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A polymorphism in the VKORC1-regulator calumenin predicts higher warfarin doses in African-Americans

D Voora[1,5], **DC Koboldt**[3], **CR King**[1], **PA Lenzini**[1], **CS Eby**[1,2], **R Porche-Sorbet**[2], **E Deych**[1], **M Crankshaw**[3], **PE Milligan**[1], **HL McLeod**[1], **SR Patel**[6], **LH Cavallari**[6], **PM Ridker**[4], **GR Grice**[1], **RD Miller**[3,*], and **BF Gage**[1]

[1]Department of Medicine, Washington University in St. Louis.

[2]Department of Pathology and Immunology, Washington University in St. Louis.

[3]Department of Genetics, Washington University in St. Louis.

[4]Center for Cardiovascular Disease Prevention and the Division of Preventive Medicine, Brigham and Women's Hospital and Harvard Medical School.

[5]Division of Cardiology, Duke University Medical Center.

[6]Department of Pharmacy Practice, University of Illinois at Chicago

Abstract

Corresponding author: Brian F. Gage, MD, MSc, telephone (314) 454-8369, fax (314) 454-5554, bgage@im.wustl.edu, Division of General Medical Sciences, Washington University School of Medicine Campus Box 8005, 660 S. Euclid Ave., St. Louis, MO, 63110.
*deceased

Authorship and conflicts of interest

Voora D: responsible for study design, analytical approach, analysis and interpretation of data, and drafting manuscript; no conflict of interest

Koboldt DC: responsible for study design, interpretation of sequence analysis, and critical revision of manuscript; no conflict of interest

King CR: responsible for study design, interpretation and performing genotype analysis, and critical revision of manuscript; no conflict of interest

Lenzini P: responsible for study design, interpretation and performing statistical analysis, and critical revision of manuscript; no conflict of interest

Eby C: responsible for study design, performing genotype and sequence analyses, and critical revision of manuscript; no conflict of interest

Porche-Sorbet R: responsible for performing genotype analysis, and critical revision of manuscript; no conflict of interest

Deych E: responsible for study design, interpretation and performing statistical analysis, and critical revision of manuscript; no conflict of interest

Crankshaw M: responsible for interpretation and performing sequence analysis and critical revision of manuscript; no conflict of interest

Milligan PE: responsible for participant recruitment and warfarin dose management, and critical revision of manuscript; no conflict of interest

McLeod HL: responsible for study design and critical revision of manuscript; no conflict of interest

Patel SR: responsible for genotyping and genotype analysis for replication cohort #2 and critical revision of the manuscript; no conflict of interest

Cavallari LH: responsible for participant recruitment and data analysis for replication cohort #2 and critical revision of the manuscript; no conflict of interest

Ridker P: responsible for providing PREVENT study samples and critical revision of manuscript; no conflict of interest

Grice GR: responsible for participant recruitment and, warfarin dose management, and critical revision of manuscript; no conflict of interest

Miller RD: responsible for study design, interpretation of sequence analysis, and critical revision of manuscript; no conflict of interest

Gage BF: responsible for study design, analytical approach, analysis and interpretation of data, obtaining funding, and critical revision of manuscript; no conflict of interest

Washington University in St. Louis may file for a patent for CALU rs339097.

Background—Warfarin demonstrates wide interindividual variability that is partly mediated by variants in *CYP2C9* and *VKORC1*. Whether variants in *CALU* (vitamin K reductase regulator) influence warfarin dose is unknown.

Methods and Results—We resequenced *CALU* regions in a discovery cohort of dose-outliers: patients with high(>90th percentile, n=55) or low(<10th percentile, n=53) dose requirements(after accounting for known genetic and nongenetic variables). One *CALU* variant, rs339097, was associated with high-doses(p=0.01). We validated this variant as a predictor of higher warfarin doses in two replication cohorts: 1)496 patients of mixed ethnicity, 2)194 African-American patients. The G allele of rs339097(African-American and Caucasian allele frequency 0.14 and 0.002, respectively), was associated with a 14.5%(SD±7%) greater therapeutic dose(p=0.03) in the first replication cohort and a higher than predicted dose in the second replication cohort(allele frequency=0.14, one-sided p=0.03).

Conclusions—*CALU* rs339097 A>G is associated with higher warfarin dose requirements independent of known genetic and nongenetic predictors of warfarin dose in African-Americans.

Keywords

anticoagulants; calumenin; genetic polymorphism; pharmacogenetics; warfarin/administration & dosage

Introduction

Warfarin sodium is a widely used anticoagulant for the prevention and treatment of venous thromboembolism, myocardial infarction, and stroke. Warfarin is characterized by a wide interindividual variability in dose response. In addition, warfarin has a narrow therapeutic index: International Normalized Ratio (INR) values greater than 3 or 4 increase the risk of bleeding while those significantly less than 2 are less effective.(1) Several single nucleotide polymorphisms (SNPs) in Cytochrome P450 2C9 and 4F2 (*CYP2C9* and *CYP4F2*, respectively) and vitamin K 2,3-epoxide reductase complex subunit 1 (*VKORC1*) are associated with dose-response, but together explain only 35% of the variation in warfarin dose requirements in Caucasians (2,3) and 10% in African-Americans(4)

Although recent whole genome association studies failed to identify novel variants outside of these three loci, they were limited to Caucasian patients.(5–7) Thus, additional genes may help predict warfarin dose, especially in African-American populations, where *VKORC1* and *CYP2C9* SNPs are not as predictive of the dose-response(8,9) and where warfarin resistance may be more common(10).

Studies of warfarin resistant rats and *in vitro* mRNA silencing experiments have identified calumenin (*CALU*) as a regulator of vitamin K 2,3-epoxide reductase (VKOR) and warfarin sensitivity.(11–13) In humans, limited studies of genetic variation in *CALU* in unselected warfarin treated patients have yielded unconvincing results. For example, an exonic *CALU* SNP, rs2290228, was found in one patient with an exceptionally high warfarin dose(14) but *CALU* SNPs have not been significantly associated with dose in other studies.(15,16)

To systematically investigate if genetic variation in *CALU* is associated with warfarin sensitivity or resistance, we chose a two-stage study design (Figure 1). In the first stage we selected patients from a well-characterized parent cohort who were at the extremes of warfarin dose requirements after accounting for known genetic and nongenetic factors from a parent cohort of patients taking warfarin chronically (Figure 1, left hand panel). In the second stage, we used two replication cohorts: 1) a larger, mixed ethnicity sample of the parent cohort (Figure 1, right hand panel) and 2) an external cohort of African-American patients. We used these

replication cohorts to validate how *CALU* SNPs correlated with higher warfarin doses while accounting for known genetic and clinical factors. This two-stage strategy has been shown to be powerful(17) and successful at identifying novel variants using candidate genes (*ABCA1* for HDL cholesterol(18) and *PCSK9* for LDL cholesterol(19)) or whole genome (*NOS1AP* for QT interval(20)) approaches. To identify novel variants in *CALU* we directly sequenced exonic regions of *CALU* as well as evolutionary conserved putative regulatory regions. To identify these regulatory elements we used a comparative genomics approach to identify noncoding elements that were conserved in the rat and mouse orthologs. We hypothesized that patients who required unusually high or low doses of warfarin (compared to their predicted dose based on a validated pharmacogenetic algorithm that incorporates genetic and nongenetic factors (9)) would be more likely to have clinically relevant SNPs in *CALU*. We then validated these associations in two larger replication cohorts.

Results

Discovery Cohort

From the parent cohort we selected 108 outliers: 53 patients formed the low-dose group and 55 patients formed the high-dose group (Figure 1, **left panel**). Despite having similar predicted doses (4.7±1.9 vs. 5.2±1.9) based on the validated pharmac algorithm (9), the average dose in the high-dose group was five times greater than in the low-dose group (Table 1). As designed, the dose ratio (therapeutic/predicted doses) was higher in the high-dose group compared to the low-dose group: 2.4 ± 1.4 vs. 0.55 ± 0.07, $p < 0.0001$. The two groups were well matched with respect to other important determinants of warfarin responsiveness. (Table 1)

The comparative genomics approach identified 10 highly conserved regions for resequencing that included all known *CALU* exons, portions of the 5' and 3' UTRs and promoter region, and several conserved transcription factor binding sites. (Supplementary Methods) Our resequencing strategy identified eight polymorphisms in *CALU*: seven previously identified in public databases (dbSNP Build ID: 130) and one novel SNP. (Table 2) All eight SNPs were in Hardy-Weinberg Equilibrium (data not shown). Among these eight SNPs, the proportion of patients carrying the minor allele of one SNP, rs339097, was significantly different between groups. (Table 2) This A>G SNP was a significant (p=0.01, false discovery rate 9%) predictor of the high-dose group among African-American outliers: it was present in 7 of 17 (41%) high-dose outliers (minor allele frequency [MAF] 20%) vs. 1 of 19 (5%) low-dose outliers (MAF = 3%). In contrast, the allele was invariant in Caucasian outliers.

Replication Cohorts

To validate these findings we first identified 496 patients from the parent cohort who had DNA and complete clinical data (replication cohort #1, Figure 1 right hand panel). We genotyped these participants for *CALU* rs339097 and three *CALU* SNPs identified in the literature (rs2290228, rs2290227, and rs1043550)(14,15,21). In a univariate analysis, only the *CALU* SNP rs339097 was significantly ($p = 0.003$) associated with therapeutic dose. Carriers of the minor (G) allele of rs339097 were more likely African-American (Table 3). In a multivariable regression model that accounted for *CYP2C9* and *VKORC1* genotypes and clinical factors including race, carriers of the G allele of *CALU* SNP rs339097 averaged (± SD) 14.5% (± 7%) higher therapeutic doses compared to noncarriers (p=0.03). In the entire parent cohort (discovery plus replication cohort #1), 25 out of the 31 carriers of the CALU rs339097 minor allele (83%) had larger therapeutic doses than predicted by the validated pharmacogenetic algorithm(9) (Figure 2). In African-Americans, rs339097 individually explained 5.7% ($R^2 = 0.057$) of the residual variation in warfarin. However, the allele was too rare in Caucasians to evaluate its influence on the R^2 in that group. There was no significant two-way interaction

between the *CALU* SNPs and either *VKORC1* (*VKORC1* T-1639A) or *CYP2C9* SNPs (*2, and *3).

Because the prevalence of the G allele for rs339097 varied significantly by race, we sought additional replication in 194 African-American patients on warfarin (replication cohort #2). The baseline characteristics of this group were similar, except for higher average dose requirements and a lower percentage of men (Table 1). In this second cohort, carriers of the G allele (MAF 14%) had a higher residual dosing error (therapeutic dose − predicted dose(9)) compared to noncarriers: mean residual (± SD) in carriers vs. noncarriers: 0.20 (± 2.0) vs. −0.41 ± 1.9, one-sided p-value = 0.03, two-sided p-value = 0.06), confirming that despite known genetic/nongenetic factors, carriers of the G for *CALU* rs339097 allele required higher warfarin doses.

In a pooled analysis of all African-American patients who participated in this study, the minor allele of *CALU* rs339097 was associated with an 11% increase in warfarin dose requirements after accounting for other factors (Table 4).

In silico validation

Because hepatic *CALU* is overexpressed in rodents with warfarin resistance(11) and rs339097 is intronic, we used publicly available gene expression data to determine whether genetic variation at rs339097 altered *CALU* gene expression in humans. In immortalized lymphoblastoid cell lines of 30 trios of African descent (parents, n = 60 and their adult children, n = 30), the number of G alleles was associated with higher *CALU* gene expression (mean normalized *CALU* expression ± SE in those with 0, 1, or 2 alleles: 10.01 ± 0.02, 10.17 ± 0.69, and 10.33 ± 0.69; respectively; p-value: 0.02).

Discussion

Warfarin is the most widely prescribed oral anticoagulant and is characterized by wide interindividual variability. Although recent studies have identified SNPs in *VKORC1* and *CYP2C9* in conjunction with nongenetic factors (9,22,23) as significant predictors of dose requirements, more than half of the variability in warfarin dose has remained unexplained. Calumenin, a recently identified regulator of VKOR(13), is overexpressed in warfarin resistant rats(11) and has been suggested to underlie warfarin sensitivity in humans(14), but had not been evaluated thoroughly.

In this pharmacogenetic study we found that carriers of the minor allele of the third intronic *CALU* SNP rs339097 required a dose that was 14.5% greater than predicted by *VKORC1*, *CYP2C9* and clinical factors. The A>G allele was rare in Caucasian (allele frequency 0.2%), but common in African-Americans (allele frequency 11%–14%).

This genetic association study had several methodological strengths: 1) use of a candidate gene approach, 2) resequencing of conserved regions to identify influential regions within this gene, and 3) employing a two-stage approach(18–20) that first examines extreme phenotypes in a population and then carries forward significant SNPs to a larger, more general samples of patients. The two–stage approach allowed us to discover a *CALU* SNP associated with a high-dose warfarin group and then demonstrate that carriers of the minor, G allele, of rs339097 had higher than predicted doses in two general populations. The decision to study *CALU* was backed by evidence of its role in rodent warfarin resistance(11) and *in vitro* evidence of calumenin inhibiting VKOR(13). We resequenced highly conserved regions of *CALU* to maximize our ability to identify influential regions outside of the coding regions, and used the false discovery rate to account for multiple comparisons.

Calumenin is a member of the CREC family of proteins and localizes in the endoplasmic reticulum,(24) where γ – carboxylase(25) and VKOR(26) also localize. In humans, two calumenin isoforms are expressed that use two different second exons (exon 2-1 and exon 2-2) and both isoforms were sequenced. In the rat, calumenin interacts with and inhibits VKOR, thereby inhibiting γ - carboxylase. Furthermore, certain warfarin-resistant rats have increased hepatic levels of calumenin that protect VKOR from inhibition by warfarin. The VKOR and γ - carboxylase isolated from these rats have normal warfarin sensitivity.(11,12) In cells genetically engineered cells to produce Factor IX or VII, *in vitro* silencing of *CALU* mRNA increases VKOR and γ - carboxylase activity and production of these VKOR dependent clotting factors.(13,27) In humans, a SNP in the 3' UTR of *CALU* was associated with increased activity of one other vitamin-K dependent protein (protein S).(15) Therefore, the available animal and *in vitro* evidence suggest that calumenin regulates VKOR and γ – carboxylase.

Although the molecular implications of rs3390907 are not known, using publicly available gene expression data from lymphoblastoid cell lines generated from trios of the YRI cohort (28) we observed that the G allele of rs3390907 is associated with higher expression of CALU. Interestingly, this association with higher gene expression in carriers of the G allele for rs339097 is consistent with the findings in warfarin resistant rats where CALU was found to be overexpressed(11). Based on HapMap linkage disequilibrium data of the YRI population, rs339097 is in the center of an 18-Kb haplotype block (Haploview(29)). This large block contains several conserved regions, particularly in the second intron, that were not examined as part of the current study. Therefore it is unclear if this SNP directly leads to alterations in calumenin expression or mRNA stability or is in linkage disequilibrium with a causative SNP within this haplotype block.

The *CALU* SNP identified in our populations, rs339097, had minor allele frequencies of 11% to 14% in African-Americans and 0.2% in Caucasians. Although we recruited subjects with a wide variety of clinical conditions, these allele frequencies are similar to those found in the YRI and CEU (Centre d'Etude du Polymorphisme Humain from Utah) populations studied by the HapMap consortium (19.5% and 0%, respectively). Thus, among patients starting warfarin, this SNP will have greater potential to benefit patients of African descent.

In the multivariable regression model that accounted for race, rs339097 remained a significant predictor of higher warfarin dose in the first replication cohort. Though the overall contribution of this SNP to the residual variance in African-Americans was modest, patients who carried this SNP had a 14.5% increase in their warfarin requirement per allele. The dose-effect is similar to the *CYP2C9*2* allele, which is associated with a 19% decrease, and to the *CYP4F2* rs2108622 allele, which is associated with a 4% to 12% increase in warfarin requirements. Identification of novel SNPs in the African-American population is important because lower allele frequencies of *VKORC1* and *CYP2C9* SNPs in this population make current pharmacogenetic models of warfarin dosing less accurate in that population.(8,9,30) Therefore, future studies of pharmacogenetic warfarin therapy, particularly those with larger numbers of patients of African descent, could use additional SNPs and perhaps initial INR response(31) to improve predictive accuracy. To facilitate these studies, we have incorporated the present findings into an online dosing algorithm: www.warfarindosing.org.

In summary, we found that carriers of the minor allele of the *CALU* SNP rs339097 require higher doses of warfarin. Because the incremental cost of adding a single SNP into commercial genotyping platforms is low, *CALU* rs339097 could be incorporated into these platforms and into clinical trials that include populations with African ancestry.

Methods

Patient Selection

Our parent cohort is comprised of approximately 700 patients on therapeutic doses of warfarin who attended an outpatient anticoagulation clinic affiliated with Barnes–Jewish Hospital at Washington University Medical Center (St. Louis), who participated in the PREVENT (**PRE**vention of **VEN**ous **T**hromboembolism) study, or who were initiated on warfarin during a hospitalization for orthopedic surgery.(2,9,32,33) These patients provided written informed consent, DNA, demographic variables, and medication histories as previously described.(9) The parent cohort is representative of most ambulatory patients on warfarin therapy (Table 1) except that it also included four patients who were referred for unusual warfarin doses (> 20 mg/day or < 1 mg/day). Because of missing data or unavailable DNA, only 604 (86%) patients were available to construct the discovery and replication cohorts. The research protocol was approved by the institutional review board at Washington University in St. Louis.

Calumenin SNP discovery cohort—For all patients in the parent cohort, the therapeutic daily warfarin dose (in mg) was compared to the dose predicted by a validated pharmacogenetic algorithm (9)). We then chose the 10% of this cohort with the largest residual dosing error (defined as therapeutic dose - predicted dose/therapeutic dose) to identify 108 outliers with DNA who were either warfarin sensitive (N = 53) or resistant (N = 55) (Figure 1, **left panel**).

Calumenin SNP replication cohorts—From the remaining patients in the parent cohort we selected the 496 patients with DNA and complete clinical data recruited at Washington University Medical Center for further genotyping (Replication cohort #1, Figure 1, right panel). Of these, three did not reach a stable dose and their therapeutic dose was estimated by two independent clinical pharmacists (PEM and GRG). The pharmacists were blinded to genotype and agreed in their estimation of therapeutic dose.

To validate our findings in an African-American population we (SRP, LHC) genotyped 194 African-American patients at the University of Illinois at Chicago (replication cohort #2)(34). We excluded those (n = 7) with missing *VKORC1* and *CYP2C9* genotypes or those on known inducers of Cytochrome P450 enzymes.

CALU in silico gene expression replication—Using software developed by Ge et al., (35) we merged publicly available gene expression data(36) from lymphoblastoid cell lines with genotype data from the HapMap project to test for an association between the minor (G) allele of rs339097 and *CALU* gene expression in trios of Yoruba people of Ibadan, Nigeria (YRI population, HapMap project).

CALU Target Region Selection

CALU is a 32Kb gene located on human 7q32 and contains six exons. In humans two *CALU* isoforms are expressed that use two different exons 2 (exon 2-1 and exon 2-2).

We used two complementary methods to target region selection for direct sequencing of *CALU*. We used two comparative genomics approaches to find 10 highly conserved (>90% across rat and mouse orthologs) regions of *CALU* and 5Kb of its 5' and 3' flanking regions using the VISTA Browser (http://genome.lbl.gov/vista/index.shtml) and the University of California at Santa Cruz (UCSC) Genome Browser's "knownGene" database file and mRNAs from GenBank (http://genome.ucsc.edu/cgi-bin/hgTrackUi?hgsid=66289578&c=chr7&g=knownGene, March 2004). These 10 regions contained all six exons and the alternatively spliced exon 2 (as expected) and a 69-bp segment of the 5'UTR. In addition, we identified two regions in the

3'UTR and one in the 5'UTR that were highly conserved. We designed primers and sequenced these 10 regions as previously described (correction to original description (37): 100 μM each of the 4 dNTPs was used). (Supplementary Methods).

Genotyping Protocols

To validate the SNPs identified by sequencing we first genotyped the 108 outliers for the SNPs identified by resequencing using PCR and Pyrosequencing® (38,39). PCR primers were designed using the UCSC Golden Path Human Genome Browser May 2004 Build (http://genome.ucsc.edu/cgi-bin/hgGateway) and *CALU* Refseq NM_001219. This confirmatory step revealed eight polymorphic *CALU* SNPs for analysis in the discovery cohort: c38412t, rs41274227, rs1043550, rs12538139, rs2290227, rs2290228, rs2307040, rs339097. For the replication cohort, we used Pyrosequencing® (40) to genotype selected SNPs.

Statistical Analyses

In the SNP discovery analysis, we used a chi-squared analysis to compare the prevalence of SNPs between the high-dose and low-dose groups and the Fisher's exact test when cell frequencies were low. As recommended,(41) we calculated and report false discovery rates.

In replication cohort #1, we tested *CALU* SNPs (rs339097, rs2290228, rs2290227, and rs1043550) in all subjects and in the subsets of Caucasians and African-Americans. We tested for Hardy-Weinberg Equilibrium using Haploview 3.32; if the SNP was in HWE (all were), we used simple linear regression to regress the therapeutic daily warfarin dose (log transformed) onto the number of minor alleles in univariate and multivariate models that included *CYP2C9* and *VKORC1* genotypes, age, smoking, body surface area, concomitant medications, target INR, venous thromboembolism indication, and race. We used an unpaired t-test to compare therapeutic doses (log transformed) of carriers and non carriers of the *CALU* minor allele. We tested for two-way interactions between SNPs in *CALU, VKORC1,* and *CYP2C9*.

In replication cohort #2, we tested for association of *CALU* SNP rs339097 A>G with residual dosing error (ln(therapeutic) − ln(predicted warfarin dose)(9)) using a one-sided (unpaired) t-test. The normality of residuals was confirmed using the Kolmogorov-Smirnov test.

For the *in silico* replication we use a generalized estimating equation to regress the normalized *CALU* gene expression onto the number of minor alleles of rs339097 (coded as 0, 1, 2) in YRI children and parents using SAS procedure GENMOD to account for the family structure of the trios. Normality of residuals was confirmed Kolmogorov-Smirnov test.

We performed all statistical analyses in SAS (Version 9.1; SAS Institute, Inc; Cary, NC) except that replication cohort #2 was analyzed in SPSS (Release 15.0; SPSS Inc., Chicago, IL). Statistical significance refers to a p-value < 0.05.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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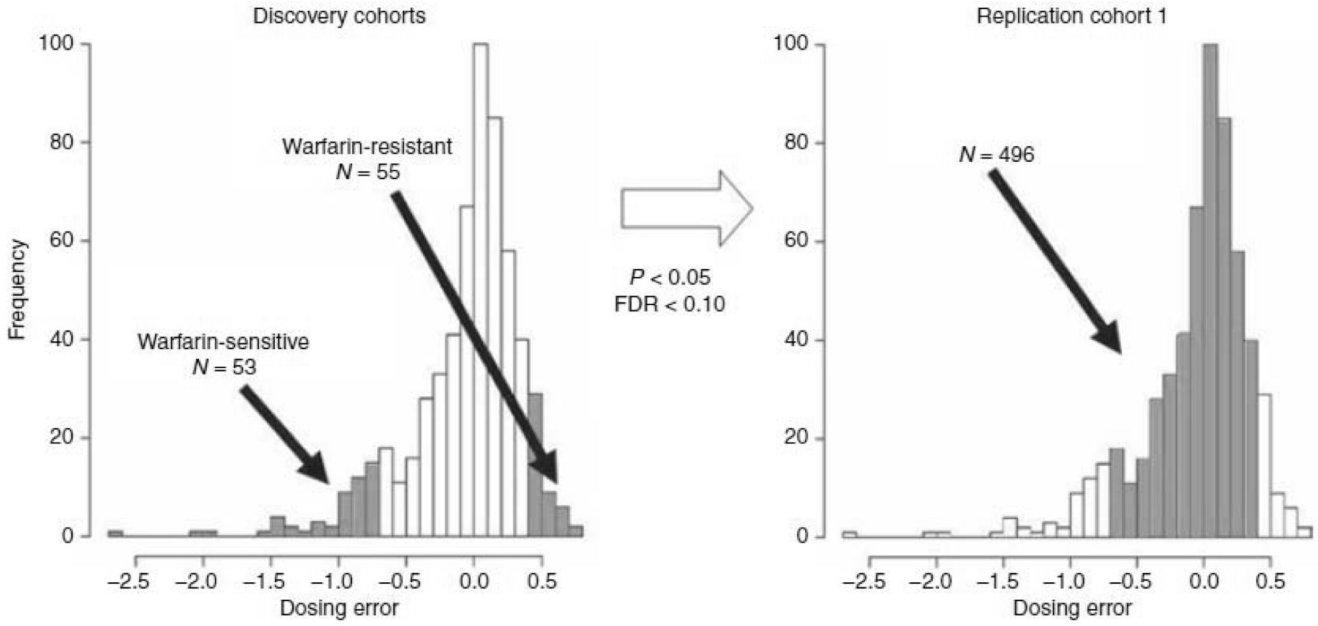

Figure 1.

Schematic of two-stage study design using a parent cohort of patients on stable warfarin therapy. In the discovery cohort (left panel, we sequenced *CALU* in patients who had the largest or smallest residual dosing errors (shaded) within the parent cohort. In the second stage, significant ($P < 0.05$, FDR < 0.010) SNPs from the discovery stage were then genotyped in the remainder of the parent cohort (shaded, replication cohort 1, right panel). Predicted doses were based on a validated pharmacogenetics algorithm.[9] *CALU*, calumenin; FDR, false-discovery rate; SNP; single-nucleotide polymorphism.

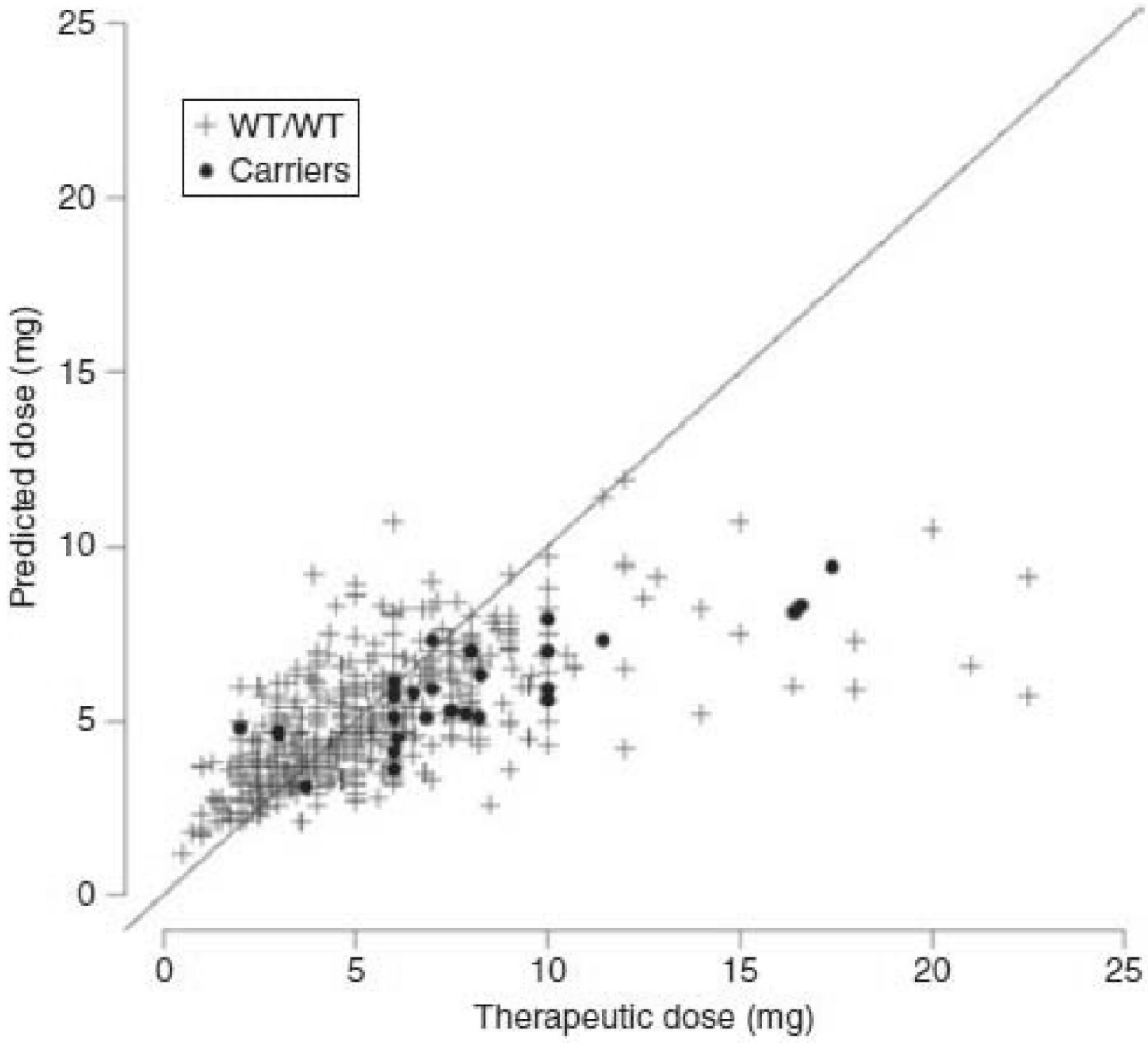

Figure 2.

Carriers of CALU SNP rs339097 require higher-than-predicted therapeutic warfarin doses in the combined discovery and replication cohort 1. Therapeutic dose vs. predicated dose (based on a validated pharmacogenetic algorithm[9]) in carriers (•) and noncarriers (+) of the G allele for CALU rs339097. In this combined cohort, 25 of 31 (83%) carriers of the G allele required a higher-than-predicated warfarin dose to achieve a therapeutic INR. CALU, calumenin; INR, international normalized ratio; SNP, single-nucleotide polymorphism; WT, wild type.

Table 1

Baseline Characteristics of Discovery and Replication Cohorts

	Discovery		Replication Cohort #1 (n = 496)	Replication Cohort #2 (n = 194)
	Low-Dose (n=53)	High-Dose (n=55)		
Therapeutic dose, mean±SD (mg/day) †	2.6±1.1	12.7±8.3	4.8 ± 1.6	5.9 ± 2.3
Age, mean±SD (years)	62±15	59±15	61 ± 15	57 ± 16
BSA, mean±SD (m^2)	2.01±0.41	2.04±0.31	2.0 ± 0.3	2.07 ± 0.33
Current Smoker, N (%)	7 (13)	11 (20)	60 (12)	35 (18)
Medications				
Amiodarone, N (%)	4 (8)	1 (2)	9 (2)	4 (2)
Simvastatin, N (%)*	4 (8)	0 (0)	56 (11)	64 (33)
Male	20 (38%)	29 (53%)	255 (51%)	47 (24%)
Race				
Black, N (%)	18 (34)	21 (38)	70 (14)	194 (100)
White, N (%)	34 (64)	33 (60)	407 (82)	0
Other, N (%)	1 (2)	1 (2)	20 (4)	0
CYP2C9*2 (% carriers)	30	23	11	5
CYP2C9*3 (% carriers)	13	15	6	2
VKORC1-1639 A (% carriers)	51	49	33	18

BSA = Body surface area; CYP2C9 = Cytochrome P450; *VKORC1* = Vitamin K epoxide reductase, complex subunit 1; SD = standard deviation; High and low dose groups based on residual dosing error = (therapeutic dose – predicted dose)/therapeutic dose; Predicted doses based on a validated pharmacogenetic algorithm.(9)

* p = 0.05 between discovery groups;

† p<0.01

Table 2

Variants discovered in *CALU* and association with high dose in outlier group (n = 108)

SNP	Region	Position	Alleles	MAF by Race in low dose group (AA/white/O,%)	MAF by Race in high dose group (AA/white/O,%)	P-value in white pts	P-value in AA pts
rs12538139	5' utr	128175777	G/A	10/23/0	8/21/50	0.7	0.9
rs2290228	exon	128175884	G/A	12/16/50	10/23/0	0.09	0.5
rs2290227	intron	128176217	G/A	0/1/0	5/6/0	0.1	0.9
rs2307040	intron	128181842	C/T	12/37/0	13/26/50	0.3	0.8
rs41274227	intron	128182044	C/T	0/3/0	5/6/0	0.1	0.9
rs339097	intron	128186460	A/G	3/0/0	20/0/0	1.0	0.01
38412	intron	128195082	C/T	6/0/0	3/0/0	1.0	0.6
rs1043550	3' utr	128196461	A/G	16/36/0	15/32/50	0.8	0.9

AA = African–American; MAF = minor allele frequency; O = Other; pts = patients; SNP = Single Nucleotide Polymorphism labeled as dbSNP reference number or internal identification number; utr = untranslated region.

Table 3

Characteristics of replication cohorts, stratified by *CALU* SNP rs339097

	Replication Cohort #1		Replication Cohort #2	
Characteristic	**rs339097 GG or AG (n = 21)**	**rs339097 AA (n= 475)**	**rs339097 GG or AG (n = 52)**	**rs339097 AA (n= 142)**
Therapeutic warfarin dose (mg/day, mean§ ± SD)	6.5 ± 1.4	4.8 ± 1.6**	6.3 ± 2.3	5.7 ± 2.3
Age (years, mean ± SD)	57 ± 19	62 ± 15	58 ± 15	56 ± 16
Race (%)				
African-American	86%	11%**	100%	100%
Caucasian	10%	85%	0%	0%
Other	5%	4%	0%	0%
BSA (m^2, mean ± SD)	2.0 ± 0.2	2.0 ± 0.3	2.0 ± 0.4	2.1 ± 0.3
VKORC1 -1639 A†	10%	37%	12%	20%
*CYP2C9*2†	2%	12%	2%	6%
*CYP2C9*3†	2%	6%	2%	1%
Current smoker	29%	14%*	10%	21%
Current amiodarone use	16%	2%*	2%	2%

BSA = Body surface area; CYP2C9*2 = Cytochrome P450 2C9*2; CYP2C9*3 = Cytochrome P450*3; SD = standard deviation; DVT = deep venous thrombosis; PE = pulmonary embolus; Comparisons to heterozygotes/homozygotes for rs339097:

* p<0.05;

** p<0.01;

† represented as percentage with minor allele;

§ geometric mean

Table 4

Influence of pharmacogenetic factors on therapeutic warfarin dose in pooled African-American patients (n = 241)

Factor		Effect on warfarin dose
Age in years, mean (SD)	58 (16)	−6% per decade
BSA, m^2, mean (SD)	2.1 (0.4)	24% per $0.5m^2$
Percent Smokers	20%	13%
Percent Using Amiodarone	3%	−16%
CALU Allele frequency	0.14	11% per allele
CYP2C9 *2 Allele Frequency	0.04	−20% per allele
CYP2C9 *3 Allele Frequency	0.01	−34% per allele
VKORC1 3673A Frequency	0.11	−24% per allele
Percent with indication of DVT or PE	43%	2%
Target INR	2.5 (0.13)	4% per 0.25 increase in target INR

SD = standard deviation; INR = international normalized ratio; BSA = body surface area; m = meter; DVT = deep venous thrombosis; PE = pulmonary embolus