Interactive Modeling for Ongoing Utility of Pharmacogenetic Diagnostic Testing: Application for Warfarin Therapy

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BACKGROUND: The application of pharmacogenetic results requires demonstrable correlations between a test result and an indicated specific course of action. We developed a computational decision-support tool that combines patient-specific genotype and phenotype information to provide strategic dosage guidance. This tool, through estimating quantitative and temporal parameters associated with the metabolism- and concentration-dependent response to warfarin, provides the necessary patient-specific context for interpreting international normalized ratio (INR) measurements.

METHODS: We analyzed clinical information, plasma S-warfarin concentration, and CYP2C9 (cytochrome P450, family 2, subfamily C, polypeptide 9) and VKORC1 (vitamin K epoxide reductase complex, subunit 1) genotypes for 137 patients with stable INRs. Plasma S-warfarin concentrations were evaluated by VKORC1 genotype (H11002G/H11022A). The steady-state plasma S-warfarin concentration was calculated with CYP2C9 genotype–based clearance rates and compared with actual measurements.

RESULTS: The plasma S-warfarin concentration required to yield the target INR response is significantly (P < 0.05) associated with VKORC1 genotype (GG, 0.68 mg/L; AG, 0.48 mg/L; AA, 0.27 mg/L). Modeling of the plasma S-warfarin concentration according to CYP2C9 genotype predicted 58% of the variation in measured S-warfarin concentration: Measured [S-warfarin] = 0.67(Estimated [S-warfarin]) + 0.16 mg/L.

CONCLUSIONS: The target interval of plasma S-warfarin concentration required to yield a therapeutic INR can be predicted from the VKORC1 genotype (pharmacodynamics), and the progressive changes in S-warfarin concentration after repeated daily dosing can be predicted from the CYP2C9 genotype (pharmacokinetics). Combining the application of multivariate equations for estimating the maintenance dose with genotype-guided pharmacokinetics/pharmacodynamics modeling provides a powerful tool for maximizing the value of CYP2C9 and VKORC1 test results for ongoing application to patient care.

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A rapid and accurate computational method for modeling the anticipated response to a drug in a specific patient would provide health care practitioners with a basis for establishing individualized therapeutic strategies. In principle, pharmacogenetics provides a mechanism-based approach for optimizing the selection and dosing of medications for a specific individual (1). As with other diagnostic tests in laboratory medicine, the application of pharmacogenetics results to general health care requires demonstrable correlations between a test result and an indicated specific course of action by a health care professional. The oral anticoagulant warfarin, one of few examples in which a genetic basis for variation in both pharmacokinetic and pharmacodynamic behavior has been identified, serves as an excellent example for proof of principle for this approach.

As of 2004, according to Wysowski et al. (2), nearly 31 million Americans had been prescribed warfarin on an outpatient basis. The correct maintenance dose for an individual cannot be anticipated a priori; it ranges from <1 mg/day to >20 mg/day (median, approximately 5 mg/day). The recommended approach to initiating dosing is to begin with doses between 5 mg and 10 mg for the first 1–2 days, with subsequent dos-
ing based on the response of the international normalized ratio (INR)\(^7\) (3). The greatest risk for bleeding events occurs during the initiation phase of therapy (4, 5). Adverse events associated with warfarin therapy are common (6), with incidences of 17.3% among patients 65 years of age and older (7) and 6.2% among the general population (8).

Recent efforts have identified 2 genes, CYP2C9\(^8\) (cytochrome P450, family 2, subfamily C, polypeptide 9) and VKORC1 (vitamin K epoxide reductase complex, subunit 1), that have been estimated to account for 35%–40% of the variation in the warfarin dose requirement (9–12). The clearance of S-warfarin is diminished in the presence of the CYP2C9*2 and CYP2C9*3 alleles (13–15). With a decreased clearance, standard daily dosing leads to an increase in the S-warfarin concentration and an increase in the time required to reach the steady state. Furthermore, individuals with variant CYP2C9 alleles will require lower maintenance dosages and will demonstrate a delayed transition to a stable response following dosage adjustments. Failure to recognize this delay will lead to misinterpretation of INR measurements (16). The −1639G>A single-nucleotide polymorphism in the VKORC1 gene is correlated with decreased VKORC1 expression (17) and a lower therapeutic interval for the steady-state plasma S-warfarin concentration (12). Consequently, patients with the VKORC1 −1639GG genotype tend to require higher doses than patients with the GA or AA genotype (12). Genetic differences in CYP2C9 and VKORC1 combined with such patient factors as age, body weight, and sex account for 45%–61% of the variation in the daily maintenance dose of warfarin. These factors have been used to derive mathematical equations for estimating the patient’s maintenance dose (12, 18–30). Although the prospective application of such equations has yielded some improvement in patient outcomes in a limited number of studies (18, 31), they have failed to provide clear and ongoing guidance for the use of such information during the various stages of warfarin therapy. To present a dynamic approach for guiding warfarin therapy based on CYP2C9 and VKORC1 genotyping, our group has conceived of a personalized medicine interface tool (PerMIT:warfarin). This tool calculates the estimated maintenance dose, graphically displays the predicted progression of plasma S-warfarin concentrations from the initiation of therapy to the steady state with reference to the VKORC1-estimated target and the steady-state S-warfarin concentration, thereby providing a time-based estimate of the INR response. Our goals were to confirm our previous observation that the relationship between the blood S-warfarin concentration and the response measured by the INR depends on the VKORC1 −1639G>A genotype, to design a dynamic decision-support tool, and to validate the accuracy of this tool through testing of an independent population data set.

Materials and Methods

Patients

The 142 predominantly Caucasian outpatients enrolled in this study were receiving maintenance warfarin therapy and had stable INR values between 2.0 and 3.0. Stable therapy was defined by the care provider and in general involved consistent therapeutic INR measurements without the necessity for a dosage modification over a period of >2 weeks. Five patients were excluded from the analysis. We excluded 3 of these patients because their volume of distribution (\(V_D\)) for S-warfarin exceeded the 97th percentile of their population and led us to question the accuracy of the data for these patients. The \(V_D\) for 1 patient was <0.038 L/kg, and the values for the other 2 patients were >0.45 L/kg. We excluded the remaining 2 patients on the basis of their CYP2C9*1/*5 genotype. The remaining 137 patients were used in the final analysis. Patients were followed at the Barnes–Jewish Hospital’s Blood Thinner Clinic in St. Louis, Missouri. The Investigational Review Board of the Washington University School of Medicine approved this protocol. All patients provided written informed consent in accordance with the Declaration of Helsinki.

Age, sex, height, weight, lean body weight (LBW), body surface area (BSA), \(V_D\), and warfarin dose were recorded for each patient (Table 1). BSA was calculated with the formula of Dubois and Dubois: \(\text{BSA} = 0.20247 \times (\text{height})^{0.725} \times (\text{weight})^{0.425}\) (32). The \(V_D\) of S-warfarin was calculated as follows: \(V_D = \text{warfarin dose} \times \text{weight}^{-1} \times [\text{plasma S-warfarin}]^{-1}\). LBW was calculated for male and female individuals as follows: \(\text{LBW}_{\text{male}} = (1.10 \times \text{weight}) - [128 \times (\text{weight}^2/(100 \times \text{height})^2)]\); \(\text{LBW}_{\text{female}} = (1.07 \times \text{weight}) - [148 \times (\text{weight}^2/(100 \times \text{height})^2)]\) (33).

Genotyping

Each sample was genotyped for single-nucleotide polymorphisms in the CYP2C9 (∗2, rs1799853; ∗3, rs1057910; ∗5; ∗6; ∗11) and VKORC1 (−1639G>A, rs9923231) genes as previously described (34).
MEASUREMENT OF PLASMA S-WARFARIN CONCENTRATION

Plasma S-warfarin concentrations were measured by HPLC with ultraviolet detection (35). The interval of assay linearity for both warfarin enantiomers was 0.1–5.0 mg/L, CVs were <15% at 0.5 mg/L and 2.0 mg/L, and the S-warfarin limit of quantification was 0.1 mg/L.

MODELING OF PLASMA S-WARFARIN CONCENTRATION

Plasma S-warfarin concentration was modeled with the following equations (36):

1. The initial peak following the first dose,

\[ C_{\text{max initial}} = \text{dose} \times \left( \frac{s \times f}{V_D \times \text{kg}} \right); \]

2. The minimum concentration just before the next dose,

\[ C_{\text{min}} = \frac{C_{\text{max}} \times 10^{-0.3(x/f_{12})}}{2}; \]

3. The subsequent maximum concentration after each successive dose,

\[ C_{\text{max}} = C_{\text{min}} + \text{dose} \times \left( \frac{s \times f}{V_D \times \text{kg}} \right). \]

\( C_{\text{max initial}} \) is the initial peak concentration; \( C_{\text{max}} \) is the peak concentration subsequent to the initial peak; \( C_{\text{min}} \) is the trough concentration; \( s \) is the fraction of S-warfarin and is equal to 0.5; \( f \) is the bioavailability and is equal to 1; \( V_D \) is the apparent \( V_D \) and is set to 0.1 L/kg; \( \text{kg} \) is the weight in kilograms; \( \lambda \) is the dose interval; and \( f_{12} \) is the mean elimination half-life of S-warfarin (set for each CYP2C9 genotype according to results described in multiple published reports: CYP2C9*1/*1, 30 h; CYP2C9*1/*2, 38 h; CYP2C9*1/*3, 51 h; CYP2C9*2/*2, 61 h; CYP2C9*2/*3, 76 h; CYP2C9*3/*3, 203 h). For the CYP2C9*1/*1 genotype, the mean S-warfarin clearance rate is 0.314 L/h, which corresponds to a mean elimination half-life of approximately 30 h. The mean S-warfarin elimination half-lives in patients with CYP2C9 variant alleles were derived from the percent reduction in metabolic clearance for each genotype, as described by Caraco et al. (31) and from supporting evidence of Hamberg et al. (37) and others (13, 14, 23, 38).

STATISTICAL ANALYSIS

Statistical analysis was performed with SigmaStat® 3.5 statistical software (Systat Software). Because the age, weight, height, LBW, warfarin dose, and VD data failed the Kolmogorov–Smirnov test for normality, these data were presented as the median and the interquartile range (25th–75th percentiles). The BSA data passed the Kolmogorov–Smirnov test for normality and were therefore presented as the mean and SD. Because the data for steady-state plasma S-warfarin concentration vs VKORC1 genotype failed the test for normality, we performed Kruskal–Wallis 1-way ANOVA on ranks followed by the Dunn method of adjustment for pairwise multiple comparisons.

Results

Seven different genotypes were detected among the patients evaluated for the CYP2C9 polymorphism; however, the 2 patients with the CYP2C9*1/*5 genotype were not included in the final analysis. Seventy patients were homozygous for the reference allele (*1/*1), and 61 patients carried only 1 of the variant alleles (n = 44 for *1/*2; n = 17 for *1/*3). Five patients were homozygous for variant alleles (n = 4 for *2/*2; n = 1 for *3/*3). One patient was compound heterozygous (*2/*3). Patients also were genotyped for the VKORC1 G>A polymorphism at position −1639. Fifty-five patients were homozygous for the reference allele (GG, 40%), 66 patients were heterozygous for the variant (AG, 48%), and 16 patients were homozygous for the variant (AA, 12%).

RELATIONSHIP BETWEEN VKORC1 −1639 GENOTYPE AND PLASMA S-WARFARIN CONCENTRATION

The median plasma S-warfarin concentration was 0.68 mg/L (interquartile range, 0.56–0.89) for the GG genotype, 0.48 mg/L (interquartile range, 0.39–0.62) for the AG genotype, and 0.27 mg/L (interquartile range, 0.15–0.42) for the AA genotype (Fig. 1). A Kruskal–Wallis 1-way ANOVA on ranks revealed statistically significant differences (P < 0.001) between all of the VKORC1 −1639 genotypes with respect to plasma S-warfarin concentration. As expected, individuals with the VKORC1 −1639GG genotype required a sig-

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**Table 1. Demographics of the study population.**

<table>
<thead>
<tr>
<th>Sex, n</th>
<th>Number (%)</th>
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</thead>
<tbody>
<tr>
<td>Male</td>
<td>80 (58%)</td>
</tr>
<tr>
<td>Female</td>
<td>57 (42%)</td>
</tr>
</tbody>
</table>

Age, years 75 (67–80)

Weight, kg 80 (68–94)

Height, m 1.7 (1.6–1.8)

LBW, kg 56 (47–65)

BSA, m² 1.9 (0.25)

Warfarin dose, mg/day 4.4 (3–6)

V₀ of S-warfarin, L/kg 0.10 (0.070–0.14)

* Data not normally distributed according to the Kolmogorov–Smirnov test for normality are presented as the median (25th–75th percentiles). BSA data were normally distributed and are presented as the mean (SD).
nificantly higher daily maintenance S-warfarin dose (5.2 mg/day) than the AG genotype (4.1 mg/day) and the AA genotype (2.4 mg/day). There were no statistically significant differences ($P = 0.057$) among the VKORC1/1639 genotypes with respect to S-warfarin clearance (data not shown).

ESTIMATING THE WARFARIN MAINTENANCE DOSE
We previously used multiple regression analysis to develop an equation for estimating the mean daily warfarin maintenance dose on the basis of VKORC1/1639 and CYP2C9 genotypes, age, sex, and body weight that accounted for up to 61% of the variance in warfarin maintenance dose (12, 39). We calculated the daily warfarin maintenance doses for each patient in this cohort and compared them with actual recorded mean daily maintenance doses. Linear regression analysis of the correlation between the estimated and actual recorded mean daily maintenance doses produced a slope of 0.72 [standard error of the estimate ($S_{y|x}$), 0.053; $P < 0.0001$], and a y intercept of 1.4 mg/day ($S_{y|x}$, 0.053 mg/day; $P < 0.0001$). The actual warfarin maintenance dose was positively correlated with the estimated warfarin maintenance dose ($r = 0.76$; Fig. 2). Fifty-eight percent of the variance in the actual daily dose was explained by the variance displayed in the estimated mean daily dose (i.e., $r^2 = 0.58$).

COMPARISON OF THE ESTIMATED AND MEASURED PLASMA S-WARFARIN CONCENTRATIONS
We next assessed the reliability of modeling the plasma S-warfarin concentration based on S-warfarin elimination adjusted for the CYP2C9 genotype (13–15, 23). The estimated plasma S-warfarin concentration was analyzed by its correlation with the measured plasma S-warfarin concentration at the time of a therapeutic INR. Ordinary linear regression analysis produced a slope of 0.67 ($S_{y|x}$, 0.059; $P < 0.0001$) and a y intercept of 0.16 mg/L ($S_{y|x}$, 0.038 mg/L; $P < 0.0001$), with an $r^2$ value of 0.58 (Fig. 3). The mean absolute difference between estimated and measured concentrations is 0.14 mg/L, and 58% of the estimated values are within 25% of the measured values.

CASE STUDIES
We have modeled the predicted S-warfarin concentration–time profile with respect to the warfarin daily-dosing history and INR response of a 40-year-old African American woman with the CYP2C9*1/*3 and VKORC1 –1639AG genotype, which were published
in a case report by LaSala et al. (40). Fig. 4A illustrates the history of the measured INR response along with the plasma concentrations of $S$-warfarin estimated with our PerMIT model incorporating the $S$-warfarin $t_{1/2}$ associated with $CYP2C9^{*1/*3}$. On the basis of this individual’s clinical and genetic characteristics, we estimated the mean daily maintenance dose to be 3.5 mg/day. This patient’s therapeutic target $S$-warfarin concentration estimated from her $VKORC1$ $-1639AG$ genotype is 0.48 mg/L (interquartile range, 0.39–0.62 mg/L) (Fig. 2). The reported dosing history for this patient is presented in Table 2. The predicted target concentration range for this patient is exceeded within the first two 5-mg doses, whereas overdosing of this patient is not revealed by INR monitoring until an INR of 3.3 is obtained on day 8. Note that the INR measurement made on day 8 is before the estimated time to reach the steady state, and although this measurement is slightly above the target INR interval (2.0–3.0), it underestimates the degree of overdosing, which is not fully reflected until the INR measurement of 5.3 is made on day 20. Of interest is that although the dose had been decreased 5 days earlier, the INR obtained on day 20 has continued to increase from 4.9. The INR thus serves as a poor indicator with respect to the eventual impact of the preceding dosage change because this measurement again was made before a steady-state $S$-warfarin concentration was achieved at the adjusted dosage. This illustration demonstrates the value of interpreting INR measurements in light of the temporal status of the plasma $S$-warfarin concentration and demonstrates the delay in INR response relative to changing plasma $S$-warfarin concentrations. This example illustrates the difficulties in managing patients that can occur when the pharmacokinetic and pharmacodynamic characteristics of the patient are unknown.

In contrast, the case illustrated in Fig. 4B demonstrates the prospective application of this approach to avoid overcompensation in dosing. A 55-year-old inpatient was referred to our anticoagulation clinic for...
Table 2. Actual dosing schedules described in case reports.

<table>
<thead>
<tr>
<th>Dosing</th>
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<tr>
<td>Case 1. Guided by INR monitoring only (Fig. 4A)</td>
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<tr>
<td>Day 1</td>
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<tr>
<td>Day 2</td>
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<tr>
<td>Day 3</td>
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<tr>
<td>Days 4–9</td>
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<td>Days 10–16</td>
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<tr>
<td>Days 17–22</td>
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<tr>
<td>Days 23–48</td>
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<tr>
<td>Days 49–90</td>
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<tr>
<td>Case 2. Guided by INR monitoring and genotype (Fig. 4B)</td>
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<tr>
<td>Day 1</td>
</tr>
<tr>
<td>Days 2–3</td>
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<tr>
<td>Days 4–8</td>
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<tr>
<td>Days 9–22</td>
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</table>

Discussion

Our overall aim was to validate the mathematical models chosen to support our clinical decision–support tool. We developed the model at one institution (University of Louisville) and validated it with data from another (Washington University School of Medicine). The critical characteristics evaluated were the relationship between the VKORC1 −1639G>A genotype and the plasma S-warfarin concentration under stable therapeutic conditions, estimation of the maintenance dose in an independent data set, and pharmacokinetic modeling of the plasma concentration of S-warfarin based on adjustments for the CYP2C9 genotype.

In an earlier report (12), we described a trend for increased plasma S-warfarin concentrations with the number of VKORC1 −1639G alleles; however, until the present study we lacked sufficient numbers of patients to demonstrate a statistically significant difference between each of the 3 VKORC1 genotypes. In the current study, we have confirmed our original observation (12) and have demonstrated significant different median plasma S-warfarin concentrations for each of the VKORC1 genotypes. The data obtained from each cohort yielded similar measures of the therapeutic plasma S-warfarin concentration for each of the 3 genotypes: VKORC1 −1639GG [mean (SD) of original study, 0.73 mg/L (0.33 mg/L); median (interquartile range) of current study, 0.68 mg/L (0.56–0.89 mg/L)], VKORC1 −1639AG [original, 0.54 mg/L (0.21 mg/L); current, 0.48 mg/L (0.39–0.62 mg/L)], and VKORC1 −1639AA [original, 0.48 mg/L (0.04 mg/L); current, 0.27 mg/L (0.15–0.42 mg/L)]. The observed consistency is important because it provides mechanistic evidence for the effect of this polymorphism on warfarin requirements. Furthermore, these data begin to describe a “therapeutic” interval for plasma concentrations of S-warfarin for patients whose VKORC1 −1639G>A genotype is known and whose target INR is between 2.0 and 3.0.

Also described in our earlier report (12) is the development of an equation for estimating the warfarin maintenance dose from clinical and genetic factors, which was subsequently validated (39). In this independent data set, this equation accounted for 58% of the variation in warfarin maintenance dose while demonstrating a minimal negative proportional bias (slope, 0.72) and a positive constant bias of 0.14 mg/L. This equation has subsequently been modified and introduced into PerMIT to include correction factors for both smoking and alternative target INR intervals. These changes do not alter the dosage estimates for the 2 cases described above because both patients were treated with a target INR of 2.5 and because neither patient was reported to use nicotine.

Genotyping and warfarin management after he had experienced a pulmonary embolism. The patient was 180 cm (71 in) tall and weighed 118 kg. Before genotyping, he had received 4 warfarin doses (5, 6, 6, and 7 mg), and his INR was 1.39 early the next morning. The patient’s genotype was CYP2C9*1/*2 and VKORC1 −1639GG. We anticipated the pharmacokinetic delay in reaching the steady state from the CYP2C9*1/*2 genotype and the pharmacodynamic resistance from the VKORC1 −1639GG genotype. After receiving five 7-mg doses (8 doses total), the patient presented to our clinic with an INR of 1.7. From a clinical point of view, we would have increased his warfarin dose, but because we knew that his warfarin concentrations were still rising (Fig. 4B), we continued dosing at 7 mg/day. We checked the INR after 6 more doses, and it was 2.0. We continued S-warfarin administration at 7 mg/day, and his INR was 2.5 after 8 additional doses. His subsequent mean INR values (still at 7 mg/day) were 2.83. Thus, our understanding of this patient’s status allowed us to avoid unnecessarily increasing the dose and thereby avoided further readjustment. This scenario illustrates one of many potential scenarios in which both the dosing and the monitoring practice can be strategically guided by placing the dosing and response information into the appropriate temporal context for the individual patient.
An important aspect of this tool is the ability to model the anticipated plasma S-warfarin concentrations after repeated dosing. To accomplish this functionality, we used traditional single-compartment, multiple-dose pharmacokinetic equations (36) driven by published values of S-warfarin clearance and elimination half-lives for each CYP2C9 genotype (31, 38). We evaluated the accuracy of this tool by comparing the calculated plasma concentration of S-warfarin to the measured S-warfarin concentration for each individual. We found the accuracy of plasma S-warfarin estimates to be comparable to the accuracy of maintenance dose estimation ($r^2 = 0.58$). The importance of this observation is that these data support the utility of published measures of S-warfarin elimination $t_{1/2}$ values for the respective CYP2C9 genotypes for incorporation into a linear pharmacokinetic model. Such models are well-established techniques for describing the sequential peak and trough concentrations of drugs caused by multiple dosing. Therefore, we can be fairly confident that the resulting drug-concentration profile generated by our tool is a reasonable approximation of the actual S-warfarin blood concentrations in each of our patients.

This data set has several limitations that should be mentioned. The validation data set is predominantly from a Caucasian population, and thus its accuracy for use with other ethnic groups cannot be assumed beyond previously demonstrated similarities or differences. Patients who took alternating doses of warfarin would have introduced random error into the measurement of the therapeutic plasma concentrations of S-warfarin, and data regarding the number of hours between the last warfarin dose and measurement of warfarin concentration were not available.

These data serve to demonstrate the feasibility of exactly modeling values of the plasma S-warfarin concentration after multiple dosing. The importance of this characteristic to a clinical decision-support tool is the mapping of the temporal changes in concentration as dosing progresses toward the steady state. This function serves the purpose of guiding both the dosing and monitoring processes and has the additional value of guiding strategies during periods in which dosing may be either accidentally or purposely suspended.

This tool is not intended to replace INR monitoring or the professional judgment of experienced clinicians, but it is intended to create an interactive framework in which the influence of genetic variation can be managed in a proactive and ongoing fashion beyond providing an estimate of a maintenance dose at a single point in time. We have demonstrated that the configuration of this tool provides an estimate of the individual’s mean daily maintenance dose, in addition to estimates of the plasma S-warfarin concentrations obtained from the actual dosing history of the patient. Finally, we have reproduced and strengthened our original finding that the steady-state S-warfarin concentration in plasma required to elicit a therapeutic INR (range, 2.0–3.0), may be estimated from knowledge of the VKORC1 $\rightarrow$ 1639G>A genotype.

**Author Contributions:** All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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**Other:** The University of Louisville has applied for patents based on the approach reported here for broad clinical applications of pharmacogenetic diagnostics (filing no. 60/798,855; May 9, 2006) (M.W. Linder and R. Valdes, Jr., inventors). An exclusive option to license this technology has been granted to PGxL Laboratories, LLC, Louisville, KY (http://www.permitwarfarin.com).

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