Genetic polymorphism in exon 4 of cytochrome P450 CYP2C9 may be associated with warfarin sensitivity in Chinese patients


CYP2C9 polymorphisms reported in Caucasians (Arg144Cys in exon 3 and Ile359Leu in exon 7) are extremely uncommon in Chinese persons. The genotype of CYP2C9 in this population was characterized to investigate its relation with the interindividual variation in warfarin dosages. Eighty-nine Chinese patients receiving warfarin were recruited. Target sequences in CYP2C9 in exons 1, 4, and 5 were amplified by polymerase chain reaction, followed by direct sequencing. Polymorphisms at 416CGT>TGT (Arg144Cys) in exon 3 and CTt (Ile359Leu) in exon 7 were found at frequencies 0.75, 0.20, 0.10, and 0.09, respectively. Seventeen patients (frequency, 0.19) were homozygous for Val208. The common genotypic combinations at these loci are Ile181/His184/Gln192/Leu208Val (n = 50), Ile181/His184/Gln192/Val208 (n = 15), Ile181/His184/Gln192/Leu208 (n = 4), Ile181/His184/Gln192Pro/Leu208Val (n = 6), Ile181/His184/Gln192Pro/Leu208Val (n = 4), and Ile181/His184/Gln192Pro/Leu208Val (n = 4). At codon 208, heterozygous Leu208Val and homozygous Val208 appeared to have a lower warfarin dose requirement than the homozygous Leu208.

Patients who are heterozygous for Ile181/Leu208 had a higher warfarin dose requirement than the homozygous Ile181. Amplified sequences in exons 1 and 5 did not exhibit polymorphism. In conclusion, Chinese patients showed genetic polymorphisms of CYP2C9 in exon 4 and at codon 208; most were heterozygous Leu208Val and homozygous Val208. Homozygous Leu208, a common allele in Caucasians, is uncommon in this cohort. The significance of these CYP2C9 polymorphic alleles remains to be determined.

© 2001 by The American Society of Hematology

From the Division of Haematology and Oncology, Department of Medicine, The University of Hong Kong.


Supported by the Kadoorie Charitable Foundation.

Reprints: Raymond Liang, Department of Medicine, Queen Mary Hospital, Pok Fu Lam Rd, Hong Kong, People's Republic of China; e-mail: rliang@hkucc.hku.hk.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

© 2001 by The American Society of Hematology

From www.bloodjournal.org by guest on June 14, 2017. For personal use only.
CTG GGC GTG CCT CCC TGC-3'; reverse, 5'-TCA GGG ATC CTT GGC CTG ACC TGG ATC CAG CAG-3'; exon 5—forward, 5'-AGC TGA ATT CGC ACA ACC AAC CAT CTG AA-3'; reverse, 5'-TCA GGG ATC CAG TCA ACT GCA GTG TTT TC-3'.

Statistical analysis

Warfarin requirements in different CYP2C9 variants were compared by the Mann-Whitney U and the Kruskal-Wallis tests. The effects of different factors are evaluated by univariate regression analysis using SPSS (Chicago, IL). P < .05 were considered statistically significant.

Results and discussion

Clinical characteristics

The mean (± 1 SEM) dose of warfarin was 3.3 ± 0.13 mg/d (range, 1.0-6.7 mg/d). To obviate the variation caused by differences in body weight, the dose of warfarin per unit body weight was calculated and used in subsequent analysis. Weight-adjusted mean dose was 58.2 ± 2.6 μg/kg per day (range, 17.6-105.1 μg/kg per day), and the mean INR achieved was 2.2 ± 0.04 (range, 1.9-2.9). Median age of the patients was 51 years (range, 26-82 years).

PCR amplification of CYP2C9 genes

Figure 1 shows the automated sequence analyses at 4 positions of exon 4, and Table 1 shows the frequencies of polymorphic alleles. Variants 608TTG→GTG (Leu208Val), 561CAG→CCG (Gln192Pro), 537CAT→CCT (His184Pro), and 527ATT→CTT (Ile181Leu) were found as heterozygotes at frequencies of 0.75, 0.20, 0.10, and 0.09, respectively. Seventeen patients (frequency, 0.19) were homozygous for Val208. Nucleotide sequences in exons 1 and 5 did not exhibit polymorphism and were identical to those in published cDNA sequences. Table 2 shows the genotypic combination at these 4 alleles. Common associations at these loci are Ile181/His184/Gln192/Leu208Val (n = 50), Ile181/His184/Gln192/Val208 (n = 15), Ile181/His184/Gln192/Leu208Val (n = 4), Ile181/His184/Gln192Pro/Leu208Val (n = 6), Ile181/His184/Gln192Pro/Leu208Val (n = 4), and Ile181/His184/Gln192Pro/Leu208Val (n = 4). Nineteen patients carried polymorphic alleles at more than one locus, and 15 of them were heterozygous at both Gln192Pro and Leu208Val loci. The rarity of other combinations, however, precluded accurate analysis of linkage disequilibrium in this study.

Table 1. Summary of the polymorphic alleles in exon 4 of cytochrome P450 2C9

<table>
<thead>
<tr>
<th>Locus</th>
<th>Homozygotes</th>
<th>Heterozygotes</th>
<th>Homozygotes for variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>527ATT→CTT</td>
<td>Ile181Leu</td>
<td>81 (91)</td>
<td>8 (9)</td>
</tr>
<tr>
<td>537CAT→CCT</td>
<td>His184Pro</td>
<td>80 (90)</td>
<td>9 (10)</td>
</tr>
<tr>
<td>561CAG→CCG</td>
<td>Gln192Pro</td>
<td>71 (80)</td>
<td>18 (20)</td>
</tr>
<tr>
<td>608TTG→GTG</td>
<td>Leu208Val</td>
<td>5 (5.6)</td>
<td>67 (75.3)</td>
</tr>
</tbody>
</table>

Correlation between warfarin dose requirement and CYP2C9 polymorphism and other clinical parameters

Patients carrying different polymorphic alleles were compared in terms of the warfarin requirement (Table 2). At codon 208, heterozygous Leu208Val (Ile181/His184/Gln192/Leu208Val) and homozygous Val208 (Ile181/His184/Gln192/Val208), which occurred at high frequencies in this cohort, appeared to require a lower warfarin dose than required for patients carrying the homozygous wild-type genotype (Ile181/His184/Gln192/Leu208). The latter occurred at a frequency of 0.04, with a warfarin dose requirement similar to that used in the Caucasian population.1 However, because of the small number of patients involved, the difference between the 3 groups of patients could not reach statistical significance (P = .2). At codon 181, heterozygous 527ATT→CTT (Ile181Leu) required a significantly higher warfarin dose than the homozygous 527ATT (Ile181) (Figure 2) (mean, 91.8 ± 3.1 vs 55.7 ± 2.9 μg/kg per day; P < .001). Interestingly, all patients carrying the heterozygous Ile181Leu alleles also carried at least one of the other polymorphic alleles; therefore, whether the higher warfarin dose requirement in this subgroup was related to leucine substitution at codon 181 or to the combined effects of amino acid substitutions at various loci could not be ascertained. To assess the contribution of other factors to the warfarin dose, age, gender, and INR were entered into univariate regression analysis together with the occurrence of polymorphic alleles. Only heterozygous 527ATT→CTT (Ile181Leu) (P = .002) was significantly associated with higher warfarin dose in the patients.

Hong Kong Chinese generally require a much lower warfarin dose than Caucasians, and the difference was not explainable entirely by variations in age, body weight, sex, dietary vitamin K intake, clinical indications for warfarin use, and target anticoagulation.1,13 We have previously shown that the common CYP2C9 variants in Caucasians, which are associated with reduced warfarin clearance and, hence, a lower warfarin dose requirement—namely, 416CGT>TGT (Arg144Cys) in exon 3 and 1061A TT (Ile359Leu) in exon 7 6-10,14 —were not found in our population.11 The current study demonstrated that exon 4 of the CYP2C9 gene in Hong Kong Chinese patients exhibited genetic polymorphisms at 4 different sites—608TTG→GTG (Leu208Val), 561CAG→CCG (Gln192Pro), 537CAT→CCT (His184Pro), and 527ATT→CTT (Ile181Leu). To our knowledge, these polymorphic alleles have not been reported in the literature. In particular, the heterozygous Leu208Val and homozygous Val208 existed at high frequencies, and patients carrying these alleles appeared to have lower warfarin dose requirements than did carriers of the wild-type (Leu208) allele, who had warfarin requirements.
(72.6 ± 14.0 μg/kg per day) in the range used in the Caucasian population.1 We speculate that this may be an important polymorphic site that contributes to a lower warfarin dose requirement in this population. Structure-activity analysis of CYP2C9 has recently been reported. 15-17 Codon 208 is located in the F helix of the CYP2C9 and is directed to the active site of the enzyme (C. M. Masimirembwa et al, personal communication, 2001). As a result, amino acid substitution at this position may affect the catalytic activity of the enzyme and, hence, the metabolism of warfarin. This may explain the lower warfarin dose requirement in patients carrying these polymorphic alleles.

In addition, some patients in this cohort exhibited heterozygosities at His184Pro and Gln192Pro. Codons 184 and 192 are located far from the active site, but proline substitutions at these locations may lead to helix breaking, thereby causing changes in the secondary structure and the enzymatic activity. However, warfarin dose requirements could not be interpreted easily because of the small number of patients involved. On the other hand, polymorphism at codon 181 (Ile181Leu) is associated with a higher warfarin dose requirement in this cohort. Codon 181 is located far from the active site of CYP2C9. Given that all patients who were heterozygous for Ile181Leu also carried other polymorphic alleles, the higher warfarin dose requirement in these patients might be attributed to the combined effects of amino acid substitutions at these codons on the enzymatic activity of the CYP2C9. This speculation would have to be confirmed in vitro studies. It is also interesting to note that 19 patients in this cohort carried more than one locus with polymorphic alleles, of whom 15 were heterozygous at both Gln192Pro and Leu208Val loci. Whether this represented true linkage disequilibrium would require confirmation with a larger number of patients.

The significance of CYP2C9 genetic polymorphism is uncertain. In routine clinical practice, the intensity of maintenance anticoagulation is often guided by INR, and knowledge of a patient’s genotype is not a prerequisite for warfarin dosage adjustment. However, characterization of CYP2C9 polymorphic alleles may identify patients at risk, so that warfarin can be given more cautiously, especially during the induction phase of anticoagulation. In fact, CYP2C9 genetic polymorphisms at the Arg144Cys and Ile359Leu loci have been associated with increased risk for bleeding complication during anticoagulation.18 In addition, because CYP2C9 is involved in the metabolism of various commonly used drugs,19 the demonstration of genetic polymorphisms of this enzyme may have implications on the interethnic and interindividual variations of drug pharmacokinetics.

In conclusion, novel polymorphic alleles at 4 positions (Ile181Leu, His184Pro, Gln192Pro, and Leu208Val) of exon 4 of the CYP2C9 gene were identified in Hong Kong Chinese. At codon 208, polymorphic alleles existed at high frequency and appeared to have lower warfarin dose requirements. Further studies are needed to delineate the effects of these amino acid substitutions on the activity of the enzyme.

Acknowledgments

We thank Drs C. M. Masimirembwa, M. Ridderström, and T. B. Andersson (Department of Drug Metabolism and Pharmacokinetics and Bioanalytical Chemistry, AstraZeneca R&D, Mölndal, Sweden) for performing 3-dimensional analysis of CYP2C9 at the reported loci.

Table 2. Summary of genetic polymorphism in exon 4 of cytochrome P450 and mean daily warfarin dose (μg/kg per day) in 89 Chinese patients

<table>
<thead>
<tr>
<th>Codon 181</th>
<th>Codon 184</th>
<th>Codon 192</th>
<th>Codon 208</th>
<th>Mean dose ± SEM</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ile</td>
<td>His</td>
<td>Gln</td>
<td>Leu</td>
<td>72.6 ± 14.0</td>
<td>4*</td>
</tr>
<tr>
<td>Ile</td>
<td>His</td>
<td>Gln</td>
<td>Leu/Val</td>
<td>53.8 ± 2.9</td>
<td>50*</td>
</tr>
<tr>
<td>Ile</td>
<td>His</td>
<td>Gln</td>
<td>Val</td>
<td>48.1 ± 3.5</td>
<td>15*</td>
</tr>
<tr>
<td>Ile</td>
<td>His</td>
<td>Gln/Pro</td>
<td>Leu/Val</td>
<td>57.1 ± 5.5</td>
<td>6</td>
</tr>
<tr>
<td>Ile</td>
<td>His</td>
<td>Gln/Pro</td>
<td>Val</td>
<td>63.6 ± 0.0</td>
<td>1</td>
</tr>
<tr>
<td>Ile</td>
<td>His/Pro</td>
<td>Gln/Pro</td>
<td>Leu/Val</td>
<td>47.4 ± 16.5</td>
<td>4</td>
</tr>
<tr>
<td>Ile</td>
<td>His/Pro</td>
<td>Gln/Pro</td>
<td>Val</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td>Ile/Leu</td>
<td>His</td>
<td>Gln/Pro</td>
<td>Leu/Val</td>
<td>87.6 ± 4.9</td>
<td>4</td>
</tr>
<tr>
<td>Ile/Leu</td>
<td>His/Pro</td>
<td>Gln</td>
<td>Leu/Val</td>
<td>95.3 ± 4.4</td>
<td>2</td>
</tr>
<tr>
<td>Ile/Leu</td>
<td>His/Pro</td>
<td>Gln/Pro</td>
<td>Leu</td>
<td>91.0 ± 0.0</td>
<td>1</td>
</tr>
<tr>
<td>Ile/Leu</td>
<td>His/Pro</td>
<td>Gln/Pro</td>
<td>Leu/Val</td>
<td>100.8 ± 0.0</td>
<td>1</td>
</tr>
</tbody>
</table>

Codons denoted by two amino acids represent heterozygosities at those loci. Warfarin dose of one patient (denoted by —) was unavailable.

*At codon 208, heterozygous 608TTG (Val208) appeared to require lower warfarin doses than the wild-type carriers (Leu208). However, the difference was not statistically significant (P = .2). N indicates number of patients in each category.

Figure 2. Correlation between warfarin dose (μg/kg per day), INR, and genotypes at codon 181 of exon 4. The warfarin dose requirement (left axis) was significantly higher in patients carrying the heterozygous ATT/CTT alleles (lane 2) than in those carrying the homozygous ATT/ATT alleles (lane 1) (P < .001, Mann-Whitney U test). There was, however, no difference in INR between these 2 groups of patients (right axis).
References


Genetic polymorphism in exon 4 of cytochrome P450 CYP2C9 may be associated with warfarin sensitivity in Chinese patients