article

A Rapid-ACCE review of *CYP2C9* and *VKORC1* alleles testing to inform warfarin dosing in adults at elevated risk for thrombotic events to avoid serious bleeding

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Purpose: Summarize evidence regarding genetic testing in adults to inform warfarin dosing to reduce adverse drug events such as serious bleeding. **Methods:** Review published (and selected gray) literature using the Rapid-ACCE structure that addresses analytic validity, clinical validity, clinical utility, and ethical, legal, and social implications. **Results:** Preliminary data suggest overall analytic sensitivity and specificity will be 98% or higher for *CYP2C9* genotyping, but strength of evidence for analytic validity is low, especially for *VKORC1* testing. Strength of evidence is high for the clinical validity of both genes in predicting stable warfarin dose, an intermediate outcome, but is low for the association between *CYP2C9* testing and severe bleeding events (clinical sensitivity 46% (95% CI 32–60%); specificity 69% (95% CI 62–75%) and absent for bleeding events associated with *VKORC1* testing. No data are available to document clinical utility of genotyping before warfarin dosing. **Conclusions:** The most important gaps identified are: which variants should be included in a testing panel, lack of data from external proficiency testing, lack of validated dosing algorithm incorporating genetic and nongenetic factors, evidence of clinical utility, reliable economic analyses, and methods to address several ethical, legal, and social implications issues. ***Genet Med* 2008:10(2):89–98.**

Key Words: *evidence review, warfarin,* CYP2C9*,* VKORC1*, severe bleeding*

The main aim of this rapid-ACCE (Analytic validity, Clinical validity, Clinical utility, and Ethical, legal, and social implications) review[1] is to systematically collect and evaluate the evidence regarding the efficacy of identifying cytochrome P-450 2C9 (*CYP2C9*) and vitamin K epoxide reductase complex 1 (*VKORC1*) alleles to guide warfarin dosing on the basis of this information, as a way to prevent occurrences of severe bleeding. The clinical scenario focuses on adult candidates for warfarin treatment, as a result of being at high risk for future thrombotic events. The clinical disorder(s) under consideration is a severe bleeding episode associated with warfarin treatment, such as hemorrhagic stroke, of sufficient severity to produce serious morbidity and mortality. Hemorrhagic events are a complication of warfarin drug treatment, because of the narrow therapeutic range. Thrombotic events are also a consequence of the narrow therapeutic range, but this evidence review was limited to the hemorrhagic events. The target range for monitoring warfarin therapy is an International Normalized Ratio (INR) value between 2.0 and 3.0 (slightly lower or higher for some conditions), which is a standardized measure of the patient's prothrombin time, such that results are comparable across laboratories and test reagents.[2] The risk for serious bleeding increases when INR values are 4.0 or higher, and such elevations are more likely to occur within the first few weeks after initiating warfarin treatment, before a stable dose and INR are achieved. It is likely that maintenance of warfarin doses will continue to be primarily based on INR measurements, but genotyping may be of help with initial dosing and obtaining stable INR more quickly.

The objectives of the present 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* alleles testing to guide warfarin dosing, (3) provide information to be used in developing patient/physician education materials, and (4) identify gaps in knowledge from which a research agenda can be developed. Understanding the extent of benefit to be gained by testing is important, because: (1) a large number of new warfarin patients per year might have genetic tests performed (hundreds of thousands to as many as 2 million), (2) up to 800 reportable adverse drug events associated with warfarin usage per year occur in the United States,[3] (3) the Food and Drug Administration has re-

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cently revised the Coumadin® label (and will revise the generic warfarin label) to include genomic test information without mandating genetic testing, and (4) *CYP2C9/VKORC1* testing services may soon be readily available. The complete evidence report is available at http://www.acmg.net.

MATERIALS AND METHODS

The ACCE methodology is specifically designed to facilitate the appropriate transition of genetic tests from investigational settings to clinical and public health practice.[1] The 44-question format was developed as part of a Centers for Disease Control and Prevention-sponsored project (CCU119356-01) to assess the availability, quality, and usefulness of existing data on DNA-based tests and testing algorithms. This methodology is both time consuming and expensive, with a full review taking many months to complete, at a cost of $50,000–$100,000, or more. In an effort to provide an evidence-based review in a more timely fashion, the rapid-ACCE methodology was recently developed.[4] Examples of when this methodology is appropriate include: topic areas with a small evidence base and topics that are narrowly defined (e.g., stakeholders require a review of only a limited set of ACCE questions or a review for a specific population or assay methodology). 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. This review utilizes the rapid-ACCE approach.

Before assessment begins, ACCE requires that the disorder, clinical scenario, and test(s) be clearly defined and agreed upon by all stakeholders. For this review, the stakeholder was an American College of Medical Genetics panel whose charge was to issue a recommendation for *CYP2C9/VKORC1* pharmacogenetic testing for warfarin/Coumadin administration (page 139, this issue).[5] This panel was composed of 14 experts in the area of pharmacology, molecular genetics, clinical genetics, bioethics, economics, and anticoagulation. The ACCE questions are divided into four major sections, defined as follows. Analytic validity refers to the ability of laboratory testing to correctly identify the genotypes of interest (analytic sensitivity and specificity) in the analytic, as well as preanalytic and postanalytic phases. Other areas included are robustness, repeatability, and quality control. Clinical validity refers to the test's ability to correctly identify the phenotype of interest. For example, clinical sensitivity for *CYP2C9* would be defined as the proportion of all severe bleeding events that occurred among nonwild genotypes. Clinical validity would also include the positive and negative predictive values (PPV and NPV), genotype/phenotype associations and penetrance. Clinical utility assesses risks and benefits of testing. For a test to have clinical utility, the results must be used to impact some aspect of patient care which leads to a measurable improvement in outcome that matters to the patient. Clinical utility also can include information regarding implementation issues such as pilot trials, needed resources, validated educational materials, and economic implications. Some of the ethical, legal, and social implications (ELSI) are included in other sections (e.g., informed consent, addressed as part of clinical utility). Other topics, such as discrimination, health disparities, patents, obligation to disclose, and the existence of effective safeguards, are addressed under this section.

Data used in each of these sections were obtained by literature searches (e.g., PubMed) and reference lists from retrieved articles. Meta-analyses were particularly sought as a way to identify existing analyses and methodology. 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.

Each study was evaluated for the strength of the study design (randomized trial being the highest), sample size, avoidance/identification of biases, description of population, and comparison to a gold standard. The ratings are as follows: marginal—multiple deficiencies that cast doubt on the conclusions, gray literature; adequate—deficiencies identified, but conclusions likely to be reliable; and good—few, if any deficiencies in study evaluation. The strength of evidence combines the available studies with formal (or informal) tests of heterogeneity of effect, with the following ratings: 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; and 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).

RESULTS

Analytic validity

The cytochrome P450 complex is a group of hepatic microsomal enzymes responsible for the oxidative metabolism of various substrates (pharmacokinetics). Thirty-seven *CYP2C9* haplotypes containing over 100 variants have been identified, but the literature focuses on two of these that are associated with reduced metabolism of warfarin. These are designated as *2 (R144C, 3608C>T) and *3 (I359L, 42614A>C) variants. *1 is the designation for the wild-type allele. The frequencies of the *2 and *3 variants are approximately 12.2% and 7.9%, respectively, in the European Caucasian population.[6] Individuals with the wild genotype reach a warfarin steady state in 3–5 days. Heterozygotes for *2 and *3 require 6–8 days and 12–15 days, respectively.[7] 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 <1%.[8] Table 1

Table 1
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		
Genotype	Enzyme production	Nomenclature
BB	Low (higher warfarin dose)	Normal, wild
AB	Medium	Heterozygote
AA	High (lower warfarin dose)	Homozygote

shows the most common *CYP2C9* genotypes, their associated warfarin metabolic rates and nomenclature.

Variants in the gene encoding *VKORC1* have also been determined to affect the response to warfarin via reduced enzyme activity (pharmacodynamics). 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 nomenclature by Rieder et al.[9] Table 1 shows the relationship between *VKORC1* genotype and warfarin dose. The frequencies of these genotypes have been estimated from data reported by several studies,[10–13] using a random effects model. Among non-Hispanic Caucasians, these frequencies are 35%, 47%, and 18% for the BB, AB, and AA genotypes, respectively. Other studies have reported wide variation of these frequencies by race/ethnicity (Question 22).[8,9,14–18] While *VKORC1* variants are considerably more prevalent than those of *CYP2C9*, there are fewer data available that characterize their analytic validity and clinical validity.

Nearly all available data for analytic validity refer to the detection of two variants in the *CYP2C9* gene; few data are available about detecting the variants in the *VKORC1* gene. Based on seven studies reporting performance in the analytic phase of testing (Table 2), assays for the common *CYP2C9* genotypes (*1/*2 and *1/*3) have an analytic sensitivity of 100% (95% CI 96.7–100%).[19–25] The analytic specificity is also 100% (95% CI 98.2–100%). 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 75–100%).[20,22,23,25] The bottom of Table 2 also contains information from the gray literature regarding both *CYP2C9* and *VKORC1* testing. No published information is available to directly estimate preana-

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

				CYP2C9 Analytic sensitivity (test result/referent result)					Analytic specificity
Reference	Year	Assay method	Referent method	(*1, *2)	(*2, *2)	(*1, *3)	(*3, *3)	(*2, *3)	(*1, *1)
Hillman et al.[22]	2004	LightCycler	Sequencing	2/2	1/1	—	1/1	1/1	4/4
Pickering et al.[23]	2004	Luminex, eSensor	Sequencing	15/15	1/1	13/13	—	2/2	70/70
Wen et al.[24]	2003	Microarray	Sequencing	—	—	7/7	—	—	13/13
Zainuddin et al.[25]	2003	Nested PCR	Sequencing	3/3	—	5/5	2/2	2/2	28/28
Eriksson et al.[21]	2002	Pyrosequencing	PCR-RFLP	9/9	—	5/5	—	—	9/9
Aquilante et al.[19]	2004	Pyrosequencing	PCR-RFLP	—	—	—	—	—	—
Burian et al.[20]	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*
Third Wave Tech	2006	Invader, Tag-It, Pyro	Sequencing	9/9	3/3	6/6	2/2	6/6	9/9
ARUP Laboratory	2006	Invader, Tag-It	Sequencing	9/9	—	1/1	—	—	21/21
LabCorp	2006	Invader, Tag-It	PCR-RFLP	6/6	1/1	5/5	1/1	4/4	5/5
				VKORC1					
				AB	AA				BB
Third Wave Tech	2006	Invader, Pyro	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

ARUP, Associated Regional and University Pathologists.

Table 3
Relative risk of INR values above 3.0 during warfarin induction, stratified by *CYP2C9* genotype

	Week after induction	Lindh et al., 2005[32]	Peyvandi et al., 2004[33]	All
Relative risk (*2 vs. *1/*1)	1	2.8 (1.2–6.7)[a]		
	2	2.1 (1.2–3.7)	1.9 (1.3–2.3)	1.8 (1.3–2.3)
	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)	2.5 (1.3–4.5)
	3	1.1 (0.6–2.0)		

*2 includes *1/*2 and *2/*2; *3 includes *1/*3, *3/*3, and *2/*3.
[a]95% confidence interval.

lytic or postanalytic errors. Depending on the methodology, sample type and sample condition, 1–5% of samples may experience repeated assay failures resulting in inconclusive test results[19] (see Question 16 in the full review for personal communications containing additional data). These failures can be viewed as reducing the analytic sensitivity and specificity.

Based on other molecular tests that have been studied in more detail (cystic fibrosis gene[26] and hereditary hemochromatosis gene[27,28]), working estimates of overall analytic sensitivity and specificity for the common *CYP2C9* genotypes are 98–99% and 99.5–99.75%, 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,[29] 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 200) among any genotype group. At least 12 laboratories in the United States now offer *CYP2C9* and/or *VKORC1* genotyping for clinical use (see full report, Table 3). Several manufacturers offer reagents to test for variants in both genes.

It appears that the methodologies used to identify *CYP2C9* and *VKORC1* variants can easily be completed in a day. Thus, turn-around-time $>$ 2 or 3 days will be because of slow transport of samples, or that the laboratory does not run the assay every day. Neither of these issues would be expected to impact analytic validity (other than to perhaps improve the quality of samples by shortening transport time). On at least one website offering testing, the laboratory turn-around-time is stated to be 1 day (http://www.kimballgenetics.com/tests.html).

The Genetic Testing Quality Control Materials Program at the CDC assists genetic testing laboratories in obtaining validated quality control materials. As part of this program, 96 samples from Coriell Cell Repositories (Camden, NJ) 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. Laboratories validating new assays can purchase these samples with known genotypes.

The College of American Pathologists has established a working group consisting of members from the College of American Pathologists/American College of Medical Genetics, Biochemical and Molecular Genetics, Special Chemistry, Toxicology, and Coagulation Committees, to develop a Pharmacogenomics (PGx) Survey for 2007. This PGx Survey will ship twice per year (April and September). Each shipment will contain two different vials of 25 μg each of extracted DNA, which participants will be able to test for genetic variations in the *CYP2C19, CYP2C9, CYP2D6, UGT1A1*, and *VKORC1* genes (see www.cap.org for updates).

Gaps in knowledge include: (1) which *CYP2C9/VKORC1* variants should be part of a clinical panel, (2) poorly defined analytic validity for the less common *CYP2C9* genotype (e.g., *3/*3), (3) published data on analytic validity for *VKORC1* against a "gold standard," (4) whether clinical laboratories are able to offer an appropriately validated test (e.g., variants included, turn-around time, costs, sample types, internal analytic validity studies), (5) limited information on long-term performance/consistency of methods (within-laboratory variability), (6) data showing between-laboratory consistency, (7) overall estimate of analytic performance including preanalytic and postanalytic error rates, (8) method-specific and sample-specific failure rates, and (9) data from the external proficiency testing program.

Clinical validity

Clinical validity was examined using one intermediate outcome (elevated INRs), as well as the health outcome of severe bleeding. INR values above 3.0 are twice as likely among *CYP2C9* heterozygotes (relative risk 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 (Table 3). This information is based on only two studies that were designed and analyzed differently.[34,35] A third study found a weak correlation between the rate of change in the INR values (slope) and *CYP2C9* genotype (nonwild genotypes had a higher slope, $P = 0.05$).[36]

Clinical sensitivity is defined as the proportion of individuals with the outcome of interest (severe bleeding) that have a genotype other than wild (i.e., *1/*2, *2/*2, *2/*3, *1/*3, *3/*3). This is synonymous with the detection rate. With nonwild

Table 4
Clinical sensitivity, clinical specificity, relative risk, and attributable risk for severe bleeding events (wild vs. nonwild *CYP2C9* genotype)

Study	Clinical sensitivity (%)	Clinical specificity (%)	Relative risk (%)	Attributable risk (%)
Ogg et al.[30a]	23	87	1.85	7
Margaglione et al.[31b]	67	53	1.91	12
Higashi et al.[32]	50	72	2.19	15
Wadelius et al.[33]	33	66	0.96	0
Summary of Higashi and Wadelius (95% CI)	46 (32–60)	69 (62–75)	1.7 (0.8–3.6)	7 (0–15)

[a]Considered only *3 genotypes (these estimates are not included in the summary line).
[b]Wild *CYP2C9* genotype frequency in Italy is low (these estimates are not included in the summary line).

CYP2C9 genotypes grouped together from two studies, the clinical sensitivity of *CYP2C9* to identify serious bleeding events is 46% (95% CI 32–60%),[32,33] indicating that about half of all serious bleeding events occur among *CYP2C9* wild-type individuals (Table 4). Clinical specificity is defined as the proportion of individuals with no severe bleeding that have the wild (*1/*1) genotype. One minus the clinical specificity is the false positive rate. The false positive rate indicates the proportion of individuals without a bleeding event that have a nonwild genotype. Overall, the clinical specificity of *CYP2C9* is 69% (95% CI 62–75%). The correspondingly high false positive rate (31%) is because nonwild *CYP2C9* genotypes are relatively common and most will not experience serious bleeding. The relative risk for serious bleeding in nonwild versus wild individuals is 1.7 (95% CI 0.8–3.6), consistent with an occasional warfarin overdose in the presence of a nonwild genotype.

Figure 1 shows the relationship between these parameters in a population with a serious bleeding rate of 5%. The prevalence of serious bleeding among populations varies widely (<1–17%) depending on many factors[37–46] (e.g., indication for warfarin, age, comorbidities, definition of serious bleeding, and other drug use). The PPV is estimated to be 7% (i.e., 1 in 14 patients with a nonwild *CYP2C9* genotype will suffer a bleeding event). Because nonwild *CYP2C9* genotypes are relatively common and the prevalence of serious bleeding is low, most will not experi-

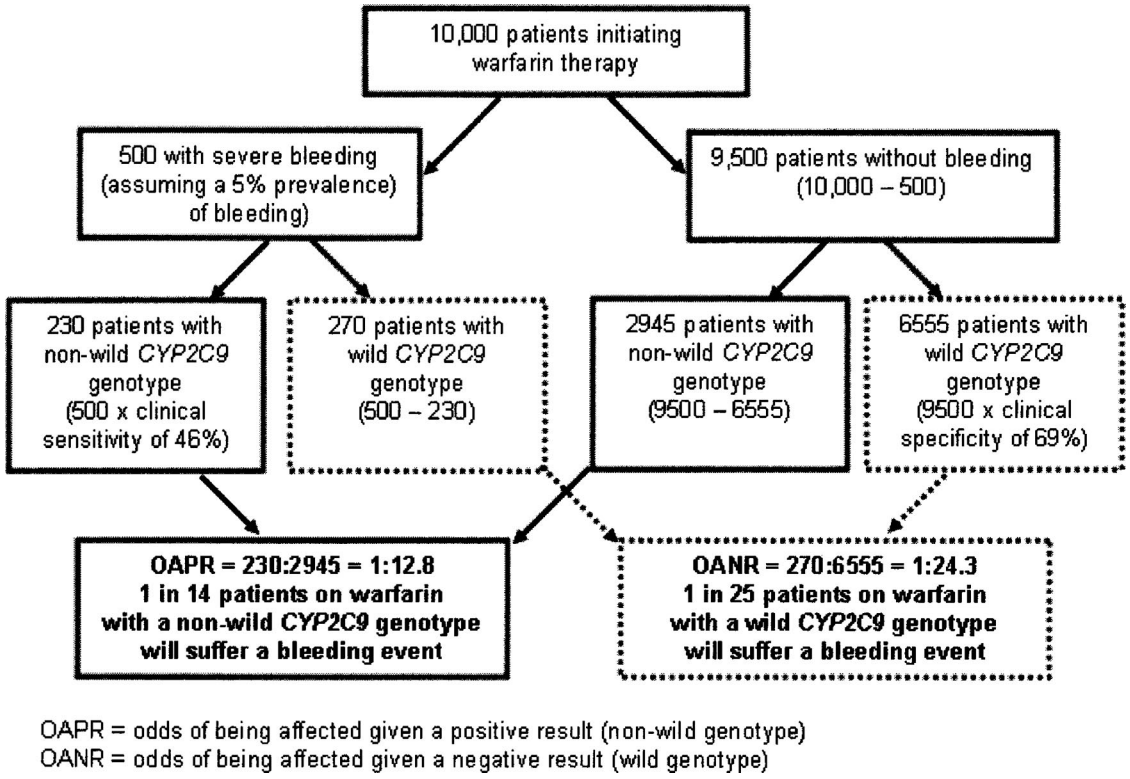

Fig. 1. Flow diagram showing episodes of severe bleeding in a hypothetical cohort of 10,000 individuals initiating warfarin treatment, stratified by *CYP2C9* genotype. The estimates used in this Figure were derived from published literature summarized in this evidence-based review. The solid bordered boxes are used to calculate the odds of being affected given a positive result (non-wild genotype), whereas the dotted bordered boxes are used to calculate the odds of being affected given a negative result (wild genotype).

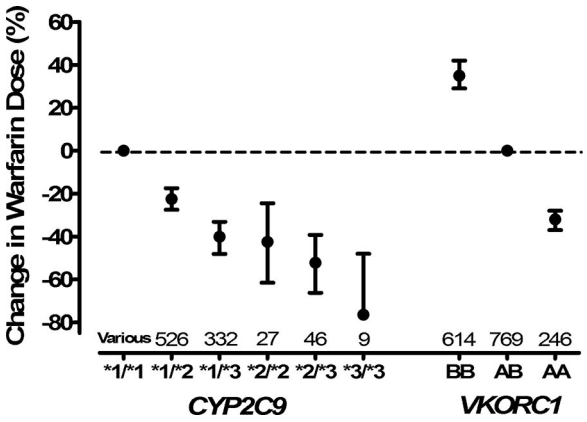

Fig. 2. Change in warfarin dose at stable INR by *CYP2C9* or *VKORC1* genotype. This meta-analysis includes 10 datasets for *CYP2C9* genotyping and seven datasets for *VKORC1* genotyping. The referent categories (horizontal dotted line) were chosen because they included the largest proportion of the population for each gene. The numbers above each genotype indicate the number of samples included in the analysis. For the *CYP2C9* reference category, the number varied from a high of 1757 for the comparison with the *1/*3 genotype to a low of 476 for the *3/*3 genotype comparison.

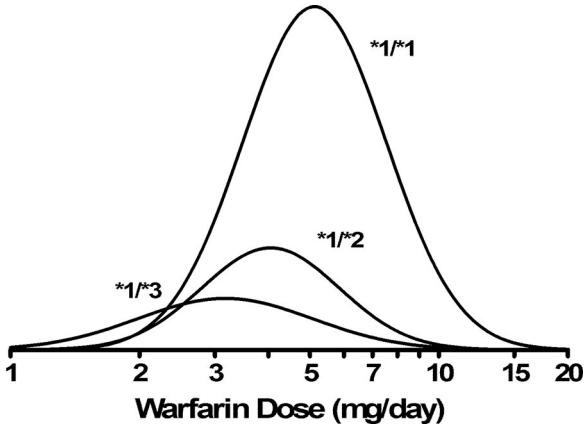

Fig. 3. Overlapping distributions of warfarin dose at stable INR for three *CYP2C9* genotypes. The modeled distributions of warfarin dose are shown on a logarithmic horizontal axis. The areas of the three distributions are in direct relation to their prevalence (*1/*1 being the most common). Although the reduction in stable warfarin dose is clearly visible for the *1/*2 and *1/*3 genotype, there is considerable overlap of the three distributions.

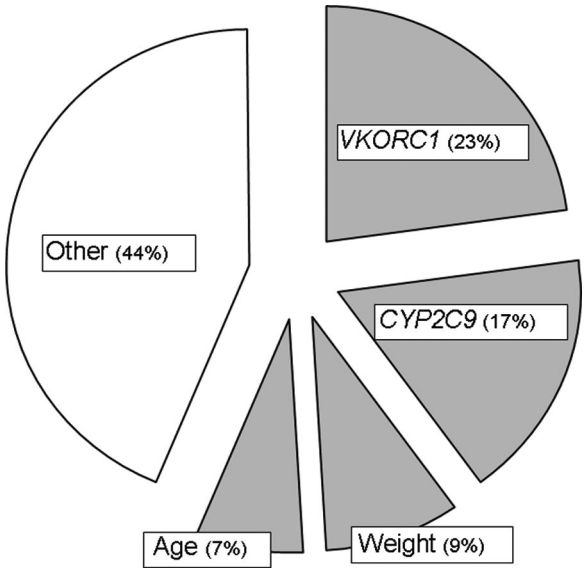

Fig. 4. Pie chart showing the known sources of variability in warfarin dose needed for a stable INR. Each estimate is based on a summary analysis of partial r^2 values from multivariate regression analysis reported in six studies that included genotyping on both *CYP2C9* and *VKORC1*.

Table 5
Estimates of warfarin dose (mg) at stable INR, stratified by *CYP2C9* and *VKORC1* genotype

	CYP2C9 genotype					
			Poor			
VKORC1 genotype	Rapid *1/*1	Inter *1/*2	*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

From www.WarfarinDosing.org, for a 65-year-old Caucasian non-Hispanic man with a body surface area of 1.96 m^2 (weight = 180 lbs, height = 5'8'') with an initial INR of 0.75 and a target INR of 2.75. He is a nonsmoker with no liver disease and is taking no relevant drugs (e.g., amiodarone, statin). The indication for warfarin is atrial fibrillation.

ence serious bleeding. Figure 1 also shows that the NPV is estimated to be 96% (i.e., 24 of 25 patients with a wild *CYP2C9* genotype will not suffer a bleeding event).

Although not considered a direct measure of clinical validity, *CYP2C9* genotypes are strongly related to warfarin dose, once the INR has stabilized. 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, (Fig. 2).[11,12,22,31,32,47–52] Compared with the heterozygote *VKORC1* genotype (indicated by AB), warfarin dose is increased by 35% among individuals with the BB genotype and reduced by 32% among those with an AA genotypes.[9–13,53] Figure 3 displays modeled distributions of stable warfarin dose for the three most common *CYP2C9* genotypes, derived using data from one study.[22] Although there are clear reductions in the average levels, there is considerable overlap among these three groups. The three *VKORC1* genotypes also have considerable overlap of stable warfarin dose (similar to Figure 10b in full report). *CYP2C9* and *VKORC1* genotypes contribute relatively independent information about stable warfarin dose (Fig. 4).[10,12] Based on six studies that involved testing for both genes in a population with a steady state INR, *VKORC1* haplotyping explains a slightly higher proportion of overall variability in warfarin dose (23%) than *CYP2C9* genotyping (17%).[9–13,53] This is because the *VKORC1* genotypes associated with changes in 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,[8,10,12,13] but none include both an appropriate transformation for warfarin dose (e.g., logarithmic) and allow for observed difference in warfarin doses for the *1/*2 vs.

Table 6
Relative adjustments to warfarin dose at stable INR, stratified by *CYP2C9* and *VKORC1* genotype and estimated frequency per 1000

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

The bolded entries (*1/*1; AB) are the most common combination of *CYP2C9/VKORC1* genotypes (300/1000) and are considered the referent group (100% dose). Other entries are represented as a percentage of this dose (e.g., 140% indicates a 40% increase in predicted dose to achieve a stable INR).
Frequencies are derived from the allele frequencies for *CYP2C9* of 12.2% and 7.9% for *2 and *3, respectively, and for the BB, AB, and AA genotype frequencies of 35%, 47%, and 18%, respectively. The two sets of allele frequencies are considered to be independent.
From www.warfarindosing.org, for a 65-year-old Caucasian with a body surface area of 1.96 m^2 (weight = 180 lbs, height = 5'8'') with a target INR of 2.75, who is a nonsmoker and is taking no other relevant drugs.

*1/*3 genotypes. Table 5 shows a comprehensive (but unpublished) warfarin dosing model (www.WarfarinDosing.org) that accounts for both *CYP2C9* genotyping and *VKORC1* genotyping, as well as several other known covariates. Since the completion of our review, two additional studies have been published that provide warfarin dosing models incorporating both *CYP2C9* and *VKORC1* variants.[54,55] Both of these models include logarithmic transformation for warfarin dose and both *CYP2C9* *2 and *3 genotypes. In addition, one of these models includes early warfarin doses and INR values.[54] Table 6 shows these same warfarin doses relative to the most common subgroup (*CYP2C9* = *1/*1, and *VKORC1* = AB) comprising 30% of the Caucasian population. The display highlights that individuals with certain genotypes will actually receive a higher warfarin dose (e.g., 40% higher dose in *1/*1, BB), compared to those with the most common genotype.

Gaps in knowledge include: (1) the clinical sensitivity, clinical specificity, relative risk, and attributable risk of severe bleeding in the *VKORC1* genotypes and in *CYP2C9* and *VKORC1* genotypes combined, (2) the contribution of genetic versus other influences toward bleeding in various racial/ethnic populations, (3) PPV and NPV for severe bleeding in the *VKORC1* genotypes and *CYP2C9* and *VKORC1* genotypes combined, (4) how the difference in dosage would 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, and (5) the roles of other genes in the pharmacokinetics and pharmacodynamics of warfarin and their impact on warfarin dosage requirements.

Clinical utility

Clinical utility is defined as the benefits and risks associated with the introduction of a test into clinical practice, and includes economic analyses to determine the financial impact of such testing. This section begins by discussing the natural history of the disorder (severe bleeding).

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, such that results are comparable across laboratories and test reagents.[2] The target INR depends on the indication for anticoagulation, but the range is 2.0–3.0 for most patients. INR monitoring usually begins 2–3 days after the initial dose.[56] In an acute, hospital setting, patients may be monitored daily; in an outpatient setting, two to three times weekly is recommended. If the INR remains stable, the interval can be gradually increased up to every 4 weeks. A steady state is usually achieved in 6–12 days (affected by *CYP2C9* variants).[7] If a *CYP2C9* *3 variant is involved, the time to reach steady state may be two to three times longer than the expected 3–5 days in wild-type individuals.

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 over-the-counter medications and nutraceuticals), health status changes that affect warfarin metabolism 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.

The intended action of *CYP2C9* and *VKORC1* alleles testing is to predict an individual's maintenance warfarin dose by incorporating demographic, clinical, and gene variant data (both *CYP2C9* and *VKORC1*). This can be used as the initial dose to limit high INR values (over-anticoagulation) that are associated with serious bleeding events, and to decrease time to stable INR. 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. One small pilot randomized trial enrolled 38 patients and found six serious bleeding events among the 20 patients with standard warfarin dosing versus two bleeding events among the 18 receiving model-based dosing using *CYP2C9* genotyping.[57] These results are not statistically significant, but show acceptability of the randomized design. Several large randomized trials are underway to determine the clinical effectiveness of *CYP2C9* genotyping and *VKORC1* haplotyping to inform warfarin dosing. Some of these trials are using severe bleeding as the outcome, whereas others are targeting intermediate measures such as reducing the time to achieve stable INR, and the percentage of time in range during dose stabilization.

Using estimates of clinical validity described earlier (Fig. 1), along with several assumptions of clinical utility (e.g., cost of testing and the effectiveness of targeted warfarin dose to avoid serious bleeding), the number of individuals that must be tested 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 targeted warfarin dose (range 80–20% in a sensitivity analysis) and the cost of genetic testing (range $300–$500).

Economic outcomes and decision analysis studies on genetic and pharmacogenetic testing have been published.[58–60] One recently released analysis suggests that genetic testing before warfarin dosing will avoid many severe bleeding events and result in large cost savings.[60] However, close examination of this study reveals that the authors made several assumptions that may not be valid. These include: targeted dosing by genotype will be 100% effective in reducing bleeding events to the level of that in individuals with the wild genotype, more effective dosing will reduce the rate of strokes, a rate of bleeding events that is higher than expected, and a relatively high estimate of new warfarin users per year.

Gaps in knowledge include: (1) the clinical utility of genotyping before warfarin dosing (e.g., is there a reduction in time to stable INR, is there a reduction in severe bleeding events?), (2) cost-effectiveness of *VKORC1* testing alone, or in combination with *CYP2C9*, (3) the impact of the timing of genotyping (e.g., before initial dose or 2–3 days after initial warfarin treatment), (4) validated educational materials for patients and providers, (5) long-term monitoring plans, and (6) guidelines for evaluating program performance.

Ethical, legal, and social implications (ELSI)

Pharmacogenomic testing might be perceived as carrying less serious ELSI than other types of genetic testing. For example, a variant that alters response to a drug (e.g., a *CYP2C9* or *VKORC1* genotype) might carry less potential for discrimination, privacy/confidentiality, and stigmatization than a mutation that is predictive of a debilitating and/or fatal disease (e.g., Huntington disease). However, a premise does exist that pharmacogenomic tests may be used to classify groups that face discrimination in health care, resulting in prejudice and stigmatization.[30,61] Furthermore, stratifying the population into genetic subgroups could mean that the costs of developing new drugs tailored to the needs of a given small subgroup might be prohibitively expensive and might not be developed. Even if this premise should bear out, an individual will still receive benefit, if found to be in a genetic subgroup for which an existing therapy is known to be harmful, in that inappropriate treatment will be avoided.

The Nuffield Council on Bioethics Report[62] suggests that "the likelihood that pharmacogenomic data will be of relevance to family members is low." Although single nucleotide polymorphisms (SNPs) are heritable, SNP testing has not been widely studied, and it may be too early to decide definitively whether 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.[63,64] It is likely that *CYP2C9* and *VKORC1* testing will also be offered directly to consumers in the near future.

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.[62] Even if it is decided that consent is not required, written information (e.g., education materials) might be appropriate.

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 dispensing other drugs utilizing the same metabolic pathway? Finally, the new FDA-revised warfarin label may make the conduct of randomized controlled trials more difficult.

The issues discussed in this section are all considered gaps in knowledge and will require further monitoring and documentation to further describe the ethical, legal, and social implications of pharmacogenomic testing.

DISCUSSION

Justifications for performing a Rapid-ACCE review at this time include: the potential for a large number of genetic tests to

be performed (as many as 1 or 2 million new warfarin patients per year), the high rate of adverse drug events associated with warfarin usage (800/year in the United States), the FDA-revised Coumadin® label that includes genomic test information, and the availability of *CYP2C9/VKORC1* testing services. The structure of the rapid-ACCE evidence review can be applied to other emerging tests. The objectives of this review were to: (1) briefly evaluate and summarize existing knowledge, (2) provide information to aid in developing clinical and laboratory guidelines for *CYP2C9* and *VKORC1* alleles testing to guide warfarin dosing, (3) provide information to be used in provider and patient education materials, and (4) identify gaps in knowledge from which a research agenda can be developed.

We found data showing the analytic validity of *CYP2C9* allele testing to be acceptable, but data are lacking for that of *VKORC1* allele testing. Results from the new College of American Pathology proficiency testing program will improve the evidence base. There exists compelling evidence for the association between *CYP2C9* and *VKORC1* genotypes and stable warfarin dose. Fewer data are available to evaluate the association between *CYP2C9* genotype and stable INR during the induction phase, when the risk of severe bleeding is highest. There are very limited data on the clinical validity of *CYP2C9* genotyping to predict severe bleeding events, and no data for *VKORC1* genotypes. The clinical utility of DNA testing in this clinical scenario is to "personalize" 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. There are several ethical, legal, and social implications that need to be monitored to ensure equitable, nondiscriminatory, and confidential *CYP2C9* and *VKORC1* testing.

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