Relative contribution of CYP2C9 and VKORC1 genotypes and early INR response to the prediction of warfarin sensitivity during initiation of therapy

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Short title: Genes, early INRs, and warfarin sensitivity

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Scientific category: Hemostasis, thrombosis, and vascular biology
Abstract

Genetic variants in CYP2C9 and VKORC1 strongly affect steady-state warfarin dose. However, these variants also affect early INR values during warfarin initiation. We examined whether CYP2C9/VKORC1 genotypes provide information about warfarin sensitivity additional to that provided by early INR responses. In 214 patients starting warfarin with INR-guided dose adjustments, we determined whether CYP2C9 and VKORC1 genotypes were associated with early measures of warfarin sensitivity (time to INR ≥ lower limit of therapeutic range; time to INR > 4; and first stable warfarin dose) after adjusting for early (day 4-6) and week 1 (day 7-9) INR values. Early INRs were associated with all outcomes (all P<0.001) and were more informative than genotypes. For time-to-INR ≥ lower-limit-of-therapeutic-range, adding either early INRs or genotypes to a baseline model (clinical variables only) increased the goodness-of-fit (R^2) from 0.05 to 0.42 and 0.19, respectively (full model, R^2=0.46). For first-stable-warfarin-dose, adding either early INRs or genotypes to the baseline model increased the R^2 from 0.08 to 0.32 and 0.27, respectively (full model, R^2=0.40). After inclusion of week 1 INRs, CYP2C9 (P=0.08) and VKORC1 (P=0.30) were not associated with stable warfarin dose. Thus, much of the information provided by CYP2C9 and VKORC1 genotypes during warfarin initiation is captured by the early INR response.
Introduction

Oral anticoagulation with warfarin is the most common therapy for the treatment and prevention of thromboembolic events. However, warfarin therapy is associated with considerable morbidity and mortality because it has a narrow therapeutic range and at least 10-fold interindividual variability in drug sensitivity. Warfarin therapy is monitored and the dose adjusted according to its pharmacodynamic effects on clotting, measured as the international normalized ratio (INR). The INR value is closely related to the risk of thrombosis with underanticoagulation, and that of bleeding with overanticoagulation.1-3

Warfarin therapy is usually started with initial doses of 5-10 mg on the first two to three days, and subsequently titrated according to the INR response with the goal of achieving an INR within the therapeutic range within 4-6 days.3 Since warfarin dose requirements vary widely, this standard regimen often results in subtherapeutic INR values in more resistant patients, and overshooting the therapeutic range in more sensitive patients. The risk of adverse effects associated with over-anticoagulation is high, especially in the first few weeks of therapy.2,4,5 Therefore, strategies to individualize the initial warfarin dose have been sought.

Several factors are associated with an individual’s sensitivity to warfarin, and thus the dose required for stable anticoagulation. These include age, ethnicity, comorbidities, concomitant medication, and genetic variation.6-19 Two genes have been extensively studied: CYP2C9, encoding the enzyme (cytochrome P450 2C9) primarily responsible for the metabolism of the more active S-enantiomer of warfarin, and VKORC1, encoding the subunit 1 of the vitamin K epoxide reductase complex, the target of warfarin. Variants in these two genes affect warfarin dose requirements at steady state substantially;6-17,20,21
moreover, at the start of warfarin therapy, \textit{VKORC1} and, to a lesser extent, \textit{CYP2C9} genotypes also affect early INR responses.\textsuperscript{22,23}

The clinically useful contribution of genotype to individualizing warfarin dose will likely be greatest in the initiation phase of therapy, before an individual’s dose requirement has been determined empirically by titration according to INR response. However, the genetic contribution to warfarin dose requirements has most often been studied using steady-state dose requirements in patients already receiving stable doses of warfarin, without considering the information provided by the early INR responses during initiation of warfarin therapy. In fact, in a recent study in orthopedic patients starting genotype-guided warfarin therapy, \textit{VKORC1} genotype did not contribute significantly to the prediction of early stable warfarin dose when the INR value on the 4\textsuperscript{th} day of treatment was included in the prediction model.\textsuperscript{24}

In 2007, the Food and Drug Administration updated the warfarin label to include information for prescribers about genotype,\textsuperscript{25} and there has been debate about the introduction of genotype-guided warfarin dosing into clinical practice.\textsuperscript{26-30} A fundamental question is whether genotyping provides information about warfarin sensitivity that is additional to that provided by the early INR response.\textsuperscript{29} This question is especially important since early INR values are routinely determined in current clinical practice, whereas genotyping before starting therapy would require extra time and cost. We therefore examined the hypothesis that \textit{VKORC1} and \textit{CYP2C9} genotypes predict warfarin sensitivity after taking into account the information provided by early INR responses.
Methods

**Study design and subjects** Details of the study design and patient recruitment have been published.\(^2\) Briefly, this was a prospective, observational study conducted between 2002 and 2004 at three outpatient clinics (Pharmacy, Cardiology, and Arthritis and Joint Replacement Center) at Vanderbilt University Medical Center that provide anticoagulation services. The study was approved by the Vanderbilt University Institutional Review Board, and all subjects gave written informed consent in accordance with the Declaration of Helsinki. Consecutive patients starting warfarin were eligible if they were older than 18 years, had complete information on doses and dates of warfarin initiation, did not suffer from alcoholism, and were not receiving cancer chemotherapy. Ethnicity was determined by self report. Since the great majority of patients were African-Americans or Caucasians, we excluded patients of other ethnicities from analysis. The initial INR response is often measured on day 4 of warfarin treatment (i.e., after three daily doses); however, in practice, a substantial fraction of patients will have their initial INR response measured on day 5 or 6 due to logistic reasons. Thus, we studied subjects who had at least one INR value determined on days 4-6 of warfarin treatment.

**Warfarin anticoagulation** The individual target INR range, frequency of INR measurements, and warfarin dosing were determined by the treating physicians, who were unaware of *CYP2C9* or *VKORC1* genotypes during the whole treatment period.

**Data collection and follow-up** Demographic characteristics (age, sex, ethnicity) and details of medical history (concomitant diseases and medications) and warfarin therapy (indication, start date, range of therapeutic INR, initial and subsequent doses and
INR values) were obtained through patient interviews and a review of electronic medical records by a trained study nurse. A venous blood sample was drawn for DNA extraction. Information on warfarin dose and INR measurements was collected from the initiation of warfarin until the end of continuous follow-up, defined as date of the last record for an INR and warfarin dose, provided that previous records were available at intervals <60 days.

**Genotyping**  
*CYP2C9* *2* and *3* alleles were determined by a fluorescent allele-specific oligonucleotide ligation assay.\(^{22}\) *VKORC1* genotyping for the variants G-1639A [rs17878363], T497G [rs17882154], C1173T [rs9934438], G1542C [rs17886369], C2255T [rs2359612]) was performed by TaqMan SNP assays with the 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA).

**Outcomes**  
To assess the contribution of genotypes and early INR values to the early phase of anticoagulation, we assessed the following outcomes: (a) time to INR ≥ the lower limit of the therapeutic range; (b) time to first INR greater than 4.0; (c) first stable warfarin dose, defined as the first unchanged daily dose given for ≥7 consecutive days that yielded an INR value within the therapeutic range.\(^{24}\)

**Data analysis and statistics**  
For *VKORC1*, group A haplotype was assigned by using the statistical software PowerMarker based on the 5 variants as described.\(^{22}\) All other haplotypes, which in white Americans belong almost entirely to haplotype group B, were termed haplotype non-A. For the analysis, *VKORC1* genotype was coded as numbers of copies (0-2) of haplotype A, and *CYP2C9* genotypes were coded as number of variant alleles (0-2), assuming additive allele effects.
Patients with at least one INR value on days 4-6 of warfarin treatment (day 1 = day of first warfarin dose) were included (n=214). For analysis, we created a variable for the first recorded INR value after three warfarin doses, \( \text{INR}_{\text{early}} \) (for 162 patients on day 4, 27 on day 5, and 25 on day 6). We also examined the additional clinical benefit of considering information provided by subsequent INR values after the first week of treatment for the 145 patients who also had at least one INR value on days 7-9. Analogous to \( \text{INR}_{\text{early}} \), \( \text{INR}_{\text{week1}} \) represented the earliest available INR after a week of treatment (for 63 patients on day 7, 34 on day 8, and 48 on day 9). In secondary analyses, we also replaced the variable \( \text{INR}_{\text{early}} \) by three variables: \( \text{INR}_4 \) (set equal to the INR value on day 4 if available, and 0 otherwise), \( \text{INR}_5 \) (set equal to the INR value on day 5 if available but 0 if \( \text{INR}_4 \) available or \( \text{INR}_5 \) unavailable), and \( \text{INR}_6 \) (day 6 INR value if available but 0 if either \( \text{INR}_4 \) or \( \text{INR}_5 \) available). This allowed us to evaluate the effects of \( \text{INR}_4 \), \( \text{INR}_5 \), and \( \text{INR}_6 \) separately. Similarly, we defined variables \( \text{INR}_7 \), \( \text{INR}_8 \), and \( \text{INR}_9 \) to replace \( \text{INR}_{\text{week1}} \) to evaluate their effects separately. Cumulative warfarin dose was calculated as the sum of all warfarin doses from day 1 to the day before the respective INR measurement.

To assess the effect of early INR response and genotypes on the outcomes, we performed Cox regression analyses to determine the hazard ratio (HR) of achieving (1) an \( \text{INR} \geq \) the lower limit of the therapeutic range (2) a first \( \text{INR} > 4 \). We also performed multiple linear regression analyses to assess the effect of genotypes and early INR values on the first stable warfarin dose. All models were adjusted for age, sex, ethnicity, use of amiodarone, target INR, and cumulative warfarin dose. Additionally, to assess their independent contribution to the outcome variables, we sequentially excluded early INR
measurements, genotypes, or both from the model, and compared the adjusted $R^2$ of the fitted models and carried out likelihood ratio tests to evaluate their significance. All analyses were performed in the statistical software R (www.r-project.org). All tests were two-tailed, and a P-value of <0.05 was considered significant.
Results

Subject characteristics  Of 325 patients who met the inclusion criteria, 297 patients were recruited, 81 of whom were excluded from analysis since they did not have an INR value recorded on days 4-6, and two were excluded because they were neither African-American nor Caucasian. Demographic and clinical data for the remaining 214 patients are presented in Table 1. For 210 patients (98%), the target INR range was between 1.7 and 3.0. Demographic, clinical, and genotype data for the excluded patients did not differ significantly from the included patients except a small difference in target INR (mean difference, 0.11; 95% CI, 0.07 to 0.15; P<0.001).

Genotyping results  VKORC1 and CYP2C9 genotype could not be determined in one and two subjects, respectively. Allele frequencies were in the range expected for the respective ethnic group, and genotype distributions conformed to Hardy-Weinberg equilibrium (Table 2).

Early INR values and outcomes  Of the 214 patients, 191 (89%) received an initial dosage of 5 mg, and 203 (95%) continued the initial daily dose unchanged at least until the first INR value. The mean INR4 was 1.60±0.72 (range, 0.90 to 5.00; n=163), and the summary early INR response (INR<sub>early</sub>) averaged 1.63±0.71 (range, 0.90 to 5.00). Age (P=0.007) and VKORC1 haplotype (P<0.001), but not CYP2C9 genotype (P=0.18) or cumulative dose (P=0.30) were significantly associated with INR<sub>early</sub>. Four patients had an INR<sub>early</sub> > 4.0 (all on day 4); their ages ranged from 63 to 80 years, their warfarin dose on days 1-3 was 5 mg daily, and none of them received amiodarone. All four patients were heterozygous carriers of VKORC1 haplotype A, and one patient also carried one CYP2C9 *2 allele.
Early INR response was significantly associated with outcomes characterizing warfarin sensitivity. Of the 214 patients, 200 achieved an INR $\geq$ the lower limit of the therapeutic range after a median time of 8.5 days (inter-quartile range [IQR], 5 to 13 days). Time to INR $\geq$ the lower limit of the therapeutic range was strongly associated with early INR response (unadjusted HR for a one unit increase in INR$_{\text{early}}$, 3.07; 95% confidence interval, 2.54 to 3.71; P<0.001). This association remained significant after adjustment for age, sex, ethnicity, use of amiodarone, target INR, cumulative dose, and VKORC1 and CYP2C9 genotypes (HR for INR$_{\text{early}}$, 3.34; 95% CI, 2.67 to 4.18; P<0.001; Table 3). Over-anticoagulation, defined as INR>4, occurred in 59 patients after a median of 15 days (IQR 10 to 22 days) of treatment. Time to over-anticoagulation was strongly associated with early INR values both before (P<0.001) and after adjustment for the covariates and VKORC1 and CYP2C9 genotypes (HR for INR$_{\text{early}}$, 2.12; 95% CI, 1.47 to 3.06; P<0.001; Table 3).

Early INR values were also associated with the first stable warfarin dose. Of the 214 subjects, 132 reached a first stable warfarin dose that yielded a therapeutic INR after $\geq$7 days of unchanged dosing. The median time to reach a stable warfarin dose was 18 days (IQR, 12 to 32 days; overall range, 8 to 140 days). After adjustment for age, sex, ethnicity, amiodarone use, target INR, cumulative warfarin dose, and VKORC1 and CYP2C9 genotypes, higher INR$_{\text{early}}$ values predicted lower therapeutic warfarin doses (β-coefficient -1.3 mg/d; 95% CI, -1.8 to -0.8 mg/d; P<0.001; Table 4).
Incremental value of genotypes in prediction of outcomes

When *VKORC1* and *CYP2C9* genotypes were added to the regression models that included INR*early*, *VKORC1* haplotype A was significantly associated with a shorter time to the INR \( \geq \) the lower limit of the therapeutic range (HR=1.64, \( P<0.001 \)), while *CYP2C9* genotype was not (HR=1.10, \( P=0.48 \); Table 3). However, the contribution of genotypes to the final model was modest and smaller than that of INR*early*. Excluding genotypes from the final model reduced the goodness of fit only modestly (reduction in \( R^2 \) from 0.46 to 0.42, \( P<0.001 \); Figure 1a), whereas exclusion of INR*early* reduced it substantially (reduction in \( R^2 \) from 0.46 to 0.19, \( P<0.001 \); Figure 1a).

When genotypes were included in the regression model for time to first INR>4, neither *VKORC1* (\( P=0.08 \)) nor *CYP2C9* genotype (\( P=0.97 \)) was associated with over-anticoagulation (Table 3). In contrast, INR*early* remained strongly associated with time to first INR>4 (\( P<0.001 \); Table 3). Accordingly, excluding genotypes from the analysis did not significantly reduce goodness of model fit (reduction in \( R^2 \) from 0.16 to 0.15; \( P=0.21 \)) while excluding INR*early* reduced it from 0.16 to 0.11 (\( P<0.001 \); Figure 1b).

When genotypes were included in the regression model predicting the first stable warfarin dose, both *VKORC1* (\( P=0.004 \)) and *CYP2C9* genotypes (\( P=0.002 \)) were significantly associated with the outcome (Table 4). Excluding genotypes from the analysis reduced the adjusted \( R^2 \) from 0.40 to 0.32 (\( P<0.001 \); Figure 1c). Again, exclusion of early INR response resulted in a larger reduction in goodness of fit (adjusted \( R^2 \) from 0.40 to 0.27, \( P<0.001 \); Figure 1c).

When the analyses were repeated using the three INR values separately (i.e., INR4, INR5, and INR6 individually, rather than as the combined variable INR*early*), the
predictive value of the three INR variables was largely comparable in magnitude and statistical significance, suggesting that using any of the three values yielded concordant results. Similarly, using three separate INR variables did not appreciably affect the predictive value of genotypes on the outcomes.

**INR\textsubscript{week1} values, genotypes, and therapeutic warfarin dose**

First stable warfarin dose was achieved after a median of 18 days; therefore we examined whether the predictive value of genotypes for the first stable warfarin dose was maintained after adjusting for INR values obtained at the end of the first week of treatment (INR\textsubscript{week1}, i.e., INR responses on day 7-9). In the full model including age, sex, ethnicity, amiodarone use, target INR, cumulative dose, genotypes, and INR\textsubscript{week1}, neither \textit{CYP2C9} (P=0.08) nor \textit{VKORC1} (P=0.30) genotypes was significantly correlated with the first stable warfarin dose (Table 4). In contrast, in this model there was a strong negative association between INR\textsubscript{week1} and the steady-state warfarin dose (P<0.001; Table 4). This was also reflected in the $R^2$ values for the different models. Excluding genotypes from the full model resulted in a non-significant reduction of $R^2$ from 0.38 to 0.36 (P=0.12), while excluding INR\textsubscript{week1} from the full model reduced $R^2$ from 0.38 to 0.18 (P<0.001).
Discussion

This study provides novel information about the value of pharmacogenetic determinants in the prediction of warfarin sensitivity beyond that provided by the pharmacodynamic measure of early INR response during warfarin induction by conventional dose titration. Our major findings are that a considerable part, but not all, of the predictive information provided by VKORC1 and CYP2C9 genotypes about warfarin sensitivity early in therapy was, in fact, reflected in the early INR response. After a week of dose titration guided by the INR response, pharmacogenetic factors did not contribute significantly to prediction of first stable dose of warfarin beyond what could be inferred from the clinical information provided by the relationship between warfarin dose and INR response.

Genetic variants in the enzyme that metabolizes warfarin, CYP2C9, and in the target of warfarin, VKORC1, strongly affect warfarin sensitivity and thus warfarin dose requirements at steady state, i.e., when therapeutic INRs have been achieved after chronic stable dosing.31 Thus, rather than starting all patients on the same dose of warfarin, selection of an initial dose based on an individual’s genotypes would be expected to improve INR control during warfarin initiation.32-37 Indeed, several prospective studies are underway to test this hypothesis.27,28 However, the pharmacodynamic measure of warfarin sensitivity, INR response, also differs early in therapy according to genotypes, particularly VKORC1 genotype,22,23 and it is possible that the information provided by genotypes about stable warfarin dose requirements is in large part captured by the information provided by the early INR response.29 If that were the case, one could argue that genotyping would contribute little additional information if one paid close attention
to the early INR response and predicted future warfarin dose requirements accordingly. Moreover, the early INR response may also reflect additional unmeasured individual factors contributing to warfarin sensitivity that are not captured by genotype. The relative contributions of VKORC1/CYP2C9 genotypes and early INR response to prediction of sensitivity to warfarin have not been well characterized.

Millican et al. studied patients receiving warfarin after orthopedic surgery. In this cohort, however, the first 3 warfarin doses had already been individualized based on CYP2C9 and/or VKORC1 genotypes and other clinical predictors. In a recent study examining the relationship between early phenotypic markers of warfarin sensitivity and genotypes, the INR on day 4 explained 31% of the variability in warfarin dose at day 14, whereas CYP2C9-VKORC1 genotypes explained only 6.5%. However, this cohort consisted entirely of hospitalized patients with significant comorbidities receiving multiple medications, a clinical situation that is likely to have increased the contribution of non-genetic factors to variability in warfarin sensitivity, and thus attenuated the contribution of genotype.

In our study, we systematically quantified the contribution of CYP2C9 and VKORC1 genotypes to the assessment of early warfarin sensitivity after adjustment for early INR response. In addition to the first stable warfarin dose, we chose several measures of warfarin sensitivity in the induction phase that are associated with clinically important endpoints. The time to a first INR value ≥ the lower limit of the therapeutic
range affects the length of concomitant heparin therapy, and thus the duration of hospitalization, for patients who require immediate anticoagulation. Time to INR>4 identifies patients with over-anticoagulation who are at greater risk for bleeding. Finally, the early steady state warfarin dose\textsuperscript{24} reflects subsequent stable warfarin steady state dose requirements. For all outcomes, the predictive power of early INR values on days 4, 5 or 6 was greater than that of genotypes, and the additional contribution of genotypes to the predictive models was generally small.

After adjustment for early INR values (day 4-6), first stable warfarin dose was the only outcome to which genotypes made a contribution that was meaningful in magnitude. The independent contribution of genotyping to prediction of warfarin sensitivity is likely to decrease over time because it can be increasingly estimated from the relationship between INR responses and cumulative warfarin dose. Thus, we also examined whether genotypes maintain their predictive contribution to early steady state warfarin dose after the first week of treatment. After adjustment for INR values on days 7-9, genotypes were no longer independent predictors of first stable warfarin dose. These findings suggest that a large part of the predictive value of \textit{CYP2C9} and \textit{VKORC1} genotypes is reflected in early INR measurements, and that as early as 1 week after the start of treatment, genotypes may contribute little additional predictive information about warfarin sensitivity beyond that reflected in INR and cumulative dose.

However, although much of a patient's warfarin sensitivity is reflected in the INR values obtained during the first week of treatment, in clinical practice it is often difficult to utilize this information optimally, since this requires frequent INR determinations and dosing instructions by specifically trained health care providers, often in specialized
anticoagulation clinics. Thus, a number of prospective clinical trials are planned or underway to test the value of \textit{CYP2C9 / VKORC1}-genotype guided warfarin therapy compared to conventional INR-guided dose titration during warfarin initiation. Our findings have important implications for these studies. Since much of the predictive information of genotypes is in fact reflected in early INR response, our results suggest that the benefit of genotype-guided therapy will be smallest in a clinical setting with closely monitored therapy, where expert dose titration includes close attention to the early INR response, and greatest in the clinical setting of non-expert warfarin dosing, where the majority of warfarin-treated patients are followed. Secondly, algorithms for genotype-based warfarin dosing should allow for inclusion of early INR response to improve the prediction of warfarin sensitivity.\textsuperscript{28} Such an algorithm that provides dosing suggestions after accounting for clinical and genetic factors and early INR values is available (www.warfarindosing.org).\textsuperscript{21}

For subjects who are very sensitive to warfarin, empiric dosing during days 1-3 may already result in overanticoagulation on day 4. In our study, four patients (1.9\%) reached an INR value \(> 4.0\) (range, 4.6 to 5.0) after three daily warfarin doses of 5 mg. Clinical variables would not have predicted this sensitivity to warfarin. Yet, all four patients were carriers of genetic variants conferring increased sensitivity; thus, genotype-guided dosing may well have reduced the risk of overanticoagulation in these subjects.

Our study has a number of strengths, including the combination of a number of outcomes with potential clinical relevance to assess warfarin sensitivity, a heterogeneous study population in a real-life environment treated with similar warfarin starting doses, and a systematic statistical analysis of the predictive value of different variables for the
assessment of warfarin sensitivity. As expected in an observational study, not all patients had INR determined on the same day, and dosing was regulated by different clinics. As we have pointed out, variability in management is likely to favor the contribution of genotype, thus enhancing the relevance of our findings. Additionally, our study focuses on the induction phase of warfarin therapy and thus does not provide information about the predictive value of genotyping and early INR measurements on stability during long-term anticoagulation.

In conclusion, in this observational study much of the information provided by CYP2C9 and VKORC1 genotype about warfarin sensitivity was captured by the early INR response. Prospective studies on the value of genotype-guided warfarin initiation should be evaluated against the background of the study’s setting, and include algorithms that incorporate early INR response.
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Author contributions: C.L. designed the research, analyzed data, and edited the manuscript. U.I.S and M.D.R. contributed to data collection and analysis and edited the manuscript. D.M.R. designed the research, revised the manuscript, and obtained funding. C.M.S. designed the research, supervised the study, obtained funding, and edited the manuscript. D.K. conceived the research, analyzed the data, and wrote the manuscript.

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References


Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>All Patients</th>
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</tr>
<tr>
<td>Female, no. (%)</td>
<td>104 (49%)</td>
</tr>
<tr>
<td>European - Americans, no. (%)§</td>
<td>197 (92%)</td>
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<td>African - Americans, no. (%)§</td>
<td>17 (8%)</td>
</tr>
<tr>
<td>Age in years, mean (SD)</td>
<td>61 (14)</td>
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<tr>
<td><strong>Indication, no. (%)</strong></td>
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<tr>
<td>Atrial Fibrillation/ Atrial Flutter</td>
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<td>26 (12%)</td>
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<td>Concomitant amiodarone medication, no. (%)</td>
<td>25 (12%)</td>
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<td>Median follow-up, days (Range)</td>
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Table 2. *VKORC1* Haplotype and *CYP2C9* Genotype Frequency

<table>
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<tr>
<th>Gene</th>
<th>Caucasian (n=197)</th>
<th>African-Americans (n=17)</th>
<th>All (n=214)</th>
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<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
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<tr>
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<tr>
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<tr>
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<td>*1/*2;*1/*3</td>
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<td>9</td>
<td>4.6</td>
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*VKORC1* genotype could not be determined in 1 Caucasian subject, and *CYP2C9* genotype could not be determined in 1 Caucasian and 1 African-American subject
Table 3. Cox regression analysis for time to INR $\geq$ the lower limit of the therapeutic range and time to INR>4

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Time to INR within or above the therapeutic range</th>
<th>Time to INR &gt;4</th>
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<td>Male sex</td>
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<td>0.48 to 0.90</td>
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<tr>
<td>Caucasian ethnicity</td>
<td>0.70</td>
<td>0.40 to 1.23</td>
</tr>
<tr>
<td>Target INR</td>
<td>0.16</td>
<td>0.06 to 0.45</td>
</tr>
<tr>
<td>Amiodarone use</td>
<td>0.96</td>
<td>0.61 to 1.51</td>
</tr>
<tr>
<td>Cumulative warfarin dose†</td>
<td>1.01</td>
<td>0.97 to 1.04</td>
</tr>
<tr>
<td>INR$\text{early}^*$</td>
<td>3.34</td>
<td>2.67 to 4.18</td>
</tr>
<tr>
<td>VKORC1 A haplotype</td>
<td>1.64</td>
<td>1.29 to 2.11</td>
</tr>
<tr>
<td>CYP2C9, any variant</td>
<td>1.10</td>
<td>0.84 to 1.44</td>
</tr>
</tbody>
</table>
N=211 due to 3 missing genotypes. * INR_{early} represented INR on day 4, 5, and 6 in 162, 27, and 25 patients, respectively; † In each patient, cumulative dose was calculated up to the day preceding the respective INR used in the analysis.
Table 4. Multiple linear regression analysis for first stable warfarin dose* including early and week 1 INR values

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Including early INR values (day 4-6)</th>
<th>Including late INR values (day 7-9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-coefficient</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age</td>
<td>-0.03</td>
<td>-0.05 to -0.01</td>
</tr>
<tr>
<td>Male Sex</td>
<td>0.42</td>
<td>-0.18 to 1.02</td>
</tr>
<tr>
<td>Caucasian ethnicity</td>
<td>0.57</td>
<td>-0.52 to 1.67</td>
</tr>
<tr>
<td>Target INR</td>
<td>1.05</td>
<td>-0.92 to 3.03</td>
</tr>
<tr>
<td>Amiodarone use</td>
<td>-0.92</td>
<td>-1.72 to -0.13</td>
</tr>
<tr>
<td>Cumulative warfarin dose†</td>
<td>0.03</td>
<td>-0.04 to 0.09</td>
</tr>
<tr>
<td>INR values</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INR_{early} **</td>
<td>-1.29</td>
<td>-1.79 to -0.80</td>
</tr>
<tr>
<td>INR_{week1} **</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>VKORC1 A haplotype</td>
<td>-0.71</td>
<td>-1.19 to -0.23</td>
</tr>
<tr>
<td>CYP2C9 dose-reducing variant</td>
<td>-0.87</td>
<td>-1.42 to -0.32</td>
</tr>
</tbody>
</table>
*First stable dose was defined as the first unchanged daily dose given for ≥7 days that yielded an INR value within the therapeutic range; **INRearly represented INR on day 4, 5, and 6 in 162, 27, and 25 patients, respectively; INRweek1 represented INR on day 7, 8, and 9 in 63, 34, and 48 patients, respectively; †In each patient, cumulative dose was calculated up to the day preceding the respective INR used in the analysis.
Panel A

Time to INR \geq \text{lower limit of therapeutic range}

Panel B

Time to INR > 4

Panel C

Stable warfarin dose
Figure 1. Comparison of goodness of fit ($R^2$) among four models for each of the
three outcome variables: Time to INR $\geq$ lower limit of therapeutic range (panel A);
Time to INR $> 4$ (panel B); and early stable warfarin dose (panel C). In each panel,
the first bar (white) represents the baseline model (including age, ethnicity, sex,
amiodarone use, target INR, and cumulative warfarin dose), the second bar (red) the
baseline model + VKORC1 / CYP2C9 genotypes, the third bar (blue) the baseline model
+ early INR values, and the fourth bar (purple) the full model. The difference between the
last two bars (purple and blue) in each panel represents the contribution of genotypes
after adjustment for baseline covariates and INR$_{\text{early}}$. 
Relative contribution of CYP2C9 and VKORC1 genotypes and early INR response to the prediction of warfarin sensitivity during initiation of therapy

Chun Li, Ute I. Schwarz, Marylyn D. Ritchie, Dan M. Roden, C. Michael Stein and Daniel Kurnik