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Pharmacogenetic Warfarin Dose Refinements Remain Significantly Influenced by Genetic Factors after One Week of Therapy

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Summary

Introduction—By guiding initial warfarin dose, pharmacogenetic (PGx) algorithms may improve the safety of warfarin initiation. However, once INR response is known, the contribution

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of PGx to dose refinements is uncertain. This study sought to develop and validate clinical and PGx dosing algorithms for warfarin dose refinement on days 6–11 after therapy initiation.

Materials and Methods—An international sample of 2,022 patients at 13 medical centers on 3 continents provided clinical, INR, and genetic data at treatment days 6–11 to predict therapeutic warfarin dose. Independent derivation and retrospective validation samples were composed by randomly dividing the population (80%/20%). Prior warfarin doses were weighted by their expected effect on S-warfarin concentrations using an exponential-decay pharmacokinetic model. The INR divided by that "effective" dose constituted a *treatment response index*.

Results—Treatment response index, age, amiodarone, body surface area, warfarin indication, and target INR were associated with dose in the derivation sample. A clinical algorithm based on these factors was remarkably accurate: in the retrospective validation cohort its R^2 was 61.2% and median absolute error (MAE) was 5.0 mg/week. Accuracy and safety was confirmed in a prospective cohort (N=43). *CYP2C9* variants and *VKORC1*-1639 G→A were significant dose predictors in both the derivation and validation samples. In the retrospective validation cohort, the PGx algorithm had: R^2= 69.1% (P<0.05 vs. clinical algorithm), MAE= 4.7 mg/week.

Conclusions—A pharmacogenetic warfarin dose-refinement algorithm based on clinical, INR, and genetic factors can explain at least 69.1% of therapeutic warfarin dose variability after about one week of therapy.

Keywords

warfarin; *VKORC1*; *CYP2C9*; pharmacogenetic

Introduction

Despite the 2010 US Food and Drug Administration (FDA) label for warfarin (1) and the increasing enthusiasm for pharmacogenetic (PGx) testing,(2–4) the relevance of genotype after the first several days of therapy is unknown. At least a dozen studies (2,5–15) demonstrate that PGx algorithms utilizing genotype (especially *VKORC1* and *CYP2C9*) and clinical information explain about half of the variability of warfarin dose when warfarin is initiated. Some experts report that genotype contributes relatively little once the International Normalized Ratio (INR) response is available,(14,16,17) but others suggest that combining INR response and genotype could result in highly accurate warfarin algorithms.(8,12,18–20) Recently, we demonstrated the significance of PGx in an algorithm that incorporates an INR measured after 4 or 5 days of therapy,(18) but whether genotype should be used in subsequent dose revisions remains unclear.

This issue is important to resolve because warfarin causes more serious adverse events than almost any other drug.(21–23) The potential cost of genotyping, though, is also substantial: with nearly 2 million people initiating warfarin annually and the cost of genotyping being ~ $100 USD (and perhaps considerably more), expenditures on genotyping alone may exceed $200 million per year. Whether this investment justifies its costs depends on how long genotype remains a significant predictor of warfarin dose after INR values become available because better INR control can improve outcomes.

The present study quantified the contribution of genotype to warfarin response after approximately one week of therapy. We developed and validated practical clinical and PGx dosing algorithms that can be used to refine warfarin dose on days 6–11 of therapy, a period during which patients return for INR testing.

Materials and Methods

Population

After Institutional Review Board approval at each of the contributing sites (Supplemental Table S1), we obtained clinical and genetic data on 2,022 patients across 3 continents. All participants at each site provided written informed consent. Patients were excluded (n=338) if they were missing key data (a therapeutic dose; an INR on day 6, 7, 8, 9, 10, or 11; warfarin doses prior to INR draw; or genotype), if their baseline (pre-warfarin) INR was above 1.4, or if they were prescribed fresh frozen plasma or vitamin K prior to their INR measurement. We randomly sampled 80% of the final dataset for derivation (N=1342), setting aside 20% for internal validation (N=342). Genotyping quality control was performed as previously described.(15,24) An external prospective validation cohort (N=43) of orthopedic patients was utilized as a second confirmation of the safety and accuracy of the clinical algorithm (genetic testing information was not available for this external cohort).

Study Outcomes

The outcome was the therapeutic maintenance warfarin dose, defined as the dose that led to stable therapeutic anticoagulation levels. To have stable therapeutic anticoagulation levels, therapeutic INR values on at least two to three consecutive visits were required (see Supplemental Table S1).

Model Development

Using forward stepwise entry and backward stepwise elimination in linear regression, we quantified the relationship between therapeutic warfarin doses and a patient's genetic and clinical information available on days 6–11 of warfarin therapy. To preserve linearity, we transformed therapeutic doses by the natural log (ln). Variables were allowed to remain in the multivariable linear regression model if they achieved statistical significance ($p<0.05$) or were marginally significant ($0.05 \leq p \leq 0.20$) with biological plausibility. To prevent collinearity, height and weight were combined into body surface area (BSA) using the classic formula,(25) and previous doses were combined with INR in a “treatment response index” (described below in Pharmacokinetic Calculations). Warfarin initiation was variable: 20% (n=338) of participants were started using PGx dosing algorithms, as previously detailed.(2,8,12) Of those participants who initiated warfarin by PGx algorithm, n=47 began with a dose that was twice their estimated therapeutic dose and n=291 began with their estimated therapeutic dose. Stratification by protocol did not affect model performance. For patients who had multiple INR values measured on days 6–11, we selected one INR (at random) to determine which variables were independent predictors of therapeutic dose. Then, to use all of the INR data, we repeated this procedure 1000 times using a bootstrap procedure and averaged the resulting coefficients from the 1000 samples.

We quantified the predictive ability of demographics (gender and race/ethnicity), warfarin indication (atrial fibrillation, orthopedic surgery, venous thromboembolism, cardiac valve, and stroke), current medications (amiodarone, some CYP inducers and statins), comorbidities (diabetes or liver disease), genotype, INR values, and doses (see Pharmacokinetic Calculations below). Categorical variables were coded `1' if present and `0' if absent. In the final (pooled) algorithm, we included the *CYP2C9* inducers rifampin/rifamycin, carbamazepine, barbiturates, phenytoin/diphenylhydantoin when available (for unknown inducer status [n=794], we assumed the participant was not taking an inducer). Fluvastatin, simvastatin, lovastatin, rosuvastatin, and atorvastatin were tested individually and in combination. If diabetes status, smoking status, statin use, or amiodarone use was unknown (n=148, n=399, n=352, and n=97, respectively), their probabilities were estimated

using logistic regression and those probabilities used as the risk factor quantity in linear regression.

Pharmacokinetic Calculations

Due to correlation between INR and dose, ln(INR/effective dose) was entered in the model instead of separate terms for INR and warfarin doses. The use of this transformation, which we termed the *treatment response index*, resulted in a more stable model over the multiple days of therapy: the relationship between treatment response index and ln(therapeutic dose) was consistent over each day of therapy. This variable was used to model the effect of previous warfarin doses on the current INR.

The effective dose used in the treatment response index was calculated by summing weighted prior doses, with weightings based on empirically-derived data commensurate with their expected effect on S-warfarin concentration. In the derivation cohort, PK-PD decay functions were employed to refine the weighting structure corresponding to a half-life of 40 hours. Specifically, the concentration at time t, C(t), was modeled as a exponentially-decaying function of peak concentration C_0, time t, and first-order rate constant *r* that was genotype-specific. Linder et al (26) showed that C_0=(dose/2)/[(0.1/L/kg)*wt(kg)], such that $C(t)=C_0*\exp(-rt)$ could be solved for r, knowing the half-lives of the genotypes to be as follows (in hours): *1/*1 half-life: 30; *1/*2 half-life: 38; *1/*3 half-life: 51; *2/*2 half-life: 61; *2/*3 half-life: 76 and *3/*3 half-life: 203. The weights for each dose were calculated by fitting a log normal curve to points of the exponential decay, and the constraint of weight zero at time 0. Though we also derived weights in consideration of genotype, the data better supported combining the resulting gene-based weight structures (in consideration of allele frequencies) into a single clinical `average' weight structure. This average structure was used for the final model in the derivation cohort and then we re-derived the structure using the average 40 hour half-life for the pooled model. The final relative weights of doses that were prescribed 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 days ago in the effective dose calculation were 0.306, 0.804, 0.555, 0.357, 0.229, 0.149, 0.099, 0.067, 0.047, and 0.033, respectively (Supplemental Figure S1).

We confirmed that the effective dose led to better predictive accuracy (in terms of R^2 and median absolute error [MAE]) than average prior doses, cumulative prior doses, and individual prior doses. In the pooled model we also recalculated the dose-weighting structure using 60- and 80-hour half-lives (as opposed to gene-based half-lives) to determine the best overall fit. Again, the average half-life of S-warfarin in the general population (40 hours) yielded the most accurate results.

Statistical Analysis of Validation Cohorts

In the retrospective validation cohort, we used a simulation approach to randomly select one measurement from each patient who had more than one INR measurement and then bootstrapped the resulting sample (using 200 resamples). We averaged the resulting bootstrapped distributions (n=6) to compare accuracy (R^2 and MAE) between the PGx and clinical refinement models. We made comparisons overall and on specific days of therapy. In the prospective cohort, we used the pooled clinical algorithms in 43 patients starting warfarin therapy and report R^2 and MAE.

Genotyping

Genomic DNA was obtained from blood or buccal cells as previously described.(24) Genotyping was performed using polymerase chain reaction, pyrosequencing, solid phase minisequening, eSensor Warfarin Sensitivity Test (GenMark Diagnostics, Pasadena, CA), Infiniti Platform (Autogenomics, Carlsbad, CA), Homogeneous Mass Extend and iPLEX

assays (Sequenom, Hamburg, Germany), or ABI PRISM 7500 Sequence Detection System and TaqMan® Pre-Developed Assay Reagent kits for Allelic Discrimination (Applied Biosystems, CA, USA). We coded *CYP2C9*2* (rs1799853) and *CYP2C9*3* (rs1057910) single nucleotide polymorphisms as 0 if absent, 1 if heterozygous, and 2 if homozygous. To improve accuracy in African ancestry populations, we also included genotyping results for *CYP2C9*5* (rs28371686) and *CYP2C9*6* (rs9332131) from the two sites which had collected this information (Alabama and St. Louis). Three individuals (all African ancestry) were known to carry these variants in the derivation cohort, but no carriers were present in the retrospective validation cohort. We followed the lead of Cavallari and included C*YP2C9*5 and CYP2C9*6* in the count for the number of *CYP2C9*3* alleles,(27) because of their similar effects on CYP2C9 metabolism.(28)

Likewise, *VKORC1*–1639 G>A (rs9923231, formerly called *VKORC1* 3673) was coded 0 (homozygous GG), 1 (heterozygous AG), or 2 (homozygous AA).(29) If *VKORC1*–1639 G>A genotype was missing (N=298), we inferred it from *VKORC1* 1173/6484 C>T (rs9934438) or *VKORC1* 1542/6853 G>C (rs8050894), which are in high linkage disequilibrium.(30)

Results

The average age was 60 years in the derivation cohort and 58 years in the retrospective validation group, while just over half of participants were male in both samples (Table 1).

Derivation

In the derivation cohort (N = 1,342), therapeutic dose was inversely associated with the treatment response index, as well as *VKORC1-1639 A*, *CYP2C9*2*, and *CYP2C9*3* ($P<0.001$). Other significant, independent predictors of therapeutic dose were age, BSA, stroke history, target INR, amiodarone, use of fluvastatin or simvastatin, valve indication, use of inducers, and day of therapy. Other statins were not significant predictors. Significant predictors of therapeutic dose in the clinical refinement algorithm were the same except that genotype was not offered into the model. Overall, the clinical refinement algorithm explained 61.9% of the variation in the derivation cohort and had a MAE of 5.2 mg/week. The PGx refinement algorithm explained 69.8% of the variation in the derivation cohort and had a MAE of 4.9 mg/week. For both algorithms, accuracy increased with subsequent days of therapy (data not shown).

Retrospective Validation

In the retrospective validation cohort (N = 342), the clinical refinement algorithm explained 61.2% of the variation in therapeutic dose and the PGx refinement algorithm explained 69.1% ($P<0.05$). While the MAE was less with the PGx algorithm (4.7 mg/week) than with the clinical algorithm (5.0 mg/week), with a similar difference to that of the derivation cohort, this difference was not significant overall, but was on multiple individual days (Table 2). The correlation between predicted and therapeutic dose ranged from 57.5–90.3%, depending on day of therapy (Table 2, supplemental Figure S2). PGx dosing was most helpful in patients whose therapeutic dose was ≤21 mg/week or >55 mg/week (supplemental Figure S3).

All of the clinical and PGx algorithms were more accurate than previously validated algorithms, including initiation (10) and day 4 algorithms (18). The variance explained (and MAE) calculated among this study's retrospective validation population for a previously-reported warfarin initiation protocol (10) was 13.7% (8.5 mg/week) for the clinical algorithm and 48.7% (6.2 mg/week) for the PGx algorithm. For a post-initiation protocol

(calculated on day 4, after 3 doses) (18) the clinical and PGx results herein were 43.1% (6.8 mg/week) and 57.2% (5.3 mg/week), respectively.

Pooled Algorithms

Variables and their relationship to therapeutic dose in the final pooled analyses were similar to the derivation cohort (Tables 3 and 4), except the valve indication for warfarin was not significant in either clinical or pharmacogenetic pooled models. The equations for dose prediction based on the pooled data were:

> PGx algorithm dose (mg/week) = EXP (2.59853 − 0.47578 × Treatment Response Index − 0.17132 × VKORC1 − 0.23385 × CYP2C9*3 − 0.10696 × CYP2C9*2 − 0.00549 × Age in years + 0.16491 × BSA − 0.09091 × Simvastatin Use − 0.251 × Fluvastatin Use − 0.11994 × Amiodarone Use + 0.3319 × Inducer Use + 0.08796 × Target INR − 0.13902 × Stroke + 0.01028 × Day of Therapy)
>
> Clinical algorithm dose (mg/week) = EXP (2.19023 − 0.66327 × Treatment Response Index − 0.00379 × Age in years + 0.1095 × BSA − 0.06548 × Simvastatin Use − 0.2809 × Fluvastatin Use − 0.08761 × Amiodarone Use + 0.2612 × Inducer use + 0.04189 × Target INR − 0.13717 × Stroke + 0.01292 × Day of Therapy).

The pooled PGx algorithm had R^2=71.8% and MAE=4.7 mg/week, while the clinical algorithm had R^2=64.8%, MAE=5.1 mg/week.

Prospective Validation of Clinical Algorithm

In the prospective validation cohort (N = 43), the pooled clinical refinement algorithm explained 58% to 79% of the variation in therapeutic dose, depending on day of therapy (Table 5). The percentage of time in the therapeutic range during days 11–30 was 62%, with clinical outcomes of 8 patients (18.6%) having an INR > 4 during the first 30 days of treatment and one patient having a minor bleed. The new pooled clinical algorithm was significantly more accurate than previously validated algorithms (Table 5).

Discussion

Because of its low cost, excellent oral bioavailability, and proven efficacy, warfarin remains the most popular oral anticoagulant worldwide. The US FDA revised the label for Coumadin™/warfarin to support the use of genotyping because warfarin is widely used, genetic variation contributes to high inter-individual variability in dose requirements, and the high variability is associated with increased risk of hemorrhage. Although the current (2010) label provides information for tailoring the initial dose to genotype,(1) it does not describe how to adjust warfarin once the INR is available. With an inherent delay in genotyping (4,24) and a high incidence of warfarin-related hemorrhages during traditional warfarin initiation using “trial-and-error” dosing,(21,23) accurate dosing algorithms incorporating genetic and clinical factors—including early INR response data—are crucial to safety for millions of people who initiate warfarin each year.

Herein we developed and validated very accurate warfarin dosing algorithms. Clinical, INR, and PGx factors were included in predictive models that also utilized pharmacokinetic principles to estimate the effective blood concentration of warfarin that was expected based on dosing history. This demonstrated that the use of genetic factors for dose refinements 6–11 days after treatment initiation resulted in important, significant improvements in the prediction of maintenance warfarin dose compared with a clinical algorithm. The PGx algorithm also had a greater R^2 (69.1% in the retrospective validation cohort, 71.8% when pooled) and lower MAE (4.7 mg/week) than previous estimates that evaluated dose

variability at earlier time points (e.g., IWPC (15): R^2=47.0%, MAE=8.3 mg/week; Lenzini *et* al (18): R^2=63%, MAE=5.5 mg/week). The clinical algorithm had a similar or better R^2 (61.2% in the retrospective validation cohort, 58–79% in the prospective validation cohort) and MAE than prior PGx algorithms, possibly revealing the added value of the second INR data point incorporated into the *treatment response index*. The treatment response index provided substantially ability to predict warfarin dose in both the clinical and PGx algorithms by integrating prior warfarin doses, weighted according to time since administration of the dose, and by utilizing information from the INR.

Generally, warfarin algorithms (16) avoid the need for prescribing an agnostic dose (e.g., 5–10 mg) on the initial days of therapy, a regimen that often overdoses petite or elderly patients.(31,32,33) Traditional methods of dose adjustment, though, have not provided optimal outcomes, even after the use of a PGx dose-initiation algorithm.

Most prior warfarin PGx modeling has addressed individualizing the warfarin initiation dose. Although those models estimate the therapeutic dose,(2,5–15) they provide no explicit guidance about refining the dose once the INR is measured. Three small trials using PGx warfarin initiation algorithms found no significant benefit compared to empiric approaches.(2,34,35) A fourth trial of similar size (N=191) found fewer minor bleeds (although not powered for this outcome), perhaps because dose revisions were tailored to *CYP2C9* genotype.(3) Thus, use of genotype information after therapy initiation may be important, especially because most bleeds occur beyond the first days of warfarin therapy.(13,36–39)

Further, although evidence indicates that PGx warfarin dosing is most impactful at the onset of treatment, current genotyping turnaround times limit the ability to initiate dosing based on PGx information. Laboratory analysis alone requires several hours,(24) and it can be days between physician order and genotype availability.(4) This suggests that algorithms that refine warfarin dosing based on PGx and clinical characteristics remain relevant in clinical settings even when the initial dose is not tailored to a patients genetic-driven needs. PGx algorithms that incorporate INR values measured after 3 or 4 warfarin doses are more accurate than clinical algorithms that ignore genotype.(8,12,18) Based on the data herein, a dose-refinement algorithm between 6–11 days may be useful to resolve initial INR instability, especially in the outpatient setting where INR data often become available only after approximately 5 or more warfarin doses.

These new PGx and clinical algorithms should guide warfarin during and after the second week of warfarin therapy—the period of time associated with the highest rate of hemorrhage.(36–39) Furthermore, because of the high accuracy of both algorithms, they may minimize the need to modify the warfarin dose during the subsequent weeks of therapy. We postulate that the greater R^2 of the genetic algorithms vs. the clinical ones will translate into fewer adverse events in patients initiating warfarin therapy, and this hypothesis is being tested in several clinical trials, as noted below. Although the PGx improvement in MAE was modest for the retrospective validation population, this should not be interpreted as typical improvement for each patient. A better interpretation is that the clinical and PGx algorithms are similarly accurate for patients with typical genotypes, but the PGx algorithm is more accurate among the subset with an unusual genotype. A less accurate pharmacogenetic algorithm was able to correct the genetic propensity to an elevated INR in patients with less common genotypes.(40)

While some variation in warfarin dose remains unexplained and the improvement in the overall MAE for the PGx algorithm was not statistically significant in the smaller retrospective validation cohort, the improvements over previous algorithms may translate to better overall INR control and enhanced safety of warfarin therapy. However, because costs

were not addressed in this study, the question remains whether PGx warfarin treatment is cost-efficient.(41) A prior decision analysis (41) concluded that routine PGx testing was unlikely to be cost effective in the atrial fibrillation population. However, that analysis did not consider using genotype throughout the induction period (as proposed here), which should improve cost effectiveness. Likewise, although dabigatran appears cost effective compared to warfarin,(42) dabigatran has not been compared to PGx-based dosing of warfarin. At the present time, PGx-guided warfarin therapy is most useful in patients with higher risk for poor outcomes.

Large randomized trials are underway to quantify how the combination of PGx dose initiation and refinement affects laboratory and clinical outcomes. On the one hand, the novel approach using pharmacokinetic modeling, a treatment response index, and genetic information will improve the relevance of genotype, but, on the other hand, the accuracy of the clinical algorithm might suggest that PGx may not be necessary to accurately predict warfarin dose in clinical practice. The benefit of PGx and clinical algorithms applied during the first few days of dosing is being quantified currently in several multi-centered, randomized trials—COAG, GIFT of Warfarin, and EU-PACT (ClinicalTrials.gov Identifiers, NCT00839657, NCT01006733, NCT01119300, respectively).

Other limitations of the study, including for the clinical algorithm, are issues faced by many observational studies. For example, incomplete adjustment for measured variables by regression modeling may have affected the accuracy of the clinical or PGx algorithms. Race was not a significant predictor of dose and not all races were represented similarly in the study (the majority were Caucasian), thus the algorithms may not apply as well to other populations. Finally, the assumptions behind the modeling, including in composing the treatment response index, may be simplifications of the actual mathematical relationships.

Conclusions

A pharmacogenetic warfarin dose-refinement algorithm that incorporates clinical, INR, and genetic factors explained 69.1% or more of the variability in therapeutic dose. Even after a week of therapy, this novel approach that includes the use of pharmacokinetic modeling, a treatment response index, and genetic data, significantly improved dose prediction over an approach that did not use genetic data, although the MAE was not significantly reduced. The use of these dose-refinement algorithms for warfarin dose adjustments during days 6–11 of therapy may improve the safety of warfarin initiation, and we have made them publicly available at www.WarfarinDosing.org. Because of the relative size of the clinical benefit for the PGx algorithm, further evaluations compared to a clinical algorithm and compared to new anticoagulants (e.g., dabigatran, rivaroxaban, apixaban) are needed to determine whether coupling the wealth of practical warfarin experience to PGx dose revision is clinically superior for each patient's care. PGx dosing may be most useful among patients initiating warfarin who are at high risk for an adverse event.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Potential conflicts of interest include that Dr. Kimmel reports receiving an honorarium from Ortho-McNeil for a talk on warfarin and grant funding from the Aetna Foundation for warfarin-related research and Dr. Burmester reports a patent for *CYP4F2*-based warfarin pharmacogenetic dosing that is licensed to Osmetech (a gene not studied here) and grant funding from Third Wave and Osmetech for a warfarin study not related to this manuscript.

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Table 1

Baseline characteristics of the derivation and validation populations.

	Derivation Cohort (N=1,342)	Retrospective Validation Cohort (N=342)	Prospective Validation Cohort (N=43)
Demographic Variables			
Male, N (%)	734 (54.7)	186 (54.4)	18 (41.9)
African-American, N (%)	64 (4.8)	17 (5)	6 (14.0)
Caucasian, N (%)	1,115 (83.1)	281 (82.2)	36 (83.7)
Asian Race, N (%)	146 (10.9)	36 (10.5)	0 (0)
Other/Unknown Race, N (%)	17 (1.3)	8 (2.3)	1 (2.3)
Hispanic Ethnicity, N (%)	7 (0.5)	3 (0.9)	0 (0)
Unknown Ethnicity, N (%)	73 (5.4)	15 (4.4)	0 (0)
Indication			
Orthopedic (Hip or Knee), N (%)	229 (17.1)	65 (19)	43 (100)
DVT or PE, N (%)	487 (36.3)	118 (34.5)	0 (0)
Atrial Fibrillation/Flutter, N (%)	375 (27.9)	88 (25.7)	0 (0)
Stroke, N (%)	17 (1.3)	5 (1.5)	0 (0)
Valve, N (%)	140 (10.4)	41 (12)	0 (0)
Other/Missing Indication, N (%)	56 (4.2)	16 (4.7)	0 (0)
Allele Frequecies			
CYP2C9*2	0.103	0.115	N/A
CYP2C9*3	0.064	0.064	N/A
VKORC1	0.418	0.402	N/A
Clinical Variables			
Age , mean (SD), years	59.7 (14.2)	58.4 (14.1)	72.8 (6.0)
Height, mean (SD), in	67 (4.3)	67 (4.3)	66.4 (4.6)
Weight, mean (SD), lbs	186 (50.5)	189 (51)	196 (49.4)
Therapeutic Warfarin Dose, geometric mean (SD)	4.6 (1.49)	4.7 (1.54)	3.8 (1.6)
Target INR, mean (SD)	2.3 (0.3)	2.3 (0.3)	2.2 (0.1)
INR6, geometric mean (SD)	2.12 (1.36)	2.14 (1.37)	1.83 (1.33)
INR7, geometric mean (SD)	2.11 (1.35)	2.11 (1.40)	2.06 (1.54)
INR8, geometric mean (SD)	2.11 (1.33)	2.13 (1.37)	2.32 (1.48)
1st Warfarin Dose, mean (SD), mg	7.2 (3.5)	7.5 (3.4)	4.4 (0.7)
2nd Warfarin Dose, mean (SD), mg	6.1 (2.6)	6.4 (2.7)	4.0 (1.4)
3rd Warfarin Dose, mean (SD), mg	4.8 (2.4)	4.9 (2.4)	4.5 (1.4)
4th Warfarin Dose, mean (SD), mg	4.4 (2.5)	4.5 (2.4)	3.6 (1.8)
5th Warfarin Dose, mean (SD), mg	4.4 (2.4)	4.5 (2.2)	4.3 (1.8)
6th Warfarin Dose, mean (SD), mg	4.4 (2.4)	4.6 (2.2)	4.4 (1.6)
7th Warfarin Dose, mean (SD), mg	4.5 (2.5)	4.5 (2.3)	4.1 (2.1)
Statin Use, N (%)	270 (20.1)	71 (20.8)	13 (30.2)
Simvastatin Use, N (%)	147 (11)	37 (10.8)	4 (9.3)
Fluvastatin Use, N (%)	8 (0.6)	2 (0.6)	0 (0)
Lovastatin Use, N (%)	11 (0.8)	1 (0.3)	2 (4.7)

	Derivation Cohort (N=1,342)	Retrospective Validation Cohort (N=342)	Prospective Validation Cohort (N=43)
Atorvastatin Use, N (%)	92 (6.9)	28 (8.2)	1 (2.3)
Rosuvastatin Use, N (%)	7 (0.5)	2 (0.6)	1 (2.3)
Pravastatin Use, N (%)	3 (0.2)	0 (0)	3 (7.0)
Amiodarone Use, N (%)	40 (3)	9 (2.6)	0 (0)
Inducer Use, N (%)	7 (0.5)	1 (0.3)	0 (0)
Current Smoker, N (%)	143 (10.7)	31 (9.1)	3 (7.0)
Liver Disease, N (%)	13 (1)	2 (0.6)	1 (2.3)
Diabetes, N (%)	78 (5.8)	25 (7.3)	6 (14.0)

Table 2

Accuracy of clinical and pharmacogenetic algorithms in the retrospective validation cohort for days 6–11 of therapy.

Day of therapy	n*	Clinical R^2†	Pharmacogenetic R^2†	Clinical MAE‡	PGx MAE‡	*p-value*§
6	114	58.8%	70.6%	6.19	5.52	0.0408
7	142	60.4%	69.1%	5.18	5.00	0.1248
8	193	59.6%	67.9%	4.87	4.97	0.0264
9	106	65.3%	73.7%	5.12	4.23	0.0086
10	77	57.5%	59.0%	4.65	3.72	0.2263
11	27	84.4%	90.3%	5.46	4.46	0.2564

*Note that individual participants may be included in more than one row (i.e. if they had more than one INR value), thus this column does not sum to the overall sample size;

†R^2 is percent variation explained by the model;

‡MAE is median absolute dosing error (mg/week);

§Wilcoxon Signed Rank test for paired difference between PGx MAE and Clinical MAE.

Table 3

Predictors in the pharmacogenetic algorithm in the pooled cohort and their effect (percent change) on warfarin dose requirements.

Variable	Coefficient	SE	Partial R^2	Unit	Effect on dose* (95% CI)	P-value
Intercept	2.598	0.096	-----	-----	-----	<0.001
Treatment						
Response Index†	−0.476	0.016	0.619	0.1	−5% (−5%, −4%)	<0.001
VKORC1	−0.171	0.011	0.025	1	−16% (−17%, −14%)	<0.001
Age in years	−0.005	0.0005	0.025	10	−5% (−6%, −4%)	<0.001
CYP2C9*3	−0.234	0.018	0.021	1	−21% (−24%, −18%)	<0.001
CYP2C9*2	−0.107	0.014	0.007	1	−10% (−13%, −8%)	<0.001
BSA	0.165	0.025	0.007	0.5	+9% (6%, 11%)	<0.001
Simvastatin Use	−0.091	0.020	0.003	1	−9% (−12%, −5%)	<0.001
Inducer Use	0.332	0.077	0.002	1	+39% (20%, 62%)	<0.001
Fluvastatin Use	−0.251	0.080	0.002	1	−22% (−34%, −9%)	<0.01
Amiodarone Use	−0.120	0.038	0.002	1	−11% (−18%, −4%)	<0.01
Target INR‡	0.088	0.024	0.002	0.25	+2% (1%, 3%)	<0.01
Stroke Indication	−0.139	0.054	0.001	1	−13% (−22%, −3%)	<0.05
Day of Therapy	0.010	0.005	0.0008	1	+1% (0%, 2%)	<0.05

*% Effect on the estimate of the maintenance dose is calculated per variant allele, per 0.1 units of the treatment response index, per decade of age, per 0.5 m^2 of body surface area, and per 0.25 unit increase in target INR;

†The treatment response index is calculated as ln(INR/effective dose), where the effective dose is the PK-PD weighted average dose of the doses preceding the INR, based on the expected concentrations of these doses (see text);

‡INR is International Normalized Ratio.

Table 4

Predictors in the clinical algorithm in the pooled cohort and their effect (percent change) on warfarin dose requirements.

Variable	Coefficient	SE	Partial R^2	Unit	Effect on dose* (95% CI)	P-value
Intercept	2.19023	0.10433	-----	-----	-----	<0.001
Treatment						
Response Index†	−0.663	0.015	0.619	0.1	−6% (−7%, −6%)	<0.001
Age in years	−0.004	0.0005	0.016	10	−4% (−5%, −3%)	<0.001
BSA	0.110	0.028	0.003	0.5	+6% (3%, 9%)	<0.001
Fluvastatin Use	−0.280	0.090	0.002	1	−24% (−37%, −10%)	<0.01
Simvastatin Use	−0.065	0.022	0.002	1	−6% (−10%, −2%)	<0.01
Inducer Use	0.261	0.086	0.002	1	+30% (10%, 54%)	<0.01
Stroke Indication	−0.137	0.061	0.001	1	−13% (−23%, −2%)	<0.05
Day of Therapy	0.013	0.006	0.001	1	+1% (0%, 2%)	<0.05
Amiodarone Use	−0.088	0.042	0.0009	1	−8% (−16%, 0%)	<0.05
Target INR‡	0.042	0.026	0.0005	0.25	+1% (0%, 2%)	0.2

*% Effect on the estimate of the maintenance dose is calculated per 0.1 units of the treatment response index, per decade of age, per 0.5 m^2 of body surface area, and per 0.25 unit increase in target INR;

†The treatment response index is calculated as ln(INR/effective dose), where the effective dose is the PK-PD weighted average dose of the doses preceding the INR, based on the expected concentrations of these doses (see text);

‡INR is International Normalized Ratio.

Table 5

Accuracy of clinical algorithms in the prospective validation cohort.

Day of Therapy	# of patients	R^2	MAE (mg/week)	Algorithm
1	43	0.24	7.49	(10)
4	42	0.39	7.28	(18)
7	26	0.58	5.67	Table 4
10	35	0.79	2.66	Table 4

MAE= median absolute error. Not all patients had an INR to use to calculate the predicted dose at day 4, so the prediction from day 3 or 5 was used if needed. If no INR was available from day 7, the value from day 6 or 8 was used. Likewise, if no INR was available from day 10, the value from day 9 or 11 was used.