Building Individualized Medicine: Prevention of Adverse Reactions to Warfarin Therapy

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ABSTRACT

Warfarin is the most widely used oral anticoagulant in the world for patients with venous thrombosis, pulmonary embolism, chronic atrial fibrillation, and prosthetic heart valves. Approximately 30 genes contribute to therapeutic effects of warfarin, and genetic polymorphisms in these genes may modulate its anticoagulant activity. In contrast to monogenic pharmacogenetic traits, warfarin drug response is a polygenic trait, and development of diagnostic tools predictive of adverse reactions to warfarin requires a novel approach. A combination of two strategies, biochemical isolation of allelic variants and linkage disequilibrium association studies, was used to find an association between genetic polymorphisms in the candidate genes and warfarin response. A strong association was found between genetic polymorphisms in six genes, including VKORC1, CYP2C9, PROC, EPHX1, GGCX, and ORM1, and interindividual variability in the anticoagulant effect of warfarin; the strongest predictors were VKORC1 and CYP2C9. Generation of single nucleotide polymorphism (SNP)-based dense genetic maps made it possible to identify haplotypes associated with drug-response phenotypes. Discrimination between haplotypes associated with warfarin dose phenotypes can be achieved by a limited set of informative polymorphisms (tag SNPs). The use of tag SNPs in pharmacogenomic analysis provides a promising tool for dissecting polygenic traits of drug response.

The human genome is variable both within generations (more than 7 million SNPs with a minor allele frequency at least 5%) and between generations (175 mutations per diploid genome per generation) (Kruglyak and Nickerson, 2001). Genetic variations make us unique in many senses, including our response to drug therapy. Pharmacogenomics uses the tools of human genetics to tailor medicinal treatment to an individual's genetic makeup. To this end, phenotypic manifestations (a therapeutic outcome or an adverse drug event, ADE) are considered in relation to the underlying genetic background of a patient.

In a pregenome era, these studies were based on biochemical isolation and characterization of drug-metabolizing enzymes and their genes, followed by sequence and functional analysis of possible mutant variants. Characterization of mutations led to development of a genotyping assay that was used to perform genetic analysis of patient DNA. Initial achievements of pharmacogenomics were related to detection of simple monogenic traits, e.g., abrogation of drug metabolism due to inactivation of a single enzyme. Characterization of inactivating mutations in arylamine-N-acetyltransferase 2, cytochrome P450 2D6, and thiopurine S-methyltransferase provided molecular mechanisms of adverse drug events caused by isoniazid, debrisoquine, and mercaptopurine (Gonzalez et al., 1988; Vatsis et al., 1991; Krynetski et al., 1995). Biochemical analysis does not rely on statistical evaluation of genetic markers and therefore works equally well for common and rare genotypes. Such functional studies require extensive knowledge of the biotransformation, transport, and physiological activity of the drug in question.

In contrast, linkage disequilibrium (LD) association studies rely on dense maps of human genome where nearly every human gene and genomic region is marked by a sequence variation. With development of massive parallel methods for SNP detection, linkage analysis and association studies are increasingly used to effectively characterize complex poly-

ABBREVIATIONS: SNP, single nucleotide polymorphism; LD, linkage disequilibrium; ADE, adverse drug event(s); INR, international normalized ratio; VKOR, vitamin K epoxide reductase; ORM, orosomucoid.
genic traits of drug response. The association studies make use of statistical analysis of genetic markers (e.g., SNPs) in large groups of patients stratified according to their dose requirements. This approach uses genetic markers rather than functional polymorphisms and therefore does not provide information about possible mechanisms behind alterations in drug response. Instead, it relies on the assumption that a mutant allele is to be correlated with an allele of an assayed SNP.

Ideally, both approaches should result in identification of the same set of functionally important polymorphisms, which in reality is rarely achieved. Whereas functional studies proved to be efficient in analysis of single genes, association studies hold great promise in identifying multiple alleles contributing to polygenic traits. A clinically important example for these two strategies has been provided by recent dissection of warfarin-pharmacogenomic determinants. Metabolism, transport, and pharmacological effects of warfarin are determined by interplay of approximately 30 genes, and genetic analysis of this pharmacological pathway is still in progress (Wadelius and Pirmohamed, 2006).

Warfarin is the most widely used oral anticoagulant in the world for patients with venous thrombosis, pulmonary embolism, chronic atrial fibrillation, and prosthetic heart valves (Wadelius and Pirmohamed, 2006). The therapeutic index for individual patients is narrow; therefore, patients are closely monitored by international normalized ratio (INR) for prothrombin time. Clinical use of warfarin is complicated due to variations in individual response, and prescribed doses may vary 20-fold or more because dose requirements, the stability of anticoagulation, and risk of bleeding are determined by a complex combination of environmental and genetic factors. Whereas nongenetic factors, such as intake of vitamin K, disease, age, gender, concurrent medications, and body surface area, have been taken into consideration by clinicians, the genetic analysis still remains an underused tool, and clinicians must be further educated on how their patients can benefit from timely recognition of pharmacogenetic variations (Krynetskiy and Evans, 2004).

Recent progress in elucidating pharmacogenetic variables contributing to variance in warfarin dose made it practical to implement genotyping of patients before beginning warfarin therapy (Geisen et al., 2005; Voora et al., 2005). A rational selection of genetic markers for genotyping poses a problem for applied pharmacogenomics, and the choice of informative polymorphisms predictive of altered warfarin pharmacokinetics or pharmacodynamics still remains to be a matter of discussion. The current article aims to describe available genotyping assays for evaluating patients’ genotype as a risk factor in warfarin treatment-related complications.

**Warfarin Therapy and Clinical Complications**

An ADE is any undesired and harmful effect of a drug that usually falls in one of two categories: 1) adverse drug reactions (ADR) defined as “any response to a drug that is noxious and unintended and occurs at doses normally used in man for the prophylaxis, diagnosis, or therapy of diseases” (World Health Organization, 1969); or 2) therapeutic failure, i.e., the failure to achieve the desired therapeutic outcome, with an event that can harm the patient.

The goal of warfarin therapy is to administer the lowest possible dose of anticoagulant to prevent clot formation or expansion while trying to avoid unintended ADE from over-anticoagulation. Warfarin-related ADR result in unintended hypoprothrombinemia, with or without frank hemorrhage. ADE are common with warfarin, thus being a leading cause of drug-induced hospital admissions (McDonnell and Jacobs, 2002). The incidence of these ADR ranges from 6 to 39% of warfarin patients annually and is directly related to the intensity of anticoagulation (Levine et al., 1995). By contrast, therapeutic failure of warfarin treatment is an event that produces unintended thromboembolism due to suboptimal anticoagulation in these patients.

Warfarin has both anticoagulant and antithrombotic activity. The anticoagulant activity of warfarin depends on the clearance of functional clotting factors from the systemic circulation. The clearance is determined by half-lives of clotting factors, with earliest changes in prothrombin time (24 to 26 h after warfarin administration) due to the clearance of factor VII (half-life of approximately 6 h). Early changes in prothrombin times do not reflect the full antithrombotic effects of warfarin, which are achieved by the 5th day in most patients after the functional clearance of prothrombin (half-life of approximately 50 h) (Hirsh et al., 1995). Therefore, the maximal anticoagulant effect is achieved after 72 to 96 of exposure.

Pharmacogenomic interpatient differences in warfarin response are an important group of factors that need to be considered for warfarin dosing. Mutations in genes involved in the therapeutic effect of warfarin cause two distinct phenotypes—increased risk of hypoprothrombinemia and warfarin resistance. In many cases, genotype can be a critical variable in determining the optimal dosing to achieve anticoagulant and antithrombotic effects (Voora et al., 2005).

**Molecular Basis for Warfarin-Induced Adverse Drug Reactions**

The biochemical basis of warfarin action is reasonably well understood (Fig. 1). The anticoagulant effect of warfarin is achieved through inhibition of vitamin K epoxide reductase (VKOR) activity, resulting in a decrease of the reduced form of vitamin K. The deficiency of the reduced form of vitamin K results in abrogation of post-translational modification of the blood coagulation components, including clotting factors II, VII, IX, and X; proteins C, S, and Z; and several other proteins, thus reducing their activity (Rost et al., 2005). Untreated hypoprothrombinemia in a genetically predisposed subpopulation of patients most often originates from reduced metabolic inactivation of warfarin by CYP2C9 or decreased expression of the drug target (VKOR).

CYP2C9 is the major isof orm of human liver cytochromes P450 that modulates the physiological effect of warfarin. Low rate of oxidative metabolism catalyzed by CYP2C9 hypomorphic alleles *2 and *3 results in slow metabolic inactivation of warfarin, which in turn leads to undesired hypoprothrombinemia (Daly and King, 2003; Palkimas et al., 2003). The decrease in CYP2C9 activity is most often caused by the nonsynonymous SNPs present in CYP2C9*2 (rs1799853: T; R144C) and CYP2C9*3 (rs1057910: C; I359L). The reduced activity of CYP2C9*2 mutant (R144C) may reflect its diminished affinity for cytochrome P450 reductase, which interacts with the positively charged residues on the surface of
CYP2C9. The mutation I359L, although conservative, leads to a near-complete abrogation of CYP2C9 activity both in vivo and in vitro. Structural analysis revealed that Ile359 lies outside the active site in the vicinity of an important residue Phe476 (Williams et al., 2003); the proximity of Leu359 to Phe476 supposedly abrogates substrate binding.

Several variants of VKORC1 gene associated with decreased enzymatic activity and resistance to warfarin have been described (Table 1). The carriers of these alleles either require enhanced warfarin dose or they do not respond to warfarin treatment at all (Rost et al., 2004, 2005). Although the effect of some of these mutations on enzymatic activity and warfarin-induced inhibition had been demonstrated in vitro, significance of other mutations still remain to be confirmed (Harrington et al., 2005; Loebstein et al., 2006). On the other hand, recently described coding polymorphism 113A→C (D38S) in Japanese population was linked to low warfarin dose (Obayashi et al., 2006).

The presence of rs9923231:A in the promoter region of the VKORC1 gene correlates with decreased VKORC1 transcription, presumably resulting in lower level of VKOR and therefore limiting the reduced vitamin K recycling. Lower doses of warfarin are needed phenotypically to attain the same INR in the group of patients with this genotype (D’Andrea et al., 2005; Rieder et al., 2005; Yuan et al., 2005).

Although the three-dimensional structure of VKOR is currently unavailable, topology mapping and bioinformatics analysis were used to identify transmembrane segments and their cytoplasmic/exoplasmic orientation in this protein (Tie et al., 2005). In parallel, site-mutagenesis of all cysteines and two highly conserved residues (Ser57 and Arg98) helped to define functionally important regions within VKOR (Rost et al., 2004).

**TABLE 1**

<table>
<thead>
<tr>
<th>Position in ORF</th>
<th>Substitution</th>
<th>Effect</th>
<th>Dose</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>47G→C</td>
<td>C16S</td>
<td>No change</td>
<td>2.7 mg/day</td>
<td>Obayashi et al., 2006</td>
</tr>
<tr>
<td>85G→T</td>
<td>V29L</td>
<td>Resistance</td>
<td>100 mg/week</td>
<td>Rost et al., 2004</td>
</tr>
<tr>
<td>106G→T</td>
<td>D36Y</td>
<td>Resistance</td>
<td>80 mg/week</td>
<td>Loebstein et al., 2006</td>
</tr>
<tr>
<td>112G→T</td>
<td>D38Y</td>
<td>No change</td>
<td>5.3 mg/day</td>
<td>D’Andrea et al., 2005</td>
</tr>
<tr>
<td>121G→T</td>
<td>A41S</td>
<td>Resistance</td>
<td>15.5 mg/day</td>
<td>Rieder et al., 2005</td>
</tr>
<tr>
<td>129C→T</td>
<td>C43C</td>
<td>N.A.</td>
<td>N.A.</td>
<td>Rost et al., 2004</td>
</tr>
<tr>
<td>134T→C</td>
<td>V45A</td>
<td>Resistance</td>
<td>No response</td>
<td>Rost et al., 2004</td>
</tr>
<tr>
<td>172A→G</td>
<td>R58G</td>
<td>Resistance</td>
<td>220 mg/week</td>
<td>Rost et al., 2004</td>
</tr>
<tr>
<td>196G→A</td>
<td>V68M</td>
<td>Resistance</td>
<td>25 mg/day</td>
<td>Harrington et al., 2005</td>
</tr>
<tr>
<td>203A→G</td>
<td>H68R</td>
<td>No change</td>
<td>3.8 mg/day</td>
<td>Obayashi et al., 2006</td>
</tr>
<tr>
<td>292C→T</td>
<td>R98W</td>
<td>Inactive VKOR</td>
<td>N.A.</td>
<td>Rost et al., 2004</td>
</tr>
<tr>
<td>358C→T</td>
<td>L120L</td>
<td>N.A.</td>
<td>N.A.</td>
<td>Rost et al., 2004</td>
</tr>
<tr>
<td>383T→G</td>
<td>L128R</td>
<td>Resistance</td>
<td>45 mg/day</td>
<td>Bodin et al., 2004</td>
</tr>
<tr>
<td>452G→A</td>
<td>R151Q</td>
<td>No effect</td>
<td>3.7 mg/day</td>
<td>D’Andrea et al., 2005</td>
</tr>
</tbody>
</table>

N.A., not available.

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**Fig. 1.** The vitamin K-dependent γ-carboxylation system. In newly synthesized proteins, the γ-glutamyl carboxylase (the product of GGDX gene) converts glutamic residues (Glu) to γ-carboxyglutamic residues (Gla). The reduced cofactor vitamin K hydroquinone (vitamin KH₂) is consumed in this reaction; the resulting vitamin K epoxide is recycled through catalysis by VKOR (the product of VKORC1 gene) and vitamin K reductase. Warfarin inhibits VKOR, thus interrupting the vitamin K cycle. Adapted with changes from Stafford (2005), and chemical structures are given according to Stafford (2005).
al., 2005). Cys51 and Ser57 located in the cytoplasmic extramembrane domain were absolutely necessary for VKOR activity and presumably formed components of the active or the binding center for the substrate vitamin K epoxide. Mutagenesis of another pair of cysteine residues located within the C132XXC135 motif abrogated VKOR activity, suggesting that this motif may serve as the redox center for VKOR (Tie et al., 2005).

Interestingly, mutagenesis of Tyr139 resulted in warfarin-resistant VKOR, providing a mechanistic explanation for warfarin-resistant rat strains (Rost et al., 2004, 2005). The Y139F variant is completely resistant to warfarin. Two groups (Rost et al., 2004; Bodin et al., 2005) reported warfarin-resistant phenotype of heterozygotic patients with L128R mutation in VKOR. Both Y139F and L128R are located within the same transmembrane domain surrounding the C132XXC135 motif, suggesting involvement of Tyr139 and Leu128 in warfarin binding.

A possible role in warfarin dose requirement was suggested for a number of genes (e.g., EPHX1, ORM1/2, GGCX, PROC) by association studies. The inclusive list of genes coding for proteins involved in interaction with warfarin pharmacological pathway can be found in a recent review (Wadelius and Pirmohamed, 2006).

Genotype as a Warfarin-Related Risk Factor

The role of genetic polymorphism in warfarin metabolism was first demonstrated in 1994 when Rettie et al. (1994) identified CYP2C9*2 as an isof orm catalyzing (S)-7-hydroxylation of S-warfarin at low rate. This discovery was further corroborated by genetic association between expression of CYP2C9*2 and *3 and sensitivity to warfarin. Comparison of daily warfarin doses in patients homozygous for the wild-type (*1) and hypomorphic alleles (*2 and *3) demonstrated that reduced CYP2C9 activity was associated with lower daily doses (Stewart et al., 1997; Aithal et al., 1999).

This finding was confirmed multiple times in different populations (for recent works, see Herman et al., 2005; Voora et al., 2005; Loebstein et al., 2006; Oyabashi et al., 2006; Tham et al., 2006); several review articles summarized these observations (Daly and King, 2003; Palkimas et al., 2003; Wadelius and Pirmohamed, 2006). Although CYP2C9 is the major catalyst responsible for biotransformation of warfarin and a closely related acenocoumarol, CYP2C9-catalyzed metabolism of another warfarin analog, phenprocoumon, is less important. Consequently, the effect of CYP2C9 polymorphism on phenprocoumon metabolism and anticoagulant response is moderate. Surprisingly, in a study performed in 201 patients, mostly of Swedish origin, an association between the CYP2C9*2 polymorphism (rs1799853) and decrease in warfarin dose was found to be not significant by univariate analysis (Palkimas et al., 2003; Wadelius et al., 2006). The hypomorphic allele CYP2C9*3 (rs1057910), which abolishes CYP2C9-catalyzed metabolism of S-warfarin, was strongly associated with warfarin dose, in accordance with previous findings (Margaglione et al., 2000). Interestingly, the minor allele of rs4917639 was in perfect LD ($r^2 = 1$), with a composite minor allele formed by aggregating CYP2C9*2 and CYP2C9*3 into a single allele, suggesting that the rs4917639 mutation occurred first, with rs1799853 (*2) and rs1057910 (*3) arising later independently on the same parent allele (Wadelius et al., 2006).

Inhibition of vitamin K epoxide reductase is the major mechanism of warfarin action, and the VKORC1 is the single gene most strongly associated with warfarin dose (see below). Existence of a mutation in an autosomal gene causing resistance to the hypoprothrombinemic effects of coumarin drugs was first described in seven persons in three generations (O'Reilly et al., 1964). The gene for vitamin K epoxide reductase (VKORC1) was cloned in 2004 by Rost et al. (2004) and Li et al. (2004).

The role of VKORC1 genetic polymorphism in warfarin therapy was demonstrated in a study on 147 patients where an association between VKORC1 mutant alleles and warfarin dose requirement was found (D’Andrea et al., 2005). A common polymorphism 1173C>T (rs9934438) was detected in intron 1 of VKORC1 gene, which correlated with higher warfarin dose required for carriers of VKORC1 1173CC genotype compared with 1173TT genotype. Statistical models confirmed that the VKORC1 1173C>T polymorphisms, along with CYP2C9 haplotypes, significantly correlated with different average doses of warfarin prescribed to attain the anti-coagulation target. Because no functional role 1173C>T was found in this study, this polymorphism was hypothesized to be in LD with other allelic variants that modulate sensitivity to warfarin therapy (D’Andrea et al., 2005).

This hypothesis was confirmed in a study on 104 Asian patients receiving warfarin where a novel polymorphism (-1639G>A) (rs9923231) in VKORC1 promoter region was detected (Yuan et al., 2005). This polymorphism was in LD with the VKORC1 1173C>T intronic polymorphism reported by D’Andrea et al. (2005) and the affected transcription of VKORC1 gene, thus confirming its functional significance.

In a retrospective study, the effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose was evaluated in 186 European-American patients receiving long-term warfarin maintenance therapy (Rieder et al., 2005). Ten common noncoding SNPs in the VKORC1 gene with frequencies greater than 5% had been identified, and seven of these SNPs were significantly associated with warfarin dose ($P < 0.001$). Among these seven SNPs, five SNPs (at positions 381, 3673, 6484, 6853, and 7566) strongly correlated with each other (LD $r^2 > 0.9$). Stepwise regression analysis identified these five highly correlated SNPs to be predictive of approximately 25% variance in warfarin dose.

Five haplotypes with >5% frequency were inferred from ten common SNPs, and four of these haplotypes were independently associated with warfarin dose. Of these four haplotypes, two were associated with low warfarin dose requirement (2.9–3 mg per day) and two with increased requirement (5.5–6.0 mg per day). A minimal set of four SNPs (861, 5808, 6853, and 9041) distinguished these groups. Patients were further stratified according to CYP2C9 genotype. Stepwise regression analysis showed that VKORC1 and CYP2C9 genotypes accounted for 21 and 6% of the variance in warfarin dose correspondingly. Significant differences in frequency of VKORC1 haplotypes were found among different ethnic groups, which correlated with tendency to require low, intermediate, and high doses of warfarin for Asian, European, and African populations (Rieder et al., 2005).

In a study of 200 blood donors from Germany, a complete SNP map of the VKORC1 locus had been generated (Fig. 2)
A total number of 28 SNPs were identified within the VKORC1 genomic sequence, including 1.8 kilobases of the 5′-flanking regions and 1.5 kilobases of the 3′-flanking regions, most of them in noncoding regions. Six of these SNPs formed three haplotypes in complete LD, which covers more than 99% of the genetic variability of VKORC1 in Europeans. A strong association was found between VKORC1 haplotypes and phenotypes (increased warfarin resistance and increased warfarin sensitivity). For example, among patients with increased coumarin sensitivity, 93% was found to be homozygous carriers of the VKORC1*2 allele (rs9923231:A). On the contrary, 86% of patients with partial coumarin resistance were homozygous for the rs9923231:G allele (non-VKORC1*2 haplotype). Interethnic differences in VKORC1 haplotype frequencies also provided a good explanation for differences in coumarin requirements. The VKORC1*2 haplotype frequencies in Han Chinese and Malay correlate well with the reported low-dose warfarin requirement. In line with this observation, the warfarin-sensitive haplotype VKORC1*2 is less frequent in Africans, which is in perfect agreement with significantly higher average warfarin requirement in African Americans. Thus, the VKORC1 haplotype distribution in three ethnic groups (Europeans, Africans, and Asians) is in complete concordance with the observed differences in warfarin response (Geisen et al., 2005).

In a comprehensive study, 29 genes in the warfarin pharmacological pathway were tested for approximately 900 SNPs (Wadelius et al., 2006). This association study made in 201 patients, mostly of North European descent, revealed that the VKORC1 was the single gene most strongly associated with warfarin dose (Wadelius et al., 2006). Mapping of the VKORC1 region on chromosome 16 revealed that three SNPs (rs2359612, rs9934438, and rs9923231) were in strong LD and accounted for approximately 30% of the warfarin dose variance. A warfarin dose prediction model was prepared by combination of all genes nominally associated with warfarin dose, and nongenetic factors (age, body weight, interaction with other drugs, and indication for treatment). Stepwise removal of variables with individual P values above 0 and low-explanatory value ($R^2$) resulted in a model containing six genes, age, body weight, and drug interactions, which explained 73% variance in warfarin dose. In the 29 genes tested, three SNPs in VKORC1 and alleles CYP2C9*2 and CYP2C9*3 were genetic factors most strongly associated with warfarin dose (Table 2).

Because frequencies of haplotypes vary in ethnic groups, some associations are difficult or impossible to find in certain populations. In a study by Yuan et al. (2005), the 3730G→A allele was found in LD with 1639A→G allele and 1173C→T allele in the Chinese population, both important predictors of VKOR activity. However, this LD was not detected in the Italian study (D’Andrea et al., 2005), probably because of the difference in haplotypes. Several other polymorphisms, reasonably well characterized as contributing factors to warfarin dose requirement, were not found in association studies.

### Table 2

Multiple regression model of major determinants of the variance in warfarin dose generated from association studies (adapted from Wadelius et al., 2006)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>SNP</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VKORC1</td>
<td>rs9923231</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>rs1799853 (*2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>rs1057910 (*3)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>rs2069919</td>
<td>0.0416</td>
</tr>
<tr>
<td>PROC</td>
<td>rs4653436</td>
<td>0.1016</td>
</tr>
<tr>
<td>Body weight</td>
<td>rs1271415</td>
<td>0.0878</td>
</tr>
<tr>
<td>EPHX1</td>
<td>rs1687390</td>
<td>0.0571</td>
</tr>
<tr>
<td>Drug interaction</td>
<td>rs1271415</td>
<td>0.0260</td>
</tr>
</tbody>
</table>

VKORC1, vitamin K epoxide reductase complex subunit 1 gene; CYP2C9, cytochrome P450 2C9 gene; PROC, protein C gene; EPHX1, microsomal epoxide hydrolase 1 gene; GGCX, γ-glutamyl carboxylase gene; ORM1, orosomucoid 1 gene or α-1-acid glycoprotein 1 gene.
or were excluded due to monomorphic genotype and low frequency of minor allele (Wadelius et al., 2006).

**Other Polymorphisms Associated with Warfarin Dose Requirement**

Additional genes associated with warfarin dose requirement are indicated in Table 2: *PROC* (rs2069919), *EPHX1* (rs4653436), *GGCX* (rs12714145), and *ORM1* (rs1687390) (Wadelius et al., 2006). Human α,β-acid glycoprotein (or ORM) is a major binding protein in blood plasma. ORM is coded by two genes, *ORM1* and *ORM2*, tightly linked on chromosome 9. Whereas *ORM2* is monomorphic in most populations, *ORM1* is highly polymorphic (Li et al., 2002). *ORM1* and *ORM2* were significantly associated with warfarin dose based on univariate regression analysis and multiple regression model (Wadelius et al., 2006). Warfarin enantiomers were found to bind differently to ORM genetic variants, although clinical significance and warfarin-dosing requirements in carriers of genetic variants still remain to be elucidated (Nakagawa et al., 2003; Hazai et al., 2006).

Genetic polymorphisms in genes coding for the components of coagulation cascade account for altered sensitivity to warfarin (D’Ambrosio et al., 2004; Shikata et al., 2004). Of seven vitamin K-dependent proteins (factors II, VII, IX, and X and protein C, were significantly associated with warfarin dose based on univariate regression analysis and multiple regression model (Wadelius et al., 2006). Warfarin enantiomers were found to bind differently to ORM genetic variants, although clinical significance and warfarin-dosing requirements in carriers of genetic variants still remain to be elucidated (Nakagawa et al., 2003; Hazai et al., 2006).

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Several groups reported an association between warfarin dose and genetic variants in γ-glutamyl carboxylase gene (*GGCX*) (Shikata et al., 2004; Chen et al., 2005; Loebstein et al., 2005; Herman et al., 2006; Kimura et al., 2006; Wadelius et al., 2006). γ-Glutamyl carboxylase is a 758-amino acid long, multi-pass, transmembrane domain protein in endoplasmic reticulum membrane involved in the γ-carboxylation of all vitamin K-dependent proteins, which act downstream of VKOR in clotting cascade (Fig. 1). The binding site for the substrate protein has been localized to the first 250 amino acids, whereas the oxidation of reduced vitamin K to its epoxide form occurs in the C-terminal region.

A highly polymorphic protein microsomal epoxide hydrolase (mEH, *EPHX1*) is hypothesized to be a component of the vitamin K epoxide reduction system and therefore provides a candidate for a association studies with warfarin dose requirement (Guenthner et al., 1998). Two association studies indicate a possible role for *EPHX1* in modulating warfarin dose requirement, although this remains to be confirmed by biochemical experiments (Loebstein et al., 2005; Wadelius et al., 2006).

**Molecular Diagnostics of Enhanced Risk for Warfarin-Induced ADR**

After a strong association between genetic polymorphism in *VKORC1* gene and interindividual variability in the anti-coagulant effect of warfarin was demonstrated, several studies further confirmed that genotype can be a predictor of variance in warfarin dose (Geisen et al., 2005; Tham et al., 2006; Wadelius et al., 2006). Inclusion of *VKORC1* polymorphisms as co-variates to the prediction algorithm accounted for more than 50% warfarin dose variability (Sconce et al., 2005; Tham et al., 2006). In *VKORC1* gene, of 28 SNPs identified in a sample of 200 volunteers, six SNPs formed three main haplotypes. There was a strong association between haplotypes distinguished by SNP (rs9923231) and coumarin dose phenotypes (\( P = 7.1 \times 10^{-18} \)). This SNP is in strong LD with other SNPs, including rs9934438, rs2359612, and rs7294 (Geisen et al., 2005).

Several algorithms have been proposed to achieve safer anticoagulation based on demographic and environmental factors, such as body weight, age, diet, and concomitant drugs (Kovacs et al., 2003). Based on analysis of 369 patients, an algorithm, including age, body surface area, *CYP2C9* genotype, concomitant drug administration (amiodarone and simvastatin), target INR, and race (Caucasian versus Afro-American), was developed that explained 39% variance in the maintenance of warfarin dose (Gage et al., 2004). Prospective validation of the dosing algorithm was performed that demonstrated feasibility of prediction of warfarin dosing based on pharmacogenetic information in combination with nongenetic parameters. By using genotype-based dosing algorithm, patients with a *CYP2C9* variant achieved a stable warfarin dose without excessive delay (Voora et al., 2005).

Given the low genetic diversity in *VKORC1* and *CYP2C9* haplotypes in an Asian population of Singapore, a simplified model for warfarin daily dose requirement was developed that included age, weight, *CYP2C9*-*3* allele, and *VKORC1* 381CC and TT genotypes (Tham et al., 2006). This model was validated in a separate cohort of 108 subjects and could accurately predict warfarin dose requirements in Asian population.

**Search for Informative SNPs**

Although acceptable for initial screening, routine analysis of all known SNPs within the candidate genes may be impractical. In a recent study (Wadelius et al., 2006), approximately 900 SNPs in 29 genes have been genotyped. In the setting where the causal SNPs (i.e., SNPs associated with phenotype) remain unknown, a more rational approach is selection of a subset of SNPs representing haplotypes (haplotype tagging SNPs, hSNPs) (Johnson et al., 2001). Besides tagging common haplotypes, other algorithms for selecting SNPs based on LD properties have been described (Goldstein et al., 2003; Carlson et al., 2004). Among all possible SNPs within a gene, a subset of tag SNPs can be selected for genotyping analysis with minimal loss of information (Carlson et al., 2004). Selection of tag SNPs can significantly reduce the volume of genotyping needed to find an association with a phenotype. Several programs for tag SNP selection are now available, including Tagger, HapBlock, and WCLUSTAG (de Bakker et al., 2005; Zhang and Jin, 2003; Sham et al., 2007). One of the limitations of this approach is that tag SNPs identified in one population may not perform well in another.
Shifting the Paradigm toward Polygenic Traits

Genetic analysis of variability in drug response is a rapidly expanding area, mainly due to two factors. First, the knowledge of mechanisms of drug action and proteins involved in metabolic activation/inactivation, transport, and drug-target interactions in many cases is complete enough to identify candidate genes. Second, the available technology provides tools to rapidly detect genetic markers in specific loci associated with drug response. Together, these two factors make it possible to revisit a huge collection of data pertinent to drug interaction with human body. Genetic analysis has become simple and fast enough to move it from scientific laboratories to diagnostic medical centers; one can envision it to become even more straightforward and automated to be a practical instrument in pharmacies.

We already have a substantial set of pharmacogenomic assays capable of predicting simple monogenic traits of altered metabolism. In a story with warfarin, up to six genes have been associated with dose requirement. The efficient use of this information will demand further genetic analysis in populations with different racial backgrounds and models to accurately predict dose requirement based on a combination of genetic and nongenetic factors.

Conclusions

Pharmacogenomics of warfarin is the next step in the direction of personalized medicine, in which polygenic rather than monogenic traits are considered to make a rational choice of the medication dose. Construction of dense genetic maps based on single nucleotide polymorphisms made association studies an important instrument to dissect polygenic traits of drug response. In combination with nongenetic factors, several polymorphic genes define warfarin dose-response phenotype. Of these genes, the strongest correlation is between warfarin dose requirement and VKORC1 haplotypes in combination with CYP2C9*2 and *3 genetic variants. Discrimination between haplotypes associated with warfarin dose phenotypes can be achieved by a limited set of informative polymorphisms (tag SNPs). The use of tag SNPs in pharmacogenomic analysis provides a promising tool for dissecting polygenic traits of drug response.

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References

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