Randomized Trial of Genotype-Guided Versus Standard Warfarin Dosing in Patients Initiating Oral Anticoagulation

Jeffrey L. Anderson, MD; Benjamin D. Horne, PhD, MPH; Scott M. Stevens, MD; Amanda S. Grove, BS; Stephanie Barton, PharmD; Zachary P. Nicholas, BS; Samera F.S. Kahn, BS; Heidi T. May, MSPH; Kent M. Samuelson, MD; Joseph B. Muhlestein, MD; John F. Carlquist, PhD; for the Couma-Gen Investigators

Background—Pharmacogenetic-guided dosing of warfarin is a promising application of “personalized medicine” but has not been adequately tested in randomized trials.

Methods and Results—Consenting patients (n=206) being initiated on warfarin were randomized to pharmacogenetic-guided or standard dosing. Buccal swab DNA was genotyped for CYP2C9 *2 and CYP2C9 *3 and VKORC1 C1173T with a rapid assay. Standard dosing followed an empirical protocol, whereas pharmacogenetic-guided dosing followed a regression equation including the 3 genetic variants and age, sex, and weight. Prothrombin time international normalized ratio (INR) was measured routinely on days 0, 3, 5, 8, 21, 60, and 90. A research pharmacist unblinded to treatment strategy managed dose adjustments. Patients were followed up for up to 3 months. Pharmacogenetic-guided predicted doses more accurately approximated stable doses (P<0.001), resulting in smaller (P=0.002) and fewer (P=0.03) dosing changes and INRs (P=0.06). However, percent out-of-range INRs (pharmacogenetic=30.7%, standard=33.1%), the primary end point, did not differ significantly between arms. Despite this, when restricted to wild-type patients (who required larger doses; P=0.001) and multiple variant carriers (who required smaller doses; P<0.001) in exploratory analyses, results (pharmacogenetic=29%, standard=39%) achieved nominal significance (P=0.03). Multiple variant allele carriers were at increased risk of an INR of ≥4 (P=0.03).

Conclusions—An algorithm guided by pharmacogenetic and clinical factors improved the accuracy and efficiency of warfarin dose initiation. Despite this, the primary end point of a reduction in out-of-range INRs was not achieved. In subset analyses, pharmacogenetic guidance showed promise for wild-type and multiple variant genotypes. (Circulation. 2007;116:2563-2570.)

Key Words: anticoagulation ■ clinical trial ■ genetics ■ pharmacogenetics ■ warfarin

Completion of the human genome project has raised the possibility of medical practice based on individual genetic characteristics.1,2 Pharmacogenetics, the study of interactions of genetics with pharmacotherapy, promises to be a premiere application of genetics to “personalized medicine.”3–7

Clinical Perspective p 2570

Warfarin is prescribed to >2 million patients for the prevention of thromboembolic events associated with atrial fibrillation, prosthetic heart valves, orthopedic surgery, or a history of vascular thrombosis. Unfortunately, clinical management is difficult because of a narrow therapeutic index and marked interpatient variability in metabolism leading to unpredictable and variable (up to 20-fold) dosing requirements.8 Anticoagulation trials for nonrheumatic atrial fibrillation have determined the optimal prothrombin time international normalized ratio (INR) range to be 2 to 3, with ratios <2 increasing thrombotic events and those >4 increasing hemorrhagic events.9,10

Genotypes of the cytochrome p450 isoform CYP2C9 and the vitamin K epoxide reductase complex subunit 1 VKORC1 conjointly determine warfarin dose requirements.11–20 The *2 (R144C) and *3 allele (I359L) variants of CYP2C9 cause reductions in enzymatic activity of 30% and 80%, respectively, and increase bleeding risk.13 Ten VKORC1 single nucleotide polymorphisms, many tightly linked, and 5 inferred haplotypes determine low-, intermediate-, and high-
dose requirements.15,19 Together, these genotypes plus clinical characteristics predict approximately one half of interindividual dose variability.18–22

These observations have raised interest in CYP2C9 and VKORC1 genotyping for clinical application. Indeed, the US Food and Drug Administration recently revised product labeling for warfarin to include information on the potential effect of genetic makeup on drug dosing and to highlight the opportunity for healthcare providers to use genetic testing to improve the initial estimate of warfarin dose for individual patients with the intent to lower the risk of bleeding complications.23 However, genotype-guided warfarin dosing algorithms have not been adequately tested for their impact on clinical outcomes in prospective, controlled trials.

Methods

Study Design
This study was designed as a prospective, randomized study comparing pharmacogenetic-guided and standard empirical dosing in patients being initiated on oral anticoagulation. The study was approved by the LDS Hospital institutional review board and registered on ClinicalTrials.gov (NCT00334464).

Objectives
Study objectives were prospectively to validate a pharmacogenetic-guided dosing algorithm20 and to assess its impact on INR-based efficacy and safety end points.

Inclusion and Exclusion Criteria
Inclusion required age ≥18 years, an indication for anticoagulation with a target INR of 2 to 3, and written informed consent. Women who were pregnant, lactating, or of child-bearing potential; those participating in other investigational trials within 30 days; those taking rifampin within 3 weeks; or patients with comorbidities precluding standard dosing (eg, advanced physiological age, renal insufficiency/creatinine >2.5 mg/dL, hepatic insufficiency, terminal disease) were excluded.

Enrollment, Randomization, and Blinding
Qualifying, consenting patients underwent buccal swab testing and randomization (in permuted blocks of 5) to the pharmacogenetic or standard arm. The randomization arm assignment was blinded to patients and clinicians/investigators and known only to a designated research assistant and pharmacist.

Warfarin Dosing
The designated anticoagulation management service pharmacist (who was unblinded to randomization arm) managed all warfarin dosing. In both arms, twice the standard (control) arm or twice the individual predicted (pharmacogenetic-guided) maintenance dose was given on days 1 and 2, followed by the respective daily maintenance dose, which was subject to subsequent modification based on INR monitoring.

Standard dosing followed the 10-mg warfarin nomogram of Kovacs et al.24 In a randomized study comparing 10- and 5-mg initiation nomograms for the treatment of acute venous thromboembolism, Kovacs et al found the 10-mg initiation nomogram to be superior because it allowed more rapid achievement of a therapeutic INR without an increase in major bleeding or number of INR measurements.>5. Thus, for this largely impatient-initiated study, in which time to therapeutic INR was an important factor and INRs were followed up closely, we selected the 10-mg initiation algorithm as standard. Specifically, 10-mg doses were given on days 1 and 2 and were followed initially by 5 mg daily (given in the afternoon or evening). INRs were routinely measured on days 0, 3, 5, 8, 21, 60, and 90; additional INRs were drawn as clinically indicated. Based on the day 5 INR, an adjusted daily dose was given on days 5, 6, and 7 (online-only Data Supplement, Appendix A).24 Day 8 and later INRs guided dose modification based on the Intermountain Healthcare protocol (online-only Data Supplement, Appendix B).

Pharmacogenetic-arm dosing was determined with a regression equation developed from our previous observational study on a distinct cohort of local patients on stable maintenance therapy with warfarin20 and on a weighted overview of literature studies.15,18–25 and included CYP2C9 (*1, *2, *3) and VKORC1 (C1173T) genotypes, age, weight, and sex; estimated weekly dose (y) = 1.64 + exp[(3.98 + *1 + *2 + *3 + 1.97) + 0.36 + 0.97 + 0.025 * (0.974 + 0.025 * (0.265 + 0.363 * 1.892 + 0.304 + (0.569 + 0.025 * (0.009 + female sex)/weight in kg)] + 0.00334464), where exp is the exponential to base e, *1, *2, *3 refer to CYP2C9 wild-type (*1) or variant (*2, *3) genotypes, respectively; and Vf refers to VKORC1 with variants CT, TT, or CC. Scores were categorized into 14 dose increments (from 1 to 8 mg/d, with twice this dose on days 1 and 2; online-only Data Supplement, Appendix C). Subsequent modification was based on INR by multiplying standard-arm changes by the pharmacogenetic algorithm coefficient. The pharmacogenetic algorithm coefficient, used to determine INR-based adjustments in dose through day 7, was defined as the ratio of the estimated individual maintenance weekly dose determined with the pharmacogenetic algorithm above to the standard weekly dose (ie, 35 mg). An example of this adjustment algorithm is shown in Appendix D of the online-only Data Supplement. A 25% dose reduction was imposed on patients receiving amiodarone (n = 2).26 Similar, standard dietary instructions were given to both groups.

Study Duration
Study duration was 3 months or to the end of warfarin therapy, if shorter (orthopedic patients generally were treated for 1 month).

DNA Extraction and Genotyping
Buccal swab DNA was extracted with the BuccalAmp DNA Extraction Kit (EPICENTRE Biotechnologies, Madison, Wis). DNA was amplified by the polymerase chain reaction using the Rapid Cycler 2 (Idaho Technology, Salt Lake City, Utah). The CYP2C9 *2 and *3 and VKORC1 C1173T polymorphisms were detected by high-resolution melting profile analysis employing Simple Probes (Idaho Technology) and the HR-1 melting curve analyzer (Idaho Technology). Genotypes were identified using the derivative peak analysis technology. Genotype analysis and for clinical dose adjustment, as VK-CT(-0.304 + Vf-CC(-0.569 + Vf-CC(0) + age(-0.009) + male sex(0.094) + female sex(0)/weight in kg(0.003))], where exp is the exponential to base e, *1, *2, *3 refer to CYP2C9 wild-type (*1) or variant (*2, *3) genotypes, respectively; and Vf refers to VKORC1 with variants CT, TT, or CC. Scores were categorized into 14 dose increments (from 1 to 8 mg/d, with twice this dose on days 1 and 2; online-only Data Supplement, Appendix C). Subsequent modification was based on INR by multiplying standard-arm changes by the pharmacogenetic algorithm coefficient. The pharmacogenetic algorithm coefficient, used to determine INR-based adjustments in dose through day 7, was defined as the ratio of the estimated individual maintenance weekly dose determined with the pharmacogenetic algorithm above to the standard weekly dose (ie, 35 mg). An example of this adjustment algorithm is shown in Appendix D of the online-only Data Supplement. A 25% dose reduction was imposed on patients receiving amiodarone (n = 2).26 Similar, standard dietary instructions were given to both groups.

Sample Size
On the basis of a retrospective analysis of patients in the anticoagulation clinic database of Intermountain Healthcare, we hypothesized that INR would be out of range 40% of the time using standard dosing14,20 and that pharmacogenetic-guided therapy would decrease this proportion to 20%. For a power of 80% and an α of 0.05, using a 2-sided χ2 test, we calculated that a sample size of 200 patients would be required to test this assumption, allowing for a 15% dropout rate.

End Points
The primary end point was the comparison between the pharmacogenetic and standard arms of the per-patient percentage of out-of-range INRs. Specifically, the number of out-of-range INR values for each individual patient was divided by the total number of INRs for that patient to give an individual percent out-of-range value. These individual values were then averaged over all patients in each group, and the group averages were compared.

Although the target INR range was 2 to 3, we prospectively defined an out-of-range INR value, for purposes of end-point analysis and for clinical dose adjustment, as <1.8 or >3.2 to allow for measurement error and to avoid problems inherent in overcor-
vention. Below therapeutic INRs were counted after day 4. Subset analysis of the primary end point by variant carrier status was prospectively proposed.

Secondary end points were (1) the time to the first supratherapeutic INR (or use of vitamin K), (2) the proportion of time within the therapeutic INR range using the method of linear interpolation,28 (3) the proportion of patients reaching therapeutic INR on days 5 and 8, (4) the total number of INR measurements and number of dose adjustments made, and (5) the proportion of patients with serious adverse clinical events in each group, defined as an INR>4, use of vitamin K, major bleeding events (after the Thrombolysis in Myocardial Infarction29 and Columbus Investigators30), thromboembolic events, stroke (all cause), myocardial infarction, and death (all cause). Prospectively defined subgroups of interest included genotypic subset, diagnosis, inpatient versus outpatient initiation, and bridging parenteral anticoagulant therapy.

Determination of Stable Maintenance Dose
A stable maintenance dose was determined for study patients as the dose achieved on day 8 or after that was associated with ≥2 (within 15%) INRs measured ≥1 week apart. Cases with abbreviated or unstable dosing patterns during the study were excluded from this analysis. For cases that closely approached stability by the final study dose, a stable dose was estimated by interpolation/extrapolation of the INR pattern by a single investigator blinded to treatment arm.

Safety and Clinical Events Committees
An independent Data and Safety Monitoring Committee tracked unblinded safety data. A separate independent Clinical Events Committee adjudicated key clinical adverse events blinded to study arm.

Statistical Analysis
Comparisons between the 2 groups for the primary and secondary end points were made using unpaired, χ², or log-rank tests as appropriate. Odds ratios (and 95% confidence intervals [CIs]) comparing the pharmacogenetic and standard groups were calculated for discrete variables through logistic regression. Hazard ratios (and CIs) were calculated for time-to-event data with Cox regression. Heterogeneity between subgroups for the primary end point was assessed by use of MANOVA. Linear regression was used to generate multivariable models for the observed stable maintenance warfarin dose for patients in this prospective study entering the genetic variants, age, weight, and sex. Primary and secondary analyses were by intention to treat in evaluable patients (n=200). Significance for the primary end point was set at P<0.05. In case of a nonsignificant primary end point, results for secondary end points and subset analyses were to be considered exploratory and nominally significant at P≤0.05.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
Patient Enrollment and Demographics
A total of 206 patients consented and were randomized. Of these, 6 were enrollment failures—3 orthopedic patients had surgery canceled, and 3 were withdrawn after a single dose of warfarin (2 physician withdrawals on delayed recognition of a contraindication to anticoagulation and 1 self-withdrawal)—before INR assessment. A total of 200 patients who received warfarin and had at least 1 follow-up INR (average, 7.6; median, 7; range, 1 to 18) formed the intention-to-treat evaluable population. Entry characteristics and allelic frequencies are summarized in Table 1. Clinical characteristics were balanced except for older age and greater prevalence of hyper tension in pharmacogenetic patients. Follow-up to last study INR averaged 46 days (SD, 32 days): 26 days after orthopedic surgery and 75 days with other diagnoses.

The overall allelic frequency distribution (Table 1) was similar to our previously studied cohort20 and to literature reports.8 Somewhat more patients in the standard arm carried a variant allele (ie, VKORC1 1173). The VKORC1 1173 variant was in complete (100%) linkage association with the commonly studied VKORC1-1639 promoter variant (data not shown).

Primary End Point
The study failed to achieve its primary end point of demonstrating a reduction by pharmacogenetic-guided dosing in the per-patient average percentage of INRs outside the therapeutic range, which averaged 30.7% and 33.1% in the pharmacogenetic and standard groups, respectively, a difference that was not significant (P=0.47)(Table 2). Of interest, more than one half (54%) of these out-of-range INRs were subtherapeutic. No significant interactions were observed on the primary end point by indication (orthopedic versus other), inpatient versus outpatient initiation, use of parenteral anticoagulant therapy, age, sex,

Table 1. Baseline Patient Characteristics and Allelic Variant Frequencies

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PG Arm</th>
<th>STD Arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>101</td>
<td>99</td>
</tr>
<tr>
<td>Age, y, mean (range)</td>
<td>63.2* (25–86)</td>
<td>58.9* (18–82)</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>49.5</td>
<td>56.6</td>
</tr>
<tr>
<td>Weight, kg, mean±SD</td>
<td>92.1±24.6</td>
<td>94.7±24.2</td>
</tr>
<tr>
<td>Preoperative orthopedic, %</td>
<td>65.3</td>
<td>54.5</td>
</tr>
<tr>
<td>Deep vein thrombosis and/or pulmonary embolism, %</td>
<td>18.8</td>
<td>28.3</td>
</tr>
<tr>
<td>Atrial fibrillation, %</td>
<td>12.9</td>
<td>15.2</td>
</tr>
<tr>
<td>Other diagnosis, %</td>
<td>3.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Ethnicity/race, % white</td>
<td>94.1</td>
<td>94.9</td>
</tr>
<tr>
<td>History of diabetes mellitus, %</td>
<td>22.8</td>
<td>18.2</td>
</tr>
<tr>
<td>History of hypertension, %</td>
<td>63.5†</td>
<td>47.5†</td>
</tr>
<tr>
<td>History of smoking, %</td>
<td>6.9</td>
<td>5.1</td>
</tr>
<tr>
<td>History of CAD, %</td>
<td>14.9</td>
<td>11.1</td>
</tr>
<tr>
<td>In-patient initiation</td>
<td>87.1</td>
<td>79.8</td>
</tr>
<tr>
<td>CYP 2C9*2, %</td>
<td>82.0</td>
<td>76.5</td>
</tr>
<tr>
<td>CYP 2C9*3, %</td>
<td>89.0</td>
<td>87.6</td>
</tr>
<tr>
<td>CYP 2C9 WT (CC)</td>
<td>10.0</td>
<td>11.3</td>
</tr>
<tr>
<td>VKORC1 1173, %</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>VKORC1 WT (CC)</td>
<td>50.5</td>
<td>34.7</td>
</tr>
<tr>
<td>VKORC1 CT</td>
<td>35.4</td>
<td>50.0</td>
</tr>
<tr>
<td>VKORC1 TT</td>
<td>14.1</td>
<td>15.3</td>
</tr>
<tr>
<td>Any variant, %</td>
<td>61.0†</td>
<td>79.6†</td>
</tr>
</tbody>
</table>

PG indicates pharmacogenetic; STD, standard; CAD, coronary artery disease; and WT, wild type.

*P<0.02, †P<0.01 between groups.

Downloaded from http://circ.ahajournals.org/ on June 4, 2017
or weight (P for interaction=0.12 to 0.99). Adjustment of the primary end point for the proportion of patients with any variant, given the imbalance between randomization groups, resulted in only a minor improvement in the outcome (P=0.41, MANOVA). This result was unchanged by further adjustment for differences in age and hypertension.

### Subset Analyses of the Primary End Point

The analysis plan called for an assessment of the primary end point by variant allele carrier status. However, in view of the negative primary end point, these subset analyses are considered exploratory. When analyzed by genotypic subset, an interaction between randomization assignment and number of variants was suggested (P=0.06). Specifically, much larger differences favoring pharmacogenetic-guidance were noted for multiple variant allele carriers and for wild-type patients (Table 2). Together, these 2 subsets experienced a 10% reduction in out-of-range INRs with pharmacogenetic guidance (from 39% to 29%; P=0.03). In contrast, single variant carriers were not advantaged by pharmacogenetic guidance.

### Secondary End Points

Pharmacogenetic guidance significantly decreased the secondary end point of the number of required dose adjustments (by 0.62 dose adjustments per patient; 95% CI, 0.04 to 1.19; P=0.035) and the associated secondary end point of number of INRs drawn (by 0.84 draws per patient; 95% CI, −0.04 to 1.73; P=0.06) (Table 3).

Differences in the secondary end points of time to first supratherapeutic INR, time in therapeutic range, and achieving therapeutic range on days 5 and 8 numerically favored pharmacogenetic guidance but were not statistically significant (Table 3). In multiple allele carriers, pharmacogenetic guidance tended to reduce the hazard of a first out-of-range INR (by 28%) (Table 3), but the comparison did not achieve significance.

### Table 2. Primary End Point Results Overall and by Genotypic Subsets of Interest

<table>
<thead>
<tr>
<th>End Point</th>
<th>PG Group (n=101), %</th>
<th>STD Group (n=99), %</th>
<th>Absolute Percent Reduction</th>
<th>Relative Percent Reduction</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Out-of-range INRs,* all patients, mean (SD)</td>
<td>30.7 (22.9)</td>
<td>33.1 (22.9)</td>
<td>2.4</td>
<td>7.3</td>
<td>0.47</td>
</tr>
<tr>
<td>Out-of-range INRs, multiple variant patients</td>
<td>31.0 (21.4)</td>
<td>40.4 (25.4)</td>
<td>9.4</td>
<td>22.3</td>
<td>0.14</td>
</tr>
<tr>
<td>Out-of-range INRs, wild-type patients</td>
<td>28.1 (24.8)</td>
<td>36.9 (25.3)</td>
<td>8.8</td>
<td>23.4</td>
<td>0.21</td>
</tr>
<tr>
<td>Out-of-range INRs, wild-type and multiple variant patients</td>
<td>29.3 (23.4)</td>
<td>39.1 (25.2)</td>
<td>9.8</td>
<td>25.1</td>
<td>0.03</td>
</tr>
<tr>
<td>Out-of-range INRs, single variant patients</td>
<td>33.6 (22.1)</td>
<td>27.0 (17.8)</td>
<td>−6.6</td>
<td>−24.4</td>
<td>0.14</td>
</tr>
</tbody>
</table>

PG indicates pharmacogenetic; STD, standard. *Primary end point, intention-to-treat population.

### Table 3. Secondary End Point Results

<table>
<thead>
<tr>
<th>End Point</th>
<th>PG Group (n=101)</th>
<th>STD Group (n=99)</th>
<th>OR/HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to first supratherapeutic INR, all patients, d, mean (median)</td>
<td>53.4 (73)</td>
<td>47.1 (24)</td>
<td>HR=0.88 (0.60–1.3)</td>
<td>0.53</td>
</tr>
<tr>
<td>Time to first supratherapeutic INR, multiple variant patients, d, mean (median)</td>
<td>39.4 (21)</td>
<td>23.8 (16)</td>
<td>HR=0.72 (0.38–1.4)</td>
<td>0.30</td>
</tr>
<tr>
<td>Time within therapeutic range,* %, mean (SD)</td>
<td>69.7 (24.3)</td>
<td>68.6 (24.3)</td>
<td>...</td>
<td>0.74</td>
</tr>
<tr>
<td>Therapeutic INR by day 5, %</td>
<td>69.7</td>
<td>68.3</td>
<td>OR=1.07 (0.56–2.04)</td>
<td>0.85</td>
</tr>
<tr>
<td>Therapeutic INR by day 8, %</td>
<td>68.8</td>
<td>63.0</td>
<td>OR=1.29 (0.71–2.36)</td>
<td>0.41</td>
</tr>
<tr>
<td>Total INRs, n/patient, mean (SD)</td>
<td>7.2 (2.3)</td>
<td>8.1 (3.5)</td>
<td>...</td>
<td>0.06</td>
</tr>
<tr>
<td>Dose adjustments per patient, mean (SD)</td>
<td>3.0 (2.0)</td>
<td>3.6 (2.0)</td>
<td>...</td>
<td>0.035</td>
</tr>
<tr>
<td>Adverse events (clinical plus INR ≥4), n (% patients)</td>
<td>34 (34.7)</td>
<td>42 (42.4)</td>
<td>OR=0.72 (0.41–1.28)</td>
<td>0.26</td>
</tr>
<tr>
<td>Serious adverse events (clinical only), n (% patients)</td>
<td>4 (4.0)</td>
<td>5 (5.1)</td>
<td>OR=0.78 (0.20–2.98)</td>
<td>0.71</td>
</tr>
</tbody>
</table>

HR indicates hazard ratio; OR, odds ratio. *Therapeutic range defined as 1.8 to 3.2, consistent with primary end point; results were similar when using target INR range of 2 to 3 (49.8% [24.6%] vs 51.9% [24.5%]; P=0.54, respectively).
### Pharmacogenetic-Guided Versus Standard Dose Prediction

The pharmacogenetic-guided algorithm selected an average initial weekly dose (35.5 mg) similar to that of standard dosing (35 mg; \( P=0.60 \)), but it individualized initial dose over an 8-fold range (from 7 to 56 mg) across patients compared with the single initial dose for all standard patients.

A stable maintenance dose could be determined in 175 patients and varied by an average of 5- to 6-fold across all categories based on the well of all allele carriers (Figure 1). Average doses by specific genotype are shown in Table 4. Multivariable modeling incorporating genotypes at all 3 loci, age, weight, and sex data from the present study was highly significantly predictive of final stable dose requirements (\( P<0.001 \)) (Table 5). Repeating our previous experience,\(^{20} \) these factors explained almost one half (47%) of interpatient dose variability (32% by genotype, 15% by clinical factors).

Both algorithms closely predicted the final average titrated dose for their overall arms (36.5 mg/wk). However, the pharmacogenetic-guided algorithm much more accurately predicted individual doses in each variant allele subset (wild type, single variant, multiple variant), whether assessed by comparing average stable/final doses with initial/assigned doses in these 3 subsets (\( P<0.001 \); Figure 2) or by comparing the absolute changes required on average (ie, correcting for both underdosing and overdosing) for individual patients in each subset to achieve their final/stable dose (\( P=0.002 \); Figure 3 and Table 4). Pharmacogenetic guidance was particularly more effective in predicting the higher required average doses in wild-type patients (\( P=0.001 \) and the substantially lower doses in multiple variant allele carriers (\( P<0.001 \); Figure 2); pharmacogenetic-selected initial doses required only one half (\( P=0.035 \)) and one third (\( P<0.001 \)) of the absolute magnitude of dose adjustment in these 2 subgroups, respectively, compared with standard dose selection (Figure 3). Standard dosing most nearly approximated eventual requirements in single variant patients, the most common genetic subset (41% of patients) (Figure 2), although even these patients tended to require smaller individual dose adjustment with pharmacogenetic guidance (Figure 3).

### Safety Observations

Multiple variant allele carriers were at significantly increased risk of an INR of \( \geq 4 \) (46% compared with 29% of other

---

**Table 4.** Predicted (Initial) and Actual Weekly Maintenance Dose Selections by PG-Guided and STD Empirical Dose Algorithms

| Genotype Group | PG-Guided Arm Predicted, mg | PG-Guided Arm Actual, mg | PG-Guided Arm | STD Arm Assigned, mg | STD Arm Actual, mg | STD Arm | P |
|----------------|-----------------------------|--------------------------|----------------|----------------------|---------------------|---------|
| All patients   | 35.5                        | 36.4                     | 7.1            | 35.0                 | 36.6                | 11.5    | 0.002 |
| All WT         | 43.3                        | 42.9                     | 9.1            | 35.0                 | 48.6                | 16.1    | 0.035 |
| Any variant    | 30.6                        | 32.3                     | 5.7            | 35.0                 | 36.6                | 10.3    | 0.002 |
| 2C9 *2 WT (CC) | 37.0                        | 38.6                     | 7.8            | 35.0                 | 39.0                | 11.8    | 0.02  |
| 2C9 *2 Var (CT)| 28.8                        | 28.5                     | 4.3            | 35.0                 | 27.8                | 10.7    | 0.009 |
| 2C9 *3 WT (AA) | 36.5                        | 37.3                     | 6.8            | 35.0                 | 37.4                | 12.0    | 0.001 |
| 2C9 *3 (AC, CC)| 27.7                        | 30.3                     | 10.2           | 35.0                 | 27.5                | 7.5     | 0.58  |
| VKORC1 (CC)    | 41.2                        | 42.1                     | 8.8            | 35.0                 | 42.2                | 11.5    | 0.27  |
| VKORC1 (CT)    | 31.7                        | 34.4                     | 5.5            | 35.0                 | 33.9                | 12.3    | 0.001 |
| VKORC1 (TT)    | 24.3                        | 20.7                     | 4.7            | 35.0                 | 28.9                | 9.2     | 0.07  |

*Actual (final) stable maintenance dose, as defined in Methods, could be determined in 175 patients (pharmacogenetic=92, standard=83).

†Absolute change scores (\(|\text{Change}|\)) calculated on paired measures.

---

**Figure 1.** Average stable maintenance warfarin doses (mg/wk) by number of variant alleles. Numbers of patients in each group: wild type (no variants), 56 (30%); 1 variant, 75 (43%); 2 variants, 36 (21%); 3 variants, 7 (4%); and 4 variants, 1 (0.6%). SEM is 2.0 for wild type and 1.4 for 1-, 2-, and 3-variant groups. Dose differences across groups are highly significant (\( P<0.001 \)).

**Figure 2.** Average changes in dose from initial to final in subsets defined by genotype and dosing algorithm. Change in weekly maintenance dose (in mg; mean with SEM) between initial (algorithm-predicted) and final (stable, titrated) dose in paired samples of wild-type allelic patients (pharmacogenetic [PG] arm=38, standard [STD] arm=18), patients with 1 variant allele (pharmacogenetic arm=33, standard arm=42), and patients with >1 (range, 2 to 4) variant alleles (pharmacogenetic arm=21, standard arm=23) (\( P<0.001 \) across categories).
patients; P=0.029 [both arms combined]), and this excess risk was driven primarily by patients who carried both CYP2C9 and VKORC1 variants (INR ≥4 in 26 of 57 [53%]; P=0.01). Total adverse events (clinical events plus INR ≥4) were numerically fewer in the pharmacogenetic than standard arm (34 versus 42), although the difference was not significant (P=0.26; Table 3). Serious clinical events were infrequent (pharmacogenetic=4, standard=5) and were unrelated to out-of-range INRs.

**Discussion**

**Study Summary**

We report a randomized study of personalized, pharmacogenetic-guided warfarin dosing using both CYP2C9 and VKORC1 variants plus clinical factors in a prospective clinical trial of moderate size. Despite replicating our earlier successful experience in dose prediction,^{20} pharmacogenetic guidance failed to achieve its primary end point of demonstrating a reduction in the per-patient average percentage of INRs outside the therapeutic range. Despite the negative primary end point, promise was suggested in exploratory analyses for 2 genotypic subsets poorly modeled by standard dosing: wild-type patients (whose dose requirements are greater than average) and, especially, carriers of multiple variant alleles (whose dose requirements are substantially less than the average). In these subsets, pharmacogenetic guidance yielded a 10% reduction in out-of-range INRs (from 39% to 29%; P=0.03), a finding of interest that should be independently validated.

**Previous Studies**

Previous observational studies, including our own, have reported on the predictive ability of CYP2C9 and/or VKORC1 genetic variants for warfarin maintenance dose.^{11–20} Studies initially focused on CYP2C9, and by 2005, they represented >1000 patients.^{25}

Attention subsequently turned to the gene for VKORC1, the target of warfarin activity. D’Andrea et al^{15} reported that a variant in intron 1 (1173 C to T) was associated with dose.

Table 5. Multivariable Predictive Model for Stable Maintenance Dose*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standardized β Coefficient</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>...</td>
<td>18.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VKORC1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>−0.331</td>
<td>−5.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TT</td>
<td>−0.447</td>
<td>−7.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age, y</td>
<td>−0.321</td>
<td>−5.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CYP2C9 *2</td>
<td>−0.232</td>
<td>−3.90</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CYP2C9 *3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>−0.206</td>
<td>−3.58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CC</td>
<td>−0.155</td>
<td>−2.62</td>
<td>0.010</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>0.135</td>
<td>2.20</td>
<td>0.029</td>
</tr>
<tr>
<td>Sex (male=1)</td>
<td>0.101</td>
<td>1.65</td>
<td>0.10</td>
</tr>
</tbody>
</table>

*Dose was natural logarithmically transformed. Model R²=0.683; model R²=0.47.

Rieder et al identified 10 common noncoding VKORC1 single nucleotide polymorphisms and inferred 5 major haplotypes.^{19} However, tight linkage among single nucleotide polymorphisms and haplotypes enables most of the associated dose variability to be captured by single selected single nucleotide polymorphisms (eg, VKORC1 C1173T or VKORC1-1639). Subsequent studies,^{18,21,22} including our own,^{20} have confirmed the important independent contribution of VKORC1 to dose variability. Together, variants in CYP2C9 and VKORC1 plus age, a measure of body size, and sex have been reported to account for approximately one half of dose variability.^{15,20–22}

Also of recent interest, Millican et al^{31} genotyped CYP2C9 and VKORC1 in 92 patients undergoing orthopedic surgery and used stepwise regression to develop a model for refining warfarin dose after the third dose. The algorithm explained 79% of residual dose variability.

Despite these multiple observational reports, few data are available from randomized, controlled trials. Hillman et al^{32} reported on a small (n=38) randomized pilot trial of model-based warfarin dose initiation using clinical information and CYP2C9 genotype. Model-based initial dosing matched final stable dose modestly better than standard dosing, but percent of time of INR in range and percent INR ≥4 did not differ between groups. Very recently, Caraco et al^{33} reported on a randomized study incorporating CYP2C9 genotyping in 191 patients. Times to first therapeutic INR and first stable INR were achieved earlier by pharmacogenetic-guided therapy, driven primarily by the higher daily dose assigned to wild-type patients in the pharmacogenetic-guided group.

**Mechanistic Considerations**

The reasons for the failure to clearly demonstrate a beneficial impact of pharmacogenetic-guided warfarin dosing on INR-based outcomes may have included better-than-expected responses in the standard arm, smaller-than-hypothesized differential effects in the pharmacogenetic arm, and unaccounted-for genetic and nongenetic factors and compliance issues.^{34–37} Standard dosing approximated dose require-
ments in single variant carriers as well as pharmacogenetic guidance, and by chance, a greater percentage of standard arm patients were single variant carriers (56% versus 44%). However, adjustment for this imbalance still did not yield a significant result. Of note, more than one half of out-of-range INRs were subtherapeutic rather than supratherapeutic, the primary focus.

For purposes of sizing future studies, these results suggest a “1 for 1” rule: ie, a 1% reduction in percent out-of-range INRs for every 1-mg/wk improvement by pharmacogenetic-guided initial dosing over empirical dose initiation in predicting final stable maintenance dose (Figure 3). Thus, in the future, an adequately powered, randomized, 2-arm trial of generally similar design should consider an enrollment target of at least 2000 patients.

Despite the key roles of CYP2C9 and VKORC1, ≈50% of dose variability remains unexplained. Variants in genes for protein C (PROC), microsomal epoxide hydrolase-1 (EPHX1), gamma-glutamyl carboxylase (GGCX), orosomucoid 2 (ORM2), calumenin (CALU), and apolipoprotein E (APOE), for example, have been reported to add modestly or moderately to dose prediction.53,54 Specifically, Wadelius and colleagues58 recently reported a predictive algorithm that accounted for 73% of interindividual dose variability using 4 distinct genetic variants in addition to the 3 VKORC1 and CYP2C9 variants plus clinical factors. This promising result should be prospectively validated. Additional clinical factors (eg, smoking, diabetes mellitus), interacting drugs, dietary factors, and dietary supplements represent potential, although likely modest, sources of variability between the randomized arms.54

Finally, careful management of dosing by a dedicated anticoagulation service and by inpatient initiation in the great majority of patients (>80%), for whom initial daily INR measurement was common, likely contributed to better-than-expected outcomes in the standard arm. Empirical therapy might be less successful in less closely managed and outpatient-based initiation programs.

Study Strengths and Limitations
Study strengths include its prospective, randomized design; its oversight by safety and events committees; and its independent funding. In addition, the rapid genotyping assay enabled pharmacogenetic guidance to be applied to the first dose of warfarin in clinical “real time.” However, the intermediate power of the study would preclude the detection of small differences in the primary and secondary end points and in serious clinical adverse events. A single, relatively aggressive standard dosing algorithm was tested24; however, a less aggressive regimen would be expected to yield smaller rather than larger intergroup differences in out-of-range INRs and other safety measures. Results apply strictly to patients primarily of white, European-American descent, although results in other racial/ethnic groups have been reported to be generally similar after accounting for differing variant frequencies.56

Conclusions
This study demonstrates the feasibility of randomized trials to test pharmacogenetic-based algorithms. An algorithm guided by pharmacogenetic and clinical factors selected an initial dose more closely predictive of the stable maintenance dose, led to fewer and smaller dose adjustments, and required fewer INR measurements. However, the study failed to achieve its primary end point of a reduction in out-of-range INRs. In exploratory subset analyses, those with wild-type genotype (whose dose requirement was greater than average) and carriers of multiple variant alleles (whose dose requirements were substantially less than average) showed promising reductions in out-of-range INRs. These initial randomized trial findings will be valuable as the basis for further evaluation of pharmacogenetic-guided warfarin therapy, including the design and sizing of additional randomized trials.39

Acknowledgments
We gratefully acknowledge the clinical and laboratory assistance of the following individuals: Todd Allen, MD, William A. Hinz, MD, A.G. Kfoury, MD, Dale G. Renlund, MD, Jason T. McKinney, PhD, Jessica L. Clarke, BS (study design and data acquisition); Andrew Buckley, RPH, Rose Chilton, RPH, Criissa Mower, Nazila Naderi, and Zachary W. Terry, PAC, LDS Hospital (data acquisition); and Raymond L. Woosley, MD, PhD, University of Arizona and C-Path Institute (study design and funding). The Data and Safety Monitoring Committee consisted of Frank Yanowitz, MD (chair), Gregory Elliott, MD, and Robert Jenson, PhD. The Clinical Events Committee consisted of Donald L. Lappé, MD (chair), Robert E. Fowles, MD, and James Revenaugh, MD.

Sources of Funding
The study was funded by grants from the Deseret Foundation and the Heart and Lung Institute, LDS Hospital, Intermountain Healthcare, Salt Lake City, Utah, and the Critical-Path Institute, Tucson, AZ (not-for-profit organization).

Disclosures
None.

References

CLINICAL PERSPECTIVE
Pharmacogenetics promises to contribute importantly to the future of “personalized medicine.” Warfarin, prescribed to >2 million patients, is subject to marked (up to 20-fold) intersubject dosing differences. Variants in 2 genes (2 in CYP2C9, 1 in VK0RC1), together with age and weight, explain approximately one half of this variability. Indeed, the US Food and Drug Administration recently revised the product label of warfarin to recognize the potential effect of genetic makeup on drug dosing. However, pharmacogenetic-guided dosing has not been adequately tested in prospective trials. This study randomized 206 patients being initiated on warfarin to pharmacogenetic-guided or standard dosing and followed them up for up to 3 months. The pharmacogenetic-guided algorithm selected an initial dose more closely predictive of the stable maintenance dose, led to fewer and smaller dose adjustments, and required fewer international normalized ratio measurements. Despite this, percent out-of-range prothrombin time international normalized ratios (pharmacogenetic = 30.7%, standard = 33.1%), the primary end point, did not differ significantly between arms. In exploratory subset analyses, those with the wild-type genotype (who had greater-than-average dose requirements) and carriers of multiple variant alleles (who had much lower requirements) showed promising reductions in out-of-range international normalized ratios with pharmacogenetic guidance. Inpatient initiation with careful management by a dedicated anticoagulation service, leading to rapid correction of inappropriate doses, likely contributed to better-than-expected outcomes in the standard arm. These findings should be valuable in assisting in the design of future randomized trials and in determining the clinical role of pharmacogenetic guidance of warfarin dosing.
Randomized Trial of Genotype-Guided Versus Standard Warfarin Dosing in Patients Initiating Oral Anticoagulation

for the Couma-Gen Investigators

Circulation. 2007;116:2563-2570; originally published online November 7, 2007;
doi: 10.1161/CIRCULATIONAHA.107.737312
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/116/22/2563

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2007/11/15/CIRCULATIONAHA.107.737312.DC1