VKORC1 and CYP2C9 genotypic data-based dose prediction alone does not accurately predict warfarin dose requirements in some Malaysian patients

Yung An CHUA¹*, Wan Zaidah ABDULLAH², Zurkurnai YUSOF³, Siew Hua GAN⁴

¹Department of Pharmacology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia
²Department of Hematology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia
³Invasive Cardiology Laboratory, Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia
⁴Human Genome Center, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia

* Correspondence: yungan.chua@gmail.com

1. Introduction
Warfarin is one of the most important drugs in personalized medicine. Many clinical conditions may affect the potency of warfarin over time, thus requiring patients to regularly visit a healthcare center for dose adjustment. Traditionally, warfarin dosage is determined by the international normalized ratio (INR) values, which are derived from prothrombin time (PT) (1). The difficulty of achieving the ideal warfarin dose is compounded by genetic variations that may affect the pharmacokinetics and pharmacodynamics of warfarin in different individuals.

Research has shown that cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase complex 1 (VKORC1) gene polymorphisms are significantly associated with warfarin sensitivity (2–5), including among Asian patients. Several dosing guidelines and algorithms based on genetic data have been proposed in response to reports that the addition of genetic data into a nongenetic dosing model may increase the predictability of the model by up to 16% (6–9). The strong association of these genes with warfarin sensitivity compelled the US Food and Drug Administration to recommend conducting genetic tests before determining the most suitable warfarin dose range (http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2007/ucm108967.htm.) Although a few studies have established strong associations between genotypic data and warfarin dose requirements, the comprehensive use of these genotypic data in clinical practice is generally hindered by the lack of evidence of the overall efficacy of this approach (10), which suggests that there are other factors that play a role in warfarin dose variation. This situation is compounded by high...
genetic variability across the world. Although Asians and Caucasians have different genetic backgrounds, many drug doses for Asians are still based on data from studies of Caucasian populations. Furthermore, the inclusion of additional laboratory tests, such as genetic screening, in addition to conventional INR is generally not preferred due to the extra cost involved (11).

In this study, we evaluated an existing warfarin dosing algorithm developed for Malaysian patients that was based only on the VKORC1 and CYP2C9 genes.

2. Materials and methods

Ethical approval was obtained from the Universiti Sains Malaysia Research Ethics Committee (USMKK/PPP/JEPEM 197.3(6)). Written informed consent was acquired from all patients before the study. Blood samples (3 mL) were obtained from 5 Malay patients, between 44 and 81 years old, who had received warfarin treatment. DNA was extracted from the blood according to the DNA extraction kit manufacturer's standard guidelines (QIAGEN, Hilden, Germany). Each patient was tested for INR and the activity of the vitamin K-dependent (VKD) clotting factors II, VII, IX, and X using an STA Compact Benchtop Hemostasis Analyzer (Diagnostica Stago, Parsippany, NJ, USA). The CYP2C9*2, CYP2C9*3, and VKORC1-1639G>A genotypes were determined by using previously reported polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods (12,13).

The demographic data, medical records, mean daily warfarin doses, and INR values 2 years before and after the measurement of VKD clotting factor activities were acquired from both the patient's folder and the hospital database. The mean warfarin doses were considered to be the doses required by the patients to maintain their respective stable INR ranges (2.5–3.5 for atrial valve replacement or between 2 and 3 for other indications). The mean dose was then compared to the predicted daily warfarin dose calculated using an Asian population-based warfarin dosing model (7). In the previously developed dosing model for Asian patients (n = 108), age, weight, and CYP2C9*3 and VKORC1 381 genotypes were processed by the algorithm and yielded a correlation coefficient of 0.73. In our study, we replaced the VKORC1 381 genotype with the VKORC1-1639G>A genotype because these genotypes have been confirmed to be closely linked with each other and therefore interchangeable (5). The percentage difference between the actual and predicted daily warfarin doses was then recorded.

3. Results

The induction of warfarin therapy and the concomitant drugs prescribed during a single hospital visit before the measurement of the current INR and the VKD clotting factor activities are shown in Table 1. Genotyping results for the CYP2C9 and VKORC1 polymorphisms are presented in gel electrophoresis images (Figures 1–3). In agreement with several findings that support VKORC1 as having a stronger association with warfarin sensitivity than any other genetic or clinical data (4,14–17), all patients who had the VKORC1-1639 AA genotype had a lower warfarin dose requirement compared to patients with the GA genotype, with the exception of Patient C (Table 2). However, further investigation of the medication history of Patient C confirmed that he was also taking celecoxib, a nonsteroidal antiinflammatory drug that is also metabolized by CYP2C9 and thus may interact with warfarin to cause an increase in warfarin sensitivity (18).

The purported “warfarin-sensitive” CYP2C9*2 genotype was not proven to be the cause of warfarin sensitivity because Patient E, who had a “warfarin-sensitive” CYP2C9*2 T allele, actually had the highest mean warfarin dose compared to other patients. Patient E also had the VKORC1-1639 G allele, which represents a “warfarin-resistant” allele. These differences further

### Table 1. Related diseases and concomitant drugs prescribed to the patients during 1 hospital visit before the assessment of the INR and activities of the VKD clotting factors.

<table>
<thead>
<tr>
<th>Indication of warfarin prescription</th>
<th>Concomitant drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient A, AVR</td>
<td>Captopril, propranolol</td>
</tr>
<tr>
<td>Patient B, CCF, IHD</td>
<td>Aspirin, bisoprolol, digoxin, enalapril, pravastatin, ranitidine, trimetazidine</td>
</tr>
<tr>
<td>Patient C, AF, CCF, IHD</td>
<td>Aspirin, atorvastatin, celecoxib, digoxin, enalapril, furosemide</td>
</tr>
<tr>
<td>Patient D, AF, CCF, IHD</td>
<td>Aspirin, digoxin, furosemide, potassium chloride</td>
</tr>
<tr>
<td>Patient E, AF, CRHD, history of TIA</td>
<td>Digoxin, furosemide, potassium chloride, propranolol</td>
</tr>
</tbody>
</table>

AVR, Atrial valve replacement; CCF, congestive cardiac failure; IHD, ischemic heart disease; AF, atrial fibrillation; CRHD, chronic rheumatic heart disease; TIA, transient ischemic attack.
confirm the fact that the VKORC1 genotype is a stronger determinant of warfarin sensitivity among Asians than the CYP2C9*2 genotype because the presence of the “warfarin-resistant” VKORC1 allele can mitigate the effect of the CYP2C9*2 alleles. These results also explain why the incorporation of the CYP2C9*2 genotype into a dosing prediction model was not favored in a univariate analysis of warfarin dosing models developed for Asian patients as proposed by Tham et al. (7).

The opposite result was observed for the CYP2C9*3 genotype. Patient A, who had the warfarin-sensitive CYP2C9*3 C allele, received the lowest warfarin dose of all 5 patients. The low warfarin dose may be a result of the combined effects of the warfarin-sensitive genotypes of the CYP2C9*3 AC and VKORC1-1639 AA alleles. All of the clotting factor activities in Patient A were also lower than in other patients. With the exception of Patient C, all patients with the VKORC1-1639 GA genotype had higher activities of clotting factors II, IX, and X when compared to patients who had the VKORC1-1639 AA genotype.

There were significant variations in the dose differences between actual and predicted mean daily doses among the 5 investigated patients. Patient D had the largest dose difference, with a predicted dose deviation of 54.24% from the actual mean dose. Apparently, Patient D would be overdosed if the warfarin dose was based on the recommended genetic-based dosing algorithm. Therefore, a dosing algorithm that is based solely on genetic data is inaccurate due to other confounding factors, such as environmental and clinical conditions. For example, drug–drug interactions, intake of supplements, intake of vitamin K, health status, smoking status, and compliance to warfarin may confound dosing predictions (19–22). Unlike genetic data, these factors are in a state of constant change and therefore should be incorporated in the dosing model. Therefore, genetic data alone can only serve as a guide, especially during the initiation of warfarin therapy, because patient response to this anticoagulant is dynamic and dependent on multiple factors.

4. Discussion
The dosing algorithm could not be applied to all patients. For example, administration of drugs that may interact
with warfarin's pharmacokinetics may render purported strong predictors of warfarin such as $\text{VKORC1}$-1639 GA less useful. Therefore, a clinical trial on a larger population is needed in order to validate the findings and to compare the algorithm with a conventional dosing method (23).

Because genetic tests are not routinely practiced by the local medical clinics in Malaysia, the measurement of VKD clotting factor activities can serve as a useful and relatively easy method to predict warfarin sensitivity in patients. Additionally, hemostatic tests can be performed simultaneously with other routine applications, such as INR tests. Each individual VKD clotting factor has a distinct diagnostic value. Factor VII is particularly sensitive to warfarin administration and may serve as an indicator for bleeding risk in addition to, or instead of, INR (24). Because the activity of factor IX is not detected by a PT test, it is determined independently of the INR result and can thus be used as a secondary measure for the determination of warfarin dosing, especially for clinical situations in which recommendations based solely on INR data are not effective (25).

A dynamic parameter such as INR must also be integrated into warfarin dosing models so that the dose can be adjusted for any changes in a patient's medical condition. Some studies also considered the use of drugs that may interact with warfarin, such as amiodarone, in the warfarin dosing model (6,9,26). Incorporation of concomitant drugs into the dosing algorithm is also advantageous for improved prediction accuracy, as evidenced by the effects of celecoxib on Patients C's warfarin dose requirement.

Although there are no studies that correlate $\text{VKORC1}$ polymorphisms with VKD clotting factor activities, several studies have indicated that $\text{VKORC1}$ polymorphisms may cause combined VKD clotting factors deficiency when plasma levels of VKD clotting factors are congenitally

![Figure 3](image)

**Figure 3.** Representative gel electrophoresis image of PCR-RFLP analysis for $\text{VKORC1}$-1639G>A for Patient D. The first lane contains a 100-bp DNA marker ladder, while -ve and +ve are the negative and positive controls, respectively. The rest of the numbered lanes are the genotype results for patients who are not discussed in this study. The mutant allele (A) was resistant to restriction enzyme digestion; therefore, it is represented as a 290-bp fragment. The wild-type allele (G) was digested into 2 fragments of 168- and 122-bp. The presence of all 3 bands in a single lane indicates that the patient is heterozygous.

<table>
<thead>
<tr>
<th>CYP2C9*2</th>
<th>Patient A</th>
<th>CC</th>
<th>Patient B</th>
<th>CC</th>
<th>Patient C</th>
<th>CC</th>
<th>Patient D</th>
<th>CC</th>
<th>Patient E</th>
<th>CT</th>
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<tbody>
<tr>
<td>CYP2C9*3</td>
<td></td>
<td>AC</td>
<td></td>
<td>AA</td>
<td>AA</td>
<td>AA</td>
<td>AA</td>
<td>AA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{VKORC1}$-1639</td>
<td></td>
<td>AA</td>
<td></td>
<td>AA</td>
<td>GA</td>
<td>GA</td>
<td>GA</td>
<td>GA</td>
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<tr>
<td>Factor II (%)</td>
<td>9</td>
<td>16</td>
<td>16</td>
<td>30</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Factor VII (%)</td>
<td>22</td>
<td>32</td>
<td>37</td>
<td>26</td>
<td>36</td>
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<td>Factor IX (%)</td>
<td>20</td>
<td>48</td>
<td>50</td>
<td>59</td>
<td>51</td>
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<tr>
<td>Factor X (%)</td>
<td>9</td>
<td>13</td>
<td>11</td>
<td>18</td>
<td>23</td>
<td></td>
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<td>Actual mean dose (mg daily)</td>
<td>2.00</td>
<td>2.30</td>
<td>2.02</td>
<td>3.30</td>
<td>3.48</td>
<td></td>
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<tr>
<td>Predicted dose based on algorithm (mg daily)</td>
<td>2.15</td>
<td>2.20</td>
<td>2.72</td>
<td>5.09</td>
<td>4.74</td>
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<td>Dose difference (%)</td>
<td>7.50</td>
<td>−4.35</td>
<td>34.65</td>
<td>54.24</td>
<td>36.21</td>
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The CYP2C9*2 C, CYP2C9*3 A, and $\text{VKORC1}$-1639 G alleles are alleles with higher warfarin dose requirements, and their respective variants have lower dose requirement. A negative dose difference percentage indicates that the predicted warfarin dose is lower than the actual mean warfarin dose in the patient.
References


