0.1, 0.5)	5.5 (4.7, 6.3)*	37.5 (35.8, 39.2)***	0.7 (0.4, 1.0)	0.0	1.6 (0.8, 2.4)	0.9 (0.4, 1.4)	
Chinese	800	25.9	11.1	0.1	4.7	52.2	1.3	0.0	3.3	1.4	Qin et al. (2008)
	200	21.5	14.0	1.0	7.0	51.0	1.5	ND	4.0	0.5	Zhou et al. (2009)
	796	28.5	11.0	1.1	6.1	48.4	1.1	0.0	3.8	ND	Man et al. (2010)
	458	22.7	13.8	0.0	5.0	58.5	ND	ND	ND	ND	Dong et al. (2015)
	402	29.9	4.7	0.2	2.0	55.0	ND	ND	3.2	5.0	Goh et al. (2017)
Sum of Chinese	2656	26.4 (24.7, 28.1)***	10.8 (9.6, 12.0)	0.5 (0.2, 0.8)	4.9 (4.1, 5.7)	52.5 (50.6, 54.4)***	1.2 (0.7, 1.7)***	0.0	3.5 (2.7, 4.3)***	2.3 (1.5, 3.1)***	
Caucasian	450	45.6	22.4	19.1	2.9	0.9	ND	0.0	6.9	2.2	Rasmussen et al. (2006)
	694	41.8	22.9	19.7	3.3	2.2	ND	0.4	8.1	1.6	Gaedigk et al. (2008)
	7552	43.2	20.9	21.0	2.3	1.1	ND	0.3	9.8	1.5	de Leon et al. (2009)
	908	38.2	25.1	18.8	3.3	2.8	0	0.1	11.7	ND	Man et al. (2010)
	1376	43.3	20.9	20.0	2.8	1.5	ND	0.2	11.2	ND	McGrane and Loveland (2016)
	1714	45.9	32.3	10.3	2.2	1.9	ND	0.1	3.1	4.2	Pietarinen et al. (2016)

Table 1 continued

Populations	n	*CYP2D6* allele frequency (%)									References
		**1*	**2*	**4*	**5*	**10*	**14*	**17*	**41*	**X* × *N*	
Sum of Caucasians	12,694	43.3 (42.4, 44.2)***	22.9 (22.2, 23.6)***	19.1 (18.4, 19.8)***	2.5 (2.2, 2.8)***	1.4 (1.2, 1.6)***	0.0*	0.2 (0.1, 0.3)	9.0 (8.5, 9.5)***	2.0 (1.7, 2.3)***	
African	386	43.7	10.9	7.0	6.0	3.1	ND	27.7	ND	1.6	Griese et al. (1999)
	308	35.1	26.9	7.8	6.2	7.5	ND	14.6	ND	1.9	Wan et al. (2001)
	228	47.0	13.0	2.0	4.0	ND	ND	34.0	ND	ND	Dandara et al. (2001)
	212	27.9	40.1	1.8	6.1	3.8	ND	17.0	ND	3.3	Wennerholm et al. (2001)
	444	37.8	11.0	8.6	5.6	4.3	ND	15.1	15.1	2.5	Cai et al. (2006)
	502	31.3	28.7	5.4	6.6	3.6	0.0	21.3	ND	3.1	Gaedigk et al. (2002)
	544	52.8	14.0	0.2	3.9	2.9	ND	19.1	1.8	5.3	Gaedigk et al. (2008)
	500	32.5	30.9	6.1	1.0	5.7	0.0	20.7	3.1	ND	Man et al. (2010)
	150	30.7	28.7	8.0	8.7	2.7	0.0	17.3	4.0	ND	Yee et al. (2013)
Sum of Africans	3274	38.6 (36.9, 40.3)*	21.6 (20.2, 23.0)***	5.1 (4.3, 5.9)***	4.9 (4.1, 5.7)	4.2 (3.5, 4.9)***	0.0**	20.5 (19.1, 21.9)***	6.0 (4.8, 7.2)***	3.1 (2.4, 3.8)***	

Values in parentheses represent the 95% confidence intervals. Difference between frequency data was calculated using Chi square test

n number of alleles, *ND* not determined

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared with sum of Koreans

Comparison of *CYP2D6* allele frequencies with Caucasian and African populations

In Caucasian population, the frequency of *CYP2D6*1* allele was very similar to Japanese populations, but it was higher than Korean and Chinese populations ($P < 0.001$). The frequency of *CYP2D6*2* allele was about twice higher in Caucasian population than in Koreans, Chinese and Japanese populations ($P < 0.001$). The frequency of *CYP2D6*4* allele was very low in East Asian populations (0.3–0.5%), but in Caucasians, there was a very high frequency (19.1%, $P < 0.001$). The *CYP2D6*5* allele was present in 4.5–5.5% of East Asian populations, but was present in 2.5% of Caucasian population ($P < 0.001$). *CYP2D6*10* allele was present at a very high frequency of 37.5–52.5% in East Asian populations but very low (1.4%) in Caucasian population ($P < 0.001$). *CYP2D6*14* and *17 existed at a very low frequency in East Asian and Caucasian populations. *CYP2D6*41* was present in 1.4–3.5% of East Asian populations, but in 9.0% of Caucasian population ($P < 0.001$) (Table 1).

Compared to the African population, the frequency of *CYP2D6*1* allele was higher in Japanese population, but lower in Korean and Chinese populations. The frequency of *CYP2D6*2* allele was about twice higher in African population than in Koreans, Chinese and Japanese populations ($P < 0.001$). The frequency of *CYP2D6*4* allele was higher in African population (5.1%) than in East Asian populations (0.3–0.5%, $P < 0.001$). The *CYP2D6*5* allele frequency was similar between East Asian populations and African population. *CYP2D6*10* allele was present at a very high frequency of 37.5–52.5% in East Asian populations but very low (4.2%) in African population ($P < 0.001$). *CYP2D6*17* existed at a very low frequency in East Asian populations, but at higher frequency (20.5%) in African population ($P < 0.001$). *CYP2D6*41* was present in 1.4–3.5% of East Asian populations, but in 6.0% of African population ($P < 0.001$) (Table 1).

Frequencies of *CYP2D6* genotypes in Korean population

In 3417 Koreans, *CYP2D6*1/*1*, *1/*2* and *2/*2 genotypes with normal enzyme activity were present in 12.1% (CI 11.0–13.2%), 8.6% (CI 7.6–9.6%) and 1.4% (CI 1.0–1.8%), respectively. Number of subjects with *CYP2D6*5/*5*, *5/*14*, and *14/*14* genotypes, who are classified as poor metabolizer (PM), were only 4, 2, and 1, respectively. Therefore, the frequency of CYP2D6 PM in Korean population was about 0.2%. Mutant genotypes with frequencies of more than 1% were *CYP2D6*1/*10* (32.0%, CI 30.4–33.6%), *10/*10* (22.3%, CI 20.9–23.7%), *2/*10* (11.7%, CI 10.6–12.8%), *5/*10* (3.7%, CI 3.1–4.3%), *1/*5* (2.5%, CI 2.0–3.0%), and *10/*41* (1.2%, CI 0.8–1.6%). Any other genotypes were present in frequencies of less than 1% (Table 2).

Comparison of metabolic activity of *CYP2D6* genotypes

The difference in the enzymatic activity of the *CYP2D6* genotypes was observed by comparing the pharmacokinetic parameters of metoprolol, a prototype substrate drug of CYP2D6. Relative values of each genotype were calculated based on the average value of pharmacokinetic parameters of *CYP2D6*wt/*wt* (*wt = *1* or *2) genotype. The relative clearance of *CYP2D6*1/*10*, *1/*5*, *10/*10*, *5/*10*, and *5/*5* genotypes were 69%, 57%, 24%, 14% and 9% of *CYP2D6*wt/*wt* genotype, respectively. The relative C_{max} of *CYP2D6*1/*10*, *1/*5*, *10/*10*, *5/*10*, and *5/*5* genotypes were 122%, 152%, 221%, 349% and 341% of *CYP2D6*wt/*wt* genotype, respectively. The relative AUC_{inf} of *CYP2D6*1/*10*, *1/*5*, *10/*10*, *5/*10*, and *5/*5* genotypes were 139%, 163%, 388%, 651% and 1062%

Table 2 *CYP2D6* genotype frequency in Korean population

Genotype	n	%	95% CI	Expected frequency
*1/*1	413	12.1	11.0, 13.2	11.7
*1/*2	294	8.6	7.6, 9.6	8.5
*2/*2	49	1.4	1.0, 1.8	1.5
*1/*10	1092	32.0	30.4, 33.6	32.4
*2/*10	400	11.7	10.6, 12.8	11.7
*1/*5	87	2.5	2.0, 3.0	2.6
*2/*5	31	0.9	0.6, 1.2	0.9
*1/*14	8	0.2	0.0, 0.4	0.4
*2/*14	5	0.1	0.0, 0.2	0.1
*1/*41	26	0.8	0.5, 1.1	0.7
*2/*41	5	0.1	0.0, 0.2	0.3
*10/*10	761	22.3	20.9, 23.7	22.4
*10/*14	18	0.5	0.3, 0.7	0.5
*10/*41	41	1.2	0.8, 1.6	1.0
*5/*10	128	3.7	3.1, 4.3	3.6
*14/*14	1	0.0	0.0, 0.1	0.0
*5/*14	2	0.1	0.0, 0.2	0.0
*5/*5	4	0.1	0.0, 0.2	0.1
*1 × N/*1	4	0.1	0.0, 0.2	0.4
*1 × N/*10	22	0.6	0.3, 0.9	0.5
*1 × N/*2	9	0.3	0.1, 0.5	0.1
*2 × N/*10	12	0.4	0.2, 0.6	0.1
*2 × N/*2	4	0.1	0.0, 0.2	0.1
*2 × N/*5	1	0.0	0.0, 0.1	0.0

CI confidence interval

of *CYP2D6*wt/*wt* genotype, respectively (Table 3, Fig. 1).

Discussion

When medication is administered, some patients do not respond to the drug, while some patients have serious adverse drug reactions. The proportion of these patients ranges from 40% to 70% depending on the drug (Eichelbaum et al. 2006; Lauschke and Ingelman-Sundberg 2016). 15–30% of these individual drug reactions are known to be caused by polymorphisms of genes encoding proteins that affect drug response such as drug metabolizing enzymes and drug transporters (Eichelbaum et al. 2006). In particular, the genetic polymorphisms of CYP enzymes have the greatest effect on individual differences in drug response, and among CYP enzymes, CYP2D6 is the most polymorphic enzyme (https://www.pharmvar.org/gene/CYP2D6).

This study reported the allele and genotype frequencies of *CYP2D6* measured in the largest scale in Korean population. *CYP2D6*10* was found to be the most frequent allele (47.3%), followed by *CYP2D6*1* (34.2%), **2* (12.4%), **5* (3.8%), and **41* (1.1%). Allele frequencies were slightly different from the previous reports (Lee et al. 2006, 2009; Man et al. 2010; Park et al. 2011), but these differences were not meaningful.

Among East Asian populations (Koreans, Japanese and Chinese), the frequencies of *CYP2D6*1* and **10* alleles were significantly different among ethnic groups. Because the enzyme activity of the *CYP2D6*10* allele is greatly reduced, these differences can lead to differences in the drug action of CYP2D6 substrate drugs among the three East Asian populations.

Recently, an article provided a global distribution map of alleles with clinical importance by integrating whole-genome and exome sequencing data 56,945 unrelated individuals of five major populations (Zhou et al. 2017). In this article, the frequencies of *CYP2D6*1* (13.6%), **2* (14%), **5* (6.5%), **10* (58.7%), **14* (1.6%), and **41* (3.0%) in East Asians were reported. However, the frequency of *CYP2D6*1* seemed to be abnormally low, whereas the frequency of *CYP2D6*10* seemed to be too high compared to the other studies including this study (Table 1).

Alleles with a significant frequency difference between East Asians and Caucasians were *CYP2D6*2* (10.8–11.8% vs. 22.9%), **4* (0.3–0.5% vs. 19.1%), and **10* (37.5–52.5% vs. 1.4%). The functional alleles (*CYP2D6*1* and **2*) were in higher frequency in Caucasians than in East Asians. In addition, a nonfunctional allele *CYP2D6*4* was also higher in frequency in Caucasians compared with East Asians. However, a decreased functional allele *CYP2D6*10* was lower in frequency in Caucasians compared with East Asians. The frequency difference of the *CYP2D6*10* allele between the two races was most characteristic.

The most distinctive difference between East Asians and Africans was the frequency difference of *CYP2D6*10* (37.5–52.5% vs. 4.2%) and **17* alleles (0% vs. 20.5%). *CYP2D6*4* allele also was in lower frequency in East Asians than in Africans (0.3–0.5% vs. 5.1%).

In Korean population, the frequency of *CYP2D6* genotypes with normal enzyme activity was 22.1%, and only 7 of the 3417 subjects had CYP2D6 PM genotypes. Unlike Caucasians (8.45%) or Africans (2.57%) with a significant frequency of CYP2D6 PM (Gaedigk et al. 2017), CYP2D6 PM was very rare in Koreans, with frequency of about 0.2%. Most of the other subjects had genotypes with one nonfunctional allele, or one or two reduced functional alleles.

Generally, CYP2D6 enzymatic activity can be expressed in terms of four phenotypes: PM, intermediate metabolizer (IM), extensive metabolizer (EM), and ultra-rapid metabolizer. Genotype analysis has become the method of choice to predict a person's metabolic status. However, there can be substantial differences in the number of genetic variants interrogated as well as differences in test interpretation. Furthermore, there is no standardized process of how a

Table 3 Comparison of clearance, AUC_{inf}, and C_{max} ratio of metoprolol in subjects with different *CYP2D6* genotypes

PK parameters	*CYP2D6* genotype (n)					
	**wt/*wt* (13)	**wt/*10* (9)	**wt/*5* (4)	**10/*10* (9)	**5/*10* (5)	**5/*5* (2)
CL/F ratio	1.00 ± 0.32	0.69 ± 0.17	0.57 ± 0.08	0.24 ± 0.05*	0.14 ± 0.01*	0.09 ± 0.01***
C_{max} ratio	1.00 ± 0.34	1.22 ± 0.24	1.52 ± 0.51	2.21 ± 0.63***	3.49 ± 0.66***	3.41 ± 1.14***
AUC_{inf} ratio	1.00 ± 0.32	1.39 ± 0.29	1.63 ± 0.25	3.88 ± 0.77*	6.51 ± 0.69*	10.62 ± 1.31***

Individual ratio values were calculated as individual parameter value/mean of parameter values in *CYP2D6*wt/*wt* (**wt* = **1* or **2*) genotype. Each value represents the mean ± SD. Statistical analysis for AUC_{inf}, C_{max}, and CL/F ratio among genotype groups was performed using one-way analysis of variance (ANOVA) with Bonferroni post hoc test or Kruskal–Wallis one-way ANOVA with Dunn's post hoc test after normality test and equal variance test. *P < 0.05, **P < 0.01, ***P < 0.001

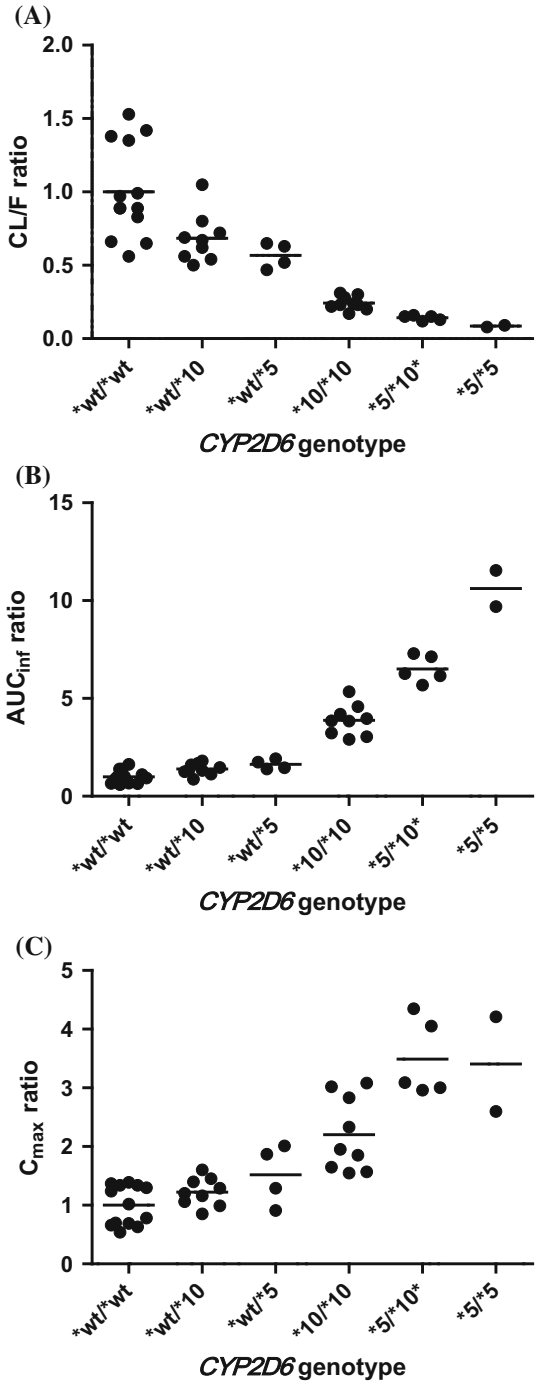

Fig. 1 Individual clearance (**a**), AUC_{inf} (**b**), and C_{max} (**c**) ratio of metoprolol in subjects with different *CYP2D6* genotypes. Individual ratio values were calculated as individual parameter value/mean of parameter values in *CYP2D6*wt/*wt* (**wt* = **1* or **2*) genotype. Each horizontal line indicates the mean of individual ratios

CYP2D6 genotype result is translated into a phenotype assignment (Hicks et al. 2014; LLerena et al. 2014; Fricke-Galindo et al. 2016).

The *CYP2D6*10* allele is associated with reduced substrate affinity (Johansson et al. 1994) and many in vivo studies have shown that the metabolic capacity of homozygous *CYP2D6*10* is between the capacities of EMs and PMs. Thus, the *CYP2D6*10/*10* genotype is commonly categorized as an IM phenotype (Wang et al. 1993; Dahl et al. 1995; Owen et al. 2009). Variations in the activity of the CYP2D6 enzyme results from various combinations of functional alleles (activity = 1), nonfunctional alleles (activity = 0), and reduced functional alleles (activity = 0.5). Steimer et al. (2004) assigned a phenotypic classification based on pure allele activity, i.e., the semiquantitative gene dose. The classification of a semiquantitative gene dose is based on the sum of two allele activities, leading to six different phenotypes, i.e., semiquantitative gene doses of 0, 0.5, 1, 1.5, 2, and > 2. The clinical relevance of distinguishing allele activities (semiquantitative gene doses of 0.5, 1.0, and 1.5) remains open to question. The semiquantitative gene dose classification of 1.5 (1.0 + 0.5) for CYP2D6 is defined as EMs. Thus, subjects with the *CYP2D6*1/*10* genotype are usually defined as EMs. However, Steimer et al. (2004) and ter Laak et al. (2010) suggested that this group should be defined as an IM in accordance with their clinical findings.

In this study, the *CYP2D6*wt/*10* subjects demonstrated 1.22-fold higher C_{max}, 1.39-fold higher AUC_{inf} values and 31% lower CL/F than did *CYP2D6*wt/*wt* subjects. Although the differences are small, they support the hypothesis of ter Laak et al. (2010). Previous studies have also demonstrated the pharmacokinetic characteristics of diverse CYP2D6 substrates in heterozygous CYP2D6 EMs. In one study of healthy Korean volunteers, *CYP2D6*1/*10* subjects showed a 1.9-fold higher C_{max} and a 2.2-fold higher AUC_{inf} of metoprolol compared to those of *CYP2D6*1/*1* subjects after a single oral dose of 100 mg of metoprolol tartrate (Jin et al. 2008). Another study showed that, after administration of a single 100 mg oral dose of tramadol hydrochloride, healthy Chinese subjects with the *CYP2D6*2/*10* genotype had significantly higher $t_{1/2}$ and AUC values and lower oral clearance of tramadol and showed the opposite effect on the pharmacokinetics of its metabolite compared to the levels in subjects with the *CYP2D6*1/*1* genotype (Li et al. 2010). Therefore, *CYP2D6*wt/*10* was considered separately from *CYP2D6*wt/*wt*, depending on the substrate.

In this study, the *CYP2D6*10/*10* subjects demonstrated 2.21-fold higher C_{max} and 3.88-fold higher AUC_{inf} values. The CL/F in the *CYP2D6*10/*10* subjects was only about a quarter of that in *CYP2D6*wt/*wt*. This shows that the enzyme activity of *CYP2D6*10/*10* genotype is much lower than the mean value in IM subjects. The frequency of *CYP2D6*5/*5* genotype was very low in Korean population, so only two subjects with this genotype participated in the pharmacokinetic study. In the *CYP2D6*5/*5* genotype, CL/F was only 9% of that in *CYP2D6*wt/*wt* genotype,

and AUC_{inf} was 10.62 times higher than that in *CYP2D6*wt/*wt* genotype.

In conclusion, in 3417 Korean population, the functional allele **1* and **2* were present in frequencies of 34.6 and 11.8%, respectively. In decreased functional alleles, **10* was the most frequent with 46.2% and **41* allele was present in 1.4%. The nonfunctional alleles **5* and **14* were present at 4.5% and 0.5% frequency, respectively. The **X* × N allele was present at a frequency of 1.0%. *CYP2D6*1/*1*, **1/*2* and **2/*2* genotypes with normal enzyme activity were present in 12.1, 8.6 and 1.4% of the subjects, respectively. *CYP2D6*5/*5*, **5/*14*, and **14/*14* genotypes classified as poor metabolizer were only present in 4, 2, and 1 subjects, respectively. Mutant genotypes with frequencies of more than 1% were *CYP2D6*1/*10* (32.0%), **10/*10* (22.3%), **2/*10* (11.7%), **5/*10* (3.7%), **1/*5* (2.5%), and **10/*41* (1.2%). The relative clearance of metoprolol in *CYP2D6*1/*10*, **1/*5*, **10/*10*, **5/*10*, and **5/*5* genotypes were 69%, 57%, 24%, 14% and 9% of *CYP2D6*wt/*wt* genotype, respectively. These results will be very useful in establishing a strategy for precision medicine related to the genetic polymorphism of CYP2D6.

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Compliance with ethical standards

Conflict of interest The authors declare no potential conflict of interest with respect to the authorship and/or publication of this article.

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