Evaluation of Pharmacogenetic Algorithm for Warfarin Dose Requirements in Japanese Patients

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**Background:** Warfarin dosing is difficult to establish because of considerable interindividual variation. Thus, warfarin pharmacogenetics have attracted particular interest in relation to appropriate control of anticoagulation.

**Methods and Results:** The 200 eligible subjects were chosen from participants in a hospital cohort. Performance of a pharmacogenetic algorithm recently developed by the International Warfarin Pharmacogenetics Consortium (IWPC) was tested and compared with a clinical algorithm (without genotype data) by calculating the percentage of patients for whom the predicted dose deviated by less than 7 mg/week (1 mg/day) from the actual dose. The pharmacogenetic algorithm accurately identified a significantly (P<0.05) larger proportion of patients to achieve the target international normalized ratio than did the clinical algorithm (68% vs 36% for a low-dose group; and 21% vs 0% for a high-dose group). Also, an increase in warfarin dosage was found to be appropriate for the current status of alcohol drinking (4 mg/week, as against non-drinking) and smoking (3.3 mg/week, as against non-smoking).

**Conclusions:** The IWPC pharmacogenetic algorithm has clinical application, particularly in identifying Japanese patients who require a low dosage of warfarin and are at greater risk of excessive anticoagulation. (Circ J 2010; 74: 977–982)

**Key Words:** Anticoagulation; CYP2C9; Pharmacogenetics; VKORC1; Warfarin

Warfarin is the most commonly prescribed oral anticoagulant drug for the prophylaxis and treatment of thromboembolic disorders, but the appropriate dose can be difficult to establish because it can vary substantially (>10 fold) among patients, in part because of differences in each patient’s age, diet, race and genotype. Incorrect doses contribute to a high incidence of adverse effects (ie, bleeding and thromboembolic events) when the effectiveness of warfarin, expressed as the international normalized ratio of prothrombin time (PT-INR), is above or below the therapeutic range.

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During the initial dosing period (ie, the first few months), patients are at the greatest risk of overanticoagulation. To reduce this risk, a number of warfarin dosing algorithms and regimens have been proposed, mostly incorporating clinical factors, demographic variables, and molecular variations in 2 genes: the warfarin metabolic enzyme CYP2C9 and the warfarin target enzyme, vitamin K epoxide reductase complex subunit 1 (VKORC1). Regarding genetic factors, of note is the fact that, in 2007, the US Food and Drug Administration (FDA) added pharmacogenetic information to the warfarin product label. Along this line, the International Warfarin Pharmacogenetics Consortium (IWPC) has recently developed a pharmacogenetic dose algorithm for warfarin using a large data set (involving a total of 5,052 patients) from diverse ethnic groups. The IWPC algorithm appears to provide better predictive accuracy than the one that uses only clinical variables or a fixed-dose (5 mg/day) strategy.

In general, patients of Asian descent require a lower maintenance dose of warfarin for a similar degree of anticoagulation than patients of European descent. Moreover, it has been reported that compared with Europeans, the incidence of thromboembolism is low in Japan, despite the less intensive regimen; which indicates that adjusted low-dose warfarin (eg, PT-INR 1.6–2.6) is optimal for prevention of thromboembolism in Japanese patients.

Considering these racial differences in the anticoagulation therapy, we attempted to validate the IWPC pharmacogenetic dose algorithm for warfarin in Japanese patients under low-
and gave written informed consent for participation in the study. The ethics committee of IMCJ approved the study protocol.

**Genotyping of CYP2C9 and VKORC1 SNPs**
Among the genetic variants of CYP2C9 used for the IWPC algorithm (⁎1, ⁎2 and ⁎3), the CYP2C9⁎2 allele (I359L) has not been reported in Asian populations.5,15,16 Accordingly, we genotyped the ⁎3=rs1057910 polymorphism in relation to the wild-type allele ⁎1, thereby determining ⁎1⁎1, ⁎1⁎3 and ⁎3⁎3 genotypes at the CYP2C9 locus. At the VKORC1 locus, on the other hand, the −1639 G→A=rs9923231 polymorphism was genotyped, following the IWPC algorithm.8 Both SNPs were characterized with the use of TaqMan assays (Applied Biosystems, Foster City, CA, USA).

**Statistical Analysis**
First, we performed multiple regression analysis to test the effects of predictor variables on interindividual variability of warfarin dose, with the square root of the warfarin dose in mg/week being used as a dependent variable, which was in accordance with the IWPC study.8 We then evaluated the potential clinical value of 2 algorithms (the IWPC pharmacogenetic algorithm and a clinical algorithm without including genotype data) by calculating the percentage of patients whose predicted dose of warfarin was within 7 mg/week (1 mg/day) of the actual stable therapeutic dose. The IWPC pharmacogenetic algorithm for Japanese was: 5.4952-(0.2614×[age in decades])+(0.0208×[weight in kg])+(0.0508×[VKORC1 A/G])+(0.0104×[CYP2C9⁎1⁎1])+(0.0012×[CYP2C9⁎1⁎3])+(0.0251×[VKORC1⁎3⁎3])+(0.0026×[CYP2C9⁎3⁎3])/2. Where N=number of patients and G=genotype, the potential clinical value of 2 algorithms (the IWPC pharmacogenetic algorithm and a clinical algorithm without including genotype data) was assessed in 3 dose groups: low dose (0–10.5 mg/week), high dose (≥31.5 mg/week), and intermediate doses (between 10.5 and 31.5 mg/week) for stable therapeutic anticoagulant treatment of thromboembolic diseases. Characteristics of the 200 participants in the present study are summarized in Table 1. Among them, the most common indications for warfarin use were atrial fibrillation (119 patients (59.5%) primary indication for warfarin treatment, the stable therapeutic dose. Besides assessing the overall performance of the IWPC algorithm, we calculated the percentage of patients for whom the predicted dose according to each algorithm was at least 7 mg/week higher than the actual dose (overestimation) or at least 7 mg/week lower than the actual dose (underestimation). Here, we adopted 7 mg/week (1 mg/day) as a difference that clinicians would be likely to define as clinically relevant. With consideration of warfarin dose distribution in the study sample (Figure S1), the performance of the IWPC algorithm was assessed in 3 dose groups: low dose (≤10.5 mg/week), high dose (≥31.5 mg/week), and intermediate doses (between 10.5 and 31.5 mg/week) for stable therapeutic anticoagulation. These thresholds of 10.5 mg and 31.5 mg/week bracket the usual maintenance dose of 17.5–24.5 mg/week (2.5–3.5 mg/day) in Japanese patients.5,15,17,18 The overall performance was measured as the coefficient of determination, \( R^2 \), which was the square of the sample correlation “R” between the predicted and therapeutic doses. Besides assessing the potential benefit of using the pharmacogenetic algorithm instead of the clinical algorithm, we computed the number needed to genotype (NNG: the number of patients who must be genotyped in order for 1 patient to have an improved dose estimate).

Furthermore, we evaluated the effects of alcohol drinking and smoking on warfarin dose requirements by multiple regression analysis in which 3 numerical models (2 categorical and 1 continuous trait models) were tested for each behavior.

**Results**
The characteristics of the 200 participants in the present study are summarized in Table 1. Among them, the most common indications for warfarin use were atrial fibrillation.

| Table 1. Characteristics of Study Subjects |
|--------------|------|
|             | n=200 |
| M/F, n       | 136/64 |
| Age at entry, years | 67.8±10.3 |
| Height, cm   | 160.3±14.6 |
| Body weight, kg | 61.4±12.7 |
| Daily warfarin dose, mg | 3.05±1.20 |
| Primary reason for anticoagulation, n (%) |
| Atrial fibrillation | 119 (59.5) |
| Prosthetic valve replacement | 27 (13.5) |
| Deep vein thrombosis/pulmonary embolism | 12 (6) |
| Other | 42 (21) |
| Amiodarone use, n (%) | 11 (5.5) |

VKORC1, vitamin K epoxide reductase complex subunit 1.

For the categorical smoking status. All subjects were Japanese.

### Methods

**Study Population**
A total of 200 eligible subjects were chosen from participants in the Hospital-Based Cohort Study in the International Medical Center of Japan (IMCJ), which was designed to investigate clinical epidemiology, pharmacogenetics and genetic susceptibility of lifestyle-related disorders such as diabetes, hypertension and cardiovascular diseases.14 We collected information on demographic characteristics, the primary indication for warfarin treatment, the stable therapeutic dose of warfarin, the treatment INR (the INR achieved with a stable warfarin dose), the use of concomitant enzyme inducers (carbamazepine, phenytoin, rifampin, or rifampicin) and amiodarone. Anticoagulation of patients was stably controlled with a target PT- INR of 1.6–2.6 for the prevention or treatment of thromboembolic diseases. Characteristics of the patients are shown in Table 1. We largely divided them into 3 categories of alcohol drinking (never-drinker; ex-drinker; current drinker) and 3 categories of smoking status (never-smoker; ex-smoker; current smoker). Participants were asked to report their daily alcohol consumption, using a structured questionnaire that ascertained the consumption of typical alcoholic beverages (beer, wine, Japanese sake, shochu and spirits). Alcohol intake was denoted in terms of servings of sake (1 gou [180 ml] of Japanese rice wine is considered equal to 22 g of ethanol). As a variable of smoking conditions, the Brinkman Index was calculated from [the number of cigarettes smoked daily]×[smoking period] in addition to the categorical smoking status. All subjects were Japanese.
The effects of predictor variables on warfarin dose were first examined in the ordinary regression model (Table 2). The effect sizes of individual variables thus estimated were applied to the Japanese patients’ data, the IWPC pharmacogenetic algorithm identified significantly (P<0.05) larger effects of predictor variables on warfarin dose were almost comparable to those in the IWPC algorithm. In the table comparing the predicted dose and actual dose of warfarin (R²=0.28, P=1.5×10⁻¹⁵) (Figure).

A significant benefit of using genetics was further verified with the NNG analysis (Table 4). The NNG can be computed using the number needed to treat (NNT) method; the NNT is the inverse of the absolute risk reduction (ARR). The ARR was calculated as the absolute difference between the event rate (ER) for the pharmacogenetic algorithm and the ER for the clinical algorithm (ER = ratio of the number of patients for which an algorithm estimates a poor dose (more or less than 7 mg/week than the actual therapeutic dose) over the total number of patients). Despite different criteria for a poor dose estimate (ie, the criteria in the IWPC study were >20% above or below the actual therapeutic dose), the NNG was in good agreement between the studies: 13.3 in the present study and 13.2 in the IWPC study.

The effects of predictor variables on warfarin dose were first examined in the ordinary regression model (Table 2). The effect sizes of individual variables thus estimated were almost comparable to those in the IWPC algorithm.

**Table 2. Effect Size of Predictors on Warfarin Dose Among Japanese Subjects**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Regression of warfarin dose</th>
<th>Effect in the IWPC algorithm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>4.940 (3.404, 6.477)</td>
<td>1.6E-09</td>
</tr>
<tr>
<td>Age in decades</td>
<td>−0.215 (−0.334, −0.095)</td>
<td>5.0E-04</td>
</tr>
<tr>
<td>Height in cm</td>
<td>0.001 (−0.009, 0.012)</td>
<td>0.82</td>
</tr>
<tr>
<td>Weight in kg</td>
<td>0.012 (−0.001, 0.025)</td>
<td>0.07</td>
</tr>
<tr>
<td>VKORC1 rs9923231 AG vs AA</td>
<td>0.862 (0.545, 1.178)</td>
<td>2.2E-07</td>
</tr>
<tr>
<td>CYP2C9 rs1057910</td>
<td>1.677 (0.714, 2.640)</td>
<td>7.3E-04</td>
</tr>
</tbody>
</table>

*n=119, 59.5%,* prosthetic valve replacement (n=27, 13.5%), and deep vein thrombosis or pulmonary embolism (n=12, 6%). The minor allele frequencies of rs1057910 (CYP2C9) and rs9923231 (VKORC1) were 0.013 and 0.093, respectively, which were comparable to those previously reported in Japanese patients or HapMap JPT (http://hapmap.ncbi.nlm.nih.gov/): 0.016–0.049 for rs1057910 and 0.075–0.088 for rs9923231. Each SNP was in Hardy-Weinberg equilibrium (P>0.05).

The effects of predictor variables on warfarin dose were first examined in the ordinary regression model (Table 2). The effect sizes of individual variables thus estimated were almost comparable to those in the IWPC algorithm. When applied to the Japanese patients’ data, the IWPC pharmacogenetic algorithm identified significantly (P<0.05) larger proportions of patients who required 10.5 mg or less per week (low-dose group) or those who required 31.5 mg or more per week (high-dose group) to achieve the target PT-INR than did the clinical algorithm (68% vs 36% in low-dose group; and 21% vs 0% in high-dose group; Table 3). We depicted the fair performance of the IWPC pharmacogenetic algorithm in the plots comparing the predicted dose and actual dose of warfarin (R²=0.28, P=1.5×10⁻¹⁵) (Figure).

A significant benefit of using genetics was further verified with the NNG analysis (Table 4). The NNG can be computed using the number needed to treat (NNT) method; the NNT is the inverse of the absolute risk reduction (ARR). The ARR was calculated as the absolute difference between the event rate (ER) for the pharmacogenetic algorithm and the ER for the clinical algorithm (ER = ratio of the number of patients for which an algorithm estimates a poor dose (more or less than 7 mg/week than the actual therapeutic dose) over the total number of patients). Despite different criteria for a poor dose estimate (ie, the criteria in the IWPC study were >20% above or below the actual therapeutic dose), the NNG was in good agreement between the studies: 13.3 in the present study and 13.2 in the IWPC study.

Our data on Japanese patients also indicated that both
alcohol drinking and smoking significantly influence warfarin dose (Table 5). In the multiple regression model, the current status of drinking or non-drinking (ex-drinker + never-drinker) and that of smoking or non-smoking (ex-smoker + never-smoker) exerted approximate warfarin dose effects of 4 mg/week (P=9.5×10^{-5}, R^2=0.06) and 3.3 mg/week (P=0.03, R^2=0.02), respectively. With these predictor variables being incorporated into the IWPC algorithm, its performance was augmented (R^2=0.33, P=5.5×10^{-19}) (Figure S2).

**Discussion**

We have evaluated the IWPC pharmacogenetic algorithm in 200 Japanese patients under low-dose warfarin treatment. Although the target PT-INR (1.6–2.6) in the present study was slightly lower than the range (2.0–3.0) set in the IWPC study, the performance of the tested algorithms in the 2 studies proved almost comparable: R^2=0.28 in the present study and R^2=0.33–0.34 for Asians in the IWPC study. Besides the reproducible performance in the whole study sample, of particular note is the fact that among patients in a low-dose group (≤10.5 mg/week), the percentage of overestimation was significantly smaller when the warfarin dose was predicted with the pharmacogenetic algorithm (32%) than with the clinical algorithm (64%) (Table 3), thus enabling us to appreciably reduce the risk of overanticoagulation.

Furthermore, we demonstrated the substantial influence of alcohol drinking and smoking on warfarin dose requirements, which used to be anticipated but has not been evaluated in detail thus far. The incidence of major bleeding (eg, intracranial hemorrhage) has been reported as higher in Japanese patients (6.6% per year) than in European patients (1.6–2.5% per year) with adjusted standard-dose warfarin therapy: a target PT-INR of 2.2–3.5 in the Japanese and 2.0–4.5 in Europeans. Because of such racial differences in bleeding tendency under warfarin, the necessity of customizing warfarin therapy has been argued. Recently, a prospective study of 4,202 patients

### Table 4. NNG Analysis: Clinical vs Pharmacogenetic Algorithm With ±7 mg/week Criterion

<table>
<thead>
<tr>
<th>compared with</th>
<th>no ±7 mg/week than actual</th>
<th>NNG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>21</td>
<td>13.3</td>
</tr>
<tr>
<td>Pharmacogenetic</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Absolute risk reduction</td>
<td>0.075</td>
<td></td>
</tr>
</tbody>
</table>

NNG, number needed to genotype.

### Table 5. Effects of Alcohol Drinking and Smoking on Warfarin Doses in Different Regression Models

<table>
<thead>
<tr>
<th>Model</th>
<th>Tested predictors</th>
<th>Approximate effect on warfarin dose in mg/week*</th>
<th>Effect (95%CI)</th>
<th>P value</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alcohol drinking</strong></td>
<td></td>
<td></td>
<td></td>
<td>---------</td>
<td>-----</td>
</tr>
<tr>
<td>Model 1</td>
<td>Stopped vs Yes</td>
<td>–4.1 (–6.6, –1.5)</td>
<td>–0.03</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>No vs Yes</td>
<td>–3.9 (–6.0, –1.7)</td>
<td>6.7E-04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 3</td>
<td>Stopped/No vs Yes</td>
<td>–4.0 (–5.8, –2.1)</td>
<td>9.5E-05</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
<td></td>
<td>---------</td>
<td>-----</td>
</tr>
<tr>
<td>Model 1</td>
<td>Stopped vs Yes</td>
<td>–3.5 (–6.4, –0.4)</td>
<td>0.03</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Model 2</td>
<td>No vs Yes</td>
<td>–2.9 (–6.0, 0.4)</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 3</td>
<td>Stopped/No vs Yes</td>
<td>–3.3 (–6.1, –0.3)</td>
<td>0.03</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brinkman index</td>
<td>–0.02 (–0.1, 0.1)</td>
<td>0.56</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

Alcohol drinking was categorized into 3 groups: current drinker (yes), abstainer (stopped), and never-drinker (no), based on the self-reported questionnaire. Likewise, smoking status was categorized into 3 groups: current smoker (yes), ex-smoker (stopped) and never-smoker (no).

*Predictors in Table 2 were included as covariates in the tested regression model.

†The square root of the value was used for the regression analysis.

CI, confidence interval.
showed an optimal PT-INR of 3.0–3.5 in the Dutch,23 whereas the Japanese Guidelines for Pharmacotherapy of Atrial Fibrillation24–26 (JCS 2008) have set a PT-INR of 2.0–3.0 as the therapeutic range, except for the elderly (>70 years of age), in whom a lower dose of warfarin (PT-INR 1.6–2.6) is recommended for prevention of thromboembolism and safety from bleeding complications.27 The intensity of warfarin control (ie, optimal PT-INR) in the Japanese remains to be further defined according to the balance between risks and benefits under individual conditions. Among the primary indications for warfarin use, the optimal therapeutic range has been debated for patients with prosthetic valve replacement,13 corresponding to 13.5% of the current subjects (Table 1).

Partly because of the risk of eventual valve failure of bioprosthesis, and resultant reoperation, there seems to be a tendency for increased use of prosthetic valves in Japan, with its population’s long life expectancy, as compared with the USA and Europe. Including patients with prosthetic valve replacement, because the target PT-INR is often set at 1.6–2.6 in outpatient clinics in Japan,3 our findings obtained in equivalent clinical setting should encourage clinicians to apply the IWPC algorithm to their patients. Nevertheless, in cases where the optimal therapeutic range is set differentially according to the primary disease condition, some modification of the IWPC algorithm may be required.

We have found that the average dose of warfarin in the Japanese patients is 21 mg/week (3 mg/day), which is less than the standard dose (35 mg/week) in Europeans, and in the present study one-eighth (12.5%) of the participants were categorized into a low-dose group (≤10.5 mg/week). If a fixed dose of 3 mg/day is given to these patients without conscientious monitoring of PT-INR, there is a high risk of overanticoagulation, leading to fatal bleeding events. The use of the IWPC algorithm will enable clinicians to detect approximately two-thirds (68%) of the Japanese patients in this low-dose group, which is twice as large as the proportions (36%) attainable with the clinical algorithm (Table 3). Although the value of adding genotype to clinical (and demographic) information seems to be less modest, benefits also accrue to Japanese patients in the high-dose group (≥31.5 mg/week); 21% of the patients were identified with the pharmacogenetic algorithm, but none (0%) with the clinical algorithm (Table 3).

Since the US FDA changed the labeling of warfarin to suggest that clinicians consider using genetic tests to guide dosing,3 warfarin pharmacogenetics has drawn substantial attention towards “personalized” patient care. Although more than 30 genes may contribute to the net warfarin effect, CYP2C9 and VKORC1 are known to exert the most influence.28–30 The pharmacogenetic algorithm involving these polymorphisms, developed by the IWPC, can predict approximately one-third of all dosing variations at most.8 The question of whether the knowledge of genetic information is cost-effective in reducing bleeding and thrombotic complications is under debate.31 Many clinical factors influence warfarin dose requirements, including diet (in particular, the vitamin K content) and concomitant drug administration, besides a list of variables that have been incorporated into the IWPC algorithm. As the clinical factors often change in individual patients, appropriate alterations in warfarin dosing must be made, regardless of genetic information. In this respect, it is important to quantitatively evaluate the individual contribution of clinical factors, as has been performed for alcohol drinking and smoking in the present study, toward refining warfarin pharmacogenetic testing for not only initial but also maintenance dose requirements.

In summary, we report the usefulness of the IWPC pharmacogenetic algorithm in its clinical application, particularly for identifying Japanese patients who require a low dose of warfarin. However, it has to be kept in mind that considering the current limitations of its application to clinical medicine, this testing alone does not make conscientious PT-INR monitoring unnecessary. When the genotype cost falls to a more reasonable level, we expect that the use of pharmacogenetic-based initial dosing will become routine clinical practice.

Acknowledgments

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