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Effect of the *VKORC1* D36Y variant on warfarin dose requirement and pharmacogenetic dose prediction

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Summary

Pharmacogenetic dosing algorithms help predict warfarin maintenance doses, but their predictive performance differs in different populations, possibly due to unsuspected population-specific genetic variants. The objectives of this study were to quantify the effect of the *VKORC1* D36Y variant (a marker of warfarin resistance previously described in 4% of Ashkenazi Jews) on warfarin maintenance doses and to examine how this variant affects the performance of the International Warfarin Pharmacogenetic Consortium (IWPC) dose prediction model. In 210 Israeli patients on chronic warfarin therapy recruited at a tertiary care center, we applied the IWPC model and then added D36Y genotype as covariate to the model (IWPC+D36Y) and compared predicted with actual doses. Median weekly warfarin dose was 35 mg (interquartile range [IQR], 24.5 to 52.5 mg). Among 16 heterozygous D36Y carriers (minor allele frequency = 3.8%), warfarin weekly dose was increased by a median of 43.7 mg (IQR, 40.5 to 47.2 mg) compared to non-carriers after adjustment for all IWPC parameters, a greater than 2-fold dose increase. The IWPC model performed suboptimally (coefficient of determination $R^2=27.0\%$; mean absolute error (MAE), 14.4 ± 16.2 mg/week). Accounting for D36Y genotype using the IWPC+D36Y model resulted in a significantly better model performance ($R^2=47.2\%$, MAE= 12.6 ± 12.4 mg/week). In conclusion, even at low frequencies, variants with a strong impact on warfarin dose may greatly decrease the performance of a commonly used dose prediction model. Unexpected discrepancies of the performance of universal prediction models in subpopulations should prompt searching for unsuspected confounders, including rare genetic variants.

Keywords

Warfarin; pharmacogenetics; *VKORC1*; dose prediction; ethnicity

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Conflicts of Interest

Dr. Gak holds a 40% stake in patent PCT no. IL2007/000405, filed in March 2007, entitled "Methods and kits for determining predisposition to warfarin resistance", which includes the *VKORC1* D36Y variant. None of the other authors reported a potential conflict of interest relevant to this article.

Introduction

Initiation of warfarin therapy is notoriously difficult, mainly because of the large (>20-fold) interindividual variability in dose requirements, the narrow therapeutic index, and the substantial morbidity and mortality associated with lack of therapeutic control (1). Genetic variants in *CYP2C9*, the enzyme that metabolizes warfarin, and *VKORC1*, the drug target for coumarins, strongly affect sensitivity to warfarin and therefore the dose of warfarin required for stable anticoagulation (2). Thus, dosing algorithms that include information about genetic variants have been developed and validated to improve prediction of an individual patient's warfarin dose (3–6). These algorithms greatly improve the accuracy of the predicted stable warfarin dose compared to approaches that use fixed doses or doses determined by clinical determinants only (such as age, height, weight, and concomitant medications), and thus may reduce fluctuations of anticoagulation control during warfarin initiation and possibly the considerable attendant morbidity and mortality (7–10). The efficacy and cost-effectiveness of using pharmacogenetic information to guide the selection of warfarin doses at initiation are currently being examined in large multicenter studies (11); nevertheless, physicians have been encouraged to use genetic information, if available, to better predict warfarin dose in individual patients (2, 12).

Prediction algorithms derived in a specific population are often less informative in populations of different ancestry (13–15). Racial differences in warfarin sensitivity are well-recognized (16), and much of this variability is explained by differences among races in the prevalence of common *CYP2C9* and *VKORC1* variants used to predict warfarin doses (3, 4, 13, 16). Thus, multiracial and international cohorts with thousands of patients have been studied to develop pharmacogenetic dose prediction models that are globally applicable (3, 4). These models are more robust across multiracial populations (15) and are therefore widely recommended for the implementation of pharmacogenetics-based warfarin dosing (2).

We recently found the previously identified (17) rare *VKORC1* coding variant D36Y (rs61742245), that results in the substitution of tyrosine for aspartic acid at amino acid 36, in 8% of unselected patients receiving warfarin at our institution (18). The D36Y variant is associated with warfarin resistance and has been identified mainly in sporadic case reports of patients with high warfarin dose requirements (17, 19, 20). However, due to the small sample sizes in these previous reports, the magnitude of the independent effect of D36Y on warfarin maintenance dose has not been adequately quantified. Moreover, little is known about how carriers of this variant in a given population affect the performance of warfarin dose-prediction algorithms.

Accordingly, the two objectives of the present study were (a) to quantify the independent effect of the D36Y *VKORC1* variant on warfarin dose requirements in a cohort large enough to allow adjustment for confounders, and (b) to examine its effect on the performance of a standard pharmacogenetic dose-prediction algorithm.

Methods

Subjects

The study was approved by the Sheba Medical Center Institutional Review Board, and all patients gave written informed consent. The population consisted of two cohorts enrolled prospectively in 2004 (cohort 1) and 2010–11 (cohort 2). Subjects from cohort 1 (n=104) contributed to an earlier report of D36Y (18) and other warfarin pharmacogenetic studies (21, 22). Consecutive patients on chronic warfarin anticoagulation attending the Sheba Medical Center anticoagulation clinic or admitted to an internal medicine ward for reasons

unrelated to the warfarin treatment were eligible if they had been on warfarin treatment for 3 months and had reached a stable warfarin dose, defined as having all INR values (a minimum of 3 determinations) within the therapeutic range (2.0–3.0 or 2.5–3.5, according to indication) for the preceding 2 months and at the index visit, without any change in warfarin dose. Exclusion criteria were the use of strong CYP2C9 inducers (rifampicin, phenytoin). Blood samples were taken for INR determination and DNA extraction. We recorded demographic and clinical information including age, sex, ethnicity, body weight, height, and use of concomitant medications. Height was not recorded for cohort 1, thus we imputed it by estimating the relationship between height and weight and sex using multiple linear regression analysis with data from cohort 2.

Genotyping

Genotyping for the 3 variants commonly used in pharmacogenetic dosing algorithms (*VKORC1*-1639G>A (rs9923231), *CYP2C9**2 (rs1799853) and *3 (rs1057910) and additionally for *VKORC1* 106G>T (D36Y; rs61742245) were performed using Sequenom multiplex iPLEX assays (Sequenom, San Diego, CA, USA) (18). Presence of D36Y was further confirmed by restriction fragment length polymorphism (RFLP) analysis using *RsaI* (18). Sequenom and RFLP genotyping of D36Y yielded identical results in all cases. Genotyping for *CYP4F2* rs2108622 C>T (V433M), a common polymorphism variably associated with increased warfarin dose requirements, was performed by allelic discrimination with TaqMan 5'-nuclease assays on an ABI 7900 HT real-time PCR system (Applied Biosystems, Foster City, CA) using validated TaqMan probes.

Data and statistical analysis

IWPC algorithm

Our reference pharmacogenetic dose prediction algorithm was the International Warfarin Pharmacogenetics Consortium (IWPC) model, derived from an international cohort of 4,043 patients from 9 countries, including Israel, in 4 continents (3). We used this model because it was based on a large and heterogeneous patient population and performed well in other predominantly Caucasian cohorts (23), and is thus one of two widely recommended pharmacogenetic warfarin dosing algorithms (2, 11, 24). The IWPC algorithm predicts weekly warfarin doses using clinical parameters (age in decades, race, height and weight, and concomitant treatment with amiodarone or enzyme inducers) and genetic markers (*CYP2C9**2 and *3 and *VKORC1* rs9923231). We also included *CYP4F2* rs2108622 C>T as a covariate in our analysis since some recent studies have found this variant to be associated with increased warfarin doses (25, 26).

IWPC+D36Y algorithm

We first tested the IWPC algorithm in our cohort and then used linear regression analysis to add the D36Y genotype as a covariate, leaving the intercept and coefficients for the other predictors unchanged (IWPC+D36Y model). This allowed us to estimate the contribution of D36Y to the weekly warfarin dose. For non-carriers of D36Y, the IWPC + D36Y and the IWPC models predicted the same warfarin dose. For the carriers, we calculated the mean and median increase in warfarin dose as follows: Let a_i be the IWPC algorithm prediction for carrier i ($i = 1, \dots, 17$). Then the IWPC warfarin dose will be a_i^2 because the IWPC algorithm uses a square root transformation of warfarin dose. The warfarin dose predicted by the model with D36Y will be $(a_i + b)^2$, where b is the coefficient of the D36Y covariate in the new model. The increase in warfarin dose attributable to the D36Y variant is thus $(a_i + b)^2 - a_i^2 = 2a_i b + b^2$.

As a measure of model performance, we determined the coefficient of determination R^2 , the total variability in warfarin dose requirements explained, on the square root scale, and the mean absolute error with standard deviation (MAE $\pm$ SD) on the warfarin dose scale. We used the following formula to calculate R^2 : $1 - \frac{(y_i - \hat{y}_i)^2}{(y_i - \bar{y})^2}$, where y_i is the square root of warfarin dosage, $\bar{y}$ is their average, and $\hat{y}_i$ is the predicted value on the square root scale.

Simulation of different D36Y prevalences

In order to model the effect of the prevalence of D36Y in populations, we divided our cohort into D36Y carriers (n=16) and non-carriers and then used resampling techniques to randomly add k D36Y carriers to the cohort of non-carriers, with $k=1$ to 17. For each k , we performed 200 random resampling iterations, and calculated R^2 and the mean of MAE $\pm$ SD.

Values are presented as mean $\pm$ standard deviation (SD) or median and interquartile range (IQR), as appropriate. We tested whether genotype distributions conformed to Hardy-Weinberg equilibrium using a chi-squared test with 1 degree of freedom. All tests were two-tailed, and P-values < 0.05 were considered significant. All analyses were performed using the statistical software R (www.r-project.org).

Results

Subjects and genotypes

We studied 210 patients (104 in cohort 1 and 106 in cohort 2). Demographic and clinical parameters are presented in Table 1. There was no statistical difference in any of these between the two cohorts (data not shown). Genotyping for the two *CYP2C9* and two *VKORC1* variants yielded call rates of 100%. Genotyping for *CYP4F2* rs2108622 was performed at a later stage, and DNA was available for 191 patients (91%). Genotyping results are presented in Table 1. Among the 16 carriers of D36Y, none carried the *CYP4F2* TT genotype. Minor allele frequencies were in the expected range, and genotype distributions conformed to Hardy-Weinberg equilibrium.

D36Y variant effect on warfarin dose

The 16 heterozygous carriers of the D36Y variant required a median weekly warfarin dose of 71.2 mg (IQR, 60.6 to 102.5 mg), compared to 35.0 mg (22.5 to 47.5 mg) in the remaining cohort ($P<0.001$; Figure 1). Using a model in which the D36Y covariate was added to the IWPC algorithm, we calculated that a single D36Y allele increased the weekly warfarin dose by 43.7 ± 3.7 mg (median [IQR], 43.7 [40.5 to 47.2]) after adjustment for all IWPC covariates (age, race, weight and height, use of amiodarone and enzyme inducers, and three standard genetic markers [*CYP2C9**2 and *3 and *VKORC1* rs9923231]). Thus, the presence of a single D36Y allele more than doubled the maintenance warfarin dose.

Effect of D36Y on performance of the IWPC algorithm

For the cohort as a whole, the standard IWPC model performed suboptimally, with a coefficient of determination R^2 of 27.0% and a mean standard error of 14.4 ± 16.2 mg warfarin/week (Table 2). For D36Y carriers, the model consistently underestimated the weekly warfarin dose (Figure 2, left panel). When the prediction model was modified to include the D36Y variant (IWPC+D36Y model), performance improved significantly: R^2 increased to 47.2% and MAE was reduced to 12.6 ± 12.4 mg warfarin/week, a mean reduction of 12.5%. In D36Y carriers, the new model significantly reduced the discrepancy between actual and predicted dose (Figure 2, right panel).

In simulated populations with a varying prevalence of D36Y, the R^2 for the IWPC prediction model declined and MAE increased as the prevalence of D36Y carriers increased (Figure 3). In contrast, using the IWPC+ D36Y model, R^2 increased with increasing D36Y prevalence while MAE increased only slightly (Figure 3).

Effect of *CYP4F2* genotype on warfarin dose

Median daily warfarin maintenance doses were 35.0 mg (IQR, 22.5 to 51.2 mg), 32.5 mg (IQR, 22.5 to 60.0 mg), and 37.2 mg (IQR, 35.0 to 51.5 mg) in patients with *CYP4F2* CC, CT, and TT genotypes, respectively ($P=0.58$). After adjustment for all the relevant IWPC covariates (age, weight, height, amiodarone use, and genotypes of *CYP2C9* and *VKORC1*) and the D36Y genotype, *CYP4F2* genotype was not associated with warfarin dose requirement ($P=0.70$). A sensitivity analysis in the subgroup of non-carriers of D36Y ($n=194$) yielded comparable results.

Discussion

This is the first report to quantify the independent effect of the *VKORC1* D36Y variant on warfarin dose requirement after accounting for other genetic and non-genetic covariates. Patients with one D36Y allele required more than twice the dose of warfarin compared to non-carriers, a much greater effect than that of common genetic variants currently included in pharmacogenetic dosing algorithms. More importantly, our results demonstrate that the presence of D36Y, even at comparatively low population prevalence, greatly reduces the performance of the prediction algorithms, and that dose prediction can be improved by the integration of D36Y into the prediction algorithm.

D36Y prevalence and mechanism

The D36Y variant was first described in an African patient with a weekly warfarin dose requirement of >70 mg (17). Subsequently, this variant was identified mainly among Ashkenazi Jews with minor allele frequencies (MAF) around 4% (18, 20, 27), Ethiopians (MAFs around 15%) (28), and sporadically in other Caucasians (19, 29, 30). D36Y was not identified among 240 patients in Southeast India (31) or among 213 black patients from South Africa (32). In contrast, in an international cohort of patients with unexplained warfarin resistance, D36Y was the most common *VKORC1* variant identified by sequencing (19). The molecular mechanism for warfarin resistance in D36Y carriers has not been fully elucidated.

The recently modified topological model of *VKORC1* within the endoplasmatic reticulum membrane localizes the D36Y substitution to the luminal loop of *VKORC1*, between transmembrane domains 1 and 2 (19). Other rare genetic mutations in this region also result in warfarin resistance (e.g., Val29Leu, Val45Ala, Arg58Gly and Val66Met), presumably due to their structural effect on a proposed accessory coumarin binding site (19, 33). Thus, decreased affinity of the *VKORC1* D36Y variant for warfarin has been the suggested mechanism accounting for increased dose requirements. Moreover, D36Y is in linkage disequilibrium with a promoter variant at -679 predicted to affect transcription rate, suggesting that D36Y may also be a marker for *VKORC1* overexpression (20, 30).

D36Y dose effect

Higher warfarin dose requirements in D36Y carriers (ranging from 50–180 mg weekly) have been described in sporadic case reports (17, 20), small case series of warfarin-resistant patients (19, 29), and two small cohort studies, including cohort 1 also reported here (18, 30). However, these studies did not assess the independent effect of the variant after adjusting for genetic and non-genetic confounders. Our study provides information derived

from the largest number of D36Y carriers to date. We found that after adjustment for genetic and non-genetic confounders, a single D36Y allele increased the weekly warfarin dose by a median of 43.7 mg (IQR, 40.5 to 47.2 mg). Thus, the magnitude of the effect of D36Y, a more than doubling of the warfarin dose requirement, is 4–6-fold larger than that of variants commonly included in pharmacogenetic algorithms. Therefore, even at comparatively low prevalence, the presence of D36Y carriers affects the performance of dose prediction algorithms significantly.

D36Y effect on IWPC model

The IWPC prediction model includes covariates for “Asian race” and “Black or African-American race” since genetic and clinical covariates do not completely account for racial differences in warfarin doses. Nonetheless, the model performs differently in different racial groups, generally best in Caucasians, the population forming the majority of the IWPC derivation cohort (3, 13). In our cohort of Israeli Caucasians, the IWPC model performed suboptimally, with a large mean absolute error and a low coefficient of determination R^2 . In particular, the IWPC prediction significantly underestimated actual warfarin doses in D36Y carriers. Inclusion of the D36Y covariate in the prediction model greatly improved overall prediction, achieving a coefficient of determination similar to that in the IWPC derivation cohort. Thus, in our population, the IWPC algorithm performs suboptimally, largely because it does not account for the strong effect of D36Y. In addition, simulation of populations with differing prevalences of D36Y carriers showed that even at low prevalence (e.g., carrier prevalence of 4%, corresponding to a minor allele frequency of 2%), the effect of undetected D36Y carriers on the prediction model will be substantial. Our findings are supported by a recent report from a cohort of patients from Canada, in whom three D36Y carriers were identified among 176 mostly Caucasian patients (MAF=0.9%). In that study, a prediction model that also included common genetic and clinical predictors, the D36Y covariate contributed a partial R^2 of 10.7% (30). Thus, even at low prevalence, the presence of D36Y in a population can have important effects on the overall performance of prediction models. Moreover, dose prediction by pharmacogenetic models has the greatest value in patients at the two tails of the warfarin dose distribution, i.e., patients with very low or high dose requirements (3). Thus, adding genetic variants with a large impact on the maintenance dose to a pharmacogenetic model will improve dose prediction precisely in those patients who are most likely to benefit from pharmacogenetic dosing.

Our findings raise important questions about the applicability of internationally derived pharmacogenetic models to specific sub-populations even within a given race (34). Beyond the commonly used *VKORC1* tagSNP -1639 G/A (rs9923231) (or related variants in nearly complete linkage disequilibrium), two genome-wide association studies in Caucasians did not identify additional independent *VKORC1* variants affecting warfarin dose. Thus, rs9923231 genotype is generally considered sufficient to represent *VKORC1* variability in warfarin dosing algorithms (13). However, our study illustrates that unsuspected variants, even at a low prevalence, can greatly affect the performance of a prediction model in a subpopulation. Thus, while algorithms derived from large international cohorts may represent the “average global patient”, they may be less precise in a subpopulation. Indeed, unexpectedly large deviations from the IWPC performance parameters in a subpopulation should prompt a search for genetic and non-genetic determinants unaccounted for by the IWPC model.

In addition to the IWPC covariates, we also examined the effect of *CYP4F2* rs2108622, since this common variant has been recently identified as marker for increased dose requirements in some (25, 26, 35) but not all studies (10, 36, 37). In our cohort, this variant was not associated with warfarin doses and did not explain the high warfarin dose requirements in D36Y carriers. Our findings are compatible with the generally small

contribution of *CYP4F2* rs2108622 to the interindividual variability in warfarin maintenance doses in European Americans and Israelis ($R^2=0.5\text{--}2\%$), requiring large cohorts to demonstrate such an independent effect (25, 26, 38).

Our study has a number of strengths. We recruited consecutive patients receiving chronic warfarin therapy at a large tertiary care hospital, so that our cohort is representative of the population at large rather than patients selected for high warfarin doses (although patients with particularly high warfarin requirements may be overrepresented in specialized anticoagulation clinics). We used the IWPC algorithm rather than an algorithm optimized to our population, because physicians are likely to use one of the globally recommended prediction models, and because using the IWPC model allows direct comparison with other populations. Lastly, our cohort included enough D36Y carriers to provide a reliable estimate of the effect of D36Y on dose requirements.

Our study also has a number of limitations. The IWPC algorithm does not account for other covariates known to affect warfarin sensitivity, including genetic factors [e.g., genetic variants of gamma-glutamyl carboxylase (*GGCX*) (39)] and non-genetic factors such as vitamin K status. However, dietary vitamin K intake was shown in our previous study in an Israeli population to be similar to that of its racial reference group (18, 40, 41). Moreover, the contribution of these covariates to the interindividual variability in warfarin doses is generally small, and their exclusion has only a minor impact on the performance of dose prediction algorithms in Caucasians (5, 21, 26, 39, 42, 43). Lastly, ours is a cross-sectional cohort study in patients on chronic maintenance treatment. Accordingly, we cannot provide information about the effect of D36Y on warfarin induction, such as time to achieving therapeutic INR, or on clinical outcomes (bleeding and thrombotic complications) and its interaction with other known risk factors (44, 45).

In conclusion, our study provides a robust estimate of the adjusted near-doubling effect of *VKORC1* D36Y on warfarin dose requirement. Furthermore, our findings exemplify how variants prevalent in a sub-population can substantially reduce the performance of an internationally derived dosing algorithm and illustrate the limitations of extrapolating the findings of such global models to specific subpopulations. We propose that the performance of global models should be evaluated in specific subpopulations; if large deviations from the findings in the original derivation cohort are found, this should prompt a search for genetic and non-genetic predictors unique to the given subpopulation.

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References

1. Kurnik D, Loebstein R, Halkin H, et al. 10 years of oral anticoagulant pharmacogenomics: what difference will it make? A critical appraisal. *Pharmacogenomics*. 2009; 10:1955–65. [PubMed: 19958094]
2. Johnson JA, Gong L, Whirl-Carrillo M, et al. Clinical Pharmacogenetics Implementation Consortium Guidelines for CYP2C9 and VKORC1 genotypes and warfarin dosing. *Clin Pharmacol Ther*. 2011; 90:625–9. [PubMed: 21900891]
3. Klein TE, Altman RB, Eriksson N, et al. Estimation of the warfarin dose with clinical and pharmacogenetic data. *N Engl J Med*. 2009; 360:753–64. [PubMed: 19228618]
4. Gage BF, Eby C, Johnson JA, et al. Use of pharmacogenetic and clinical factors to predict the therapeutic dose of warfarin. *Clin Pharmacol Ther*. 2008; 84:326–31. [PubMed: 18305455]

5. Wadelius M, Chen LY, Lindh JD, et al. The largest prospective warfarin-treated cohort supports genetic forecasting. *Blood*. 2009; 113:784–92. [PubMed: 18574025]
6. Gong IY, Schwarz UI, Crown N, et al. Clinical and genetic determinants of warfarin pharmacokinetics and pharmacodynamics during treatment initiation. *PLoS One*. 2011; 6:e27808. [PubMed: 22114699]
7. Caraco Y, Blotnick S, Muszkat M. CYP2C9 genotype-guided warfarin prescribing enhances the efficacy and safety of anticoagulation: a prospective randomized controlled study. *Clin Pharmacol Ther*. 2008; 83:460–70. [PubMed: 17851566]
8. Anderson JL, Horne BD, Stevens SM, et al. Randomized trial of genotype-guided versus standard warfarin dosing in patients initiating oral anticoagulation. *Circulation*. 2007; 116:2563–70. [PubMed: 17989110]
9. Epstein RS, Moyer TP, Aubert RE, et al. Warfarin genotyping reduces hospitalization rates results from the MM-WES (Medco-Mayo Warfarin Effectiveness study). *J Am Coll Cardiol*. 2010; 55:2804–12. [PubMed: 20381283]
10. Gong IY, Tirona RG, Schwarz UI, et al. Prospective evaluation of a pharmacogenetics-guided warfarin loading and maintenance dose regimen for initiation of therapy. *Blood*. 2011; 118:3163–71. [PubMed: 21725053]
11. Carlquist JF, Anderson JL. Using pharmacogenetics in real time to guide warfarin initiation: a clinician update. *Circulation*. 2011; 124:2554–9. [PubMed: 22144632]
12. [Last accessed on January 31, 2012] FDA approves updated warfarin sodium label. 2010. Available at <http://www.fda.gov/Safety/MedWatch/SAfetyInformation/ucm201100.htm>
13. Limdi NA, Wadelius M, Cavallari L, et al. Warfarin pharmacogenetics: a single VKORC1 polymorphism is predictive of dose across 3 racial groups. *Blood*. 2010; 115:3827–34. [PubMed: 20203262]
14. Botton MR, Bandinelli E, Rohde LE, et al. Influence of genetic, biological and pharmacological factors on warfarin dose in a Southern Brazilian population of European ancestry. *Br J Clin Pharmacol*. 2011; 72:442–50. [PubMed: 21320153]
15. Shin J, Cao D. Comparison of warfarin pharmacogenetic dosing algorithms in a racially diverse large cohort. *Pharmacogenomics*. 2011; 12:125–34. [PubMed: 21174627]
16. Takahashi H, Wilkinson GR, Nutescu EA, et al. Different contributions of polymorphisms in VKORC1 and CYP2C9 to intra- and inter-population differences in maintenance dose of warfarin in Japanese, Caucasians and African-Americans. *Pharmacogenet Genomics*. 2006; 16:101–10. [PubMed: 16424822]
17. D'Ambrosio RL, D'Andrea G, Cafolla A, et al. A new vitamin K epoxide reductase complex subunit-1 (VKORC1) mutation in a patient with decreased stability of CYP2C9 enzyme. *J Thromb Haemost*. 2007; 5:191–3. [PubMed: 17059426]
18. Loebstein R, Dvoskin I, Halkin H, et al. A coding VKORC1 Asp36Tyr polymorphism predisposes to warfarin resistance. *Blood*. 2007; 109:2477–80. [PubMed: 17110455]
19. Watzka M, Geisen C, Bevans CG, et al. Thirteen novel VKORC1 mutations associated with oral anticoagulant resistance: insights into improved patient diagnosis and treatment. *J Thromb Haemost*. 2011; 9:109–18. [PubMed: 20946155]
20. Bodin L, Perdu J, Diry M, et al. Multiple genetic alterations in vitamin K epoxide reductase complex subunit 1 gene (VKORC1) can explain the high dose requirement during oral anticoagulation in humans. *J Thromb Haemost*. 2008; 6:1436–9. [PubMed: 18532998]
21. Vecsler M, Loebstein R, Almog S, et al. Combined genetic profiles of components and regulators of the vitamin K-dependent gamma-carboxylation system affect individual sensitivity to warfarin. *Thromb Haemost*. 2006; 95:205–11. [PubMed: 16493479]
22. Loebstein R, Vecsler M, Kurnik D, et al. Common genetic variants of microsomal epoxide hydrolase affect warfarin dose requirements beyond the effect of cytochrome P450 2C9. *Clin Pharmacol Ther*. 2005; 77:365–72. [PubMed: 15900282]
23. Shaw PB, Donovan JL, Tran MT, et al. Accuracy assessment of pharmacogenetically predictive warfarin dosing algorithms in patients of an academic medical center anticoagulation clinic. *J Thromb Thrombolysis*. 2010; 30:220–5. [PubMed: 20204461]

24. [Last accessed on January 31] IWPC algorithm. Available at <http://www.pharmgkb.org/drug/PA451906IWPCalgorithm>
25. Caldwell MD, Awad T, Johnson JA, et al. CYP4F2 genetic variant alters required warfarin dose. *Blood*. 2008; 111:4106–12. [PubMed: 18250228]
26. Ramirez AH, Shi Y, Schildcrout JS, et al. Predicting warfarin dosage in European-Americans and African-Americans using DNA samples linked to an electronic health record. *Pharmacogenomics*. 2012; 13:407–18. [PubMed: 22329724]
27. Scott SA, Edelmann L, Kornreich R, et al. Warfarin pharmacogenetics: CYP2C9 and VKORC1 genotypes predict different sensitivity and resistance frequencies in the Ashkenazi and Sephardi Jewish populations. *Am J Hum Genet*. 2008; 82:495–500. [PubMed: 18252229]
28. Aklillu E, Leong C, Loebstein R, et al. VKORC1 Asp36Tyr warfarin resistance marker is common in Ethiopian individuals. *Blood*. 2008; 111:3903–4. [PubMed: 18362220]
29. Harrington DJ, Gorska R, Wheeler R, et al. Pharmacodynamic resistance to warfarin is associated with nucleotide substitutions in VKORC1. *J Thromb Haemost*. 2008; 6:1663–70. [PubMed: 18680536]
30. Shuen AY, Wong BY, Fu L, et al. Evaluation of the warfarin-resistance polymorphism, VKORC1 Asp36Tyr, and its effect on dosage algorithms in a genetically heterogeneous anticoagulant clinic. *Clin Biochem*. 2012; 45:397–401. [PubMed: 22266406]
31. Pavani A, Naushad SM, Rupasree Y, et al. Optimization of warfarin dose by population-specific pharmacogenomic algorithm. *Pharmacogenomics J*. 2011
32. Mitchell C, Gregersen N, Krause A. Novel CYP2C9 and VKORC1 gene variants associated with warfarin dosage variability in the South African black population. *Pharmacogenomics*. 2011; 12:953–63. [PubMed: 21635147]
33. Harrington DJ, Siddiq S, Allford SL, et al. More on: endoplasmic reticulum loop VKORC1 substitutions cause warfarin resistance but do not diminish gamma-carboxylation of the vitamin K-dependent coagulation factors. *J Thromb Haemost*. 2011; 9:1093–5. [PubMed: 21362126]
34. Suarez-Kurtz G. Population diversity and the performance of warfarin dosing algorithms. *Br J Clin Pharmacol*. 2011; 72:451–3. [PubMed: 21575037]
35. Cooper GM, Johnson JA, Langae TY, et al. A genome-wide scan for common genetic variants with a large influence on warfarin maintenance dose. *Blood*. 2008; 112:1022–7. [PubMed: 18535201]
36. Zhang JE, Jorgensen AL, Alfirevic A, et al. Effects of CYP4F2 genetic polymorphisms and haplotypes on clinical outcomes in patients initiated on warfarin therapy. *Pharmacogenet Genomics*. 2009; 19:781–9. [PubMed: 19741565]
37. Perini JA, Struchiner CJ, Silva-Assuncao E, et al. Impact of CYP4F2 rs2108622 on the stable warfarin dose in an admixed patient cohort. *Clin Pharmacol Ther*. 2010; 87:417–20. [PubMed: 20182420]
38. Bejarano-Achache I, Levy L, Mlynarsky L, et al. Effects of CYP4F2 polymorphism on response to warfarin during induction phase: a prospective, open-label, observational cohort study. *Clin Ther*. 2012; 34:811–23. [PubMed: 22417713]
39. King CR, Deych E, Milligan P, et al. Gamma-glutamyl carboxylase and its influence on warfarin dose. *Thromb Haemost*. 2010; 104:750–4. [PubMed: 20694283]
40. Cushman M, Booth SL, Possidente CJ, et al. The association of vitamin K status with warfarin sensitivity at the onset of treatment. *Br J Haematol*. 2001; 112:572–7. [PubMed: 11260056]
41. Braam L, McKeown N, Jacques P, et al. Dietary phylloquinone intake as a potential marker for a heart-healthy dietary pattern in the Framingham Offspring cohort. *J Am Diet Assoc*. 2004; 104:1410–4. [PubMed: 15354158]
42. Loebstein R, Yonath H, Peleg D, et al. Interindividual variability in sensitivity to warfarin-nature or nurture? *Clin Pharmacol Ther*. 2001; 70:159–64. [PubMed: 11503010]
43. Lurie Y, Loebstein R, Kurnik D, et al. Warfarin and vitamin K intake in the era of pharmacogenetics. *Br J Clin Pharmacol*. 2010; 70:164–70. [PubMed: 20653669]
44. Garcia DA, Lopes RD, Hylek EM. New-onset atrial fibrillation and warfarin initiation: high risk periods and implications for new antithrombotic drugs. *Thromb Haemost*. 2010; 104:1099–105. [PubMed: 20886196]

45. Lip GY, Andreotti F, Fauchier L, et al. Bleeding risk assessment and management in atrial fibrillation patients. Executive Summary of a Position Document from the European Heart Rhythm Association [EHRA], endorsed by the European Society of Cardiology [ESC]. Working Group on Thrombosis Thromb Haemost. 2011; 106:997–1011.

Figure 1. Weekly warfarin doses in carriers and non-carriers of D36Y

Warfarin dose requirements were significantly higher in carriers of D36Y (median [IQR] = 71.2 mg [60.6 to 102.5 mg]; $n=16$) compared to non-carriers (35 mg [22.5 to 47.5 mg]; $n=194$; $P<0.001$). Horizontal mid-lines represent the median, and bars the interquartile range.

Figure 2. Predicted vs. actual weekly warfarin doses for the IWPC model (left panel) and for the IWPC+D36Y model (right panel)
Carriers of D36Y are represented by closed red circles.

Figure 3. Model performance, assessed by coefficient of determination R^2 (upper panel) and the mean absolute error (MAE, lower panel), for increasing numbers of D36Y carriers
 For the IWPC model (red circles), R^2 decreased and MAE increased with increasing prevalence of carriers of the D36Y variant in the population. In contrast, for a model that included the D36Y covariate in addition to the IWPC algorithm (blue triangles), R^2 increased substantially and MAE only slightly with an increasing prevalence of D36Y carriers. Error bars represent standard error of the mean.

Table 1

Demographic, clinical, and genotyping information for 210 patients

Parameter	Value
Age, years	61.3 ± 16.2
Female sex	110 (52.4%)
Race: Asian, black or African American	0
Weight, kg	76.5 ± 15.9
Height, cm	170 ± 14
Amiodarone use	12 (5.7%)
Enzyme inducers	0
Weekly warfarin dose	
Median, IQR	35.0 mg (24.5 to 52.5 mg)
Range	7.0 to 150.0 mg

Genotypes	n for 0, 1, and 2 variant alleles	Minor allele frequency, %
CYP2C9 *2	158/51/1	12.6
CYP2C9 *3	166/41/3	11.2
CYP4F2 rs2108622 *	89/82/20	31.9
VKORC1 rs9923231	74/94/42	42.4
VKORC1 D36Y (rs61742245)	194/16/0	3.8

* Genotyping results were not available for 19 subjects.

Table 2

Model performance for the IWPC prediction model and the IWPC + D36Y model. Adding D36Y as covariate to the IWPC model improved model fit and decreased mean prediction error in the whole cohort. For non-carriers, both models yielded equivalent values for R^2 and mean absolute error

Group/Subgroup	Algorithm	R^2 , %	Mean absolute error $\pm$ SD, mg warfarin/week
Whole cohort n=210	IWPC	27.0	14.4 $\pm$ 16.2
	IWPC + D36Y	47.2	12.6 $\pm$ 12.4
Non-carriers of D36Y n=194	IWPC or IWPC+D36Y	35.6	11.8 $\pm$ 11.3