

CYP2C9 polymorphism: prevalence in healthy and warfarin-treated Malay and Chinese in Malaysia

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ABSTRACT

Introduction: Genetic polymorphisms of *CYP2C9* among different populations in different geographical regions could be different. *CYP2C9* has been reported to be the enzyme responsible for the metabolism of many drugs, including warfarin and other drugs with a narrow therapeutic index. Realising the importance of inter-individual differences in the genetic profile in determining the outcome of a drug therapy, this study was conducted to explore the types and frequencies of *CYP2C9* alleles in healthy and warfarin-treated Malays and Chinese, the two major ethnic groups in Malaysia. We aimed to evaluate the prevalence of the types and frequencies of common *CYP2C9* alleles (*1, *2, *3 and *4) among the healthy unrelated individuals and diseased patients prescribed with warfarin.

Methods: A total of 565 Malay and Chinese subjects, including 191 patients prescribed warfarin, were recruited into the study. The healthy unrelated volunteers were also blood donors and they were confirmed to be physically fit before participating in the study. For the patients group, their medical records were reviewed for the relevant clinical data. 5 ml of blood was taken from each subject, and DNA was isolated and used for identification of the *CYP2C9* allele *1, *2, *3 and *4 using nested-allele-specific-multiplex-polymerase chain reaction.

Results: *CYP2C9**1,*2 and *3 were detected among the healthy unrelated individuals but only *CYP2C9**1 and *3 were found in the diseased patients. Among the healthy Malays, 92.8 percent had *CYP2C9**1/*1, 2.6 percent had *CYP2C9**1/*2 and 4.6 percent had *CYP2C9**1/*3 genotypes. Among the Chinese, 92.3 percent had *CYP2C9**1/*1 and 7.7 percent had *CYP2C9**1/*3, but *CYP2C9**2 and *4 were not found in the Chinese. Among the warfarin-treated group, only *CYP2C9**1 and *3

were detected. Even though some alleles were not detected among the patients, suggesting the possible role of *CYP2C9* in certain disorders, the sample size of the current study is too small to be able to arrive at any conclusive results.

Conclusion: Based on the above-observed genotypes, the prevalence of *CYP2C9**2 and *3 was low in healthy and warfarin-treated Malays and Chinese in Malaysia. Further studies are required to support the clinical effectiveness of pharmacogenomics testing.

Keywords: *CYP2C9* frequency, genetic polymorphism, pharmacogenomics testing, warfarin

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INTRODUCTION

CYP2C9 is one of the major drug-metabolising enzymes in humans. It has been shown to be polymorphic. The human *CYP2C9* gene is located on chromosome 10 with a length of approximately 55 kb.⁽¹⁾ *CYP2C9* is a major enzyme that belongs to the *CYP2C* subfamily. It constitutes about 20% of the hepatic cytochrome P450 enzyme expressed in humans and thus is responsible for the metabolism of a wide spectrum of clinically important drugs.⁽²⁾ *CYP2C9* polymorphism has contributed to the wide inter-individual pharmacokinetic variability in terms of drug metabolism, for instance, S-warfarin,⁽³⁾ diclofenac,⁽⁴⁾ losartan,⁽⁵⁾ phenytoin⁽⁵⁾ and tolbutamine.⁽⁶⁾ Thus far, 12 different variants have been identified. The wild type of allele is identified as *CYP2C9**1 and the mutant variants are known according to an ascending numerical order of *CYP2C9**2 to *CYP2C9**12. The mutations of two amino acids, Arg144Cys and Ile359Leu, have been shown to cause reduced enzyme activity in the *CYP2C9* variants compared to the wild type of allele.⁽⁴⁾ The examples are a substitution of C430 → T nucleotide in exon 3 (*CYP2C9**2) and an A 1075 → C substitution in exon 7 (*CYP2C9**3). This nucleotide substitution of the gene has resulted in reduced catalytic activity of

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the hepatic cytochrome P450 enzyme. The effect of the polymorphism on warfarin metabolism has been studied extensively in the Caucasian population.⁽⁷⁾ The clinical implications of the reduced metabolism may explain the increased bleeding complication as well as the lower dose requirement in individuals with *CYP2C9* variants.^(2,3) It has also been found that the *CYP2C9**3 allele has much lower catalytic activity as compared to the *CYP2C9**2 allele.^(4,7) For example, a heterozygous variant of *CYP2C9**3 has caused a reduction in the clearance of oral S-warfarin *in vivo* by 66%, whereas the homozygote *CYP2C9**3 showed a 90% reduction in warfarin clearance relative to the wild type of the genotype (*CYP2C9* *1/*1 genotype).⁽³⁾

Although several allelic variants of *CYP2C9* have been reported and identified in various populations, the commonly-found mutant alleles are *CYP2C9**2 and *CYP2C9**3, besides the wild type of allele. *CYP2C9* polymorphism has been extensively studied in many major populations including the Caucasian,⁽⁸⁾ Russian,⁽⁹⁾ African,⁽¹⁰⁾ Turkish,⁽¹¹⁾ Japanese,⁽¹²⁾ Korean,⁽¹³⁾ and Tamilian Indian.⁽¹⁴⁾ The mutants *CYP2C9**2 and *CYP2C9**3 are relatively more frequently found in Caucasians, whereas they are rare in the Chinese and Japanese.⁽⁸⁾ There is a lack of clinical data on the distribution of this genetic polymorphism among the patients prescribed drugs that are metabolised by *CYP2C9* in Malaysia. In Malaysia, there are three major ethnic groups, the majority being the Malays (60%), followed by the Chinese (30%), Indians (8%) and others (2%). This study selected a population in Kuala Lumpur that represents a similar distribution of the ethnic groups. The Malays and Chinese are the two major ethnic groups residing in Kuala Lumpur, and thus were selected due to the feasibility of achieving adequate samples. Inter-individual differences in drug metabolism are important factors in determining the therapeutic doses as well as the safety profiles of the drugs. Therefore, we investigated the inter-ethnic differences in the genetic polymorphism of *CYP2C9* metabolism between the healthy volunteers and patients prescribed warfarin among the Malays and Chinese living in Malaysia. Genotyping was done to determine the *CYP2C9* alleles *2, *3 and *4, and this was compared to the allelic distribution between the two ethnic groups.

METHODS

Ethical approval was obtained from the local Research and Medical Ethics Committee. The warfarin-treated subjects were recruited from the anticoagulation (INR) clinic and inpatient wards of a tertiary hospital, the National University of Malaysia Hospital, from January to December 2006. The genotype data of *CYP2C9* for the healthy unrelated subjects was obtained via the method

of Zainuddin et al.⁽¹⁵⁾ A total of 565 unrelated healthy and warfarin-treated subjects were recruited after a brief physical examination and interview. Subjects were asked about their medical history and ancestral origin for up to three generations. Those individuals with mixed ethnic origins for up to three generations were excluded. 5 ml of blood was taken from each subject after written informed consent was obtained. The study protocol followed the Helsinki Declaration for studies using human subjects.

A nested polymerase chain reaction (PCR) genotyping method was developed by the Pharmacogenetic Research Group at the Universiti Institut Teknologi MARA to detect *CYP2C9**2, *3 and *4 for the DNA samples obtained from the warfarin-treated subjects, while the genotypes for the healthy volunteers were determined as reported earlier.⁽¹⁵⁾ Allele-specific PCR was performed at the same time to identify the single nucleotide differences for the variants. Allele-specific primers at the 3' ends were designed to differentiate single nucleotide changes at the specific locus during PCR amplification. In order to avoid the incompatibility of the primer sets, they were designed accordingly to have a similar annealing temperature with appropriate length and GC contents. The primers were designed manually and the initial annealing temperature was determined using the formula, $T_m = [2(A + T) + 4(C + G)]$.

The nested PCR protocol was performed in a 25 μ L reaction mixture of 1 \times PCR buffer (Biotools®, B & M Labs, SA, Madrid, Spain), 2.0 mmol/L MgCl₂, 0.2 mmol/L dNTP (Promega Corporation, Madison, WI, USA), primers (submitted for filing for patent), 200 ng (2 μ L) genomic DNA as template and 1.0 U DNA Taq polymerase (Biotool®, B & M Labs, SA, Madrid, Spain). The PCR was performed with an initial hot start at 94°C for 2 min, followed by 20 cycles of denaturation at 94°C for 90 seconds, annealing at 64°C for 30 seconds and extension at 72°C for another 30 seconds. The PCR was continued with another 18 cycles of denaturation at 94°C for 90 seconds, annealing at 54°C for 30 seconds and extended at 72°C for 30 seconds using the GeneAmp® PCR system 2700 Perkin Elmer (Applied Biosystems, Foster City, CA, USA). The PCR products were subjected to electrophoresis on an ethidium bromide stain, 3% agarose gel (LE, analytical grade; Promega Corporation, Madison, WI, USA) in 1 \times TBE (tris, borate, ethylenediaminetetraacetic acid) buffer at 100 V for 45 minutes.

The prevalence of wild and mutant alleles as well as genotypes were presented as the frequency \pm 95% confidence interval according to the following formula: $[p \pm 1.96\sqrt{p(1-p)/n}]$. The expected genotype and allele frequencies were calculated using the Hardy-Weinberg equilibrium as follows: $[p^2 + 2pq + q^2 = 1]$; where p and q are

Table I. Distribution of the genotype and allele frequencies of CYP2C9 among the healthy and warfarin-treated Malay and Chinese subjects.

Distribution	No. (%) of Malay subjects			No. (%) of Chinese subjects		
	Healthy	Warfarin-treated	Total	Healthy	Warfarin-treated	Total
Genotype						
Total no.	209	95	304 [†]	165	96	261 [‡]
*1/*1	191 (91.4)	91 (95.8)	282 (92.8)	154 (93.3)	87 (90.6)	241 (92.3)
*1/*2	8 (3.8)	ND	8 (2.6)	ND	ND	ND
*1/*3	10 (4.8)	4 (4.2)	14 (4.6)	11 (6.7)	9 (9.4)	20 (7.7)
Allele						
*1	400 (95.7)	186 (97.9)	586 (96.4)	319 (96.7)	183 (95.3)	502 (96.2)
*2	8 (1.9)	ND	8 (1.3)	ND	ND	ND
*3	10 (2.4)	4 (2.1)	14 (2.3)	11 (3.3)	9 (4.7)	20 (3.8)
*4	ND	ND	ND	ND	ND	ND

[†]p = 0.14; [‡]p = 0.42
 ND: not detected

the frequencies of the alleles. The chi-square test was used to compare the genotype and allele frequencies between the two study populations. A p-value of 0.05 or less was regarded as significant. A demographical comparison between the two study groups cannot be made as the data for the healthy blood donor is confidential and not made available for statistical analysis. All statistical tests were performed using the Statistical Package for Social Sciences version 12.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

The frequencies of the *CYP2C9*2*, *CYP2C9*3* and *CYP2C9*4* variants were analysed according to the allele-specific PCR methods described earlier. A total of 583 samples from the healthy unrelated and warfarin-treated patients were analysed, but 18 samples were excluded because of a failure to generate the PCR products using the above methods. A total of 565 subjects were available for analysis. They comprised 304 Malays (209 were healthy subjects and 95 were from the warfarin-treated group) and 261 Chinese subjects (165 were healthy subjects and 96 were warfarin-treated patients). The percentage frequencies of *CYP2C9*1*, *CYP2C9*2* and *CYP2C9*3* among the 565 subjects were 96.3%, 0.7% and 3.0%, respectively. We did not find any *CYP2C9*4* allele in our study population. Allele *CYP2C9*4* has not been previously reported in Asian populations. No individual among our study subjects was found to be homozygous for *CYP2C9*2* and *CYP2C9*3*. Among the 565 patients available for analysis, three different genotypes were detected. The percentage frequencies for genotype *CYP2C9*1/*1*, *CYP2C9*1/*2* and *CYP2C9*1/*3* were 92.6%, 1.4% and 6.0%, respectively.

The allelic variants and genotype frequencies in Malaysian Malays and Chinese among the healthy and warfarin-treated subjects are summarised in Table I. The

wild type of allele, *CYP2C9*1*, was the predominant allele found in our study population (Malays 96.4% and Chinese 96.2%). *CYP2C9*2* was only found in the healthy Malay subjects with a frequency of 1.3%. None of the Chinese in this study population were found to have *CYP2C9*2*. There were no significant differences found between the frequencies of the *CYP2C9* genotype when comparing the healthy and warfarin-treated groups of both ethnicities. The allele and genotype frequencies observed in this study were similar to those predicted by the Hardy-Weinberg equilibrium.

DISCUSSION

The frequencies of *CYP2C9*1*, *CYP2C9*2* and *CYP2C9*3* among the Malays and Chinese in Malaysia in this study were similar to those found in previous studies.^(15,16) We did not find any subjects with the *CYP2C9*4* allele in these two ethnic groups. *CYP2C9*4* has not been reported in the Asian population.^(13,14,16) No individual was found to be homozygous for *CYP2C9*2*, *CYP2C9*3* and *CYP2C9*4*. As expected, the wild type of allele, *CYP2C9*1*, was the most common allele found in our subjects. This is consistent with other published data on major ethnic groups around the world.^(9,13,14,17,18) We specifically studied the prevalence of *CYP2C9* polymorphism among the warfarin-treated patients as this gene plays a major role in the metabolism of warfarin. We found that the prevalence in this group resembled that in the general population. This is useful for the prediction of the bleeding complications of warfarin due to altered metabolism by the mutant variants.

Future studies looking at the clinical outcomes may be required to support the effectiveness of *CYP2C9* genetic testing in clinical practice. However, from our analysis, the prevalence of the mutant variants of this gene was very low in both the major ethnic groups in Malaysia.

Thus, it is difficult to justify the cost-effectiveness of this pharmacogenetic test in our population at this moment. A previous study included healthy Indian subjects, who are a minority group in Malaysia, and found that *CYP2C9* polymorphism was highly prevalent (22%).⁽¹⁶⁾ Malaysian Malays and Chinese have a lower frequency of *CYP2C9* variants when compared to the Indian and Caucasian population.^(14,15,17,18) Therefore, understanding the pharmacogenetics of warfarin metabolism that contribute to the variability of the warfarin dose response relationship may help in tailoring warfarin therapy in a safe and effective manner.

In conclusion, *CYP2C9* polymorphism exists in Malaysian Malays and Chinese healthy as well as warfarin-treated subjects. However, routine genotyping of *CYP2C9* *2 and *CYP2C9**3 allelic variants before warfarin therapy is initiated requires a randomised controlled trial to investigate the cost-effectiveness of pharmacogenotyping in clinical practice.

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