A study of correlation between CYP2C9 gene polymorphism and Warfarin maintenance dose in anticoagulant therapy among Han people in Yunnan of China

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In this study, we investigated the correlation between CYP2C9 gene polymorphism and maintenance dose of Warfarin in 300 patients who were the Han population derived from the Affiliated Yan An Hospital of Kunming Medical University in Yunnan Province of China, subjected to the operation of cardiac valve replacement and Warfarin oral administration post operation from 2008 to 2009 by detecting the genotypes and Allele Frequency at the three candidate loci (CYP2C9*2, CYP2C9*3 and CYP2C9*c_65) of CYP2C9 gene from the blood samples. Polymerase chain reaction -restriction fragment length polymorphism (PCR-RFLP) assay and DNA sequencing were used to ascertain the genotypes and their corresponding distribution rate. The maintenance dose of Warfarin administered in anticoagulant therapy among the CYP2C9*3 genotypes found in our experiment showed: A/A wild type > A/C heterozygote>C/C homozygote, suggesting that patients with C mutation need the lowest maintenance dose of Warfarin among the three genotypes found in this study. Our results will shed a new light on the personalized medicine of Warfarin and provide basic and genetic experimental data and foundation for future studies with regard to multiple genes’ effect on Warfarin dosage in anticoagulant therapy.

Key words: Warfarin, maintenence dosage, CYP2C9, gene polymorphism, single nucleotide polymorphism (SNP), restriction fragment length polymorphism (RFLP), correlation.

INTRODUCTION

As a kind of common oral anticoagulant, Warfarin is extensively applied for the anticoagulant therapy in various diseases, including valvular heart disease or pathological conditions, valve replacement, fibrillation atrial, electrical conversion, coronary heart disease, pulmonary embolism, deep vein thrombosis and stroke etc. Along with the elevation of incidence rate in chronic fibrillation atrial, cardiovascular and cerebrovascular diseases related to thrombo embolism occur more than ever before. Additionally, the popularity of artificial cardiac valve replacement results in more and more patients receiving long-term oral administration of Warfarin for anticoagulant...
therapy. However, in the clinical practice of anticoagulant therapy, it is difficult to control the effective maintenance dose of Warfarin, due to the large variation with regard to intra-individual difference in maintenance dose of anticoagulant agent, its therapeutic effect and side effect (Aithal et al., 1999).

Inadaptable dose of Warfarin administration, especially over dose may lead to some serious complications including hemorrhage or thrombosis, even a threat to life. In the early stage of Warfarin administration, the incidence rate of hemorrhage is about 12%, and death rate resulted from hemorrhage in patients is 2% (Levine et al., 2001). It is reported that in America, there are about 2 million patients who were suffered from the side effect of drugs, half of which were lethal (Lazarou et al., 1998). Therefore, to make a change for traditional medication mode into personalized medicine will shed a new light on the settlement of this difficulty by adjusting the Warfarin dose as optimal one suitable for patients and, at the same time, reducing the side effect of it.

To date, among the studies of gene polymorphism of CYP2C9, the most studied and extensive ones were involving in the correlation between mutation of either CYP2C9*2 or CYP2C9*3 and clinical Warfarin maintenance dosage (Higashi et al., 2002). Furthermore, there exists large variation in Allele Frequency of CYP2C9*2 and CYP2C9*3 among different races. However, until now, there are few reports about the roles of gene loci of CYP2C9 in Warfarin administration, and the guidance of Warfarin personalized medication needs intensive study.

In this study, we investigated the correlation between the gene polymorphism of three important gene loci (CYP2C9*2, CYP2C9*3 and CYP2C9*c_65) in CYP2C9 gene and Warfarin maintenance dosage administered in anticoagulant therapy, so as to pave a new way for the personalized medicine of Warfarin according to different genotypes of different patients.

MATERIALS AND METHODS

Object of study

Sample harvesting and Admission standard of patients

A total of 300 patients of Han population who were subjected to cardiac valve replacement from 2008 to 2009 in Affiliated Yan An Hospital of Kunming Medical University in Yunnan Province of China were registered and recruited according to the strict standard (see below) in this study. After informed consent was signed, a total of 3ml peripheral venous blood was extracted from each patient. The blood samples were anticoagulated by addition of ethylenediaminetetraacetic acid (EDTA) and preserved at -48°C.

All 300 patients, more than 18 year-old, were orally administered with Warfarin for anticoagulant therapy under strict monitor persisted for one month post of operation, whose intentional normalized ratio (INR) range from 1.5 to 3.0. In the retrospective whole therapies of the patients were performed at this Hospital. The Warfarin tablets administered to all these patients were produced from the same pharmaceuticals company (Orion Corporation). The strict monitor index consisted of normal hepatic function and obeying dietetic contraindication according to the medical order. Clinical therapeutic and laboratory data were recorded in detail.

Exclusion standard of samples

Under following circumstances, the patients were excluded outside of this study: The patients who had liver diseases at present and before, or his (her) serum transaminase was 1.5 fold more than that of normal level. Patients with impaired renal function, whose serum creatinine >120 μmol/L. In the prospective cases, Warfarin was orally administered three months prior to this test. Patients who were administered or administering the drugs influenced the metabolism of Warfarin Herman et al. (2006) Basal INR scale>1.4 - patients who were not appropriate for Warfarin administration due to other reasons.

Definition of target International normalized ratio (INR) scale and stable dosage of Warfarin

International normalized ratio (INR)

Since Professor Armand Quick (1935), 1935) set up routine prothrombin time (PT) blood coagulation assay in 1935, until now, it is still an important screen test to measure factors and associated inhibitors in exogenous blood coagulation system. However, the outcome of PT assay was influenced by various factors. Therefore, it must undergo standardization and quality control so as to elevate its precision, accuracy and reliability. In recent years, international normalized ratio (INR) detection was adopted extensively to measure the clotting time, which avoids the differential outcomes in different detections due to different reagents used in this assay.

Target International normalized ratio (INR) in this study

In this study, the scales of INR measured from blood samples of patients were screened by the standard ranging from 1.5 to 3.0, downward for 0.2 was considered as the normal. The patients whose INR were outside of this range were excluded from this study, because under this situation, the Warfarin dose could not attain stability, which is unfavorable to our study and may produce a misleading and even false outcome.

Stable dosages of Warfarin

Stable dosage of Warfarin was referred to: under the same dosage of Warfarin, sequential INR detection of patients ranged from 1.5 to 3.0. The interval of two INR tests was at least above 7 days.

Primer synthesis

The primers used in the PCR assay were synthesized by Sai Bai Sheng Gene Technology Co. Ltd. Primers of these 3 candidate gene loci were as follows:

CYP2C9*2: locates at 3rd exon
Single Nucleotide Polymorphism (SNP) name: rs1799853
Upriver primer 5'-ATGGAAACAGAGACTTACAGAGGT-3' 
Downstream primer 5' -CCAGTAAAGTCGTAGTATGGTAGGAGTAG-3'
CYP2C9*3: locates at 7th exon SNP name: rs1057910
Upriver primer 5'- CGAATTGCTACAAACAATGTGCCA-3' 
Downstream primer 5' -AGGCTGTTGGGAGAGGTGTCAC-3'
CYP2C9*c_65: locates at 3rd exon SNP name: rs9332127 Uprriver primer 5' -TTTTTGCTTGGGAGGAATTTG-3' 
Downstream primer 5' -CATTACAGGCTTGCATCCATG-3'
Table S1. Contents and quantities of PCR reaction system.

<table>
<thead>
<tr>
<th>Contents of PCR reaction system</th>
<th>Gene locus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CYP2C9*2 (quantity: μl)</td>
</tr>
<tr>
<td>Distilled water (PH8.2)</td>
<td>14</td>
</tr>
<tr>
<td>10×PCR Buffer</td>
<td>2.0</td>
</tr>
<tr>
<td>DNA template (250ng/μl)</td>
<td>1.0</td>
</tr>
<tr>
<td>dNTP</td>
<td>2.0</td>
</tr>
<tr>
<td>Upriver primer</td>
<td>0.5</td>
</tr>
<tr>
<td>Downstream primer</td>
<td>0.5</td>
</tr>
<tr>
<td>TaqDNA polymerase (5U/μl)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table S2. Contents and quantities of the digestion system of CYP2C9*2, CYP2C9*3 and CYP2C9*c_65 gene locus.

<table>
<thead>
<tr>
<th>Contents in Digestion system</th>
<th>Gene locus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CYP2C9*2 (Quantity: μl)</td>
</tr>
<tr>
<td>Distilled water (PH8.2)</td>
<td>7.7</td>
</tr>
<tr>
<td>10 × Buffer (Ava II)</td>
<td>0.8</td>
</tr>
<tr>
<td>Restrictive endonuclease (10u/ μl)</td>
<td>0.5</td>
</tr>
<tr>
<td>Products of PCR amplification</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Selection of restrict endonuclease

CYP2C9*2: restrict enzyme of Ava II recognition site: GGTC*C
CYP2C9*3: restrict enzyme of Mva1 recognition site: CCA*GG
CYP2C9*c_65: restrict enzyme of Hpa I recognition site: GTT*AAC

DNA extraction

Saturated phenol/ chloroform method was employed according to traditional procedure described previously by Joanna et al. (2012) to extract DNA from the blood samples of 300 patients who were recruited for this study.

DNA purification and concentration assaying were performed as described in Alessandra et al. (2011) report.

Primer dilution

The pairs of primers used in PCR amplification were diluted and adjusted at the concentration of 20 pmol/μl referred to following formula:

\[ \text{Volume of distilled water added (μl)} = 2 \times \text{number of nmol of synthesized primer} \times 10^3 / 40 \]

Following dilution, the primer solution was subpackaged and stored at -48°C, awaits further usage.

PCR amplification

PCR reaction systems are shown in Table S1. Pure water, 10×PCR reaction buffer, DNA template, dNTP, primers and Taq DNA polymerase was added into 0.2 ml sterile microcentrifuge tube respectively and orderly, covered by paraffin oil. The tubes were placed into the PCR amplifier (GeneAmp PCR SYSTEM 9700, AB applied Biosystems) under conditions set up as following:

- **PCR reaction conditions of CYP2C9*2**, CYP2C9*3 and CYP2C9*c_65 were shown below respectively:
  - CYP2C9*2: 97°C 5 min → 80°C 5 min → 94°C 1 min → 72°C 10 min → 72°C 10 min
  - CYP2C9*3: 97°C 5 min → 80°C 5 min → 94°C 1 min → 72°C 10 min → 72°C 10 min
  - CYP2C9*c_65: 97°C 5 min → 80°C 5 min → 94°C 1 min → 72°C 10 min → 72°C 10 min

After PCR reaction was finished, the products obtained from PCR were taken out and stored at -20°C.

Digestion by nucleate endonuclease

Restrictive endonuclease Ava II, Mva1 and Hpa I were used for digestion in target fragment of CYP2C9*2, CYP2C9*3 and CYP2C9*c_65 gene loci respectively. The contents and quantities of the digestion system of CYP2C9*2, CYP2C9*3 and CYP2C9*c_65 gene loci were shown in Table S2. The digestion reaction was performed in a thermostatic waterbath at 37°C for 14 h.

Electrophoresis test for products from PCR amplification and digestion

In this test, 8% vertical native polyacrylamide gel electrophoresis
Figure 1. Samples 1 to 5 showed the digestive products following PCR, their genotypes were all C/C wild type. Sample 6 showed PCR product of CYP2C9*2 locus, whose segment size was 309 bp. Number 1 was heterozygotemutant A/C (127, 105, 75 and 22 bp), Number 2 was homozygote mutant C/C (105, 75 and 22 bp), Number 3 to 5 was wild type A/A (127 and 75 bp), Number 6 was the product of PCR amplification of CYP2C9*2 locus, with segment size of 202 bp.

was employed. Silver Nitrate staining was used to observe the outcomes of the electrophoresis. By using 50 and 100 bp DNA ladder as standard molecular weight markers, the lengths of DNA fragments of target genes were ascertained and their genotypes were determined. Silver Nitrate staining method was used for coloration. Camera (Canon, IXY DIGITAL) was used to take pictures of the staining outcome. Dry gelatin was made so as to preserve the outcome.

DNA sequencing

DNA sequencing of three loci of CYP2C9 gene following PCR amplification

The products of PCR amplification of CYP2C9*2 (rs1799853), CYP2C9*3 (rs1057910) and CYP2C9*c_65 (rs9332127) were subjected to DNA sequencing. A total of 5 DNA samples from each locus were used for DNA sequencing in order to ascertain whether the PCR amplification products was the expectant target fragments. 3130-Genetic Analysis Apparatus (ABI Company, America) was employed to perform automatic DNA sequencing.

DNA sequencing of three loci of CYP2C9 gene following PCR amplification and digestion

The digestive products underwent DNA sequencing at CYP2C9*3 (rs1057910) locus consisted of 5 cases of A/A wild type (127 bp, 75 bp), 1 case of C/C homozygote mutation type (105 bp, 75 bp, 22 bp) and 2 cases of A/C heterozygote mutant (127 bp, 105 bp, 75 bp, 22 bp).

The DNA sequencing for the digestive products of CYP2C9*c_65 (rs9332127) locus following PCR amplification included 5 cases of G/G wild type (317 and 54 bp) and 2 cases of G/C heterozygote mutant (371, 317 and 54 bp).

Statistical analysis

Experimental data were expressed as mean±SD and analyzed by

RESULTS

Products of PCR amplification and genotypes of three loci of CYP2C9 gene

The segment size of CYP2C9*2 locus derived from the PCR and digestion in 300 DNA samples was all 309 bp (Figure 1). With the digestion by restrictive endonuclease Ava II, when restriction enzyme cutting site was at base C, fragment of 309 bp-product following PCR was digested into three fragments, whose segment size was 195, 91 and 22 bp respectively. When restriction enzyme cutting site was at base T, the fragment of product following PCR could be digested into two fragments, whose segment size was 286 and 23 bp respectively. Only one allele- C (195, 91 and 23 bp) and one kind of genotype- C/C wild type (Figure 1) was checked out in the 300 samples. Among the 300 DNA samples, there was no mutational site was detected at CYP2C9*2 gene locus. As the digestive product of 23 bp fragment at CYP2C9*2 locus was the smallest one, when polyacrylamide gel electrophoresis (PAGE) finished, this fragment have run out of the gelatin, image of this fragment could not be observed within the gelatin. Only the 195 and 91 bp DNA fragments could be observed.

The segment size of CYP2C9*3 locus obtained from the PCR and digestion in 300 DNA samples was all 202
Figure S1. Samples 1 to 5 showed the digestive products following PCR, their genotypes were all C/C wild type. Sample 6 showed PCR product of CYP2C9*2 locus, whose segment size was 309 bp.

Figure S2. Number 1 to 6 showed PCR amplified fragments of CYP2C9*c_65 locus, with segment size of 371 bp.

bp (Figure S1). With restrictive nuclease va1 digestion, when the restriction enzyme cutting site was at base A, the 202 bp DNA fragments were digested into two fragments, whose segment size was 127 and 75 bp respectively. When the restriction enzyme cutting site was at base C, the DNA fragments (202 bp) were digested into three fragments, with segment size of 105, 75 and 22 bp respectively. Among 300 samples, two kinds of alleles were detectable, which were A and C. There were three kinds of genotypes found in these 300 samples, exhibiting A/A wild type (127 bp, 75 bp), C/C homozygosis mutant (105, 75 and 22 bp) and A/C heterozygote mutant (127, 105, 75 and 22 bp) in genotypes (Figure S1). Among these 300 DNA samples, there were 276 cases of A/A wild type for CYP2C9*3 locus, which accounted for 92%. There were 22 cases of A/C heterozygote mutant, accounting for 7.3%. There were 2 cases of C/C homozygote mutant, accounting for 0.7%. The frequency of allele A was 95.67%, and that of allele C was 4.03%.

The DNA fragment of PCR and digestive product at CYP2C9*3 locus was 22 bp. As it was too small, when the gel electrophoresis finished, this fragment has run out of the gelatin, this fragment could not be seen within the gelatin. Only DNA fragments sized 127, 105 and 75 bp could be observed.

The segment size of CYP2C9*c_65 locus derived from the PCR and digestion in 300 DNA samples was all 371 bp (Figure S3). By using restrictive nuclease Hpa I, when enzyme cutting site located at base G, 371 bp sized DNA fragment obtained from PCR was digested into two fragments, whose segment size was 317 and 54 bp respectively. When the enzyme cutting site located at base C, the DNA fragment of 371 bp could not digested by Hpa I, leaving a single fragment of 371 bp. Among the 300 DNA samples, there were two kinds of alleles detectable, which were G and C. There were two kinds of genotypes found in these 3000 DNA samples, exhibiting G/G wild type and G/C heterozygote mutant (Figure S2). Among these 300 DNA samples, there were 281 cases showing G/G wild type, accounted for 93.7%. There were 19 cases showing heterozygote mutant, accounting for 6.3%. The frequency of allele G was 96.83%, and that of allele C was 3.17% (Figure S3).
Table 1. Hardy-Weinberg goodness of fit test of genotypes of CYP2C9*3 locus.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>A1/A1</th>
<th>A1/A2</th>
<th>A2/A2</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual frequency</td>
<td>276</td>
<td>22</td>
<td>2</td>
<td>4.002</td>
<td>0.177 (&gt;0.05)</td>
</tr>
<tr>
<td>HW theoretical frequency</td>
<td>274.56</td>
<td>24.87</td>
<td>0.56</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Hardy-Weinberg goodness of fit test of genotypes of CYP2C9*c_65 locus.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>A1/A1</th>
<th>A1/A2</th>
<th>A2/A2</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual frequency</td>
<td>281</td>
<td>19</td>
<td>0</td>
<td>0.321</td>
<td>0.451 (&gt;0.05)</td>
</tr>
<tr>
<td>HW theoretical frequency</td>
<td>281.30</td>
<td>18.40</td>
<td>0.30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Sex distribution of CYP2C9*3 locus.

<table>
<thead>
<tr>
<th>Gender</th>
<th>CYP2C9*3 locus Genotype frequency</th>
<th>CYP2C9*3 locus Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1/A1</td>
<td>A1/A2</td>
</tr>
<tr>
<td>Male</td>
<td>106</td>
<td>94</td>
</tr>
<tr>
<td>Female</td>
<td>194</td>
<td>182</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gender</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0.581</td>
<td>0.434 (&gt;0.05)</td>
</tr>
<tr>
<td>Female</td>
<td>0.134</td>
<td>0.714 (&gt;0.05)</td>
</tr>
</tbody>
</table>

DNA sequencing

Genetic analysis apparatus (3130 Genetic Analyzer, AB applied Biosystems) was used for sequencing the DNA fragments from PCR and restrictive nuclease digestion was conducted to confirm them as target DNA fragments. Results showed that sequencing outcome was in accordance with the standard DNA sequences of target DNA checked out from GeneBank.

Statistical analysis

Hardy-Weinberg balance test (HWSIM) statistical analysis revealed that the observed number coincided well with the expected value in CYP2C9*3 and CYP2C9*c_65 locus (P>0.05), which was in accordance with the Hardy–Weinberg balance law, suggesting that the DNA samples possessed group representativeness (Tables 1 and 2). Sex distribution of CYP2C9*3 and CYP2C9*c_65
DISCUSSION

In the present study, only the population of patients subjected to the operation of cardiac valve replacement was investigated. Because, in this population, patients routinely administered Warfarin, which provides an appropriate opportunity to examine the correlationship of genotypes of special loci in CYP2C9 gene and Warfarin maintenance dosage. As for other diseases apart from cardiovascular disorders, such as diabetes, hypertension, were not involved in this study due to their independent with our study.

Influence of CYP2C9 gene mutation on Warfarin dosage in anticoagulant therapy and possible mechanism

The molecular mechanism of CYP2C9 gene polymorphism leading to metabolic defect of Warfarin lies on the base mutation, which results in alteration of sequences of DNA base, and then, amino acid replacement occurs, ultimately changes the catalytic activity of proteins, expressing as weak metabolic pattern and enzyme deficiency pattern. So far, researches involving in the correlation between CYP2C9 mutation and maintenance dosage of Warfarin in anticoagulant therapy demonstrated that CYP2C9 gene mutation could decrease the Warfarin metabolism. Therefore, patients with CYP2C9 gene mutation needed relatively lower dosage of Warfarin.

Higashi et al. (2002) firstly reported that the correlation between genotype of CYP2C9 gene and anticoagulation or hemorrhage. Subsequently, Sconce et al. (2005) found mutation of CYP2C9*2 or CYP2C9*3 reduced the Warfarin dosage needed in patients subjected to anticoagulant therapy, and CYP2C9*3 mutation led to a more lage extent of Warfarin dosage reduction (30% reduced). There exists much variation of allele frequency in CYP2C9*2 and CYP2C9*3 loci among different races (Margaglione, 2000; Taube, 2000; Loebstein, 2001; Sanderson, 2005; Yu, 2004; Mizutani, 2003; Hong, 2005; Bae, 2005) as well. In White People, obvious ununiformity is commonly seen in the distribution of allele frequency of CYP2C9*2 gene in that the allele frequency ranges from 8 to 19% (Nakai, 2005; Scordo, 2001; Garcia-Martin, 2001). Higashi et al. (2002) reported CYP2C9 gene has high genetic polymorphism, especially in the mutation of CYP2C9*2 and CYP2C9*3, because the activity of enzymes encoded by them decreased 30 and 80% respectively when compared with that of wild type CYP2C9*1, which is the main cause of CYP2C9 mutation, leading to the lower dosage of Warfarin administered in patients with CYP2C9 mutation. Although the correlationship between CYP2C9*2 and/or CYP2C9*3 and Warfarin maintenance dose was involved in several studies by other authors, as for the correlationship between CYP2C9*2 and/or CYP2C9*3 mutation and Warfarin maintenance dose in the Han people in China was few reported .Additionally, the crucial role of gene polymorphism of CYP2C9*2 and/or CYP2C9*3 in the intra-individual variation of Warfarin dose attracts more and more attention. Therefore, in this study, our finding that the influence of CYP2C9 gene polymorphism on the substrate drug metabolism has gene dosage effect sheds a new light on the personalized medication of Warfarin by applying Warfarin according to different genotypes of patients. Importantly, results from CYP2C9*3 locus study showed that the maintenance dose of Warfarin administered in anticoagulant therapy among the CYP2C9*3 genotypes exhibited: A/A wild type >A/C heterozygote>C/C homozygote, suggesting that patients carried with C/C mutation needed the lowest maintenance dose of Warfarin among the three genotypes found in this study. Our investigation is the first time to elucidate the conclusive correlationship between the mutatant in CYP2C9*3 gene locus and Warfarin maintenance dosage in Han people for clinical practice, providing a novel, useful and effective guidance for Warfarin personalized medication according to different genotypes in different patients.

Correlation between CYP2C9*c_65 locus mutation and Warfarin maintenance dosage

In the present study, CYP2C9*c_65 locus-genotypes and their corresponding distributed rates suggesting the mutation at CYP2C9*c_65 locus may not correlated with maintenance dosage of Warfarin. However, Chern et al.
(2006) found in Chinese Taiwanese population that the mutation in CYP2C9*c_65 locus could reduce the Warfarin dosage administered, and in patients carried c_65 heterozygote or homozygote only needed one half of conventional dosage of Warfarin. To further ascertain the conclusive correlation between mutation of CYP2C9*c_65 locus and the Warfarin maintenance, we investigate whether or not this correlation exist in the Han people of China. The results of ours and other authors above mentioned were much different, it is indicative that CYP2C9*c_65 mutation has obvious variation among different populations and races. At least until now, there are no definite evidence revealing CYP2C9*c_65 mutation has conclusive correlation with Warfarin maintenance administered in anticoagulant therapy. This is another first report proposed by us from this study in Han people of China. We also found that there was no significant difference in genotype-distribution and allele frequency in CYP2C9*3 and CYP2C9 c_65 gene loci between male and female patients, suggesting CYP2C9 gene polymorphism had no definite correlation with gender variation.

Taken together, the maintenance dose of Warfarin administered in anticoagulant therapy among the CYP2C9*3 genotypes found in our experiment showed: A/A wild type> A/C heterozygote>C/C homozygote, suggesting that patients with C mutation need the lowest maintenance dose of Warfarin among the three genotypes found in this study. This will provide a conclusive and effective guidance for the Warfarin maintenance dose application according to different genotypes of patients. In future, intensive studies should focus on the correlation of more gene loci with Warfarin maintenance dose administered in the anticoagulant therapy and how to perform personalized medication of Warfarin in clinical practice.

**ABBREVIATIONS**

INR, International normalized ratio; PT, prothrombin time; SNP, single nucleotide polymorphism.

**REFERENCES**


