Using Pharmacogenetics in Real Time to Guide Warfarin Initiation

A Clinician Update

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A 75-year-old white woman is initiated on warfarin for atrial fibrillation. Empirical dosing generally would begin with 5 mg/d. If age, weight (132 kg), and height (63 in) are taken into account, the maintenance dose is estimated at 3.5 to 4 mg/d. In this case, a pharmacogenetic approach is chosen to guide initial dose selection. To augment clinical information, a rapid-turnaround assay (<4 hours) is used. Genotyping for CYP2C9 identifies the *2 CC (wild-type) and *3 AC (reduced-function heterozygote) alleles; genotyping for VKORC1 identifies an AA variant (reduced-function homozygote). With the use of a freely available Web-based algorithm (www.warfarindosing.org), a daily maintenance dose of 1.8 mg is calculated, with an optional miniloading dose of 2.7 mg/d on day 1. These calculated doses are rounded to doses of 2.5 mg on day 1 and 2.0 mg on days 2 and 3. A prothrombin time international normalized ratio (INR) measurement is planned on day 4 to guide dose adjustment if needed. Empirical dosing or dosing using only clinical characteristics would likely result in excessive anticoagulation with an increased risk of early out-of-range INRs and bleeding.

Historical Considerations

Since its introduction as the mainstay of oral anticoagulation therapy, warfarin has achieved an unfavorable reputation among physicians and patients alike. This relates to the difficulty in achieving and maintaining the narrow therapeutic range of the drug (commonly INR of 2–3), a process that requires frequent INR testing and dose adjustment. Even dedicated anticoagulation clinics achieve a time in therapeutic range of only ~60%. A major cause of this challenge is highly variable interindividual dosing requirements. Subtherapeutic anticoagulation may lead to thromboembolic events, whereas excessive anticoagulation increases the risk of bleeding. Not surprisingly, some of the most commonly reported drug-associated adverse events are warfarin related.

An important part of interindividual dose variability arises from variation in 2 genes, CYP2C9 and VKORC1. These genes are associated with the pharmacokinetics (affecting drug metabolism) and pharmacodynamics (affecting drug target) of warfarin, respectively. It was recognized almost 20 years ago that metabolism of (S)-warfarin to its inactive metabolite was mediated by the cytochrome p450 enzyme CYP2C9. Two relatively common, nonsynonymous coding variations in the CYP2C9 gene produce amino acid substitutions in the translated protein at residue 144 (coded by the CYP2C9*2 polymorphism) and residue 359 (coded by the CYP2C9*3 polymorphism). The minor variant of both of these polymorphisms produces a metabolically impaired enzyme with reduced capacity (by ~30% and 80%, respectively) to metabolize warfarin, leading to delayed and elevated warfarin steady-state levels and potentially to an increased bleeding risk.

The molecular target of warfarin is vitamin K epoxide reductase complex subunit 1 (encoded by VKORC1). The function of VKORC1 is the gamma carboxylation of glutamyl residues,
which is required for activation of multiple vitamin K–dependent clotting factors. The VKORC1 gene was discovered by positional cloning and shortly thereafter, a functional variant was found in its promoter region (−1639G>A) that alters a transcription factor binding site. In vitro studies confirmed that the A (minor) allele reduces transcription of the gene by almost 50% compared with a promoter bearing the wild-type G allele. A number of other tightly linked variants have been identified in the −1639G>A-containing haplotype.

### Genetic Polymorphisms and Stable Warfarin Dose

As early as 1995, articles began to report on pharmacokinetic interactions of variants in the CYP2C9 gene with stable warfarin dose. More than 15 reports over the ensuing 10 years were confirmatory. Subsequently, the contribution of VKORC1 genotype was recognized, increasing the percent of dose variability attributable to genetic variants plus clinical characteristics to 50%. Our own experience confirms the impact of CYP2C9 and VKORC1 variants on interindividual dose requirements (Figure 1). We have observed a range of weekly warfarin doses from 7 mg/wk for an individual carrying variants at all 3 loci to 70 mg/wk for an individual homozygous for the major (wild-type) allele at all loci. Compelled by mounting evidence and a regulatory mandate for drug safety, the Food and Drug Administration in 2007 modified the label for warfarin to include general information regarding the impact of genetic variation on warfarin dose requirements and bleeding risk. The modified label did not include recommendations for genotype-based dose modifications, an omission that caused concern for many physicians. Accordingly, the label was modified again in 2010 to include tables with specific recommended dosing ranges for the composite genotypes. Finkelman et al compared the accuracy of genetic tables and formal pharmacogenetic algorithms for warfarin dose in a retrospective cohort study from 3 anticoagulation centers. Genetic tables predicted warfarin dose better than empirical dosing, but formal pharmacogenetic algorithms were most accurate. Variants in several other genes subsequently have been studied for a potential impact on warfarin dose, but, with the possible exception of CYP4F2, their contribution appears to be minor.

### Application of Information on Genotype to Dose Prediction

Several pharmacogenetic warfarin initial dose-prediction models have been developed. These algorithms incorporate CYP2C9 and VKORC1 polymorphisms, age, sex, measures of body size, and variably race/ethnicity, smoking status, and relevant concomitant medications. Although differing in detail, several give reasonably comparable results. In addition, a pharmacogenetic model has been developed that incorporates the day 4 INR plus genetic and clinical factors for subsequent dose adjustment. These dose-initiation models indicate that the CYP2C9 and VKORC1 polymorphisms account for 18% and 30% of the observed variability of warfarin dose, respectively, with the VKORC1 variant predicting warfarin sensitivity (pharmacodynamics) and the CYP2C9*2/*3 variants affecting warfarin clearance (pharmacokinetics). Clinical factors account for an additional 12% of dose variability, so 50% to 60% of interindividual variability is accounted for overall.

Some dosing algorithms being tested also suppress CYP2C9 polymorphisms until 1 to 3 doses have been given, ie, until drug clearance becomes important; conversely, the VKORC1 genotype is captured beginning with the first dose because of its immediate effect on warfarin sensitivity. Whether this refinement will be clinically relevant remains to be determined.

### Clinical Trials Database

Several prospective studies have demonstrated the feasibility of initiating warfarin therapy on the basis of pharmacogenetics-guided dose-prediction algorithms. However, only a few were randomized, controlled clinical trials, and they have been limited by diverse issues, including study design and sample size.

Applying Pharmacogenetic Algorithms to Individualize Dosing of Warfarin (CoumaGen), the largest (n=200) trial to date to test for both CYP2C9 and VKORC1 variants, was a randomized study of pharmacogenetics-guided warfarin initiation masked to patients and investigators. Pharmacogenetics-guided dosing predicted stable maintenance doses significantly better than standard empirical dosing (5 mg/d). The primary end point of percent of out-of-range INRs and the secondary end point of time in therapeutic range were not met. However, patients in 2 prespecified genetic subgroups (ie, those with multiple variants, anticipated to require smaller doses, and those with no variants, anticipated to require larger doses) experi-

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**Figure 1.** Weekly stable dose for 216 subjects is presented by combined CYP2C9 and VKORC1 genotype. WT indicates wild-type (common variant).
enced absolute 10% and relative 26% reductions in out-of-range INRs with pharmacogenetic guidance (P<0.03). The secondary end points of the number of dose adjustments and nonprotocol INRs also were reduced. Although the importance of INR measurement during the first week of therapy is recognized,19,22 CoumaGen found that by day 3 in the standard dosing group, 11 of 18 subjects (61%) with variants in both CYP2C9 and VKORC1 had INRs above the therapeutic range, and 4 (22%) had an INR >4. In contrast, with pharmacogenetic guidance, only 3 of 15 (20%) with these genotypes had an INR >3, and only 2 (13%) had an INR >4 (Figure 2). Similar trends were noted across all genotypes, further suggesting that pharmacogenetic guidance may add to early INR guidance alone for optimal dose prediction. This concept is being tested further in CoumaGen-II (clinicaltrials.gov NCT00927862), with results expected in late 2011.

**Pharmacogenetics-Guided Dosing of Warfarin: An Option, Not a Mandate**

Pharmacogenetics-guided dosing of warfarin, although shown to most accurately predict individual warfarin dose requirements, has not demonstrated the ability to decrease out-of-range INRs, to improve time in therapeutic range, to decrease thromboembolic and bleeding events, and to be cost-effective.23 Accordingly, pharmacogenetic guidance has not been endorsed by relevant medical societies (such as the American Heart Association) or incorporated into clinical guideline recommendations. Hence, the current clinical use of pharmacogenetic guidance for warfarin initiation must be viewed as an option (based on physician preference/expertise, patient characteristics, and genotyping availability) and not a general mandate.24 We use a rapid-turnaround (in-house) genotype assay for CYP2C9 and VKORC1, incorporating genotype together with clinical factors (age, body size, medications, smoking status, etc) to derive an estimated dose before warfarin initiation.9,14,15 On the basis of the clinical scenario, a modified “loading dose” of 1.5 to 2 times the maintenance dose may be given on day 1. If an initial dose of warfarin has been given before pharmacogenetic assessment, doses on days 2 and 3 are adjusted to achieve the 3-day calculated total dose. An INR is obtained on day 4, and the dose is modified if needed on the basis of either a validated empirical dosing algorithm25 or, more recently, an algorithm incorporating INR together with genetic and clinical information.16

**Laboratory Testing, Cost, and Turnaround Time for Warfarin-Relevant Genotypes**

An assumption reflected in the study design of the major ongoing US randomized trials (CoumaGen II, Clarification of Optimal Anticoagulation Through Genetics [COAG],25 and Genetics Informatics Trial of Warfarin Therapy [GIFT]29) is that pharmacogenetic dose prediction before initiation of anticoagulation has the highest probability for patient benefit. We have observed that separation of mean INRs among genotypes with standard dosing begins after the first day and becomes significant between wild-type (common polymorphism) and double-variant (at both loci) subjects after day 2 (P<0.03; Figure 2).9 In contrast, these mean differences are not observed with pharmacogenetic guidance (Figure 2).9 These data support the concept of incorporating genotype into dose selection for the first dose whenever possible. A Medco-Mayo clinical effectiveness study reported that hospitalizations for bleeding and thromboembolism were reduced (hazard ratio=0.72) by genotyping, even though results were not available for a minimum of 11 days (median, 32 days).27 The design and results of this nonrandomized study have been criticized as being susceptible to physician treat-

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**Figure 2.** Average international normalized ratio values for days 1 to 7 after initiation of anticoagulation therapy for subjects receiving standard (Kovacs) dosing (A) and pharmacogenetics (PG) dosing (B). Data are from the Applying Pharmacogenetics Algorithms to individualize Dosing of Warfarin (CoumaGen) trial.9

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moment bias (Hawthorne effect) and lack temporal plausibility because the contribution of genotype to INR-alone guidance has been shown to diminish over time.\textsuperscript{9,16,28}

Given the greater impact early on of genetics in dose initiation, a prudent recommendation is to incorporate pharmacogenetic guidance before the first dose or, at the latest, before the second or third warfarin dose. The urgency of the clinical indication may inform the decision of whether to delay the first dose of warfarin until genotype information is available. For example, anticoagulation for venous thrombosis should not be delayed (although bridging with low-molecular-weight heparin or fondaparinux for an extra day or 2 is a therapeutic option before warfarin is instituted). Collection of genotype information for a patient undergoing elective joint replacement can be scheduled in advance of surgery.

Because warfarin-sensitivity genotyping currently is performed infrequently, turnaround time may be delayed for up to a few days, whereas in our research laboratory, an assay using a rapid-melting curve analysis has provided results within 1 hour.\textsuperscript{29} Although presently unavailable, point-of-care genotyping would greatly facilitate pharmacogenetics-guided warfarin initiation. Point-of-care molecular diagnostics are available for the detection of infectious diseases,\textsuperscript{30} and a point-of-care method for detecting single-nucleotide polymorphisms has been described.\textsuperscript{31} If results of ongoing major randomized trials (CoumaGen II, COAG,\textsuperscript{25} GIFT,\textsuperscript{26} and European Pharmacogenetics Anticoagulation Therapy [EU-PACT; clinicaltrials.gov/ct2/show/NCT01119300]) are positive and affect guideline recommendations, the demand for clinical point-of-care technology for warfarin-sensitivity genotyping will increase (the Table).

Currently, there are 4 Food and Drug Administration–approved/cleared testing options for warfarin-sensitivity genotyping platforms: Infinity Warfarin Assay (Autogenomics, Inc, Vista, CA), eSensor Warfarin Sensitivity Test (GenMark Diagnostics, Inc, Carlsbad, CA), eQ-PCR LC Warfarin Genotyping Kit (TrimGen, Sparks, MD), and Verigene Warfarin Metabolism Nucleic Acid Test (Nanosphere, NorthBrook, IL). All platforms test for CYP2C9*2 and *3; all test for VKORC1 −1639G>A except Veri-gen, which tests for the 1173C>T single-nucleotide polymorphism in high linkage disequilibrium with −1639G>A. The current charge for these commercial assays ranges from about $400 to $800.

**Considerations for Clinical Application**

Warfarin dose recommendations that incorporate genotype and clinical information can be generated for individual patients from publically available mechanisms. The 2 best validated pharmacogenetic algorithms are available online at http://warfarindosing.org\textsuperscript{14} and http://www.pharmgkb.org/do/serve?objId=PA162372936&objCls=Dataset#tabview=tab2.\textsuperscript{15} Alternatively (although less accurately\textsuperscript{13}), dose selection can be estimated by genotype (but without regard to clinical information) from a table in the current warfarin drug information brochure (2010).

Diet, concomitant medications, and a number of herbal and dietary supplements, in addition to standard clinical factors and genotype, can affect response to warfarin. Moreover, individuals of various ancestries (eg, of African descent) may have additional modifying gene polymorphisms not

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Table. Ongoing Major Pharmacogenetic Trials of Warfarin Initiation

<table>
<thead>
<tr>
<th>Study</th>
<th>Target, n</th>
<th>Date</th>
<th>Target Population</th>
<th>Study Design</th>
<th>End Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoumaGen-II</td>
<td>500+</td>
<td>2000</td>
<td>Any indication to initiate warfarin: orthopedic and valve surgery, DVT/PE, AF, heart failure</td>
<td>R, DB c/o IWPC and modified PG-guided warfarin dosing</td>
<td>%OOR INRs and TTR at 1 (primary) and 3 mo</td>
</tr>
<tr>
<td>COAG</td>
<td>1238</td>
<td>2009–2013</td>
<td>DVT/PE, AF, orthopedic surgery (≥1 mo therapy, INR 2–3)</td>
<td>R, DB, placebo-controlled PG-guided vs clinically guided initiation of warfarin</td>
<td>TTR at 1 (primary) and 3 mo, safety (INR &gt;4, SAEs)</td>
</tr>
<tr>
<td>EU-PACT</td>
<td>970</td>
<td>2010–2013</td>
<td>AF, VTE</td>
<td>R, SB comparison of modified IWPC PG-guided and standard dosing of 1 of 3 coumarins</td>
<td>TTR at 3 mo Safety, efficiency</td>
</tr>
<tr>
<td>GIFT</td>
<td>1600</td>
<td>2011–2013</td>
<td>Orthopedic surgery</td>
<td>2×2 Factorial R, DB c/o PG-guided and clinically guided dosing of warfarin</td>
<td>VTE, hemorrhage, death, or INR &gt;4 (4–6 wk) INR control (TTR)</td>
</tr>
</tbody>
</table>

CoumaGen-II (NCT 00927862), Applying Pharmacogenetics Algorithms to individualize Dosing of Warfarin; COAG (NCT 00839657), Clarification of Optimal Anticoagulation Through Genetics trial; EU-PACT (NCT01119300), European Pharmacogenetics Anticoagulation Therapy trial; GIFT (NCT 01006733), Genetics Informatics Trial of Warfarin Therapy; DVT, deep vein thrombosis; PE, pulmonary embolism; AF, atrial fibrillation; R, randomized; DB, double-blind; c/o, comparison of; IWPC, International Warfarin Pharmacogenetic Collaboration\textsuperscript{15}; PG, pharmacogenetics; %OOR, percent out of range; INR, international normalized ratio; TTR, time in therapeutic range; SAE, serious adverse events; SB, single-blind; and VTE, venous thromboembolism.
considered in the present dosing models. Poor compliance and dosing errors are additional major issues that affect patient response to warfarin. Thus, the use of pharmacogenetic dosing should not be considered a substitute for clinical and INR monitoring.

Conclusions
Warfarin dose requirements are commonly affected by genetic variation, and pharmacogenetics-guided dosing helps predict individual warfarin dose requirements. However, the ability to improve time in therapeutic range, to decrease thromboembolic or bleeding events, and to be cost-effective remains to be shown. Therefore, current clinical use of pharmacogenetic guidance for warfarin initiation is an available option but not a general mandate. Other factors, including diet, medications, supplements, and compliance, affect response to warfarin, so pharmacogenetic guidance does not eliminate the need for close clinical follow-up. With the emergence of new, fixed-dose oral anticoagulants and with the cost-effectiveness of new, fixed-dose oral anticoagulants and INR monitoring. With the emergence of pharmacogenetic guidance does not be considered a substitute for clinical and INR monitoring.

Disclosures
Drs Carliquist and Anderson are investigators of the National Institutes of Health-funded COAG and GIFT studies.

References


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