Blood Cells, Molecules, and Diseases 43 (2009) 119–128

Contents lists available at ScienceDirect

Blood Cells, Molecules, and Diseases

journal homepage: www.elsevier.com/locate/ybcmd

Influence of *CYP2C9* and *VKORC1* on warfarin response during initiation of therapy ☆

N.A. Limdi [a,*], H. Wiener [b], J.A. Goldstein [c], R.T. Acton [d], T.M. Beasley [e]

[a] Department of Neurology, University of Alabama at Birmingham, 1719 6thAvenue South, CIRC-312, Birmingham AL 35294-0021, USA
[b] Department of Epidemiology, University of Alabama at Birmingham, AL, USA
[c] Laboratory of Pharmacology and Chemistry, National Institute of Environmental Health Sciences, University of Alabama at Birmingham, AL, USA
[d] Department of Microbiology, University of Alabama at Birmingham, AL, USA
[e] Department of Biostatistics, Section on Statistical Genetics, University of Alabama at Birmingham, AL, USA

ARTICLE INFO

Article history:
Submitted 15 October 2008
Revised 27 November 2008
Available online 17 March 2009

(Communicated by R. I. Handin, M.D.,
6 February 2009)

Keywords:
CYP2C9
VKORC1
Pharmacogenetics
Percent time in target range
Over-anticoagulation
Hemorrhage
African American
European American

ABSTRACT

Background: Although multiple reports have documented the influence of *CYP2C9* and *VKORC1* variants on warfarin dose, risk of over-anticoagulation and hemorrhage, their influence on anticoagulation maintenance and individual proportion of time spent in target INR range (PPTR) is limited. Moreover the potential benefit of genotype-guided dosing implemented after initiation of therapy in a racially diverse population has not been explored. Herein we present the influence of *CYP2C9* and *VKORC1 C1173T* on warfarin response during the first 30 days of therapy.

Methods: Warfarin dose was empirically determined in 250 African Americans 271 European Americans. The influence of *CYP2C9* and *VKORC1* on rate of INR increase, anticoagulation maintenance, risk of over-anticoagulation, and change in dose over 30 days was evaluated after adjustment for socio-demographic, lifestyle and clinical factors.

Possession of variant *VKORC1* (± variant *CYP2C9*) genotype was associated with a more rapid attainment of target INR and higher frequency of dose adjustments. Patients possessing variant genotypes spent less time in target range. However adjustment for rate of INR increase rendered the association non-significant. European Americans (but not African Americans) possessing variant *VKORC1* (± variant *CYP2C9*) genotype had a higher risk of over-anticoagulation. Neither *CYP2C9* nor *VKORC1* influenced the risk of minor hemorrhage. *CYP2C9* and *VKORC1* explained 6.3% of the variance in dose change over the first 30 days of therapy demonstrating that the usefulness of genotype-guided dosing may extend beyond first day of therapy.

Conclusion: The benefit of genotype-based dose prediction may extend beyond first few days of therapy. Whether genotype-guided dosing will decrease the risk of over-anticoagulation, improve anticoagulation control and most importantly improve outcomes for chronic warfarin users remains to be proven.

Initiation of warfarin therapy in a qualifying patient has long been an iterative process, in which, initially a standard dose is prescribed and then adjusted based on observed response. Recognition and incorporation of the influence of patient-specific factors (e.g. age, weight, medications, etc) has facilitated improvements in estimating dose [1]. However despite these refinements, stabilizing therapy may take weeks to months. Even after stabilization, the International Normalized Ratio (INR) is maintained in target range only 40–60% of the time [2–5]. Therefore, during the remaining unprotected time periods, especially during initiation of therapy, patients may be at an increased risk of hemorrhagic or thromboembolic complications [6–8].

The recognition of genetic control of warfarin response has stimulated efforts to quantify this influence. The significant influence of Cytochrome *P*4502C9 (*CYP2C9*, *2 and *3) and Vitamin K epoxide reductase (*VKORC1 C1173T* and *−1639G/A*) variants on warfarin dose has been demonstrated in observational studies among patients of European [9–25] and African [26–28] descent, prospective studies [29,30] and randomized clinical trials [31,32]. This evidence served as the main impetus to the recent warfarin package insert update (http://www.fda.gov/cder/drug/infopage/warfarin/default.htm) by the United States Food and Drug Administration (FDA). Although this change may be a sign of personalized medicine making initial steps into the mainstream several key issues including feasibility of implementation, utility and effectiveness of genotype-based therapy in clinical practice need to be addressed. As genotype-based therapy is a fairly recent development, few laboratories provide such services.

☆ Supported in part by grants from the National Heart Lung and Blood Institute (RO1HL092173) and the National Institute of Neurological Disorders and Stroke (K23NS45598) and in part by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences.

* Corresponding author.
E-mail address: nlimdi@uab.edu (N.A. Limdi).

1079-9796/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcmd.2009.01.019

Therefore the availability of genotype information prior to administration of the first warfarin dose is not feasible for most patients. Delaying initiation of warfarin therapy is not an option as this will likely delay discharge in hospitalized patients, prolong the use of heparins in ambulatory patients and increase healthcare costs. Therefore it is likely that even proponents of genotype-based therapy will initiate warfarin without genotype information.

Although prospective efforts [29,31,32] demonstrated the superiority of genotype-based dosing in prediction of warfarin dose, they failed to demonstrate improvement in anticoagulation control. Millican et al. and Schwarz et al. recently reported on the influence of *CYP2C9* and *VKORC1* on warfarin response during initiation of therapy in Caucasian cohorts [29,33]. However the influence of genes on initial warfarin response in a racially diverse population is not well documented and the benefit of genotype-guided dosing implemented a few days after initiation of therapy has not been explored.

Herein we present the influence of *CYP2C9* (*2, *3, *5, *6 and *11) and *VKORC1 C1173T* (hereafter referred to as *VKORC1*) genotype on warfarin response in both African Americans and European Americans during initiation (first 30 days) of therapy. Specifically we evaluate the influence of *CYP2C9* and *VKORC1* on the rate of INR increase, percent INRs in target range, and risk of over-anticoagulation. We also evaluate whether implementation of *CYP2C9* and *VKORC1* genotyping can improve dose refinement by assessing genotype effects on change in warfarin dose over the first 30 days of therapy.

Methods

The Pharmacogenetic Optimization of Anticoagulation Therapy (POAT) is an ongoing prospective cohort study aimed at defining the influence of polymorphisms in *CYP2C9* and other genes on warfarin response over a 2-year follow-up period. Patients were enrolled from the anticoagulation clinic at The Kirklin Clinics and the Jefferson Clinic P.C., Jefferson County Health System under the approval of the respective Institutional Review Boards. Both clinics follow a standardized empiric approach to manage anticoagulation therapy [1].

Inclusion and exclusion

Patients ≥20 years of age were identified at the initiation of warfarin therapy. Patients were considered eligible if the intended duration of anticoagulation therapy was ≥2 years, therapy was managed at the anticoagulation clinic and the target INR range was 2–3.

Data collection

A structured interview form was used at the time of enrollment to obtain a detailed medical lifestyle and concomitant medication history. Information on self-reported race, indication for therapy, demographics, height and weight, medications and comorbid conditions was documented. Lifestyle and socioeconomic data included smoking, alcohol use, education, annual household income, medical insurance, physical activity, and dietary vitamin K intake. Medical history was then verified by medical records review. All patients were followed at monthly intervals for up to two years from initiation of therapy. At each visit factors influencing warfarin response such as warfarin dose, INR, concurrent medications, dietary vitamin K (number of servings of foods rich in vitamin K consumed per week) alcohol intake (number of alcoholic drinks per week), compliance and level of physical activity were documented. For this study we focused on warfarin response in the first month of therapy.

DNA extraction and genotyping

Methodology for *CYP2C9* and *VKORC1* variants were detailed in recent manuscripts [28,34]. Briefly *CYP2C9* genotyping was conducted using pyrosequencing methods and PCR-RFLP methodology. *VKORC1 C1173T* (rs9934438) genotyping was conducted using the Sequenom iPLEX technology at the Broad Institute (http://www.sequenom.com).

Outcome definitions and statistical methods

Analysis of variance was used to assess group differences for continuous variables and χ^2 test of independence for categorical variables. The assumption of Hardy Weinberg Equilibrium (HWE) was tested using the χ^2 test of independence and exact statistics obtained using a Markov Chain Monte Carlo algorithm [35]. *CYP2C9* and *VKORC1* genotypes were first categorized into two groups: variant (one or both variant alleles) versus wild-type. The resulting four genotypic groups were; wild-type for both *VKORC1* and *CYP2C9* (referent group), variant *VKORC1* and wild-type *CYP2C9*, wild-type *VKORC1* and variant *CYP2C9*, and variant *VKORC1* and variant *CYP2C9* (multiple variants). All multivariable analysis models included genetic (*VKORC1* and *CYP2C9*), socio-demographic, lifestyle and clinical

Table 1
Cohort characteristics African American ($n=250$) and European American ($n=271$) participants with follow-up of ≥30 days[a].

	Genotype* (wt = wild-type, v = variant)				*P*-value
	wtCYP2C9 wtVKORC1 $N=247$	*wtCYP2C9 vVKORC1* $N=159$	*vCYP2C9 wtVKORC1* $N=57$	*vCYP2C9 vVKORC1* $N=58$	
Age	60.6 (±15.4)	62.1 (±16.8)	61.3 (±13.6)	62.7 (±15.3)	0.70
BMI	30.3 (±7.7)	29.3 (±6.7)	30.2 (±6.3)	28.2 (±6.3)	0.17
Race					
African American	176 (71.3%)	46 (28.9%)	24 (42.1%)	4 (6.9%)	<0.0001
European American	71 (28.7%)	113 (71.1%)	33 (57.9%)	54 (93.1%)	
Gender					
Female	130 (52.6%)	81 (50.9%)	26 (45.6%)	22 (37.9%)	0.21
Male	117 (47.4%)	78 (49.1%)	31 (54.4%)	36 (62.1%)	
No alcohol intake	194 (78.5%)	122 (76.7%)	40 (70.2%)	34 (58.6%)	0.01
Current smokers	34 (13.8%)	22 (13.8%)	9 (15.8%)	4 (6.9%)	0.48
Education					
≤High school	172 (69.6%)	84 (52.8%)	32 (56.1%)	24 (41.4%)	<0.0001
>High school	75 (30.4%)	75 (47.2%)	25 (43.9%)	34 (58.6%)	
Annual household income					
<50,000	210 (85.4%)	114 (72.1%)	39 (68.4%)	32 (55.2%)	<0.0001
≥50,000	36 (14.6)	44 (27.8%)	18 (31.6%)	26 (44.8%)	
Medical Insurance	206 (83.4%)	136 (85.5%)	49 (86.0%)	53 (92.9)	0.33
Indication for warfarin**					
Arterial	91 (36.8%)	77 (48.4%)	24 (42.1%)	28 (48.3%)	0.10
Venous	108 (43.7%)	59 (37.1%)	20 (35.1%)	23 (39.7%)	0.46
Both	23 (9.3%)	15 (9.4%)	5 (8.8%)	6 (10.3%)	0.90
Other	40 (16.2%)	21 (13.2%)	8 (14.0%)	9 (15.2%)	0.86
Number of comorbid conditions					
Low (0 or 1)	82 (33.2%)	38 (24.9%)	12 (21.0%)	15 (25.9%)	0.17
Medium (2 to 4)	110 (44.5%)	75 (47.2%)	34 (59.6%)	29 (50.0%)	
High (5 or more)	55 (22.3%)	46 (28.9%)	11 (19.3%)	14 (24.1%)	
Concurrent medications					
Antiplatelet agents	83 (33.6%)	69 (43.4%)	26 (45.6%)	25 (43.1%)	0.12
CYP2C9 substrate	51 (20.6%)	33 (20.7%)	9 (15.8%)	8 (13.8%)	0.56
CYP2C9 inhibitors	29 (11.7%)	26 (16.3%)	10 (17.5%)	7 (12.1%)	0.46

**CYP2C9* Variant genotype includes *2, *3 alleles among European Americans and *2, *3, *5, *6 and *11 alleles among African Americans.
Variant *VKORC1 C1173T* (rs9934438) includes '*TT* or *CT*'.
** Arterial thromboembolism includes patients with MI, Stroke and TIA. Venous thromboembolism includes patients with DVT and PE. Both include patients with venous and arterial events. None includes patients with no thromboembolic events (e.g. Atrial Fibrillation).
Comorbid conditions include cardiomyopathy, congestive heart failure, diabetes mellitus, hyperlipidemia, hypertension, malignancy, coronary artery disease, renal insufficiency and renal failure Patients can have more than one indication for therapy and comorbid conditions.
[a] All patients had a prescribed target INR range of 2–3. Patients with orthopedic surgery excluded due to short (3–6 months) treatment duration, patients with mechanical heart valve and hypercoagulable state excluded due to higher intensity of anticoagulation required. Three Hispanic patients excluded Mean (SD) displayed for continuous variables and frequency counts (column percent) for categorical variables.

factors (age, race, gender, education, medical insurance, income, alcohol, smoking, compliance, vitamin K intake, comorbid conditions, use of interacting drugs, etc).

Rate of INR increase was calculated for each patient utilizing all INR assessments up to the time at which target INR was first attained. Patients with fewer than three assessments ($n=35$) prior to attaining target were excluded as a patient-specific rate could not be estimated. The influence of *CYP2C9* and *VKORC1* genotypes on the rate of INR increase, warfarin maintenance dose, and change in dose over the first 30 days (difference in dose = dose on day 30 − dose on day 1) was evaluated using multivariable regression analysis.

We recently reported the influence of *CYP2C9* and *VKORC1* on attainment of target INR and stable dosing [36]. Herein we assess the influence of *CYP2C9* and *VKORC1* on anticoagulation control using proportion INRs in range. Computation of this measure encompassed the period of time after attainment of target INR until the completion of 30 days. Proportion of INRs in range was calculated by dividing the number of INR's within target range by the total number of INR's during the selected time interval for each patient. Assessing genotypic differences in percent INRs in/below/above range can be misleading as it ignores three vital issues; the influence of socio-demographic, clinical and environmental factors (e.g. drug interactions), the correlation between repeat INR measurements within an individual, and the inequality in the number of INR measurements between patients. To account for this we conducted multivariable analyses using PROC MIXED (SAS version 9.1).

Over-anticoagulation was defined as episodes where the patients INR exceeded four. Hemorrhagic complications were classified as minor or major using the scheme detailed by Fihn et al. [37] Minor hemorrhages included mild nosebleeds, microscopic hematuria, mild bruising, and mild hemorrhoidal bleeding. Serious, life threatening and fatal bleeding episodes were combined into one endpoint; 'Major

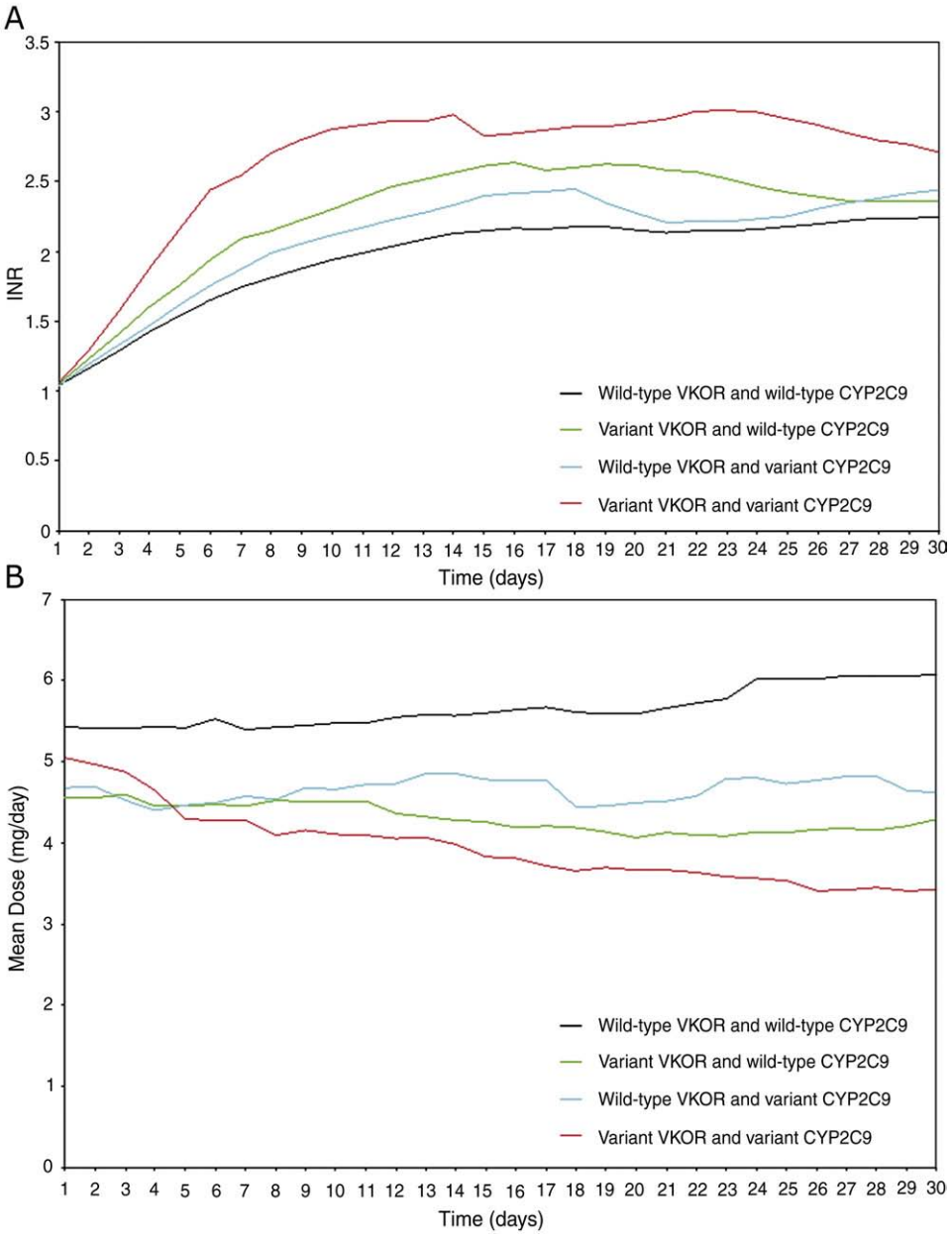

Fig. 1. The influence of *CYP2C9* and *VKORC1* genotype status on INR increase (Panel A) in response to the doses administered (Panel B) during initiation of warfarin therapy.

hemorrhage' since these events are infrequent and demand intervention. All major hemorrhagic complications were adjudicated by the Director of the Anticoagulation Clinic blinded to genotype.

In any given patient, INR deviates from target (2.5, range 2–3) over time in response to many independent perturbations. Higher intra-individual variation in INR (due to unobserved/unmeasured factors) may influence (increase) the risk of over-anticoagulation and hemorrhagic complications. To capture its effect we computed a patient-specific variance growth rate (Vscore), a cumulative measure of time-weighted variance of the INR for each time interval (between visits) as proposed by Fihn et al, [37] with minor modification [36]. This measure adjusts for the influence of the number of visits and the interval between visits on INR variation for each patient. The use of the Vscore from the preceding interval accounts for the patient-specific unobserved heterogeneity in the analyses of time to over-anticoagulation.

To assess the risk of over-anticoagulation and hemorrhagic complications the hazard ratio (HR) and 95% CI were obtained using the counting process format in the PH model. This format allows individuals to contribute more than one event. Valid confidence intervals were obtained by correction of dependence using robust variance estimation [38,39]. These multivariable analyses also included changes in medications, vitamin K and alcohol intake, and Vscore as time-varying covariates. The evaluation of gene–hemorrhage association also included INR at the time of the event. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC) at a non-directional alpha level of 0.05.

Results

Patients meeting eligibility criteria between August 2003 and April 2007 ($n=621$) were asked to participate in the study. Forty-three (6.9%) patients declined participation. Genotypic, socio-demographic and medical characteristics of the cohort ($N=578$, 47.2% African American, 51% men) have been detailed in prior publications [28,34,36,40]. Since the purpose of the study was to assess response to warfarin therapy during the first 30 days of therapy, we excluded patients ($n=57$ due to follow-up duration was <30 days, unavailable data on initial dosing and INR response, and unavailable genotype information). Additionally three Hispanic patients were excluded resulting in a final analyzable cohort of 521 participants. There were no significant differences in age, BMI, indication for therapy, number of comorbid conditions, concurrent medications, and proportion of men, current smokers and insured participants across the four genotype groups (Table 1).

Genotype distributions for *CYP2C9* and *VKORC1* were in HWE among European Americans (all *P*-values >0.5) and African Americans (all *P*-values >0.25) [36]. Of the variant *CYP2C9* alleles tested only *CYP2C9**5, *CYP2C9**6, and *CYP2C9**11 were observed among African Americans while *CYP2C9**2, and *CYP2C9**3 were observed among European Americans and African Americans [28,34,36]. Variant *CYP2C9* genotype was more common among European Americans than among African Americans (33% versus 11%, $P<0.0001$). European Americans had higher frequency of variant *VKORC1 C1173T* genotype compared to African Americans (60.4% versus 20.1%, $P<0.0001$). European Americans had a higher prevalence of multiple variants (i.e. variant *CYP2C9* and *VKORC1* genotype, Table 1).

Influence of CYP2C9 and VKORC1 on rate of INR increase during initiation of therapy

The association of warfarin dose on INR increase (Fig. 1A) in response to the doses administered (Fig. 1B) during initiation of therapy is significantly influenced by *CYP2C9* and *VKORC1* genotype status. Median time to attain target INR was 9.0 days (Inter-quartile-range- IQR: 4.2–26.4). Attainment of target INR was significantly faster among patients with variant *VKORC1* only (median 6.0, IQR 3.4–19.1) and variant *CYP2C9* and *VKORC1* (median 5.0, IQR 2.5–11.2) compared to those with variant *CYP2C9* only (median 12.7, IQR 4.3, 26.3) and no variant *CYP2C9* and *VKORC1* alleles (median 12.3, IQR 5.7, 31.7, $P<0.0001$).

The mean rate of INR increase, 0.12 U/day (median 0.1, inter-quartile range (IQR) 0.03 to 0.19), did not differ across race ($P=0.68$). The rate of INR increase was significantly influenced by possession of variant *CYP2C9* and *VKORC1* alleles in univariate and multivariable analyses ($P<0.0001$, Table 2). Neither socio-demographic factors (age, gender, race, alcohol intake, current smoking, health insurance, income or education; all *P*-values >0.15) nor concomitant medications (*CYP2C9* inducers, inhibitors or substrates, statins; all *P*-values >0.5) influenced rate of INR increase. Higher comorbidity ($P=0.1$), average loading dose ($P=0.07$) and weight (or BMI, $P=0.09$) showed marginal statistically significant influence.

Influence of CYP2C9 and VKORC1 on change in warfarin dose during initiation of therapy

As this was an observational study, dose determination was based on demographic and clinical characteristics only with adjustments based on INR assessments without knowledge of patients' genotype. Initial warfarin doses, determined by the treating physician, reflect varying dosing patterns with 61% patients receiving 5 mg (17.5% 2.5 mg, 3.5% 7.5 mg, 10.5% 10 mg and 7.5% other) per day.

Univariate analyses showed strong association between genotype and dose at the time of attainment of first target INR ($P=0.002$ European Americans, $P=0.004$ for African Americans) and on Day 30 ($P<0.0001$ for European and African Americans). Multivariable analyses demonstrated significant influence of variant genotypes in both race-adjusted (all *P*-values ≤ 0.0004) and race-stratified analyses. Adjusted initial dose, mean dose at time of attainment of target INR and mean dose on Day 30 are displayed in Fig. 2A stratified by *CYP2C9* and *VKORC1* genotype status. Among European Americans possession of lone *VKORC1* ($P<0.0001$), lone *CYP2C9* ($P=0.0004$) and *VKORC1* ± *CYP2C9* ($P<0.0001$) variants was associated with significant dose reduction (27%, 26% and 50% respectively). Among African Americans possession of lone *VKORC1* ($P=0.003$) and *VKORC1* ± *CYP2C9* ($P<0.0001$) variants was associated with significant dose reduction (24%, and 80% respectively). Although possession of lone *CYP2C9* was associated with a 17% reduction in dose requirement, it did not attain statistical significance ($P=0.17$). This is consistent with our earlier report [36].

Table 2
Influence of *CYP2C9* and *VKORC1* on rate of INR increase during initiation of warfarin therapy.

Genotype	*N*	INR increase per day (Mean, SE)			
		Unadjusted	*P*	Adjusted[a]	*P*
wtCYP2C9, *wtVKORC1*	232	0.107 (0.007)	<0.0001	0.103 (0.008)	<0.0001
wtCYP2C9, *vVKORC1*	146	0.142 (0.009)		0.150 (0.010)	
vCYP2C9, *wtVKORC1*	55	0.114 (0.015)		0.123 (0.015)	
vCYP2C9, *vVKORC1*	53	0.179 (0.015)		0.196 (0.017)	

Rate of INR increase was calculated for each patient utilizing all INR assessments up to the time at target INR was first attained. Patients with fewer than three assessments ($n=35$) prior to attaining target were excluded as a patient-specific rate could not be estimated.
wt = wild-type, v = variant.
CYP2C9 Variant genotype includes *2, *3 alleles among European Americans and *2, *3, *5, *6 and *11 alleles among African Americans.
Variant *VKORC1 C1173T* includes '*TT* or *CT*'.
[a] Adjusted for age, race, gender, BMI, vitamin K intake, alcohol, education, insurance, income, smoking, number of comorbid conditions, concomitant therapy with *CYP2C9* inhibitors and statin therapy and average loading dose.

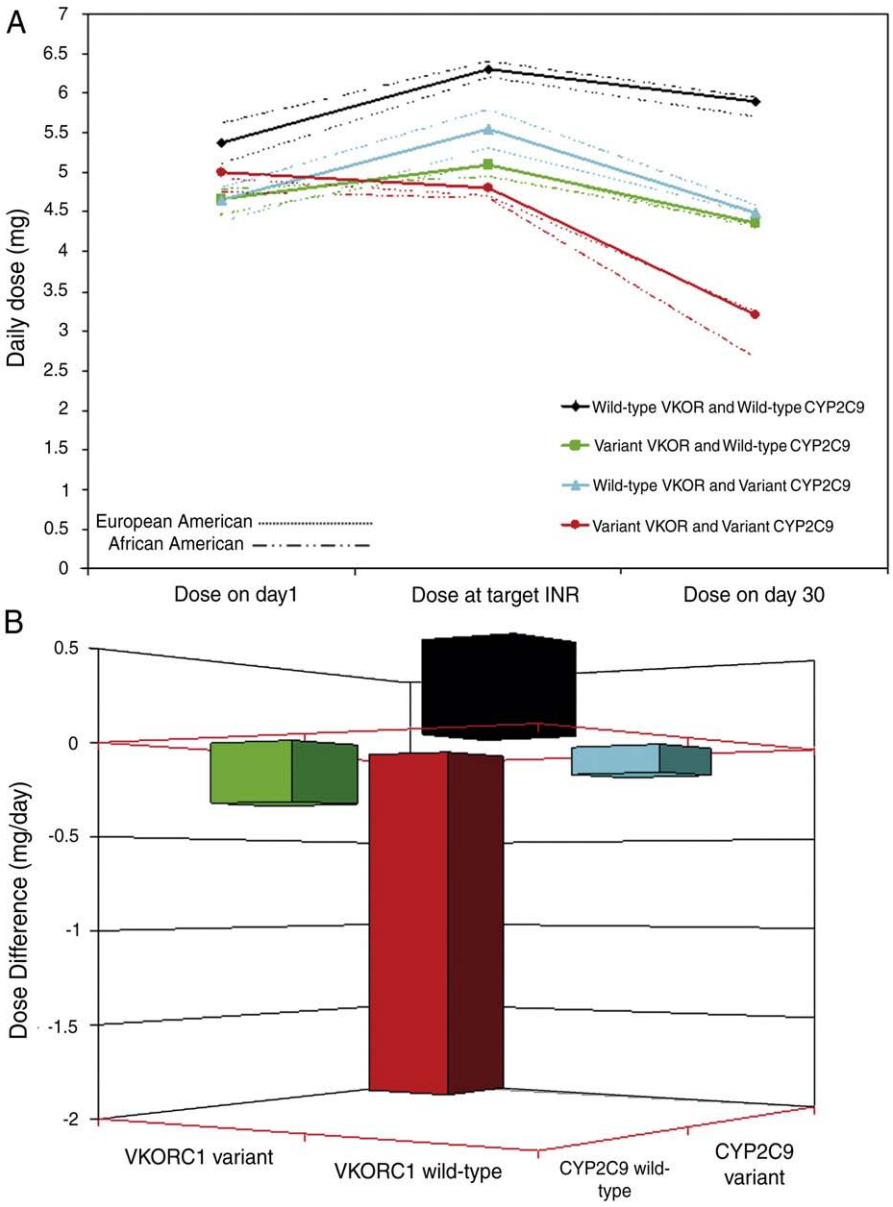

Fig. 2. (Panel A) Mean daily warfarin dose (least square mean in mg/day) by *CYP2C9* and *VKORC1* genotype status on Day 1, Day at which target INR was attained and Day 30. (Panel B) Mean dose difference (least square mean difference in mg/day; difference in dose on day 30 and dose on day 1) by *CYP2C9* and *VKORC1* genotype status. Both analyses adjusted for age, gender, race, BMI, vitamin K intake, alcohol intake, education, health insurance, income, smoking, number of comorbid conditions, concomitant therapy with *CYP2C9* inhibitors and HMG-Coenzyme A inhibitors.

To assess whether incorporation of genotype information after initiation of therapy would help refine dose adjustments we assessed the influence of *CYP2C9* and *VKORC1* on dose difference (Day30 dose − Day1 dose). Dose difference was not significantly different among patients possessing lone *CYP2C9* variant ($P=0.7$), marginally significant among those with lone *VKORC1* variant ($P=0.12$) genotype. Among patients with wild-type *CYP2C9* and wild-type *VKORC1* genotype dose on Day1 was significantly lower (by approximately 0.5 mg/day) than dose required to maintain target INR on Day30. Among patients with variant *CYP2C9* and variant *VKORC1* genotype dose on Day1 was significantly higher by approximately 2.0 mg/day) than dose required to maintain target INR (Fig. 2B,

Table 3
Daily warfarin dose requirements among patients by genotype group.

Warfarin	Genotype (wt = wild-type, v = variant)				*P*-value
Dose (mg/day)	*wtCYP2C9 wtVKORC1*	*wtCYP2C9 vVKORC1*	*vCYP2C9 wtVKORC1*	*vCYP2C9 vVKORC1*	
Day 1	5.37 [5.1, 5.6]	4.67 [4.3, 5.0]	4.65 [1.1, 5.2]	5.10 [4.5, 5.7]	0.01
Day 30	5.85 [5.5, 6.1]	4.36 [4.0, 4.7]	4.49 [3.9, 5.1]	3.20 [2.6, 3.8]	<0.0001
Difference	0.48 [0.6, 0.83]	−0.32 [−0.75, 0.11]	−0.15 [−0.83, 0.53]	−1.95 [−2.7, −1.2]	<0.0001

Least square means adjusted for age, race, gender, BMI, vitamin K intake, alcohol, education, insurance, income, smoking, number of comorbid conditions, concomitant therapy with *CYP2C9* inhibitors and HMG-Coenzyme A inhibitors.

Table 4
Univariate associations of *CYP2C9* and *VKORC1*genotypes on anticoagulation control during the first 30 days of therapy among POAT participants[a].

	Genotype (wt = wild-type, v = variant)				*P*-value
	wtVKORC1 wtCYP2C9	*vVKORC1 wtCYP2C9*	*wtVKORC1 vCYP2C9*	*vVKORC1 vCYP2C9*	
Number of patients	247	159	57	58	
African American	176	46	24	4	
European American	71	113	33	54	
Number of visits	1174	835	252	349	<0.0001
African American	875	235	98	23	
European American	299	600	154	326	
Number of dose changes	94 (8.0%)	106 (12.7%)	21 (8.3%)	37 (10.6%)	0.006
Dose increase	43 (3.7%)	21 (2.5%)	7 (2.8%)	4 (1.1%)	0.08
Dose decrease	51 (4.3%)	85 (10.2%)	14 (5.5%)	33 (9.4%)	<0.0001
Measures of anticoagulation control after attainment of first target INR (range2–3)					
Percent INRs[b]					
<2	18.8%	22.2%	18.6%	15.9%	0.26
2–3	57.7%	47.8%	51.0%	54.8%	0.026
>3	23.5%	30.0%	30.4%	29.3%	0.12
		Variant *CYP2C9* or *VKORC1* genotype			
<2	18.8%		20.0%		0.62
2–3	57.7%		50.2%		0.011
>3	23.5%		29.8%		0.016

3 Hispanic patients excluded.
CYP2C9 Variant genotype includes *2, *3 alleles among European Americans and *2, *3, *5, *6 and *11 alleles among African Americans.
Variant *VKORC1 C1173T* (rs9934438) includes '*TT* or *CT*'.
[a] All patients had a prescribed target INR range of 2–3. Patients with orthopedic surgery excluded due to short (3–6 month) treatment duration, patients with mechanical heart valve and hypercoagulable state excluded due to higher intensity of anticoagulation required.
[b] Percent INRs in target range, Percent time in target range, Percent time below range, Percent time above range) were assessed after attainment of first INR in target range.

Table 3) on Day 30. These differences were consistent across race and remained significant after adjustment for other covariates ($P<0.0001$) with *VKORC1* and *CYP2C9* explaining 6.3% ($P<0.0001$) of the variance in dose difference (Day30 dose − Day1 dose).

Influence of CYP2C9 and VKORC1 on maintenance of therapeutic anticoagulation during initiation of therapy

In the first 30 days, patients possessing variant in both *CYP2C9* and *VKORC1* and those possessing lone *VKORC1* variants had a higher frequency of clinic visits and more frequent dose adjustments (Table 4). Among patients with no variants 54% of adjustments required a decrease in dose while 46% required dose increment. Dose adjustments more often involved dose decrement among patients with variant *VKORC1* (80%), *CYP2C9* (67%) and variant *VKORC1* + *CYP2C9* (89%) genotypes.

After attainment of first target INR, percent INRs in target range (for the remainder of the 30 days) was higher among patients with no variants compared to those possessing variant *CYP2C9*, variant *VKORC1* and variant *VKORC1* + *CYP2C9* genotypes ($P=0.026$, Table 4). This finding, influenced more so by the presence of *VKORC1* variants ($P=0.02$) than presence of *CYP2C9* variant ($P=0.84$), remained consistent after adjusting other covariates ($P=0.038$). The absence of dose change between visits was a significant predictor ($P<0.0001$) of maintenance of INR in target range. Although patients with any variant (*CYP2C9*, or *VKORC1*, or both) had a high frequency of INRs above target range (INR >3, $P=0.016$) the association was not statistically significant ($P=0.16$) in multivariable analyses.

Since warfarin dosing patterns differed significantly across patients, we recognize that the rate of INR increase (time to target INR) may be influenced by doses administered in the first few days. This in turn may have influenced percent INRs in range after attainment of therapeutic anticoagulation. For example a patient may receive 10 mg/day while another (with the same genotypic and clinical characteristics) may receive 5.0 mg/day for the first two days. Although the 10 mg/day dose can shorten the time to attain target INR the maintenance of target INR may differ compared to 5 mg/day dose. Therefore time to attainment of target INR may be an independent predictor of maintenance of therapeutic anticoagulation. Moreover the different times to attain target INR would result in different length of observation period thereby allowing more or less opportunity for

Table 5
Adjusted hazard ratios (95% CI) for the association of *CYP2C9* and *VKORC1* on risk of over anticoagulation and associated complications[a].

Episodes	Genotype (wt = wild-type, v = variant)			
	wtVKORC1 wtCYP2C9 *N* = 247	*vVKORC1 wtCYP2C9* *N* = 159	*wtVKORC1 vCYP2C9* *N* = 57	*vVKORC1 vCYP2C9* *N* = 58
INR >4[b]	44 (3.7%)	67 (8.0%)	14 (5.5%)	33 (9.5%)
African American	37 (4.2%)	16 (6.8%)	5 (5.1%)	2 (8.7%)
European American	7 (2.3%)	51 (8.5%)	9 (5.8%)	31 (9.5%)
Minor hemorrhage[c]	12	18	3	4
African American	11	4	1	0
European American	1	14	2	4
Risk of over-anticoagulation (INR>4) during the first 30 days of therapy				
All patients	Ref	2.0 [1.28, 3.24]	1.4 [0.62, 3.14]	2.6 [1.50, 4.68]
African American	Ref	1.5 [0.70, 3.31]	0.9 [0.24, 3.29]	1.2 [0.34, 4.23]
European American	Ref	3.4 [1.32, 8.57]	2.6 [0.77, 8.44]	4.8 [1.83, 12.75]
Risk of minor hemorrhage during the first 30 days of therapy				
All patients	Ref	1.5 [0.43, 5.05]	1.2 [0.27, 5.81]	0.46 [0.04, 4.9]
African American	Ref	*	*	*
European American	Ref	*	*	*

3 Hispanic patients excluded.
CYP2C9 Variant genotype includes *2, *3 alleles among European Americans and *2, *3, *5, *6 and *11 alleles among African Americans.
Variant *VKORC1 C1173T* (rs9934438) includes '*TT* or *CT*'.
Hazard ratios adjusted for age, gender, Vscore, BMI, vitamin K intake, alcohol intake, number of comorbid conditions, education, insurance, income, smoking, concomitant therapy with *CYP2C9* inhibitors and HMG-Coenzyme A inhibitors after accounting for correlation between repeat episodes within the patient.
[a] All patients had a prescribed target INR range of 2–3. Patients with orthopedic surgery excluded due to short (3–6 month) treatment duration, patients with mechanical heart valve and hypercoagulable state excluded due to higher intensity of anticoagulation required.
[b] Percent of INRs above 4 calculated by dividing number of episodes of over-anticoagulation by total number of visits from Table 4.
[c] Minor hemorrhagic complications included mild nosebleeds (lasting less than 30 min) microscopic hematuria, mild bruising. The evaluation of gene–hemorrhage association also included INR at the time of the event.
* Race specific risks could not be estimated due to the low frequency of events.

INR deviation from target range. Therefore we reassessed the gene-percent-INR after incorporating time to attainment of target INR as a predictor in the multivariable model. Patients who attained target INR in less than 5 days were significantly more likely to have INR<2 ($P=0.02$), less likely to maintain INR in target range ($P=0.0002$) and more likely to have INR>3 ($P=0.04$). The influence of *CYP2C9* and *VKORC1* was not statistically significant (all *P*-values ≥ 0.12).

Influence of CYP2C9 and VKORC1 on risk of over-anticoagulation during initiation of therapy

One hundred and fifty eight episodes (6.0% of all INR measurements) of over-anticoagulation were encountered in 124 patients during the first 30 days of therapy. Over-anticoagulation was less frequent among patients with no variants compared to those possessing variant *CYP2C9*, variant *VKORC1* and variant *VKORC1* + *CYP2C9* genotypes ($P<0.0001$, Table 4) with *VKORC1* variants ($P<0.0001$) showing a stronger influence than *CYP2C9* variant ($P=0.04$).

The frequency of over-anticoagulation was higher in European Americans (98 episodes in 78 patients) than African Americans (60 episodes in 46 patients, $P=0.017$) but did not differ across gender ($P=0.53$). The frequency of over-anticoagulation did differ by genotype groups among European Americans ($P=0.0016$) but not among African Americans ($P=0.33$). However within African Americans ($P=0.08$) and European Americans ($P=0.0003$) over-anticoagulation was more frequent among patients with variant *VKORC1* genotype (Table 5). Therefore we present results of both race-adjusted and race-stratified analyses.

In multivariable analyses, after adjusting for race, possession of variant *VKORC1* ($P=0.003$) or variant *VKORC1* ± *CYP2C9* ($P=0.0008$) genotype increased the risk of over-anticoagulation while possession

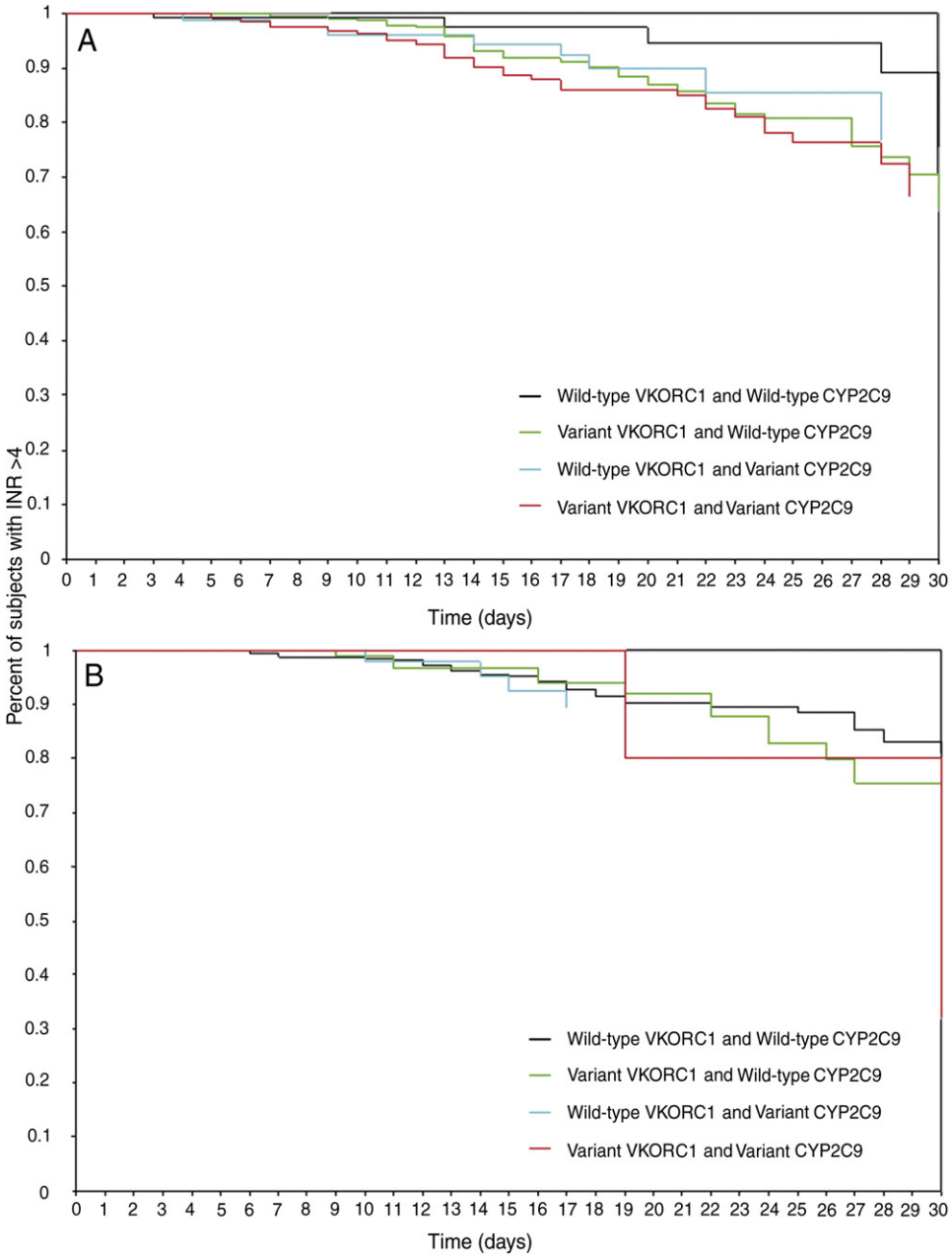

Fig. 3. Estimated survival curve from Cox PH model for time to over-anticoagulation (INR>4) in the first 30 days of therapy among European Americans (Panel A) and African Americans (Panel B). Models adjusted for age, gender, BMI, vitamin K intake, alcohol intake, education, health insurance, income, smoking, number of comorbid conditions, Vscore, concomitant therapy with *CYP2C9* inhibitors and HMG-Coenzyme A inhibitors.

of lone *CYP2C9* variant ($P=0.41$) did not (Table 5). Race-stratified analyses indicate statistically significant influence of *VKORC1* variants among European Americans ($P<0.01$, Fig. 3A) but not in African Americans ($P>0.3$, Fig. 3B). Possession of lone *CYP2C9* variant has a marginally significant effect among European Americans ($P=0.12$) but not among African Americans ($P=0.87$).

Thirty-seven minor hemorrhages were encountered in 28 patients during the first month. There was no difference in occurrence of minor hemorrhages across race ($P=0.41$). Neither *CYP2C9* nor *VKORC1* influenced the risk of minor hemorrhage (Table 5, all *P*-values >0.35). Race-stratified analyses could not be conducted due to the limited number of event during the 30 day study period. The association of *CYP2C9* and *VKORC1* could not be evaluated as only two major hemorrhages were encountered during the first 30 days.

Discussion

Our prospectively ascertained cohort study demonstrates the influence of *VKORC1* and *CYP2C9* on warfarin dose among both African Americans and European Americans. Consistent with prior reports possession of *VKORC1* variant was associated with significant dose reduction in both race groups whereas possession of *CYP2C9* variant was associated with statistically significant dose reduction only among European Americans [27,36]. Recent studies have demonstrated that pre-prescription genotype-based dosing significantly improves dose prediction [31,32,41]. This study, by assessing the influence of these genes on dose change over the first 30 days, demonstrates that the usefulness of genotype-based dose prediction may extend beyond the first day of initiation of therapy even with standard empiric dosing.

Among patients who possessed sole *VKORC1* or *CYP2C9* variants initial dose based on clinical and demographic characteristics were close approximations of dose on Day 30. Patients with no variant alleles and those that possessed both variant *VKORC1* and *CYP2C9* required significant dose adjustments over the 30 day study period. Dose prediction in these groups (comprising 60% of the cohort) could potentially be refined further by implementation of genotype-based dosing. This is consistent with the report by Anderson et al. who identified 55.6% of the cohort (wild-type for *VKORC1* and *CYP2C9* and those with multiple variants) that can potentially benefit from genotype-guided therapy. These findings have several significant implications for assessing the utility and cost effectiveness of genotype-based therapy. First, consistent with the recent report by Anderson et al., [31] genotype-guided therapy will improve dose prediction for a significant proportion (>50%) of warfarin users. Second, as reported by Millican et al. [29] genotype-based dose refinement may be beneficial even if implemented after the administration of clinically-determined dose for the first few days. The recent report by Schwarz et al. [33] indicates that both the *CYP2C9* and *VKORC1* had a significant influence on the required warfarin dose after the first 2 weeks of therapy. This latter finding, by allowing a more feasible genotyping time-frame, may facilitate implementation of such therapy in clinical practice. Incorporation of genotype information to determine warfarin dose can be facilitated through nomograms. One such dosing nomogram can be assessed at www.warfarindosing.org.

We demonstrate the larger influence of *VKORC1* in attaining target INR. In the first 30 days of therapy, possession of variant *VKORC1* was also associated with poor anticoagulation control (both percent INRs and percent time spent in target range) in multivariable analyses. However, incorporation of rate of target INR attainment rendered these associations statistically non-significant. These findings are consistent with the findings of a recent randomized clinical trail by Anderson et al. who concluded that pharmacogenetically guided therapy did not improve time spent within target INR range [31].

Our results with regard to the rate of target INR attainment are consistent with those of Schwarz et al. [33] but discordant with regard to percent INR in target range. The latter difference may be due to the differences in populations studied, the number and nature of covariates and the adjustment for time to attain target INR in our analyses. The influence of rate of INR attainment on quality of anticoagulation control needs to be assessed further. This constellation of findings suggests that genotype-guided therapy (by improved dose prediction) may allow the attainment of target INR at a rate that may favor improved maintenance of therapeutic anticoagulation.

Among Europeans, Schalekamp et al. reported an increased risk of over-anticoagulation (INR>6) among phenprocoumon users possessing variant *CYP2C9* and/or variant *VKORC1* genotype [42] and among acenocoumarol users possessing both variant *CYP2C9* and variant *VKORC1* genotype [43]. Schelleman et al., Kealey et al. and Schwarz et al. also reported a significantly increased risk of over-anticoagulation (INR>4) among European American warfarin users possessing variant *VKORC1* genotype after adjustment for *CYP2C9* and clinical covariates [26,27,33]. Our findings among European Americans concur with these earlier reports. The differences in risk ratios among European American populations in our study and prior studies may perhaps be explained by the number and nature of clinical covariates and the inclusion of a measure of intra-patient variability in INR (unobserved heterogeneity) in our analyses. Inability to account for such heterogeneity has been recognized as a limitation by several investigators [27,44]. Our results provide evidence that the gene-over-anticoagulation association is independent of such heterogeneity. The recent prospective randomized study by Anderson et al. confirms these gene-over-anticoagulation associations with the excess risk of over-anticoagulation driven mainly by the concurrent possession of *CYP2C9* and *VKORC1* variants [31].

Despite the lower frequency of variant genotypes and episodes of over-anticoagulation, the marginally significant *VKORC1* effect among African Americans ($P=0.08$) in our cohort suggests that effect of *VKORC1* variants is similar across race groups. However as reported by Schelleman et al. [27] the association of *VKORC1* on risk of over-anticoagulation among African Americans was not significant in multivariable analyses. Although we cannot explain the differential influence of variant *VKORC1* and *CYP2C9* on risk of over-anticoagulation across race groups, we can speculate on the influence and interplay of various factors:

1. Heterozygosity for *CYP2C9* (10.6% for African Americans versus 30.7% European Americans) and *VKORC1* genotype (18.2% for African Americans versus 50.2% for European Americans) varied across race. Similarly homozygosity for variant *CYP2C9* (1.2% for African Americans versus 3.0% European Americans) and *VKORC1* genotype (0.8% for African Americans versus 11.2% for European Americans) varied across race. The rarity of African Americans homozygous for the variant *CYP2C9* and *VKORC1* genotypes necessitated the categorization of genotypes as wild-type versus variant for multivariable analyses. Given the significant racial difference in prevalence of *VKORC1* and *CYP2C9* genotypes, such re-categorization may have differentially diluted the effect of gene-over-anticoagulation association across the race groups.
2. The association between the *VKORC1* polymorphisms studied and the causative polymorphism(s) that determines warfarin response is weaker in African Americans compared with European Americans because of different haplotype structures.
3. Genetic and environmental factors other then those studied influence the risk of over-anticoagulation in African Americans. This idea is supported by the higher intra-individual variation (Vscore, $P=0.004$) in INR among African Americans compared to European Americans.

To our knowledge, our cohort represents the largest population of African Americans genotyped for *CYP2C9* and *VKORC1*. Inclusion of the *5, *6 and *11 variants in the genotyping provides a robust estimate of the *CYP2C9* allele frequencies in this previously under-represented racial group. We did not assess the *−1639G/A* polymorphism (rs9923231) as studies have demonstrated that the 1173C and *−1639G* allele are in linkage disequilibrium among both African Americans [27] and European Americans [19]. We also recognize our sample-size was inadequate to detect significant *CYP2C9-VKORC1* interaction in either race group. Documentation of vitamin K intake was based on patient report using vitamin K inventory and was not quantified by assay/measurements [45]. However, all measurements were used consistently; therefore, bias if any should be non-differential. We recognize that many factors including changes in vitamin K intake can contribute to INR fluctuation [46,47]. The inclusion of the Vscore potentially accounts for the changes in unmeasured/unobserved environmental influences. We assessed the influence of only two genes (*CYP2C9* and *VKORC1*) and recognize that other genes may influence warfarin response or modify the effect of these genes. ApoE has recently been shown to influence warfarin dose among African Americans [48,49]. Other genes such as gamma-glutamyl carboxylase [15,21,50–52], calumenin [50,53], epoxide hydroxylase [23,53] may influence warfarin dose in this race group. However, the extent to which variability in other genes in the warfarin pathways influences warfarin response is yet to be resolved.

Conclusion

The benefit of genotype-based dose prediction may extend beyond first few days of therapy. Incorporation of genotype information after a few doses will allow enough time for offsite genotyping, avoid delaying initiation of therapy and make implementation of this technology more feasible in clinical practice. Whether genotype-guided dosing will decrease the risk of over-anticoagulation, improve anticoagulation control and most importantly improve outcomes for chronic warfarin users remains to be proven.

Acknowledgments

The authors thank Joyce Blaisdell for her work with *CYP2C9* genotyping. We are grateful to all the patients that participated in the study. We thank Janice Ware for her untiring efforts with patient recruitment and the staff of the Anticoagulation Clinic at The Kirklin Clinic, the Cooper Green Hospital and Jefferson Clinic P.C for their help with identification of potential participants. We also thank the physicians, especially Drs. Mark Wilson, and Melissa Baird; at the University of Alabama at Birmingham and the Health Service Foundation for their support of this research. Thanks to Steve Duncan and Darlene Green and the Office of Data Resources for their work with the POAT database and quality assurance.

This work was supported in part by grants from the National Heart Lung and Blood Institute (RO1HL092173) and the National Institute of Neurological Disorders and Stroke (K23NS45598) and in part by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences (ZO1 ES2104).

This study has contributed samples to the NINDS Human Genetics Resource Center DNA and Cell Line Repository (http://ccr.coriell.org/ninds), NINDS Repository sample numbers corresponding to the samples used are ND04466, ND04556, ND04604, ND04605, ND04626, ND04869, ND04907, ND04934, ND04951, ND05036, ND05108, ND05175, ND05176, ND05239, ND05605, ND05606, ND05701, ND05702, ND05735, ND06147, ND06207, ND06385, ND06424, ND06480, ND06706, ND06814, ND06871, ND06983, ND07057, ND07234, ND07304, ND07494, ND07602, ND07711, ND07712, ND08065, ND08596, ND08864, ND08932, ND09079, ND09172, ND09760, ND09761and ND09809.

The Broad Institute Center for Genotyping and Analysis is supported by grant U54 RR020278-01 from the National Center for Research Resources.

References

[1] O.L. Ansell JE, A.K. Wittkowsky, Managing Oral Anticoagulation Therapy: Clinical and Operational Guidelines Lippincott Williams & Wilkins, St. Louis, MO, 2005.
[2] J. Ansell, J. Hollowell, V. Pengo, F. Martinez-Brotons, J. Caro, L. Drouet, Descriptive analysis of the process and quality of oral anticoagulation management in real-life practice in patients with chronic non-valvular atrial fibrillation: the international study of anticoagulation management (ISAM), J. Thromb. Thrombolysis 23 (2007) 83–91.
[3] E. Chiquette, M.G. Amato, H.I. Bussey, Comparison of an anticoagulation clinic with usual medical care: anticoagulation control, patient outcomes, and health care costs, Arch. Intern. Med. 158 (1998) 1641–1647.
[4] A.K. Wittkowsky, Effective anticoagulation therapy: defining the gap between clinical studies and clinical practice, Am. J. Manag. Care 10 (2004) S297–S306 discussion S312–S317.
[5] A.K. Wittkowsky, E.B. Devine, Frequency and causes of overanticoagulation and underanticoagulation in patients treated with warfarin, Pharmacotherapy 24 (2004) 1311–1316.
[6] E.M. Hylek, A.S. Go, Y. Chang, N.G. Jensvold, L.E. Henault, J.V. Selby, D.E. Singer, Effect of intensity of oral anticoagulation on stroke severity and mortality in atrial fibrillation, N. Engl. J. Med. 349 (2003) 1019–1026.
[7] E.M. Hylek, D.E. Singer, Risk factors for intracranial hemorrhage in outpatients taking warfarin, Ann. Intern. Med. 120 (1994) 897–902.
[8] E.M. Hylek, S.J. Skates, M.A. Sheehan, D.E. Singer, An analysis of the lowest effective intensity of prophylactic anticoagulation for patients with nonrheumatic atrial fibrillation, N. Engl. J. Med. 335 (1996) 540–546.
[9] C.L. Aquilante, T.Y. Langaee, L.M. Lopez, H.N. Yarandi, J.S. Tromberg, D. Mohuczy, K.L. Gaston, C.D. Waddell, M.J. Chirico, J.A. Johnson, Influence of coagulation factor, vitamin K epoxide reductase complex subunit 1, and cytochrome P450 2C9 gene polymorphisms on warfarin dose requirements, Clin. Pharmacol. Ther. 79 (2006) 291–302.
[10] L. Bodin, C. Verstuyft, D.A. Tregouet, A. Robert, L. Dubert, C. Funck-Brentano, P. Jaillon, P. Beaune, P. Laurent-Puig, L. Becquemont, M.A. Loriot, Cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase (VKORC1) genotypes as determinants of acenocoumarol sensitivity, Blood 106 (2005) 135–140.
[11] P. Borgiani, C. Ciccacci, V. Forte, S. Romano, G. Federici, G. Novelli, Allelic variants in the CYP2C9 and VKORC1 loci and interindividual variability in the anticoagulant dose effect of warfarin in Italians, Pharmacogenomics 8 (2007) 1545–1550.
[12] G. D'Andrea, R.L. D'Ambrosio, P. Di Perna, M. Chetta, R. Santacroce, V. Brancaccio, E. Grandone, M. Margaglione, A polymorphism in the VKORC1 gene is associated with an interindividual variability in the dose-anticoagulant effect of warfarin, Blood 105 (2005) 645–649.
[13] B.F. Gage, C. Eby, J.A. Johnson, E. Deych, M.J. Rieder, P.M. Ridker, P.E. Milligan, G. Grice, P. Lenzini, A.E. Rettie, C.L. Aquilante, L. Grosso, S. Marsh, T. Langaee, L.E. Farnett, D. Voora, D.L. Veenstra, R.J. Glynn, A. Barrett, H.L. McLeod, Use of pharmacogenetic and clinical factors to predict the therapeutic dose of warfarin, Clin. Pharmacol. Ther. 84 (2008) 326–331.
[14] C. Geisen, M. Watzka, K. Sittinger, M. Steffens, L. Daugela, E. Seifried, C.R. Muller, T.F. Wienker, J. Oldenburg, VKORC1 haplotypes and their impact on the inter-individual and inter-ethnical variability of oral anticoagulation, Thromb. Haemost. 94 (2005) 773–779.
[15] D. Herman, P. Peternel, M. Stegnar, K. Breskvar, V. Dolzan, The influence of sequence variations in factor VII, gamma-glutamyl carboxylase and vitamin K epoxide reductase complex genes on warfarin dose requirement, Thromb. Haemost. 95 (2006) 782–787.
[16] R. Montes, E. Ruiz de Gaona, M.A. Martinez-Gonzalez, I. Alberca, J. Hermida, The c.-1639G > A polymorphism of the VKORC1 gene is a major determinant of the response to acenocoumarol in anticoagulated patients, Br. J. Haematol. 133 (2006) 183–187.
[17] T. Mushiroda, Y. Ohnishi, S. Saito, A. Takahashi, Y. Kikuchi, S. Saito, H. Shimomura, Y. Wanibuchi, T. Suzuki, N. Kamatani, Y. Nakamura, Association of VKORC1 and CYP2C9 polymorphisms with warfarin dose requirements in Japanese patients, J. Hum. Genet. 51 (2006) 249–253.
[18] K. Obayashi, K. Nakamura, J. Kawana, H. Ogata, K. Hanada, M. Kurabayashi, A. Hasegawa, K. Yamamoto, R. Horiuchi, VKORC1 gene variations are the major contributors of variation in warfarin dose in Japanese patients, Clin. Pharmacol. Ther. 80 (2006) 169–178.
[19] M.J. Rieder, A.P. Reiner, B.F. Gage, D.A. Nickerson, C.S. Eby, H.L. McLeod, D.K. Blough, K.E. Thummel, D.L. Veenstra, A.E. Rettie, Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose, New Engl. J. Med. 352 (2005) 2285–2293.
[20] H. Takahashi, G.R. Wilkinson, E.A. Nutescu, T. Morita, M.D. Ritchie, M.G. Scordo, V. Pengo, M. Barban, R. Padrini, I. Ieiri, K. Otsubo, T. Kashima, S. Kimura, S. Kijima, H. Echizen, 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 16 (2006) 101–110.
[21] M. Wadelius, L.Y. Chen, K. Downes, J. Ghori, S. Hunt, N. Eriksson, O. Wallerman, H. Melhus, C. Wadelius, D. Bentley, P. Deloukas, Common VKORC1 and GGCX

polymorphisms associated with warfarin dose, Pharmacogenomics J. 5 (2005) 262–270.
[22] H.Y. Yuan, J.J. Chen, M.T. Lee, J.C. Wung, Y.F. Chen, M.J. Charng, M.J. Lu, C.R. Hung, C.Y. Wei, C.H. Chen, J.Y. Wu, Y.T. Chen, A novel functional VKORC1 promoter polymorphism is associated with inter-individual and inter-ethnic differences in warfarin sensitivity, Hum. Mol. Genet. 14 (2005) 1745–1751.
[23] R. Loebstein, M. Vecsler, D. Kurnik, N. Austerweil, E. Gak, H. Halkin, S. Almog, Common genetic variants of microsomal epoxide hydrolase affect warfarin dose requirements beyond the effect of cytochrome P450 2C9, Clin. Pharmacol. Ther. 77 (2005) 365–372.
[24] E.A. Sconce, T.I. Khan, H.A. Wynne, P. Avery, L. Monkhouse, B.P. King, P. Wood, P. Kesteven, A.K. Daly, F. Kamali, The impact of CYP2C9 and VKORC1 genetic polymorphism and patient characteristics upon warfarin dose requirements: proposal for a new dosing regimen, Blood 106 (2005) 2329–2333.
[25] D.L. Veenstra, J.H. You, M.J. Rieder, F.M. Farin, H.W. Wilkerson, D.K. Blough, G. Cheng, A.E. Rettie, Association of Vitamin K epoxide reductase complex 1 (VKORC1) variants with warfarin dose in a Hong Kong Chinese patient population, Pharmacogenetics Genomics 15 (2005) 687–691.
[26] C. Kealey, Z. Chen, J. Christie, C.F. Thorn, A.S. Whitehead, M. Price, F.F. Samaha, S.E. Kimmel, Warfarin and cytochrome P450 2C9 genotype: possible ethnic variation in warfarin sensitivity, Pharmacogenomics 8 (2007) 217–225.
[27] H. Schelleman, Z. Chen, C. Kealey, A.S. Whitehead, J. Christie, M. Price, C.M. Brensinger, C.W. Newcomb, C.F. Thorn, F.F. Samaha, S.E. Kimmel, Warfarin response and vitamin K epoxide reductase complex 1 in African Americans and Caucasians, Clin. Pharmacol. Ther. 81 (2007) 742–747.
[28] N.A. Limdi, J.A. Goldstein, J.A. Blaisdell, T.M. Beasley, C.A. Rivers, R.T. Acton, Influence of CYP2C9 Genotype on warfarin dose among African American and European Americans, Personalized Medicine 4 (2007) 157–169.
[29] E.A. Millican, P.A. Lenzini, P.E. Milligan, L. Grosso, C. Eby, E. Deych, G. Grice, J.C. Clohisy, R.L. Barrack, R.S. Burnett, D. Voora, S. Gatchel, A. Tiemeier, B.F. Gage, Genetic-based dosing in orthopedic patients beginning warfarin therapy, Blood 110 (2007) 1511–1515.
[30] D. Voora, C. Eby, M.W. Linder, P.E. Milligan, B.L. Bukaveckas, H.L. McLeod, W. Maloney, J. Clohisy, R.S. Burnett, L. Grosso, S.K. Gatchel, B.F. Gage, Prospective dosing of warfarin based on cytochrome P-450 2C9 genotype, Thromb. Haemost. 93 (2005) 700–705.
[31] J.L. Anderson, B.D. Horne, S.M. Stevens, A.S. Grove, S. Barton, Z.P. Nicholas, S.F. Kahn, H.T. May, K.M. Samuelson, J.B. Muhlestein, J.F. Carlquist, Randomized trial of genotype-guided versus standard warfarin dosing in patients initiating oral anticoagulation, Circulation 116 (2007) 2563–2570.
[32] Y. Caraco, S. Blotnick, M. Muszkat, CYP2C9 genotype-guided warfarin prescribing enhances the efficacy and safety of anticoagulation: a prospective randomized controlled study, Clin. Pharmacol. Ther. 83 (2008) 460–470.
[33] U.I. Schwarz, M.D. Ritchie, Y. Bradford, C. Li, S.M. Dudek, A. Frye-Anderson, R.B. Kim, D.M. Roden, C.M. Stein, Genetic determinants of response to warfarin during initial anticoagulation, N. Engl. J. Med. 358 (2008) 999–1008.
[34] N. Limdi, G. McGwin, J. Goldstein, T. Beasley, D. Arnett, B. Adler, M. Baird, R. Acton, Influence of CYP2C9 and VKORC1 1173C/T genotype on the risk of hemorrhagic complications in African-American and European-American patients on warfarin, Mol. Ther. 83 (2008) 312–321.
[35] S.W. Guo, E.A. Thompson, Performing the exact test of Hardy–Weinberg proportion for multiple alleles, Biometrics 48 (1992) 361–372.
[36] N.A. Limdi, D.K. Arnett, J.A. Goldstein, T.M. Beasley, G. McGwin, B.K. Adler, A. R.T., Influence of CYP2C9 and VKORC1 polymorphisms on warfarin dose, anticoagulation attainment and maintenance among European American and African Americans, Pharmacogenomics 9 (2008) 511–526.
[37] S.D. Fihn, M. McDonell, D. Martin, J. Henikoff, D. Vermes, D. Kent, R.H. White, Risk factors for complications of chronic anticoagulation. A multicenter study. Warfarin Optimized Outpatient Follow-up Study Group, Ann. Intern. Med. 118 (1993) 511–520.
[38] C.F. Ake, and A.L. Carpenter, Extending the Use of PROC PHREG in Survival Analysis Proceedings of the 11th Annual Western Users of SAS SAS Institute Inc, Cary, NC:, 2003.
[39] T.M. Therneau, P.M. Grambsch, Multiple events per subject, in: T.M. Therneau, P.M. Grambsch (Eds.), Modeling Survival Data: Extending the Cox Model (Statistics for Biology and Health), Springer-Verlag, New York, New York, 2001, pp. 169–229.
[40] N.A. Limdi, T.M. Beasley, D.B. Allison, C.A. Rivers, R.T. Acton, Racial differences in the prevalence of Factor V Leiden mutation among patients on chronic warfarin therapy, Blood Cells Mol. Dis. 37 (2006) 100–106.
[41] M.A. Hillman, R.A. Wilke, S.H. Yale, H.J. Vidaillet, M.D. Caldwell, I. Glurich, R.L. Berg, J. Schmelzer, J.K. Burmester, A prospective, randomized pilot trial of model-based warfarin dose initiation using CYP2C9 genotype and clinical data, Clin. Med. Res. 3 (2005) 137–145.
[42] T. Schalekamp, B.P. Brasse, J.F. Roijers, E. van Meegen, F.J. van der Meer, E.M. van Wijk, A.C. Egberts, A. de Boer, VKORC1 and CYP2C9 genotypes and phenprocoumon anticoagulation status: interaction between both genotypes affects dose requirement, Clin. Pharmacol. Ther. 81 (2007) 185–193.
[43] T. Schalekamp, B.P. Brasse, J.F. Roijers, Y. Chahid, J.H. van Geest-Daalderop, H. de Vries-Goldschmeding, E.M. van Wijk, A.C. Egberts, A. de Boer, VKORC1 and CYP2C9 genotypes and acenocoumarol anticoagulation status: interaction between both genotypes affects overanticoagulation, Clin. Pharmacol. Ther. 80 (2006) 13–22.
[44] M. Wadelius, M. Pirmohamed, Pharmacogenetics of warfarin: current status and future challenges, Pharmacogenomics J. (2006).
[45] S.J. Booth, SL, J.L. Weihrauch, G. Ferland, Vitamin K (phylloquinone) content of foods. J Food Consump Anla 6 (1993) 109–120.
[46] R. Couris, G. Tataronis, W. McCloskey, L. Oertel, G. Dallal, J. Dwyer, J.B. Blumberg, Dietary vitamin K variability affects international normalized ratio (INR) coagulation indices, Int J Vitam Nutr Res 76 (2006) 65–74.
[47] L.J. Schurgers, M.J. Shearer, K. Hamulyak, E. Stocklin, C. Vermeer, Effect of vitamin K intake on the stability of oral anticoagulant treatment: dose-response relationships in healthy subjects, Blood 104 (2004) 2682–2689.
[48] S.E. Kimmel, J. Christie, C. Kealey, Z. Chen, M. Price, C.F. Thorn, C.M. Brensinger, C.W. Newcomb, A.S. Whitehead, Apolipoprotein E genotype and warfarin dosing among Caucasians and African Americans, Pharmacogenomics J. 8 (2008) 53–60.
[49] E.A. Sconce, A.K. Daly, T.I. Khan, H.A. Wynne, F. Kamali, APOE genotype makes a small contribution to warfarin dose requirements, Pharmacogenet Genomics 16 (2006) 609–611.
[50] R. Kimura, Y. Kokubo, K. Miyashita, R. Otsubo, K. Nagatsuka, T. Otsuki, T. Sakata, J. Nagura, A. Okayama, K. Minematsu, H. Naritomi, S. Honda, K. Sato, H. Tomoike, T. Miyata, Polymorphisms in vitamin K-dependent gamma-carboxylation-related genes influence interindividual variability in plasma protein C and protein S activities in the general population, Int J Hematol 84 (2006) 387–397.
[51] M.J. Rieder, A.P. Reiner, A.E. Rettie, Gamma-glutamyl carboxylase (GGCX) tagSNPs have limited utility for predicting warfarin maintenance dose, J Thromb Haemost 5 (2007) 2227–2234.
[52] R. Kimura, K. Miyashita, Y. Kokubo, Y. Akaiwa, R. Otsubo, K. Nagatsuka, T. Otsuki, A. Okayama, K. Minematsu, H. Naritomi, S. Honda, H. Tomoike, T. Miyata, Genotypes of vitamin K epoxide reductase, gamma-glutamyl carboxylase, and cytochrome P450 2C9 as determinants of daily warfarin dose in Japanese patients, Thromb. Res. 120 (2007) 181–186.
[53] M. Vecsler, R. Loebstein, S. Almog, D. Kurnik, B. Goldman, H. Halkin, E. Gak, Combined genetic profiles of components and regulators of the vitamin K-dependent gamma-carboxylation system affect individual sensitivity to warfarin, Thromb. Haemost. 95 (2006) 205–211.