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Warfarin Pharmacogenetics

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Abstract

The cytochrome P450 (CYP) 2C9 and vitamin K epoxide reductase complex 1 (VKORC1) genotypes have been strongly and consistently associated with warfarin dose requirements, and dosing algorithms incorporating genetic and clinical information have been shown to be predictive of stable warfarin dose. However, clinical trials evaluating genotype-guided warfarin dosing produced mixed results, calling into question the utility of this approach. Recent trials used surrogate markers as endpoints rather than clinical endpoints, further complicating translation of the data to clinical practice. The present data do not support genetic testing to guide warfarin dosing, but in the setting where genotype data are available, use of such data in those of European ancestry is reasonable. Outcomes data are expected from an on-going trial, observational studies continue, and more work is needed to define dosing algorithms that incorporate appropriate variants in minority populations; all these will further shape guidelines and recommendations on the clinical utility of genotype-guided warfarin dosing.

Introduction

Since its approval in 1954, warfarin has been widely prescribed for the prophylaxis and treatment of venous thromboembolism and complications associated with atrial fibrillation and cardiac valve replacement. Even with the availability of newer agents shown to be noninferior to warfarin, warfarin remains the most commonly prescribed oral anticoagulant.[1–4] Owing to its narrow therapeutic index, warfarin is also a leading cause of serious adverse drug events and is implicated in 33% of hospitalizations secondary to adverse drug events among older adults in the U.S.[5] Therapy with warfarin is further complicated by marked inter-patient variability in the dose necessary for optimal anticoagulation, defined as an international normalized ratio (INR) of 2 to 3 for most indications. Genotype is a major determinant of warfarin dose requirements and also impacts risk for over-anticoagulation and hemorrhage, especially in the initial months of therapy.[6] Guidelines and dosing algorithms are available to assist with application of genotype data to

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dose warfarin. However, clinical trials evaluating the effect of genotype-guided warfarin dosing on time in therapeutic range (TTR) produced variable results. Other trials are on-going, including one powered to detect clinical endpoints. Herein, we review the evidence for genetic associations with warfarin response, evaluate the design and results from clinical trials, and discuss evidence needed to further define the role for genotyping to guide warfarin dosing.

Overview of genetic associations with warfarin dose requirements

The primary genes contributing to warfarin dose requirements are vitamin K epoxide reductase complex 1 (VKORC1) and cytochrome P450 2C9 (CYP2C9). The CYP2C9 enzyme metabolizes the more potent *S*-enantiomer of warfarin, while VKORC1 is the target protein for warfarin. Specifically, warfarin inhibits VKORC1, thus preventing reduction of vitamin K to vitamin KH_2, a necessary co-factor for carboxylation and activation of clotting factors. The gene for CYP4F2, which metabolizes vitamin K, provides a minor contribution to dose requirements, particularly in European and Asian populations.

A single polymorphism in the *VKORC1* regulatory region, c.−1639G>A (rs9923231), predicts dose requirements across ethnic groups.[7] The minor A allele at this position is associated with approximately 2-fold lower gene expression and significantly lower warfarin dose requirements compared to the G allele.[8] The frequency of the A allele varies by ethnicity, as shown in Table 1, with the highest frequency in Asians, intermediate frequency in Europeans, and the lowest frequency in African Americans. Differences in allele frequency account for the lower dose requirements generally observed in Asian populations and higher requirements in African Africans compared to Europeans.

The majority of *CYP2C9* variants impacting warfarin dosing are nonsynonymous single nucleotide polymorphisms (SNPs) that occur in the exonic regions of the gene and lead to reduced enzyme activity against *S*-warfarin and consequently lower warfarin dose requirements.[9,10] An exception is the **6* allele (rs9332131), which results from a single nucleotide deletion, shift in the reading frame, and loss of function. The **2* (R144C, rs1799853) and **3* (I359L, rs1057910) alleles are the primary dysfunctional alleles in Europeans, but are less common in African Americans; the **2* allele rarely occurs in Asians (Table 1). Additional functional polymorphisms occurring primarily in African populations include **5* (D360E, rs28371686), **6*, **8* (R150H, rs7900194), and **11* (R335W, rs28371685). The number of individuals expected to carry a *CYP2C9* variant allele, based on allele frequency data, are shown in Table 2.

The *CYP4F2* V433M (rs2108622) SNP was first identified as a contributor to warfarin dose requirements in Europeans, with variant allele homozygotes requiring higher doses.[11] The association was subsequently replicated in Asians, but not in African Americans, in whom the variant allele is much less common (Table 1).[12,13] In functional studies, the 433M allele led to reduced hepatic concentrations of CYP4F2 and decreased vitamin K metabolism.[14] As a consequence, variant allele carriers are expected to have higher vitamin K concentrations and require higher warfarin doses to inhibit vitamin K dependent clotting factor activation.

A genome wide association study (GWAS) in African Americans revealed an additional SNP, rs12777823G>A, associated with variability in warfarin dose requirements in this population.[15] This SNP occurs in chromosome 10 near the *CYP2C18* gene, with the minor A allele correlated with reduced *S*-warfarin clearance and dose requirements. Interestingly, while the variant is common across populations (Table 1), its association with warfarin dose is only evident in African Americans, which suggest that it is likely not the causative allele, but rather inherited with one or more alleles influencing warfarin response in West African populations. Further research is required to elucidate the mechanism underlying the association between rs12777823 and dose requirements in African Americans.

Unequivocal data that genotype predicts warfarin dose requirements

Multiple candidate gene studies have consistently demonstrated that the *VKORC1* and *CYP2C9* genotypes influence the inter-patient variability in warfarin dose requirements, together explaining 10% to 45% of the overall variance depending on the population studied and genotypes detected.[16–18] This has been confirmed by several genome-wide association studies (GWAS).[12,19,20] The *CYP4F2* SNP explains approximately 1% of the overall variability in warfarin dose requirements.[12,20] Specific effects of these and other variants on dose are shown in Table 3. The *CYP2C9* genotype has also been correlated with risk for over-anticoagulation and hemorrhage during warfarin therapy. The risk for bleeding is highest in the initial 3 to 6 months following warfarin initiation, but continues with chronic therapy.[6,21] In a meta-analysis of 22 studies including over 6,000 patients, the *CYP2C9*2, *3,* and *VKORC1* −1639A alleles were associated with an increased risk for over-anticoagulation, defined as an INR>4.[22] Interestingly, the association between *VKORC1* −1639G>A genotype and over-anticoagulation was evident only in the initial month of therapy, whereas the risk conferred by *CYP2C9* extended beyond this period, suggesting that the effects of the *VKORC1* genotype are realized early on while the influence of *CYP2C9* genotypes persists into chronic therapy. Only the *CYP2C9* genotype was associated with risk for major hemorrhagic complications in this meta-analysis.

In 2010, the FDA approved the addition of a table in the warfarin label providing dose recommendations based on *VKORC1* and *CYP2C9* genotypes. In addition, a number of investigators have proposed pharmacogenetic dosing algorithms, including both genotype and clinical factors (e.g. age, body size, amiodarone use), to assist clinicians with estimating dose requirements. Algorithms more accurately predict warfarin dose requirements than the FDA-approved table since they include clinical factors, whereas the table does not.[23] The algorithms by Gage et al[24] and the International Warfarin Pharmacogenetics Consortium (IWPC)[25] were derived from large populations (n=1015 and 4043, respectively) predominately of European descent and are freely accessible via the www.warfarindosing.org internet site. However, they do not include many of the variants important for African Americans and do not perform as well in African Americans compared to Europeans.[7,26] The www.warfarindosing.org algorithm also includes is a formula for dose refinement so that therapy can be adjusted based on INR response to previous doses.[27]

Early warfarin pharmacogenetic studies suggested promise

Several studies were conducted early on to help identify the potential clinical utility of warfarin pharmacogenetic testing, with mixed results, leading to the speculation that they were too small to draw meaningful conclusions. However, a comparative effectiveness trial called COUMAGEN-II was published in 2012, and was the largest warfarin pharmacogenetics study at that time. COUMAGEN-II tested two dosing algorithms; a modified IWPC algorithm, and the IWPC algorithm with dose revision. There were no differences in the two dosing algorithm approaches,[28] and so those patients with pharmacogenetic dosing were compared against parallel controls, with all patients managed by the same anticoagulation service and protocols. They found that pharmacogenetic-guided dosing led to significantly fewer out of range INRs at 1 and 3 months and greater time in therapeutic range (TTR) over the same time periods (all $p < 0.001$). Pharmacogenetic dosing also resulted in fewer low (< 1.5) and high (> 4.0) INRs. There were also differences in 90 day medical record computed serious adverse events, with 4.5% in the pharmacogenetics group and 9.4% in the controls. Collectively, these data suggested potential promise for pharmacogenetic-guided warfarin dosing.

Randomized controlled clinical trials: COAG and EU-PACT

The data suggesting improved effectiveness in dose prediction with use of genetic information, and the positive results from the comparative effectiveness trial COUMAGEN-II led to great anticipation of the results of several randomized controlled trials. The Clarification of Optimal Anticoagulation through Genetics (COAG) and European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) trials were randomized, multi-center, controlled trials designed to assess the clinical utility of genotype-guided warfarin dosing, with results from each published in late 2013.[29,30] Table 4 outlines the design and study population for each study. There were important differences in patient populations between trials, with EU-PACT participants being over a decade older on average and more likely to have atrial fibrillation versus venous thromboembolism as an indication for warfarin. In addition, EU-PACT enrolled from the United Kingdom and Sweden, while COAG participants were from the U.S. and much more ethnically diverse. Both trials used a recommended pharmacogenetic algorithm for genotype-guided dosing; EU-PACT used a modified version of the IWPC algorithm,[25,31] with a dose revision algorithm at day 4–5,[27] and COAG used the algorithm by Gage et al24, with the same dose revision algorithm on day 4–5. The EU-PACT investigators reported a significant improvement in the primary endpoint of percent of time within the therapeutic INR range with genotype-guided dosing compared to standard dosing over the initial 12 weeks of therapy (67% versus 60%, $p<0.001$). The differences between dosing arms were apparent as early as 4 weeks (55% versus 46%, $p < 0.001$). In addition, subjects in the genotype-guided group had significantly fewer instances of supra-therapeutic anticoagulation, defined as an INR ≥4 (27% versus 37%), and reached a therapeutic INR more quickly (median of 21 versus 29 days) than those receiving standard dosing.

In contrast to the EU-PACT results, the COAG trial showed no differences in percent TTR in the initial 4 weeks of therapy between genotype-guided and clinically-guided dosing

algorithms (45% in both arms). Moreover, among African Americans, genotype-guided dosing was associated with a lower percentage TTR compared to the clinical algorithm arm (35% versus 43.5%) and a higher percent of time with an INR >3 (27% versus 22.5%). The combined outcome of any INR ≥4, major bleeding, or thromboembolism was similar between pharmacogenetic and clinical algorithm dosing (20% and 21%) in the population overall and in African Americans over the initial 4 weeks of therapy. However, there were numerically more major bleeds with clinically-guided versus genotype-guided dosing at 4 weeks (10 versus 4, p=0.13), and this difference persisted to the end of follow-up at 6 months (19 versus 7, p=0.021). Another study of EU-PACT evaluated phenprocoumin and acenocoumarol, vitamin K antagonists used in parts of Europe.[32] This trial was more similar in design to COAG, with the control arm being a clinical dosing algorithm, tested against a pharmacogenetic algorithm. In this study, the pharmacogenetic algorithm arm had significantly greater TTR than the clinical algorithm at 4 weeks, but not at 12 weeks.

Interpreting the differences in findings in COAG and EU-PACT

Despite the hope that the reasonably powered randomized controlled trials would provide clarity on the clinical utility of pharmacogenetic guidance for warfarin dosing, this was not the case. In fact, COAG and EU-PACT, which were presented in the same session at the 2013 American Heart Association Scientific Sessions, and published simultaneously in the New England Journal of Medicine, created more confusion because of the differences in their findings.

In trying to understand the results of these trials and the implications on warfarin pharmacogenetics moving to clinical practice, it is instructive to evaluate the differences in study design that might have led to the differences in outcomes between the two trials. Table 4 includes study design features of the two trials.

The most likely factor contributing to different outcomes in these trials was the use of a clinical algorithm (COAG), versus clinical dosing (usual care, EU-PACT). The IWPC previously reported that considering clinical factors, through use of a clinical algorithm, is more predictive of the stable warfarin dose than starting on a fixed dose, and genetics added into the clinical algorithm (the pharmacogenetics algorithm) just improves the prediction further.[25] Thus, it may have been predictable that it would be easier to show differences if the comparator was usual care versus a clinical algorithm. However, the EU-PACT study of other vitamin K antagonists used a clinical algorithm as their comparator and still saw improvements in TTR in the genetic arm of the study in the first four weeks (which was the primary endpoint timeline in COAG). So use of a clinical algorithm is not the only explanation for the differences in findings of the COAG and warfarin EU-PACT studies.

Another difference was the use of a loading dose in EU-PACT, while no loading dose was given in COAG. While this may have led to early attainment of the therapeutic INR in EU-PACT, it is not clear how this would have explained the difference between the genetic-guided and control arm in EU-PACT, since both treatment arms in this study had a loading dose. Lack of availability of genotype in COAG in 55% of patients for the first dose may have also contributed to the pharmacogenetic arm not showing benefit, although nearly all

patients in COAG had their second dose determined by genotype. It seems unlikely that a difference of one day contributed substantially to the differences in trial results, particularly since genotype was ignored in variant carriers for *CYP2C9* for the first dose in COAG.

Some have suggested that differences in the dosing algorithms used in the two trials explain the differences, but the dosing algorithms used in COAG and EU-PACT perform similarly, and the IWPC algorithm used in EU-PACT was derived with from a diverse population, including a large number of patients from the United States. So differences in these two algorithms seem unlikely as the explanation for different findings. COAG was double-blinded while EU-PACT was single blinded, and this could have led to the clinicians being more attentive in the genotype-guided arm in EU-PACT, though there is no evidence for this. Regional differences in anticoagulation care between the US and Europe may have also played a role, and such differences have certainly been evident in other cardiovascular trials.

The COAG trial had a diverse population, consisting of nearly 30% African Americans, while EU-PACT enrolled a homogeneous European population. This is likely an important difference between the two trials, particularly since there was suggestion of harm in the African Americans in COAG. Genotyping for both the EU-PACT and COAG trials was limited to the *VKORC1* −1639G>A and *CYP2C9*2* and **3* variants. Approximately 35% of Europeans, 4% of Asians, and 25% of African Americans carry at least one *CYP2C9* variant allele. In the case of Europeans and Asians, dose-altering *CYP2C9* variants were essentially fully represented in the COAG and EU-PACT trials. In contrast, for African Americans in the COAG trial, *CYP2C9* genotype was not appropriately considered for the majority of African Americans with a dose-altering variant allele. Specifically, approximately 6% of African Americans have a **2* or **3* allele, whereas approximately 20% have a **5*, **6*, **8*, or **11* allele, as is evident in Table 2.[33] Thus, approximately one-fifth of African Americans would have been considered to have a normal (or **1/*1*) *CYP2C9* genotype (and thus normal warfarin metabolism via the CYP2C9 enzyme), when in fact, their metabolism of warfarin would be impaired but the relevant polymorphisms were not considered. Initiation of usual warfarin doses would place them at risk for supra-therapeutic anticoagulation. Additional African Americans with an rs12777823 variant would be at similarly high risk for over-dosing of warfarin. The fact that many genotypes associated with lower dose requirements in African Americans were not accounted for in the COAG trial likely explains the greater time outside TTR and greater number of INRs over 3 with genotype-guided dosing in this population. However, the analysis of non-blacks only in COAG suggested small, but non-significant differences in the genotype versus clinical algorithm approaches. Thus, the inclusion of a large number of African Americans, who were not well served by the dosing algorithm in COAG, is not a complete explanation for the differences in trial findings between COAG and EU-PACT.

Thus, it seems that no single difference in study design contributed to the contradictory findings between COAG and EU-PACT. However, collectively the differences in the comparator arm, ethnic distribution, differences in use of a loading dose, timing of availability of the genotype data, and regional differences in care may have led to the different findings.

Warfarin pharmacogenetics in guidelines and understanding the place of warfarin pharmacogenetics in therapy after COAG and EU-PACT

There are two major sets of guidelines regarding warfarin pharmacogenetics, although both were published prior to publication of the most recent randomized controlled trials. The first are the guidelines from the American College of Chest Physicians, published in 2012, which recommended against routine genetic testing to guide warfarin dose.[34]

The Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines do not address the appropriateness of ordering a pharmacogenetic test, but whether pharmacogenetic data should be used in clinical practice if such data are available. Based on the wealth of data demonstrating genetic determinants of warfarin dose requirements and ability to more accurately predict warfarin dose with genetic data, the warfarin CPIC guidelines, published in 2011, strongly recommend warfarin dosing based on genotype when genotype information is available.[35] The guidelines further recommend application of either the Gage et al[24] or IWPC algorithm[25] to assist with dosing. The 2011 CPIC guidelines are freely available through the Pharmacogenomics Knowledgebase website (www.PharmGKB.org).

It is of interest that these two guidelines provide the framework for warfarin pharmacogenetics moving forward, namely that there are two different ways of thinking about warfarin pharmacogenetics. First, is whether the data support ordering a warfarin pharmacogenetic test to guide warfarin dosing; the second is in the scenario when warfarin pharmacogenetic data are readily available, and whether they should be used.

Should warfarin pharmacogenetic tests be ordered prior to warfarin therapy?

Collectively, the data at present do not support the clinical utility of ordering a warfarin pharmacogenetic test prior to therapy initiation. More importantly, if only using the SNPs tested in COAG, the data indicate that such an approach should be avoided in African Americans, since COAG suggested harm in this group.

Two recent meta-analyses also provide insights. One meta-analyses included 9 randomized trials of coumarins, which had enrolled 2,812 patients into genotype-guided warfarin dosing versus a comparator.[36] They compared TTR, INR > 4, major bleeding and thromboembolic events, and found no significant differences in any of these endpoints. The meta-analysis can be criticized because the TTR was not a standard time interval across studies, but whatever was reported in the trial, and the comparator arms were a mix of clinical dosing/usual care approaches and clinical algorithm-based approaches. The other meta-analysis included warfarin only, and utilized 7 clinical trials that enrolled 1,910 patients.[37] They considered control arms that were fixed dosed separately from non-fixed dose and found that the trials with fixed dosing of warfarin as the comparator (which essentially represents usual care in most settings) revealed significant improvements in TTR with genotype-guided dosing. In contrast, those with non-fixed dose comparators (e.g. clinical algorithm), there were no differences in TTR.

These meta-analyses support two points. The first, which can be inferred from evaluating COAG and EU-PACT, but is reinforced in these meta-analyses, is that use of a clinical algorithm needs to become "usual care". It seems apparent that using clinical factors that personalize a patient's warfarin dose has no cost (other than the few second it takes to enter the data into an algorithm, though this can be automated in an electronic health record), and has the potential to be an important improvement over the current fixed dose approach that is the norm with warfarin therapy initiation globally. The meta-analyses also support that there is limited clinical utility associated with ordering a pharmacogenetic test, particularly compared to a clinical algorithm. In the absence of additional clinical trial data demonstrating that genotype-guided dosing improves TTR, and more importantly, clinical outcomes, over clinically-based dosing, it seems certain that the ACCP guidelines will continue to recommend against ordering warfarin pharmacogenetic tests (likely more strongly than in the past), and in the U.S., regional Medicare providers are writing policies that will not allow reimbursement of warfarin pharmacogenetic tests.

Should warfarin pharmacogenetic data be used in clinical care if already available?

The second issue is whether warfarin pharmacogenetic data should be utilized if already available to the patient or in their electronic health record. This will become increasingly common as clinical exome and genome sequencing becomes more commonplace, as it already is in cancer. Stated another way, is it reasonable to ignore the warfarin pharmacogenetic data if it is available? This changes the context, and is the focus of CPIC guidelines. In this scenario the question becomes: is there a potential for benefit and absence of harm? In other words, it becomes a question of non-inferiority, not superiority which many argue is needed to warrant ordering a genetic test.

While the data are conflicting regarding clear benefits associated with warfarin pharmacogenetic testing, with the exception of African Americans, there are no data suggesting harm. In fact, the first meta-analysis (see above) found no significant benefits, yet for all the endpoints studied (TTR, INR > 4, major bleeding, thromboembolism), the point estimates all favored genotype-guided dosing. The most notable was major bleeding, which was 40% lower in the genotype-guided group. This is consistent with the COAG trial, where major bleeding was numerically lower at 4 weeks (4 versus 10) and significantly lower at 6 months (7 vs 19) in the genotype-guided arm.

Collectively, among those of European ancestry, some trials suggest benefit of warfarin pharmacogenetics, there is essentially no evidence of harm, the point estimates all favor using the genetic data, and in particular the data suggest there may be benefit as it relates to bleeding. These data argue that when warfarin pharmacogenetic data are already available, they should be utilized in defining a patient's initial warfarin dose.

In contrast, the data suggest that using just *VKORC1* −1639 and *CYP2C9 *2* and *3, may be harmful in African Americans, likely because this approach ignores important genetic variants in the *CYP2C9* cluster that lead to lower warfarin doses. Thus, using warfarin pharmacogenetic data, if it is only the 3 variants above, should be avoided in African Americans, even when the data are available. However, in the context of genome or exome sequencing, the other *CYP2C9* variants likely would be available. In this case, if a genotype

associated with reduced warfarin dose requirements in African Americans is present, it seems reasonable to use lower than usual doses. However, there is still a question of defining the appropriate dosing algorithm for African Americans. An algorithm was recently derived from an African American population and was shown to outperform the IWPC algorithm in an independent African American cohort.[38] However, it has not yet been tested to the extent that the IWPC and Gage algorithms have been, but does provide a basis for dose adjustment with variants that are commonly observed only in those of African ancestry. We have even fewer data on Asians or Hispanic/Latinos to understand whether use of available pharmacogenetic data is reasonable, thus much work remains to be done.

Future Research

The amount of information in the field of warfarin pharmacogenomics, especially from well conducted trials assessing clinical events, is still very low, particularly when gauged against the large scale clinical trials that are the norm in cardiovascular disease. There remain a number of important areas for research in the field, particularly in the context of using readily available data. On-going studies, especially an on-going trial assessing clinical endpoints, will further address the clinical utility of genotype-guided dosing. Additional data are expected from comparative effectiveness and observational studies, which may help inform how to use existing data.

One important area for research relates to developing dosing algorithms that sufficiently capture the genetic variability that explains dose requirements across populations. While recent work has uncovered genotypes important for predicting dose requirements in African Americans, with additional discovery efforts underway, further work is needed to define algorithms that improve warfarin dosing and to assure that such algorithms improve anticoagulation-related outcomes in this population.

Hispanics are one of the largest minority populations in the U.S. and thus represent a significant portion of the population requiring anticoagulation therapy. While data are very limited to date, available evidence points to *CYP2C9*, *VKORC1*, and *CYP4F2* genotypes as important influences of warfarin response in Hispanics of Mexican descent.[13] Extrapolating these data across populations (e.g. to Hispanics from the Caribbean Islands) is complicated however by the significant heterogeneity in genetic ancestry that exists among Hispanics depending on geographic origin.[39] There are similar challenges with extrapolating data to other admixed populations. For example, in Egyptians, some SNPs are similar in frequency to Europeans, while others are similar to Africans, and still others are different from both populations.[40] In addition, while common, the *CYP4F2* V433M polymorphisms is not associated with warfarin dose in Egyptians, as it is in Europeans and Asians.[11,12] There is also evidence of differences in genetic associations with warfarin dose requirements in ethnogeographically distinct Asian groups.[41] These data emphasize the importance of verifying genotype associations with warfarin response across populations as well as within subsets of populations.

Variants important for warfarin dosing are better defined for Asian populations. Asians were not included in the COAG or EU-PACT populations. Yet, given the high prevalence of the

variant *VKORC1* −1639A allele in this population, Asians may derive particular benefit from genotype guided dosing. A trial is underway in a Chinese population targeting individuals requiring warfarin therapy with a target INR of 1.5 to 3 (ClinicalTrials.gov Identifier: NCT01610141). Patients are being randomized to pharmacogenetic or standard warfarin dosing with a primary endpoint of percent of out-of-range INRs. The study is estimated to be completed in 2014.

An additional trial, called the Genetics Informatics Trial (GIFT), is on-going in the U.S. The trial is randomizing adults over the age of 65 years who are undergoing hip or knee arthroplasty and requiring prophylaxis for venous thromboembolism to warfarin dosing with a pharmacogenetic versus clinical algorithm, similar to the strategy used in the COAG trial. Unlike either the COAG or EU-PACT trial or the trial underway in Asians, GIFT is powered to detect a difference in thrombotic and major bleeding events with genotype-guided dosing.[42] Data from GIFT may be helpful in resolving the discrepancy between similar TTR between dosing strategies in COAG, but a signal for more adverse outcomes with clinically-guided dosing. However, the patient population (e.g. older individuals), warfarin indication (e.g. prophylaxis after orthopedic surgery versus treatment of venous thromboembolism or stroke prevention in atrial fibrillation), and anticoagulation intensity (e.g. target INR) differ between GIFT and previous trials. The importance of genotype information in dosing warfarin may vary depending on indication and patient characteristics. Older individuals, such as those enrolled in GIFT (and EU-PACT), are more likely to have multiple comorbidities and require multiple medications, which may impact bleeding risk with warfarin. Patients recently undergoing major orthopedic surgery are at especially high risk for both bleeding and thrombotic events. Thus questions regarding the usefulness of genotype-guided dosing of warfarin across warfarin indications and patient characteristics may remain after completion of GIFT. Regardless, findings from GIFT are highly anticipated given the focus on clinical outcomes versus TTR.

The investigators for GIFT are genotyping participants for *VKORC1* −1639G>A, *CYP2C9*2* and **3*, in addition to *CYP4F2* V433M. However, similar to previous trials, variants that uniquely contribute to dose requirements in African Americans will not be typed. Thus, even when GIFT is complete, we will not have a clinical trial that adequately considered the genotypes in African Americans that influence dose requirement, and thus we will remain uninformed of the full potential value of a genotype-guided approach in African Americans.

There are examples of genotype-guided warfarin dosing in clinical practice that may provide additional evidence on the utility of this approach. In particular, routine genotyping was implemented into dosing protocols for patients newly starting warfarin at an institution serving a mostly African American population.[43] Genotyping included the *CYP2C9*5, *6*, and **11* alleles, thus capturing several important dose-altering variants for African Americans that were not typed in clinical trials. Outcomes data are expected and will help define the role for pharmacogenetic dosing that extends beyond the *CYP2C9*2, *3*, and *VKORC1* variants.

Conclusion

The discovery of the gene encoding the protein target for warfarin, *VKORC1*, occurred in 2004. In the ensuing decade, substantial research has been done that defined major genetic determinants of warfarin dose requirements, led to creation of dosing algorithms that incorporate genetic and clinical information, and better predict stable dose than usual care approaches or clinical algorithms. Observational studies and randomized clinical trials then tested the clinical utility of these algorithms, with some suggesting improvements with a genotype-guided dosing approach, while others did not show significant benefit. Recent trials were powered to detect differences in TTR, not clinical endpoints (e.g. bleeding and thrombosis), which are more important for evaluating the benefit of warfarin pharmacogenomics. Previous trials suggested fewer adverse events with genotype-guided dosing, and this will be further evaluated in an on-going trial, that unlike recently published trials, is appropriately power for clinical endpoints. At present, ordering a genetic test to guide warfarin dosing cannot be recommended, although for those of European ancestry, we contend it is reasonable to use genetic information, if it is already available, to guide warfarin dosing. Such an approach cannot be recommended for other ethnic groups until ongoing research can better define appropriate dosing algorithms and the potential benefits (or risks). The only randomized controlled trial testing outcomes will be completed in the near future and could significantly impact the future clinical utility of genotype-guided warfarin dosing.

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Table 1

Minor allele frequencies by ethnicity[12,15,33182413]

Variant	Europeans	African Americans	Asians
VKORC1 −1639A	0.37	0.10	0.86
*CYP2C9*2*	0.13	0.02	ND
*CYP2C9*3*	0.06	0.01	0.02
CYP2C9*5	**ND**	**0.01**	**ND**
CYP2C9*6	**ND**	**0.01**	**ND**
CYP2C9*8	**ND**	**0.06**	**ND**
CYP2C9*11	**ND**	**0.02**	**ND**
CYP4F2 433Met	**0.29**	**0.07**	**0.29**
rs12777823	**0.14**	**0.25**	**0.32**

ND = not detected or rare

Variants shown in bold text were not included in either the COAG or EU-PACT study.

Table 2

Expected prevalence of carriers of *CYP2C9* variants by ethnicity

***CYP2C9* allele**	**Ethnicity**		
	Europeans	**African Americans**	**Asians**
**2*	0.24	0.04	ND
**3*	0.12	0.02	0.04
**5*	ND	0.02	ND
**6*	ND	0.02	ND
**8*	ND	0.12	ND
**11*	ND	0.04	ND

Carriers of variant alleles calculated from allele frequencies in Table 1. Carriers are defined as those with one (e.g. *1/*2 or *2/*3) or two (i.e. *2/*2) copies of the variant allele.

Table 3

Approximate effect of genetic variant on warfarin dose requirement[8,11,15,3321]

Variant	Approximate effect on dose requirements
VKORC1 −1639 GG	1 mg/day increase
VKORC1 −1639 AA	2–3 mg/day reduction
*CYP2C9*2*	1 mg/day reduction
*CYP2C9*3*	2 mg/day reduction
*CYP2C9*5*	2 mg/day reduction*
*CYP2C9*6*	ND
*CYP2C9*8*	1 mg/day reduction*
*CYP2C9*11*	1–2 mg/day reduction*
CYP4F2 V/M	0.5 mg/day increase
CYP4F2 M/M	1 mg/day increase
rs12777823 AG	1 mg/day reduction*
rs12777823 AA	1.5 mg/day reduction*

*Association only identified in African Americans. ND=insufficient evidence to evaluate effect on dose requirements

Table 4

Comparison of subject characteristics and study design of the COAG and EU-PACT trials

Characteristic/design	COAG	EU-PACT
Number of subjects	1015 (955 in analytic sample)	455 (427 in analytic sample)
Subject age (years)	Median age=53 in Blacks; 59 in non-Blacks	Mean age= 67
Ethnicity of subjects	27% Black; 73% non-Black	98.5% European ancestry*
Most common indication for warfarin	DVT or PE (58%)	Atrial fibrillation (72%)
Allele frequencies		
VKORC1 −1639A	0.32	0.36
*CYP2C9*2*	0.09	0.125
*CYP2C9*3*	0.04	0.05
Number of recruitment sites	18 in the United States	3 in the United Kingdom and 2 in Sweden
Comparison arm	Dosing based on clinical variables	Traditional dosing/usual care
Dosing algorithm	Gage, et al[24] with pharmacogenetic dose revision algorithm[27]	Modified IWPC[3125] with pharmacogenetic dose revision algorithm[27]
Loading dose used	No	Yes
Blinding	Double blind	Single blind
Genotype availability	45% before 1st dose	100% before 1stdose
Primary endpoint	Percent time in range from day 4 or 5 through day 28	Percent time in range during the 12 weeks after warfarin initiation

*ethnicity of remaining participants not described