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Allele and genotype frequencies of *CYP2C9* in a Korean population

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Aims
To determine the frequencies of the variant alleles and the genotypes of *CYP2C9* in a Korean population.

Methods
Three hundred and fifty-eight healthy Korean subjects were studied. *CYP2C9* alleles were detected by polymerase chain reaction-restriction fragment length polymorphism assays and direct sequencing assays.

Results
The allele frequencies were 0.934 for *CYP2C9*1*, 0.060 for *CYP2C9*3* and 0.006 for *CYP2C9*13*. The *CYP2C9*2, *4, *5* and **11* alleles were not detected. The frequencies of the *CYP2C9*1/*1*, **1/*3* and **1/*13* genotypes were 0.869, 0.120 and 0.011, respectively.

Conclusion
The frequency of the *CYP2C9*3* allele in the Korean population studied was significantly higher than reported elsewhere, and a novel allele, *CYP2C9*13*, was found at a frequency of 0.006 (95% confidence interval 0, 0.012). Only three genotypes of *CYP2C9, CYP2C9*1/*1, *1/*3* and **1/*13* were observed in this Korean population.

Introduction

Genetic polymorphisms of drug metabolizing enzymes are one of the major determinants of interindividual variability in drug response. The cytochrome P450 enzyme CYP2C9 is primarily responsible for the oxidative metabolism of drugs with a narrow therapeutic index such as warfarin, tolbutamide and phenytoin, other commonly used drugs such as glibenclamide, glimepiride, glipizide, losartan, irbesartan and torasemide, as well as many anti-inflammatory drugs [1, 2]. Twelve allelic variants of the *CYP2C9* gene have been reported, and recently a novel variant, *CYP2C9*13*, was identified in a Chinese population [3, 4]. Three alleles, *CYP2C9*1*, **2* and **3*, are present in most ethnic populations and decreased *CYP2C9* function has been reported in individuals with the *CYP2C9*2* and **3* mutant alleles [2, 5]. In addition, several other alleles may cause impaired metabolism [6], which may give rise to drug toxicity. Therefore, the doses of these drugs may need to be adjusted according to *CYP2C9* genotype. Because the frequencies of these variant alleles vary according to ethnic group, the aim of the present study was to determine the frequencies of the variant alleles and genotypes of the *CYP2C9* gene in the Korean population.

Methods

Subjects

Three hundred and fifty-eight unrelated healthy Korean subjects (290 males and 68 females; mean age 24.7 ± 2.3 years, range 20-32 years) were enrolled. Written informed consent was obtained from all subjects, and the institutional ethics committee approved the protocol.

Genotyping

For each subject, a 10-ml sample of venous blood was collected in an EDTA tube. The genomic DNA was isolated from the peripheral blood leucocytes. Genotyping for the *CYP2C9*2* (Arg144Cys, C430T) and *CYP2C9*13* (Leu90Pro, T269C) polymorphisms was performed using a previously published and validated polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay [4, 7]. Genotyping of the *CYP2C9*3* (Ile359Leu, A1075C), *CYP2C9*4* (Ile359Thr, T1076C), *CYP2C9*5* (Asp360Glu, C1080G) and *CYP2C9*11* (Arg335Thr, C1003T) alleles was performed using direct sequencing. PCR amplification of the *CYP2C9*2* and **13* alleles was carried out using the forward and reverse primer (5′-TACAAATACA ATGAAAATATCATG-3′ and 5′-CTAACAACCAGA CTCATAATG-3′) in the genomic DNA [4, 7]. Amplification was carried using a Mastercycler Gradient (Effendorf, Hamburg, Germany). After amplification, the DNA was digested with specific restriction enzymes such as *Ava*II for *CYP2C9*2* and *PspG*I for *CYP2C9*13*. The digested PCR products were separated by electrophoresis on 3% agarose gels and stained with ethidium bromide. The variant genotypes identified using PCR-RFLP were confirmed by sequence analysis.

Sequencing of CYP2C9 exon

The genomic DNA was amplified using the intron-specific primers for *CYP2C9* exon 7 (containing *CYP2C9*3*, **4*, **5* and **11* allele) and *CYP2C9* exon2-exon3 (containing *CYP2C9*2* and **13* allele) [7, 8]. The specific primers coding exon 7 of the *CYP2C9* gene were 5′-CTGAATTGCTACAACAAATGTG-3′ as the forward primer and 5′-GATACTATGAATTTGGGAC TTC-3′ as the reverse primer [8]. The PCR products were purified and sequenced on an ABI 377 automatic sequencer using a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems Inc., Foster City, CA, USA).

Statistical analysis

The data were compiled according to the genotype and allele frequencies, which were compared using a χ^2 test.

Table 1
Allele (**A**) and genotype (**B**) frequencies for *CYP2C9* in a Korean population. Expected frequencies were calculated using the Hardy-Weinberg equation

A

Alleles	*N*	Frequency	95% CI
*CYP2C9*1*	669	0.934	0.916, 0.952
*CYP2C9*2*	0	–	–
*CYP2C9*3*	43	0.060	0.043, 0.077
*CYP2C9*4*	0	–	–
*CYP2C9*5*	0	–	–
*CYP2C9*11*	0	–	–
*CYP2C9*13*	4	0.006	0.000, 0.012

B

Genotype	Number of subjects	Observed frequency	95% CI	Expected frequency
*CYP2C9*1/*1*	311	0.869	0.833, 0.904	0.872
*CYP2C9*1/*3*	43	0.120	0.086, 0.154	0.112
CYP2C9*1/*13	4	0.011	0.000, 0.022	0.011
*CYP2C9*3/*3*	0	0	–	0.004
*CYP2C9*3/*13*	0	0	–	0
*CYP2C9*13/*13*	0	0	–	0

Table 2
Allele frequencies for *CYP2C9* in different Asian populations

Populations	n	CYP2C9 allele frequency *1	*2	*3	*13	Reference
Korean	358	0.934 (0.916, 0.952)	0	0.060 (0.043, 0.077)	0.006	Present study
Korean	574	0.989 (0.983, 0.995)	0	0.011 (0.005, 0.017)	–	[9]
Sum of Korean	**932**	**0.968 (0.960, 0.976)**	**0**	**0.030 (0.022, 0.038)**	**0.002 (0, 0.004)**	
Chinese	115	0.983	0	0.017	–	[10]
Chinese	102	0.951	0	0.049	–	[11]
Chinese	394	0.963	0.001	0.036	–	[12]
Chinese	98	0.974	0	0.026	–	[7]
Chinese	133	0.959	0	0.041	–	[13]
Sum of Chinese	**842**	**0.965 (0.956, 0.974)**	**0.001 (–0.001, 0.003)**	**0.034 (0.025, 0.043)**		
Japanese	140	0.982	0	0.018	–	[14]
Japanese	123	0.955	0	0.045	–	[13]
Japanese	218	0.979	0	0.021	–	[15]
Japanese	86	0.983	0	0.017	–	[16]
Japanese	134	0.989	0	0.011	–	[17]
Sum of Japanese	**701**	**0.978 (0.970, 0.986)**	**0**	**0.022 (0.014, 0.030)**		

Values in parentheses represent the 95% confidence intervals] n*, number of subjects. Differences between gene frequencies were calculated using the* χ^2 *test.*
P < 0.001 vs. the previous data in Korean [9] (95% CI on the difference 0.023, 0.075).
P < 0.01, vs. sum of Chinese (95% CI on the difference –0.001, 0.053).
P < 0.001, vs. sum of Japanese (95% CI on the difference 0.011, 0.065).

A *P*-value < 0.05 was considered significant. The Hardy-Weinberg equilibrium was determined by comparing the genotype frequencies with the expected values using a contingency table χ^2 test.

Results

The frequencies of the allelic variants and genotypes of the *CYP2C9* gene in a Korean population are summarized in Table 1. The observed genotype frequency distribution did not show a significant deviation from the Hardy-Weinberg equilibrium. The *CYP2C9*1* allele was the most common [0.934, 95% confidence interval (CI) 0.916, 0.952]. The most frequently identified mutant allele was *CYP2C9*3* (0.060, 95% CI 0.043, 0.077). The frequency of the *CYP2C9*1/*3* genotype in this study was more than five times higher than that reported elsewhere [9] (Table 2). The frequency of the *CYP2C9*13* allele in our population was 0.006 (95% CI 0, 0.012).

There were 311 subjects (0.869, 95% CI 0.833, 0.904) with the *CYP2C9*1/*1* genotype, 43 (0.120, 95% CI 0.086, 0.154) with the *CYP2C9*1/*3* genotype and four (0.011, 95% CI 0.000, 0.022) with the *CYP2C9*1/*13* genotype (Table 1). None of the subjects had the *CYP2C9*2*, **4*, **5* or **11* allele, or was homozygous for the *CYP2C9*3* or **13* allele. The genotype results obtained by PCR-RFLP were confirmed by sequencing (data not shown).

Discussion

The allelic variants, *CYP2C9*2* and *CYP2C9*3*, code for enzymes with approximately 10–40% and 5–15% of the activity of the wild-type form *CYP2C9*1*, respectively [18]. The *CYP2C9*2* allele is the most common mutant allele among caucasians with a frequency of approximately 0.125 [18]. In contrast, *CYP2C9*2* has not been detected in Asian populations [18]. The *CYP2C9*2*, *CYP2C9*4*, *CYP2C9*5* and *CYP2C9*11* alleles were also not detected in the 358 Koreans studied in the present work.

The functional importance of *CYP2C9*3* is greater

than that of *CYP2C9*2* because the former allele appears to confer the largest decrease in enzyme activity *in vitro*, whereas the *CYP2C9*2* allele produces an intermediate fall in activity compared with *CYP2C9*1* [19]. Caucasians exhibit a wide range (0.033-0.162) in *CYP2C9*2* allele frequency, whereas the *CYP2C9*3* allele has a much lower frequency [19]. The frequency of the *CYP2C9*3* allele in the present Korean population was 0.060, which was above five times higher than that reported elsewhere (0.011) [9]. Such a discrepancy can be seen in other studies of Japanese and Chinese populations (Table 2). Although a frequency of 0.060 for the *CYP2C9*3* is significantly higher ($P < 0.01$ and $P < 0.001$, respectively) than those in the Japanese (0.022) and Chinese populations (0.034) (Table 2), the mean value (0.030) from the two Korean studies published to date [present, 9] was similar to that in the Chinese population. In the present study, all the subjects with the *CYP2C9*3* allele had the heterozygous genotype, *CYP2C9*1/*3*.

The *CYP2C9*4* allele has been detected only in a Japanese epileptic patient [20]. The *CYP2C9*5* allele has been found in African-Americans with a low frequency of 0.017 [2] and in Africans [21]. The *CYP2C9*11* allele has been detected in caucasians and Africans [22]. However, very little is known about the functional effect of these alleles. As expected, no *CYP2C9*4*, **5* and **11* alleles were identified in the present study.

A novel allele, *CYP2C9*13*, was recently identified in a Chinese population, and a large decrease in enzyme activity was observed in those with the *CYP2C9*3/*13* genotype [4]. In the present study, four out of 358 Korean subjects possessed the *CYP2C9*1/*13* genotype, but no other genotypes, including the *CYP2C9*13* allele, were found. Currently, there is no information available on the metabolic activity of the enzyme coded by *CYP2C9*11*13*.

In summary, the frequency of the *CYP2C9*3* allele in the Korean population was significantly higher than reported elsewhere, and a novel allele, *CYP2C9*13*, was found at a frequency of 0.0056 (95% CI 0, 0.011) in the Korean population. Only three genotypes of *CYP2C9*, *CYP2C9*1/*1*, **1/*3* and **1/*13*, were detected.

Competing interests: None to declare.

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