

**A Rapid ACCE¹ Review of *CYP2C9* and *VKORC1* Allele Testing
to Inform Warfarin Dosing in Adults at Elevated Risk
for Thrombotic Events to Avoid Serious Bleeding**

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¹ Alytic validity, Clinical validity, Clinical utility, and Ethical, legal, and social implications

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Introduction and Background

In a recent CDC-sponsored project (CCU119356-01), we developed and evaluated a model system to assess the availability, quality, and usefulness of existing data on DNA-based tests and testing algorithms. The methodology (referred to as ACCE) was specifically designed to facilitate the appropriate transition of genetic tests from investigational settings to clinical and public health practice. It derives its name from: a) **A**lytic validity, b) **C**linical validity, c) **C**linical utility, and d) identifying **E**thical, legal, and social implications (ELSI). Before starting any given assessment, ACCE requires that the disorder, clinical scenario, and test(s) be clearly defined.

This current project, funded by the American College of Medical Genetics (ACMG), through an award from the ACMG Foundation, uses the ACCE methodology to study *CYP2C9* and *VKORC1* allele testing prior to warfarin dosing. Tm Bioscience provided the ACMG Foundation with partial funding for this study. This review represents the first use of the ACCE process in the area of pharmacogenomic testing. Potential benefits of such testing include improving safety by avoiding adverse reactions to certain medicines, adjusting dosage of medicines, and enhancing efficacy of medicines. A summary of the rapid ACCE methodology for grading study strength and strength of evidence is contained in Appendix F. Responses to questions raised by reviewers are contained in Appendix G.

The clinical disorder that is the focus of this document is uncontrolled bleeding episodes associated with warfarin treatment (including hemorrhagic stroke), that are of sufficient severity to produce serious morbidity and mortality. The clinical scenario focuses on adult candidates for warfarin treatment, as a result of being at high risk for future thrombotic events. The purpose of testing these patients for cytochrome P-450 2C9 (*CYP2C9*) and vitamin K epoxide reductase complex 1 (*VKORC1*) alleles is to identify specific individuals who could receive lower doses of warfarin, as a way to reduce risk of serious bleeding events.

The objectives of this review are to: 1) briefly evaluate and summarize existing knowledge, 2) provide information to aid in developing clinical and laboratory guidelines for *CYP2C9* and *VKORC1* allele testing to guide warfarin dosing, 3) provide information to be used in patient education materials, and 4) identify gaps in knowledge from which a research agenda can be developed.

Justification for performing a Rapid ACCE review at this time includes: the potential for a large number of genetic tests to be performed (hundreds of thousands of new warfarin patients per year), the potential for severe adverse drug events associated with warfarin usage (800 per year in the US), the FDA advisory committee recommendation to include genomic test information in the warfarin label, and the availability of *CYP2C9/VKORC1* testing services.

EXECUTIVE SUMMARY

A Rapid ACCE Review of *CYP2C9* and *VKORC1* Allele Testing to Inform Warfarin Dosing in Adults at Elevated Risk for Thrombotic Events to Avoid Serious Bleeding

Disorder/Clinical Scenario/Test

The main aim of this Rapid ACCE (Analytic validity, Clinical validity, Clinical utility, and Ethical, legal, and social implications) review is to evaluate the efficacy of identifying *CYP2C9* and *VKORC1* alleles prior to warfarin dosing, in order to prevent severe bleeding events. The clinical disorder(s) under consideration in this document are severe bleeding episodes associated with warfarin treatment, such as hemorrhagic stroke, that are of sufficient severity to produce serious morbidity and mortality. These hemorrhagic events are a complication of drug treatment, due to the narrow therapeutic range. The clinical scenario focuses on adult candidates for warfarin treatment, as a result of being at high risk for future thrombotic events. The purpose of testing these patients for cytochrome P-450 2C9 (*CYP2C9*) and vitamin K epoxide reductase complex 1 (*VKORC1*) alleles is to identify specific individuals who could receive lower doses of warfarin, as a way to reduce risk of bleeding events.

Analytic Validity

Nearly all available data for analytic validity refer to two variants in the *CYP2C9* gene; few data are available for the variants in the *VKORC1* gene. Based on seven studies reporting performance in the analytic phase of testing, assays for the common *CYP2C9* genotypes (*1/*2 and *1/*3) have an analytic sensitivity of 100% (95% CI 96.7% to 100%). The analytic specificity is also 100% (95% CI 98.2% to 100%). Based on sparse data for the less common *CYP2C9* genotypes (*2/*2, *2/*3, and *3/*3) the analytic sensitivity of selected assay systems is still 100%, but the confidence interval is wider (95% CI 75% to 100%). No published information is available to directly estimate pre- or post-analytic errors. Depending on the methodology, 1% to 10% of samples may experience repeated assay failures resulting in inconclusive test results. These failures can be viewed as reducing the analytic sensitivity and specificity. Based on other molecular tests in wide use, working estimates of overall analytic sensitivity and specificity for the common *CYP2C9* genotypes are 98% to 99% and 99.5% to 99.75%, respectively. Too few data exist to estimate these rates for *VKORC1* genotyping. Nearly all available data are based on DNA extracted from whole blood samples. Other sample types (e.g., mouthwash) have been mentioned, but data are sparse. Using these estimates for *CYP2C9*, incorrect genotype assignments would be expected to be relatively rare (1 in 50 to 1 in 400) among any genotype group. At least 12 laboratories in the U.S. now offer *CYP2C9* and/or *VKORC1* genotyping for clinical use. Several manufacturers offer reagents to test for variants in both genes.

Clinical Validity

International normalized ratio (INR) values above 3 are more likely among *CYP2C9* heterozygotes (risk ratio of 2.0 or higher), and are more likely to occur in the first and second week (induction phase) after warfarin initiation than in the third week or later. With all non-wild *CYP2C9* genotypes grouped together, the clinical sensitivity of *CYP2C9* to identify serious bleeding events is 46% (95% CI 32% to 60%), indicating that about half of all serious bleeding events occur among *CYP2C9* wild-type individuals. The clinical specificity of *CYP2C9* is 69% (95% CI 62% to 75%). This relatively high false positive rate is because non-wild *CYP2C9* genotypes are relatively common and most will not experience serious bleeding. The relative risk for serious bleeding in wild vs. non-wild individuals is 1.7 (95% CI 0.8 to 3.6). With a bleeding rate of 5%, the positive predictive value (PPV) is estimated to be 7% (odds of 1:14); the negative predictive value (NPV) is estimated to be 96% (odds of 24:1). The prevalence of serious bleeding among populations varies widely (<1% to 17%) depending on many factors (e.g., indication for warfarin, age, comorbidities, definition of serious bleeding and other drug use).

CYP2C9 genotypes are strongly related to warfarin dose at stable INR. Compared with the wild genotype (*1/*1), warfarin dose is reduced by 22%, 36%, 43%, 53% and 76% among individuals with the *1/*2, *1/*3, *2/*2, *2/*3 and *3/*3 genotypes, respectively. Compared with the wild *VKORC1* haplotype (indicated by BB), warfarin dose is reduced by 28% and 50% among individuals with the AB and AA genotypes, respectively. *CYP2C9* genotypes and *VKORC1* haplotypes contribute relatively independent information about stable warfarin dose. *VKORC1* haplotyping explains a slightly higher proportion of overall variability in warfarin dose (23%) than *CYP2C9* genotyping (17%), mainly because the *VKORC1* haplotypes associated with reduced dosage are more common in the Caucasian population. Other important factors in predicting warfarin dose are body weight (9% of variability) and age (7% of variability). Four dosing models have been published, but none perform both the appropriate logarithmic transformation on dose and predict different doses for *1/*2 versus *1/*3.

Clinical Utility

The intended action is to compute an individual's initial warfarin dose by incorporating demographic, clinical, and gene variant data (both *CYP2C9* and *VKORC1*) as a way to limit high INR values (over-anticoagulation) that are associated with serious bleeding events. Many of these events will occur within the first few weeks of treatment. No study has yet shown this intervention to be effective in reducing the incidence of high INR values, the time to stable INR, or the occurrence of serious bleeding events. Several large randomized trials are underway to determine the clinical effectiveness of *CYP2C9* genotyping and *VKORC1* haplotyping to inform warfarin dosing. Using estimates of clinical validity described earlier, along with several assumptions of clinical utility, the number of individuals that must be tested and warfarin-dose adjusted to avoid one serious bleeding event ranges from 48 to 385. The cost per serious bleeding event averted ranges from \$14,500 to \$95,900. Key assumptions that strongly influence this cost estimate are the effectiveness of reduced warfarin dose in avoiding serious bleeding (range 80% to 20% in a sensitivity analysis) and the cost of genetic testing (range \$300 to \$500).

Ethical, Legal, and Social Implications

The Genetic Information Nondiscrimination Act prohibits a group health plan or health insurance issuer from adjusting premiums on the basis of genetic information or requesting or requiring an individual or a family member of such an individual to undergo a genetic test. This act further prohibits employment discrimination on the basis of genetic information. The Health Insurance Portability and Accountability Act of 1996 (HIPAA) (hhs.gov) required the Department of Health and Human Services to develop standards for protecting the privacy of individually identifiable health information from inappropriate use and disclosure. Genetic information is treated as all other "Protected Health Information."

Concerns regarding the use of pharmacogenomic testing include: 1) equitable distribution of the technology, 2) data protection/confidentiality, 3) possibility of discrimination and stigmatization in the workplace, by health and life insurers, and others, 4) creation of "orphan" genotypes, and 5) social consequences of using racial or ethnic categories in clinical care.

Ownership of data and/or samples submitted for clinical testing has not, as of yet, presented any cause for litigation. Legal implications may arise as pharmacogenomic testing becomes widespread. For instance, will providers and drug companies be held liable for not considering genetic information? Should pharmacies store genotype information obtained for one application and use it when dosing other drugs utilizing the same metabolic pathway? Should direct-to-consumer testing be available?

Full Rapid ACCE Report (44 Questions)

DISORDER/TEST/CLINICAL SCENARIO (INTRODUCTION)

1. What is the specific clinical disorder to be studied?

Serious bleeding occurs in up to 5% (or more depending on the definition of serious bleeding and population characteristics discussed in Question 2) of patients treated with warfarin (Coumadin®) due to over-anticoagulation. Serious bleeding events, including hemorrhagic stroke, are the clinical disorders considered in this rapid ACCE review. These complications occur even though there is extensive drug monitoring performed in association with warfarin treatment. Warfarin is commonly administered for preventing thrombotic events in high risk individuals, including those with: 1) atrial fibrillation, 2) prosthetic heart valves, 3) orthopedic surgery procedures (e.g., knee or hip replacement), or 4) a history of spontaneous venous or arterial thrombosis or thrombo-embolism. The target range for warfarin therapy is an International Normalized Ratio (INR) value between 2.0 and 3.0 (slightly lower or higher for some conditions). The risk for serious bleeding increases when the INR values are 4.0 or higher, and such elevations are more likely to occur within the first few weeks of warfarin initiation (see Appendix A for more detail concerning current standard practices). Anticoagulation with warfarin is challenging, due to the narrow therapeutic window, variable response to dosing, and frequent adverse drug events. Some of this variability is due to extrinsic factors, such as other medications or diet. Some is due to intrinsic characteristics of the patient, such as age and weight. More recently, variants in two genes, *CYP2C9* and *VKORC1*, have been demonstrated to account for a significant proportion of the remaining variability in response. This review focuses on only one disorder of interest (serious bleeding). An additional endpoint that may be of interest is the possibility of under-anticoagulation in selected genotypes and the associated reduced effectiveness of warfarin in reducing future thrombotic events.

2. What are the clinical findings defining this disorder?

There appears to be no standard definition of severe bleeding, and there is variation among studies in its definition. Major bleeding has been defined as “overt bleeding that led to the loss of at least 2.0 units in 7 days or less, or was otherwise life-threatening (e.g., intracranial bleeding,)”.¹ Intracranial bleeding is analogous to a hemorrhagic stroke. Bleeding has also been “classified as major if it was intracranial or retroperitoneal, if it led directly to death, or if it resulted in hospitalization or transfusion”.² The risk of major bleeding is related to: 1) the indication for warfarin, 2) intensity of the anticoagulant effect, 3) age of 65 years or older, 4) a history of stroke or gastrointestinal bleeding, 5) co-morbid conditions such as renal insufficiency or anemia, and 6) concomitant use of drugs that impair platelet function, produce gastric erosions, or severely impair synthesis of vitamin K-dependent clotting factors. A recent review reported that four studies have shown higher frequencies of bleeding early in the course of therapy, but not all have supported this observation.²

3. What is the clinical setting in which the test is to be performed?

This Rapid ACCE review aims to examine the evidence on whether testing for variants in the *CYP2C9* and *VKORC1* genes should be used to guide initial warfarin dosing in adults at elevated risk for thrombotic events, as a way to reduce serious drug-related morbidity and mortality (e.g. serious bleeding). This review is not aimed at investigating possible reductions in the effectiveness of warfarin due to under-dosing. Annually, approximately one million individuals in the U.S. are candidates for initial warfarin treatment. This report is, in general, focused on the non-Hispanic Caucasian population; fewer data are available for studying other racial/ethnic

groups. In order to place the possible use of DNA testing in the context of current practice, Appendix A contains the standard practices for warfarin treatment to prevent thrombotic events, including patient indications, setting, initiation of therapy, and monitoring/maintenance activities. Also of interest would be to review the reliability of INR testing, and whether improvements to that testing might lead to increased effectiveness of warfarin and a reduction in adverse drug events. It is also possible that therapeutic drug monitoring (TDM) of coumarin metabolites may have a role to play in reducing adverse drug events.

4. What DNA test(s) are associated with this disorder?

The cytochrome P450 complex is a group of hepatic microsomal enzymes responsible for the oxidative metabolism of various substrates. The *CYP2C9* isoenzyme is primarily responsible for the metabolism of a number of commonly used drugs, including warfarin. Thirty-seven *CYP2C9* haplotypes containing over 100 variants are listed by the Cytochrome P450 (CYP) Allele Nomenclature Committee (www.cypalleles.ki.se/cyp2c9.htm), and the National Center for Biotechnology Information's dbSNP maps 315 variants to the *CYP2C9* gene (www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=1559). The literature focuses on two of these that are associated with reduced gene activity and reduced metabolism of warfarin: *2 (R144C, 3608C>T) and *3 (I359L, 42614A>C). *1 is the designation for the wild type allele. The *2 and *3 variant frequencies are approximately 12.2% and 7.9%, respectively, in the European Caucasian population.³ Warfarin clearance is highly dependent on *CYP2C9* genotype. Individuals with the wild genotype reach a steady state in 3 to 5 days. Heterozygotes for *2 and *3 require 6 to 8 days and 12 to 15 days, respectively.⁴ Three additional variants [*4 or I359T or 42615T>C (Ile359Thr); *5 or D360E or 42619C>G (Asp360Alu); and *6 or 10601delA or 818delA] are sometimes mentioned for inclusion in a testing panel for African Americans or Asian Americans. However, even in these populations, the allele frequencies for *4, *5 and *6 are low.⁵

Variants in the gene encoding vitamin K epoxide reductase complex 1 (*VKORC1*) have also been determined to affect the response to warfarin, independent of *CYP2C9*. The clinically relevant variants (-1639G>A, 1173C>T, 1542G>C, 2255T>C, 3730G>A) in non-Hispanic Caucasians are in strong linkage disequilibrium. There are several conflicting nomenclatures used to refer to these variants. We have chosen to use the haplotype nomenclature used by Rieder *et al.*⁶ in this report. The AA haplotype requires a low warfarin dose; the BB haplotype, a high dose, and the AB haplotype, an intermediate dose. The prevalence of these haplotypes have been estimated using data from several studies⁷⁻¹⁰ using a random effects model. These prevalences among non-Hispanic Caucasians are 35%, 47%, and 18% for the BB, AB, and AA haplotypes, respectively (Table 1). Other studies have reported wide variation of these prevalences by race/ethnicity (Question 22).^{5, 6, 11-15} While *VKORC1* variants are considerably more prevalent than those of *CYP2C9*, they are less understood.

Together, the *VKORC1* and *CYP2C9* variants have been reported to account for one-third to one-half of the variability in warfarin dosing in European Caucasians. The reference (or gold standard) testing method is targeted bi-directional sequencing, but other methodologies are under development, and are likely to be less expensive and more rapid than sequencing. Several manufacturers have included more than the *2 and *3 *CYP2C9* alleles in their standard panel. However, the internal software application can be set to 'read and interpret' only selected assay results (e.g., *2 and *3 only, or *2 through *6). Thus, some manufacturers could accommodate the two most common alleles (or more than two) without actually modifying their assay. This would also allow the same set of reagents to be used in a clinical or a research setting. The National Association of Clinical Biochemists (NACB) Standards and Guidelines

(www.nacb.org/Impg/LMPG-pharmacogenetics.pdf) recommend testing for *CYP2C9* *2 through *6 as part of routine testing for warfarin applications.

Table 1. *CYP2C9* and *VKORC1* common allele designations and associated single nucleotide polymorphisms (SNPs) in European Caucasians

Genotype / Haplotype	Nucleotide Position (2 alleles)	Prevalence (%) ¹
<i>CYP2C9</i>		
*1/*1 (no variant)	wild + wild	63.8
*1/*2	wild + 3608C>T	19.5
*1/*3	wild + 42614A>C	12.6
*2/*2	3608C>T + 3608C>T	1.9
*2/*3	3608C>T + 42614A>C	1.5
*3/*3	42614A>C + 42614A>C	0.6
<i>VKORC1</i>		
BB (no variant)	wild + wild	35
AB	wild + (-1639G>A, 1173C>T, 1542G>C, 2255T>C, 3730G>A ²)	47
AA	2 * (-1639G>A, 1173C>T, 1542G>C, 2255T>C, 3730G>A)	18

¹ In non-Hispanic Caucasians when testing is restricted to the *2 and *3 variants

² These 5 SNPs are in strong linkage disequilibrium. Therefore, we have combined them.

Note: When discussing *CYP2C9* variants in this review, we use the genotype nomenclature shown in Table 1, which refer to the specific SNPs listed. We use the haplotype nomenclature shown in Table 1, for *VKORC1* variants, which refer to multiple SNPs.

Gap in Knowledge: Which variants in *CYP2C9* should be part of a warfarin testing panel? Some occur in selected racial/ethnic groups at low frequency. Most data relate to the two main variants *2 and *3.

5. Are preliminary screening questions employed?

Preliminary screening questions are not employed in the usual sense. In the present clinical scenario, *CYP2C9* and *VKORC1* testing is to be offered only to a select population of adults who are at elevated risk of a thrombotic event (e.g., atrial fibrillation) and who are candidates for treatment with warfarin.

6. Is it a stand-alone test or is it one of a series of tests?

7. If it is part of a series of tests, are all tests performed in all instances (parallel) or are some tests performed only on the basis of other results (series)?

Testing for *CYP2C9* and *VKORC1* variants is expected to be performed in parallel on all individuals who satisfy the initial requirement (i.e. adults with specific indications of high risk for thrombotic events and who will be or are being anti-coagulated with warfarin).

Gap in knowledge: The reliability of INR testing could also be examined to determine differences between laboratories, between point of care testing and clinical laboratory testing, and whether focusing on improving INR testing might be more important or cost-effective than pharmacogenetic testing.

ANALYTIC VALIDITY

Summary:

- Nearly all available data for analytic validity refer to two variants in the *CYP2C9* gene; fewer data are available for the variants in the *VKORC1* gene
- Most available data are based on DNA extracted from whole blood samples. Other sample types (e.g., mouthwash) have been mentioned, but data are sparse.
- At least 12 laboratories in the U.S. now claim to offer *CYP2C9* and/or *VKORC1* genotyping for clinical use.
- Based on seven studies reporting performance in the analytic phase of testing, the common *CYP2C9* genotypes have an
 - analytic sensitivity of 100% (95% CI 96.7% to 100%)
 - analytic specificity of 100% (95% CI 98.2% to 100%)
- Based on sparse data for the less common *CYP2C9* genotype (i.e., *2/*2, *2/*3, *3/*3), the analytic sensitivity of selected assay systems is 100% (95% CI 75% to 100%)
- No information is available to directly estimate pre- or post-analytic errors.
- Based on other molecular tests in wide use, working estimates of overall analytic sensitivity and specificity for the common *CYP2C9* genotypes are 98% to 99% and 99.5% to 99.75%, respectively. Too few data exist to estimate these rates for *VKORC1* genotyping
- Using these estimates for *CYP2C9*, incorrect genotype assignments would be expected to be relatively rare (1 in 50 to 1 in 200) among any genotype group.
- Depending on methodology, 1% to 10% of samples may experience repeated assay failures resulting in inconclusive test results. These failures can be viewed as reducing the analytic sensitivity and specificity

8. Is the test qualitative or quantitative?

Current methodologies employed in testing for single nucleotide polymorphisms (SNPs) that affect warfarin response or disposition in the *CYP2C9* and *VKORC1* genes are qualitative and yield at least three different general categories, as shown in Table 2. Some methods may also fail to provide a valid result (i.e., inconclusive) and is discussed later (Question 16). *CYP2C9* genotypes are associated with warfarin metabolism (pharmacokinetics) with the wild genotype (*1/*1) being the most rapid metabolizer (i.e., a given dose will have the least effect). *VKORC1* genotypes are associated with mRNA expression for the enzyme vitamin K epoxide reductase (pharmacodynamics) with the highest production found in the AA haplotype. Warfarin acts by inhibiting this enzyme.

Table 2. CYP2C9 variants and their relationship to warfarin metabolism and a VKORC1 variant and its relationship to gene expression

CYP2C9		
Genotype	Metabolism	Nomenclature
*1/*1	Extensive, Rapid, Ultra-metabolizer	Normal, Wild
*1/*2	Intermediate	Heterozygote
*1/*3	Poor, Slow	Heterozygote
*2/*3	Poor, Slow	Compound heterozygote
*2/*2	Poor, Slow	Homozygote
*3/*3	Extremely slow	Homozygote

VKORC1 (-1639G>A)		
Haplotype	Enzyme production	Nomenclature
BB	Low (higher warfarin dose)	Normal, Wild
AB	Medium	Heterozygote
AA	High (lower warfarin dose)	Homozygote

9. How often is a test positive when a mutation is present (analytic sensitivity)?

10. How often is the test negative when a mutation is not present (analytic specificity)?

Analytic sensitivity and analytic specificity of CYP2C9 assays

Table 3 summarizes published and unpublished (gray) studies relating to the analytic sensitivity and specificity of CYP2C9 assays. Analytic errors can occur in the pre-analytic, analytic and post-analytic phases. These data relate almost exclusively to the analytic phase when the correct sample is tested under optimal conditions. These studies are unblinded and, therefore, may 'leave out' samples for which one or both of the assays failed, or have run samples repeatedly until a matching genotype was obtained. This might result in an overestimation of analytic performance. The final estimates of analytic sensitivity and specificity also need to account for pre- and post-analytic errors (e.g., sample mix-up, administrative errors). Nearly all of the studies focused on identifying the *1, *2, and *3 alleles, and Table 3 is limited to these. The first four reports all relied on sequencing as the referent method and together examined the performance of four different assay methodologies. No false positive or false negative errors were identified. The next three published reports relied on PCR-RFLP (restriction fragment length polymorphism) as the referent method, examined one additional methodology, and provided supplementary information on another. Again, no errors were identified. Studies that did not use sequencing or PCR-RFLP as a referent method were not included.¹⁶

We combined these seven studies to provide an estimate of analytic sensitivity and specificity for the analytic phase of testing. Overall, 109 of 109 samples with a *2 and/or *3 allele were correctly identified, for an analytic sensitivity of 100% (95 percent confidence interval 96.7% to 100%). However, only 13 observations are available for the three less common genotypes (*2/*2, *3/*3 and *2/*3) resulting in a much wider confidence interval around the analytic sensitivity estimate of 100% (95 percent confidence interval 75% to 100%). As expected, a larger number of samples was reported with the *1/*1 genotype and, therefore, the analytic specificity will be more reliably estimated. A total of 203 of 203 samples had correct results, leading to an analytic specificity of 100% (95 percent confidence interval 98.2% to 100%). We were unable to

find any literature regarding the analytic sensitivity for *CYP2C9* alleles other than *2 and *3. Some might consider assay failure equivalent to reduced analytic performance, as individuals with no test result will likely be treated with warfarin as though they possessed a *1/*1 (wild) genotype (Question 16).

The following sections briefly review each of the seven studies used in the analysis shown in the top half of Table 3. All use DNA extracted from blood (unless otherwise specified) and all test only for the *2 and *3 variants (unless otherwise specified).

- Hillman M. *et al.* reported the results of a retrospective cohort study of 453 mainly Caucasian patients managed by an anticoagulation service.¹⁷ As part of the report of 2C9 genotyping of these individuals using LightCycler technology, they state that 10 of the patient samples were sequenced to confirm the assay results. Although the actual number of each genotype was not reported, the 10 samples included the following genotypes: *1/*1, *1/*2, *2/*2, *2/*3 and *3/*3. Only the *1/*3 genotype was not represented. Study strength: marginal, due to no sample blinding, convenience sample, limited to analytic phase and small sample size.
- Pickering J. *et al.* performed a method comparison for 2C9 and 2C19 genotyping in 101 whole genome amplified DNA samples (obtained as part of a clinical service in the U.S.) using three methodologies: 1) Luminex SNP genotyping assay, 2) eSensor SNP assay from Motorola Life Sciences and 3) bidirectional sequencing.¹⁸ Study strength: marginal, due to no sample blinding, convenience sample and limited to analytic phase.
- Wen S. *et al.* analyzed *CYP2C9* genotypes in 62 patients with high blood pressure in China using a oligonucleotide microarray.¹⁹ A subset of seven *1/*3 heterozygotes and 13 *1/*1 homozygotes were then sequenced. No other genotypes were identified in this population. Study strength: marginal, due to no sample blinding, convenience sample, limited to analytic phase, small sample size and limited to *3 alleles.
- Zainuddin A. *et al.* used an in-house nested allele-specific multiplex polymerase chain reaction to identify *CYP2C9* genotypes in 40 unrelated healthy Malaysian Indian volunteers.²⁰ All samples were also sequenced. Study strength: marginal, due to no sample blinding, convenience sample, limited to analytic phase, and small sample size.
- Eriksson S. *et al.* tested samples already genotyped via PCR-RFLP in 28 individuals in Sweden using Pyrosequencing technology.²¹ The authors reported the allele frequencies and some accompanying information, allowing us to derive the probable genotype information shown in Table 3. Study strength: marginal, due to no sample blinding, convenience sample, limited to analytic phase, no gold standard and small sample size.
- Aquilante C. *et al.* collected mouthwash samples from 253 subjects in Florida and compared genotypes derived from Pyrosequencing technology with PCR-RFLP.²² The study was aimed at comparing the costs and time requirements. Individual genotypes were not reported, but the authors report “the concordance between Pyrosequencing and RFLP genotype determinations was 100%”. However, not all 253 subjects received a genotype, due to failure of one (or both) of the assays. Study strength: marginal, due to no sample blinding, convenience sample, limited to analytic phase, no gold standard and individual genotypes not reported.
- Burian M. *et al.* obtained blood sample DNA from 118 unrelated German Caucasians and used both real-time fluorescence PCR (LightCycler) and PCR-RFLP to test for *CYP2C9*

variants.²³ The authors reported allele frequencies and additional information enabling the assignment of likely genotypes. One sample with a *3/*3 genotype was obtained from another laboratory and not subjected to referent testing. Study strength: marginal, due to no sample blinding, convenience sample, limited to analytic phase, no gold standard and individual genotypes not reported.

The following data were not peer reviewed.

- Third Wave performed a method comparison for *CYP2C9* *2 and *3 and *VKORC1* *1 and *2 genotyping in 100 samples of varied ethnicity using Invader chemistry and sequencing (in press). In addition, genotypes were generated using Invader chemistry on 22 Coriell samples and compared to sequencing and reference genotypes contained in the CDC's Genetic Testing Quality Control Material Program (GTQC) resource distributed by Coriell Cell Repositories (http://www.phppo.cdc.gov/dls/genetics/qcmaterials/pdf/CYP2C9_VKORC1.pdf). The 22 samples were selected to provide representation of each possible genotype combination for the 3 alleles. An additional 12 patient samples were analyzed and genotype assignment compared between Invader chemistry, Pyrosequencing, and GTQC.
- ARUP analyzed 31 Coriell samples and compared reference genotypes provided by GTQC for *CYP2C9* *2 and *3, and *VKORC1* *1 and *2, with genotypes generated by Invader chemistry.
- LabCorp performed a series of analyses using the same 22 Coriell samples as Third Wave to provide an indication of inter-laboratory performance. A method comparison was completed using Invader chemistry, sequencing and PCR-RFLP.

Overall strength of evidence for the analytic phase of *CYP2C9* testing is low (multiple homogeneous) marginal studies.

Several other published studies provide *CYP2C9* genotyping information, but do not include a referent method and, therefore, are not included in the analysis of analytic sensitivity/specificity. The report by Hruska and his colleagues used the Pyrosequencing method for genotyping *CYP2C9* for not only the *2 and *3 alleles, but also for *4, *5 and *6.²⁴ Among 50 volunteers in Florida, the allele frequencies for *2 and *3 were 9% (close to expectation), but no instances of the *4, *5 or *6 were found. This latter finding is likely due to the small sample size and to the majority of volunteers being Caucasian (the *4 through *6 alleles are found mainly in African Americans and Asian Americans). In addition, the authors report successful use of DNA from blood, urine, buccal swabs and mouthwash rinses.

One report provides evidence that referent methodologies may have difficulty in identifying certain variants. A novel variant allele led to erroneous assigning of genotype *3/*3 by the PCR-RFLP method.²⁵

Gap in Knowledge: There are few data for homozygote and compound heterozygote *CYP2C9* genotypes (2*/2*, 3*/3*, 2*/3*) and for non-Caucasian alleles. Study design and methodologies for analytic validity reports need to be agreed to and then followed.

Analytic sensitivity and analytic specificity of *VKORC1* assays

No published data exist to estimate analytic sensitivity/specificity for *VKORC1* assays. However, three laboratories/manufacturers were able to provide some information collected in-house concerning both *CYP2C9* and *VKORC1*. All relate to the Invader assay (Third Wave Technologies). These are contained in the bottom of Table 3. Additional data on analytic validity for *VKORC1* genotyping against a 'gold standard' referent method may exist once the data on the public databases (PharmGKB, Sabta Cruz, Japanese Gnome project and BCBI) are available.

Gap in Knowledge: There are no published peer-reviewed data concerning analytic specificity/sensitivity for *VKORC1* genotyping against a 'gold standard' referent method.

Table 3. Analytic validity of CYP2C9 (restricted to the *2 and *3 variants) and VKORC1 testing

Reference	Year	Assay Method	Referent Method	CYP2C9					Analytic Specificity (*1, *1)
				Analytic Sensitivity (test result/referent result)					
				(*1, *2)	(*2, *2)	(*1, *3)	(*3, *3)	(*2, *3)	
Hillman M. <i>et al.</i>	2004	LightCycler	Sequencing	2/2	1/1	-	1/1	1/1	4/ 4
Pickering J. <i>et al.</i>	2004	Luminex, eSensor	Sequencing	15/15	1/1	13/13	-	2/2	70/ 70
Wen S. <i>et al.</i>	2003	Microarray	Sequencing	-	-	7/7	-	-	13/ 13
Zainuddin A. <i>et al.</i>	2003	Nested PCR	Sequencing	3/3	-	5/5	2/2	2/2	28/ 28
Eriksson S. <i>et al.</i>	2002	Pyrosequencing	PCR-RFLP	9/9	-	5/5	-	-	9/ 9
Aquilante C. <i>et al.</i>	2004	Pyrosequencing	PCR-RFLP	-	-	-	-	-	-
Burian M. <i>et al.</i>	2002	LightCycler	PCR-RFLP	27/27	1/1	10/10	1/1	1/1	79/ 79
			Total	56/56	3/3	40/40	4/4	6/6	203/203

The following data were not subject to peer review

Third Wave Tech	2006	Invader, Tag-It, Pyrosequencing	Sequencing	9/9	3/3	6/6	2/2	6/6	9/9
ARUP Laboratory LabCorp	2006	Invader, Tag-It	Sequencing	9/9	-	1/1	-	-	21/21
	2006	Invader, Tag-It	PCR-RFLP	6/6	1/1	5/5	1/1	4/4	5/5

VKORC1 (Haplotype)

				(AB)	(AA)	(BB)
Third Wave Tech	2006	Invader, Pyrosequencing	Sequencing	16/16	12/12	7/7
ARUP	2006	Invader	Sequencing	10/10	4/4	17/17
LabCorp	2006	Invader	PCR-RFLP, Sequencing	10/10	5/5	7/7

11. Is an internal quality control program defined and externally monitored?

Federal regulations require that all laboratories offering *CYP2C9* and/or *VKORC1* allele testing (a high complexity test) for clinical purposes be CLIA certified. Although this is a minimum qualification, it does ensure that the laboratory meets specifications for a high-complexity test and is externally reviewed at least every two years. A more comprehensive review is required for New York State certification (although for rare tests, an exemption can be obtained). Part of laboratory certification is to have a well-documented and active internal quality control/quality assessment program consisting of the blind insertion of samples of known abnormal and normal sequences into the routine laboratory workload (positive and negative controls). Additionally, all laboratories would be required to have internally validated their assay for clinical use. These data are usually not available for review outside the laboratory, but would provide some laboratory-specific information about analytic validity. An external review might be done by the state in which the laboratory resides, the College of American Pathologists (CAP) or another organization such as Joint Commission on Accreditation of Healthcare Organizations (JCAHO). Using Google (search term “laboratory, 2C9”), we were able to identify the laboratories in Table 4 that claim to offer clinical testing for the *CYP2C9* gene (some also offer *VKORC1*) testing in the U.S. (checked August 30, 2006). This list is likely to be incomplete, and we have not verified that clinical testing is actually available.

Table 4. Laboratories that offer clinical testing in the U.S. for *CYP2C9* and/or *VKORC1* alleles.

PG _{XL} Laboratories	www.pgxl.com
ARUP Laboratories	www.aruplab.com/guides/clt/tests/clt_a211.jsp
LabCorp	www.labcorp.com/pdf/Cytochrome_P450_2C9_LabCapsule.pdf
Genomas	www.genomas.net/lph/professional/test.html
Cincinnati Children’s Hospital	www.cincinnatichildrens.org/svc/alpha/g/gps/choose.htm
Mayo Medical Laboratories	www.mayoreferenceservices.org/mrs/molecular/genetics.asp
OKU Molecular Pathology Lab	w3.uokhsc.edu/pathology/DeptLabs/Mole_Path/CYP2C9.htm
Specialty Laboratories	www.specialtylabs.com/
Esoterix Laboratory Services	www.esoterix.com/index.asp
Molecular Diagnostics Laboratories	www.mdl-labs.com/warfarin.htm
Genelex	www.healthanddna.com
Kimball Genetics	http://www.kimballgenetics.com/

If any laboratory were to test samples using sequence analysis, it is recommended by the Clinical and Laboratory Standards Institute (CLSI) that a quality score be determined for each sequence run (MM9-A). These scores could be used as a quality control measure.

Gap in Knowledge: Little information is available for laboratories currently offering *CYP2C9* and/or *VKORC1* genotyping. This includes information about sample types accepted, alleles tested, internal test validation results, turnaround time, laboratory experience, numbers of samples already tested, possible linkage with clinicians experienced in interpreting pharmacogenetic testing and content of reports.

12. Have repeated measurements been made on specimens?

Repeated DNA measurements in multiple assay runs offer the opportunity for the laboratory to assess consistency of performance and the impact on testing of external factors (e.g., quality of the sample, exposure to high temperatures). As part of some analytic validity studies, repeated measurements have been successfully made on a limited number of samples.²⁶ However,

these were short-term studies of only a few samples (5 *1/*1 samples in triplicate, 5 *1/*2 in triplicate and 1 *2/*2 with 15 replicates). These limited data do not rule out problems with longer-term repeatability, as reagents and equipment age and new reagent lots become available.

Gap in Knowledge: There are limited existing data on replicates to indicate whether the assay systems to be used are robust. Little data are available about the long-term consistency of results within a single laboratory and method.

13. What is the within- and between-laboratory precision?

This question is not applicable to the use of DNA testing for *CYP2C9* and/or *VKORC1* alleles, since such testing is qualitative. However, comparisons between/among quality scores in sequencing runs (Question 11) could provide a measure of precision. These data are not available in the literature.

Gap in Knowledge: Little data are available about consistency of results between laboratories, either within- or between-methods. An external proficiency testing program such as one proposed by the College of American Pathologists would provide some of the data needed to assess between laboratory variability and refine estimates of analytic sensitivity and specificity for *CYP2C9* and *VKORC1*.

14. If appropriate, how is confirmatory testing performed to resolve false positives in a timely manner?

Confirmatory testing (or additional follow-up testing) most often occurs when the test result is unanticipated, or might be due to a false positive test result. One way of determining the importance of confirmatory testing is to estimate the analytic positive predictive value (aPPV) and negative predictive value (aNPV). Assume the allele frequencies of *CYP2C9* to be 0.799, 0.122 and 0.079 for *1, *2 and *3, respectively.³ Then, assume analytic false negative rates of 1% and 2% (corresponding to overall analytic sensitivities of 99% and 98%) and analytic false positive rates of 0.5% and 0.25% (corresponding to overall analytic specificities of 99.5% and 99.75%). Table 5 shows the four main categories of errors, how many might occur in a hypothetical population of 1,000,000 individuals tested, the error rates, and the proportion of the population that would need to be tested to potentially identify the error. For example, the first pair of rows shows the impact of false negative *2 or *3 test among heterozygotes (incorrectly identifying these as *1/*1, or wild type). Among the 1,000,000 individuals tested, a total of 3,212 (or 6,424) errors of this type might occur, depending on the false negative rates of 1% (or 2%). Among the population of individuals assigned the wild genotype, these errors would occur once in every 400 (or 200) results. In order to have an opportunity to identify these errors, one would need to retest all individuals with a *1/*1 genotype, approximately 64% of the population (640,000 of the 1,000,000). The table shows that errors of either type are generally uncommon within any of the assigned genotypes. The likely modification in treatment needs also to be taken into account when deciding whether routine repeat testing is warranted. Other than repeating all testing, the only group of errors that might be approachable by repeat testing would be all homozygote/compound heterozygote results. They are relatively rare results (4% or all test results), and as many as 1% or more may be incorrectly classified. There do not appear to be compelling reasons for confirmatory testing, as long as the analytic sensitivity is 98% or higher and the analytic specificity is 99.5% or higher. These rates of analytic performance need to be monitored in actual clinical practice to determine whether they can be maintained.

Gap in Knowledge: Since reliable estimates of overall analytic sensitivity and specificity are not yet available, the need for confirmatory testing cannot be confidently estimated. In addition, we have assumed that both false positive and false negative results occur randomly by allele, and this may not be true. Additional information on types of error, their frequency and their causes would help clarify the possible need for confirmatory testing among one, or more, *CYP2C9* and/or *VKORC1* genotypes.

Table 5. Analytic positive and negative predictive values.

Group tested	FN Rate	FN Number	Proportion	Number to test
<u>Analytic false negatives</u>				
*1/*2 and *1/*3	1%	3,212	1 in 200	64%
incorrectly called	2%	6,424	1 in 100	64%
*1/*1				
*2/*2, *2/*3, *3/*3	1%	404	1 in 100	32%
incorrectly called	2%	808	1 in 50	32%
*1/*2 or *1/*3				
	FP Rate	FP Number	Proportion	Number to test
<u>Analytic false positives</u>				
*1/*1	0.25%	1,596	1 in 200	32%
incorrectly called	0.50%	3,192	1 in 100	32%
*1/*2 or *1/*3				
*1/*2 or *1/*3	0.25%	401	1 in 100	4%
incorrectly called	0.50%	802	1 in 50	4%
*2/*2, *2/*3 or *3/*3				

FN – False Negative, FP – False Positive

It is also important to remember that clinicians will informally assign individuals a warfarin ‘phenotype’, based on early INR values. When this impression clashes with the assigned genotype, additional testing might be ordered. For example, a *CYP2C9* *1/*1 (wild) individual whose early INR values are relatively high, might be tested for additional alleles if initial testing only included *2 and *3. If a mistake had been made, it is possible that such follow-up testing might identify an error.

15. What range of patient specimens has been tested?

Whole blood is often used to obtain DNA from patients for clinical testing. Alternative sample types include saliva, buccal scrapings, mouthwash samples and urine.²⁴ Some argue that purified DNA from blood samples is the best sample type, as the likelihood of analytic errors is reduced. Others argue that alternative samples that are not associated with blood borne pathogens have important collection advantages, and that their use will still result in acceptable analytic performance. Each laboratory would need to use established Standards and Guidelines (e.g., those available from ACMG) to validate its chosen methodology on the sample type(s) deemed by that laboratory to be acceptable for submission. Understanding the potential impact of additional substances that may be present in the samples of this particular patient population

may be a consideration. For example, candidates for warfarin therapy are often treated with heparin-containing medications.

16. How often does the test fail to give a useable result?

The failure rate of direct sequencing for laboratory testing of satisfactory specimens is very low (less than 0.1% - 1 per 1000). Poor sample quality (e.g. obvious contamination or hemolysis, exposure of sample to extreme temperature, or delay in transit) or insufficient sample quantity may result in test failure. It is relatively common for a PCR reaction to fail. However, another DNA sample can easily be obtained from the original specimen for repeat testing.

The failure rate for assays developed for *CYP2C9* and/or *VKORC1* allele testing is higher than for sequencing. TaqMan® Drug Metabolism Genotyping Assays (Applied Biosystems, Foster City, CA) was evaluated in a research setting, using about 320 samples. Two samples failed to give a result at one SNP in *VKORC1*, and 10 samples failed to give a result for another SNP (personal communication, C. Sutcliffe, Vanderbilt University School of Medicine, Nashville, TN). These same 320 samples were analyzed by oligoligation assay for *CYP2C9* SNPs. Approximately 10 to 15% failed to give a result (personal communication, C. Ingram, Vanderbilt University School of Medicine, Nashville, TN). These assay failure rates may be higher in research settings than in clinical settings, due to handling of samples (e.g. frozen vs fresh).

Another study reported failure rates for the Pyrosequencing methodology.²² Among 253 mouthwash samples collected, Pyrosequencing was able to determine the *2 allele status in 212 (84%) on the first pass. After a second pass, the *2 allele status was assigned to 243 (96%). The *3 allele status was successful on the first pass in 236 (93%) and on the second pass for 251 (99%). Using DNA extracted from blood may increase this success rate. It is also likely that the failure to assign a genotype will be method-specific and/or sample type specific. As part of an ongoing intervention trial (COUMA-GEN see Question 33), 150 saliva samples have been tested using a DNA melting curve technology (Idaho Technologies) for both *CYP2C9* and *VKORC1* genotypes, with no failures (personal communication, M. Williams, Intermountain Healthcare, Salt Lake City, UT).

Gap in Knowledge: The rates of failure to assign a *CYP2C9* genotype may be as high as 5% for some methodologies/sample types. Documenting the failure rates in routine practice by method will be helpful in refining estimates of analytic validity, clinical validity and costs.

17. How similar are results obtained in multiple laboratories using the same, or different, technology?

The Genetic Testing Quality Control Materials Program at the Centers for Disease Control and Prevention assists genetic testing laboratories in obtaining validated quality control materials. As part of this program, 96 samples from Coriell were genotyped for *CYP2C9* and *VKORC1* variants (www.phppo.cdc.gov/dls/genetics/qcmaterials/pdf/CYP2C9_VKORC1.pdf). Two laboratories used the Tag-It (TM Bioscience) methodology to analyze the *CYP2C9* gene, and both identified the same genotypes in all samples. Two other laboratories sequenced the *VKORC1* gene, and both identified the same genotypes in all samples.

The College of American Pathologists (CAP) has established a working group consisting of members from the CAP/ACMG Biochemical and Molecular Genetics, Special Chemistry, Toxicology and Coagulation Committees to develop a Pharmacogenomics Survey for 2007. The group has tentatively identified the following genes to be included in the first year: *CYP2C19*, *CYP2C9*, *CYP2D6*, *UGT1A1*, and *VKORC1* (Camille Murray, Marketing, CAP, personal com-

munication). As of November 2006, no materials are available for laboratories to sign up for this proficiency testing program (see www.cap.org for updates).

A quality assessment of SNP genotyping laboratories in Scandinavia provides some information that indirectly relates to *CYP2C9* genotyping.²⁷ Eleven experienced SNP genotyping laboratories were provided with 47 DNA samples and asked to test for 18 (or 6) randomly assigned SNPs. Overall, performance was rated 'excellent', but the organizers conclude that special attention should be paid to the quality of the DNA samples prior to genotyping. Degraded samples would have more often resulted in incorrect assignments, had the laboratories not taken a conservative approach to making an assignment. Higher accuracy may result in a higher 'no call' rate.

Gap in Knowledge: No external proficiency program is yet available for laboratories offering pharmacogenetic testing. A proposed program by the College of American Pathologists will provide useful information regarding the analytic validity of *CYP2C9* and *VKORC1* testing in clinical laboratories.

CLINICAL VALIDITY

Summary

- INR values above 3 are more likely among *CYP2C9* heterozygotes (risk ratio of 2.0 or higher), and are more likely in the first and second week (induction phase) after initiation than the third week or later.
- With all variant *CYP2C9* genotypes grouped together
 - Clinical sensitivity of *CYP2C9* to identify serious bleeding events is 46% (95% CI 32% to 60%), indicating that half of the serious bleeding occurs among wild-type individuals.
 - Clinical specificity of *CYP2C9* is 69% (95% CI 62% to 75%), indicating that non-wild *CYP2C9* genotypes are relatively common.
 - Relative risk for serious bleeding is 1.7 (95% CI 0.8 to 3.6).
 - Positive predictive value (PPV) is estimated to be 7%; the NPV is 96%.
- The prevalence of serious bleeding among populations varies widely (<1% to 17%) depending on many factors (e.g., indication for warfarin, age, comorbidities, definition of serious bleeding and other drug use). We use a rate of 5% for modeling
- *CYP2C9* genotypes are strongly related to warfarin dose at stable INR
 - among *1/*2 individuals; 22% reduction (compared with *1/*1)
 - among *1/*3 individuals; 36% reduction
 - among *2/*2 individuals; 43% reduction
 - among *2/*3 individuals; 53% reduction
 - among *3/*3 individuals; 76% reduction
- Time to steady state warfarin levels varies by genotype
 - 3 to 5 days for *1/*1
 - 5 to 8 days for *1/*2
 - 12 to 15 days for *1/*3
- *VKORC1* haplotypes are strongly related to warfarin dose at stable INR
 - among AB individuals; 28% reduction (compared with those with no variants, BB)
 - among AA individuals; 50% reduction
- *CYP2C9* and *VKORC1* genotypes contribute independent information to the warfarin dose and compared to the most common combination (*1/*1 and AB), individuals with other genotype combination (e.g., *1/*1 and BB) will need more than the usual dose, while others would require less (e.g., *1/*3 and AA).
- *VKORC1* haplotyping explains a slightly higher proportion of overall variability in warfarin dose (23%) than *CYP2C9* genotyping (17%), mainly because the *VKORC1* haplotypes associated with reduced dosage requirements are more common. Additional factors include body weight (9% of variability) and age (7%).
- Models that predict warfarin dose should consider the following characteristics
 - Use the logarithm of the warfarin dose (not warfarin dose) as the dependent variable
 - Allow different dosages for *CYP2C9* genotypes *1/*2 and *1/*3
 - Include other important factors such age, body weight (or height, BMI, BSA)

18. How often is the test positive when the disorder is present?

19. How often is the test negative when the disorder is not present?

Steady state warfarin dose is a surrogate end point for severe bleeding. However, severe bleeding does occur when the INR is in the target range. For this reason, warfarin dose at steady state stratified by genotype is discussed in Question 24 (genotype/phenotype relationships).

CYP2C9 genotypes and INR levels above 3.0 during the first 3 weeks of warfarin treatment (induction phase)

Three studies address the relationship between *CYP2C9* genotyping and INR level during the induction phase of warfarin treatment.²⁸⁻³⁰ One of these studies looked at the rate of change in the INR values (slope) during induction and found weak evidence that the non-wild genotypes had higher slopes ($p = 0.05$).³⁰ The two other studies followed the INR values over the first three weeks of warfarin treatment and performed genotyping retrospectively.^{28, 29} Both defined a 'high' INR that might be associated with severe bleeding as > 3.0 . Using the $*1/*1$ genotype as the referent category, the results are shown in Table 6. Lindh and colleagues see a clear change over time, as the dose is reduced due to higher INR levels in non-wild individuals. Peyvandi and colleagues also saw the same effect over time (not shown in Table 6), but they quantified the effect by looking at mean daily dose of warfarin.

Table 6. Proportion of INR values above 3.0 during warfarin induction, stratified by *CYP2C9* genotype

	Week	Lindh, 2005	Peyvandi 2004	All
Relative risk ($*2$ vs. $*1/*1$)	1	2.8 (1.2 – 6.7)		
	2	2.1 (1.2 – 3.7)	2.0 (1.4 – 2.7)	2.0 (1.4 – 2.7)
	3	1.0 (0.5 – 1.8)		
Relative risk ($*3$ vs. $*1/*1$)	1	5.4 (2.5 – 12)		
	2	3.5 (2.1 – 5.8)	2.0 (1.3 – 3.1)	2.4 (1.8 – 3.6)
	3	1.1 (0.6 – 2.0)		

$*2$ includes $*1/*2$ and $*2/*2$ $*3$ includes $*1/*3$, $*3/*3$ and $*2/*3$

Importantly, both groups only see differences in required dose (due to higher INR levels) beginning at about 4 days post initiation. Figure 1 is taken from Peyvandi *et al.* and shows the average warfarin dose in the three *CYP2C9* genotype groups. The three groups ($*1/*1$ = diamonds, $*2/any$ = square and $*3/any$ = open circles) do not differ significantly in mean warfarin dose (y-axis) until the fourth day. After the first week, the difference in average dose is clear, with the $*2/any$ and $*3/any$ having significantly lower warfarin dose to maintain INR.

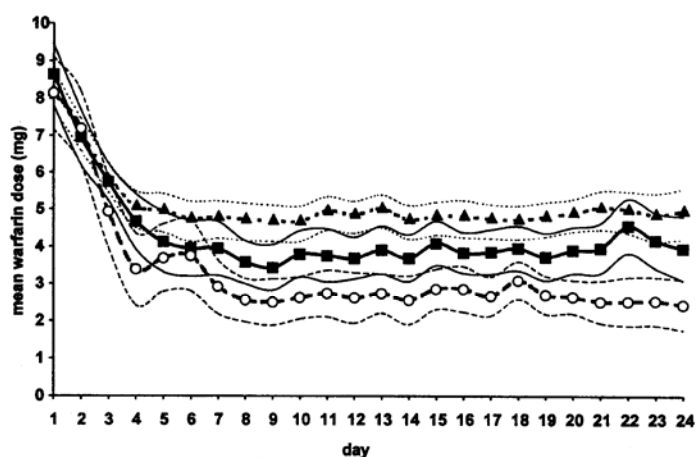


Fig 1. Changes in daily doses of warfarin required by carriers of wild-type alleles ($2C9*1/*1$) (with mean values indicated by triangles and 95% confidence intervals by dotted lines) and carriers of at least one $2C9*2$ allele (indicated by squares and solid lines) and one $2C9*3$ allele (indicated by circles and dashed lines) during the first 24 days of therapy (induction phase).

Figure 1

CYP2C9 genotypes and severe bleeding

There is limited evidence on the relationship between the *CYP2C9* genotype and major bleeding as a result of anticoagulation therapy. Clinical sensitivity is defined as the proportion of individuals with severe bleeding that has a genotype other than wild (*i.e.*, $*1/*2$, $*2/*2$, $*2/*3$, $*1/*3$,

*3/*3). This is synonymous with the detection rate. Clinical specificity is defined as the proportion of individuals with no severe bleeding that has the wild (*1/*1) genotype. One minus the clinical specificity is the false positive rate. The false positive rate indicates the proportion of individuals that has a non-wild genotype, but does not have a bleeding event.

Four studies examined the relationship between bleeding complications and *CYP2C9* genotypes. However, only two classified the bleeding events as serious and/or life-threatening and assessed all genotypes.^{31, 32} All studies are included in Table 7 and summarized later in this section.

Table 7. Clinical sensitivity, clinical specificity, relative risk, and attributable risk for severe bleeding events (wild vs. non-wild *CYP2C9* genotype)

Study	Clinical Sensitivity (%)	Clinical Specificity (%)	Relative Risk (%)	Attributable Risk (%)
Ogg <i>et al.</i> ³³ ^a	23	87	1.85	7
Margaglione <i>et al.</i> ³⁴ ^b	67	53	1.91	12
Higashi <i>et al.</i> ³¹	50	72	2.19	15
Wadelius <i>et al.</i> ³²	33	66	0.96	0
Summary (95% CI)	46 (32-60)	69 (62-75)	1.7 (0.8-3.6)	7 (0-15)

^a Considered only *3 genotypes and is not included in the summary line

^b Wild *CYP2C9* genotype frequency is much lower than others (estimates not included in summary)

The summary estimates for the studies by Higashi *et al.* and Wadelius *et al.* were combined in a random effects model using Comprehensive Meta-Analysis software (Englewood, NJ). The study by Ogg *et al.* was not included in the summary because it considered only *3 genotypes, while the study by Margaglione and colleagues was omitted because the wild *CYP2C9* genotype found in Italy is much lower than that found in the other studies (49% versus 69% and 67%). The clinical sensitivity of severe bleeding events among individuals with a non-wild *CYP2C9* genotype is 46% (95% CI 32% to 60%). Clinical sensitivity can be interpreted as the proportion of all serious bleeding events that occurs among the 'non-wild' genotypes of *CYP2C9*. The clinical specificity of no bleeding events among individuals with the wild genotype is 69% (95% CI 62% to 75%). Because these studies are heterogeneous, these results should be interpreted with caution, and the strength of evidence for these study estimates is low.

A more common approach to examining the relationship between a test and outcome is the relative risk (or risk ratio). The combined relative risk for bleeding events among those with a non-wild *CYP2C9* genotype compared with those with the wild genotype is 1.7 (95% CI 0.8, 3.6), using the bottom two studies from Table 7. In other words, the average risk of severe bleeding among individuals with a non-wild genotype is 1.7 times higher than the risk of severe bleeding among individuals with the wild *CYP2C9* genotype (*1/*1). However, the confidence interval includes 1.0 and, thus, is not statistically significant.

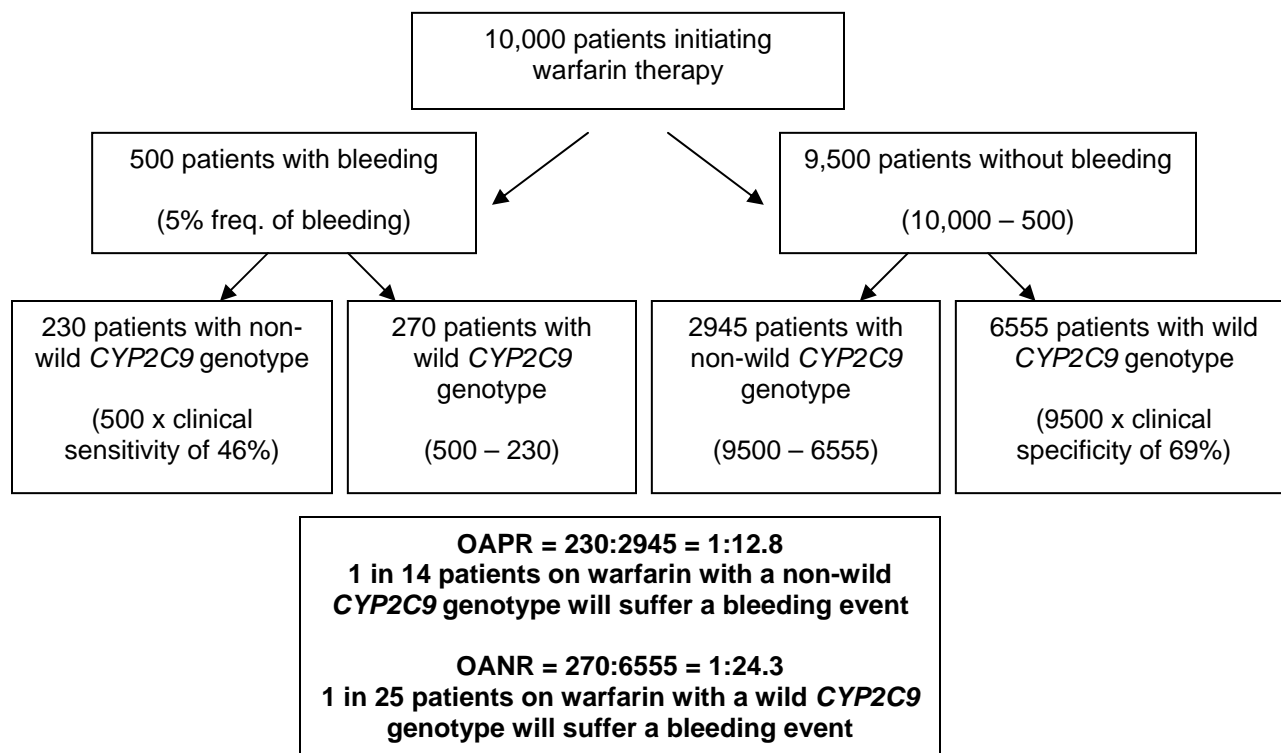
Attributable risk for bleeding events among those with a non-wild *CYP2C9* genotype (compared with the wild genotype) is 7% (again, using the bottom two studies summarized in Table 7). The attributable risk expresses the proportion of the bleeding events that is attributable to the *CYP2C9* genotype; about 7% of bleeding events among those with a non-wild genotype are directly related to that genotype. While estimates vary widely among the studies, they are not de-

terminated to be heterogeneous ($p=0.07$). Some of the variation is likely to be due to the length of follow-up, definition of bleeding events, and indication for warfarin.

Below is a brief summary of the four studies that contain clinical sensitivity and specificity data.

- Ogg *et al.* examined the effect of the *CYP2C9**3 variant in 233 men at high risk for ischemic heart disease.³³ Bleeding episodes were not limited to those described as severe. During the first 3 months of follow-up, 17 bleeding events occurred among 210 men with the wild genotype, and 5 bleeding events occurred among 32 men with a *1/*3 or *3*3 genotype. Strength of study: marginal, due to no definition of what constitutes a bleeding episode, only *3 alleles were identified.
- Margaglione *et al.* analyzed an Italian cohort of 180 patients (100 males) who were pre-scribed warfarin.³⁴ The indication for treatment for the majority of patients ($n=141$) was venous thromboembolism. Arterial disease was the indication in 25 patients; all remaining indications were collapsed into “other”. Follow-up time was at least 75 days. 59 bleeding episodes occurred in 36 patients. 10 events were classified as major, but it was not possible to categorize these events by genotype. 12 bleeding events occurred among 88 patients with the wild genotype, and 24 bleeding events occurred among 92 patients with a non-wild genotype. Strength of study: marginal, due to inability to differentiate major bleeding events by genotype.
- Higashi *et al.* recruited a U.S. cohort of 185 anticoagulation clinic patients (96% Caucasian, 64% male).³¹ The indications for warfarin were atrial fibrillation (51%), deep vein thrombosis/pulmonary embolism (DVT/PE) (22%), dilated cardiomyopathy (20%), and valve replacement (7%). Follow-up time ranged from 14 days to 11 years (median = 422 days). 28 serious and 4 life-threatening bleeding events occurred, and were combined for this analysis. 16 events occurred among the 127 patients with the wild genotype, and 16 events occurred among the 58 patients with a non-wild genotype. Strength of study: good.
- Wadelius *et al.* recruited a predominantly Caucasian cohort of 201 patients from an anti-coagulation clinic in Sweden.³² Indications for warfarin were atrial fibrillation (56%), valve replacement (24%), DVT/PE (4%), cardiomyopathy (4%), trans-ischemic attack (2%), and not given (10%). Follow-up time ranged from 2.4 months to 26 years (median = 2 years). Among these patients, 12 had a severe bleeding event. Another 24 patients with serious warfarin-related bleeding were recruited through the Swedish reporting of adverse drug reactions ($n=20$) and an ongoing study on cerebral bleeding and warfarin ($n=4$). It was not possible to ascertain the genotypes of the 12 patients from the original cohort. To adjust for the additional patients with bleeding events who were recruited, we assumed that genotype was consistent among the original 12 patients and the 24 additional patients with bleeding events ($12 \times 30\%$ [clinical sensitivity] = 4 patients with bleeding events and non-wild genotype). Thus, for the relative and attributable risk calculations, we used 4 bleeding events among the 69 patients with a non-wild genotype and 8 bleeding events among the 132 patients with the wild genotype. Strength of study: adequate, due to inability to identify genotypes for the 12 patients with a severe bleeding event who were part of the original cohort.

Figure 2 represents the relationships among the detection rate (clinical sensitivity), the false positive rate (1-specificity), and the prevalence of bleeding events among those on warfarin therapy (Question 21) in a hypothetical cohort of 10,000 patients. These data show that the odds of being affected (having a bleeding event) given a positive result (having a non-wild *CYP2C9* genotype) is nearly twice the odds of being affected given a negative result (1 in 14 versus 1 in 25, respectively).



OAPR = odds of being affected given a positive result (non-wild genotype)
 OANR = odds of being affected given a negative result (wild genotype)

Figure 2

A recent meta-analysis calculated a relative risk for bleeding of 2.26 (95% CI 1.36-3.75) for carriers of the *2 or *3 *CYP2C9* genotype compared with the wild type (*1).³ The individual bleeding risks for *2 and *3 genotypes are 1.91 (95% CI 1.16-3.17) and 1.77 (95% CI 1.07-2.91), respectively. The relative risk estimate of 1.9 from Table 6 is consistent with the 2.26 estimate by Sanderson *et al.*³

There are no published data for the relationship between *VKORC1* genotype and major bleeding as a result of anticoagulation therapy. There are also no published data on the combined *CYP2C9* and *VKORC1* genotypes and bleeding events in warfarin treated patients.

Gap in Knowledge: What are the clinical sensitivity, clinical specificity, relative risk, and attributable risk of severe bleeding in the *VKORC1* haplotypes and in *CYP2C9* and *VKORC1* genotypes combined?

20. Are there methods to resolve clinical false positive results in a timely manner?

Clinical false positive results can result when there is an analytic error (pre-analytic, analytic, or post-analytic – see Questions 9 and 10), or if an individual with a slow metabolizing *CYP2C9* *2 and/or a *3 allele or a *VKORC1* allele with decreased gene expression does not develop serious bleeding. The first of these occurrences should be rare. The second reflects that bleeding is influenced by genetic, as well as other factors such as age, comorbidities, and other medications. Various dosing algorithms have been studied that predict starting doses for warfarin based on these factors and genotype.^{9, 35-37} It is still not possible to predict which patients will

suffer from a severe bleeding episode. Current practice is to continually monitor the INR and adjust warfarin dosing based on its value.

As noted earlier in analytic validity, It is important to remember that clinicians informally assign individuals a warfarin ‘phenotype’, based on INR. When this impression clashes with the assigned genotype, additional testing might be ordered. For example, a *CYP2C9* *1/*1 (wild) individual who experiences a bleeding event, might be tested for additional alleles, if initial testing only included *2 and *3. If a mistake had been made, it is possible that such follow-up testing might identify an error.

21. What is the prevalence of the disorder in this setting?

The prevalence of major bleeding among patients treated with warfarin is dependent on the intensity of the anticoagulant effect, patient characteristics (e.g. age, comorbidities, ethnicity), the concomitant use of drugs that interfere with hemostasis, and the length of therapy. As previously discussed, there is no standard definition of major bleeding (Question 2). The rates of hemorrhagic complications due to warfarin therapy, by three indications, have been recently summarized.² The fourth indication, joint replacement surgery, was summarized by us using data gathered in a literature search (PubMed search terms = warfarin, joint replacement, bleeding).³⁸⁻⁴⁶ Warfarin therapy for joint replacement surgery had the lowest frequency range of major bleeding events at <1% to 2.2% (Table 8). This may be explained by the short-term use of anticoagulation for this indication. The range with the highest frequency of major bleeding events was among patients taking warfarin due to venous thromboembolism (<1% to 16.7%). The warfarin indications of atrial fibrillation and prosthetic heart valves had similar frequency ranges for major bleeding (<1% to 6.6% and 1% to 5.5%, respectively).

Table 8. Major bleeding frequencies among patients taking warfarin, by indication.

Indication for warfarin	Number of Patients	INR range	Bleeding frequency (%) ¹
Atrial Fibrillation	4,555	1.4 – 4.5	<1 to 6.6
Prosthetic Heart Valves	636	2.5 – 9.0	1 to 5.5
Venous Thromboembolism	1,452	2.0 – 4.5	<1 to 16.7
Joint Replacement Surgery	4,951	1.4 – 3.0	<1 to 2.2

¹ length of follow-up varied, ranging from months to 10 years or more

22. Has the test been adequately validated on all populations to which it may be offered?

Population distributions of *CYP2C9* genotype vary by ethnicity.⁴⁷ Asians and African Americans have significantly lower prevalence of the *2 and *3 alleles. *VKORC1* haplotype distributions have been reported in European-, African- and Asian-Americans.⁶ The prevalence of the AA haplotypes (predictive of low warfarin dose) is about 18% in Caucasians (Table 1). It is more common in the Asian-American population (89%), but similar in the African-American population (14%). However, allele testing methodologies should identify variants in the *CYP2C9* and *VKORC1* genes, regardless of the characteristics of the individual being tested (e.g., race or ethnicity).

Gap in Knowledge: Is the contribution of genetics versus other influences toward bleeding the same in all racial/ethnic populations?

23. What are the positive and negative predictive values?

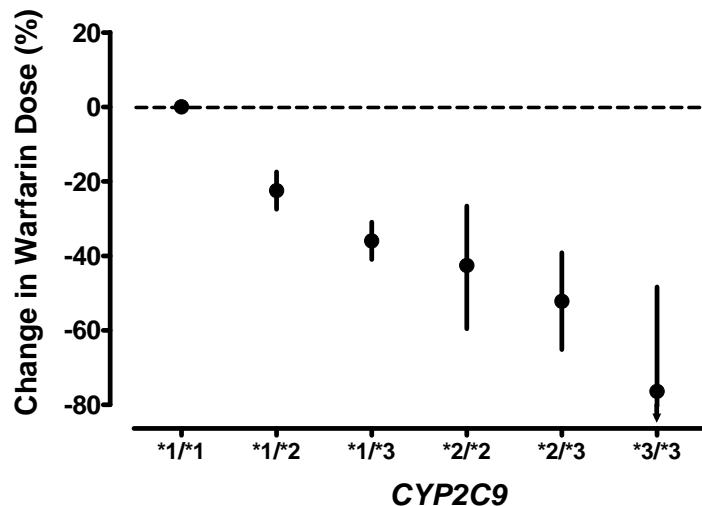
In the present scenario, the clinical positive predictive value (PPV) is the proportion of individuals with a non-wild *CYP2C9* allele that has a severe bleeding event. Referring to Figure 1, the calculation for PPV is $230 / (230 + 2945) = 7.2\%$. The clinical negative predictive value (NPV) is the proportion of individuals that has the wild *CYP2C9* genotype and does not suffer a severe bleeding event. The NPV is $6555 / (6555 + 270) = 96.0\%$. Viewed another way, the wild *CYP2C9* genotype is associated with a 4.0% absolute rate of bleeding. As in clinical sensitivity and clinical specificity, there are no data on bleeding events by *VKORC1* haplotypes, nor are there data for *VKORC1* and *CYP2C9* genotypes combined.

Gap in Knowledge: What are the positive and negative predictive values (PPV and NPV) for severe bleeding in the *VKORC1* haplotypes and *CYP2C9* and *VKORC1* genotypes combined?

24. What are the genotype/phenotype relationships?

CYP2C9 genotype and warfarin steady state dose

We identified 11 studies examining warfarin dose by *CYP2C9* genotype; few reported more than the *2 and *3 alleles.^{8, 9, 17, 31, 33, 34, 36, 48-52} Distribution of average daily (or average weekly) dose along with standard deviation for each genotype was recorded along with the number of observations. Several studies reported the mean dose of warfarin per week. This was converted to mean dose per day by dividing by seven. The summary method was similar to that used by HuGEnet in their recent report.³ Values were expressed as a percentage reduction in the mean daily warfarin dose from that found in individuals with the wild genotype (*1/*1). This is because the mean daily warfarin dose varied widely between studies, from 4.1 mg/day³³ to 7.2 mg/day³⁶. One study included in HuGEnet was omitted from ours, as it excluded patients with relatively high, or low, doses of warfarin.⁵³ Several studies have been reported since the HuGEnet report, so our analysis contains additional studies. Figure 3 shows the estimated average reduction in mean daily warfarin dose (as a percentage of the dose in *1/*1 'wild' individuals) separately for genotypes *1/*2 (478 observations), *1/*3 (309 observations), *2/*2 (27 observations), *2/*3 (44 observations) and (*3/*3) (9 observations). The data and accompanying analyses are contained in Appendix B. Analyses were performed by a random effects model using Comprehensive Meta-Analysis software (Englewood, NJ). Figure 3 also shows that, compared to the wild type (dashed line at 0), all genotypes studied have significantly reduced average daily warfarin dose. The smallest reduction is among *1/*2 heterozygotes (22%), followed by larger, but similar, reductions for *1/*3 heterozygotes (36%), *2/*2 homozygotes (43%), *2/*3 compound heterozygotes (53%). The reduction for *3/*3 homozygotes appears larger (76%), but few data are



available for the estimate. No study dominated the results and the summary reductions for each genotype changes little with the removal of any one study.

Figure 3. Average change in warfarin dose by *CYP2C9* genotype based on 11 published studies.

These mean reductions, however, tell only part of the study, as the spread of the data (estimated by the standard deviation) is also important in determining the strength of relationship between *CYP2C9* genotype and warfarin dose. Figure 4 shows the theoretical overlapping distributions of average daily warfarin dose for the six most common *CYP2C9* genotypes. This analysis relies on the mean differences shown in Figure 2 and also takes advantage of our new observation that the average ratio of the standard deviation to mean value for daily warfarin dose across studies is approximately constant at 45%. The distributions are drawn to represent the observed genotype frequency in non-Hispanic Caucasians (e.g., 68% **1/*1*, 18.5% **1/*2*). Figure 4 shows that there is still considerable overlap in stable warfarin dose after stratification by *CYP2C9* genotype.

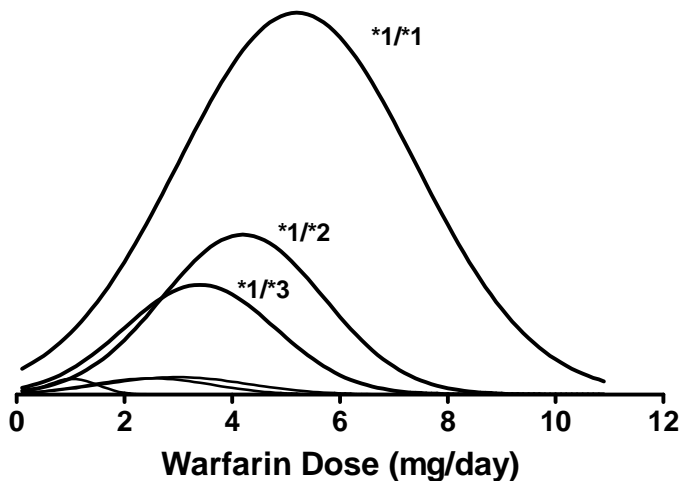


Figure 4. A theoretical distribution of stable warfarin dose based on *CYP2C9* genotype. The areas are proportional to how common the genotype is among European Caucasians.

As a way to verify the reliability of the mean and standard deviation as reliable summary statistics, Figure 5 examines, in detail, one large study that provides individual mean daily warfarin doses in 453 *CYP2C9* genotyped individuals.¹⁷ Included in the figure are horizontal lines with tic marks at the mean value (bold tick) and ± 1 standard deviation (expected to include 67% of the data) and ± 2 standard deviations (expected to include 95% of the data). The data do not appear to be Gaussian (normal or bell-shaped), as there is a 'tail' of higher doses, especially for the **1/*1* and **1/*2* genotypes and a concentration of observations at lower doses.

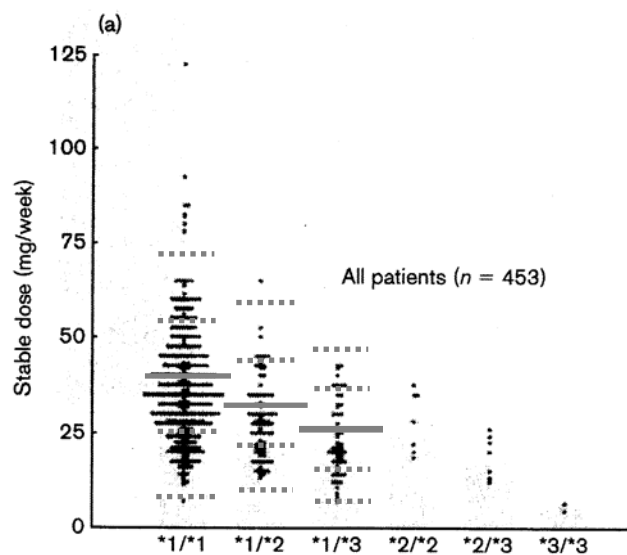


Figure 5

This finding of the distributions being non-Gaussian is confirmed in another study by Daly *et al.*⁵⁴, shown in Figure 6. We then examined whether a logarithmic Gaussian model might fit the warfarin dose better. We fitted the data for *1/*1, *1/*2 and *1/*3 genotypes from Hillman *et al.*¹⁷, and the resulting overlapping distributions for both the linear and logarithmic fits are shown in Figure 7. The warfarin data for the *1/*1 and *1/*2 genotypes fit the logarithmic distribution much better than the linear distribution, when compared to the observed data in Figure 5. Warfarin dose data for *1/*3 individuals fit the logarithmic Gaussian model less well.

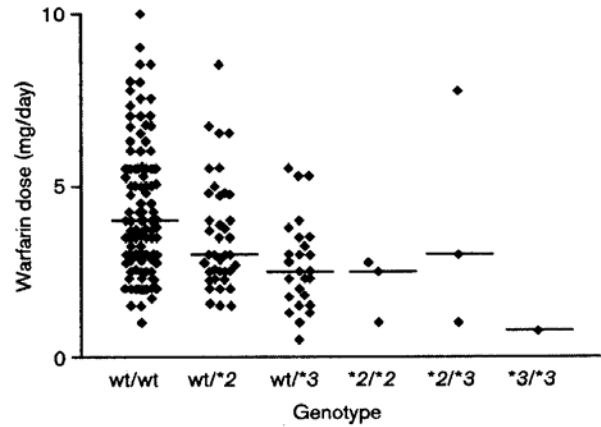


Figure 6

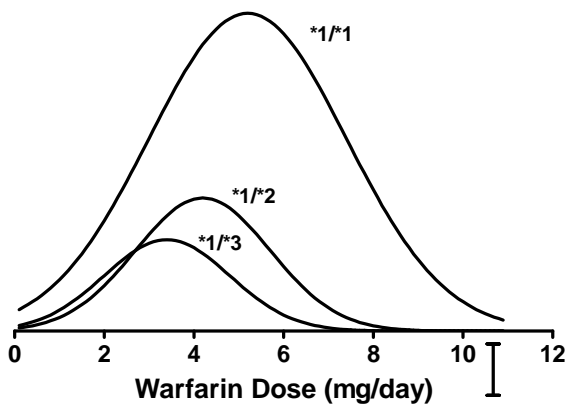


Figure 7 A (linear fit)

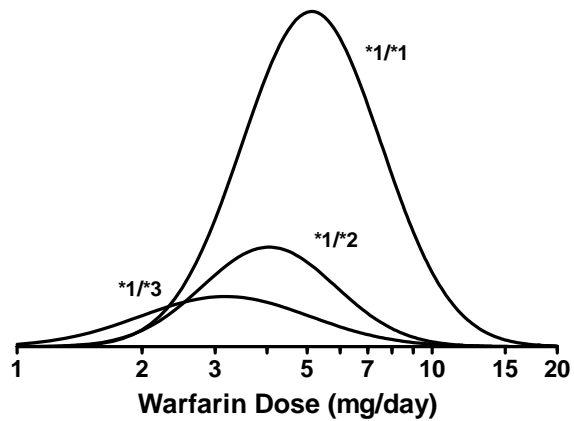


Figure 7 B (logarithmic fit)

Gap in Knowledge: Future analyses using *CYP2C9* genotyping to predict warfarin dose should explore whether a logarithmic transformation might result in a closer correspondence between predicted and observed warfarin doses. Studies of dose versus genotype should include individual observations as part of the report, to enable alternative methods of analysis (such as logarithmic transformation) to be examined.

CYP2C9 genotype and steady state s-warfarin modeling In addition to warfarin dose requirements, *CYP2C9* genotyping also provides information concerning time to steady state of warfarin plasma levels. Figure 8 shows the results of modeling time to steady state in three genotypes (*1/*1, *1/*2 and *1/*3). Each is provided with a targeted dose (5, 3 and 1.6 mg, respectively). It is clear that the wild type individuals reach steady state within 3 to 5 days. This is much faster than the 6 to 8 days for those with a *1/*2 genotype or the 12 to 15 days for a *1/*3 genotype. This delay may have long-term INR monitoring implications when warfarin doses are being modified.

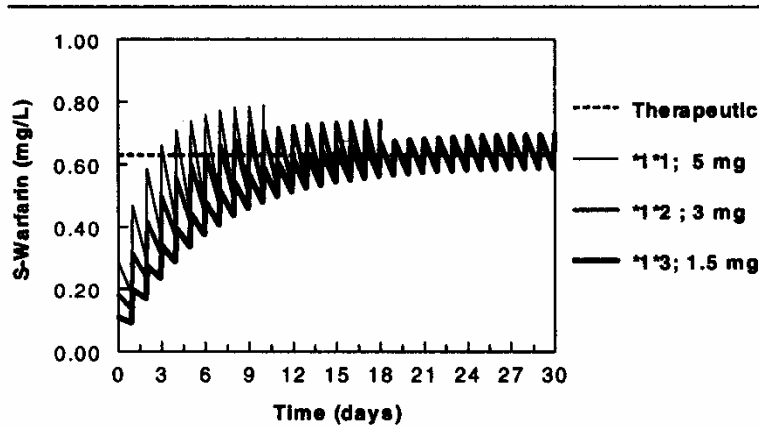
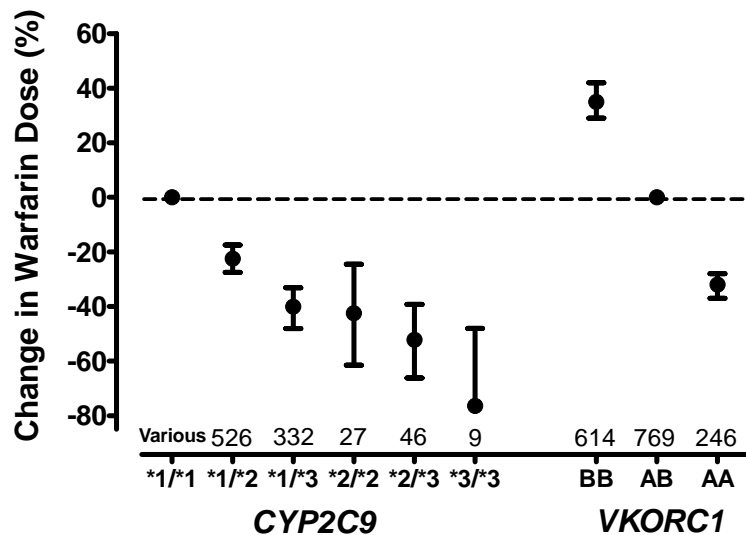


Figure 8 (from Linder MW *et al.*⁴) showing the accumulation of s-warfarin to steady state via modeling for three CYP2C9 genotypes.

VKORC1 haplotype and warfarin stable dose

We identified 6 suitable studies.^{6-10, 37} Studies that did not report information for Caucasians, or that did not study the effect on warfarin (or coumadin) were removed.^{5, 11-15, 55} For one study, the means and standard deviations were estimated from a figure.³⁷ For two studies, the numbers of individuals with each genotype were estimated using the reported allele frequencies, the total number of observations and the Hardy-Weinberg principle.^{6, 37} Values were expressed as a percentage reduction in the mean daily warfarin dose from that found in individuals with the wild genotype (normal gene expression). This is partly because the mean daily warfarin dose varied between studies, from 6.1 mg/day³⁷ to 7.3 mg/day¹⁰. We also wanted the two analyses to be comparable and, therefore, used the same methodology. Figure 9 shows the estimated average change in mean daily warfarin dose (as a percentage of the dose in the 769 *VKORC1* heterozygous individuals) separately for those with wild (“BB”) and decreased (“AA”) gene expression (188 observations). The individual studies and accompanying analyses are contained in Appendix C. Analyses were performed by a random effects model using Comprehensive



Meta-Analysis software (Englewood, NJ). Figure 9 shows that the *CYP2C9* and *VKORC1* variants have significantly reduced average daily warfarin dose, compared to heterozygotes (dot, and dashed line at 0). The dose among the wild type is 35% higher, while the homozygotes are 32% lower. For purposes of comparison, the reductions for the *CYP2C9* genotype (first shown in Figure 3) are also shown. No study dominated the results, and the summary reductions for each genotype change little with the removal of any one study.

Figure 9

Figure 10 shows the theoretical overlapping distributions of average daily warfarin dose for the three levels of *VKORC1* gene expression. It also takes advantage of the observation that the average ratio of the standard deviation to mean daily warfarin dose across studies is 38%.

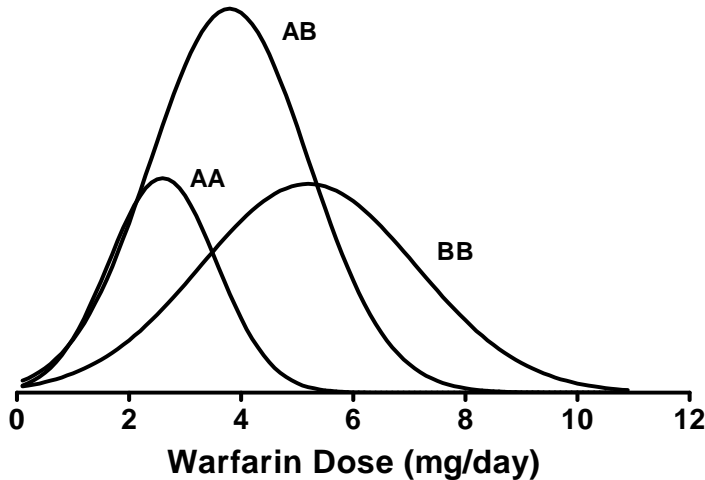


Figure 10. Estimated warfarin dose by *VKORC1* gene expression. The areas under the three curves indicates how common the genotypes are expected to be among European Caucasians (i.e., BB 35%, AB 47%, AA 18%)..

None of the studies included in our review of *VKORC1* provided individual warfarin doses, so it was not possible to generate logarithmic distributions. However, given that the distribution of warfarin doses is the same, regardless of how it is stratified, it is likely that the logarithmic distribution is appropriate for this analysis, as well.

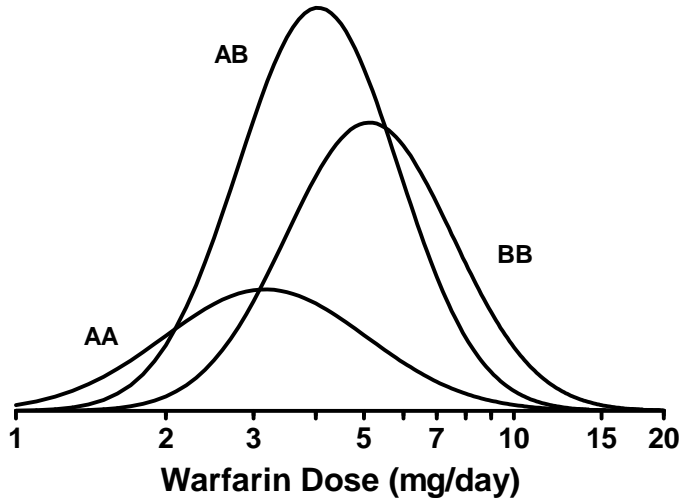


Figure 11. *VKORC1* Genotypes and Warfarin Dose on a Logarithmic Scale

Note: The six studies used to evaluate *VKORC1* genotype and warfarin stable dose collectively identified five variants (-1639G>A, 1173C>T, 1542G>C, 2255T>C, and 4931T>C) that are associated with low warfarin dose. Because of the strong linkage disequilibrium among at least 4 of these variants (-1639G>A, 1173C>T, 1542G>C, 2255T>C) and the fact that all studies used at least one of these variants, we combined the dose estimates for the analyses contained on this and the next page.

The average reduction for *CYP2C9* *1/*2 is similar to that found with intermediate *VKORC1* gene expression (Figures 3 and 8). In turn, the larger average reductions for *CYP2C9* *1/*3, *2/*2 and *2/*3 are similar to that found with decreased *VKORC1* gene expression. Given this, and the constant ratio of standard deviation to mean value, the 'best guess' for the logarithmic distribution for *VKORC1* is shown in Figure 11.

Predicting warfarin dose using both *CYP2C9* genotyping and *VKORC1* haplotyping.

In order to determine whether *CYP2C9* genotyping and *VKORC1* haplotyping are independent predictors of warfarin dose, it is necessary to determine both genotypes in the same set of patients. We identified six studies that genotyped both *CYP2C9* and *VKORC1* in patients receiving a maintenance dose of warfarin with a steady state INR.^{6-10, 37} All found testing both genes to add useful, independent information in predicting maintenance warfarin dose.

Figure 11 is from one of those studies³⁷ showing the independent effect. The box and whisker plots are grouped first by *CYP2C9* genotypes (first three are *1/*1, the next three are *1/*2, the third three are *1/*3, and the last three are various low prevalence genotypes). Within each of the groups, the distribution of warfarin dose stratified by *VKORC1* genotypes is shown for the decreased, intermediate and normal gene expression. The required warfarin dose is higher for each *VKORC1* genotype within each *CYP2C9* genotype.

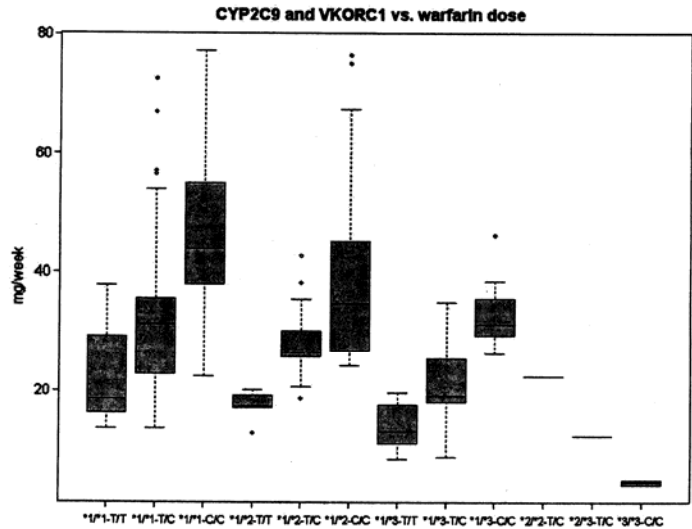


Figure 11

Three studies that had both *CYP2C9* genotyping and *VKORC1* haplotyping information provided information on warfarin dose at stable INR in Caucasians.^{6, 10, 37} Each, however, reported results in slightly different formats (Table 9). We extracted the median warfarin dose from a figure summarizing results for 12 genotype / genotype combinations for the study by Wadelius and colleagues³⁷. The remaining two studies grouped some genotypes together. Each cell in Table 9 contains the warfarin dose relative to the *CYP2C9* wild (*1/*1) / *VKORC1* wild (BB) genotypes. Also included in the cell is the absolute dose (weekly or daily) and the number of observations. In two studies^{6, 37}, the number of observations for each cell was not reported, and we estimated the numbers based on HWE. Empty cells indicate that no observations were available. Because of the way the authors chose to collapse (or not collapse) the cells, comparisons are difficult. However, it is clear that within a row (*VKORC1* haplotype), the varying *CYP2C9* genotypes influence dose. Alternatively, within a column (*CYP2C9* genotype), the *VKORC1* haplotype influences dose. This indicates that testing for the two genes may improve prediction of stable warfarin dose.

Table 9. Relative warfarin dose (w/r to the *CYP2C9* (rapid) genotypes and *VKORC1* (high) haplotypes), along with weekly or daily warfarin dose (and number of observations).

Wadelius et al.³⁷

<i>VKORC1</i> Haplotype	<i>CYP2C9</i> Genotype					
	Rapid *1/*1	Intermediate *1/*2	*1/*3	*2/*2	Poor *2/*3	*3/*3
High (BB)	100% 43.0 (39)	79% 34.0 (12)	72% 31.0 (8)	-	-	12% 5.0 (3)
Medium (AB)	72% 31.0 (52)	60% 26.0 (16)	44% 19.0 (10)	51% 22.0 (1)	28% 12.0 (1)	-
Low (AA)	44% 19.0 (20)	40% 17.0 (6)	30% 13.0 (4)	-	-	-

Vescler et al.¹⁰

<i>VKORC1</i> Haplotype	<i>CYP2C9</i> Genotype	
	Rapid *1/*1	Other All others
High (BB)	100% 7.0 (25)	88% 6.1 (12)
Med / Low (BA, AA)	74% 5.1 (27)	44% 3.1 (26)

Rieder et al.⁶

<i>VKORC1</i> Haplotype	<i>CYP2C9</i> Genotype	
	Rapid *1/*1	Other All others
High (BB)	100% 6.8 (74)	71% 48 (37)
Medium (BA)	76% 5.1 (100)	56% 3.8 (50)
Low (AA)	48% 3.2 (38)	38% 2.6 (18)

Regression models predicting warfarin dose

Four studies provided regression models that utilize both *CYP2C9* genotypes and *VKORC1* haplotypes to predict warfarin dose in Caucasians.^{5, 7, 9, 10} Takashi and colleagues⁵ provided a single regression equation that they suggest is appropriate for their entire population of Asians, Caucasians and Blacks. One regression model predicts the square root of the dose⁹; the other three predict dose on a linear scale. None of these models use the logarithmic transformation of warfarin dose suggested earlier in this review. The models did not agree on how the *CYP2C9* genotype should be coded. It appears to be important to predict separate initial warfarin doses for *1/*2 vs. *1/*3 *CYP2C9* genotypes, as the change in dose (22% vs. 36%, Figure 3) is significantly different.

- Sconce and colleagues⁹ used two variables: one for the number of *2 alleles and one for the number of *3 alleles. This allows for a different reduction in warfarin dose for each *CYP2C9* genotype.

- Vescler and colleagues¹⁰ used one variable containing the number of *2 and *3 polymorphisms identified. Thus, this model would result in the same dose reduction for *1/*2 and *1/*3 genotypes.
- Aquilante and colleagues⁷ used two variables: one for heterozygotes and one for homozygotes. Like the Vescler model, this will not distinguish between *1/*2 and *1/*3. This model will also not distinguish between *2/*2 and *3/*3, while the model by Vescler will.
- Takashshi and colleagues⁵ coded *CYP2C9* genotypes in the same way as Aquilante.

Because there are only 3 *VKORC1* haplotypes, coding is simpler. All four models allow for different warfarin doses for each haplotype. However, two of the models^{9, 10} are designed to require that the difference in dose between the BB and AB haplotypes be the same as between the AB and AA haplotypes. In a small dataset it might be necessary to provide reliable estimates, but the differences in dose may not actually be identical. The other two models do not require any specific relationship among the doses in BB, AB and AA *VKORC1* haplotypes.

It should, in theory, be straightforward to compare the predicted warfarin dose for selected genotypes between these (and other) models. However, the comparison is complicated by several factors. The models are not designed to target the same average warfarin dose. Displaying the relative warfarin doses may be useful, but that relationship between categories is not constant on the linear scale. For example, if a patient's weight was high (requiring a larger dose), then the relative difference between such individuals by genotype would be smaller than if the patient's weight was low. As a compromise, we have expressed expected warfarin dose as a percentage of the *CYP2C9* *1/*1 genotype and *VKORC1* BB haplotype. Constant values used in the analysis are noted, along with the warfarin dose equation. Lastly, the marginals contain the observed row and column proportions that can be compared to those reported earlier (Figure 3, Figure 9). In some instances, we have 'guessed' at the coding used for some variables or for the values of the regression constant that was not supplied. Because of this, and because all four of these models have important flaws, the results should only be used as a rough guide. As implemented here (Appendix D), these four models should not be used for clinical purposes.

In summary, there are two potential limitations in these published models. They do not consider dose on a logarithmic scale and they do not allow for dose differences between the *CYP2C9* *1/*2 and *1/*3 genotypes.

A publically available, but yet to be published algorithm, may address some of these limitations (available on the web at www.warfarindosing.org). Table 10 contains the variables included in an earlier version of this model, that is partially described in a powerpoint presentation available at www.fda.gov/ohrms/dockets/AC/05/slides/2005-4194S1_04_Gage.ppt (personal communication, Brian Gage, Washington University, St. Louis, MO). The variables are in the order of their impact on the overall variability of warfarin dose. The model was derived from 900 individuals and subsequently validated on a separate set of 100 (ASH abstract, Gage, BF)

Table 10. Model predicting warfarin dose at stable INR that incorporates both *CYP2C9* and *VKORC1* test results

Variable	Coding	Change in warfarin dose
VKORC1 haplotype	0=BB, 1=AB, 2=AA	-27% per variant
Body surface area (m ²)	continuous, with mean = 2.0	+12% per 0.25 m ²
CYP2C9 *3	0=none, 1=one, 2=homozygote	-33% per variant
CYP2C9 *2	0=none, 1=one, 2=homozygote	-20% per variant
Age (years)	continuous, with mean = 69	-7% per decade
African American VKOR5808	0=none, 1=one, 2=homozygote	-33% per variant
Target INR	continuous	+8% per 0.5 increase
Amiodarone	0=non/unknown, 1=yes	-24%
African American	0=non/unknown, 1=yes	-12%
Smokes	0=non/unknown, 1=yes	+9%
Simvastatin or fluvastatin	0=non/unknown, 1=yes	=5%

Table 11 contains both the absolute warfarin dose (top half) and the relative warfarin dose (bottom half) for an average new patient beginning warfarin treatment. The last column (Relative Dose) shows the relative reductions with respect to the *VKORC1* wild haplotype (BB) weighted by the expected frequency of each *CYP2C9* genotype (Table 1). These reductions of 28% and 48% are nearly identical to the univariate reductions of 28% and 50% found in the earlier meta-analyses of six studies reporting the effect of *VKORC1* haplotypes alone (Figure 9). The last row (labeled Relative Dose) shows the relative reductions with respect to *CYP2C9* genotypes, weighted by the expected frequency of each *VKORC1* haplotype (Table 1). The reductions of 29%, 38%, 39%, 46% and 55% can be compared to the univariate reductions (Figure 3) of 22%, 36%, 43%, 53% and 76%, respectively. The results are in good agreement for the more common genotype (*1/*2, *1/*3 and *2/*2), but the modeled reductions for the rare genotypes (*2/*3 and *3/*3) are less than the observed reductions. More data are needed to resolve this discrepancy. The last display of the model changes the comparative dose from the *1/*1 and BB combination to the most common combination of *1/*1 and AB. This shows that certain *CYP2C9* / *VKORC1* genotypes will need a higher dose than the usual 5 mg starting dose (in the example shown in the model, this dose is 4.8 mg). Also included in parentheses is the expected number individuals per 1000 from the Caucasian population expected with that combination of genotypes. For example, the most common combination will be *1/*1 and AB in 300 per 1000, or 30% of individuals.

Gap in knowledge: How can the difference in dosage be best presented to clinicians who are initiating treatment in warfarin naïve individuals to ensure that a targeted dose will account for all known important sources of variation.

Table 11. Absolute and relative estimates warfarin dose at stable INR, stratified by CYP2C9 genotype and VKORC1 haplotype (www.warfarindosing.org)

VKORC1 Haplotype	CYP2C9 Genotype					
	Rapid *1/*1	Inter *1/*2	Poor			
			*1/*3	*2/*2	*2/*3	*3/*3
High (BB)	6.7	5.4	4.5	4.4	3.6	3.0
Medium (AB)	4.8	3.9	3.2	3.2	2.6	2.2
Low (AA)	3.5	2.8	2.3	2.3	1.9	1.6

Where age=65, BSA 1.96 m² (weight=180 lbs, height=5'8"), non-smoker, target INR of 2.75 and no other drugs

VKORC1 Haplotype	CYP2C9 Genotype						Relative Dose
	Rapid *1/*1	Inter *1/*2	Poor				
			*1/*3	*2/*2	*2/*3	*3/*3	
High (BB)	100%	81%	67%	66%	54%	45%	100%
Medium (AB)	72%	58%	48%	48%	39%	33%	64%
Low (AA)	52%	42%	34%	34%	28%	24%	36%
Relative dose	100%	81%	67%	66%	54%	45%	63%

VKORC1 Haplotype	CYP2C9 Genotype						Number / 1000
	Rapid *1/*1	Inter *1/*2	Poor				
			*1/*3	*2/*2	*2/*3	*3/*3	
High (BB)	140% (223)	113% (68)	94% (44)	92% (5)	75% (7)	63% (2)	(350)
Medium (AB)	100% (300)	81% (92)	67% (59)	67% (7)	54% (9)	46% (3)	(470)
Low (AA)	73% (115)	58% (35)	48% (23)	48% (3)	40% (3)	33% (1)	(180)
Number / 1000	(638)	(195)	(126)	(15)	(19)	(6)	(1000)

Regression models and reduction in the overall variance of warfarin dose

As an alternative way of exploring the relationship between stable warfarin dose, genotyping and other predictive factors, we summarized the six studies that performed linear regression analysis with warfarin daily dose as the dependent variable. All also included CYP2C9 and VKORC1 genotype information, the individuals' ages at first dose, and the individuals' weights. Appendix E summarizes some of the information reported from these studies. The data were not complete and, as shown earlier, the studies were not consistent in the way genotyping was included in the analysis. Overall, conclusions were as follows: only the two genotypes, age and weight (or a close relative of weight such as height or BMI) were consistently related with an important reduction in the overall variance of the warfarin dose. The two most important predictors were CYP2C9 and VKORC1 genotypes, followed by weight and age. The relative reduction in the overall variance (as measured by the partial r^2 values) varied considerably for the CYP2C9 genotyping. This may be due to the frequency of the wild genotype within the population studied. For example, in the study of Italians⁸, where only 50% of the population has the CYP2C9 *1/*1 genotype, VKORC1 genotype was less important than the CYP2C9

genotype. On the other hand, in the study of individuals from the US⁷, where 71% of the population has the *CYP2C9* *1/*1 genotype, *VKORC1* genotype was more important in predicting warfarin dose than the *CYP2C9* genotype. Overall, the total r^2 values reported by five of the studies were consistent, ranging from a low of 51% to a high of 63%.

Figure 12 shows the overall variance of warfarin dose, divided into the component parts. The two largest identified components are the *VKORC1* and *CYP2C9* genotypes, followed by an individual's weight and age at first dose. Overall, about 56% of the variability is explained, with about 44% of the total variance remaining unexplained. One point to remember, however, is that the spread of data is determined by the standard deviation, which is the square root of the variance. Thus, if the overall warfarin dose were to be 6 mg/day, with a standard deviation of 2.5 mg/day (variance of 2.5×2.5 or 6.25), then taking into account these four variables in a regression analysis would result in a new expected dose (e.g., 4.2 mg/day) with a reduced standard deviation of 1.7 mg/day (standard deviation is the square root of the variance 6.25 time 0.44). This is a reduction in the standard deviation of $(2.5-1.7)/2.5$ or 32%, considerably smaller than the 56% reduction in variance.

Appendix D contains the summary of the 11 studies used in the *CYP2C9* analysis and the six studies used in the *VKORC1* analyses. Overall, the strength of evidence for the association between *CYP2C9* genotypes and maintenance dose of warfarin is strong (multiple adequate/good studies without heterogeneity). Overall, the strength of evidence for the association between *VKORC1* genotypes and maintenance dose of warfarin is strong (multiple adequate/good studies without heterogeneity).

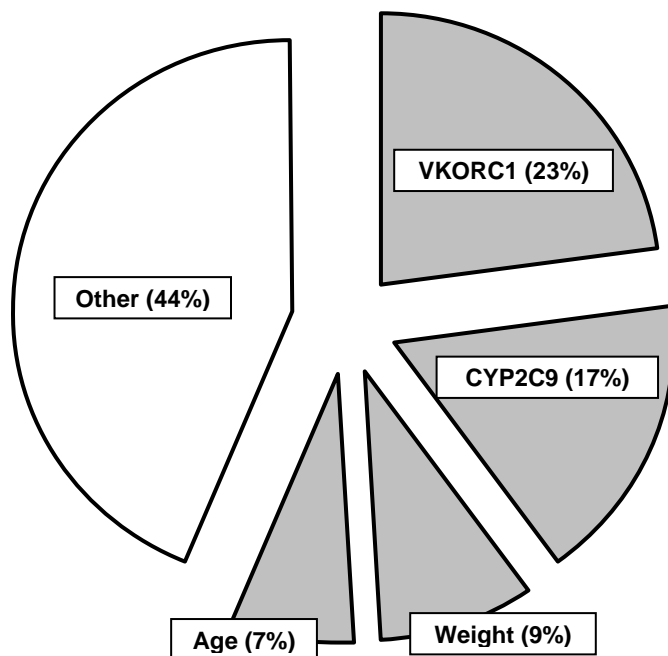


Figure 12. Pie chart showing the known sources of variability in warfarin dose needed for a stable INR. Each estimate is based on a summary analysis of partial r^2 values from multivariate regression analysis reported in six studies that included genotyping on both *CYP2C9* and *VKORC1*

25. What are the genetic, environmental or other modifiers?

Environmental factors that affect warfarin dose requirements include:

- Age
- Weight (or height, body mass index (BMI), body surface area (BSA))
- Gender
- Hepatocellular damage
- Cardiac disease
- Pyrexia
- Drug interactions (inhibitors and inducers)
- Body weight
- Dietary vitamin K intake
- Alcohol consumption
- Cigarette smoking

In addition, sensitivity to warfarin varies by ethnicity (see Question 22).

Gap in Knowledge: What are the roles of other genes in the pharmacokinetics and pharmacodynamics of warfarin and what is their impact on warfarin dosage requirements?

CLINICAL UTILITY

SUMMARY

- The clinical utility of DNA testing in this clinical scenario is to compute an individual's initial warfarin dose by incorporating demographic, clinical, and genotype data (*CYP2C9* and *VKORC1*), as a way to limit high INR values (over-anticoagulation) that are associated with an increased risk of serious bleeding events. No large study has yet shown this to be acceptable or effective.
- Several randomized trials are underway to determine the clinical effectiveness of *CYP2C9* and *VKORC1* genotyping to inform warfarin dosing to reduce serious bleeding.
- Number needed to treat to avoid one serious bleeding event ranges from 48 to 385.
- Using estimates of clinical validity described earlier, along with several assumptions of clinical utility, the cost per serious bleeding event avoided is likely to range from \$14,500 to \$95,900. Key assumptions that strongly influence the cost are:
 - Effectiveness of individualized reduced warfarin dose in avoiding serious bleeding (range 80% to 20%)
 - The fee for genetic testing (range \$300 to \$500)

26. What is the natural history of the disorder?

Warfarin anticoagulation must be sufficient to avoid thrombotic events. However, excessive anticoagulation can result in severe, possibly fatal, bleeding events. The therapeutic window is narrow, and therapy is monitored by the international normalized ratio (INR), which is a standardized measure of the patient's prothrombin time (PT) such that the result is comparable across laboratories and test reagents.⁵⁶

The target INR range is 2.0-3.0 for most patients; exceptions include a slightly higher target range (e.g. 2.5-3.5) for patients with certain types of prosthetic heart valves⁵⁷ and a lower target (1.5) for patients with coronary artery disease at particularly high risk of coronary events⁵⁸. INR monitoring usually begins 2 to 3 days after the initial dose. In an acute, hospital setting, patients may be monitored daily; in an outpatient setting, 2 to 3 times weekly is recommended. If the INR remains stable, the interval can be gradually increased up to every 4 weeks. Although a pharmacokinetic steady state is usually achieved in 6 to 12 days, pharmacodynamic stability is highly variable and may take much longer. This is especially true if, for example, a *3 variant is involved where the time to reach steady state may be two to three times longer than the expected 3 to 5 days in wild-type individuals.

The intensity of anticoagulant therapy, as measured by the INR, is strongly related to the risk of major bleeding. The frequency of major bleeding in patients enrolled in clinical trials and assigned an INR target range of 2.0 to 3.0 is less than in those assigned to INR >3.0.² The risk of intracranial hemorrhage increases rapidly with INR >4.0. The risk of thrombotic events as a result of under-anticoagulation depends on the patient indication and the risk prior to treatment and must be balanced against the risk of bleeding when considering and adjusting anticoagulation therapy. Bleeding risk can be predicted using published models^{1, 59, 60} and can help in decision-making.

There is general agreement that the frequency of bleeding is somewhat higher early in the course of therapy, when INR fluctuations are more common.² Thus, the greatest concern is the possibility of over-anticoagulation at the first INR after initiation of therapy. In general, reducing

INR fluctuations outside the target range should reduce the frequency of adverse events. Primary physician education and adherence to monitoring guidelines, referral to anticoagulation clinics, or patient self-testing/management may improve anticoagulation control.

The goal of long-term anticoagulation monitoring is to maintain the patient in the INR target range; success is measured as percent time in the therapeutic range and avoidance of adverse events. The stability of therapy over time may be influenced by changes in concomitant medications (including “alternative” and over-the-counter medications), health status changes that affect warfarin disposition or vitamin K-dependent coagulation factors, dietary or gastrointestinal factors affecting vitamin K (e.g., alcohol use, irregular ingestion of vitamin K-rich foods, changes in intestinal absorption capacity). It is important that the health care provider monitor at appropriate intervals, consider any changes in status, and make necessary and appropriate dose adjustments to maintain INR in the target range. In addition, patient communication, education, and compliance are important determinants of success. Finally, active intervention may be required when the INR is excessively prolonged and the patient has active bleeding or is at high risk for bleeding.

27. What is the impact of a positive (or negative) test on patient care?

The premise of *CYP2C9* and *VKORC1* testing prior to warfarin administration is that, by knowing a patient’s genotype, this information can be incorporated into estimating a starting dose and/or identifying those patients who may benefit from closer monitoring, especially during the induction phase. Genotype information may also potentially impact the warfarin dose adjustments until stable dosing is achieved. These actions would be taken to lessen the chance for a serious bleeding event. In addition, it may be important to consider the *CYP2C9* genotype when scheduling INR testing to help ensure that the steady state has been reached. A theoretical negative impact is that some patients who test ‘positive’ for a low-dose genotype may feel that warfarin is unsafe for them and stop taking the drug.

28. If applicable, are diagnostic tests available?

Not applicable.

29. Is there an effective remedy or acceptable action, or other measurable benefit?

Actions include adjusting the starting doses of warfarin and/or closely monitoring the INR among patients who have a *CYP2C9* and/or *VKORC1* allele associated with slow warfarin metabolism. Vitamin K therapy may be considered in patients with warfarin overdosing. Genotype information may also potentially impact the warfarin dose adjustments until stable dosing is achieved. The effectiveness and/or patient benefits of these actions have not been documented as being effective. They are currently under investigation (see Question 33).

30. Is there general access to that remedy or action?

The availability of anticoagulation clinic services varies geographically. Those who live in remote, rural areas, as well as those who lack health insurance or are under-insured, are likely to have less access to these services. In community practice, warfarin may be initiated and managed by an internist or internist-specialist (e.g. cardiologist), or by a family practice physician, depending on the patient indication for treatment and geographic location. Portable coagulometers are also available for patient self management. Access to these community practitioners and/or portable coagulometers is also dependent on health insurance and/or transportation. A recent randomized trial has shown the performance of self-managed oral anticoagulant therapy to be at least as good as conventional management when properly implemented.^{61, 62}

31. Is the test being offered to a socially vulnerable population?

In the present scenario, those being offered testing already have been determined to require anticoagulation. Patients are, therefore, undergoing testing only to learn about how they might respond to the anticoagulant, and would not be considered socially vulnerable.

32. What quality assurance measures are in place?

For the laboratory component, see Questions 11 to 17. The College of American Pathologists (CAP) offers proficiency testing for coagulation. Included in this survey is an INR challenge. At present, there are no special regulations that apply to pharmacogenomic tests to ensure the overall quality of test results.

33. What are the results of pilot trials?

The optimal study design to evaluate the effectiveness of using *CYP2C9* and *VKORC1* genotype to inform warfarin dosing is a randomized trial. A small prospective trial has been published that randomized 38 patients to either a standard 5 mg initiation dose of warfarin (n=20) or a model-based dose that included *CYP2C9* genotype (n=18).³⁵ Six bleeding events occurred among 20 patients who received standard dosing compared with 2 bleeding events in the model-based dosing group. While these sample sizes are too small to demonstrate the efficacy of a model-based treatment algorithm, the study demonstrates that the design is feasible and acceptable to patients and providers.

Studies of effectiveness

The COUMA-GEN Study (NCT00334464) is a prospective, randomized study of patients who are to begin chronic warfarin therapy. Qualifying patients will be consented and randomized to an individualized, genotype-based warfarin-dosing regimen that includes both *CYP2C9* and *VKORC1* or to standard care (without knowledge of genotype). The objectives of this study are to determine whether the pharmacogenomic guided arm can a) maintain patients for a greater time in therapeutic range (as assessed by INR), b) achieve a higher proportion of therapeutic warfarin levels by days 5 and 8 of therapy, and c) reduce the need for unplanned dose adjustments and additional INR measurements because of excessive or insufficient INR level and clinical adverse events. PI: Jeffrey Anderson

As part of the “Genotyping to optimize individualized drug therapy” study (2P01GM031304-200021), a prospective, randomized study in the U.S. will test the hypothesis that initiating warfarin therapy based on an individual’s *CYP2C9* genotype will improve clinical outcomes compared to the current standard care approach. PI: C.M. Stein

A study that is scheduled to start in September, 2006, will randomize up to 1000 hip and knee replacement patients to a standard warfarin dose group or to a genotype-based dose group that includes both *CYP2C9* and *VKORC1* variants. Outcomes will include time required to achieve therapeutic INR (secondary) and major bleeding events, thrombosis, and death (primary). PI: Gwen McMillin

The “Pharmacogenetic optimization of anticoagulation therapy” study, which will be completed in 2008, will recruit 500 stroke patients. Multivariate analysis will evaluate the association of *CYP2C9* genotype-warfarin dose and genotype-INRs outside target range and associated complications both hemorrhagic and thromboembolic. Confounding variables - drug interactions, comorbid conditions, and compliance will be statistically controlled. PI: Nita Limdi

Study of association

Another ongoing study is taking place in the UK. The “Variability in response to warfarin: a prospective analysis of pharmacogenomic and environmental factors” study (www.genres.org.uk/prp/projects/liverpool2.htm) is recruiting 2000 patients to investigate the genetic and environmental factors involved in determining the response to warfarin. The findings will then be validated in another group of 400 patients. The result of these studies will be the development of an algorithm that determines the dose required to maintain a safe level of anticoagulation. PI: Munir Pirmohamed

Gap in Knowledge: Although several funded and well-designed trials are underway, the results of these trials are not available, and the endpoints of the trials may not include the reduction of serious adverse drug events, as they are relatively rare. Thus, the clinical utility is still unknown.

34. What health risks can be identified for follow-up testing and/or intervention?

There is no follow-up testing to *CYP2C9* and *VKORC1* testing. Interventions for those found to have a variant allele(s) include lowering the warfarin dose and/or close monitoring of the INR. In the current clinical scenario, there are no additional health risks from either of these interventions.

35. What are the financial costs associated with testing?

36. What are the economic benefits associated with actions resulting from testing?

Clinical laboratories quote charges of about \$250 for a single CYP 450 enzyme test. The economic impact of such a test is best evaluated in a formal cost-effectiveness analysis of total costs and outcomes resulting from medical intervention.^{63, 64} Several factors influence the cost-effectiveness analysis of a pharmacogenomic test; absence of critical information makes it difficult to accurately determine overall benefits. These factors include⁶⁵:

- Disease/condition prevalence and severity
- Prevalence and penetrance of genetic variants
- Availability and effectiveness of interventions based on genetic test result
- Indication (future risk; immediate diagnosis)
- Test cost, accuracy; can information be used more than once?
- Cost of genetic counseling, if necessary
- Indirect costs/benefits (e.g. impact on family members; loss of privacy, etc.)

Higashi and Veenstra⁶⁶ published a basic cost evaluation of *CYP2C9* to guide warfarin dosing, resulting in a number needed to screen of 44 and a cost of approximately \$6,000 (44 multiplied by the cost of test) to prevent one bleeding event. This analysis was based on acknowledged assumptions derived from limited data, including: an estimate of the bleeding rate in patients with variant alleles; ability to reduce the bleeding rate in patients with variant alleles to that of patients with wild-type alleles; a test cost of \$135. As the authors point out, not included in the analysis were potential costs of genetic counseling, of managing bleeding events, surveillance, etc.

More recently, a decision analysis was published by You *et al.* to evaluate the potential clinical and economic outcomes of *CYP2C9* genotyping to guide anticoagulation therapy for use in designing prospective, comparative trials.⁶⁷ The authors noted limited clinical data to guide and support several assumptions (including test cost, estimated at \$100, range \$50-200) but, in the context of their assumptions, estimated a marginal cost per additional major bleeding event averted in genotyped patients of \$5,778. They reported that the model was sensitive to the cost

and effectiveness of bleeding reduction in the intensified coagulation service proposed for patients with *CYP2C9* functional variants. The marginal cost increased sharply if the difference in bleeding rates between standard and intensified services was less than 30%.

A recent report from the AEI-Brookings Joint Center for regulatory studies entitled “Health care savings from personalizing medicine using genetic testing: the case of warfarin” (<http://www.aei.brookings.org/wnew/index.php?menuid=2>) concludes that testing results in large cost savings.

“We estimate that formally integrating genetic testing into routine warfarin therapy could allow American warfarin users to avoid 85,000 serious bleeding events and 17,000 strokes annually. We estimate the reduced health care spending from integrating genetic testing into warfarin therapy to be \$1.1 billion annually, with a range of about \$100 million to \$2 billion”

After review, it appears that these authors made several choices in their modeling that may not be warranted, or may need further documentation:

- The authors assume that targeted dosing based on genotyping will be 100% successful in reducing bleeding events to the level in individuals with the wild genotype. No data are available to support this, and it should be subject to sensitivity analysis with a wide range (e.g., 10% to 90% effectiveness).
- The authors assume that more effective dosing will reduce the rate of strokes. There are no data to support this assumption, and it should be removed as a supposed benefit.
- The rates of bleeding used are far too high. A summary of serious bleeding rates among patients receiving warfarin found the overall rates to be at about 5%. The report used a cumulative risk of serious and life-threatening bleeding of 27.6% for the variant group and 12.6% in the wild group, based on data contained in Higashi.³¹ Higashi et al., did not report cumulative risks, instead, they reported rates per 100 person years (12.5% and 5.6%) that are more appropriate for modeling. It is likely that the bleeding events that are preventable by genotyping and targeted dosing will occur in the first year after initiating treatment.
- The authors report 2,000,000 new warfarin users each year, but this is a difficult number to estimate, and we could find no definitive source for a reliable number. Some experts suggest the numbers are lower (perhaps 200,000 to 400,000 per year in the U.S.).

These comments lead to the following set of more plausible assumptions: 1) there are 300,000 new patients each year, 2) blood collection and genotyping costs are \$350 per patient, 3) genotyping is 50% effective in reducing serious bleeding, 4) strokes should not be included, and 5) first year or annualized bleeding rates (as reported by Higashi) should be used, rather than cumulative risks. Under these assumptions, the costs of testing are \$105 million for the 300,000 patients. About 3,465 serious bleeding events might be prevented. At an estimated cost of \$13,500 per event avoided, the health costs avoided are \$47 million. Thus, the costs of testing may be more than twice the expected health care savings under this scenario. This last finding (testing expenditures being higher than health care savings) does not depend on the number of new patients each year.

Brief analysis of cost per serious bleeding event avoided by modifying warfarin dose due to *CYP2C9* genotypes and *VKORC1* haplotypes

The following is a simplified analysis of cost per bleeding event avoided. It is based on the data from this Rapid ACCE review, along with several assumptions that need to be made in the light of specific gaps in knowledge. The following assumptions have been made:

- The *CYP2C9* genotypes and *VKORC1* haplotypes are independent
- The risk ratio for *VKORC1* and serious bleeding is the same as for *CYP2C9* (1.7)
- Stratifying the population into wild/non-wild genotypes is reasonable
- The risk ratios for *VKORC1* and *CYP2C9* are independent (Question 18)
- The effectiveness of reduced initial warfarin dose in avoiding serious bleeding ranges from 100% (level in non-wild reduced to the level in wild genotype individuals) to 10% (only a slight reduction in serious bleeding events)
- Costs of testing are between \$250 and \$500 (for both *CYP2C9* genotyping and *VKORC1* haplotyping)

Table 12 shows the proportion of a hypothetical population of 10,000 individuals initiating warfarin therapy that has specific *CYP2C9* genotypes and *VKORC1* haplotypes (refer to Figure 2). In this analysis, genotyping is simplified into wild (*1/*1 or BB) and non-wild (all genotypes other than *1/*1 or BB). Assuming the charge for testing both genes is \$250, the costs per bleeding event avoided range from \$13,900 (80% effectiveness) to \$55,700 (20% effectiveness). If the testing charges were \$500, these costs would increase to \$27,800 and \$111,000, respectively. The number needed to treat to avoid one serious bleeding event depends on the effectiveness of individualized warfarin dosing. If individual dosing were highly effective (80%), then 43 individuals would need to have tailored dosing to prevent one severe bleeding event. If the effectiveness were to be low (20%), the number needed to treat would be 173.

According to a published economic analysis of low molecular weight heparin, costs for a non-cerebral major bleeding event are about \$5,000, while the cost of an acute hemorrhagic stroke is about \$13,000 (data from the Centers for Medicare and Medicaid).⁶⁸ Follow-up care after an acute hemorrhagic stroke can be \$16,000 per year, and about 40% of individuals with hemorrhagic stroke die within one year.

It is also possible that cost savings will be realized if INR values are more rapidly stabilized as inpatient time is reduced. However, we did not find any data to support this assumption.

Gap in Knowledge: No reports of cost or cost-effectiveness of testing for *VKORC1* alone, or for *CYP2C9* plus *VKORC1* together were found. There is insufficient information for an accurate cost-effectiveness analysis of a combined test at this time. Missing critical information includes: cost of combined *CYP2C9* and *VKORC1* testing; actual method of intervention (e.g. description of initial dosing algorithm based on test result, intensity of monitoring); time to stable dose; and degree of reduction of adverse events (effectiveness).

Table 12. Distribution of genotypes, number and rate of serious bleeding, bleeding events avoided via genotype-specific warfarin dose, costs per serious bleeding event avoided and number needed to treat, in a hypothetical cohort of 10,000 individuals initiating warfarin therapy¹

Genotype		Number	Serious Bleeding		Effectiveness of reduced dose in avoiding serious bleeding events					
<i>CYP2C9</i>	<i>VKORC1</i>		Events	Rate (%) ²	100%	80%	60%	40%	20%	10%
*1/*1	BB	2,236	61	2.4	0	0	0	0	0	0
*1/*1	not BB	4,153	193	4.5	79	63	48	35	18	8
not *1/*1	BB	1,265	59	4.5	24	19	14	11	5	2
not *1/*1	not BB	2,346	185	8.6	121	97	73	58	29	12
Any	Any	10,000	497	5.0	224	180	135	90	45	22
Cost per case avoided if testing charges are \$250					\$11,200	\$13,900	\$18,500	\$27,800	\$55,600	\$111,000
Cost per case avoided if testing charges are \$500					\$22,300	\$27,800	\$37,100	\$55,700	\$111,000	\$222,000
Number needed to treat to avoid one serious bleeding event					35	43	58	86	173	346

¹ Assumptions necessary for these analyses are provided in the preceding text.

² Risk ratios between the second/first, and third/first rows are 1.7. The risk ratio between the fourth & first rows is 1.7 x 1.7.

37. What facilities/personnel are available or easily put in place?

A list of testing laboratories that offer clinical *CYP2C9* and/or *VKORC1* gene testing is found in Table 4. This list may be incomplete, and other laboratories may be adding this service in the near future. Several of these laboratories are national and are likely to be able to handle a large volume of testing. However, it is not known if laboratories can provide the genotype result within three days, which is when differences in required dose by genotype (due to higher INR levels) have been shown (Figure 1). The anticoagulation clinics and community practitioners are already in place.

Gap in knowledge: Although for the application of warfarin dosing, genotyping can be incorporated successfully by the second or third day, this will not be the case for all pharmacogenetic testing. It is also possible that clinicians will be more likely to use pharmacogenetic testing for this application if it is available at the time of initial dosing and, therefore, incorporated into care from the beginning. Ongoing studies might be able to examine the issue of turn-around-time and acceptability to both patients and physicians.

38. What educational materials have been developed and validated, and which of these are available?

Educational materials need to be available for two separate but overlapping audiences: providers and patients. Because pharmacogenomic testing is likely to be implemented in settings where expertise in genetics and genetic testing may be limited, it is important that providers have access to information about how to offer *CYP2C9* and *VKORC1* testing and to interpret the results. In addition, they should be able to answer most of the patient questions that will arise. There also needs to be access to expert genetic resources, in the event that difficulties arise. The Secretary's Advisory Committee on Genetic Testing (SACGT) has suggested a genetic test template to inform and educate health professionals (Federal Register, 2000). The seven elements include the purpose of testing, clinical condition addressed, specific laboratory measurements, analytic validity, clinical validity, clinical utility and cost of testing. Additional elements might include laboratory-specific components such as sample type and volume, turn-around-time, and content of reports.

Equally important are high quality, validated educational materials for patients to help them make informed decisions. Content of patient education materials has been discussed, and includes the intended purpose of testing, test performance, risks, limits and benefits, rights of patients, description of condition, genetic counseling, test interpretation, treatment options and costs to the patient.⁶⁹ Because the average literacy level of the population in the United States is between the 6th and 8th grade, it is important to consider reading level for patient materials. Simple and clear language is necessary so that the message is understood. The Suitability Assessment of Materials (SAM), a well-tested evaluation system for health-related educational materials for patients, is now in widespread use. It provides ratings based on 22 factors in six categories: content, literacy demands, graphics, layout and typography, learning stimulation and cultural appropriateness.⁷⁰

Gap in Knowledge: There is a lack of existing validated educational materials for both patients and providers for use in pharmacogenetic testing in general, and for *CYP2C9* and *VKORC1* allele testing in particular.

39. Are there informed consent requirements?

There are currently no formal consent requirements for *CYP2C9* and/or *VKORC1* allele testing, although at least one clinical testing laboratory recommends having consent (ARUP Laboratories). These variant alleles provide information on how the patient may respond to warfarin, along with other drugs. It is possible, however, that in the future, these variants (SNPs) may be

found to have other clinical implications (e.g. responses to other medicines or susceptibility to an unrelated disease).

A new set of standards and guidelines for pharmacogenetic testing, currently under consideration by the National Academy of Clinical Biochemistry (NACB), suggest that informed consent be given for genotyping information to be released to drug-dispensing organizations to be used as part of its safety verification procedures (www.nacb.org/lmpg/LMPG-pharmacogenetics.pdf). The American College of Medical Genetics is currently studying the issue of informed consent for genetic testing (Quality Assurance Committee) and may offer specific guidance for pharmacogenetic testing.

New York State regulations require that the laboratory make a reasonable effort to document consent prior to testing. This can be in the form of a patient or physician signature. Existing CLIA regulations do not require that laboratories document informed consent, but current CLIA recommendations do include this requirement. The NCCLS Molecular Guidelines state that the referring clinician has the primary responsibility for informing patients of the risks, costs and benefits of testing. Whether or not the laboratory is required to document consent is left to the discretion of the laboratory and any applicable federal, state or local requirements.

40. What methods exist for long term monitoring?

When introducing a new technology such as this, it is reasonable to establish a monitoring scheme that tracks implementation issues, tests performed, and impact on patient care. In the early stages, funded pilot trials are likely, and their reports are useful. If such testing were to be expanded to the general population, long term monitoring helps to document and ensure quality of service delivery, assess public health impact and identify areas of concern. Because of the complexity of pharmacogenomic testing, laboratories may be wary in making dosing recommendations; this may prove to be a barrier to use in some practices. Long-term monitoring programs should be capable of identifying practice patterns, arising problems, and effectiveness. The process requires cooperation between health care providers and screening laboratory personnel. Oversight by professional organizations and/or governmental agencies would be beneficial. A successful monitoring strategy involves ensuring that the data collected are useful.

Gap in Knowledge: If long-term monitoring schemes are to be established, what information will they collect, how will it be obtained, and how will it be supported?

41. What guidelines have been developed for evaluating program performance?

No specific guidelines for long-term program evaluation of pharmacogenomic testing have been published. The National Academy of Clinical Biochemistry (NACB) has promulgated clinical and laboratory guidelines for such testing, but these guidelines do not include program evaluation (http://www.nacb.org/lmpg/LMPG_Pharmacogenetics.pdf).

Gap in Knowledge: What guidelines should be developed for evaluating program performance of *CYP2C9* and *VKORC1* allele testing, and who should develop them?

ETHICAL, LEGAL AND SOCIAL IMPLICATIONS

42. What is known about stigmatization, discrimination, privacy/confidentiality and personal/family social issues?

The Genetic Information Nondiscrimination Act of 2005 was passed by the Senate in February 2005 and is supported by the President. This bill will need to complete the legislative process before it can be signed into law. This act prohibits a group health plan or health insurance issuer from adjusting premiums on the basis of genetic information or requesting or requiring an individual or a family member of such an individual to undergo a genetic test. This act further prohibits employment discrimination on the basis of genetic information. Absent a national standard, many states have enacted legislation to specifically define how genetic information can be used.

A premise exists that, through the use of pharmacogenomic tests, existing or newly formed groups face discrimination in healthcare.^{71, 72} The use of biological markers may enhance prejudice and may lead to stigmatization.⁷² The stratification of the population into genetic subgroups may mean that the costs of developing new drugs for small populations may be prohibitively expensive. Therefore, effective therapies for these groups might not be developed.

The Health Insurance Portability and Accountability Act of 1996 (HIPAA) (hhs.gov) required the Department of Health and Human Services to develop standards for protecting the privacy of individually identifiable health information from inappropriate use and disclosure. The resulting Privacy Rule came into effect on April 14, 2003. Within the Privacy Rule, genetic information is treated as all other "Protected Health Information." The Privacy Rule does not preempt more stringent state law; therefore, there are many state laws that prevail over the Privacy Rule.

The Nuffield Council on Bioethics Report⁷³ suggests that "the likelihood that pharmacogenomic data will be of relevance to family members is low". However, since SNP testing has not been widely studied and SNPs are heritable, it may be too early to decide definitively if this statement will be upheld.

Pharmacogenetic testing for *CYP2D6*, in the context of tamoxifen use, is already being marketed directly to consumers (www.DNAdirect.com). Stand-alone *CYP2D6* testing for generalized drug metabolism is advertised, but not yet available. The issues of direct-to-consumer marketing of genetic tests have been discussed elsewhere.^{74, 75} It is likely that *CYP2C9* and *VKORC1* testing will also be offered directly to consumers in the near future.

43. Are there legal issues regarding consent, ownership of data and/or samples, patents, licensing, proprietary testing, obligation to disclose, or reporting requirements?

It has been recommended that, if information about unrelated medicines or diseases is likely to be obtained from pharmacogenomic testing, or if the results of the test will have a significant impact on the health or lifestyle of the patient, written consent may be appropriate.⁷³ Even if it is decided that consent is not required, written information (e.g. education materials) should be supplied.

Ownership of data and/or samples submitted for clinical testing has not, as of yet, presented any cause for litigation.

A number of biotechnology companies have developed or are developing unique testing methodologies for *CYP2C9* and *VKORC1* alleles. Some of these technologies have been or are likely to be patented.

Legal implications may arise as pharmacogenomic testing becomes widespread. For instance, will providers and drug companies be held liable for not considering genetic information? The Privacy Rule (Question 42) prohibits inappropriate disclosure of genetic information.

44. What safeguards have been described and are these safeguards in place and effective?

There are limited safeguards for pharmacogenomic testing. Proficiency testing for clinical laboratories is scheduled to start in 2007. If implemented, this will provide some level of oversight on the analytic validity of the testing process. The National Academy of Clinical Biochemists (NACB) is in the process of establishing "Guidelines and Recommendations for Laboratory Analysis and Application of Pharmacogenetics to Clinical Practice" http://www.nacb.org/Impg/LMPG_Pharmacogenetics.pdf. The Food and Drug Administration's Center for Devices and Radiological Health has issued draft guidance for industry entitled "Pharmacogenetic tests and genetic test for heritable markers" that contains non-binding recommendations for developing and validating pharmacogenetic tests in the U.S. (<http://www.fda.gov/cdrh/oivd/guidance/1549.pdf>).

The Food and Drug Administration Clinical Pharmacology Subcommittee of the Advisory committee on Pharmaceutical Science has recommended that DNA genotyping for *CYP2C9* and *VKORC1* variants be performed for all patients starting warfarin.

Coverage position statements by several health care providers state that drug metabolizing enzyme genotyping systems are considered experimental, investigational or unproven (<http://www.aetna.com/cpb/data/CPBA0715.html>, http://www.cigna.com/health/provider/medical/procedural/coverage_positions/medical/mm_0381_coveragepositioncriteria_AmpliChip.pdf). These statements call for randomized controlled trials to document the clinical utility of genotyping to improve health outcomes. The Canadian Coordinating Office for Health Technology Assessment (CCOHTA) concluded that prospective studies are needed to assess the benefits and potential risks of this technology (AmpliChip CYP450 test) in guiding drug selection and dose adjustment. Until such studies are available, drug metabolizing enzyme test results can only supplement other tools for therapeutic decision making, with routine monitoring by a physician (http://www.cadth.ca/media/pdf/375_armplichip_cetap_e.pdf).

Gap in Knowledge: If the proposed revision in the warfarin label is accepted, will the possibility of conducting randomized controlled trials be affected?

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A Rapid ACCE¹ Review of *CYP2C9* and *VKORC1* Allele Testing to Inform Warfarin Dosing in Adults at Elevated Risk for Thrombotic Events to Avoid Serious Bleeding

APPENDICES

Appendix A – Anticoagulation Therapy: Standard Practices

Appendix B - *CYP2C9* genotype and average daily warfarin dose in individuals with stable INR
(Data tables and summary figures)

Appendix C - *VKORC1* genotype and average daily warfarin dose in individuals with stable INR
(Data tables and summary figures)

Appendix D – Published models predicting warfarin dose at steady state INR using both
CYP2C9 genotype and *VKORC1* haplotype

Appendix E – Summary of the *CYP2C9* and *VKORC1* studies used in genotype/phenotype
analyses

Appendix F - Rapid ACCE Methodology

Appendix G – Reviewer’s comments and responses

Appendix A. ANTICOAGULATION THERAPY: STANDARD PRACTICES

The major indications for warfarin therapy are:

- Patients with atrial fibrillation, particularly those at high risk of stroke⁷⁶;
- Patients recovering from major orthopedic surgery, e.g. total hip or knee arthroplasty;
- Patients with a mechanical prosthetic heart valve;
- Patients with a history of venous thromboembolic event e.g., deep vein thrombosis (DVT) or pulmonary embolism (PE);
- Patients with a history of cardioembolic stroke.

General Information

Warfarin may be administered in either an acute or outpatient setting. Hospitalization is necessary for an acute condition requiring a rapid anticoagulation effect e.g. for acute atrial fibrillation (presenting either with symptoms alone or as a result of cardiac surgery), major orthopedic surgery, or a venous thromboembolic event. In non-urgent situations warfarin can be initiated in an outpatient setting. For example, warfarin therapy may be initiated in patients presenting with a long history of symptoms of atrial fibrillation but who are not hemodynamically unstable.

Warfarin initiation and maintenance (including hospitalization and post-discharge followup) may be managed by referral to an anticoagulation clinic service (ACS) or by the individual attending physician (community practice). In community practice, warfarin may be initiated and managed by an internist or internist-specialist (e.g. cardiologist), or by a family practice physician, depending on the patient indication for treatment and geographic location.

There are different ACS models; most are directed by a clinical pharmacist with or without a physician medical director (in many states pharmacists may enter into written agreements with physicians in order to manage drug therapy for patients). Staff is likely to include other clinical pharmacists, nurse specialists and/or physician assistants. Other ACS models include primary management by nurse specialists. Where present, ACS medical directors tend to reflect the patient case mix and may be hematologists, internist/specialists, surgeons, or family practice physicians.

Several articles have compared the results of long-term anticoagulation management in these two settings. Most recently, a systematic review and meta-regression indicates that patient populations in community practices have poorer control (i.e. spend less time in their INR target range, see below for INR information) than those attending anticoagulation clinics or enrolled in clinical trials (-12.2%; 95% CI, -19.5 to -4.8%; $p < 0.0001$).⁷⁷ Witt *et al.*⁷⁸ conducted a retrospective, observational cohort study (n=6,645) in a group model health maintenance organization and found that patients managed by an anticoagulation service were 39% less likely to experience an anticoagulation-related adverse event than were patients managed by their personal physicians (hazard ratio, 0.61; 95% confidence interval, 0.42 to 0.88). However, some comparative studies indicate modest⁷⁹ or no difference⁸⁰ in outcomes between management settings.

Portable coagulometers are also available for patient self management and several small studies have suggested that long-term self-management may be feasible and comparable to physician or clinic management. Larger studies have generated contradictory results^{62, 81} and a large comparative study under the auspices of the Department of Veterans Affairs Cooperative Studies Program is currently underway⁸².

Several published guidelines comprehensively address the initiation and management of anti-coagulation therapy for a variety of patient indications. The Seventh ACCP Conference on Anti-thrombotic and Thrombolytic Therapy^{2, 57, 58, 76} series of evidence-based guidelines describe the process, recommended anticoagulation for various patient indications, and adverse events of anticoagulation. A listing can be found at http://www.chestjournal.org/content/vol126/3_suppl/. Additional comprehensive guidelines include the American Heart Association/American College of Cardiology⁸³; the Institute for Clinical Systems Improvement (<http://www.icsi.org/knowledge/detail.asp?catID=29&itemID=151>), and the British Committee for Standards in Haematology⁸⁴. Several other guidelines address specific patient indications.⁸⁵

Initiation of Therapy

Starting dose. Warfarin is usually initiated at a fixed dose, commonly 5 mg daily. A lower starting dose (e.g. 2.5) may be selected for elderly patients and for those with severely impaired hepatic function, other relevant comorbidities, or severe malnutrition. In contrast, young, healthy patients with newly diagnosed thrombotic conditions may be started at higher doses up to 10 mg daily. Adjustments may also be made for concomitant medications that inhibit warfarin metabolizing enzymes (*CYP2C9*), those that compete with warfarin for metabolism, or Vitamin K disrupters (e.g. prolonged course of certain antibiotics). Loading doses are not recommended. Algorithms to determine the starting dose based on patient clinical characteristics have been published but are seldom used for initiation. Algorithms for dose adjustment within the first several days of therapy may be employed when hospitalized patients are monitored daily.

Heparin bridging. When warfarin is initiated while patients are in an active thrombogenic state (e.g. post-operative), levels of certain coagulation factors (protein C and S, and factor VII) decline more rapidly than those of other factors, creating the potential for thrombosis during the first few days of treatment. This procoagulant effect of unopposed warfarin can be counteracted by overlapping heparin or low molecular weight heparin (LMWH) treatment with warfarin for 3-5 days, allowing heparin to act as a bridge to the full anti-coagulant effect of warfarin. Heparin bridging may also be applied when warfarin is discontinued.

Monitoring. Warfarin anticoagulation must be sufficient to avoid thromboembolic events. However, excessive anticoagulation can result in severe, possibly fatal bleeding events. The therapeutic window is narrow and therapy is monitored by the international normalized ratio (INR), which is a standardized measure of the patient's prothrombin time (PT) such that the result is comparable across laboratories and test reagents.⁵⁶

The target INR range is 2.0-3.0 for most patients; exceptions include a slightly higher target range (e.g. 2.5-3.5) for patients with certain types of prosthetic heart valves⁵⁷ and a lower target (1.5) for patients with coronary artery disease at particularly high risk of coronary events⁵⁸.

INR monitoring usually begins 2-3 days after the initial dose. In an acute, hospital setting, patients may be monitored daily; in an outpatient setting, 2-3 times weekly is recommended. When the target INR is achieved and sustained for at least 2 consecutive days, 2-3 times weekly is recommended for 1-2 weeks. If the INR remains stable, the interval can be gradually increased up to every 4 weeks. Although a pharmacokinetic steady state is usually achieved in 6-12 days, pharmacodynamic stability is highly variable and may take much longer.

Adverse events. The intensity of anti-coagulant therapy, as measured by the INR, is strongly related to the risk of major bleeding. The frequency of major bleeding in patients enrolled in clinical trials and assigned an INR target range of 2.0-3.0 is less than in those assigned to INR>3.0.² The risk of intracranial hemorrhage increases rapidly with INR>4.0. The risk of

thromboembolic events as a result of under-anticoagulation depends on the patient indication and the risk prior to treatment and must be balanced against the risk of bleeding when considering and adjusting anticoagulation therapy. Bleeding risk can be predicted using published models^{1, 59} and can help in decision-making.

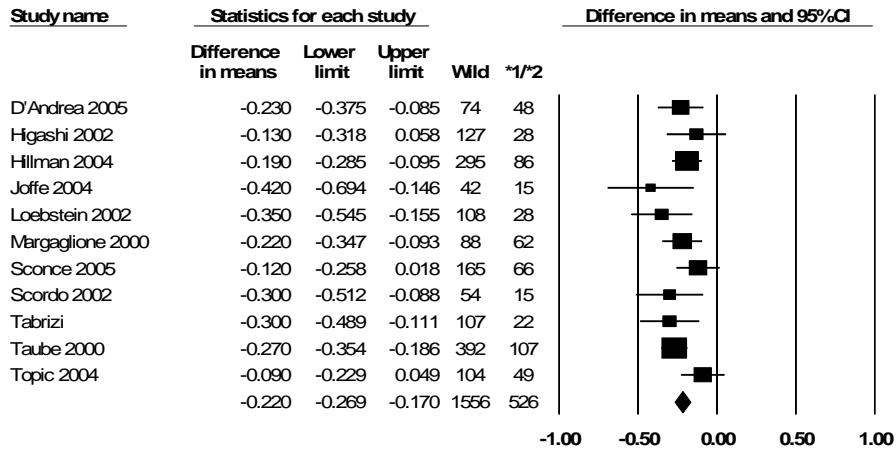
There is general agreement that the frequency of bleeding is higher early in the course of therapy, when INR fluctuations are more common.² Thus, the greatest concern is the possibility of over anti-coagulation at the first INR after initiation of therapy. In general, reducing INR fluctuations outside the target range should reduce the frequency of adverse events. Primary physician education and adherence to monitoring guidelines, referral to ACSs, or patient self-testing/management may improve anticoagulation control.

Maintenance

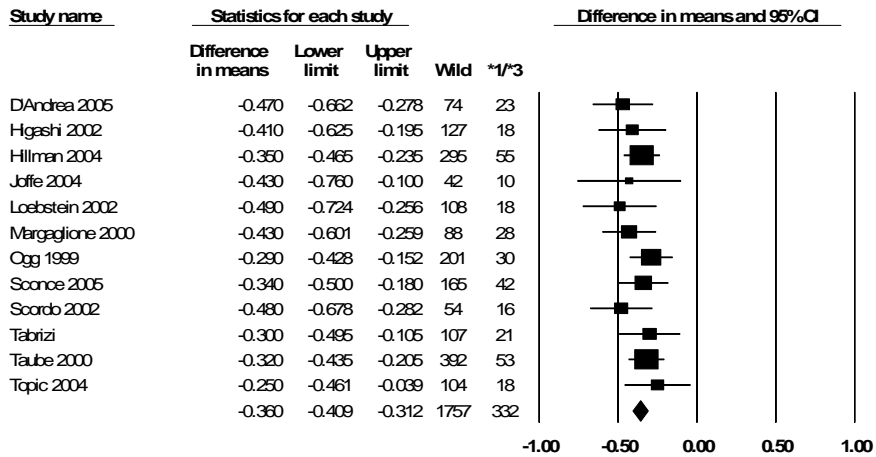
The goal of long-term anticoagulation monitoring is to maintain the patient in the INR target range; success is measured as percent time in the therapeutic range (TTR) and avoidance of adverse events. The stability of therapy over time may be influenced by changes in concomitant medications (including “alternative” and over-the-counter medications), health status changes that affect warfarin disposition or vitamin K-dependent coagulation factors, dietary or gastrointestinal factors affecting vitamin K (e.g. alcohol use, irregular ingestion of vitamin K-rich foods, changes in intestinal absorption capacity). It is important that the health care provider monitor at appropriate intervals, consider any changes in status, and make necessary and appropriate dose adjustments to maintain INR in the target range. In addition, patient communication, education, and compliance are important determinants of success. Finally, active intervention may be required when the INR is excessively prolonged and the patient has active bleeding or is at high risk for bleeding.

Appendix B. CYP2C9 genotype and average daily warfarin dose in individuals with a stable International Normalized Ratio (INR)

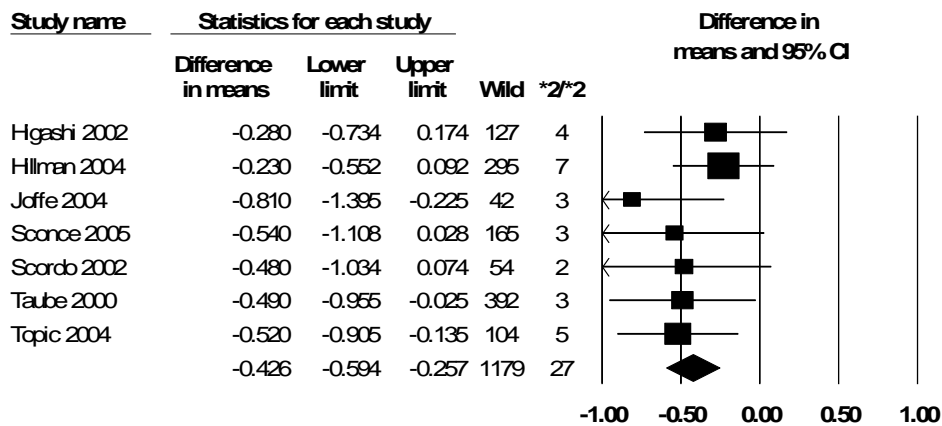
CYP2C9 Genotype and Warfarin Dose



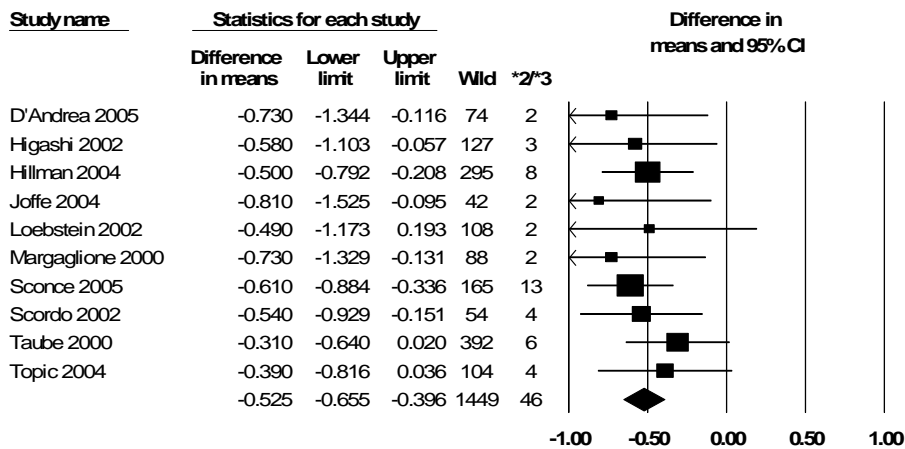
CYP2C9 Genotype and Warfarin Dose



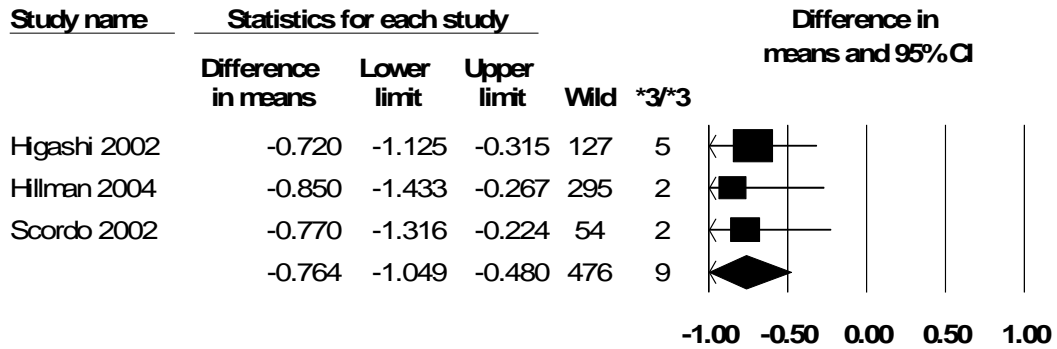
CYP2C9 Genotype and Warfarin Dose



CYP2C9 Genotype and Warfarin Dose

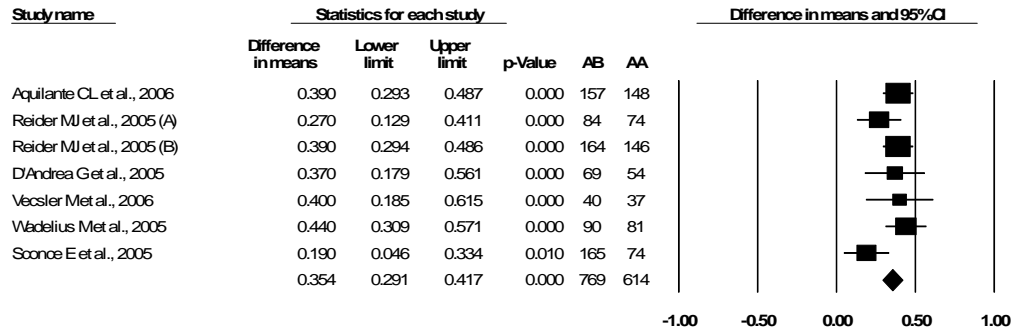


CYP2C9 Genotype and Warfarin Dose

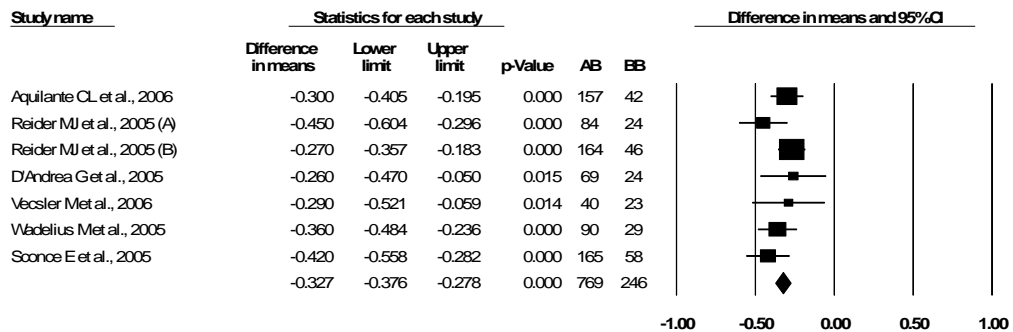


Appendix C. VKORC1 genotype and average daily warfarin dose in individuals with a stable International Normalized Ratio (INR)

VKORC1 Haplotype and Warfarin Dose



VKORC1 Haplotype and Warfarin Dose



Appendix D. Relative warfarin dose from four models, by CYP2C9 & VKORC1 test results

Sconce et al.⁹							
VKORC1 Haplotype	CYP2C9 Genotype						Relative Dose
	Rapid	Inter	Poor				
	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3	
High (BB)	100%	80%	75%	62%	58%	53%	100%
Medium (AB)	80%	62%	57%	46%	42%	39%	79%
Low (AA)	62%	46%	41%	33%	30%	27%	60%
Relative Dose	100%	78%	73%	59%	54%	50%	67%
$\text{Warfarin dose} = (0.628 - 0.0135 \cdot \text{age} - 0.240 \cdot \text{CYP}^*2 - 0.307 \cdot \text{CYP}^*3 - 0.241 \cdot \text{VKOR} + 0.0162 \cdot \text{ht})^2$ Where age=65, height=170 cm, CYP*2=0, 1 or 2, CYP *3=0, 1 or 2 and VKOR = 0, 1 or 2							

Takashshi et al.⁵							
VKORC1 Haplotype	CYP2C9 Genotype						Relative Dose
	Rapid	Inter	Poor				
	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3	
High (BB)	100%	74%	74%	57%	57%	57%	100%
Medium (AB)	80%	54%	54%	38%	38%	38%	78%
Low (AA)	56%	30%	30%	13%	13%	13%	51%
Relative dose	100%	69%	69%	49%	49%	49%	62%
$\text{Warfarin dose} = 6.656 - 0.035 \cdot \text{age} + 0.031 \cdot \text{weight} - 1.706 \cdot \text{CYP_he} - 2.815 \cdot \text{CYP_ho} - 1.316 \cdot \text{VKOR_he} - 2.941 \cdot \text{VKOR_ho}$ Where age=65, weight=74 kg, CYP_he=0 or 1, CYP_ho=0 or 1, VKOR_he=0 or 1, and VKOR_ho=0 or 1							

Vescler et al.¹⁰							
VKORC1 Haplotype	CYP2C9 Genotype						Relative Dose
	Rapid	Inter	Poor				
	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3	
High (BB)	100%	92%	92%	85%	85%	85%	100%
Medium (AB)	91%	84%	84%	76%	76%	76%	91%
Low (AA)	83%	75%	75%	68%	68%	68%	82%
Relative dose	100%	92%	92%	84%	84%	84%	62%
$\text{Warfarin dose} = \text{constant} - 0.252 \cdot \text{age} + 0.281 \cdot \text{weight} - 0.397 \cdot \text{CYP} + 0.455 \cdot \text{VKOR}$ Where constant=2, age=65, weight=70 kg, CYP=0, 1 or 2, VKOR=0, 1 or 2, other genes = 0, drugs = 0, and Vit K = ?							

Aquilante et al.⁷							
VKORC1 Haplotype	CYP2C9 Genotype						Relative Dose
	Rapid	Inter	Poor				
	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3	
High (BB)	100%	77%	77%	72%	72%	72%	100%
Medium (AB)	67%	44%	44%	39%	39%	39%	64%
Low (AA)	41%	18%	18%	13%	13%	13%	36%
Relative dose	100%	69%	69%	62%	62%	62%	66%
$\text{Dose} = (19.9 - 23.3 \cdot \text{VKOR_he} - 13.2 \cdot \text{VKOR_ho} + 0.21 \cdot \text{age} - 8.9 \cdot \text{CYP_he} - 10.9 \cdot \text{CYP_ho} - 0.23 \cdot \text{age} + 9.9 \cdot \text{mlINR} - 6.2 \cdot \text{glINR} + 0.03 \cdot \text{Vit K}) / 7$ Where age=69, weight=75 kg, VKOR_he & ho=0 or 1, CYP_h & ho=0 or 1, other genes=neg, mean INR=2.5, goal INR=1 or 2, smoking=0, CYP inhib=0, CYP induc=0							

These models are presented only as examples and should not be considered to be correct implementation. The authors often did not provide sufficient information to fully implement their model. We attempted to construct as complete a model as possible but may not have succeeded.

Appendix E: Study summaries for genotype/phenotype analyses

Below are short summaries and study grade for the 11 studies providing information about CYP2C9 genotype and stable daily warfarin dose. All studies reported genotyping occurring after stable INR was achieved.

- Ogg MS *et al.*³³, published a short letter reporting genotyping results of patients in the U.K. enrolled in the double-blind placebo-controlled 'Thrombosis Prevention Trial'. DNA testing could be completed for 1392 of the 5499 men in the study. Of the 1392, 233 received warfarin or warfarin plus aspirin. Summary means and SD's were provided only for Leu359 (*3). Study strength: marginal, because only 25% of the population could be tested and only the *3 variant was tested.
- Taube J *et al.*⁵¹, collected 683 patients submitting samples for INR testing at a single laboratory in the U.K. Additional information collected included warfarin maintenance dose and target INR. The 561 patients with a target INR of 2.5 were selected for genotyping. Summary means and SD's were provided for both *2 and *3 alleles. Study strength: marginal, due to opportunistic sample collection, arbitrary criteria for genotyping.
- Margaglione M *et al.*³⁴, approached 203 individuals attending a coagulation clinic in Italy. 199 were prescribed warfarin, 15 refused participation and 4 had DNA assay failures. All possible *2 and *3 genotypes were reported along with mean and standard deviation of warfarin dose. Study strength: good.
- Loebstein R *et al.*⁴⁸, identified 174 consecutive patients in Israel receiving warfarin who had a stable INR for 4 clinic visits while receiving the same dose. Population represented a good cross-section of indications. Both *2 and *3 variants were identified, but the two *3/*3 individuals were grouped with the *1/*3 heterozygotes. Study strength: good.
- Scordo MG *et al.*⁴⁹, studied 93 Italian outpatients all suffering from cardiovascular disease. Mean and standard deviation of warfarin dose was reported for genotypes that included the *2 and *3 variants. Study strength: adequate, due to opportunistic method of identifying patients and relatively small sample size.
- Tabrizi AR *et al.*⁵⁰, studied 153 patients in the US receiving warfarin therapy for cardiovascular disease. Most (78%) were Caucasian; the remaining patients were African American. Both the *2 and *3 variants were identified. Mean and standard deviation of warfarin dose was reported by genotype, but not by race (authors reported no significant difference in dose by race). Study strength: adequate, due to opportunistic method of identifying patients.
- Higashi MK *et al.*³¹, determined there were 286 eligible patients and randomly approached 213; 200 provided consent. Subsequently, five were excluded and PCR failed in another 10 leaving 185 patients to be studied. All were European Caucasians attending clinic in Seattle. Mean, median, inter-quartile range and standard deviation of warfarin maintenance dose were reported by race. Study strength: good.
- Topic E *et al.*⁵², studied 181 patients in Croatia receiving warfarin therapy for cardiovascular disease. Both the *2 and *3 variants were identified. Mean, median, standard deviation and range were provided for warfarin dose by genotype and for males and females. Study strength: adequate, due to opportunistic method of identifying patients.
- Joffe HV *et al.*³⁶, identified 135 consecutive patients receiving warfarin who had a stable INR value. Those with warfarin doses between 2-4 mg/day and between 6-10 mg/day were excluded. Both the *2 and *3 variants were identified. Mean and standard deviation of warfarin dose was reported by genotype. Study strength: marginal, due to opportunistic method of identifying patients, exclusion of patients by required dose and small sample size.
- Hillman MA *et al.*¹⁷, approached 600 patients and 453 agreed to participate. Both the *2 and *3 variants were identified and warfarin dose (mean and standard deviation) were provided

for all genotypes separately. A scatterplot of individual warfarin dose by genotype was also provided. Study strength: good.

- D'Andrea G *et al.*⁸, report on 147 of the 180 patients already reported by Margaglione *et al.*, 2000 and was not included in the *CYP2C9* analysis. The *VKORC1* (and accompanying *CYP2C9*) results are used in later analyses of reduced variance (see below).
- Sconce EA *et al.*⁹, include 121 patients reported in Kamali *et al.*, 2004, along with an additional 176 patients from the U.K. Both *2 and *3 variants were genotyped. Mean and standard deviations for warfarin dose by genotype were reported. A second cohort of 38 was used to study the effect of targeted individual dosing and was not included in this analysis. Study strength: adequate, due to opportunistic method of identifying patients.

Overall strength of evidence is high (multiple adequate or higher studies with homogeneity).

Below are short summaries and study grade for the six datasets providing information about *VKORC1* genotype and stable daily warfarin dose. All studies looked the interaction of *CYP2C9* and *VKORC1* genotypes.

- Rieder MJ *et al.*⁶, studied a 'primary' cohort that had been recruited as part of an earlier study (Higashi *et al.*, 2002 – see *CYP2C9* review above). *VKORC1* genotypes were defined as AA, AB and BB. Mean and standard error for the stable warfarin dose were reported for each genotype. Study strength: adequate.
- Rieder MJ *et al.*⁶, also studied a 'replication' population.
- D'Andrea G *et al.*⁸, (summarized above). Of the original 180 subjects studied, 147 had sufficient DNA to be tested for *VKORC1* genotypes. Mean and standard deviation of the maintenance warfarin dose were reported for the 1170C->T variant. Study strength: adequate, due to initial design and loss of 33 samples.
- Wadelius M *et al.*³⁷, enrolled 204 Swedish patients. Mean and standard deviation (along with box and whisker plots) for stable warfarin dose for the rs2359612 SNP.
- Aquilante CL *et al.*⁷, ran a multi-center study in Florida that identified 350 patients (mostly Caucasian males from VA hospitals). Mean, median, standard deviation and range of stable warfarin dose were reported for the 3676G->A variant. Study strength: adequate, due to opportunistic method of identifying patients.
- Vecsler M *et al.*¹⁰, identified 100 Israeli Jews with a stable warfarin dose seen at an Anticoagulation Clinic. Mean and standard error for the maintenance warfarin dose were reported for the 1542G->C variant. Study strength: adequate, due to opportunistic method of identifying patients.

Overall strength of evidence is high (multiple adequate or higher studies with homogeneity).

Information from six studies reporting both *CYP2C9* and *VKORC1* genotyping and performing regression analysis to determine the proportion of variability explained by genetic testing, along with a summary of those studies

Source	Country	Proportion of the population with the wild genotype (%)		Approximate proportion of overall variability in warfarin dose explained by				
		<i>CYP2C9</i>	<i>VKORC1</i>	<i>CYP2C9</i>	<i>VKORC1</i>	Weight	Age	All
Aquilante CL <i>et al.</i> , ⁷	US	71	40	14		11	4	51
Takahashi H <i>et al.</i> , ⁵	Japan			NR	NR	NR	NR	57
D'Andrea G <i>et al.</i> , ⁸	Italy	50	37	22	14	NR	6	57
Vecsler M <i>et al.</i> , ¹⁰	Israel	62	37	20	21	12	10	63
Wadelius M <i>et al.</i> , ³⁷	Sweden	69	37	11	29	5	10	56
Rieder MJ <i>et al.</i> , ⁶	US		37	8	25	NR	NR	NR
Summary ¹				17	23	9	7	56

¹ weighted mean value

Appendix F: Rapid ACCE Methodology

Difference between a Rapid and Standard ACCE Review: Both types of ACCE reviews are evidence-based with clear methodological and grading criteria. A full ACCE review is a comprehensive overview of all relevant data that is usually based on original publications. There is no restriction on topics that can be selected for a full ACCE review. Efforts are made to search both published and gray literature. It should be expected that a full ACCE review would take many months to complete and take at least \$50,000 to \$100,000 or more to complete. In contrast, a Rapid ACCE review generally is more applicable to an emerging application that has less literature and will usually ‘trim’ the question. For example, the current review on warfarin does not attempt to quantify all data on racial/ethnic groups other than non-Hispanic Caucasians. Some questions may not be dealt with in detail, especially some of those relating to contextual issues relating to implementation. Lastly, the Rapid ACCE review is somewhat more lenient with the interpretation of limited data as it tries to provide as much guidance as possible based on what may be very limited data. Depending on the amount of literature and availability of experts to aid the interpretation of complex issues, Rapid ACCE reviews might be completed for between \$10,000 and \$40,000 within a few months. More information about ACCE methodology and description of the 44 targeted questions has been published.⁸⁶

Identification of relevant publications: Targeted MedLine searches were made for each questions (or group of questions) for which published literature might be available. Reference lists from retrieved articles were also scanned for relevant publications. Meta-analyses were sought as a way to identify existing analyses and methodology.

Identification and use of gray literature: When published data were not available, or did not cover the question adequately, data were sought via the gray literature. This included FDA submissions, laboratory web-site information, abstracts and materials distributed at meetings. In some instances, individuals who likely held the relevant information were directly contacted and asked to collaborate. Data from the gray literature were labeled as such to avoid confusion with published literature.

Nomenclature for strengths: The following names and definitions were used throughout the review to summarize study strength and strength of evidence.

- Study strength evaluations accounted for study design (randomized trial being the highest), sample size, avoidance/identification of biases, description of population, comparison to a gold standard,
 - marginal – multiple deficiencies that cast doubt on the conclusions, gray literature
 - adequate – deficiencies identified, but conclusions likely to be reliable
 - good – few, if any deficiencies in study evaluation
- Strength of evidence combines the available studies with formal (or informal) tests of heterogeneity of effect
 - low – one, or several marginal to adequate studies with heterogeneity
 - medium – multiple adequate studies (or multiple studies with at least one good study) with homogeneity, or multiple good studies with heterogeneity
 - high – multiple good studies with homogeneity

Strength of effect is independent of strength of evidence. For example, there can be a high strength of evidence rating for a weak measure of effect (e.g., confident that the odds ratio is 1.3)

Appendix G Response to Comments of Reviewers

Thank you for the thoughtful comments that we received. Minor edits that were suggested have been made. Below are responses to the more substantive suggestions and/or questions. They have been ordered by major heading and by ACCE question number.

Disorder/Clinical scenario/Test

Question 1. The review explicitly focuses on bleeding events but we should recognize throughout that this is not all that is being considered clinically and that any real risk:benefit assessment would also have to include thrombotic events due to under-anticoagulation also. I recognize that the immediate focus is a safety one, but this cannot be at the expense of efficacy. We agree that warfarin efficacy is critical in the care of patients. However, in the proposal that this review committee accepted, it is specifically stated that “This review is not aimed at investigating possible reductions in the effectiveness of warfarin due to under-dosing.” In addition, one cost/benefit analysis included under-anticoagulation as an outcome in its model, but used the same rate in both the genotyping and non-genotyping arm.

Question 3. There is always the dichotomy between those who want to genotype and those who want to phenotype. For quite a number of years INR outliers were assessed by coumarin metabolite TDM testing. How the warfarin and metabolite tests are used and what the evidence is for the basis of the total warfarin metabolites, and free warfarin testing, would be helpful if discussed in the context of the proposed PGx testing. Are they complementary, would phenotyping be a better choice, will PGx replace much of the TDM, and so forth? What is the evidence for using phenotyping? Would this be a useful approach for CYP2C9 non- *1,*2, *3 individuals? These are great questions, but have to be considered outside of the targeted review that we undertook. However, we have added a sentence or two in this section noting these possible avenues of research for the future under a new ‘gap in knowledge’.

Question 4. I strongly disagree that which CYP2C9 variants should be tested represents a significant gap in knowledge. The principal variants affecting function *2 and *3 represent 90% of the variability in enzyme activity that is genetic in a wide range of studies of CYP2C9 phenotype using losartan, tolbutamide, flurbiprofen and a range of other probe drugs over the last 20 years. While there may be other functional variants that affect induction or inhibition of CYP2C9 in minor ways, these constitute the vast majority of the genetic effect on function. What you say may be true, but three of the manufacturers provide reagents to detect more than the 2 SNP’s for CYP2C9 and the 1 SNP for VKORC1. Also, the NACB Standards and Guidelines for Pharmacogenetics specifically recommends testing for CYP2C9 *2, *3, *4, *5 and *6 for warfarin applications. This information has been added to the text to place the gap in knowledge in context.

Question 4. There may be some discomfort from the use of wild or wild-type when referring to patients. Suggest conferring with Michael Watson on the recommended terminology from ACMG. Wild is the term used for the common allele/genotype in virtually all of the publications reviewed. It seems reasonable to keep with the convention.

Question 7. There should be a small discussion, as background information, of the INR and the problem with INRs: that they may have different results on the same patient depending where they are done and how they are done, considering a mobile population in

the USA and the problems of harmonizing laboratory vs POC INRs. Also a small paragraph on the adequacy/inadequacy of INR without considering a PGx component. This has been added as a gap in knowledge.

Analytic Validity (Question 8 through 17)

Question 8: In Table 2 (as well as Fig 8 and 10) - Designation of VKORC1 allele types: Rapid, intermediate and slow are used to describe VKORC1 which is a pharmacodynamic target, not a pharmacokinetic enzyme. Normal, intermediate and decreased expression of VKORC1 would be more appropriate, or a sentence is needed linking VKORC1 haplotype to warfarin metabolizer phenotype. We have changed the terminology in the figures and added some explanation in Question 8, Table 2 regarding pharmacokinetics and pharmacodynamics.

Question 9/10. I would endorse the gap in knowledge indicating that there are few data for homozygote and compound heterozygote CYP2C9 genotypes (2*/2*, 3*/3*, 2*/3*) and for non-Caucasian alleles, but would argue that these are very rare genotypes and that they are even more rare in Asian populations. The gap might therefore be in African populations where genetic variability is always greater, but even in this case there exist more than 100 resequenced individuals within the Coriell sample sets. Although these genotypes are rare, it is important to document that the testing methods used are analytically valid when they do occur.

Question 9/10. The gap in knowledge as relates to no published peer-reviewed data concerning analytic specificity/sensitivity for VKORC1 genotyping against a 'gold standard' referent method may not exist once the data on the public databases (PharmGKB, Sabta Cruz, Japanese Gnome project and BCBI) are incorporated. We have added this information to the review but have retained the gap in knowledge.

Question 11. Did you contact the group in Louisville (Valdez et al) as they do a fair bit of testing? Yes, their laboratory is PG_{XL} laboratories. I contacted Dr. Mark Linder and he had agreed to send data for the analytic validity section of this report, but I never received them.

Question 14. Comments regarding confirmatory testing are out of the clinical context. If we were in a purely genetic context where no phenotype of effect was available, this would be a reasonable discussion. In reality unexpected changes in the INR would likely guide retesting and protect against the rare errors that might occur. The same points would apply to Question 20 on methods to resolve clinical false positive results in a timely manner. This concept of identifying false positive (or false negative) results via clinical phenotype is interesting and it has been included in both questions 14 and 20.

Question 16. Is the DNA used in some of these research studies more degraded than when used for the first time in clinical testing? We seem to have better first time call rates with our clinical assays than with research samples sent on to us. This is possible. The lab that reported these failure rates does not provide clinical testing, so there is no direct comparison. We have added the following text: "These assay failure rates may be higher in research settings versus clinical settings due to handling of samples (e.g. frozen vs fresh)."

Clinical Validity (Question 18 through 25)

Question 18/19. Table 7: relative risks and sensitivities are discussed but I think a more useful approach would be the same one that we normally use to compare drugs, based on the ABSOLUTE risk. Relative risks hide the incident rates of an event and the number needed to treat is therefore the clinically preferred metric used by the American College of Physicians, ASCPT and the AMA and FDA ...the number needed to test (referred to later in the review) to prevent a serious bleed would therefore be a valuable number to have. The number needed to treat is more of a clinical utility question, as it requires a measure of treatment effectiveness. For this reason, we have included estimates of NNT under Question 35/36 that deal with economics.

Question 24: On page 25, I don't understand at all the gap in knowledge cited as : **What are the roles of novel variants in CYP2C9 and VKORC1 in warfarin metabolism? What are the roles of other genes in the warfarin metabolism pathway on the impact of warfarin dosage requirements?"** This has been modified to include possible additional gene impact on both the kinetics and dynamics of warfarin dosage requirements.

Clinical Utility (Question 26 through 41)

Question 28. Would the PT testing not be the equivalent of a diagnostic confirmation in that they diagnose the events being monitored and managed? The fact that not everyone with mutations will have elevated INRs and those without the mutations may have high INRs requiring adjustments obviously complicates this. Did you consider susceptibility tests when you developed the ACCE criteria? It seems that there should be some correlate to confirmatory testing in this setting. The ACCE questions are designed to be widely applicable and, occasionally, there are questions that don't seem appropriate for a given disorder/clinical scenario/test. Confirmatory testing was covered earlier

Question 30. Here you mention that most community anticoagulation is done by internists. Is this what the articles say, or is this speculation? Margaret Piper received that information from the MDs with whom she spoke. We were unable to find any specific utilization data in the literature. The text has been changed to read "In community practice, warfarin may be initiated and managed by an internist or internist-specialist (e.g. cardiologist), or by a family practice physician, depending on the patient indication for treatment and geographic location."

Question 36. In this question, I think we should at least mention that the cost per case avoided does not include any offset for costs to the medical system avoided when a patient doesn't have a severe bleeding event. A brief cost per major bleeding event and cost per hemorrhagic stroke (acute and long-term) have been added with appropriate reference.

Question 36. While estimates of cost are inevitably speculative and highly assumption-dependant, the cost described of a single bleeding episode (many of which are fatal and result in the complete loss of any contribution to society) would appear low, and the cost of genetic testing would appear high. The costs of tests are likely to soon be in the \$100 range with the cost of the clinical advice bundled into the routine cost of care without a special counseling fee. We would agree that the true costs of testing might be in the \$100

range. However, in talking with manufacturers, laboratories and providers, it is clear that the costs over the short term will be much higher (discussions at recent ASHG meeting).

Question 36. I assume there was no data on whether reduced inpatient times due to more rapid stabilization of INR led to cost savings. I suspect that this will be one of the more significant places where cost savings will occur. This is a good point that we have added to the discussion, but we did not find any data on the topic.

Question 37. One concept that needs to be addressed is the timing of the testing. This will have much to do with where testing is done. If the goal is to have the information in the hands of the providers at the time of the first Warfarin dose, it is unlikely that a national referral laboratory would be able to turn the test around fast enough to be useful. This will definitely affect uptake and utility of the testing. We have added the following text: “However, it is not known if laboratories can provide the genotype result within three days, which is when differences in required dose by genotype (due to higher INR levels) have been shown (Question 18).”

Question 38. We may need to decide if patient education materials are necessary for this type of genetic testing. We don't generally talk about other tests, such as renal function studies, that we might do to inform warfarin dosing. In this context, should pharmacogenetic testing be treated differently? It is possible that patient education materials may not be as important as for other genetic tests. However, the ELSI section of this review raises some issues that should be discussed.