Current pharmacogenetic developments in oral anticoagulation therapy: The influence of variant VKORC1 and CYP2C9 alleles

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Summary
For decades coumarins have been the most commonly prescribed drugs for therapy and prophylaxis of thromboembolic conditions. Despite the limitation of their narrow therapeutic dosage window, the broad variation of intra- and inter-individual drug requirement, and the relatively high incidence of bleeding complications, prescriptions for coumarins are increasing due to the aging populations in industrialised countries. The identification of the molecular target of coumarins, VKORC1, has greatly improved the understanding of coumarin treatment and illuminated new perspectives for a safer and more individualized oral anticoagulation therapy. Mutations and SNPs within the translated and non-translated regions of the VKORC1 gene have been shown to cause coumarin resistance and sensitivity, respectively. Besides the known CYP2C9 variants that affect coumarin metabolism, the haplotype VKORC1*2 representing a frequent SNP within the VKORC1 promoter has been identified as a major determinant of coumarin sensitivity, reducing VKORC1 enzyme activity to 50% of wild type. Homozygous carriers of the VKORC1*2 allele are strongly predisposed to coumarin sensitivity. Using individualized dose adaptation, a significant reduction of bleeding complications can be expected, especially in the initial drug saturation phase. Furthermore, concomitant application of low dose vitamin K may significantly reduce intra-individual coumarin dose variation and, thus, may stabilize oral anticoagulation therapy. The use of new pharmacogenetics-based dosing schemes and the concomitant application of low-dose vitamin K with coumarins will decidedly influence the current practice of oral anticoagulation and greatly improve coumarin drug safety.

Keywords
VKORC1, CYP2C9, acenocoumarol, phenprocoumon, warfarin, genotype

Introduction
Since 1950, coumarins such as warfarin and phenprocoumon have been widely used as orally administered anticoagulants for therapy and prophylaxis of thromboembolic conditions. They rank among the most prescribed drugs worldwide and millions of patients begin coumarin therapy each year. Although oral application is convenient for the patient and the annual cost of administration is low, clinical use of coumarins is complicated by their narrow effective therapeutic concentration ranges and broad variation in required individual dosage (1). Nevertheless, vitamin K antagonists still remain the therapy of choice for short-term through lifetime anticoagulation treatment and are used intensively in prevention of both venous and arterial thromboembolic disorders including deep vein thrombosis, pulmonary embolism and ischaemic stroke (2, 3). Clinical management of coumarin therapy has, thus far, been demanding, as potentially serious adverse effects such as intracerebral bleeding can lead to severe morbidity or death. Especially in the first weeks of treatment, risk of haemorrhagic events exceeds 10–17%, including 2–5% major bleedings (4). The risk of bleeding during this initial treatment timeframe is ten times greater compared to that by the twelfth month of treatment (5). These difficulties, chiefly due to highly variable interindividual response to coumarins, excarer-...
bate selection of correct initial dosage. Furthermore, the final adjusted maintenance dose shows high interindividual variability. To minimize the bleeding risk, known influencing factors including weight, gender, age, and race have been historically considered when setting initial dosage (6, 7). Additionally, frequent monitoring of the INR, especially during the initial phase of oral anticoagulation therapy, is regularly used to indicate onset of over-anticoagulation in order to inform dosage adjustment.

Recently, the human vitamin K epoxide reductase (VKORC1) was successfully cloned. This enzyme sustains the vitamin K-cycle and is inhibited by coumarins (8, 9). Its influence on coumarin dose is significant, as the common haplotype group VKORC1*2 is responsible for a VKORC1-mRNA expression level decrease of approximately 50% compared to wild type transcriptional levels (10, 11). Furthermore, several rare amino acid mutations in the translated VKORC1 primary sequence have been identified that result in coumarin resistance (see Table 1; [8, 12–15]). Combining these newly discovered pharmacogenetic marker with previously used environmental and patient-specific factors such as the CYP2C9 genotype, age, weight, liver function, magnitude of dietary intake of vitamin K and interaction with other drugs, new dosing algorithms have been developed (16–20). Compared to classical algorithms (21, 22), these new dosing regimens were shown to predict coumarin maintenance dose quite accurately and could contribute to improved drug safety. Thus, the outlook is currently optimistic for improving individualized therapeutic regimens based on previously published exploratory pharmacodynamic and phar-

Table 1: Variant VKORC1 and CYP2C9 alleles and their influence on warfarin dosage adjustment in humans. Notational clarifications: **) Warfarin dosage adjustment for each mutation from a single patient case given in n-fold increase over a population-averaged mean dose of 5 mg/day. †) For SNPs affecting VKORC1 mRNA transcriptional levels, dosage adjustments are given as percentage relative to the warfarin dosage requirement in homozygous VKORC1*1. ‡) For SNPs affecting the CYP2C9 enzyme activity, dosage adjustments are given as percentage relative to the warfarin dosage requirement in homozygous CYP2C9*1.

<table>
<thead>
<tr>
<th>Haplotype or aa mutation</th>
<th>Warfarin dosage adjustment**</th>
<th>Comments (ref.)</th>
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<tbody>
<tr>
<td>Seven known human VKORC1 ORF polymorphisms (single aa mutations)</td>
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<tr>
<td>VKORC1 V29L</td>
<td>+3- to +5-fold (patient data) 96.5% wt activity (measured in vitro)</td>
<td>warfarin resistance, rare (8)</td>
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<tr>
<td>VKORC1 D36Y</td>
<td>+2-fold (patient data)</td>
<td>warfarin resistance, rare (14, 84), 15% in Jewish ethnic groups of Ethiopian origin (15)</td>
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<tr>
<td>VKORC1 V45A</td>
<td>“complete resistance” (max. dosage unreported) 23.0% wt activity (measured in vitro)</td>
<td>warfarin resistance, rare (8)</td>
</tr>
<tr>
<td>VKORC1 R58G</td>
<td>+6- to +8-fold (patient data) 20.7% wt activity (measured in vitro)</td>
<td>warfarin resistance, rare (8)</td>
</tr>
<tr>
<td>VKORC1 V66M</td>
<td>&gt;+5-fold (patient data)</td>
<td>warfarin resistance, rare (12)</td>
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<tr>
<td>VKORC1 R98W</td>
<td>not a WR phenotype</td>
<td>VKCFD2 disease, rare (8)</td>
</tr>
<tr>
<td>VKORC1 L128R</td>
<td>“complete resistance” +9-fold (patient data) 5.2% wt activity (measured in vitro)</td>
<td>warfarin resistance, rare (8, 13)</td>
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<tr>
<td>Human VKORC1 non-coding region SNPs affecting transcriptional level †</td>
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<tr>
<td>VKORC1*1 (wt) homozygous</td>
<td>Reference warfarin dosage level</td>
<td>10% Africans, 0% Chinese, 0% Caucasians (80)</td>
</tr>
<tr>
<td>VKORC1*1 (wt) heterozygous</td>
<td>0 or -25% dependent on 2nd allele</td>
<td>31% Africans, 0% Chinese, 0% Caucasians (80)</td>
</tr>
<tr>
<td>VKORC1*2 homozygous</td>
<td>−50%</td>
<td>14% Africans, 95% Asians, 42% Caucasians (80)</td>
</tr>
<tr>
<td>VKORC1*2 heterozygous</td>
<td>−25%</td>
<td>14% Africans, 95% Chinese, 42% Caucasians (80)</td>
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<tr>
<td>VKORC1*3 hetero- &amp; homozygous</td>
<td>As for †1</td>
<td>43% Africans, 4% Chinese, 38% Caucasians (heterozygous) (80)</td>
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<tr>
<td>VKORC1*4 hetero- &amp; homozygous</td>
<td>As for †1</td>
<td>12% Africans, &lt;1% Chinese, 20% Caucasians (heterozygous) (80)</td>
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<tr>
<td>Human CYP2C9 SNPs affecting anticoagulant metabolism (single aa mutations) ‡</td>
<td></td>
<td></td>
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<tr>
<td>CYP2C9*1 homozygous wild-type</td>
<td>Reference warfarin dosage level</td>
<td>(16)</td>
</tr>
<tr>
<td>CYP2C9*2 R144C</td>
<td>−20 % warfarin for each *2 allele</td>
<td>2.0–8.7% Africans, 0 % Asians, 9.2–26.7% Caucasians (16, 86)</td>
</tr>
<tr>
<td>CYP2C9*3 I359L</td>
<td>−40 % warfarin for each *3 allele</td>
<td>1.0–4.6% Africans, 2.3–8.2% Asians, 8.6–26.7% Caucasians (16, 86)</td>
</tr>
<tr>
<td>CYP2C9*5 D360E</td>
<td>not reported</td>
<td>3.0% Africans, 0% Asians, 0% Caucasians (82, 89, 90)</td>
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<tr>
<td>CYP2C9*6 B188delA</td>
<td>not reported</td>
<td>0.6% Africans, 0% Asians, 0% Caucasians (90, 91)</td>
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<tr>
<td>CYP2C9*11 R335T</td>
<td>not reported</td>
<td>1% African, 1% Caucasian (70)</td>
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macokinetic analyses of worldwide patient populations. In the following pages we review these recent findings together with recent advances in understanding the enzymatic pathways at the molecular level that are directly affected by the genetic profile of patients relying on oral anticoagulation therapy based on coumarin derivatives.

Recent developments in understanding VKORC1 protein structure and function

Based on experimental evidence, VKORC1 is a membrane intrinsic protein localized to the endoplasmic reticulum (ER) with a proposed topology beginning with a short ER-localized N-terminus, three transmembrane α-helices with a solvent-accessible cytoplasmic loop between the first two helices, a short turn consisting of only a few amino acids between the second and third helices, and a short cytoplasmic C-terminus (23). Notably, four cysteine residues at the amino acid positions 43, 51, 132 and 135 were shown by sequence alignment to be completely conserved among all species analysed thus far including archaea, eubacteria, insects, vertebrates, and plants (24). Due to their high degree of conservation, these residues have been assumed to form part of the active catalytic center of VKORC1. Especially cysteins 132 and 135 have been proposed to be the primary residues of the redox center, catalysing the reduction of vitamin K epoxide to the quinone form. This assumption was confirmed by site-directed mutagenesis and recombinant expression of VKORC1 variants in HEK 293 (25). In these studies, variants Cys132Ser, Cys135Ser, Cys51Ser, and Ser57Ala exhibited highly impaired VKOR activity. In a recent article, Jin et al. (26) determined that the same Cys132/Cys135 pair is responsible not only for the first reaction reducing vitamin K epoxide to the quinone form, but additionally is catalyzing the second reaction that converts vitamin K quinone to the fully reduced quinol form that is required as a substrate for the subsequent γ-carboxylation reactions activating the necessary clotting factors. Interestingly, in making this discovery, Jin et al. were able to confirm by mass spectroscopic analysis of purified VKORC1, the existence of a previously unknown element of tertiary structure, a native disulfide bond between Cys 43 and Cys 51. Although usually presumed to be a stabilizing structural element, decisive experiments designed to mutate both of these residues to alanines, or even delete the nine amino acid sequence joined by this disulfide, resulted in VKOR enzymatic activity levels of 112% and 85%, respectively, compared to that of the wild-type enzyme, suggesting – at least by in vitro experiments – that this newly found structural element is not part of the active enzymatic site and is not crucial for maintaining the active site function. However, mutations in this region that are associated with a warfarin resistant phenotype suggest a significant biological role of these amino acids.

Another key residue (Tyr 139), which is found to be changed to phenylalanine in warfarin resistant rats, provides evidence for the TYA motif at amino acid positions 138–140 as the coumarin binding site in VKORC1, as this linear tripeptide was previously identified as the coumarin binding site in NAD(P)H quinone dehydrogenase 1 (NQO1; [27, 28]).

Although there has been a recent flourish of research results concerning VKORC1 and its role in oral anticoagulation therapy, we would like to point out that research developments leading to the current understanding actually began over 70 years ago. We have included some of the historical investigative highlights in Figure 1 and refer to the original publications.

Recently, the biological mechanism of vitamin K reduction by VKORC1 has been enlightened by Wajih et al. (46). In this in vitro study, oxidative protein folding by protein disulfide isomerase (PDI), an oxidoreductase containing a thioredoxin-like domain, was proposed to provide reducing equivalents for VKORC1. When reduced RNase was used as a substrate, PDI-mediated oxidative protein folding was able to provide electrons for vitamin K epoxide reduction. The thio-oxidoreductase motif CIVC in VKORC1, reminiscent of an identical primary sequence element from an unrelated family of highly conserved sodium transporting NADH:ubiquinone oxidoreductase complex C subunits (NqrC) in archaean and pathogenic organisms (25), might play a central role in the reduction of vitamin K epoxide, as we (25), Wajih et al. (46), and more recently Jin et al. (26) have demonstrated that mutation of these cystein residues leads to complete loss of VKOR activity. Furthermore, Wajih et al. could show by immunoprecipitation that VKORC1 forms a protein-protein complex with PDI, suggesting a multi-protein complex within the vitamin K cycle (46). Contrary to this, Chu et al. found no evidence for a VKOR complex, as recombinant expressed, purified, and reconstituted VKORC1 protein exhibited greater activity than microsomal VKORC1 preparations (45). Therefore, discussion of whether VKORC1 is part of a multi-protein complex or performs vitamin K recycling without additional protein binding partners is still open, and further data are necessary to clarify the reaction mechanism. One of the important experimental goals remaining is to overexpress and purify VKORC1 to homogeneity in its native, functional state for further biochemical and structural studies. Since the initial cloning reports in 2004, human VKORC1 has been heterologously expressed in a number of eukaryotic systems both at low levels for biochemical and functional studies (8, 9, 25, 45, 46) and at high production levels for structural studies (47, 48). A great stride has been made in this direction by Chu et al. as they have now reported advances in purifying and reconstituting human VKORC1 expressed as an affinity tag fusion protein in baculovirus transfected insect cells (45). Still, a remaining problem to overcome was recently pointed out by Wajih et al. (46) in that VKORC1 has still not been purified to homogeneity as assessed by SDS-PAGE data. The lack of being able to obtain pure, homogeneous VKORC1 protein could prove refractory to further structural studies. However, Chu et al. (45) were able to identify, by mass spectroscopic analysis, the trace protein contaminants in their highly enriched VKORC1 isolates and to conclude that none of these proteins likely play a role in the vitamin K cycle. After reconstitution of the highly enriched VKORC1 with defined DOPC lipid in the presence of deoxycollate, VKORC1 was demonstrated to regain full enzymatic activity in the presence of lipid and tris(hydroxypropyl)phosphine (THP), a lipophilic and pH-insensitive reductant used in place of DTT in these experiments.
Pharmacodynamics and pharmacokinetics of coumarins

In overview, orally administered coumarin-based anticoagulants function by directly inhibiting reduction of vitamin K epoxide by VKORC1 and, thus, block the recycling of vitamin K epoxide and reduced vitamin K (quinone) to the fully reduced quinol form required, along with CO$_2$ and O$_2$, by the vitamin K-dependent (VKD) hepatic γ-glutamyl carboxylase (GGCX) for the biosynthesis of doubly anionic carboxyglutamate (gla) residues on various VKD coagulation factors in the liver. These coagulation factors, including factors II, VII, IX and X, protein C and protein S become activated only through the enzymatic transformation of numerous glutamic acid side chains to gla residues which, in turn, can bind calcium ions to complex with and anchor to anionic phospholipids on platelet surfaces where clotting can proceed (49). Due to the limited availability of vitamin K in tissues in vivo, the rapid recycling of vitamin K epoxide to reduced vitamin K quinol has been demonstrated, in fact, to be the rate-limiting step of the vitamin K cycle (50).

In practice, the standardly administered coumarins worldwide include three approved drugs: acenocoumarol, phenprocoumon, and warfarin. These lipid-soluble coumarin derivatives are well absorbed with a bioavailability of over 95%, and a high percentage is bound to plasma albumin for transport through the circulatory system and distribution to internal tissues. Because of non-stereoselective synthesis, coumarins have generally been administered as a racemic mixture of R- and S-enantiomers. As is the case for many other drugs, the stereoisomers effect different actions and undergo different fates.

In general, the anticoagulant effect of the S-enantiomer of coumarin derivatives is about three- to five-fold greater compared to that of the R-enantiomer, but clearance of the S-enantiomer is more rapid (51, 52). Most coumarins such as warfarin or acenocoumarol are eliminated in the liver. Warfarin has a measured circulating half-life of 24–33 hours (h) and 35–58 h for its S- and R-enantiomers, respectively (53). In contrast, acenocoumarol S- and R-enantiomers are cleared much more rapidly with half-lives of 1.8 h and 6.6 h, respectively. Phenprocoumon exhibits the slowest clearance kinetics with mostly unmetabolised S- and R-enantiomers being excreted in the urine with respective circulating half-lives of 110–130 h and 110–125 h. The S-enantiomers are metabolised by CYP2C9 and the R-enantiomers by various other cytochrome P450 isoenzymes (54–56). Accordingly, the following discussion concerning CYP2C9 vari-

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**Figure 1: Historical research milestones and PubMed citation trends for vitamin K epoxide reductase.** Boxed inset lists key discoveries and achievements leading up to the discovery of the VKORC1 gene and biochemical studies of the encoded enzyme. Numbers in parentheses cite original articles and sources. The bar chart shows, by year, the complete PubMed citations for “vitamin K epoxide reductase OR VKORC1 OR VKOR” through June 2007 (black bars). Blue, green and red bars represent the fraction of publications for each year grouped under the categories of medical research, basic research and review/commentary, respectively. Interestingly, while the broad peak in publication between the mid-1970s and the early 1990s is dominated by basic research publications, the recent surge in publication since the cloning of VKORC1 in 2004 exhibits a majority of medical literature.
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ants applies mainly to warfarin and acenocoumarol, but is not of major importance for phenprocoumon.

Although the VKORC1-inhibitory effect of R-warfarin is considerably less than that of S-warfarin, it has been suggested that both enantiomers, when co-administered as a racemic mixture, effect a relatively steady combined anticoagulant effect until they are metabolically cleared. The pharmacokinetic profile is shaped by a combination of S-warfarin reaching its peak concentration faster, followed by a longer steady state, but less potent, R-warfarin plasma level (52). For racemic acenocoumarol, this effect is even more pronounced, as the S-enantiomer is so quickly metabolised by CYP2C9, that the anticoagulant effect is almost completely achieved by circulating R-acenocoumarol (57, 58).

As is true for nearly every enzyme encoded in a genome, occasional genetic mutations, if they do not entirely compromise expression of the enzyme, may lead to subtle or even robust change in expression level or function. CYP2C9, which is the most abundant enzyme of the CYP2C family (59), displays several polymorphisms which have been associated with impaired enzyme activity (for a comprehensive listing of human cytochrome P450 mutants with URL links to reference citations and abstracts in PubMed see [60]). In Caucasians, CYP2C9*2 (Arg144Cys) and CYP2C9*3 (Ile359Leu) are found with up to 15% and 7% frequency, respectively. In other ethnicities, the prevalence of CYP2C9*2 and CYP2C9*3 is considerably lower (61–63). For both variants, enzymatic degradation of S-warfarin and S-acenocoumarol is dramatically reduced. Therefore, in homozygous or compound heterozygous patients with combinations of these variant alleles, plasma levels of the more potent S-enantiomer quickly rise to supratherapeutic levels, leading to increased risk for bleeding events (51, 52, 64). Takahashi et al. (65) demonstrated that homozygous CYP2C9*3 is associated with a reduction of warfarin maintenance dose to only 25% of the average amount required by warfarin insensitive patients. This effect is less pronounced for phenprocoumon, as clearance of this drug is primarily by direct renal excretion (66). Other rare variants with impaired enzymatic activity (e.g. CYP2C9*11) have been described, but prevalence is so low, that these polymorphisms do not show up often in routine clinical practice (65, 67, 68).

Metabolism of the less potent R-enantiomers is performed by cytochrome P450 enzymes CYP1A2, CYP2C8, CYP3A4, or CYP3A5 (55, 56, 69, 70), but polymorphisms in these genes have, thus far, not been identified as modulators of coumarin dosage.

Recently, polymorphisms of other proteins which influence vitamin K transport and metabolism including apolipoprotein E (APOE), calumenin (CALU), γ-glutamyl carboxylase (GGXC) and microsomal epoxide hydrolase (EPHX1) were shown to affect coumarin dose only marginally (71–73). Thus, the effects from these interactions on dosage are small compared to the effects of VKORC1 and CYP2C9 polymorphisms which, together with age and body mass, account for nearly 63% of variance in coumarin dosage (72).

Although it was known by 1985 that cytochrome P450 was responsible for metabolizing coumarin derivatives (74), cytochrome gene polymorphism effects on coumarin derivative dosing were not characterized until 1994 and later (75–79). A first VKORC1 intrinsic polymorphism influencing median warfarin dose in human patients was identified only recently by D’Andrea et al. shortly after the initial reports of cloning (44). This polymorphism was shown to be part of the complex haplotype VKORC1*2 (10, 80). In this haplotype, a single promoter SNP (VKORC1 c.-1639 G>A, rs9923231) alters a transcription factor binding site, leading to reduced mRNA expression. As shown by in-vitro and in-vivo investigations, mRNA expression and subsequent total VKOR enzyme activity of haplotype VKORC1*2 is reduced to approximately 30–50% (10, 11). Concerning the mode of action of this genetic variant, an almost linear dose-response relationship would be expected. This is reflected by a dose reduction of 25% and 50% when comparing homozygous wt VKORC1 to heterozygous VKORC1*2, and homozygous VKORC1*2 genotypes, respectively. This correlation has been confirmed by several other studies, and even the inter-ethnic differences in coumarin dosage can be explained by VKORC1 haplotype frequency distributions (10, 11, 80). Accordingly, this haplotype is responsible for the largest part of interindividual coumarin dose variation. While dose-reducing haplotype VKORC1*2 is highly prevalent in patients of Asian origin (up to 95%), in African populations VKORC1*2 represents approximately 15% of alleles. In European cohorts, VKORC1*2 shows a prevalence of about 40%. This population-specific distribution is reflected by low coumarin requirement in Asian, intermediate doses in European, and high doses in African populations as has been shown by several independent studies (10, 80, 81).

Coumarin resistance

The patient population exhibiting a clinical phenotype of partial or complete coumarin resistance can be subdivided into essentially two groups depending on genotype. The first group is partially warfarin resistant and characterized by a combination of homozygous non VKORC1*2 and wt CYP2C9 alleles, which is incident in Caucasians at a rate of approximately 20% (10, 16, 80). In these patients, coumarin dose is elevated by ~50% compared to the average dose for the whole Caucasian population. These patients correspond to the upper quarter of the normal range for coumarin dosage. As non VKORC1*2 is infrequent in Asians, partial coumarin resistance is rarely observed among this population. For Africans where non VKORC1*2 is frequent, average coumarin dose is comparably higher than for Caucasians (10, 44, 65, 80, 82) (see Fig. 2).

The second genotype, resulting in a clinical phenotype ranging from elevated dose to complete coumarin resistance, is caused by single amino acid mutations (8, 12–14, 83, 84). Although these cases are rare, the phenotype can be quite dramatic. For some afflicted patients, warfarin doses up to 40 mg/day are necessary to reach therapeutic effect, while other patients failed to respond at these doses (8).

Interestingly, mutations in VKORC1 have been observed in two specific regions of the protein primary sequence. The first region comprising the C-terminal transmembrane alpha helix includes two functional domains: the CIVC redox motif (aa 132–135; [85]) and, adjacent by just one turn further along the helix,
the TYA warfarin binding motif (aa 138 – 140; [27]). Mutations targeting this region are thought to impair warfarin binding and therefore result in a reduced coumarin effect. The second region, where mutations in VKORC1 are observed to cluster, is the large (50 to 70 amino acid residues long depending on precise topological assignment) extra-membranous loop that joins the first two N-terminal transmembrane α-helices. In this loop, three highly conserved amino acid residues (Cys43, Cys51, Ser67) were shown by Rost et al. to be essential for VKORC1 activity (25), suggesting the loop is somehow involved in VKOR catalysis and that its overall structure and function are very sensitive to local mutational perturbations. However, as previously discussed, Jin et al. found Cys43 and Cys 51 do not have a large impact on VKOR activity in their experimental system. These contradictory results may be due to differences in the expression system and experimental design used by each group. Mutations in this loop region, although distant by linear primary sequence from the warfarin binding site, also lead to coumarin resistance (8, 12, 14, 15). A mechanistic explanation for this effect is still unclear and will likely rely on obtaining high resolution structural data in order to understand, but indirect interaction of the loop with the proposed redox catalytic center of VKORC1 can not be excluded.

Recently, an Asp36Tyr mutation was found to be common in Jewish ethnic groups of Ethiopian and European origin and associated with an increased coumarin dosage requirement. Here, a prevalence of 15% and 4%, respectively, in the normal population was reported (15). In other Israeli Jewish populations originating from North Africa and Yemen, the frequency was only 0.5% (15). Asp36Tyr seems to be present in Caucasians at an even lower level, as this mutation has to date not been observed in several hundred alleles derived from the normal population, but only in a single case of a coumarin-resistant patient (10, 80, 84). Furthermore, Asp36Tyr is in allelic association with the putative ancestral wild-type haplotype VKORC1*1 (15), which is almost absent from Caucasians and Asians (80). Therefore, Asp36Tyr seems to represent a mutation with distinct impact in specific populations.

**Coumarin sensitivity**

Coumarin sensitivity is observed as the normal phenotype in Asians. Here, dose reducing haplotype VKORC1*2 has a prevalence of 95% (see Table 1). In Caucasians and Africans, this haplotype is less prevalent and, hence, average coumarin doses are greater. Nevertheless, coumarin sensitivity due to homozygous VKORC1 *2 haplotype is also observed in these populations (10, 16, 80). In patients treated withacenocoumarol or warfarin, but not with phenprocoumon, defective CYP2C9 alleles including *2, *3, or *11 can amplify this effect (16). Especially patients combining genotypes with VKORC1 and CYP2C9 alleles predisposing for low coumarin dose were shown to have an elevated risk for severe over-anticoagulation and subsequent bleeding complications (90–92). In particular, CYP2C9*3 has been identified as a major risk factor in warfarin/acenocoumarol treatment, as overdosing is rapidly achieved due to the impaired metabolism with subsequent accumulation of the more effective S-enantiomer (58, 93, 94).

In rather rare cases of dramatically increased coumarin sensitivity, mutations in genes of the later clotting cascade have been found to be responsible for coumarin-sensitive phenotypes. Such cases result from mutations in exon 2 of the clotting factor IX (FIX) gene. These mutations in the FIX propeptide affecting the Ala10 residue (Ala [GCC] > Val [GTC] and Ala [GCC] > Thr [ACC]) cause a reduced affinity of the γ-glutamyl carboxylase for the FIX precursor, leading to an isolated and dramatic decrease in FIX activity mimicking a severe haemophilia B phenotype in the presence of coumarins (95, 96). In daily life in the absence of coumarins, the phenotype of these patients is, however, completely normal.

**New dosing algorithms**

To improve management of oral anticoagulation, several studies on genotype dependent coumarin dosage have been undertaken and new dosing algorithms have been developed (16–18, 20, 97). These all include previously known factors which influence coumarin dose such as age, gender, and weight, but additionally, genotype of both VKORC1 and CYP2C9 were introduced. Consistently, all studies report on VKORC1 genotype as the main predictor of coumarin dosage, whereas the influence of CYP2C9 genotype was found to be of lesser impact. As combinations of polymorphisms in VKORC1 and CYP2C9 have been shown to lead to an elevated risk for bleeding events, genotype-dependant dosage selection as a tailored, personalized adaptation might lead to a significant reduction of initiation phase related complications (90, 92, 98–100).

Cost-effective screening of prospective anticoagulation therapy patients in order to better determine initial coumarin loading and long-term maintenance dosages is highly desirable. This might identify patients at high risk for bleeding complications and improve the safety of oral anticoagulant therapy and save lives. Further studies are needed to directly compare current standard dosing and pharmacogenetic factor-inclusive dosing.
regimens on separate patient populations. Thus, pharmacogenetic testing to determine patient genotype in advance of initiating coumarin-based anticoagulant therapy could improve the overall efficacy and safety in oral anticoagulation therapy.

New insight into concomitant administration of coumarins and vitamin K

Another aspect to reconsider in the use of coumarins to target VKORC1 in anticoagulation therapy, possibly the basis for a new principle in oral anticoagulation, is the concomitant administration of coumarins together with a small, defined dosage of vitamin K in order to reduce the incidence of undesirable bleeding (101). The concomitant application of agonist and antagonist might seem contraindicated at first consideration, but nevertheless appears to be a reasonable strategy to consider in oral anticoagulation upon a second look. The primary complications of current coumarin therapy are consecutive bleeding complications correlated with measured international normalized ratio (INR) values above the therapeutic range. The difficulty in maintaining an effective therapeutic coumarin dosage without encountering complications can have multiple causes: i) variable dietary vitamin K intake, ii) lack of bodily vitamin K reserves, iii) autocatalytic dependence of the GGCX enzyme for vitamin K, and iv) VKOR activity as the rate limiting step in vitamin K recycling. A couple of recently published articles strongly support this view. Schurgers et al. (102) could demonstrate in healthy volunteers, that supplementation of 150 µg per day of vitamin K did not affect INR significantly in oral anticoagulation. Supportingly, Sconce et al. (103) reported on a generally lower daily vitamin K intake in patients with time-unstable INR compared to patients with stable INR courses. Most recently, first experiences concerning this new approach have been published by this group in which they present results from a randomized, double-blind study probing the effect of patients receiving either a daily dose of 150 µg vitamin K or a placebo over a period of six months (104). They concluded that vitamin K supplementation resulted in a significantly greater increase in standard deviation of INR compared with placebo (-0.24 ± 0.14 vs. –0.11 ± 0.18; P < 0.001) and a significantly greater increase in percentage time within target INR range (28% ± 20% vs. 15% ± 20%; P < 0.01). Their data point towards a higher stability of anticoagulation, but cohorts were too small to draw final conclusions. Although they did not elucidate the mechanism by which vitamin K supplementation improved stability of warfarin response, they suggested day-to-day variability in vitamin K intake as a potential cause of instability in the case of unsupplemented treatment with warfarin. In light of the frequency of 0.25% deadly bleeding complications among all patients undergoing treatment per year worldwide (1, 4, 5), it is worth examining this hypothesis by further prospective randomised double-blind studies.

Conclusion

The identification of VKORC1 has greatly advanced understanding of the vitamin K cycle. The VKORC1 protein has been identified as the molecular target of coumarin anticoagulants and represents the rate-limiting enzyme of the vitamin K cycle and possibly even the sole component of VKOR activity. Mutations and SNPs within the translated and non-translated regions of the VKORC1 gene cause coumarin resistance and sensitivity. Testing for these variants might be helpful for more rapidly adjusting patient coumarin dosage to an effective and safe level. Additionally, the concomitant oral administration of low-dose vitamin K might change the paradigm of oral anticoagulation treatment, and likely lead to less intra-individual variation in coumarin anticoagulant dosage requirement. These findings – just three years after the discovery of VKORC1 – have illuminated new perspectives for a safer and more individualized oral anticoagulation therapy standard in the near future.

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References


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