

Research Article

Genetic determinants of response and adverse effects following vitamin K antagonist oral anticoagulants

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ABSTRACT

Background: Vitamin K antagonist anticoagulants (warfarin/acenocoumarol) are commonly used anticoagulants that require careful clinical management to balance the risks of over anticoagulation and bleeding with those of under anticoagulation and clotting. Genetic variants of the enzyme that metabolizes vitamin K antagonist anticoagulant, cytochrome P-450 2C9 (*CYP2C9*), and of a key pharmacologic target of vitamin K antagonists anticoagulant, vitamin K epoxide reductase (*VKORC1*), contribute to differences in patients' responses to various anticoagulant doses.

Methods: In thirty patients on oral vitamin K antagonist anticoagulant therapy, presented with either clotting manifestations (valve thrombosis, pulmonary embolism and DVT) or prolonged INR/bleeding manifestations, we assessed *CYP2C9* genotypes, *VKORC1* haplotypes, clinical characteristics, response to therapy (as determined by the international normalized ratio [INR]), and bleeding events.

Results: Of the thirty patients, thirteen patients INR was high and four patients presented with major bleeding and four with minor bleeding manifestations. Out of thirteen patients with high INR, ten patients showed *CYP2C9* polymorphism (*1/*3 and *2/*3) of poor metabolizer genotype. Most of the high INR patients were recently started on oral vitamin K antagonist anticoagulant. Most patients presented with clotting manifestations with below therapeutic INR are noncompliant with anticoagulants.

Conclusions: The results of this study suggest that the *CYP2C9* polymorphisms are associated with an increased risk of over anticoagulation and of bleeding events among patients on vitamin K antagonists' anticoagulant setting. Screening for *CYP2C9* variants may allow clinicians to develop dosing protocols and surveillance techniques to reduce the risk of adverse drug reactions in patients receiving vitamin K antagonist anticoagulants. However the cost-effectiveness of genotyping of patients must be considered.

Keywords: Vitamin K antagonists, *CYP2C9* polymorphism, *VKORC1* haplotypes, International normalized ratio

INTRODUCTION

Oral anticoagulation with the vitamin K antagonists (warfarin or acenocoumarol) also called coumarin anticoagulants, reduces the rate of thromboembolic events for patients in a variety of clinical settings.¹ However, vitamin K antagonist anticoagulants therapy is challenging because there is wide variation among patients in response and therefore in dose requirement.

To achieve and maintain an optimal warfarin dose, the prothrombin time and the international normalized ratio (INR) are monitored, and doses are adjusted to maintain each patient's INR within a narrow therapeutic range.^{1,2}

The management of warfarin therapy is challenging because of variability in patient response due to a multitude of factors including drug, diet, and disease-state interactions.² An INR of less than 2 is associated

with an increased risk of thrombo-embolism, and an INR of 4 or more is associated with an increased risk of bleeding.^{3,4}

In addition, genetic variation of the hepatic microsomal cytochrome P450-2C9 enzyme (CYP2C9), are known to contribute to variability in sensitivity to vitamin K anticoagulant. CYP2C9 is the enzyme primarily responsible for the metabolic clearance of the S-enantiomer of vitamin K antagonist warfarin.^{5,6}

Patients with certain common genetic variants of CYP2C9 require a lower dose of vitamin K antagonists and a longer time to reach a stable dose. They are also at higher risk for over-anticoagulation and serious bleeding.^{7,8} Two common variant alleles (polymorphisms) of CYP2C9 have been identified.^{9,10} The *2 allele (R144C) and the *3 allele (I359L) cause decreased enzymatic activity of 30% and 80%, respectively.^{11,12} The frequencies of the *2 and *3 alleles have been estimated at 11% and 7%, respectively.¹³

Several studies have evaluated the association of these polymorphisms with clinical phenotypes in patients treated with vitamin K antagonists' anticoagulants. Aithal et.al compared individuals having at least 1 variant CYP2C9 allele with individuals having the wild-type (*1/*1) genotype.¹⁴ They reported significant associations between variant CYP2C9 genotype and low-dose requirements for vitamin K antagonists, and between low-dose vitamin K antagonist anticoagulants requirements (but not genotype) and major but not minor bleeding events.

Taube J, et al also found a significantly lower maintenance dose in patients with variant allele vs patients having the wild-type genotype, but did not find evidence of an association between genotype and anticoagulation status as assessed via INR.¹⁵ Loebstein et.al using multiple regression analysis reported that CYP2C9 genotype was independently associated with vitamin K antagonist anticoagulant maintenance doses.¹⁶

Vitamin K epoxide reductase (VKORC1) recycles vitamin K epoxide to the reduced form of vitamin K, an essential cofactor in the formation of the active clotting factors II, VII, IX, and X through γ -glutamyl carboxylation.¹⁷ VKORC1 is the target of coumarin anticoagulants, and its common genetic variants result in altered sensitivity to vitamin K antagonist anticoagulant.¹⁰ VKORC1 polymorphisms are associated with a need for lower doses of warfarin during long-term therap. In some studies, were found to contribute to the variation in dose requirement more than CYP2C9 variants especially in initial period of anticoagulation.¹⁸

On the basis of these observations, the Food and Drug Administration (FDA) approved a labeling change for vitamin K antagonist anticoagulant warfarin that describes the reported effects of VKORC1 and CYP2C9

on dose requirements.¹⁹ The package insert as of August 2007 states that lower initiation doses should be considered for patients with certain genetic variations in CYP2C9 and VKORC1 enzymes. The FDA also approved clinical tests for these genetic variants.²⁰ The first months of anticoagulant treatment are particularly problematic, since the safe and effective dose for an individual patient is not known and is determined empirically. Consequently, the risk of over-anticoagulation, with the potential for hemorrhagic complications, is higher during this time than subsequently.^{21,22}

Although medical management practices are crucial in determining anticoagulation state, but variant CYP2C9 alleles would also play a role and result in a longer time to therapeutic INR, a higher rate of out-of-range INRs, a longer time to achieve stable dosing, and a higher risk of major or life-threatening bleeding events.⁷ Therefore, the current study was designed to evaluate the association between variant CYP2C9 alleles, VKORC1 variants and clinical outcomes such as anticoagulation status and bleeding events.

METHODS

The study was conducted after the institutional ethical clearance and informed consent from all the patients. Patients on vitamin K antagonist anticoagulant (warfarin/acenocoumarol) for various indications, presenting with either prolonged INR/bleeding manifestations or clotting manifestations (e.g. prosthetic valve thrombosis, pulmonary embolism or DVT) were enrolled in the study after written informed consent from the patient.

Patients diagnosed for active cancer requiring, or with the potential to require concurrent chemotherapy as well as active alcoholism were excluded. The target therapeutic INR range was determined in each patient and varied according to the indication for vitamin K antagonist anticoagulant treatment.

Each patient's age, sex, indication for anticoagulant therapy, date of initiation of anticoagulant, target INR range, initial anticoagulant dose, and concomitant medications were recorded. Data regarding subsequent INR values and anticoagulant dose the frequency of INR monitoring, and bleeding events were obtained from medical records. All patients were tested with CBC, ECG, ECHO, LFT, RFT, and in pulmonary embolism patients CTPA. CYP2C9 and VKORC1 genotyping for coumarin anticoagulants was done by polymerase chain reaction (PCR). CYP2C9 genotyping was performed with the use of a fluorescent allele-specific oligonucleotide ligation assay after initial polymerase-chain-reaction amplification with the use of primers for the major variant alleles of CYP2C912. VKORC1 genotyping for known variants with functional importance within the promoter and intronic regions was performed.

Statistical analysis: The data obtained in the present study were represented as percentages.

RESULTS

A total of thirty patients were included in the study. Out of which 40% were males and 60% were females. The

CYP2C9 *1/*1 represents the genotype for the normal metabolisers who can be administered standard drug dose. Intermediate metabolisers (*1/*2) may require than average drug dose for optimal therapeutic response. Poor metabolisers (*1/*3,*2/*2,*2/*3,*3/*3) are at increased risk of drug induced side effects due to impaired drug elimination and hence requires significant reduction in vitamin K antagonist dosage (Table 1).

Table 1: The minimum time required to reach INR depending on CYP2C9 genotype and type of metaboliser (N=30).

CYP2C9 Genotype	Type of metaboliser	Time to reach INR
*1/*1 Genotype	Normal metaboliser	4-5 days
*1/*2 Genotype	Intermediate metaboliser	8-10 days
*1/*3,*2/*2,*2/*3,*3/*3	Poor metaboliser	2-4 weeks

Table 2: Range of expected therapeutic warfarin doses based on CYP2C9 and VKORC1 Genotype as per FDA.

VKORC1	CYP2C9					
	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3
GG	5-7mg	5-7mg	3-4mg	3-4mg	3-4mg	0.5-2mg
GA	5-7mg	3-4mg	3-4mg	3-4mg	0.5-2mg	0.5-2mg
AA	3-4mg	3-4mg	0.5-2mg	0.5-2mg	0.5-2mg	0.5-2mg

Table 3: Number of patients on the basis of indications for anticoagulation with vitamin K antagonists.

Indications	Number of patients	Percentage
Prosthetic valve related indications	23	76.6%
DVT/Pulmonary embolism related indications	06	20%
Atrial fibrillation	01	3.4%

Table 4: Number of patients on the basis of presenting manifestations.

Indications	Number of patients	Percentage
Valvular thrombosis	15	50.0%
Prolonged INR/Bleeding manifestations	08	26.6%
DVT	07	23.4%

Table 5: PCR analysis of patients recruited in the study.

Genotype	Number of patients	Percentage
genotype *1/*3	10	33.4%
genotype *2/*3	08	26.6%
genotype *1/*1	12	40.0%

The VKORC1 test identifies how sensitive an individual is to vitamin K antagonist anticoagulants. Low sensitivity (GG), intermediate sensitivity (GA) and high sensitivity (AA) belongs to CYP2C9 *1/*1 require higher dosage than those belongs to CYP2C9 *3/*3 genotypes (Table 2). Out of twenty three prosthetic valve patients, fifteen patients presented with Valve thrombosis, eight patients presented with prolonged INR/ bleeding manifestations.

Two patients in prosthetic valve group who presented with valve thrombosis, later developed prolonged INR, these two patients were recently underwent valve replacement less than three months duration (Table 3). Total numbers of patients presented with bleeding manifestations are eight. Major bleeding manifestations occurred in four patients. Minor bleeding occurred in four patients as defined by second Copenhagen atrial

fibrillation, aspirin and anticoagulation (Table 4). In thirty patients PCR analysis showed ten patients are poor metaboliser (genotype *1/*3 and *2/*3) for vitaminK anticoagulant and twenty are normal metaboliser (*1/*1 genotype).So CYP2C9*1/*1 genotype in this study is 67%, CYP2C9 *1/*3 genotype is 30% , and CYP2C9 *2/*3 genotype is 3% (Table 5).

DISCUSSION

In this study, patients who were on vitamin K antagonist anticoagulants therapy investigated the relationship between VKORC1 haplotypes and CYP2C9 genotypes and the INR responses that are used to adjust doses.²³ A study by Schwarz UI et al showed that in their study major finding was that genetic variation in VKORC1, but not in CYP2C9, modulates the early response to warfarin.²⁴ Most of the patients with prolonged INR are GG variant VKORC1.However most of the patients with prolonged INR were recently started on vitamin K antagonist anticoagulant.

The results of the present study suggest that CYP2C9 genotype is associated with warfarin maintenance dose, time to stable warfarin dosing, rate of above-range INRs, and bleeding events in patients taking warfarin. During the initiation period of warfarin therapy, it appears that patients with variant CYP2C9 alleles become over-anticoagulated at a faster rate and must undergo additional dose adjustments, thus translating into a longer time until stable dosing is achieved. When these patients do become stable, their daily maintenance dose of warfarin is significantly lower than that of patients without genetic impairment of warfarin metabolism.

Patients with variant CYP2C9 alleles also experience a higher risk of serious and major bleeding events.²⁵ Fihn SD et al found that recent initiation (first 90 days) of warfarin therapy compared with any time thereafter was an independent predictor of first-episode serious bleeding.²⁶ We found CYP2C9 genotype is an important predictor of a first bleeding event during the initiation phase of therapy.

This increased risk may be caused by the administration of loading doses that are too high for patients with genetic impairment of CYP2C9. The incidences of major bleeding events in our study are similar to those found in other studies.²⁶ The frequencies of the CYP2C9 *1/*1 genotype was 67%, CYP2C9 *1/*3 genotype 30% and CYP2C9 *2/*3 genotype 3%, compared to other studies CYP2C9 *1/*3 genotype patients were more.

Most important finding in the present study was that those patients who were on vitamin K antagonist anticoagulant, presented with clotting manifestations (valve thrombosis, DVT or pulmonary embolism) are noncompliant to anticoagulant and poor periodic INR checkup. Yasar U et al genotyped 430 Swedish

volunteers and found *2 and *3 frequencies of 11% and 7%.²⁷

As genomic information becomes more readily available, it is likely that clinicians will need to consider new guidelines for patient management, especially when administering drugs with narrow therapeutic indexes such as warfarin.²⁸ Variant CYP2C9 genotype could be considered a "sensitivity factor" for low-dose requirements when initiating warfarin therapy, and patients with a variant genotype could be considered candidates for increased surveillance for bleeding risk.

The prognostic specificity of CYP2C9 genotype was also an important factor in its clinical usefulness. Significant additional medical care resources could be consumed unnecessarily by patients with variant genotypes despite low risk of bleeding. It was necessary to demonstrate the value of the genotype information as being useful in the maintenance phase.⁷

Understanding the pharmacogenetics that contributes to variability in the vitamin K antagonist anticoagulant and dose-response relationship may help in tailoring drug therapy to patients in a safe and effective manner. This study confirms the dose-genotype association found in previous studies.⁷ We found that patients with a variant genotype experienced a higher rate of above-range INRs, less stability on maintenance therapy, and a higher risk of bleeding events. The use of CYP2C9 testing may be a method to identify high-risk patients who are candidates for lower vitamin K antagonist anticoagulant doses, more frequent monitoring, or treatment with alternate therapies as they become available.

CONCLUSION

The results of our study suggest that the CYP2C9 polymorphisms are associated with an increased risk of over anticoagulation/ prolonged INR and of bleeding events among patients in on vitamin K antagonist anticoagulant. Screening for CYP2C9 variants may allow clinicians to develop dosing protocols and surveillance techniques to reduce the risk of adverse drug reactions in patients receiving oral vitamin K antagonist anticoagulant. However the cost-effectiveness of genotyping patients must be considered.

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