Frequency of cytochrome P450 2C9 mutant alleles in a Korean population

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Aims To determine the frequencies of CYP2C9 variants in the Korean population and compare them with the frequencies in other ethnic populations.

Methods Genotyping of *CYP2C9*2* and *CYP2C9*3* allelic variants was carried out in 574 Korean subjects by PCR and restriction fragment length pattern analysis.

Results Thirteen of 574 subjects (2.3%) were heterozygous for *CYP2C9*3* ($Ile_{359}Leu$), but no subjects with a *CYP2C9*2* allele or homozygous for *CYP2C9*3* were identified. The allele frequency of *CYP2C9*3* in Korean subjects (0.0113, 95% CI 0.0066–0.0193) was similar to that of other East Asian populations, but was considerably lower than that of Caucasian populations.

Conclusions *CYP2C9*3* seems to be an allelic variant related to the functional polymorphism of CYP2C9, but this variant is rarely seen among Koreans compared with Caucasians. Routine genotyping of the *CYP2C9*2* allele is considered to be unnecessary in Korean and East Asians, because this allele appears to be extremely rare or absent in these populations.

Keywords: CYP2C9, genotype, Korean

Introduction

Cytochrome P450 2C9 (CYP2C9) is a genetically polymorphic enzyme that is involved in the metabolism of phenytoin, S-warfarin, tolbutamide, losartan, torasemide, and many nonsteroidal anti-inflammatory drugs, including diclofenac, ibuprofen, and flurbiprofen [1]. Several different CYP2C9 cDNA sequences have been reported. Two amino acid variants, $Arg_{144}Cys$, which result from a $C_{430}\rightarrow T$ nucleotide substitution in exon 3 (*CYP2C9*2*), and $Ile_{359}Leu$, produced by an $A_{1075}\rightarrow C$ substitution in exon 7 (*CYP2C9*3*) have reduced catalytic activity compared with the wild type *CYP2C9*1* [2, 3]. The catalytic activity of the *CYP2C9*3* encoded enzyme is much lower than those of *CYP2C9*1* and *CYP2C9*2* [4]. The unbound oral clearance of S-warfarin *in vivo* was reduced by 66% and 90% among subjects heterozygous and homozygous for the *CYP2C9*3* allele, respectively, compared to homozygous *CYP2C9*1* subjects [5].

The frequency of *CYP2C9* allelic variants has been reported to differ among Caucasian, African, and Asian populations. The allele frequencies of *CY2C9*2* and *CYP2C9*3* tend to be greater in Caucasian populations than in African-American and Asian populations [3, 6–8, 10]. In contrast to Caucasians and African-Americans, the *CYP2C9*2* allelic variant was not found in East Asians including Chinese and Japanese [8, 10]. However, no information on the genotype of *CYP2C9* allelic variants is available for Korean populations. Since subjects with *CYP2C9* allelic variants are rare, especially in East Asian populations, study population size can affect the accuracy of estimations of the frequency of allelic variants. This is the first report on the frequency of *CYP2C9* allelic variants in a Korean population, and is the largest *CYP2C9* genotyping study published to date.

Methods

Subjects

A group of 574 unrelated Korean subjects (388 males and 186 females) were recruited for genotyping after obtaining

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Received 17 March 2000, accepted 7 November 2000.

informed written consent. Whole blood (7 ml) was drawn from 480 healthy subjects and 94 outpatients who took phenytoin for epilepsy. The aliquot of whole blood was stored at −70°C until used for *CYP2C9* genotyping. This study was approved by the Institutional Review Board of Inje University Pusan Paik Hospital, Pusan, Korea.

Identification of subjects with a mutant CYP2C9 allele

The CYP2C9 genotype was determined by PCR-RFLP as described by Nasu *et al.* [8] with slight modification. DNA was prepared from 600 μl of whole blood using a standard phenol/chloroform extraction method [9] and PCR was performed in 20 μl volumes with 1.5 mM $MgCl_2$, 1 × PCR buffer (TaKaRa Shuzo Co., Ltd, Shiga, Japan), 200 μM dNTP (TaKaRa Shuzo Co., Ltd), 10 pmol of the forward and reverse primers for *CYP2C9*2* or *CYP2C9*3,* 1 U of *Taq* DNA polymerase (Amersham Pharmacia Biotech, Uppsala, Sweden), and 60 ng of genomic DNA. The sequences of the forward and reverse primers used were 5′-GTA TTT TGG CCT GAA ACC CAT A-3′ and 5′-GGC CTT GGT TTT TCT CAA CTC-3′ and for the *CYP2C9*2* genotype, and 5′-TGC ACG AGG TCC AGA GGT AC-3′ and 5′-ACA AAC TTA CCT TGG GAA TGA GA-3′ for the *CYP2C9*3* genotype. PCR amplification to detect *CYP2C9*2* was performed using a Gene Amp PCR System 2400 (Perkin Elmer, Foster City, CA, USA) with an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 64°C for 45 s, and extension at 72°C for 1 min. A final 5-min extension at 72°C followed. To genotype *CYP2C9*3,* 35 PCR cycles were used and the annealing temperature was 60°C. Aliquots of each PCR product (15 μl) were digested with restriction enzymes (*Ava*II for *CYP2C9*2*, *Kpn*I for *CYP2C9*3*, Boehringer Mannheim GmbH, Mannheim, Germany) at 37°C for 1.5 h. The DNA fragments were separated by 2.5% agarose gel electrophoresis and were detected by ethidium bromide staining. Alleles for $Tyr_{358}Cys$ and $Gly_{417}Asp$ were also determined using polymerase chain reaction with restriction fragment length polymorphism as described by Nasu *et al.* [8]. Because of absence of these alleles in 310 Korean subjects screened, no further screening of these alleles were continued to other subjects.

Statistical analysis

Data were compiled according to the genotype and allele frequencies were estimated from the observed numbers of each specific allele. The population frequency of each allele is given together with the 95% confidence intervals.

Table 1 Frequency distribution of the Ile359Leu variants in CYP2C9 observed in the 574 Korean subjects.

	*CYP2C9*1/*1 (Ile359Ile)*	*CYP2C9*1/*3 (Ile359Leu)*	*CYP2C9*3/*3 (Leu359Leu)*	*Total*
Observed number	561 (97.7 %)	13 (2.3 %)	0	574
Expected number★	561.1 (97.75 %)	12.83 (2.24 %)	0.07 (0.01 %)	574

★Calculated by the Hardy-Weinberg law. ★One subject homozygous for CYP2C9★3 is expected in every 7,700 Korean subjects.

Results

No mutant *CYP2C9*2* allele was found in any of the 574 Korean subjects genotyped in this study. In addition, no subject homozygous for *CYP2C9*3* was found. Only 13 subjects (2.3%) heterozygous for *CYP2C9*3* (Ile_{359}leu) were identified (Table 1). Using the Hardy–Weinberg law, the frequency of the homozygous *CYP2C9*3* genotype (q^2) was estimated to be 0.013% (about 1 of 7700 subjects) in the Korean population. The allele frequency of the *CYP2C9*3* in the 574 Korean subjects was 0.0113 (95% CI 0.0066–0.0193, Table 2).

Discussion

In this study, no subjects with a mutant *CYP2C9*2* allele were identified in 574 Korean subjects. This is consistent with the previous studies of the CYP2C9 genotype in other East Asian subjects, including Japanese [8], Taiwan Chinese [3], and Han Chinese [10] populations. These results suggest that the *CYP2C9*2* genotype is absent or at least very rare in Koreans and other East Asian populations, and routine genotyping for this mutant allele is not necessary to determine the effects of this variant on catalytic activity of CYP2C9 in these Asian populations. In addition, many authors have failed to find $Tyr_{358}Cys$ and $Gly_{417}Asp$, other reported allelic variants of CYP2C9, in Asian or Caucasian subjects [3, 8, 10, 11]. In our study, we tested 310 of the 574 Korean subjects for the $Tyr_{358}Cys$ and $Gly_{417}Asp$ alleles and did not find subjects with these mutant alleles (data not shown), suggesting that these allelic variants are not involved in the genetic polymorphism of CYP2C9.

For the *CYP2C9*3* genotype, 13 subjects (2.3%) were heterozygous for the $Ile_{359}Leu$ allele, but none of the 574 Korean subjects was homozygous for this allele. The frequencies of the Ile_{359} and Leu_{359} alleles in this Korean population were 0.989 and 0.011, respectively. This result was in good accordance with an expected frequency of 0.011 for the Leu_{359} allele, calculated by the

Table 2 Comparison of allele frequencies of CYP2C9 reported from different ethnic populations.

Population	n*	*Ile359*	*Leu359*	n*	*Arg144*	*Cys144*	*Reference*
Asian							
Korean	1148	0.987 (0.981–0.993)	0.011 (0.007–0.019)	620	1	0	Present study
Japanese	436	0.979 (0.966–0.992)	0.021 (0.008–0.034)	436	1	0	[8]
Han Chinese	230	0.983 (0.956–0.993)	0.017 (0.007–0.044)	106	1	0	[10]
Taiwan Chinese				164	1	0	[10]
Taiwan Chinese	196	0.974 (0.942–0.989)	0.026 (0.011–0.058)	196	1	0	[3]
Caucasian							
American	200	0.940 (0.907–0.973)	0.060 (0.027–0.093)	200	0.920 (0.882–0.958)	0.080 (0.042–0.118)	[3]
British	200	0.915 (0.876–0.954)	0.085 (0.046–0.124)	200	0.875 (0.829–0.921)	0.125 (0.079–0.171)	[13]
Swedish	860	0.926 (0.906–0.941)	0.074 (0.056–0.091)	860	0.893 (0.872–0.913)	0.107 (0.086–0.127)	[7]
Turkish	998	0.900 (0.880–0.917)	0.100 (0.079–0.123)	998	0.894 (0.873–0.911)	0.106 (0.089–0.127)	[12]
African							
African-American	200	0.995 (0.985–1.005)	0.005 (−0.005–0.015)	200 (0.976–1.004)	0.990 (−0.004–0.024)	0.010	[3]

*Number of alleles studied.

Hardy–Weinberg equation (Table 1). On average one subject homozygous for *CYP2C9*3* is expected in every 7700 Korean subjects. This finding is similar to that for a Japanese population, in which the frequency of subjects homozygous for *CYP2C9*3* allele is also expected to be extremely low (about 1 in 2200) [8]. No subject homozygous for *CYP2C9*3* was identified in our 574 Korean subjects or in any other East Asian subjects, including 218 Japanese, 98 Taiwan Chinese and 115 Han Chinese [3, 8, 10]. Unlike East Asian populations, several subjects homozygous for *CYP2C9*3* have been identified in Caucasian and Turkish populations [3, 6, 7, 12].

The allele frequency of *CYP2C9*3* among 574 Korean subjects was 0.011, a value slightly lower than in Japanese (0.021) [8] and in Chinese (0.017–0.026) [3, 10], but higher than in African-Americans (0.005) [3] (Table 2). The allele frequencies of *CYP2C9*3* in Koreans and East Asians (0.015, a value pooled from Korean, Japanese, and Chinese populations) [present study, 3, 8, 10] is considerably lower than in Caucasians (0.085, a value pooled from American, British, Swedish, and Turkish populations) [3, 7, 12, 13].

The allele frequency of *CYP2C9*2* in Caucasian populations is 0.106 (a value pooled from references 3, 7, 12, 13). In contrast, no subject with a CYP2C 9*2 allele has been found in 761 East Asian subjects from four different studies [present study 3, 8, 10].

In summary, *CYP2C9*3*, an allelic variant related to the functional polymorphism of CYP2C9, is relatively rare in Koreans and seems to occur at a lower frequency than in Caucasians. The *CYP2C9*2* variant appears absent in East Asians including Koreans or present at an even lower frequency than the *CYP2C9*3* allele, suggesting that routine genotyping for the *CYP2C9*2* allele is unnecessary in these populations.

This work was supported in part by a 1998 Inje University research grant.

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