The Effects of CYP2C9 and VKORC1 Genotypes

In November of 2005 an FDA Advisory Committee voted to change warfarin's label to include a recommendation for genetic testing. This recommendation is based on the current body of evidence linking genetic variations in CYP2C9 and VKORC1 to inter-individual variations in warfarin therapy. A member of the Cytochrome P450 family of enzymes, CYP2C9 metabolizes warfarin. The vitamin K epoxide reductase complex (VKORC1) protein is the drug target for warfarin, and is important in the blood clotting cascade. This collection provides a list of relevant papers on the effects of CYP2C9 and VKORC1 genotypes on warfarin dose and adverse events associated with warfarin therapy, as well as economic analyses of CYP2C9 genotyping applied to warfarin therapy.

Key Points

- Polymorphisms in the CYP2C9 and VKORC1 genes have been shown in retrospective studies to be important genetic determinants of warfarin dose.
- Prevalence of CYP2C9 and VKORC1 variant alleles: 3 to 20 percent of individuals, depending on ethnicity, have variant CYP2C9 alleles that affect warfarin dose. Fifty-five percent of individuals in the US have VKORC1 genotypes that affect warfarin dose.
- Patients receiving warfarin who have CYP2C9 variant alleles have an increased risk of overanticoagulation and bleeding, TABLE OF CONTENTS and take longer to attain stable dosing.
Prevalence of Adverse Drug Reactions with Warfarin


This paper describes a meta-analysis of studies on bleeding complications of oral anticoagulants. In most studies, the anticoagulant used was warfarin. The meta-analysis covered 10,757 patients undergoing 4374 patient years of anticoagulation. The rate of major bleeding was 7.22 per 100 patient years, and the rate of fatal bleeding was 1.31 per 100 patient years. The case fatality rate for major bleeding was 13.4 percent. The authors describe a front-loading of major bleeding episodes occurring shortly after the initiation of anticoagulation, with as many major bleeding episodes in the first three months of anticoagulation as during the entire year after initiation of therapy.

“The rate of major bleeding was 7.22 per 100 patient years, and the rate of fatal bleeding was 1.31 per 100 patient years.”


Palareti et al. prospectively assessed bleeding in outpatients receiving oral anticoagulation therapy. Sixty-four percent of the patients were receiving warfarin, and 36 percent were receiving acenocoumarol. Patients were within, below and above a therapeutic INR 68.0, 26.1 percent and 5.9 percent of the time, and these numbers were not significantly different in patients with bleeding complications compared to those with no bleeding. The incidence of bleeding did not vary according to sex, coumarin type, target INR (below 2.8 versus above 2.8), or size of the enrolling center. The rate of bleeding was higher in older patients and in patients receiving anticoagulation for arterial disease. The incidence of bleeding overall was 7.6 bleeding events per 100 patient years. Most of the bleeding events were classified as minor, with an incidence of 6.2 per 100 patient years. The rate of fatal bleeding was 0.25 per 100 patient years, and the rate of major bleeding was 1.1 per 100 patient years. Rates of bleeding were higher when the target INR was greater than 4.5, during the first 90 days of treatment, and when the indication for warfarin was arterial disease.

“Rates of bleeding were higher when the target INR was greater than 4.5, during the first 90 days of treatment, and when the indication for warfarin was arterial disease.”

Note about VKORC1 haplotypes: There is currently no standard nomenclature for either the VKORC1 polymorphisms or the VKORC1 haplotypes.
Effects of CYP2C9 and VKORC1 Genotypes on Warfarin Dose


Genetic and non-genetic factors that influence warfarin dose were investigated. Three hundred and fifty patients receiving stable doses of warfarin, mostly Caucasian, were investigated for polymorphisms in the factor II, factor VII, factor X, VKORC1, and CYP2C9 genes. The most important determinants of dose were polymorphisms in VKORC1 and CYP2C9, and non-genetic factors. Factors contributing to lower warfarin doses included VKORC1 3673AA or GA genotype (the 3673 SNP is also called -1639 in some papers), one or two CYP2C9 variant alleles, increasing age, concomitant CYP2C9 inhibitors, and lower goal INR. Individuals with the VKORC1 3673GA or 3673AA genotypes were 5.2 times or 33.2 times more likely respectively to require low-dose warfarin. Individuals with one or two CYP2C9 variant alleles were 3.5 times or 10.3 times more likely respectively to require low-dose warfarin. Factors contributing to higher warfarin doses included increasing weight, being a current smoker, concomitant CYP2C9 inducers, higher goal INR, factor X insertion/deletion genotype, factor X insertion/insertion genotype, factor VII deletion/deletion genotype, and vitamin K intake.


A mutation in the VKORC1 gene was found in a patient resistant to warfarin, acenocoumarol, fluindione, and phenprocoumon. This patient had a heterozygous point mutation that caused a leucine to arginine substitution at amino acid 128. This mutation was also observed by Rost et al. in another warfarin resistant patient.


D’Andrea et al. describe an association between a common polymorphism in VKORC1 and the warfarin dose. The 1173C>T polymorphism had an allele frequency of 39.8 percent. Patients with the 1173CC genotype had a mean daily warfarin dose of 6.2 mg, patients heterozygous at this locus (1173CT) had a mean daily warfarin dose of 4.8 mg, and patients with the 1173TT genotype had a mean daily warfarin dose of 3.5 mg.


A new allele of CYP2C9 was identified by Dickmann et al., CYP2C9*5. This allele contained a cytosine to guanine change at nucleotide 1080, which resulted in an aspartic acid to glutamic acid change at amino acid 360 (D360E). The activity of CYP2C9*5 was shown to be impaired in vitro: CYP2C9*5 had decreased catalytic activity for the CYP2C9 substrates warfarin, diclofenac, and lauric acid. The CYP2C9*5 allele was found in 3 percent of African Americans and no European Americans.


The effect of several variables on warfarin dose was assessed. Variables significantly associated with lower doses were advanced age, lower body surface area, one or more CYP2C9 variant alleles, lower target INR, use of amiodarone or simvastatin, white race, and male gender. A pharmacogenetic dosing algorithm was developed based on these variables. It was estimated that for the patients in this study, only 6.5 percent would have been overdosed using dosing based on the algorithm, but 16 percent would have been overdosed based on a standard daily dose of 5 mg.

A warfarin resistant individual was found to have a mutation in the VKORC1 gene. The patient had a heterozygous point mutation causing a valine to methionine substitution at amino acid 66 (Val66Met). This patient had two asymptomatic family members with the same mutation who had never received warfarin.


A method for genotyping CYP2C9 alleles *1, *2 and *3 involving high resolution melting analysis was developed. Eighty-four patients were genotyped using this new method, and the mean maintenance warfarin doses correlated with CYP2C9 genotype: patients heterozygous for a CYP2C9 variant allele (n=25) had warfarin doses 7 percent lower than wild-type, and patients homozygous for a CYP2C9 variant allele (n=2) had 30 percent lower warfarin doses. This association of CYP2C9 genotype with mean maintenance warfarin dose has been observed in many previous studies.


A prospective randomized single-blinded pilot clinical trial was performed to assess the feasibility of pharmacogenetics-based dosing for warfarin. The outcomes measured were patient willingness to participate, physician willingness to refer, sample processing time, ability to administer calculated dosage and adequacy of follow-up. The authors concluded that pharmacogenetics-based dosing is feasible. Clinical outcomes of the different dosing regimens (pharmacogenetics-based or standard) were analyzed, but the statistical power was insufficient to draw conclusions because of the small number of patients enrolled. However, in this small sample of patients, 6 adverse events occurred in 5 patients out of 20 patients total in the standard dosing group, and 2 adverse events occurred in 2 patients out of 18 patients total in the pharmacogenetics-based dosing group.


Chinese and Malay patients have been reported to require lower warfarin doses than Indians; however, this was not explained by CYP2C9 polymorphisms. This paper describes VKORC1 haplotypes associated with low and high warfarin doses whose frequencies in Chinese, Malays and Indians explain the interethnic variability of warfarin doses in these populations. The H1 VKORC1 haplotype, associated with low warfarin dose, was common in Chinese and Malay subjects, with frequencies of 87 percent and 65 percent respectively. This haplotype was not common in Indians, with a frequency of only 12 percent. The H7, H8, and H9 haplotypes were associated with high warfarin doses, and were rare in Chinese (frequency of 9 percent), intermediate in Malays (frequency of 30 percent) and common in Indians (frequency of 82 percent). This paper also makes the point that genotypic differences exist between different Asian ethnic populations, despite the fact that different Asian populations are often grouped together as one ethnic group in studies of ethnicity and genotype.


This is a retrospective study examining the association between SNPs in VKORC1 and CYP2C9 and the average weekly warfarin dose. Ninety-three European-Americans were genotyped for 6 VKORC1 SNPs, and CYP2C9 *1, *2, and *3. Five out of six VKORC1 SNPs were associated with warfarin dose: the three most strongly associated were 1173C>T, 1542G>C, and 2255T>C. These three SNPs were frequent in strong linkage disequilibrium and four different haplotypes were described. Two haplotypes in the VKORC1 gene were associated with lower mean warfarin doses, and
two were associated with higher mean warfarin doses. The four haplotypes were all relatively common, with frequencies ranging from 13 percent to 35 percent. This study found no association of CYP2C9 genotype with warfarin dose, but because the *2 and *3 alleles of CYP2C9 were much less common than the VKORC1 SNPs, the study had lower statistical power for CYP2C9.


Two cases of patients stabilized on low warfarin doses of approximately 10 mg weekly were reported. Genotyping of these patients revealed that one patient was a heterozygote for CYP2C9*3, and the other was a homozygote for CYP2C9*6. In both patients the warfarin S:R ratios were elevated, indicating impairment of warfarin metabolism. The genotypes of these patients provided an explanation for the low warfarin dose requirements.


Variants in the VKORC1 gene were shown in a retrospective study to affect the response to warfarin. Five common haplotypes were identified, divided into two haplotype groups by association with warfarin dose (A is the low dose haplotype group, and B is the high dose haplotype group). The mean maintenance daily dose of warfarin differed among the three haplotype group combinations, with 2.7 mg per day for A/A, 4.9 mg per day for A/B, and 6.2 mg per day for B/B. Twenty-five percent of the variance in warfarin dose was explained by the VKORC1 haplotype.

“The mean maintenance daily dose of warfarin differed among the three haplotype group combinations, with 2.7 mg per day for A/A, 4.9 mg per day for A/B, and 6.2 mg per day for B/B. Twenty-five percent of the variance in warfarin dose was explained by the VKORC1 haplotype.”


Rost et al. identify the gene for VKORC1 and show that mutations in this gene are involved in two human diseases: combined deficiency of vitamin K-dependent clotting factors type 2 (VKCFD2), and warfarin resistance. A homozygous point mutation, arginine to tryptophan at amino acid 98, was found in two individuals with VKCFD2. Warfarin resistance has autosomal dominant inheritance, and four different heterozygous point mutations were found, and all of these mutations caused amino acid substitutions: valine to leucine at amino acid 29 (Val29Leu), valine to alanine at amino acid 45 (Val45Ala), arginine to glycine at amino acid 58 (Arg58Gly), and leucine to arginine at amino acid 128 (Leu128Arg). The four warfarin resistance mutations showed decreased VKOR activity in cell culture.


Sanderson et al. describe a meta-analysis of nine different studies looking at the relationship of CYP2C9 genotype (*2 and *3 alleles) to warfarin dose and risk of bleeding on warfarin. Twenty percent of patients in the studies carried at least one variant. Decreases in doses for patients carrying *2 or *3 were seen: the average decrease was 17% for the *2 allele and 37% for the *3 allele. The risk of bleeding was approximately doubled in patients with a *2 or *3 allele. The relative risk of bleeding was 1.91 for carriers of *2 compared with noncarriers, and 1.77 for carriers of *3.

A warfarin dosing algorithm based on age, height, CYP2C9 genotype, and VKORC1 genotype is described. This dosing algorithm was assessed in a population of patients on warfarin therapy and there was a close relationship between the doses calculated using the algorithm, and the actual doses.


The effect of polymorphisms in VKORC1 and CYP2C9 on the maintenance dose of warfarin in different ethnic groups was examined. Patients receiving stable warfarin doses were genotyped. The frequencies of variant alleles of both VKORC1 and CYP2C9 differ between Japanese, Caucasians, and African Americans, and the frequencies of the various alleles may help explain interethnic and interindividual differences in warfarin sensitivity. The VKORC1 1173C>T polymorphism is most common in Japanese, with a frequency of 89.1 percent. The frequency in Caucasians is 42.2 percent and in African Americans is 8.6 percent. This polymorphism has a gene dose effect on warfarin dose: 1173TT patients have the lowest dose, 1173CT patients have an intermediate dose, and 1173CC patients have the highest dose. CYP2C9 genotype, VKORC1 genotype, age and body weight were all found to contribute to the variability of warfarin dose, and together explained 57 percent of the variance in dose.


Vecsler et al. examined the influence of polymorphisms in several genes on warfarin dose requirements. The genes studied were CYP2C9, VKORC1, calumenin (CALU), gammaglutamyl carboxylase (GGCX), and microsomal epoxide hydroxylase (EPHX1). The warfarin dose was predominantly determined by VKORC1 and CYP2C9 polymorphisms, which together explained 63 percent of the variance in dose. CALU genotype also had a significant effect.

“The warfarin dose was predominantly determined by VKORC1 and CYP2C9 polymorphisms, which together explained 63 percent of the variance in dose.”


Voora et al. prospectively validate a warfarin dosing algorithm that incorporated CYP2C9 genotype, age, gender, and other factors. This algorithm was predicted to explain 39 percent of the variance in warfarin dose and, in this patient cohort, was found to predict 42 percent of the variance. The time to stable, therapeutic INR was similar for patients with wild-type and variant genotypes. However, patients with a variant genotype were 3.6 times more likely than wild-type patients to have an INR greater than 4 despite pharmacogenetics-based dosing. These findings need to be expanded in a larger, randomized study with a control group of patients not receiving pharmacogenetics-based dosing.


Analysis of polymorphisms in the VKORC1 and GGCX genes determines which polymorphisms are associated with warfarin dose. Five polymorphisms in VKORC1 co-vary with warfarin dose. Four of these are in linkage disequilibrium and have an allele frequency of 40 percent, and one additional polymorphism has an allele frequency of 4 percent. One polymorphism with an allele frequency of 40.8 percent in the GGCX gene showed an association with warfarin dose. The VKORC1 polymorphisms explained 29 to 30 percent of the variation in warfarin dose, and the GGCX polymorphism explained 12 percent of the variation in dose.
A new VKORC1 polymorphism associated with low warfarin doses was identified. A homozygous VKORC1 promoter polymorphism, -1639G>A, was found in 16 out of 16 warfarin sensitive Chinese patients. Among 104 Chinese patients on warfarin, AA homozygotes had lower doses than AG heterozygotes, or GG homozygotes. The frequencies of the genotypes in Chinese were 82 percent for -1639AA, 18 percent for -1639AG, and 0 percent for -1639GG. In Caucasians, the genotype frequencies were 14 percent for -1639AA, 47 percent for -1639AG, and 39 percent for -1639GG. This polymorphism affects VKORC1 promoter activity: the -1639G promoter had 44 percent higher activity than the -1639A promoter. The -1639G>A polymorphism may help explain interindividual differences in warfarin sensitivity, as well as interethnic differences in warfarin sensitivity as it has been established that Chinese patients generally require lower warfarin doses than Caucasians.


These six papers retrospectively examine the relationship between CYP2C9 genotype and warfarin dose. Patients with one variant allele (*2 or *3) require lower mean maintenance doses than homozygous wild-type patients, and patients with two variant alleles require even lower doses. The *3 allele has a larger effect on dose than the *2 allele.

“Patients with one variant allele (*2 or *3) require lower mean maintenance doses than homozygous wild-type patients, and patients with two variant alleles require even lower doses.”
Effect of CYP2C9 Genotypes on Adverse Events during Warfarin Therapy


The association of warfarin dose with CYP2C9 genotype and the risk of bleeding was examined. Patients were classified into low dose and control groups. The odds ratio for a patient having one or more variant alleles in the low dose group was 6.21. The incidence of minor bleeding was not significantly different between the two groups, but the incidence of serious or lifethreatening (major) bleeding was significantly higher in the low dose group. The incidence of major bleeding in the low dose group was 11 events in 132.8 patient years, and the incidence of major bleeding in the control group was 7 events in 311.1 patient years (odds ratio 3.68).

“Bleeding events were more common in patients with variant genotypes: the hazard ratio for serious or life-threatening bleeding events was 2.39 for variant genotypes compared to wild-type genotypes.”


This retrospective study examines the effect of CYP2C9 genotype (*2 or *3 allele) on maintenance warfarin dose, time to stable dosing, and the risk of serious or life-threatening bleeding events. The maintenance dose was lower for patients with variant genotypes compared to wild-type, and patients with variant genotypes took longer to achieve stable dosing (the median difference was 95 days). Bleeding events were more common in patients with variant genotypes: the hazard ratio for serious or life-threatening bleeding events was 2.39 for variant genotypes compared to wild-type genotypes. The bleeding rates were 10.92 per 100 patient years and 1.56 per 100 patient years for serious and life threatening bleeding in patients with variant genotypes, and 4.89 per 100 patient years and 0.7 per 100 patient years serious and life threatening bleeding in patients with a wild-type genotype.


In addition to examining the relationship between warfarin dose and CYP2C9 genotype (see above), this paper retrospectively evaluates whether variant CYP2C9 genotypes are associated with a higher risk of overanticoagulation and bleeding. The rates of overanticoagulation are higher in patients with one variant allele, and even higher in patients with two variant alleles, compared to wild-type. However, an increased risk of bleeding was not seen in patients with variant genotypes. This result differs from other studies, which did find an increase in bleeding; however, the authors point out that the bleeding risk could vary depending on how closely the INR is monitored.


A prospective study was conducted to examine the impact of the CYP2C9*2 and *3 alleles on the risk of overanticoagulation during induction of warfarin therapy. The risk of overanticoagulation (at least 1 INR value above the therapeutic level) was higher for carriers of a *2 allele than *1/*1 patients, and even higher for carriers of a *3 allele. The study was not large enough to examine the impact of CYP2C9 genotype on bleeding; although bleeding can occur with overanticoagulation, it is a relatively rare event and no bleeding episodes occurred during the course of the study.


Margaglione et al. investigate whether CYP2C9 variants affect the warfarin dose (see section 1) and the risk of bleeding. The odds ratio for bleeding was 2.57 for carriers of a CYP*2 or *3 allele compared to *1 homozygotes.

Sanderson et al. describe a meta-analysis of 9 different studies looking at the relationship of CYP2C9 genotype (*2 and *3 alleles) to warfarin dose and risk of bleeding on warfarin. Twenty percent of patients in the studies carried at least one variant. Decreases in dose for patients carrying *2 or *3 were seen: the average decrease was 17 percent for the *2 allele and 37 percent for the *3 allele. The risk of bleeding was approximately doubled in patients with a *2 or *3 allele. The relative risk of bleeding was 1.91 for carriers of *2 compared with noncarriers, and 1.77 for carriers of *3. The paper also explains that differences in treatment and monitoring regimens, selection criteria for studies, and distribution of interacting factors in study populations may lead to an underestimation of bleeding risk or conflicting results between studies.

“Decreases in dose for patients carrying *2 or *3 were seen: the average decrease was 17 percent for the *2 allele and 37 percent for the *3 allele. The risk of bleeding was approximately doubled in patients with a *2 or *3 allele.”

Economics of CYP2C9 Genotyping for Warfarin


Fanikos et al. describe a study of bleeding complications in 2460 patients receiving warfarin therapy. Both the incidence and cost of bleeding episodes were analyzed. A low incidence of major bleeding events was observed: 0.32 events per 100 patient years. The average cost per bleeding event was $15,988, with an average length of hospital stay of six days. The cost per hospital admission ranged from $2,707 to $64,446. The high end of the range resulted from a patient with an intracranial hemorrhage requiring two neurosurgeries. When this patient was excluded, the average cost per bleeding event was $11,142.

“The average cost per bleeding event was $15,988, with an average length of hospital stay of six days.”


Criteria that can be used to evaluate the cost effectiveness of genetic testing are described. The criteria described are: 1. There should be an association between the variant gene and clinical outcomes. 2. The prevalence of the variant should be considered. 3. The severity of clinical outcomes must be defined. 4. An effective intervention to improve clinical outcomes for patients with a variant gene must exist. 5. There must be a sensitive, specific, and rapid genetic test. These criteria are applied to the example of CYP2C9 genetic testing for patients on warfarin therapy. The conclusions are that 44 patients need to be screened to prevent one adverse event, at a screening cost of $5,940 (assuming a cost per test of $135). Whether this is cost-effective depends on the cost of one adverse event, which was not reported. The effect of VKORC1 was not factored in to this analysis.
Review Article


This paper is a review primarily of the effects of CYP2C9 variants on warfarin metabolism, dosing requirements, and clinical outcomes (overanticoagulation and bleeding). Warfarin dosing requirements are reduced in carriers of a CYP2C9*2 allele, and even more reduced in carriers of a CYP2C9*3 allele. Carriers of CYP2C9 variant alleles have been found to be at greater risk for complications of warfarin therapy including supratherapeutic INR, longer hospital stays, and bleeding. Wittkowsky et al. state that knowledge of the CYP2C9 genotype could be used to guide the frequency of INR monitoring, response to overanticoagulation, and dosing adjustments. Warfarin resistance through mutations in VKORC1 is also briefly discussed. The four mutations identified by Rost et al. [Nature 427(6974): 537-41] in VKORC1 are summarized.
Terms Used

INR: International Normalized Ratio: A system for reporting the results of blood coagulation (clotting) tests. All results are standardized using the international sensitivity index for the particular thromboplastin reagent and instrument combination utilized to perform the test. The target range for warfarin therapy depends on the indication but is generally between 2.0 and 3.0. Supratherapeutic INRs are associated with overanticoagulation and an increased risk of bleeding.

Prothrombin time (PT): A blood test that measures how long it takes blood to clot. A prothrombin time test can be used to screen for bleeding abnormalities. PT is also used to monitor treatment with medication that prevents the formation of blood clots.

Meta-analysis: A statistical analysis of a large collection of results from multiple individual studies for the purposes of integrating the findings.

Odds ratio (also called relative risk, risk ratio, or hazard ratio): The odds ratio is a way of comparing whether the probability of a certain event is the same for two groups. An odds ratio of one implies that the event is equally likely in both groups. An odds ratio greater than one implies that the event is more likely in the first group. An odds ratio less than one implies that the event is less likely in the first group.

Maintenance daily dose for Warfarin: Varies widely depending on many factors. The range for patients with a wild-type CYP2C9 genotype is 3 mg to 7 mg.