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GENETIC VARIANTS INFLUENCING VITAMIN K

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Abstract – *There are two forms of natural vitamin K, phylloquinone and menaquinone. Phylloquinone, vitamin K1, is the major type of dietary vitamin K, which is present in high amounts in green vegetables, while menaquinone, vitamin K2, is synthesized by the human intestinal bacteria. Furthermore, a synthetic type of vitamin K is known: vitamins K3. Vitamin K-dependent proteins require carboxylation of certain glutamate residues for their biological functions such as blood coagulation (factor II, VII, IX, X and proteins C, S and Z), bone metabolism (osteocalcin) and vascular biology. Without vitamin K, blood coagulation is impaired, and uncontrolled bleeding occurs. Low levels of vitamin K also weaken bones and promote calcification of arteries. The aim of this study is to examine how genetic variations influence responses to oral anticoagulant therapy. There is a large variability in response to anticoagulant therapy, and this can modify the benefit/risk ratio of drugs. This variability can be explained by clinical factors such as age, sex, demographic and environmental factors, inter- and intra-individual variability and genetic variants. In recent years, several genetic polymorphisms have been associated with variable biological responses to oral anticoagulants.*

KEYWORDS: *CYP2C9, VKORC1, CY4F2, Pharmacogenomics.*

INTRODUCTION

Vitamin K is an essential vitamin. It is one of the four fat-soluble vitamins as well as vitamins A, D, E. Vitamin K, phylloquinone and menaquinone, includes some derivatives naftoquinonic soluble. Phylloquinone, vitamin K_1, is the major type of dietary vitamin K, which is present in high amounts in green vegetables (spinach, cabbage, tomatoes). Menaquinone, vitamin K_2, is produced by intestinal microbiota. The intestinal production of Vitamin K_2 is usually enough to cover the daily requirements, and it is the main form stored in the liver (about 90% of the total). Another synthetic type of vitamin K is known as vitamins K3 or menadione, which is partially soluble in water. Menadione is a synthetic naphthoquinone without the isoprenoid side chain and biological activity, but it can be converted to active vitamin K2 by alkylation in vivo.

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Vitamin K participates in the ordinary process of blood clotting, and its deficiency causes bleeding. The blood clotting requires the carboxylation of certain glutamate residues of the protein prothrombin into γ-carboxyprothrombin so that it can bind to other factors. Carboxylation is catalyzed by the enzyme y-glutamyl carboxylase (GGCX) which requires three co-substrates: reduced vitamin K, CO_2, and O_2. Carboxylation requires the abstraction of a proton from the 4-carbon of glutamate by reduced vitamin K and results in the conversion of vitamin K to vitamin K epoxide. The vitamin K epoxide must be recycled to vitamin K before being reused, and this reaction is catalyzed by the enzyme vitamin K epoxide reductase (VKOR).

The four vitamin K-dependent procoagulants (prothrombin, and factors VII, IX, and X) are serine proteases that are synthesised in the liver and then secreted into the circulation as inactive forms (zymogens). The vitamin K in the diet, mainly as phylloquinone, is absorbed in the proximal intestine due to bile salts contained in pancreatic juice. At the level of the intestinal mucosa, vitamin K is incorporated into chylomicrons, secreted into the lymph and introduced into the bloodstream.

Circulating phylloquinone is associated with lipoprotein (LDL and VLDL), and only a small part is transported in free form. The liver is responsible for the reactivation of the vitamin by the action of the hepatic vitamin K epoxide reductase. So, if liver function is normal, the vitamin is effectively recovered and, since the intestinal microbiota synthesizes vitamin K, the daily requirements are satisfied.

Deficiencies can occur due to inadequate lipid absorption, intestinal dismicrobism and liver disease. Moreover during fetal development, placental transfer of vitamin K is low, leading to an exogenous deficiency of vitamin K in newborns secondary to low substrate intake. Newborns' main dietary intake of vitamin K is either through breast milk or commercially available infant formula; breast milk contains significantly lower levels of vitamin K than does commercial formula. Therefore, insufficient dietary intake of vitamin K continues after birth, especially in exclusively breastfed infants. Recommended prophylaxis of vitamin K by injection in the newborn period replaces physiologically low vitamin K1 levels and decreases the risk of vitamin K deficiency associated hemorrhage[1]. In addition to these classical applications, recently vitamin K_3 or menadione is used to protect the skin from the secondary toxicity caused by radiation therapy with EGFR inhibitors (Epidermal growth factor receptor). In this regard, we want to bring back two case studies. A case study of the effectiveness of vitamin K3 in radiotherapy with EGFR inhibitors erlotinib and cetuximab, and a case study on the usefulness of vitamin K3 (menadione) as against of pancreatic cancer.

VITAMIN K METABOLIC FATE

Vitamin K was discovered fortuitously in 1929 as part of experiments on sterol metabolism and was immediately associated with blood coagulation. It was named vitamin K because of the German word "koagulation".

The four vitamin K-dependent procoagulants (factor II or prothrombin, and factors VII, IX, and X) are serine proteases synthesized in the liver and then secreted into the circulation as inactive forms (zymogens)[2]. Their biologic activity is dependent on the normal complement of Gla residues, which are capable chelators of calcium ions. In the presence of Gla and calcium ions these proteins bind to the phospholipids of surface membrane of platelets and endothelial cells where, together with other cofactors, they form membrane-bound enzyme complexes. The zymogens of the four vitamin K-dependent clotting factors are cleaved to yield the active protease clotting factors[3]. The protein S and protein C (other vitamin K-dependent proteins) play a narrow role in the inhibition of coagulation. The role of protein C is to degrade phospholipid-bound of activated factors V and VIII in the presence of calcium. Protein S acts as a synergistic cofactor to protein C by enhancing the binding of activated protein C to negatively charged phospholipids. Another vitamin K-dependent protein described in human in 1984 is Protein Z[4]. Protein Z (PZ) is a vitamin K-dependent factor but it has no enzymatic activity. The structure of protein Z shows wide homology with many coagulation factors, such as VII, IX, X, and protein C. However, in contrast to other vitamin K-dependent coagulation factors, protein Z is not a serine protease because of the lack of the active centre in its amino acid sequence. It is a cofactor of a serpin, the protein Z-dependent protease inhibitor (ZPI), and the complex PZ/ZPI inhibits activated factor X on phospholipid surfaces[5,6]. Its plasma concentration spans a very wide range in normal individuals and its plasma concentration is greatly reduced during oral anticoagulation[7]. Despite conflicting results, a recent meta-analysis indicated that PZ deficiency could be a risk for venous and arterial thrombosis and early fetal loss[8]. However, these conclusions are drained from case-control studies of small size, constituting a significant limitation. Recently, it has been shown that PZ and/or ZPI are

synthesised by normal kidney and different cancer cells, suggesting that the complex PZ/ZPI might play a role in inhibiting the tissue deposition of fibrin[9-11]. The physiopathological consequences of these observations remain to be recognized.

VITAMIN K AND ANTICOAGULATION

Warfarin, a coumarin derivative, produces an anticoagulant effect by interfering with the interconversion cyclic of vitamin K and its 2,3 epoxide (vitamin K epoxide). Vitamin K is a cofactor for the carboxylation of glutamate residues to γ-carboxyglutamates (Gla) on the N-terminal regions of vitamin K-dependent proteins. By inhibiting the vitamin K conversion cycle, warfarin induces hepatic production of partially decarboxylated proteins with reduced coagulant activity[12]. Oral anticoagulant therapy is a widely used treatment of subjects with increased thrombosis risk; it is based on the daily intake of 4-hydroxycoumarins (warfarin, acenocoumarol, phenprocoumon) which bind to the VKORC1 enzyme and thus inhibit recycling of vitamin K. Consequently, the carboxylation of coagulation factors is inhibited resulting in the formation of inactive, non-carboxylated species also known as PIVKAs (proteins induced by vitamin K absence or antagonists). It was normally assumed that this was the only effect of oral anticoagulants. The discovery of new Gla-proteins not involved in blood coagulation initiated the search for side effects of oral anticoagulant treatment. Analysis of bone mineral density (BMD) in patients on long-term anticoagulation revealed that coumarin anticoagulants are associated with accelerated bone loss and low bone mass[12]. Therefore, long-term use of oral anticoagulants is considered as a risk factor for developing osteoporosis. Similarly, impairment of MGP must be regarded as a risk factor for arterial calcification. Indeed, two independent studies have demonstrated that subjects on long-term anticoagulation have much more arterial and heart valve calcification than age- and sex-matched control population.

GENETIC VARIANTS INFLUENCING VITAMIN K PLASMA LEVELS AND THE ROLE OF ORAL ANTICOAGULANT THERAPY

The new era of pharmacogenomics, which integrates individual genetic profile with the pharmacokinetic and the pharmacodynamic of a drug, provide greater safety and efficacy in drug therapy[13]. The difficulty in managing oral anticoagulants is strongly associated to the narrow therapeutic index range of Warfarin and acenocoumarol and to the great inter- and intra-individual variability in response to the treatment. This is estimated by measuring the International Normalized Ratio (INR), sensitive to clotting factors deficiencies (factors II, VII and IX, three of the VK-dependent clotting factors). Beside with demographic and environmental factors, genetic polymorphisms have also been identified, explaining the reason for the unpredictability in response to oral anticoagulant therapy[14]. To date, genetic variants influencing the vitamin K pathway include the key genes (Figure 1): a) vitamin K Epoxide reductase C1 (VKORC1), b) cytochrome-P450 2C9 (CYP2C9); and c) cytochrome-P450 4F2 (CYP4F2).

The VKORC1 gene encodes a dithiol-dependent reductase that converts VK epoxide to VK quinone. This enzyme appears to be one of the target enzymes of oral anticoagulants (ie Warfarin). Irreversible inhibition of VKORC1 by oral anticoagulants blocks VK regeneration, resulting in non-functional pro-coagulation factors.

VKORC1 gene is located on chromosome 16 and various polymorphisms have been described, most of them grouped into 4 major haplotypes. Among them, VKORC1*2 haplotype seems to be the most important in relation to the variability in response to oral anticoagulants and the risk of excessive bleeding[15].

The VKORC1*2 haplotype is labelled by the G-1639A polymorphism located in the promoter region of the VKORC1 gene, indicating the presence of a low amount of active VK (Table 1). Several studies report that the VKORC1*2 haplotype is related to the risk of bleeding in acenocoumarol average dose. Also, the C-1173T polymorphism in intron 1 of the VKORC1 gene is a representative for the VKORC1*2 haplotype, because it is in complete linkage disequilibrium with G-1639A polymorphisms. VKORC1 C-1173T polymorphism, whose T-allele frequency is 45% in Caucasians, meaning that almost half of the individuals belonging to this population could be at risk of sensitivity to acenocoumarol. The G-1639A polymorphism recorded a frequency of 57.8% and frequency of 42.2% for G and A allele, respectively. It seems that the VKORC1*2 haplotype has a greater contribution (40%) to the inter-individual and inter-ethnic variability in response to acenocoumarol than the CYP2C9 variants. CYP2C9 variants have 14% of contribution so the variability in response to acenocoumarol is over 50% determined by CYP2C9 and VKORC1 variants[16].

Rare mutations in VKORC1 gene relate with anticoagulant resistance. Therefore, we need higher

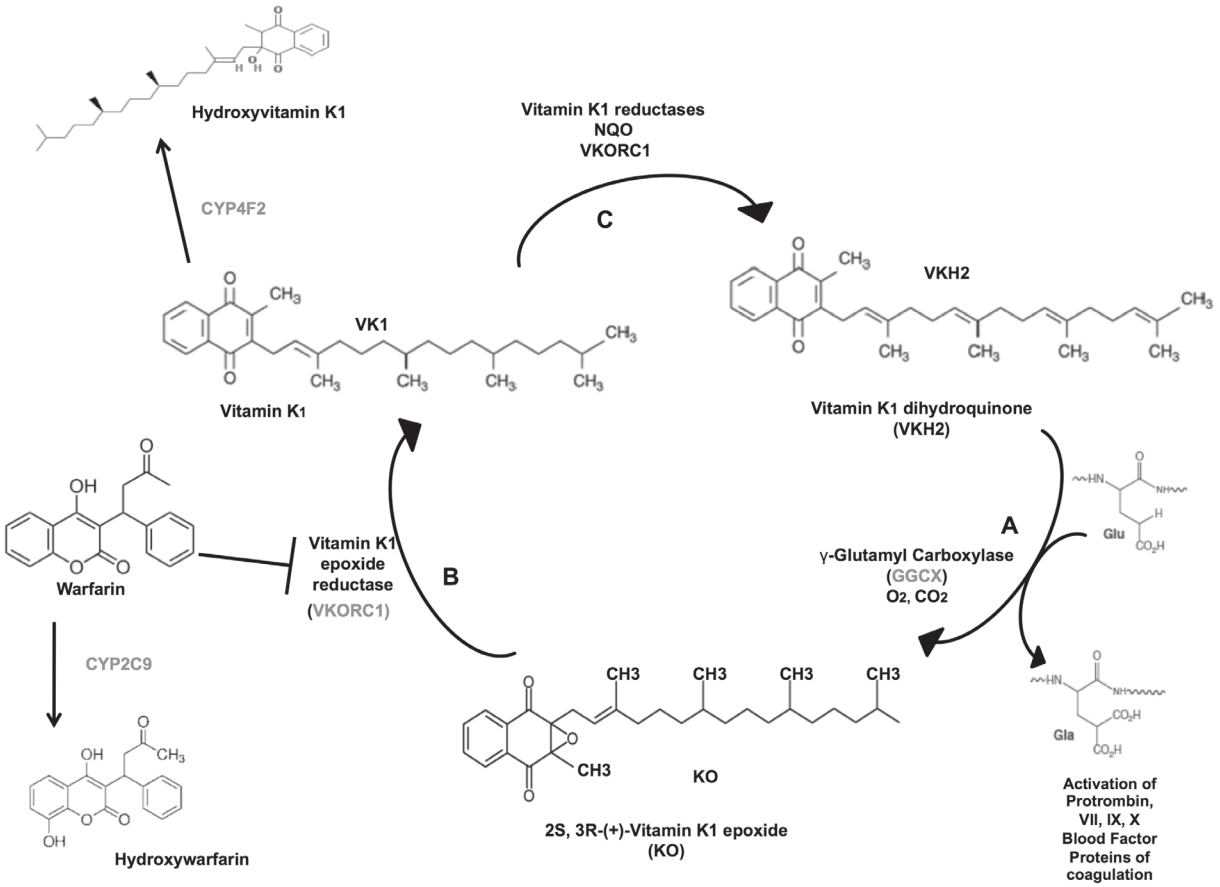

Fig. 1. H

doses of anticoagulant to win this resistance. The mentioned mutation is a G5417T transversion, which results in the substitution of an aspartate with a tyrosine at codon 36 (Asp36Tyr) of the VKORC1[17].

D'Andrea et al[18] have identified 1173C>T polymorphism as an independent factor influencing the mean daily maintenance dose in a study accounting 147 patients treated with warfarin: it is significantly lower in 1173TT patients (3.5 mg; $p<0.001$) than in 1173CT patients (4.8 mg; $p=0.002$) and 1173CC patients (6.2 mg). Militaru et al[19] have shown the influence of the c.-1639G>A polymorphism on the time to therapeutic INR.

TABLE 1. Clinically validated polymorphysms involved in vitamin K plasma levels.

Allele	*Nucleotide changes*	*Exon location*	*Protein variation*	*Activity compared with wild type alleles*	*Reference*
VKORC1*2	-1639A>G	5'UTR	—	Low-dose warfarin	Buzoianu AD 2013
VKORC1*2	-1173C>T	Intron 1	—	Low-dose warfarin	D'Andrea G 2005
VKORC1	5417G>T	Exon 1	Asp36Tyr	Higher warfarin dose	Loebstein R 2007
CYP2C9*2	430C>T	Exon 3	Arg144Cys	Decrease	Higashi MK 2002, Di Francia R 2015, Militaru FC 2014
CYP2C9*3	1075A>C	Exon 7	Ile359Leu	Decrease	
CYP2C9*4	1076T>C	Exon 7	Ile345Thr	-	
CYP2C9*5	1080C>G	Exon 7	Asp360Glu	Decrease	
CYP2C9*6	818delA	Exon 5	Null allele	No activity	
CYP2C9*8	449G>A	Exon 3	Arg150His	Increase	
CYP2C9*9	752A>G	Exon 5	His251Arg	Decrease	
CYP2C9*11	1003C>T	Exon 7	Arg335Trp	Decrease	
CYP2C9*12	1465C>T	Exon 9	Pro489Ser	Decrease	
CYP4F2	1297G>A	–	Val433Met	VK plasma level variabilty	Hirai K 2015

Montes et al[20] have also demonstrated that the A allele of the -1639G>A polymorphism in the VKORC1 gene is associated with the need for a lower dose of acenocoumarol.

The polymorphisms found in CYP2C9 gene vary according to ethnicity (African, Asian, Caucasian). For example, the most widespread variants in Caucasians are CYP2C9*2 (Arg 144-Cys) and CYP2C9*3 (Ile 359-Leu), present in 8-19% and 6-10% respectively. The mutant enzymes of these polymorphisms reduce the metabolism of coumarin derivatives. Individuals carrying at least one mutant allele have an increased sensitivity to warfarin and they are known as "poor metabolizers" (PM). These conditions have dramatic implications for drug-drug interaction in concomitant therapies[21]. Several studies have shown a relationship between the genotype and the mean warfarin maintenance dosing: in mutated patients, compared to patients carrying two normal alleles(CYP2C9*1/*1).As calculated by international algoritm warfarin dosing the dose is reduced by:

– 13-22%; in patients carrying 2C9*1/*2 alleles
– 18-40% in patients carrying 2C9*2/*2 alleles;
– 21-49%; in patients carrying 2C9*1/*3 alleles;
– 18-73%; in patients carrying 2C9*2/*3, and

71% or more in patients carrying 2C9*3/*3 alleles (Table 1). Higashi et al[22], in a retrospective study conducted on 185 patients receiving warfarin therapy, have revealed that the patients bearing at least one CYP2C9 mutant allele is associated with a significantly increased dosage and risk of bleeding (*hazard ratio* [HR] 1.40 [95% CI: 1.03-1.90] $p<0.001$), with an increase in the maintenance dose range (HR 0.65 [95% CI: 0.45-0.94] $p<0.001$). The risk of warfarin overdose is comparable to the results obtained in similar studies of acenocoumarol and phenprocroumon based therapy[23]. CYP4F2 enzyme is known to influence plasma vitamin K concentration[24]. A recent study conducted on 217 Japanese patients treated with oral anticoagulant, demonstrated that the plasma vitamin K1 (VK1) and menaquinone-4 (MK-4) concentrations are significantly influenced by CYP4F2 genetic polymorphism but not associated with warfarin therapy. Patients with the CYP4F2 (rs2108622) at nucleotide 1297G>A, the AA genotype had significantly higher plasma VK1 and MK-4 concentrations than those with GG and GA genotypes. The multiple linear regression model including VKORC1, CYP4F2, and CYP2C9 gene tic variants, age, and weight could explain 42% of the variability in warfarin dosage. The contribution of CYP4F2 polymorphism has been estimated to be 2.2%[25].

DISCUSSION

The management of VKA is complex and it depends on several mutations for CYP2C9 and VKORC1 genes. The risk of bleeding and thromboembolic events is really tangible; there have been attempts to develop algorithms in order to establish the therapeutic dose that would protect the patient from these risks[26]. The algorithms developed to estimate the adequate therapeutic doses of warfarin, acenocoumarol or phenprocoumon are based on clinical (age, gender, body mass index liver failure, kidney failure); concomitant therapy (amiodarone, statins, antifungals, antibiotics, ACE inhibitors,) and on mutations that directly or indirectly influence the therapy with VKA (polymorphisms in the VKORC1, CYP2C9, CYP4F2, and GGCX genes)[27]. Instead, the algorithms establishing the stable dose of VK antagonists have shown good results in reducing the frequency of adverse reactions. To date, the literature isn't still indicating about the cost-efficiency ratio. Furthermore, a detailed knowledge of pharmacology is a prerequisite for application in clinical practice, and physicians might find it difficult to interpret the clinical value of pharmacogenetic test results[28].

Conflict of Interests:
The Authors declare that they have no conflict of interests.

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