Association of Apolipoprotein E Genotype with Duration of Time to Achieve a Stable Warfarin Dose in African-American Patients

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Study Objective. To test the hypothesis that genotypes for proteins affecting vitamin K availability influence the duration of time required to achieve a stable warfarin dose in African-American patients.

Design. Retrospective cohort study.

Setting. Pharmacist-managed antithrombosis clinic.

Patients. Ninety-two African-American adults whose warfarin therapy was initiated between September 2, 1999, and July 8, 2009.

Measurements and Main Results. During a routine anticoagulation clinic visit, a sample was collected from each patient for genetic analysis. Genotyping was performed for the following variants: apolipoprotein E ε2, ε3, and ε4; NAD(P)H:quinone oxidoreductase (NQO1)*2; cytochrome P450 (CYP) 4F2 V433M; CYP2C9*2, *3, *5, *8, and *11; and vitamin K epoxide reductase complex 1 (VKORC1) –1639G>A. Patients’ medical records were then reviewed, and data were collected retrospectively for each anticoagulation clinic visit during the first 6 months of warfarin therapy or until dose stabilization. The median time required to reach a stable warfarin dose, defined as the dose that produced therapeutic anticoagulation for three consecutive clinic visits, was 83 days. Compared with the 46 patients who achieved a stable warfarin dose within 83 days, the 46 patients who required longer durations for dose stabilization had a higher frequency of the apolipoprotein E ε3/ε3 genotype (37% vs 59%, p=0.037). Sixty-one percent of patients with the ε3/ε3 genotype versus 40% of those with an ε2 or ε4 allele had a delay in achieving a stable dose (p=0.037). Neither the CYP4F2 nor NQO1 genotype was associated with warfarin dose stabilization.

Conclusion. Our data support the hypothesis that the apolipoprotein E genotype is associated with duration of time to reach a stable warfarin dose in African-American patients. Further insight into the genetic effects on warfarin dose stabilization could reveal novel methods to improve anticoagulation control during the warfarin initiation period.

Key Words: warfarin, genotype, African-American, apolipoprotein E, vitamin K.

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during the initial months of warfarin therapy.\textsuperscript{3, 4} Thus, it is imperative to efficiently achieve therapeutic anticoagulation after warfarin initiation.

Warfarin exerts its anticoagulant effects by inhibiting vitamin K oxidoreductase (VKOR), thus preventing formation of vitamin K\textsubscript{1} (Figure 1). Vitamin K\textsubscript{1} is subsequently reduced to vitamin KH\textsubscript{2}, a necessary cofactor for generation of functional clotting factors.\textsuperscript{5-12} Much less is known about genes influencing warfarin dose stabilization. Although results of some studies support the CYP2C9 gene as a contributor to dose stabilization, the data are inconsistent.\textsuperscript{5, 8, 13, 14} Most studies show no association between VKOR (VKORC1) and time to attain a stable dose.\textsuperscript{8, 14}

The apolipoprotein E, NAD(P)H:quinone oxidoreductase (NQO1), and CYP4F2 genes are involved in vitamin K distribution and metabolism (Figure 1). As such, these genes could influence response to vitamin K antagonism with warfarin. Specifically, apolipoprotein E is involved in the hepatic uptake of vitamin K\textsubscript{1}. Two apolipoprotein E single nucleotide polymorphisms, C130R (rs429358 T>C) and R176C (rs7412 C>T), result in three common alleles, designated as the \(e2\) (130C/176C), \(e3\) (130C/176R), and \(e4\) (130R/176R) alleles. Low, intermediate, and high clearance of vitamin K\textsubscript{1} from the plasma have been reported with the \(e2\), \(e3\), and \(e4\) alleles, respectively.\textsuperscript{15} Increased hepatic uptake of vitamin K with the \(e4\) allele is proposed to increase vitamin K–dependent clotting factor activation.\textsuperscript{16} The NQO1 enzyme reduces vitamin K\textsubscript{1} to vitamin KH\textsubscript{2}. A common CYP4F2 single nucleotide polymorphism, V433M (rs2108622 C>T), decreases CYP4F2 concentration.\textsuperscript{19} Thus, the CYP4F2 genotype influences the quantity of vitamin K\textsubscript{1} available for reduction to vitamin KH\textsubscript{2} by NQO1.

We hypothesized that through their effects on vitamin K availability, the apolipoprotein E, NQO1, and CYP4F2 genes influence warfarin dose stabilization. Thus, we sought to determine whether the apolipoprotein E, NQO1, and CYP4F2 genotypes influence the time required to achieve a stable warfarin dose. We focused our analysis on African-American patients, a population largely underrepresented in warfarin pharmacogenomic studies, yet at particularly high risk for recurrent thromboembolism and adverse sequelae from thromboembolism.\textsuperscript{20, 21}

**Methods**

**Study Setting**

All study participants were patients at the pharmacist-managed antithrombosis clinic at the University of Illinois Medical Center at Chicago...
The antithrombosis clinic operates under a collaborative agreement protocol with oversight provided by a medical director. Approximately 450 warfarin-treated patients are managed at the clinic, most of whom are African-American (60%) or Hispanic (25%). Clinic staffing is provided by eight clinical pharmacists, with two or three pharmacists providing coverage at any given time. Clinic policies and procedures, including a protocol for warfarin dose changes based on international normalized ratio (INR) values during both warfarin initiation and maintenance therapy, are approved by the UIMCC pharmacy and therapeutics committee and followed by each clinician. This ensures consistency in following and adhering to dosing guidelines for all patients seen at the antithrombosis clinic. According to these policies and procedures, patients with stable warfarin doses are seen at the clinic at least once every 4 weeks, whereas patients whose therapy is not yet stabilized or patients newly starting warfarin may be seen as often as once or twice/week. The INR is determined at each clinic visit through point-of-care testing with use of the ProTime monitor (QAS, Orlando, FL); patients with INR values greater than 4.5 at point-of-care testing are sent to the medical center's central laboratory for verification.

At the initial clinic visit, all new patients receive intensive counseling on warfarin therapy, including dietary counseling with regard to vitamin K intake, and literature on foods with high vitamin K content. Average vitamin K intake, in terms of servings of food with high vitamin K content, is assessed at this visit, and patients are counseled to maintain a consistent intake of such foods during warfarin therapy. In addition to INR testing, a clinic pharmacist assesses basic criteria for each patient, regardless of INR results, at each clinic visit with documentation of such in the medical record. These criteria include adherence to warfarin therapy, status of medical problem(s) warranting anticoagulation, and recent alterations in drugs or intake of foods with high vitamin K content.

Study Cohort

Eligible patients had to be African-American by self-report, aged 18 years or older, and had their warfarin therapy initiated at the UIMCC between September 2, 1999, and July 8, 2009. Patients with a history of liver dysfunction or serum transaminase levels greater than 3 times the upper limit of normal were excluded. All patients provided written informed consent for the collection of a buccal cell sample for genetic analysis and for retrospective review of their medical record, as previously described. The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional review board at the University of Illinois at Chicago.

Data Collection and Study Outcomes

Patients' medical record data were collected from each clinic visit during the 6 months after warfarin was started or until a stable warfarin dose was achieved, whichever was longer. A stable dose was defined as the same dose for three consecutive clinic visits spanning a period of 4 or more weeks for which the INR value at each visit was within 0.1 unit of the therapeutic range (e.g., 1.9–3.1 for a goal range of 2–3). The extended INR range was used because values within 0.1 unit of the therapeutic range do not usually elicit a dose change. Data were collected on the date of warfarin initiation, warfarin doses, INR values, missed or extra warfarin doses, and dietary changes.

The primary outcome of the study was the time required to achieve a stable warfarin dose. This was defined as the time between starting warfarin therapy until the first of the three consecutive visits when the warfarin dose was stable. In addition, adherence to warfarin therapy was assessed. Adherence was expressed as the percentage of visits with 100% patient-declared adherence to warfarin (no missed or extra warfarin doses) relative to the total number of clinic visits during the 6-month period after warfarin initiation.

Genotyping

Genomic DNA was isolated from buccal cells by using a commercially available kit (PureGene; Qiagen, Valencia, CA). The apolipoprotein E C130R and R176C; CYP2C9 R144C (*2), I359L (*3), and D360E (*5); and VKORC1 rs9923231 (–1639G>A) genotypes were determined by polymerase chain reaction (PCR) and pyrosequencing methods, as previously described. The NQO1 P187S, CYP4F2 V433M, and CYP2C9 R335W (*11) single nucleotide polymorphisms were also determined by PCR and pyrosequencing. The CYP2C9 R150H (*8) allele was determined by PCR and capillary sequencing. Primers are shown in Table 1. Each PCR reaction consisted...
of 25 µl of HotStarTaq Master Mix (Qiagen), 25 pmol of primers, 15 µl of water, and 20–100 ng of DNA. Thermocycling consisted of denaturation for 15 minutes at 95°C, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing for 30 seconds at 62°C for NQO1*2, 58°C for CYP4F2 V433M, 61°C for CYP2C9*8, and 61°C for CYP2C9*11, and extension at 72°C for 45 seconds, with a final extension of 72°C for 10 minutes. All genotypes were assigned by investigators blinded to clinical data.

The frequencies of patients with each genotype and their demographic and warfarin-related characteristics were compared between those patients who achieved a stable warfarin dose within the median time frame for the study population and those requiring longer than the population median to achieve a stable dose. Other patient characteristics related to warfarin therapy were also evaluated based on genotype.

Subgroup Analysis

To evaluate the effect of vitamin K intake on the time needed to reach a stable warfarin dose, apolipoprotein E genotype distribution was compared between those patients reaching and those not reaching a stable dose within 83 days in a subgroup of patients who reported alterations in vitamin K intake at any point during their first 6 months of therapy.

Statistical Analysis

For the comparison of genotype and other characteristics between those patients who achieved a stable warfarin dose within the median time frame for the study population and those requiring longer than the population median to achieve a stable dose, the χ² analysis for categoric data and the Student unpaired t test or Mann Whitney U test for continuous data were used. In addition, a binary logistic regression analysis was used to estimate the apolipoprotein E contributions to dose stabilization when accounting for the potential interaction with VKORC1 and CYP2C9 genotypes. Time to attain a stable dose was compared between genotypes by using χ² analysis or the Fisher exact test. The Hardy Weinberg equilibrium assumption was tested by χ² analysis. Data are expressed as number (percentage), mean ± SD, or median (interquartile range).

Results

A total of 97 unrelated African-American patients were initially included; however, one patient had the apolipoprotein E ε2/ε4 genotype and was subsequently excluded given the divergent effects of the ε2 and ε4 alleles on vitamin K clearance. Four other patients moved, were lost to follow-up, or discontinued warfarin before reaching a stable warfarin dose. The mean age of the remaining 92 patients was 57 years (range 24–86 yrs). Most were female (76%) and taking warfarin for secondary prevention of venous thromboembolism (55%).

The median time to reach a stable dose in the study population was 83 days (mean 136 days, range 7–541 days). Characteristics, including warfarin adherence rate, vitamin K intake, frequency of dietary changes, and VKORC1 and CYP2C9 genotypes, were similar between patients reaching and those not reaching a stable warfarin dose within 83 days (Table 2). The number of clinic visits required to reach a stable dose and percentage of clinic visits with INR

Table 1. Polymerase Chain Reaction and Sequencing Primers

| Variant     | Primers (5’ to 3’)                                                      |
|-------------|--------------------------------------------------------------------------|
| NQO1 P187S  | PCR forward: GCATTTCTGTTGGCTTCCAAGTC  
|             | PCR reverse: biotin-GTGTCGCAATGCTATGTCAG  
|             | Sequencing: TGGCTTCCAAGTCTTAG |
| CYP4F2 V433M| PCR forward: GAGGAAGGTGATGGATACT  
|             | PCR reverse: TTCTCTCCCAACAGGCTTAC  
|             | Sequencing: CCCATCAACACCCAG |
| CYP2C9*8    | PCR forward + sequencing: CCTCTTTGTTGTTCTCCTCC  
|             | PCR reverse: TGAGCTAAACAGGACTCA |
| CYP2C9*11   | PCR forward: GAACGTGATGGCAGAAGAC  
|             | PCR reverse: biotin-GCATCTGTGAGGCATGTG  
|             | Sequencing: CGTGTATGGGCAGAA |

NQO1 = NAD(P)H:quinone oxidoreductase; PCR = polymerase chain reaction; CYP = cytochrome P450.
values greater than 0.1 unit outside the therapeutic range during the first 6 months of therapy were significantly higher among those patients with a delay in reaching a stable dose.

None of the genotypes deviated from Hardy Weinberg equilibrium. Allele frequencies were 0.18 for $NQO1^*2$; 0.06, 0.72, and 0.22 for apolipoprotein E $\epsilon2$, $\epsilon3$, and $\epsilon4$, respectively; and 0.08 for $CYP4F2$ 433M. Figure 2A shows the genotype distributions between those patients achieving and those not achieving a stable warfarin dose within 83 days. Patients with a delay in achieving a stable dose had a significantly higher frequency of the apolipoprotein E $\epsilon3/\epsilon3$ genotype ($p=0.037$). Carriers of the $\epsilon2$ or $\epsilon4$ allele were similarly distributed between groups. No significant association was noted between time required to achieve a stable dose and the $NQO1$ or $CYP4F2$ genotype. Logistic regression analysis showed that the association between apolipoprotein E and time required to achieve a stable dose remained significant, regardless of the inclusion of $VKORC1$ and $CYP2C9$ genotypes in the model (odds ratio 2.6, 95% confidence interval 1.1–6.0, $p=0.033$).

In the subgroup analysis of 72 patients who reported alterations in vitamin K intake during their first 6 months of warfarin therapy, the difference in apolipoprotein E genotype distribution between those patients reaching and those not
reaching a stable dose within 83 days became more pronounced (Figure 2B).

When comparing the apolipoprotein E genotypes in the overall patient cohort, we found that significantly more ε3 homozygotes required greater than 83 days to achieve a stable warfarin dose than did ε2 or ε4 allele carriers (61% vs 40%, p=0.037). In the subgroup of patients with dietary changes, the differences by genotype were more pronounced, with 68% of ε3 allele homozygotes versus 38% of ε2 or ε4 carriers achieving a stable dose after 83 days (p=0.013).

A previous pharmacogenetic study that evaluated the influence of the CYP2C9 and VKORC1 genes on warfarin response in African-Americans used a definition of time required to achieve a stable dose that was similar to that in our study, except that the difference between consecutive stable warfarin doses was allowed to vary up to 10%. When analyzing our data according to this less stringent definition, the median time required to achieve a stable dose was shortened to 67 days. The apolipoprotein E ε3/ε3 genotype remained associated with a delay in dose stabilization. The apolipoprotein E ε3/ε3 genotype was especially overrepresented among those requiring longer than 90 days to achieve a stable dose compared with those reaching a stable dose in half that time (45 days; 65% vs 36%, p=0.020).

Discussion

Alterations in vitamin K intake can affect anticoagulation response and influence warfarin dose stabilization. Previous investigators have observed significant interpatient variability in anticoagulant response to alterations in dietary vitamin K.22 We found that the apolipoprotein E ε3/ε3 genotype was associated with a delay in achieving a stable warfarin dose in African-American patients. Given that apolipoprotein E mediates vitamin K uptake into the liver, we believe that the most likely explanation for our findings is that apolipoprotein E genotype influences INR response to dietary vitamin K.13 Specifically, our data suggest that alterations in vitamin K intake during the warfarin initiation phase have a greater impact on the INR value in apolipoprotein E ε3 allele homozygotes compared with patients with other genotypes. As vitamin K uptake is already increased in the presence of an ε4 allele and decreased in the presence of the ε2 allele, carriers of these alleles may have little further change in vitamin K uptake with alterations in vitamin K intake. However, those with the apolipoprotein E ε3/ε3 genotype and “normal” uptake of vitamin K could be more susceptible to changes in vitamin K intake. We believe that the more pronounced association between apolipoprotein E genotype and duration of time required to achieve a stable warfarin dose among those reporting dietary changes supports differential response to dietary alterations by apolipoprotein E genotype.

Neither CYP4F2 nor NQO1 genotype was associated with the time required to achieve a stable warfarin dose in our study. There are in vitro data to suggest that the NQO1 enzyme plays a smaller role in vitamin K recycling than previously thought, and this may account for our negative findings with regard to the NQO1 genotype. Our negative findings with regard to the CYP4F2 433M allele may be reflective of its low frequency in African-American individuals. The CYP4F2 433M variant is more common in

![Figure 2.](image-url)
Caucasians, and its effects on warfarin stabilization in this population remain to be examined.

Although not a primary focus of our study, we also genotyped for VKORC1 and CYP2C9 alleles. Similar to previous findings, neither genotype was associated with warfarin dose stabilization in our African-American population. Numerous studies have demonstrated that CYP2C9 and VKORC1 influence warfarin dose requirements. However, the ability of these genotypes to predict warfarin dose requirements declines over time. Whether the effect of apolipoprotein E genotype on dose stability changes over time remains to be determined. However, it is conceivable that patients who experience large fluctuations in INR values with dietary changes may become more conscientious about maintaining consistent vitamin K intake. This, in turn, could minimize genetic influences of INR response to diet over time. Other investigators have reported associations between apolipoprotein E genotype and warfarin dose requirements. However, the data are inconsistent and, in some cases, conflicting.

We found no association between apolipoprotein E genotype and warfarin dose in a previous study of over 200 patients. The definition we used for a stable dose was more conservative than that used in previous studies and may account for the relatively long time experienced until a stable dose was achieved. For example, other studies used the time to the first therapeutic INR value rather than the time required to achieve a stable warfarin dose, defined a stable dose using fewer than three INR values, allowed the warfarin dose to vary somewhat rather than requiring it to remain unchanged, allowed greater variability in INR values (e.g., 0.2 unit outside the target range), or required very little time between INR measurements. We believe our definition was appropriate since it is the same definition commonly used to define a stable warfarin dose during long-term therapy. In addition, using a less stringent definition that allowed stable warfarin doses to vary by up to 10% did not significantly influence our results.

Limitations

One limitation of our study is that the retrospective method of data collection did not allow for more thorough assessment of vitamin K intake. In addition, we were unable to prospectively assess INR response to dietary changes by genotype. Thus, the mechanism underlying the associations we observed requires prospective confirmation. The influence of genotype on vitamin K supplementation to improve anticoagulation stability or use of vitamin K to treat overanticoagulation would be of particular interest. However, data such as ours are important to inform which candidate genes to prospectively study for their effects on vitamin K response. Another limitation is that our study population was limited to African-American patients. Although our study provides important pharmacogenomic data in African-Americans, a population underrepresented in pharmacogenomic studies, it may not be applicable to other populations given racial differences in allele frequencies and vitamin K intake.

Conclusion

The apolipoprotein E genotype was associated with the duration of time required to achieve a stable warfarin dose among African-American patients. The mechanism underlying this association requires investigation but may relate to response to dietary changes. Given the retrospective nature of our data collection, particularly with regard to assessment of vitamin K intake, our results should be considered hypothesis generating and require confirmation in a prospectively designed study. Insight into genetic influences of the time required to achieve a stable warfarin dose could ultimately reveal novel methods of improving anticoagulation control during the warfarin initiation period.

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