Pharmacogenetic Aspects of Coumarinic Oral Anticoagulant Therapies

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Received: 17 April 2011 / Accepted: 17 April 2011 / Published online: 1 May 2011
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Abstract Coumarinic oral-anticoagulants (COAs) are commonly used for treatment of thromboembolic events. However, these medications have a narrow therapeutic range and there are large inter-individual variations in drug response. This is especially important in the initial phases of oral-anticoagulant therapy. Recent advancements in pharmacogenetics have established that clinical outcomes in oral-anticoagulant therapy are affected by genetic factors. The allelic variants of genes like cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase complex subunit 1 (VKORC1) are closely associated with maintenance dose of oral anti-coagulants. In addition, GGCX (Gamma-glutamyl carboxylase) polymorphism at position 12970 (rs11676382), CYP4F2 (rs2108622; V433M; 1347 C > T) and Apolipoprotein E (APOE) variants have been shown to explain a small but significant influence on dose requirements. There are large differences in the frequencies of these polymorphisms between different world populations which are also related to the requirements of oral anticoagulants. However, the final drug dosage in an individual is determined by complex sets of genetic and environmental factors

and several dosing algorithms which combine clinical and genetic parameters to predict therapeutic COA doses have also been developed. The algorithm based dose prediction shows the importance of pharmacogenetic testing in patients undergoing oral anticoagulant therapies.

Keywords Coumarinic oral anticoagulants · Pharmacogenetics · Vitamin K cycle · Drug metabolism · Polymorphism · Dosing algorithm

Introduction

A large number of 4-hydroxycoumarins, with aromatic substituent at the 3' position, possess anticoagulant properties and collectively called coumarinic oral-anticoagulants (COAs). The commercially available coumarins include warfarin, acitrom, acenocoumarol and phenprocoumon. In India, acitrom is the popular drug of choice for oral anticoagulant therapy in many indications like calf deep vein thrombosis, proximal deep vein thrombosis, pulmonary embolism, recurrent venous thromboembolism, cardioversion, atrial fibrillation, antiphospholipid syndrome and mechanical heart valves. In the western world, warfarin is given as oral anticoagulant. Both acitrom and warfarin belong to the same class of acenocoumarol drugs and exist in R- and S- enantiomeric forms.

Although, COAs are widely prescribed for treatment of thromboembolic events but these medications have a narrow therapeutic index indicated by the fact that administration and use of these COAs is associated with clot formation and bleeding when the dose is subtherapeutic and supratherapeutic respectively. Therefore, dosage adjustments are frequently required as there is large inter-individual variation in drug response. This is especially important in the initial

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phases of oral-anticoagulant therapy. The safe and effective use of these oral-anticoagulants is closely monitored by maintaining prothrombin time within a therapeutic range. Prothrombin time is expressed as International Normalised Ratio (INR). Traditional methods for dose adjustments include trial and error methods especially in case of new patients. The frequent monitoring of INR is expensive as well as responsible for patient discomfort.

Recent advancements in pharmacogenetics have established that clinical outcomes in oral-anticoagulant therapy are affected by genetic factors. The allelic variants of genes like cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase complex subunit 1 (VKORC1) are closely associated with maintenance dose of oral anti-coagulants. However, the final drug dosage in an individual is determined by complex sets of genetic and environmental factors. Therefore, several dosing algorithms which combine clinical and genetic parameters to predict therapeutic COA dose have also been developed. The algorithm based dose prediction shows the importance of pharmacogenetic testing in patients who are likely to undergo COA therapy.

Effect of Coumarins on Coagulation Cascade

The blood coagulation cascade requires biologically active forms of the calcium-dependent clotting factors II, VII, IX and X and the regulatory factors protein C, protein S, and protein Z. Synthesis of these factors is inhibited by COAs [1, 2]. Gamma-glutamyl carboxylase enzyme carboxylates the glutamic acid residues of the precursors of clotting factors. This makes the coagulation factors to bind to phospholipid surfaces inside blood vessels, on the vascular endothelium. The carboxylase enzyme simultaneously converts a reduced form of vitamin K (vitamin K hydroquinone) to vitamin K epoxide which is converted back to vitamin K and vitamin K hydroquinone by vitamin K epoxide reductase (VKOR).

The coumarinic anticoagulants target the vitamin K epoxide reductase enzyme in the vitamin K cycle. The cycle is necessary for the generation of biologically active coagulation factors and proper functioning of coagulation cascade. Specifically, COAs act on vitamin K epoxide reductase subunit 1 [3–5] and reduce the available vitamin K and vitamin K hydroquinone in the tissues. As a consequence, the carboxylation activity of glutamyl carboxylase is quenched. This results in the inability of the coagulation factors to get carboxylated at glutamic acid residues rendering them biologically inactive. The inactive factors replace the previously produced active factors over several days and the anticoagulation becomes effective. The under-carboxylated and biologically inactive coagulation factors are known as PIVKAs (proteins induced by

vitamin K absence/antagonism). The COAs, therefore, cause hindrance of coagulation cascade in the patient Fig. 1.

Genetic Variants in Components of the Coagulation Cascade

VKOR is the target enzyme for COAs, therefore the identification and molecular characterization of the gene coding for VKOR had been a landmark in the pharmacogenetics of COA therapy [4]. This enzyme is involved in the vitamin K cycle and hence genetic variations in the gene coding for this enzyme have a role in the functioning of this cycle. Lower VKOR activity is found to be associated with certain single nucleotide polymorphisms (SNPs) in the gene encoding VKORC1 [5]. There are many studies reporting the influence of SNPs in the VKORC1 activity. These SNPs include 3462C > T or 8773C > T, 2255C > T or 7566C > T, 1542G > C or 6853G > C, 1173C > T, 6009C > T or 698C > T [6, 7]. However, the VKORC1 –1639 G > A, C6484T, 1173C > T, G3673A, G9041A and 3730 G > A polymorphisms are important variants that influence the dosage requirements to achieve the therapeutic INR [8]. The SNPs 1173C > T in intron 1 and 3730 G > A in 3' UTR region are in linkage disequilibrium with –1639 G > A polymorphism in the promoter region which is responsible for the low dose phenotype [9]. Therefore, VKORC1 –1639 G > A has emerged as the primary SNP in pharmacogenetic dose requirements for coumarinic oral-anticoagulants.

It has been found that patients with VKORC1 –1639 G > A variant AA genotype have least VKOR enzyme functionality causing low availability of vitamin K and vitamin K hydroquinone in the tissues. This results in reduced efficacy in blood clotting and decrease in mean

Fig. 1 Vitamin K cycle and action of COAs in its inhibition. COAs inhibit the VKORC1 enzyme involved in the regeneration of oxidized form of vitamin K. This indirectly stops the production of biologically active forms of clotting factors II, VII, IX, X and proteins C/S/Z

daily drug requirement for anticoagulation. In persons with heterozygous GA genotype, the VKOR enzyme shows intermediate activity and therefore higher maintenance mean daily drug dose requirement. The highest requirement of oral anticoagulant has been observed with wild type GG genotype where the enzyme is not defective at all and is fully functional (Table 1) [10].

There is difference in the distribution of VKORC1 –1639 G > A genotypes between different world populations. The AA genotype is more frequent in Chinese than that in the Caucasians. [11]. The frequency of VKORC1 –1639A allele was higher in Chinese (CHB, 94.6%), Japanese (JPT, 90.1%) and European (CEU, 39.8%) populations [11]. The VKORC1 –1639A allele frequency in the northern Indian population was 14.22% [12]. The dose requirements of oral anticoagulants were also found to be in accordance with the trend shown by the genotypes [13]. In a study involving Chinese, Malay and Indians living in Singapore [14], the mean weight-normalized COA dose was lower for Chinese and Malays than for Indians. In our study, we genotyped 180 patients of northern Indian origin, who were on COA therapy with stabilized INR range values between 2.0 and 3.5, for VKORC1 –1639 G > A polymorphism and observed that the VKORC1 –1639G > A minor allele frequency in the northern Indian patient group was 22% [10]. The genotyping for VKORC1 –1639 G > A polymorphism was done by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) using the restriction enzyme Msp1 (Fig. 2).

Other Genes in Coagulation Cascade

Another enzyme of vitamin K cycle, Gamma-glutamyl carboxylase (GGCX) also exhibits polymorphism in its coding genetic sequence. In a study on a European population, a SNP at position 12970 (rs11676382) explained a

Fig. 2 VKORC1 –1639 G > A Genotyping

small but significant 2% variance in warfarin dose requirement [15].

Prothrombin gene has a G to A transition at nucleotide position 20210. This is termed as the factor II G 20210 A mutation. The association of factor II G20210A mutation with an increased risk of venous thrombosis was reported by Poort et al. [16]. Several studies confirmed this initial observation [17, 18]. The patients with this genetic change show increased prothrombin levels and therefore more thrombotic activity [19]. This may cause greater requirement of COAs for effective anticoagulation.

Genetic Variants in Drug Metabolizing Enzymes

Many foreign chemicals, drugs and endogenous compounds including oral coumarins are metabolised by cytochrome P450 (CYP) enzymes. CYP2C9 is important in the metabolism and inactivation of the COAs. COAs exist in the form of a racemic mixture of two active enantiomers R- and S- forms. Both of these enantiomeric forms are metabolized by different pathways and their pharmacodynamic properties are also different. S- acenocoumarol is several times less potent as an anticoagulant than R-acenocoumarol [20].

After ingestion, the coumarins are first biotransformed. The biotransformation of coumarins occurs by at least two

Table 1 Relationship between VKORC1 -1639G > A, CYP2C9*2 and CYP2C9*3 polymorphisms with mean weight normalized maintenance doses of Acitrom

VKORC1-1639G > A	Acitrom mean daily dose, mg/kg body weight (SD)
GG	0.064 (0.027)
GA	0.046 (0.017)
AA	0.024 (0.010)
CYP2C9*2	Acitrom mean daily dose, mg/kg body weight (SD)
CC	0.057 (0.026)
CT	0.051 (0.021)
CYP2C9*3	Acitrom mean daily dose, mg/kg body weight (SD)
AA	0.058 (0.025)
AC	0.049 (0.028)

metabolic pathways—oxidation and reduction to pharmacologically inactive metabolites. Oxidation and reduction result into two hydroxylated metabolites and two different alcohol metabolites respectively. Reduction of the nitrite group produces an amine metabolite which is further transformed to the corresponding acetamide metabolite.

Polymorphisms due to single base substitutions in the coding region of CYP gene cause expression of proteins with differential catalytic activities [21]. The wild-type reference allele is designated as CYP2C9*1 (Arg144/Tyr356/Ile359/Gly417). CYP2C9*2 and CYP2C9*3 are the two most common variant alleles with amino acid substitutions Arg144Cys and Ile359Leu respectively.

The variant alleles of CYP2C9 result in expression of enzymes with decreased activity. The *2 genotype shows a decreased enzyme activity of 30% and the *3 genotype shows a decrease of 80%. The activity of CYP2C9 enzyme is directly associated with the metabolism of COAs. The *3 allele in homozygous condition shows poorest enzyme activity. Furuya et al. [22] have done first in vivo experiment to report that warfarin dose requirement is affected CYP2C9 polymorphisms. In this study, up to 20% lower warfarin doses were required in patients with the CYP2C9*1*2 genotype to maintain a target INR between 2 and 4 as compared to the anticoagulated patient population studied. 90% of patients requiring the lowest warfarin dose were heterozygous for *2. The odds ratio for an individual on low-dose warfarin having one or more of the variant CYP2C9 alleles as compared with the general patient population was found to be 6.21 (95% CI 2.48–15.6) [23]. The patients in the low dose subgroup were also prone to develop bleeding complications. Association between CYP2C9 polymorphism and warfarin dose requirement has also been confirmed by Scordo et al. [24].

The CYP2C9*2 frequency is higher in European population (CEU, 10.4%) but 0% in Chinese, Japanese and African populations [11]. We have reported the CYP2C9*2 and *3 allele frequencies in northern Indian population as 4.9 and 3.9%, respectively [12] (Table 2). However, the CYP2C9*2 allele frequency in North Indians and South Indians was found to be comparable.

We have also looked for genotypic effect on dosage requirements in northern Indian patients in case of CYP2C9*2 and *3 polymorphisms. In 180 patients of northern Indian origin, who were on COA therapy with stabilized INR range values between 2.0 and 3.5, were genotyped for CYP2C9*2 and *3 polymorphisms. The patients with wild type genotype (CYP2C9*1) required higher doses in comparison to their heterozygous counterparts with CYP2C9*2 and *3 polymorphisms (Table 1). This can be explained by decreased functionality of the drug metabolizing enzyme due to presence of variant

Table 2 Minor allele frequencies of VKORC1 –1639 G > A, CYP2C9*2, CYP2C9*3, CYP4F2 V433M:C > T and GGCX 12970 C > G polymorphism in North Indian and the HapMap selected populations

SNP ID	Major/Minor allele	Minor allele frequencies (%)					
		NI	CEU	CHB	GIH	JPT	AFR
rs9923231 (VKORC1)	G/A	14.2	39.8	94.6	19.3	90.1	2.2
rs1799853 (CYP2C9*2)	C/T	4.9	10.4	0	n.r.	0	0
rs1057910 (CYP2C9*3)	A/C	3.9	5.8	5.4	13.1	2.3	n.r.
rs2108622 (CYP4F2)	C/T	n.r.	23.2	21.7	44.1	23.2	5.1
rs11676382 (GGCX)	C/G	n.r.	11.5	0	n.r.	0	0

NI North Indian (the present data); CEU, CEPH (Utah residents with ancestry from Northern and Western Europe); GIH Gujarati Indians in USA; JPT Japanese in Tokyo, Japan; CHB Han Chinese in Beijing, China; AFR African. n.r. not reported [11]

alleles and thereby greater available drug concentrations for effective anticoagulation.

A SNP in CYP4F2 (rs2108622; V433M; 1347 C > T) was reported by Caldwell et al. [25]. This SNP accounted for a difference in drug dose of ~1 mg/day between TT and CC genotypes in three US patient cohorts. CYP4F2 polymorphism affects COA dose requirement and it has been confirmed by other investigators also. The exact role of CYP4F2 enzyme in COA pharmacokinetics/dynamics has not been found yet.

Genetic Variations in Other Genes

Apart from the polymorphisms present in the cytochrome P450 and VKORC1 genes, there are others which are implicated to affect therapeutic doses of COAs. These include Apolipoprotein E (APOE) variants encoded by three alleles ϵ 2, ϵ 3, and ϵ 4 [26]. This protein is a major part of plasma lipoproteins which mediate receptor-specific uptake of vitamin K-rich lipoproteins into the liver. The three variants are responsible for the functional difference between the proteins coded by them. Frequency of these allelic variants is different in different races and therefore their influence on dose requirement is relevant only in those populations in which they are more frequent. For example, the ϵ 4 genotype frequency is very low in Asians in comparison to that in Caucasian and African Americans so it will not contribute much to warfarin dose requirement in Asian populations [27].

Genome-Wide Association Studies and COA Dose

Till recently, most genetic studies were carried out using candidate gene approaches. However, in recent years, availability of higher density microarray has made it possible to carry out Genome-Wide search for genes involved in pharmacogenetics of coumarins. These genome wide Association Studies (GWAS) confirm VKORC1, CYP2C9, and CYP4F2 as principal genetic determinants of Warfarin dose [28].

Environmental Factors Affecting the COA Dosage

The COA anticoagulation is affected by certain environmental factors, either alone or in combination. These include: drug dose, compliance, liver disorders, age, intercurrent illness other medications (including high doses of acetaminophen), diet, herbs, or supplements, smoking status and alcohol intake. For example, heavy or highly uncertain alcohol intake leads to liver dysfunction which may increase the effect of COAs. The patients on COA treatment should not completely inhibit consumption of vitamin K1 rich foods, but they should eat a diet with consistent vitamin K1 content. Many of the herbs containing coenzyme Q, ginseng, Gingko biloba, and vitamin E may influence the COA effect. The excessive intake of food supplements may result in fluctuating INR values. Any medication that affects platelet function increases the risk of hemorrhage and clinical outcome of COA treatment.

Pharmacogenetics Guided Dosing Algorithms for COAs

With the advancements in pharmacogenetics and identification of genetic markers primarily responsible for therapeutic dose variations, there have been efforts to develop algorithms based on clinical as well as genetic information to predict the correct dose for an individual patient. This approach is leading towards personalised medicine in the coming era of pharmacogenetic therapy. Most of these algorithms calculate drug dose by considering clinical and genetic factors such as patient's age, sex, height, body surface area, smoking status, interacting medicines (amiodarone and simvastatin), race, CYP2C9*2 and *3 genotypes and VKORC1-1639 GG/GA/AA genotypes (Table 3).

Gage et al. [29, 30] developed and validated a pharmacogenetic algorithm in a cohort of 1,015 participants using the following independent predictors of therapeutic dose: VKORC1 –1639/3673 G > A polymorphism, body surface area (BSA), use of statins and azole drugs, CYP2C9*3, CYP2C9*2, CYP2C9*5, CYP2C9*6, age, target INR, amiodarone use, smoker status, race, and

current thrombosis. Up to 53–54% of the variability in the warfarin dose could be explained by this algorithm. Authors also compared it with a clinical equation and found that only 17–22% of the dose variability ($P < 0.001$) is explained by the later one. They have developed a non-profit webpage, <http://www.WarfarinDosing.org>. This internet resource can be used by anyone to use the clinical and pharmacogenetic parameters to predict COA doses in follow-up as well as prospective patients.

Sconce et al. [31] have tried to study the contribution of age, CYP2C9 and VKORC1 genotype, and body size to warfarin-dose requirements. This study was carried out on 297 patients with stable anticoagulation as reflected by target INR of 2.0 to 3.0. The patients with CYP2C9 homozygous wild type genotype required highest mean warfarin daily doses as compared with those having variant *2 and *3 alleles. Similar trend was also observed in patients with VKORC1 (position –1639) GG genotype compared with those with the GA genotype and the AA genotype. Authors used the multivariate regression model and included age, CYP2C9 and VKORC1 genotypes, and height as variables to generate the best model for estimating warfarin dose.

In an attempt to develop a pharmacogenetic algorithm to predict warfarin maintenance doses, Zhu et al. [32] studied 65 patients with stable anticoagulation. They genotyped these patients for the CYP2C9*2 and *3 as well as for VKORC1 –1639 G > A polymorphism. They reported that the VKORC1 –1639A allele accounts for low dosage requirements of most patients without a CYP2C9 variant. In this study also VKORC1 and CYP2C9 genotypes, age, sex, and body weight were found to be the key players in warfarin dose variations.

A large prospective warfarin treated cohort study was done by Wadelius et al. [33] in Sweden. They genotyped 183 polymorphisms in 29 candidate genes in 1,496 Swedish patients starting warfarin treatment and looked for their association with response. A multivariate regression model was applied to develop an algorithm which used the predictors CYP2C9, VKORC1, age, sex, and drug interactions. The results of this study strongly promote the initiation of warfarin guided by pharmacogenetics in the improvement of clinical outcome.

A multicentre large study involving 4,043 patients from more than one population cohorts has been done by the International Warfarin Pharmacogenetics Consortium [34]. In this study two dose algorithms were designed: one used the clinical and genetic data both and the other one uses only clinical variables. Age, height, weight, VKORC1 –1639 G > A polymorphism, CYP2C9*2 and *3 alleles, race, and interacting medications were the variables used in these algorithms. The results of this study showed that the initial dose predicted by the pharmacogenetic algorithm

Table 3 Pharmacogenetics based COA dosing algorithms and the different variables used

Variables	Algorithms (<i>n</i> = Sample Size)					
	Gage et al. [29] (<i>n</i> = 369)	Sconce et al. [31] (<i>n</i> = 297)	Zhu et al. [32] (<i>n</i> = 65)	Gage et al. [30] (<i>n</i> = 1015)	IPWC [34] (<i>n</i> = 4043)	Wadelius et al. [33] (<i>n</i> = 1496)
Target INR	Yes	No	No	No	No	No
Interacting drugs	Yes	No	No	Yes	Yes	Yes
CYP2C9 *2 and *3 genotype	Yes	Yes	Yes	Yes	Yes	Yes
VKORC1 –1639 genotype	No	Yes	Yes	Yes	Yes	Yes
Age	Yes	Yes	Yes	Yes	Yes	Yes
Sex	Yes	No	Yes	No	Yes	No
Height	No	Yes	No	No	No	Yes
Weight	No	No	Yes	No	No	Yes
BSA	Yes	No	No	Yes	No	No
Enzyme inducer ^b	No	No	No	No	No	Yes
Race	Yes	No	No	Yes	No	Yes
Smoking status	No	No	No	Yes	No	No
Disease (DVT/PE) ^a	No	No	No	Yes	No	No

^a DVT deep vein thrombosis; PE pulmonary embolism

^b Cytochrome P450 enzyme inducers (phenytoin, carbamazepine, and rifampin)

was significantly closer to the therapeutic stable doses predicted by the clinical algorithm or fixed dose approach.

Shaw et al. [35] evaluated the accuracy of 6 pharmacogenetic dosing algorithms in predicting drug doses in a retrospective cohort study on 71 patients. These included algorithms by Gage et al. [29, 30], Sconce et al. [31], Zhu et al. [32], Wadelius et al. [33] and International Warfarin Pharmacogenetic Consortium (IWPC) [34] algorithm. The algorithms by Zhu et al. [32], Gage et al. [30], and IWPC [34] were similar in the primary accuracy endpoints with mean absolute error (MAE). Zhu et al. [32] algorithm was found to severely over-predict the dose as compared with Gage et al. [30] and IWPC [34] algorithms. In this study the algorithms published by Gage et al. [30] and the IWPC [34] were found to be the two most accurate pharmacogenetic equations in predicting therapeutic COA dose. The authors have, however, stated that the degree of accuracy obtained in this study does not still warrant the frequent use of genotyping to predict doses for all patients newly prescribed for COA therapy.

A recent study from southern India has been done by Pavani et al. [36] to optimize the warfarin dose. Vitamin K uptake, CYP2C9*2, CYP2C9*3, VKORC1*3, VKORC1*4, D36Y and -1639 G > A polymorphisms were used to develop a pharmacogenomic algorithm using multiple linear regression models. This new algorithm was validated by correlating it with the Wadelius et al. [33], International Warfarin Pharmacogenetics Consortium [34] and Gage et al. [30] algorithms with the therapeutic doses. The accuracy,

sensitivity and specificity of this new algorithm was claimed to be better than the other algorithms. By using this new algorithm, the rate of over or under-estimation of drug dose was claimed to be significantly reduced.

The US Food and Drug Administration (FDA) has approved and relabelled warfarin with indication of CYP2C9 and VKORC1 genotyping for dosage management in 2007. The revised label for warfarin stated that patients with variations in one or both of these genes should be prescribed with lower doses of warfarin. At present there is lack of sufficient randomized data from prospective studies, so there is a need to go for such studies for necessary changes in the guidelines [37].

Future of Pharmacogenetics Based COA Therapy

In the next decade it may be possible that large randomized trials will generate results that will show impact of pharmacogenetics on COA dosing and its incorporation in routine clinical practice [38]. Rapid and cost effective accurate genotyping will facilitate dosage management prior to the initiation of therapy. More genetic studies may identify additional genetic polymorphisms responsible for dosage variations among individuals. These studies will improve the predictive ability of dosing algorithms. Although the availability of high-throughput genotyping capabilities have facilitated pharmacodynamics based pharmacogenetic studies, pharmacoproteomic studies based

on proteomics may provide additional information regarding variability in COA dose requirements.

The burden of health systems is relieved by pharmacogenetics guided COA therapy as there will be reduced risk of complications due to over- or under-dosing. In the global scenario, clinical application of pharmacogenetically-guided oral anticoagulants is round the corner, but in India, there is an urgent need to initiate the background work for identifying various genetic and environmental factors to formulate India-specific algorithms for oral anticoagulant therapies.

Acknowledgment The study was supported by financial assistance from Department of Biotechnology (DBT) Government of India, New Delhi.

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