

ORIGINAL ARTICLE

## Effect of *VKORC1* Haplotypes on Transcriptional Regulation and Warfarin Dose

Mark J. Rieder, Ph.D., Alexander P. Reiner, M.D., M.P.H.,  
Brian F. Gage, M.D., M.Sc., Deborah A. Nickerson, Ph.D., Charles S. Eby, M.D.,  
Howard L. McLeod, Pharm.D., David K. Blough, Ph.D.,  
Kenneth E. Thummel, Ph.D., David L. Veenstra, Pharm.D., Ph.D.,  
and Allan E. Rettie, Ph.D.

### ABSTRACT

#### BACKGROUND

The management of warfarin therapy is complicated by a wide variation among patients in drug response. Variants in the gene encoding vitamin K epoxide reductase complex 1 (*VKORC1*) may affect the response to warfarin.

#### METHODS

We conducted a retrospective study of European-American patients receiving long-term warfarin maintenance therapy. Multiple linear-regression analysis was used to determine the effect of *VKORC1* haplotypes on the warfarin dose. We determined *VKORC1* haplotype frequencies in African-American, European-American, and Asian-American populations and *VKORC1* messenger RNA (mRNA) expression in human liver samples.

#### RESULTS

We identified 10 common noncoding *VKORC1* single-nucleotide polymorphisms and inferred five major haplotypes. We identified a low-dose haplotype group (A) and a high-dose haplotype group (B). The mean ( $\pm$ SE) maintenance dose of warfarin differed significantly among the three haplotype group combinations, at  $2.7\pm 0.2$  mg per day for A/A,  $4.9\pm 0.2$  mg per day for A/B, and  $6.2\pm 0.3$  mg per day for B/B ( $P<0.001$ ). *VKORC1* haplotype groups A and B explained approximately 25 percent of the variance in dose. Asian Americans had a higher proportion of group A haplotypes and African Americans a higher proportion of group B haplotypes. *VKORC1* mRNA levels varied according to the haplotype combination.

#### CONCLUSIONS

*VKORC1* haplotypes can be used to stratify patients into low-, intermediate-, and high-dose warfarin groups and may explain differences in dose requirements among patients of different ancestries. The molecular mechanism of this warfarin dose response appears to be regulated at the transcriptional level.

From the Departments of Genome Sciences (M.J.R., D.A.N.), Epidemiology (A.P.R.), Pharmacy (D.K.B., D.L.V.), Pharmaceutics (K.E.T.), and Medicinal Chemistry (A.E.R.), University of Washington, Seattle; and the Departments of Medicine (B.F.G., C.S.E., H.L.M.) and Pathology and Immunology (C.S.E.), Washington University, St. Louis. Address reprint requests to Dr. Rieder at the Department of Genome Sciences, University of Washington, Box 357730, Seattle, WA 98195, or at [mrieder@u.washington.edu](mailto:mrieder@u.washington.edu).

N Engl J Med 2005;352:2285-93.

Copyright © 2005 Massachusetts Medical Society.

**C**OUMARIN-BASED ANTICOAGULANT drugs are the definitive treatment worldwide for the long-term prevention of thromboembolic events. In 2003, a total of 21.2 million prescriptions were written for the oral anticoagulant warfarin (a derivative of coumarin) in the United States alone.<sup>1</sup> However, management of warfarin therapy is challenging in two respects: first, a safe and effective stabilization dose must be determined during the early months of therapy, and second, maintenance doses must be adjusted to compensate for changes in patients' weight, diet, disease state, and concomitant use of other medications. In addition, studies indicate that genetic factors affect outcomes, despite adjustment for these factors. Specifically, patients with the common, functionally defective \*2 and \*3 allelic variants of the cytochrome P-450 enzyme 2C9 (CYP2C9) require significantly lower maintenance doses, have longer times to dose stabilization, and are at higher risk for serious and life-threatening bleeding than are patients without these variants.<sup>2</sup> Such warfarin sensitivity is easily rationalized because CYP2C9 is responsible for the metabolic clearance of the more pharmacologically potent S-enantiomer of warfarin.<sup>3</sup>

In contrast to genetically determined cases of warfarin sensitivity, such as those described above, are rare cases of warfarin resistance. A potential pharmacodynamic mechanism underlying warfarin resistance has been elucidated with the recent discovery of the warfarin target gene, which encodes vitamin K epoxide reductase complex 1 (VKORC1).<sup>4,5</sup> This complex recycles reduced vitamin K, which is essential for the post-translational gamma-carboxylation of vitamin K-dependent clotting factors II (prothrombin), VII, IX, and X. Several rare mutations that lead to amino acid changes in the VKORC1 protein have been discovered in warfarin-resistant patients but not in the general population,<sup>4</sup> suggesting that coding-region variants of VKORC1 are extremely detrimental and that they probably do not explain the typical variability in warfarin dose (2 to 10 mg per day) among individual patients. Recently, a single, noncoding polymorphism was found to be associated with warfarin dose across the normal dose range,<sup>6</sup> suggesting that other regulatory polymorphisms in VKORC1 might influence the pharmacodynamic response to warfarin.

The purpose of this study was to determine whether other polymorphisms in noncoding re-

gions or their unique haplotype combinations contribute to the variability in the maintenance dose of warfarin. Additional goals were to probe population-specific differences in warfarin dose requirements and to investigate the molecular mechanisms underlying significant VKORC1 effects.

---

## METHODS

---

### PATIENTS

The study was approved by the human subjects review committees of the University of Washington, Seattle, and Washington University, St. Louis. All the patients who participated in the study provided written informed consent.

The primary study population, from the University of Washington Medical Center, consisted of the same patients previously studied to assess the association between CYP2C9 variants and anticoagulation-related outcomes.<sup>2</sup> Patients were recruited from pharmacist-run anticoagulation clinics affiliated with the center. Inclusion criteria were a confirmed date of the initial exposure to warfarin, current anticoagulation therapy, and an age of 18 years or older. Exclusion criteria were Asian or African descent (36 patients), management by telephone rather than in person (185), absence of verbal and written consent (5), absence of a blood specimen (3), and absence of a confirmed date of initial exposure to warfarin (11). A total of 186 patients from this population were eligible for the study.

The secondary patient population consisted of patients 18 years of age or older whose warfarin therapy was managed at one of the anticoagulation clinics affiliated with Barnes-Jewish Hospital at Washington University Medical Center, as previously described.<sup>7</sup> Exclusion criteria for this population were non-European ancestral origin (139 patients) and absence of verbal and written consent (17 patients). We prospectively followed 47 patients who had recently begun warfarin therapy until they were taking their maintenance dose. A total of 368 patients from this population were eligible for the study.

### COLLECTION OF CLINICAL DATA

Collection of data from the primary patient population consisted of a review of inpatient and outpatient medical records. Two trained abstractors collected data with the use of standardized abstract forms. The anticoagulation database of the University of Washington Medical Center was used to ob-

tain information on the international normalized ratio (INR), daily warfarin dose, and use of prescription drugs and over-the-counter drugs. The daily maintenance dose of warfarin was defined as the dose at three consecutive clinic visits at which the INR measurement was within therapeutic range. The electronic medical-records database of the University of Washington Medical Center was used to obtain information on bleeding events, coexisting conditions, and demographic variables. Blood samples were collected from patients during regularly scheduled office visits.

Data from the secondary patient population were collected by means of structured patient interviews, as previously described in detail,<sup>7</sup> from 2001 to 2004. In brief, patients provided a 5-ml blood sample, demographic and dietary information, a comprehensive list of prescription and over-the-counter drugs, and access to information about their warfarin doses and INR measurements.

#### POPULATION-SPECIFIC DNA DIVERSITY PANELS

DNA panels consisting of samples from American persons of European, Asian, or African descent were purchased from the Coriell Cell Repository (<http://locus.umdnj.edu/nigms>). The Asian-American panel consisted of samples from 96 persons from the HD100CHI set (Han People of Los Angeles), 10 from the HD13 set (Southeast Asians), 7 from the HD32 set (Chinese), and 7 from the HD07 set (Japanese). Samples from 96 European-American persons were selected from the HD100CAU set and from 23 European-American persons from the parental generation of the families in the Centre d'Etude du Polymorphisme Humain collection ([http://pga.gs.washington.edu/data/sample\\_description.html](http://pga.gs.washington.edu/data/sample_description.html)). Samples from 96 African-American persons were selected from the HD100AA set.

To explore the functional mechanism of the variability in warfarin dose, we measured *VKORC1* messenger RNA (mRNA) levels in human liver specimens selected from a tissue bank maintained by the University of Washington School of Pharmacy. Basic demographic information on the individual organ donors and methods of tissue procurement have been reported previously.<sup>8,9</sup> All 53 liver specimens used in this study came from European-American donors.

#### DNA AND mRNA ANALYSES

Because the *VKORC1* gene was identified only recently as the gene encoding the primary warfarin-sensitive component of vitamin K epoxide reduc-

tase,<sup>4,5</sup> limited information on polymorphisms within this gene was available. We therefore carried out DNA sequence analysis across the entire genomic region (approximately 11 kb) in samples from our primary patient population to catalogue single-nucleotide polymorphisms (SNPs) comprehensively and to establish the haplotype structure of the *VKORC1* gene. All clinical samples from the primary patient population were subjected to sequence analysis across the extended genomic sequence, which included 5 kb in the upstream promoter region, 4.2 kb of intragenic (intron and exon) sequence, and 2 kb of the 3' downstream region. Ten common SNPs were identified, at positions 381, 861, 2653, 3673, 5808, 6009, 6484, 6853, 7566, and 9041 of the *VKORC1* reference sequence (GenBank accession number AY587020).

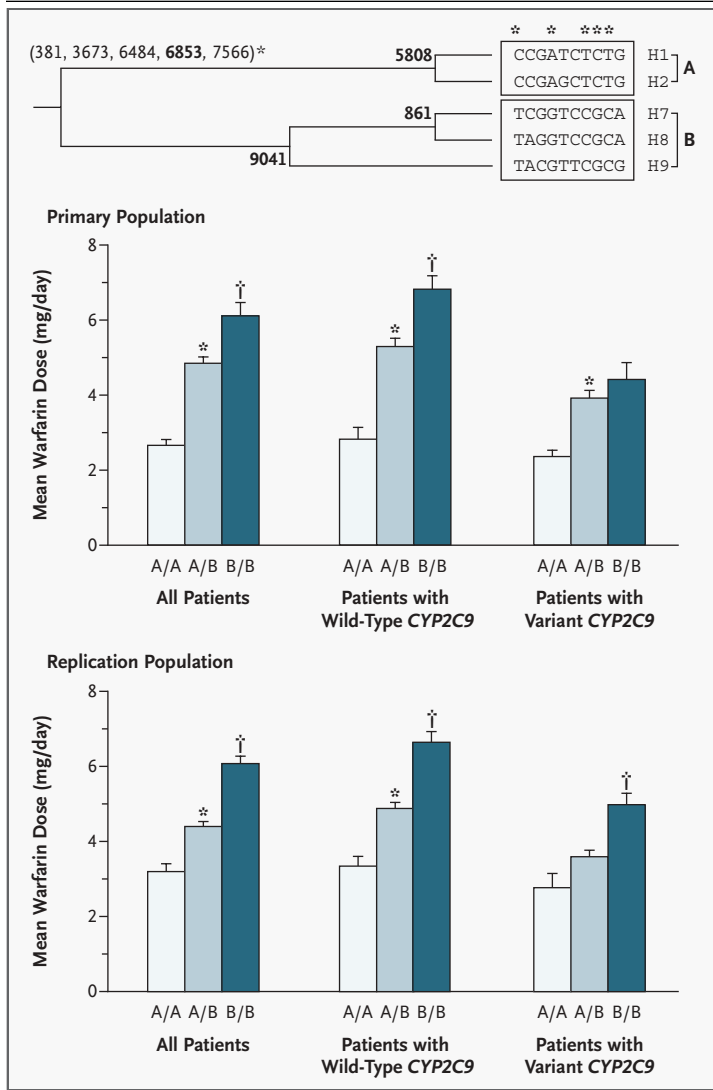
The population-specific diversity samples (European American, African American, and Asian American) were genotyped for the 10 common SNPs identified in the European-American clinical population, with the use of the same method of sequence analysis. In the secondary patient population, four informative SNPs (at positions 861, 5808, 6853, and 9041) were genotyped to differentiate haplotypes H1, H2, H7, H8, and H9, according to the genealogic tree shown in Figure 1.

Total RNA and DNA were extracted from human control liver specimens with Trizol reagent. RNA was reverse-transcribed to yield complementary DNA with the use of poly-dT primers (as described in the Supplementary Appendix, available with the full text of this article at [www.nejm.org](http://www.nejm.org)). Quantitative polymerase-chain-reaction analysis was performed (also as described in the Supplementary Appendix). DNA extracted from liver samples was genotyped at each of the 10 common *VKORC1* SNPs by DNA resequencing (as described above). Haplotypes were inferred, and each sample was classified as one of three haplotype group combinations (A/A, A/B, or B/B, as described in the Supplementary Appendix).

#### STATISTICAL ANALYSIS

All SNPs identified were tested for deviations from Hardy-Weinberg disequilibrium with the use of a chi-square test. The significance level for all statistical tests was set at  $P < 0.05$ . Haplotypes for each individual sample were estimated with use of the PHASE program (version 2.0),<sup>10</sup> and independent runs were performed for clinical and population-specific samples from each population studied.

We performed multiple linear-regression analy-



**Figure 1. Effect of VKORC1 Haplotype Combination on Clinical Warfarin Dose.**

As shown in the upper panel, common haplotypes (H1, H2, H7, H8, and H9) were clustered with use of the UPGMA method (unweighted pair group method with arithmetic mean); they formed two distinct evolutionarily distant groups, designated A (comprising H1 and H2) and B (comprising H7, H8, and H9). Eight single-nucleotide polymorphisms (SNPs) are labeled at the nodes of the tree, and four SNP sites (shown in boldface) were used to discriminate between each branch and to distinguish groups A and B. Asterisks indicate correlated SNP sets that were significantly associated with warfarin dose. Group A was associated with a low warfarin dose and group B a high warfarin dose. As shown in the middle panel, patients in the primary population were genotyped and assigned a VKORC1 haplotype combination (A/A, A/B, or B/B). The patients were further classified according to CYP2C9 genotype (the wild type or either the \*2 or \*3 variant). The total numbers of patients having a group A combination, a group B combination, or both were 182 (all patients), 124 (wild-type CYP2C9), and 58 (variant CYP2C9). Four patients could not be assigned either to group A or to group B. As shown in the bottom panel, 357 patients from the replication sample were genotyped and grouped as were those in the primary patient population; 233 had wild-type CYP2C9 and 124 variant CYP2C9. The asterisks in the bottom two panels denote P<0.05 for the comparison with combination A/A and the daggers P<0.05 for the comparison with combination A/B. The T bars represent standard errors.

sis of the log-transformed maintenance dose, with all patient covariates initially considered. Significant covariates contributing to warfarin dose were age, sex, use or nonuse of amiodarone, use or nonuse of losartan, and CYP2C9 genotype. The effect size associated with each predictor was calculated as the percentage of the variation in warfarin dose explained by the predictor, divided by the total variance in the regression model.

Genealogic trees were constructed on the basis of the number of differences between haplotypes and with use of the UPGMA clustering method (unweighted pair group method with arithmetic mean). The Kruskal–Wallis test, a distribution-free analysis of variance, was used to assess differences in the warfarin maintenance dose among patients according to their haplotype combination (A/A, A/B,

or B/B) and CYP2C9 genotype (wild-type or variant). Other than the grouping of patients according to CYP2C9 genotype, no other adjustment for clinical covariates was used. After the overall chi-square test for differences among the three groups had been performed, pairwise comparisons of groups were carried out with use of the asymptotic normality of the total ranks within each group. We applied the Bonferroni correction for each of the three comparisons (A/A vs. A/B, A/B vs. B/B, and A/A vs. B/B).

Data on liver mRNA expression were analyzed after log transformation, and the overall test for group differences was performed by analysis of variance. Pairwise comparisons between groups for significance were performed with the use of Tukey’s Studentized range test. Significance levels were set at P<0.05. Additional information on the statistical methods used is provided in the Supplementary Appendix.

RESULTS

We found 28 VKORC1 noncoding SNPs in the primary population (comprising 186 patients) and a single heterozygous, nonsynonymous SNP in the

coding region (genomic position G5432T, encoding Ala41Ser). The patient heterozygous for this polymorphism had the highest overall maintenance dose of warfarin among the patients in the primary population (15.5 mg per day) and was excluded from the other analyses. No other previously reported coding-region SNPs were identified.<sup>4</sup> Of the 28 noncoding SNPs, 10 occurred at a frequency of greater than 5 percent. No deviations from the expected population genotype proportions (predicted by Hardy–Weinberg equilibrium) were detected at these common SNP sites.

Individual tests of each SNP and adjustments for significant covariates revealed that seven SNPs (at positions 381, 3673, 5808, 6484, 6853, 7566, and 9041) were significantly associated with the warfarin dose ( $P < 0.001$ ); the strength of the association between the warfarin dose and the other three SNPs (at positions 861, 2653, and 6009) was less significant ( $P = 0.01$ ,  $P = 0.02$ , and  $P = 0.02$ , respectively). Of the seven highly significant SNPs, five (at positions 381, 3673, 6484, 6853, and 7566) were strongly correlated with one another (linkage disequilibrium  $r^2 \geq 0.9$ ), and two others (at positions 5808 and 9041) were not correlated with any other SNP in this region. Stepwise regression analysis identified the five highly correlated SNPs as those that were most predictive of the approximately 25 percent variance in warfarin dose. In the same analy-

sis, the *CYP2C9* genotype accounted for 10 percent of the variance in warfarin dose.

The 10 common SNPs were used to infer *VKORC1* haplotypes from the primary sample and the three diversity samples, yielding nine haplotypes (H1 through H9). These, in turn, were used to assign haplotype pairs to each patient or member of a diversity panel. We identified five common haplotypes (those with  $>5$  percent frequency) in the primary sample: H1, H2, H7, H8, and H9 (Table 1).

In the multiple linear regression analysis adjusted for clinically important covariates, four of the five common haplotypes were found to be independently associated with the warfarin dose ( $P \leq 0.05$ ) (Table 1). We identified two haplotypes (H1 and H2) associated with a low warfarin dose requirement (2.9 and 3.0 mg per day) and two haplotypes (H7 and H9) associated with an increased requirement (6.0 and 5.5 mg per day). Results obtained with the use of a generalized linear-score model for haplotypes were similar, as were the results of secondary analyses that excluded the 24 patients receiving amiodarone.

The genealogic tree showing the relationship among the five common haplotypes indicates the emergence of two distinct, highly divergent haplotype groups (Fig. 1). We designated these groups A (comprising haplotypes H1 and H2) and B (comprising H7, H8, and H9) and were able to assign

**Table 1.** *VKORC1* Haplotype Frequency and Effect on Warfarin Dose among 186 European-American Patients.\*

Haplotype Identification Code	Haplotype Sequence†	Frequency of Haplotype in Primary Patient Population		Mean Maintenance Dose among Homozygous Patients (95% CI)‡	P Value
		proportion	no. of persons		
H1	CCGATCTCTG	0.12	43	2.9 (2.2–3.7)	<0.0001
H2	CCGAGCTCTG	0.24	88	3.0 (2.5–3.6)	<0.001
H3	CCGGTCCCCG	0.01	2	NA	NA
H4	CCGGTCCGTG	<0.01	1	NA	NA
H5	TCGAGCTCTG	<0.01	1	NA	NA
H6	TCGGTCCGCG	0	0	NA	NA
H7	TCGGTCCGCA	0.35	132	6.0 (5.2–6.9)	<0.001
H8	TAGGTCCGCA	0.08	28	4.8 (3.4–6.7)	0.76
H9	TACGTTCCGCG	0.21	77	5.5 (4.5–6.7)	0.05

\* CI denotes confidence interval, and NA not analyzed.

† For each haplotype sequence, the single-nucleotide polymorphisms are listed in sequential order along the *VKORC1* gene, at positions 381, 861, 2653, 3673, 5808, 6009, 6484, 6853, 7566, and 9041.

‡ Analyses were adjusted for age, sex, use or nonuse of amiodarone, use or nonuse of losartan, and *CYP2C9* genotype. The mean warfarin dose among all the patients was 5.15 mg per day (95 percent confidence interval, 4.78 to 5.51).

haplotype group combinations to 182 patients. According to the regression analysis, this higher-order clustering showed that group A contained the haplotypes associated with a low dose of warfarin and group B the haplotypes associated with a high dose of warfarin. As shown in Figure 1, a minimal SNP set composed of four SNPs (at positions 861, 5808, 6853, and 9041) distinguished each of the groups and haplotypes at the terminal ends of this tree.

Patients were assigned a *VKORC1* haplotype group combination (A/A, A/B, or B/B) and then grouped according to *CYP2C9* genotype (the wild type in 124 patients and the \*2 or \*3 variation in 58). In this analysis, we made no other adjustments for clinical covariates. The warfarin maintenance dose differed significantly among the three *VKORC1* haplotype combinations, at  $2.7 \pm 0.2$  mg per day for A/A,  $4.9 \pm 0.2$  mg per day for A/B, and  $6.2 \pm 0.3$  mg per day for B/B ( $P < 0.001$ ), both within the entire primary patient population and among the patients who were not carriers of *CYP2C9* functional variants (Fig. 1). In the primary population, the overall mean maintenance dose of warfarin ( $5.1 \pm 0.2$  mg per day) and range of maintenance doses were typical of those that have been reported in other clinical studies.<sup>7</sup> The average INR was approximately 2.5 and did not differ significantly among the patients classified according to *VKORC1* haplotype combination (lowest  $P$  value = 0.22) (data not shown).

We carried out a replication study involving a larger, independent population of warfarin-treated European-American patients. We genotyped these patients using the four informative SNP sites that resolved the five common haplotypes. Haplotypes were inferred, major haplotype combinations assigned, and patients subclassified according to *CYP2C9* genotype in the same manner as those in the primary patient population (Fig. 1). These data have been deposited in the Pharmacogenetics and Pharmacogenomics Knowledge Base (accession number PS204853). Stepwise regression analysis indicated that *VKORC1* and *CYP2C9* genotypes accounted for 21 percent and 6 percent, respectively, of the variance in warfarin dose. For all 357 patients in whom a *VKORC1* haplotype could be assigned, there was a significant additive effect: warfarin doses were  $3.2 \pm 0.2$  mg per day for the A/A combination,  $4.4 \pm 0.1$  mg per day for A/B, and  $6.1 \pm 0.2$  mg per day for B/B ( $P < 0.05$  for the comparisons between A/A and A/B and between A/B and B/B). We observed a similar additive effect with significant differences between combinations among the 233

patients with wild-type *CYP2C9*. The average INR among these patients was also approximately 2.5 and was not significantly different between any of the haplotype combinations.

The five haplotypes predictive of the warfarin dose accounted for 99 percent and 96 percent of the total haplotypes in the European-American clinical and diversity populations, respectively; there was no significant difference between these populations in the distribution of the two major haplotype groups (group A, 35 percent vs. 37 percent, respectively; group B, 64 percent vs. 58 percent). The five common haplotypes within the European-American population accounted for only 62 percent of the more diverse African-American haplotypes (Table 2). The African-American and Asian-American populations showed significant differences in the frequencies of groups A and B when compared with the European-American population ( $P < 0.001$ ). The frequency of group A haplotypes (predictive of a low warfarin dose) was significantly higher in the Asian-American population (89 percent) and lower in the African-American population (14 percent) than in the European-American population (37 percent) ( $P < 0.001$  for both comparisons).

To explore the mechanism of the association between warfarin doses and *VKORC1* polymorphisms, we assayed *VKORC1* mRNA levels in human liver tissue and also determined the major *VKORC1* haplotype group (A/A, A/B, or B/B) of each tissue specimen. A graded and highly significant gene-dose effect was evident ( $P = 0.002$ ). mRNA levels in the B/B (high-dose) group were about three times as high as those in the A/A (low-dose) group ( $P < 0.05$ ) (Fig. 2).

---

## DISCUSSION

---

In our primary study population, we found that approximately 25 percent of the variance in warfarin dose was explained by the *VKORC1* haplotype alone. Independent verification of a genetic association is critical for determining its validity and importance,<sup>11</sup> so we replicated the association in a second clinical population, in which 21 percent of the variance was explained by the *VKORC1* haplotype. Since *CYP2C9* explained 6 to 10 percent of the variability in these two patient samples, the *VKORC1* genotype appears to be the most important genetic factor determining variability in warfarin dose: in both clinical populations its effect was approximately three times that of the *CYP2C9* genotype.

**Table 2. Distribution of VKORC1 Haplotypes in European-American, African-American, and Asian-American Populations.**

Haplotype Identification Code or Group	Haplotype Sequence	Frequency of Haplotype in American Populations*		
		European (N=119)	African (N=96)†	Asian (N=120)‡
proportion (number of haplotypes)				
Haplotype distribution				
H1	CCGATCTCTG	0.12 (28)	0.07 (14)	0.89 (213)
H2	CCGAGCTCTG	0.26 (61)	0.06 (12)	0
H7	TCGGTCCGCA	0.21 (49)	0.42 (80)	0.10 (25)
H8	TAGGTCCGCA	0.14 (34)	0.01 (2)	0
H9	TACGTTCCGCG	0.24 (56)	0.06 (11)	0
Other haplotypes	—	0.04 (10)	0.38 (73)‡	0.01 (2)
Group distribution				
Group A (H1, H2)	—	0.37 (89)	0.14 (26)	0.89 (213)
Group B (H7, H8, H9)	—	0.58 (139)	0.49 (93)	0.10 (25)
Total of groups A and B	—	0.96 (228)	0.62 (119)	0.99 (238)

\* The total number of haplotypes analyzed is twice the number of persons assessed.

† There were significant differences between the African-American and European-American populations and between the Asian-American and European-American populations, both in terms of haplotype ( $P<0.001$ ) and in terms of group distribution (A or B) ( $P<0.001$ ).

‡ The haplotypes consisted largely of H3 and H6.

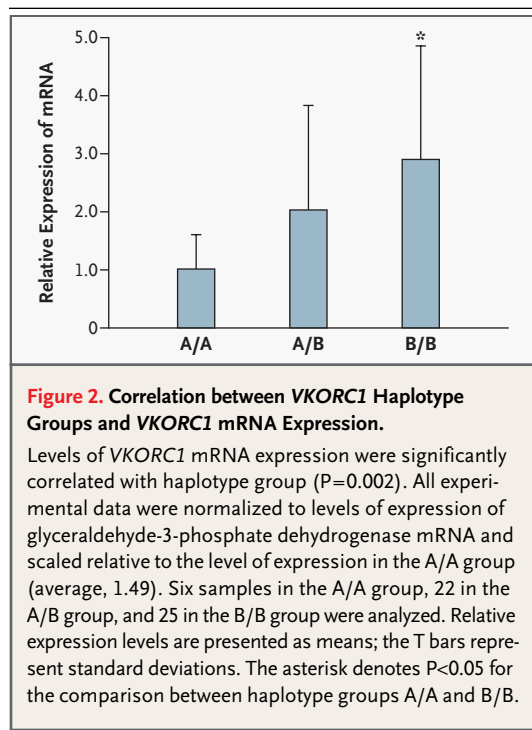
We found that haplotypes of *VKORC1* are no more informative than a single segregating SNP chosen from among those at positions 381, 3673, 6484, 6853, and 7566. This finding is consistent with data from a previous study that also showed an association between a SNP in intron 1 of *VKORC1* (C1173T, the SNP at position 6484 in the current study) and the warfarin dose.<sup>6</sup> However, our results explain a larger portion of the interindividual variations in warfarin dose (21 to 25 percent, vs. 14 percent in the previous study) and indicate that *VKORC1* has a proportionally larger effect than *CYP2C9*. (Our results show a threefold effect of *VKORC1* variants when compared with *CYP2C9* variants \*2 and \*3, whereas the previous study reported that *CYP2C9* had a greater effect than *VKORC1*.) Furthermore, only 2 of our 10 SNPs have been studied previously.<sup>6</sup>

Evidence from various clinical and population studies suggests that persons of Asian, European, and African ancestry tend to require, on average, lower, intermediate, and higher doses of warfarin (approximately 3.0, 5.0, and 6.5 mg per day, respectively).<sup>7,12-14</sup> Because group A haplotypes predicted the low-warfarin-dose phenotype and were relatively common in the Asian-American population,

it is likely that the association between ancestral origin and dose is, in part, an effect of the *VKORC1* haplotype. Conversely, the prevalence of group B haplotypes was relatively high in the African-American population, potentially giving rise to the increased dose requirement in this population.

The more diverse distribution of haplotypes among African Americans is consistent with the higher genomic sequence diversity found in populations of African descent.<sup>15,16</sup> These population-specific haplotype differences may be due to demographic effects, such as geographic selective pressures, migration, or population bottlenecks and have been observed for other medically relevant genes, such as *ADRB2*.<sup>17</sup> Additional studies involving patients of African and Asian descent who are receiving warfarin will be required to confirm the associations between *VKORC1* haplotype and warfarin dose in these populations, including the influence of haplotypes other than those of groups A and B.

The associations between the A haplotype and reduced mRNA expression and between the B haplotype and increased mRNA expression parallel the effect of these haplotypes on warfarin dose, as would be predicted by a simple, noncompetitive



model of enzyme inhibition by this anticoagulant.<sup>18</sup> We hypothesize that the level of VKORC1 mRNA is directed by each haplotype and determines the level of protein synthesis of the vitamin K epoxide reductase complex, which in turn accounts for differences among these patients in their warfarin maintenance-dose requirements. The primary SNP candidates that explain this effect would be those that designate the major haplotype split (the SNPs at positions 381, 3673, 6484, 6853, and 7566) and predict the warfarin maintenance dose. We mapped these SNPs to homologous regions in rats, mice, and dogs to identify potentially conserved, non-coding sequences that encompass these sites. Only two SNPs (at positions 6484 and 6583) from the informative group are conserved; they flank exon 2 but fall outside the canonical regions required for exon splicing. Presumably, these regions act as regulatory sequences that may bind transcription-factor-binding sites, but additional studies will be required to elucidate the mechanism underlying altered VKORC1 transcription.

The merits of genotyping before or during treatment involving drugs such as warfarin, irinotecan, and thiopurine — the effectiveness of which depends on genetic variants of *CYP2C9* (and now *VKORC1*), *UGT1A1*, and *TPMT*, respectively — is an area of active debate between regulatory authorities and the clinical community.<sup>19</sup> Recently published guidelines suggest initial warfarin doses of 5 to 10 mg per day,<sup>20</sup> but our results suggest that this strategy may expose patients with the A/A *VKORC1* haplotype, who require a low dose of warfarin, to unnecessarily high doses of drug. Because the initial warfarin dose is already individualized according to other clinical data and the dose subsequently adjusted according to the anticoagulation status, it could be inferred that *VKORC1* and *CYP2C9* genotyping may not provide a clinically significant improvement over current practice. However, in our retrospective<sup>2</sup> and prospective<sup>21</sup> studies, we found a significant effect of *CYP2C9* variants on a variety of anticoagulation-related outcomes, despite individualized dosing and frequent monitoring in a specialized anticoagulation clinic at an academic medical center.<sup>1</sup> The current data strongly suggest that analysis of *VKORC1* should be an essential component of prospective studies aimed at investigating the value of genotyping for warfarin therapy. In addition, they provide the detailed genetic information necessary to ensure that such studies are designed appropriately.

Supported by grants from the National Heart, Lung, and Blood Institute (U01 HL66682, from the Program for Genomic Applications, to Drs. Rieder and Nickerson; and R01 HL074724, to Drs. Gage, McLeod, and Eby); by General Medical Sciences grants from the National Institutes of Health (GM068797 and GM32165, to Drs. Rettie, Thummel, Blough, and Veenstra); by a grant from the National Institute of Environmental Health Sciences (P30ES07033, to the Center for Ecogenetics and Environmental Health, University of Washington, where Drs. Rettie and Thummel are research core directors); and a grant from Pharmacogenetics Research Network (GM63340, to Dr. McLeod).

We are indebted to M. Ahern for technical assistance in the sequencing; to N.C. Hastings, S. Marsh, C. King, and R. Porche-Sorbet for analysis of *VKORC1* SNPs; and to C. Baier and J.D. Smith for other technical assistance.

Drs. Rieder and Rettie report having applied for a patent (application serial no. 10/967,879) on the use of *VKORC1* haplotypes and SNPs. Dr. Thummel, an associate dean of the School of Pharmacy at the University of Washington, reports that the school receives financial support from Bristol-Myers Squibb. Dr. McLeod reports having served as a consultant to Veridex, Precision Therapeutics, and Orion Genomics.

## REFERENCES

1. Marketos M. The top 200 generic drugs in 2003 (by units). *Drug Topics* 2004;148:76. (Accessed May 9, 2005, at <http://www.drugtopics.com/drugtopics/article/articleDetail.jsp?id=109800>.)
2. Higashi MK, Veenstra DL, Kondo LM, et al. Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. *JAMA* 2002;287:1690-8.
3. Rettie AE, Korzekwa KR, Kunze KL, et al. Hydroxylation of warfarin by human cDNA-expressed cytochrome P-450: a role for P-4502C9 in the etiology of (S)-warfarin-drug interactions. *Chem Res Toxicol* 1992;5:54-9.
4. Rost S, Fregin A, Iwaskevicius V, et al. Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2. *Nature* 2004;427:537-41.
5. Li T, Chang CY, Jin DY, Lin PJ, Khvorova A, Stafford DW. Identification of the gene for vitamin K epoxide reductase. *Nature* 2004;427:541-4.
6. D'Andrea G, D'Ambrosio RL, Di Perna P, et al. A polymorphism in the VKORC1 gene is associated with an interindividual variability in the dose-anticoagulant effect of warfarin. *Blood* 2005;105:645-9.
7. Gage BF, Eby C, Milligan PE, Banet GA, Duncan JR, McLeod HL. Use of pharmacogenetics and clinical factors to predict the maintenance dose of warfarin. *Thromb Haemost* 2004;91:87-94.
8. Paine MF, Khalighi M, Fisher JM, et al. Characterization of interintestinal and intra-intestinal variations in human CYP3A-dependent metabolism. *J Pharmacol Exp Ther* 1997;283:1552-62.
9. Lin YS, Dowling AL, Quigley SD, et al. Co-regulation of CYP3A4 and CYP3A5 and contribution to hepatic and intestinal midazolam metabolism. *Mol Pharmacol* 2002;62:162-72.
10. Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet* 2003;73:1162-9.
11. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. *Genet Med* 2002;4:45-61.
12. Yu HC, Chan TY, Critchley JA, Woo KS. Factors determining the maintenance dose of warfarin in Chinese patients. *QJM* 1996;89:127-35.
13. Chenhsu RY, Chiang SC, Chou MH, Lin MF. Long-term treatment with warfarin in Chinese population. *Ann Pharmacother* 2000;34:1395-401.
14. Absher RK, Moore ME, Parker MH. Patient-specific factors predictive of warfarin dosage requirements. *Ann Pharmacother* 2002;36:1512-7.
15. Przeworski M, Hudson RR, Di Rienzo A. Adjusting the focus on human variation. *Trends Genet* 2000;16:296-302.
16. Crawford DC, Carlson CS, Rieder MJ, et al. Haplotype diversity across 100 candidate genes for inflammation, lipid metabolism, and blood pressure regulation in two populations. *Am J Hum Genet* 2004;74:610-22.
17. Drysdale CM, McGraw DW, Stack CB, et al. Complex promoter and coding region beta 2-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. *Proc Natl Acad Sci U S A* 2000;97:10483-8.
18. Fasco MJ, Principe LM, Walsh WA, Friedman PA. Warfarin inhibition of vitamin K 2,3-epoxide reductase in rat liver microsomes. *Biochemistry* 1983;22:5655-60.
19. Lesko LJ, Woodcock J. Translation of pharmacogenomics and pharmacogenetics: a regulatory perspective. *Nat Rev Drug Discov* 2004;3:763-9.
20. Ansell J, Hirsh J, Poller L, Bussey H, Jacobson A, Hylek E. The pharmacology and management of the vitamin K antagonists: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest* 2004;126:Suppl:204S-233S. [Erratum, *Chest* 2005;127:415-6.]
21. Voora D, Eby C, Linder MW, et al. Prospective dosing of warfarin based on cytochrome P-450 2C9 genotype. *Thromb Haemost* 2005;93:700-5.

Copyright © 2005 Massachusetts Medical Society.

**PHYSICIAN-JOURNALIST**

The *Journal* is seeking a physician with substantial reporting experience to write occasional articles on timely topics in medicine and society for the Perspective section. Send curriculum vitae and writing samples to Perspective Editor, *New England Journal of Medicine*, 10 Shattuck St., Boston, MA 02115, or at [writer@nejm.org](mailto:writer@nejm.org).