Association between *CYP2C9, VORC1, VDR,* and *APOE* genotypes on warfarin maintenance and response during initial anticoagulation for Chinese patients with heart valve replacement

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To the Editor: For patients with mechanical and bioprosthetic heart valve replacement, vitamin K antagonist (VKA) therapy is recommended according to guidelines. Effective and safe therapy with warfarin requires continuous monitoring of prothrombin time and international normalized ratio (INR) levels to adjust the dose of warfarin.

Many articles have concluded that cytochrome P450 (*CYP*)*2C9***2,***3,* and vitamin K epoxide reductase *(VKORC1)* variants might contribute to a lower warfarin dose and higher risk of bleeding with above-normal levels of INR in addition to body mass index, age, comorbidities, concomitant drugs, and dietary intake.[1,2]

Several members of the nuclear hormone receptor family, a group of ligand-dependent transcription factors, have been shown to be responsible for endobiotic- and xenobiotic-mediated induction of *CYP2C* genes. These nuclear hormone receptors include vitamin D receptor (VDR) and apolipoprotein E (APOE), which are not routinely detected in the clinic.[3,4] However, until now, there has not been enough evidence to associate genetic changes in nuclear receptors with warfarin sensitivity. Our research aimed to develop a pharmacogenetic algorithm for accurate evaluation of the efficacy of warfarin based on clinical and genetic factors.

In total, this prospective cohort enrolled 148 patients who received heart valve replacement operation and underwent effective oral anticoagulation therapy with continuous VKA with a target INR range (2.0–3.0). Notably, most enrolled patients were medicated with a median of four drugs (interquartile range from two to eight drugs). The mean stable warfarin dose required for anticoagulation was 2.83 ± 0.67 mg/day, ranging from 1.5 to 4.5 mg/day, with a high inter-individual variation dose among the study patients.

To decipher the genetic polymorphism associated with warfarin maintenance dose in this Chinese population with heart valve replacement, we genotyped nine genetic variants, *CYP2C9***2*, **3*, *VKORC1*, *VDR,* and *APOE,* that were related to anticoagulation in a previous study. The DNA of each patient was isolated by using the QIAamp DNA Blood Kit (Qiagen, Hilden, Germany) according to standard company recommended procedure, and single-nucleotide polymorphisms (SNPs) were conducted by polymerase chain reaction and TaqMan genotyping assays (Light Cycler 480, Roche, Pleasanton, CA, USA). All the studied genotype frequencies of all variants were found to be in Hardy–Weinberg equilibrium ($P > 0.05$).

In our study, individuals carrying loss-of-function alleles with *CYP2C9***2,***3*, and *VKORCI* variants (rs9923231 and rs7294) required significantly lower mean daily warfarin maintenance doses than non-carriers, and our results showed a gene-dose effect ($P < 0.05$).

Together with some candidate gene studies, we have revealed many genetic polymorphisms with large effects on warfarin doses, including genotypes *VDR* and *APOE*. Carriers of the G variant allele in *VDR* (rs11168292) required significantly higher doses of warfarin (2.87 mg for GG and 3.06 mg for CG variants) than carriers of the normal genotype (2.72 mg for CC, $P = 0.023$). In addition, for genotype *VDR* (rs7975232), patients carrying a variant allele received a higher dose (2.70 mg for AA and 3.01 mg for AC variants) than wild type patients (2.68 mg for CC, $P = 0.015$). The wild-type *APOE* patients required a higher warfarin daily maintenance dose, whereas patients carrying one or two variant T alleles required a higher dose ($P = 0.042$), as shown in Supplementary Table 1, http://

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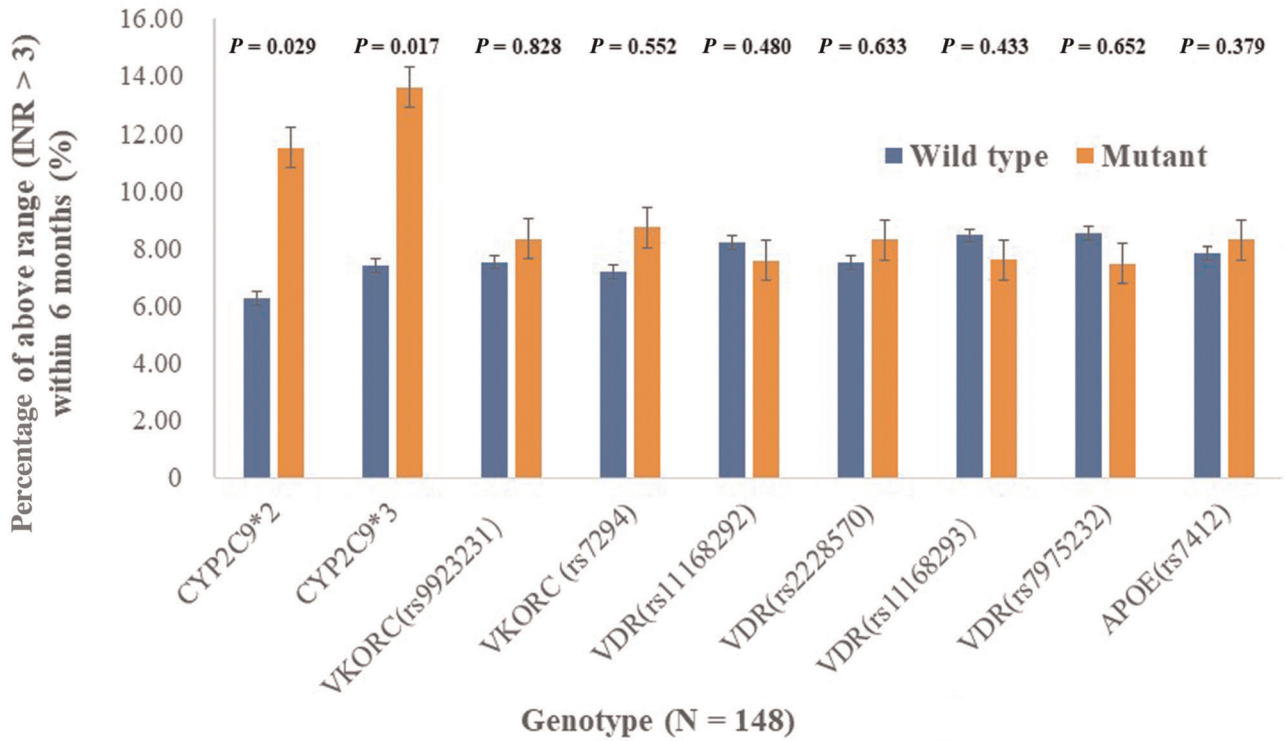

Figure 1: Comparison of above-range INRs analysis among genotypes. APOE: Apolipoprotein E; CYP: Cytochrome P450; INR: International normalized ratio; VDR: Vitamin D receptor; VKORC: Vitamin K epoxide reductase.

links.lww.com/CM9/A955. The multivariate regression analysis revealed that genetic factors together contributed 49.2% of the variation in the daily maintenance dose of warfarin in our study. The *VKORC1* (rs9923231) T allele, *VKORC1* (rs7294) C allele, and *CYP2C9**2 (rs1799853) T allele variants accounted for 38.4%, 7.2%, and 3.6% of warfarin maintenance dose variability, respectively.

Of the whole 6-month cohort, the average time in therapeutic range (TTR) was 62.6% ± 11.0%, ranging from 38.8% to 87.1%. Among all the patients, approximately 99 patients (66.9%) achieved a TTR ≥58%, and 49 patients (33.1%) achieved a TTR <58%. Multivariate regression analysis for inter-variants in anticoagulation activity was performed by considering the significant variables identified by a previous study. The results revealed that clinical and genetic factors together contributed 39.6% (adjusted $r^2 = 0.370$, $P = 0.001$) of the variation in the anticoagulation effect of warfarin. In this analysis, *CYP2C9* contributed most to the inter-individual variability in anticoagulation activity, accounting for 14.9%. *VDR* (rs11168292, rs2228570, rs11168293, and rs7975232) was a minor but significant factor, which could explain approximately 5.4%, 3.8%, 6.0%, and 6.6% of individual differences in anticoagulation clinical effects. A logistic regression model was established by using the equation {Logit(P) = −0.499–0.942 × (stable maintenance dose) – 1.543 × (*CYP2C9**2), where *CYP2C9**2, yes = 1, no = 0)} was based on the independent risk factors, followed by calculating the combined predictor. ROC analysis indicated a moderate performance in the prediction of anticoagulation activity (area under curve = 0.751, $P < 0.01$, 95% confidence interval: 0.673–0.830).

The ratio of an INR >3 was significantly higher for *CYP2C9**2 and *3 wild types than for variants during the 6 months of warfarin anticoagulation therapy ($P = 0.029$ and $P = 0.017$, respectively). *CYP2C9**2 and *3 variant carriers were more likely to be overanticoagulated than wild-type carriers, with an incidence of INR >3 during the 6 months of therapy (11.4% *vs.* 6.2% and 13.6% *vs.* 7.3%, respectively). The incidence rates of INR >3 did not reach significant differences among *VDR* and *APOE* genotypes, as shown in Figure 1.

Although warfarin-associated genotype studies demonstrated that inter-patient variability in warfarin dose requirements was influenced by *CYP2C9* and *VKORC1,* the large variability of individualized warfarin dose requirements remains unexplained. We identified *VDR* and *APOE* as new genetic variations that are significantly associated with the warfarin maintenance dose and anticoagulation activity in this study.

Many studies have indicated that patie nts with at least one copy of the variant allele (*CYP2C9**2 and *CYP2C9**3) might have reduced metabolism, leading to higher warfarin concentrations.[1] As a result, they required a lower daily warfarin dose than patients who were homozygous for wild-type *CYP2C9* alleles.

We observed a contribution up to 38.4% of the variability of *VKORC1* in warfarin daily dose. This result was in agreement with previous research showing that *VKORC1* accounted for the variation in the warfarin dose requirement.[5] *VKORC1* was identified as a putative miRSNP in the *VKORC1* gene that showed varying

degrees of linkage disequilibrium with the clinical variant *VKORC1* across populations. It could influence the epigenetic regulation of the pharmacogene *VKORC1* by binding miRNAs.

Our results suggest that VDR polymorphisms might be associated with inter-individual variability in warfarin dose requirements independent of the *CYP2C9* and *VKORC1* genotypes. A previous study reported that a stable warfarin dose was associated with *VDR* SNPs for East Asian patients with mechanical cardiac valves.[3] The mechanism might be that the *VDR* SNP genes were associated with expression quantitative trait loci (eQTL) effects on the neighboring gene SLC48A1, with the minor alleles being associated with a decrease in eQTL gene expression levels. Our findings also contributed to the general understanding of the role that *VDR* polymorphisms can influence anticoagulation via the warfarin metabolism pathway.[6] The results might be explained partly by considering that *VDR* polymorphism could modulate *CYP2C9* expression; therefore, one could speculate that compared with homozygotes of the wild-type allele, carriers of *VDR* polymorphisms could have an altered warfarin metabolism and, consequently, lead to the anticoagualtion influence.

Our study data also confirm the pharmacogenetic impact of *APOE* SNPs on the warfarin daily maintenance doses, in keeping with recently published reports. We found that patients carrying the *APOE* T allele required a relatively higher average warfarin dose ($P < 0.05$), and these findings are likely to be clinically significant.[1] Recent studies have shown that *APOE* genotypes have a potential anticoagulation effect by affecting the uptake of vitamin K. Therefore, it seems that *APOE* T alleles might be associated with lower required doses.

In this study, we observed that compared with normal metabolizers, patients who inherit one or two copies of *2 or *3 are more sensitive to warfarin. They required lower doses and were at a greater risk of bleeding during warfarin initiation. The approximate reason might be that patients with the variants continued to have a higher risk throughout the duration of therapy without warfarin dose adjustment, including over anticoagulation across the therapy. Patients with *VKORC1* variant status were not found to be at greater risk for supratherapeutics. Thus, knowledge of *VKORC1* status may improve warfarin initiation by allowing clinicians to more aggressively treat patients with the variant, thus decreasing bleeding risk and potentially saving costs by allowing patients to achieve therapeutic INR more quickly.

This study provided evidence of an association of genotypes including *CYP2C9, VKORC1, VDR,* and *APOE* and anticoagulation activity. We found that *CYP2C9*2, VDR,* and *VKORC1* had relatively high percentages of TTR. This finding may be a result of the pharmacokinetics and pharmacodynamics alterations of genotype variants. Genetic-based warfarin prescription might provide a safe and effective anticoagulation therapy for heart valvular replacement operation patients. We must acknowledge many limitations of our study. First, there was a sample size limitation, as we enrolled only 148 patients. Second, we lacked clinical follow-up outcomes, such as thrombosis and bleeding location. Research studies with large sample sizes and longer follow-ups are still needed to provide evidence-based data.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published, and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Conflicts of interest

None.

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