Systematic Review

Influence of the CYP4F2 polymorphism on the risk of hemorrhagic complications in coumarin-treated patients

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ABSTRACT

الأهداف: تقييم آثار تعدد أشكال CYP4F2 على مضاعفات النزف وتخثر الدم المفرط بسبب الكومارين.

الطريقة: أُجري بحث أدبي شامل للبحث عن دراسات مؤهله نُشرت قبل شهر فبراير عام 2015 في EMBASE وPubMed وقد حُددت المراجع بدقة باستخدام معايير الإنتقاء والإقصاء وجرى التشاور مع مؤلفي الدراسات الأولية للحصول على معلومات وبيانات إضافية. اُستخدم برنامج Revman 5.3 لتحليل آثار تعدد أشكال CYP4F2 على حالات مضاعفات النزيف وتخثر الدم المفرط (النسبة المعيارية الدولية 4>).

النتائج: باستخدام معايير الإنتقاء أُجريت 8 دراسات اشتملت على 3,101 عينة وجرت المقارنة مع جين بري النمط (CYP4F2*1*1) وحاملات CYP4F2*3 المتنوعة فكانت النتيجة عدم وجود آثار هامة على جميع حالات النزيف (نسبة الأرجحية OR: 0.86 وفترة الثقة 95%CI: 0.71-1.05، p=0.15)، مضاعفات النزف الرئيسية في عدد مستخدمي الكومارين (OR: 0.80; 95% CI: 0.64-1.01; p=0.06) ولم تكن هناك ارتباطات هامه بخطر تخثر الدم المفرط للمرضى حاملي CYP4F2*3 (الخطر النسبي RR: 079 و95%CI: 0.59-1.06; p=0.12). قد وجدنا مخاطر أقل في المرضى الذين يحملون CYP4F2*3 متماثل لكن لم يكن هناك أهمية إحصائية (RR: 0.66; 95% CI: 0.43-1.01; p=0.05).

الخاتمة: أشار هذا التحليل التلوي أثر تعدد أشكال CYP4F2 على مضاعفات النزيف وتخثر الدم المفرط على المرضى الذين عولجوا بالكومارين ولم يصلوا إلى مستوى الدلالة الإحصائية، مع ذلك هناك حاجة لدراسات واسعة النطاق وجيدة الإعداد ليحسم العلاقة بين تعدد أشكال CYP4F2 وخطر النزيف.

Objectives: To evaluate the impact of the CYP4F2 polymorphism on bleeding complications and over-anticoagulation due to coumarin.

Methods: A comprehensive literature search was performed to look for eligible studies published prior to February 2015 in EMBASE and PubMed. References were strictly identified by inclusion and exclusion criteria, and authors of primary studies were consulted for additional information and data. Revman 5.3 software was used to analyze the impact of the CYP4F2 polymorphism on hemorrhagic complications and over-anticoagulation events (international normalized ratio >4).

Results: Eight studies involving 3,101 samples met the specified inclusion criteria. Compared with wild-type homozygotes (CYP4F2*1*1), carriers of the CYP4F2*3 variant had no significant effects on total bleeding events (odds ratio [OR]: 0.86; 95% confidence interval [CI]: 0.71-1.05; p=0.15), major hemorrhage complications in coumarin users (OR: 0.80; 95% CI: 0.64-1.01; p=0.06). Patients carried CYP4F2*3 also had nonsignificant associations with the risk of over-anticoagulation (relative risk [RR]: 079; 95% CI: 0.59-1.06; p=0.12). We found a lower risk in patients with homozygotes for CYP4F2*3, but there was no statistical significance (RR: 0.66; 95% CI: 0.43-1.01; p=0.05).

Conclusion: This meta-analysis indicated the impact of the CYP4F2 polymorphism on bleeding complications and over-anticoagulation in coumarin-treated patients failed to reach the level of statistical significance. However, large-scale and well designed studies are necessary to determine conclusively the association between the CYP4F2 polymorphism and hemorrhage risk.

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Coumarin drugs (warfarin, acenocoumarol, and phenprocoumon) are widely used for the prevention of thrombotic events in patients diagnosed with atrial fibrillation, pulmonary embolism, deep vein thrombosis, or mechanical heart valve. These drugs decrease the vitamin K-dependent clotting factors by inhibiting vitamin K epoxide reductase.[1] In spite of its definite treatment effects,[2,3] the application of coumarin anticoagulants is largely hampered by several limiting conditions, including the narrow therapeutic range,[4] high interindividual variations in dose requirements,[5] frequent monitoring,[6] and especially, bleeding risks.[7] Anticoagulation intensity for coumarin is measured by international normalized ratio (INR), which is now used to normalize the prothrombin time ratio by correcting for differences in reagent responsiveness.[8] The target range for INR depends on the condition being treated, and a moderate intensity INR of 2-3 is effective for most indications.[6] Large observational studies have identified 2 genes: cytochrome P450 2C9(CYP2C9), and vitamin K epoxide reductase complex subunit 1(VKORC1), which are associated with variation in coumarin maintenance doses.[9,10] In order to predict required coumarin initial dosage and increase its effectiveness and safety, regression models incorporating both clinical and genetic factors in different ethnic groups have also been constructed.[11-13] However, these pharmacogenetic dosing algorithms collectively account for only 40-60% of dose variability, and the results of randomized controlled trials remain discrepant and controversial.[14-17] In 2008, Caldwell et al[18] first reported a possible association between carriers of CYP4F2*3 (rs2108622, c.1297G>A, p.V433M) and an increase in warfarin dosage requirement in 3 independent white populations. Then, functional studies[19] showed that the CYP4F2 mediates the metabolism of vitamin K1, and the variant protein is related to a reduced capacity to metabolize vitamin K1 relative to the wild-type. The association between the CYP4F2 genotype and coumarin maintenance dosage has been demonstrated by a large number of subsequent studies.[20] The CYP4F2 polymorphism was also incorporated into pharmacogenetics-based coumarin dosing algorithms to enhance the prediction accuracy.[21,22] Compared with Caucasian and Asian, the effect of the CYP4F2 genotype on therapeutic dosage of coumarin in African descent populations is largely unknown, most likely because of their low frequency of CYP4F2*3.[23-25] Although there are abundant literature evaluating the influence of CYP4F2 polymorphisms on coumarin dose requirement, few studies has focused on the relationship between this polymorphism and safety outcomes of coumarin. An increase in INR above the therapeutic window leads to a predisposition to hemorrhagic complications during anticoagulation treatment,[26,27] which is a common cause of emergency hospitalizations.[28] In the meta-analysis, we used INR>4 as the over-anticoagulation criteria to select individual study, because INR>4 is most likely to be appropriate classification as excess anticoagulation.[7,29] By integrating the accumulated information from genetic association studies, we contribute to this effort by specifically investigating the relationship between the CYP4F2 polymorphism and the risk for coumarin adverse events, including bleeding complications and over-anticoagulation.

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Methods.

Search strategy. A systematic search for published literature was conducted in PubMed and EMBASE computerized database. The language was limited to English. The search algorithm integrated 3 categories for "cytochrome", "drug," and "gene". We used the following search terms: (coumarin, or coumadin, or rodenticide, or warfarin, or acenocoumarol, or phenprocoumon), and (CYP4F2*3, or CYP4F2*, or 4F2*, or rs2108622), and (gene, or genotype, or genetic, or allele, or polymorph, or pharmacogenetic, or cytochrome). Reference lists of all primary studies were scrutinized. We collaborated with experts and authors of studies, in order to obtain relevant data and other information.

Study selection and data collection. Two reviewers performed initial evaluation of potential articles for eligibility. Discrepancies were resolved by discussion with a third reviewer. The studies selected had to meet the following major inclusion criteria: 1) prospective and retrospective cohort studies, case control studies in coumarin-treated patients; 2) a study with at least one of the outcomes: bleeding events and over-anticoagulation (INR>4) events; 3) CYP4F2 genotyping performed in all patients, or randomly selected patients; and 4) outcomes presented separately for each CYP4F2 genotype groups. We did not impose restrictions on the inclusion criteria with respect to indication for coumarin use, target INR range, concomitant medication, ethnic groups, and patient demographic characteristic. However, we excluded animal studies, case reports, review articles, conference reports, meeting abstracts, and notes. We also excluded prospective studies, in which participants

received initial coumarin dose on the basis of genotypes. Next, the investigators extracted relevant data using the methods of the Cochrane Handbook.

Assessment of study quality. To explore the risk of bias in studies, we evaluated the epidemiologic quality of the primary literature referring to the Newcastle-Ottawa Scale,[30] a grading system with a maximum score of 9 points for case-control and cohort studies used for systematic review. If a study was graded a score of 7 points or greater, we assumed the study is of high quality. Deviation from Hardy-Weinberg Equilibrium (HWE) was checked for CYP4F2 genotype frequencies of each study separately using Michael H. Court's (2005-2008) online calculator (http://www.tufts.edu/~mcourt01/Documents/). If $p<0.05$, we considered it as departure from HWE, and excluded that study in a sensitivity analysis.

Statistical analysis. We defined CYP4F2*1*1 as wild-type genotype, and CYP4F2*3 heterozygote and homozygote as variant genotype. Subgroup analyses were carried out according to each homozygous or heterozygous of genetic variant, classification of bleeding complications, and coumarin drugs. Sensitivity analyses were performed by deselecting studies one by one, especially excluded study with a small sample size or low quality, in order to detect the potential impact of pooled results.

The heterogeneity across studies was tested by chi-squared Q test (Mantel-Haenszel chi-squared test). Meanwhile, the measure of total variance attributable to inconsistency among studies was evaluated using the statistic of I^2.[31] If $p<0.1$, or $I^2>50\%$, the heterogeneity was regarded as significant, and a random-effects model (the Der Simonian and Laird method) was used. Otherwise, a fixed-effects model (the Mantel-Haenszel method) was selected. All analyses in this meta-analysis were conducted with RevMan 5.3 software (Cochrane Collaboration). All p-values were 2-sided with $p<0.05$.

Results. *Identification and characteristics of studies.*

As shown in **Figure 1**, a total of 374 records were identified by searching online databases. We reviewed 13 full-text articles and assessed it for eligibility, of which we eliminated 8 unavailable publications. Then, 3 studies were re-included as additional data were obtained through contact with the authors, resulting in 8 studies finally were included in our systematic review.[32-39] The 8 studies, which assessed the relationship between the CYP4F2 genotype and the risk of bleeding involved a total of 3,101 patients. Of these studies, one study was performed in a mixture of Caucasians and Asians,[37] and one study was in a mixture of Caucasians and African-Americans,[34] one study in Asians,[35] and 5 studies in Caucasians. Regarding coumarin drugs, 6 studies evaluated this association for warfarin (2,032 participants),[32,34-37,39] and 2 for acenocoumarol (1,069 participants).[33,38] The Newcastle-Ottawa Scale score and Hardy-Weinberg Equilibrium test for individual studies are showed in **Table 1**.

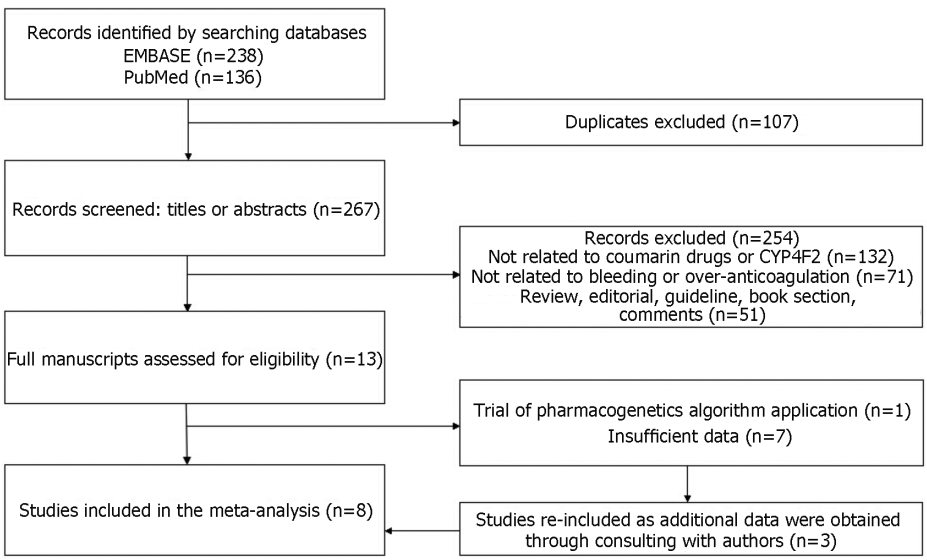

Figure 1 - Flow diagram showing the number of citations identified, retrieved, extracted, and included in the final analysis.

Table 1 - A summary of the 8 articles included in the meta-analysis and its characteristics.

Study	Population	n	Men, %	Age, year mean	Indication of coumarin	Target INR	Medicine	Coumarin dose, mean mg/day	Follow-up period month	Gene frequencies, % CC	CT	TT	HWE	NOS score
Zhang et al,[39] 2009	Caucasian	311	59.0	66 (NA)	AF, PE, DVT, CVA, TIAs, MI, MHVR	2.0-3.0	Warfarin	4.25	6	45.6	42.8	11.6	0.57	7
Bejarano-Achache et al,[32] 2012	Caucasian	241	47.7	55.2 (19.4)	DVT, PE, AF, other	2.0-3.0	Warfarin	6.18	NA	46.5	43.1	10.4	0.90	8
Ma et al,[35] 2012	Asian	312	50.6	56.6 (16.0)	AF, VR, DVT, PE, other	1.6-3.0	Warfarin	3.0	3-15	56.1	CT+TT=43.9		N/A	6
Shaw et al,[37] 2014	Caucasian Asian	89	55.9	4.8 (NA)	FP, VR, DVT, PE	NA	Warfarin	3.0	4.5	45.0	49.4	5.6	0.11	7
Jimenez-Varo et al,[38] 2014	Caucasian	128	45.3	73 (9.0)	AF, VTE	2.0-3.0	Acenocoumarol	NA	7	36.7	50.0	13.3	0.51	8
Cerezo-Manchado et al,[33] 2014	Caucasian	941	45.8	73 (0.8)	AF, DVT, PE, other	NA	Acenocoumarol	7.0	3	37.5	48.9	13.6	0.27	7
Roth et al,[36] 2014	Caucasian	570	54.4	70.2 (NA)	AF, VR, DVT, PE, JR, Stroke, MI, CABG, other	NA	Warfarin	NA	40-45	53.8	39.2	7.0	0.96	9
Kawai et al,[34] 2014	Caucasian African-American	509	54.2	63.2 (NA)	AF, DVT, PE, Stroke, HCS, JR, VR	NA	Warfarin	4.8	NA	50.7	40.1	9.2	0.47	7

AF - atrial fibrillation, DVT - deep vein thrombosis, VR - mechanical heart valve replacement, PE - pulmonary embolism, JR - joint replacement, MI - myocardial infarction, CABG - coronary artery bypass graft, FP - Fontan procedure, VTE - venous thrombus embolism, CVA - cerebrovascular accident, TIAs - transient ischemic attacks, HCS - hypercoagulable state, NA - no available data, HWE - Hardy-Weinberg Equilibrium, NOS - Newcastle-Ottawa Scale

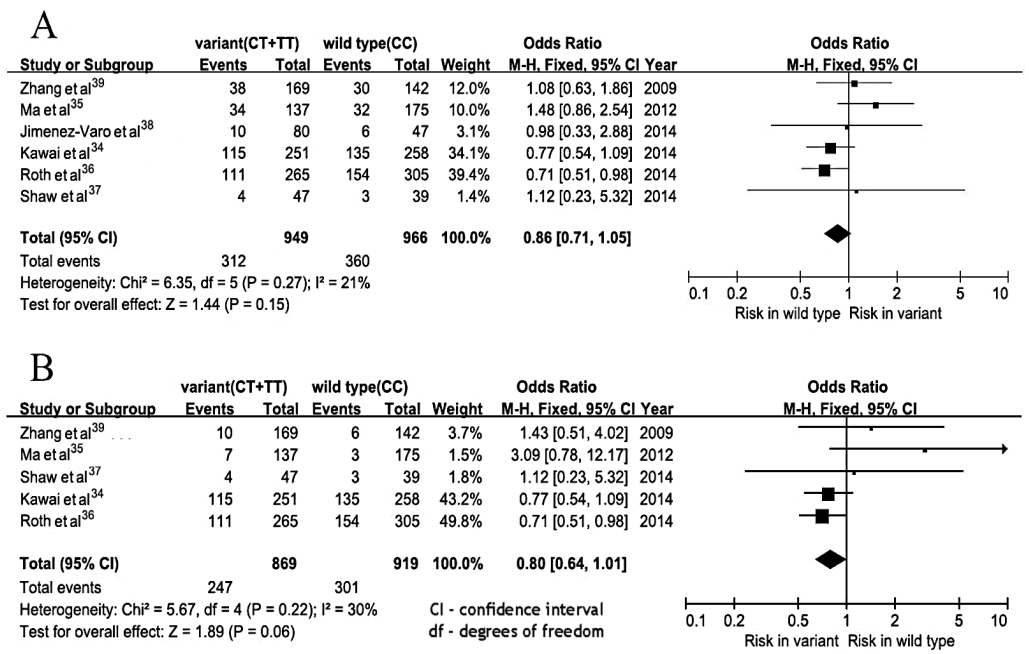

Figure 2 - Forest plots of bleeding in carriers of CYP4F2*3 compared with CYP4F2*1*1 in: **A**) showing the association between CYP4F2*3 and total hemorrhages; and **B**) showed the association between CYP4F2*3 and major hemorrhage. Events/total - the numbers of patients with events/the numbers of total patients.

Impact of the CYP4F2 gene on hemorrhagic complications. Six studies investigated the relationship between the CYP4F2 genotype and total hemorrhagic complications of coumarin, including a total of 1915 samples.[34-39] One of the 6 studies showed the CYP4F2*3 variant as a protective factor for major bleeding events.[36] In the Forest plot (**Figure 2**), compared with wild type, the CYP4F2*3 variant is non significantly associated with decreased risk of total hemorrhage (odds ratio (OR): 0.86; 95% confidence interval [CI]: 0.71-1.05; p=0.15). Furthermore, 5 studies containing a total of 1788 samples provided data on the CYP4F2 polymorphism

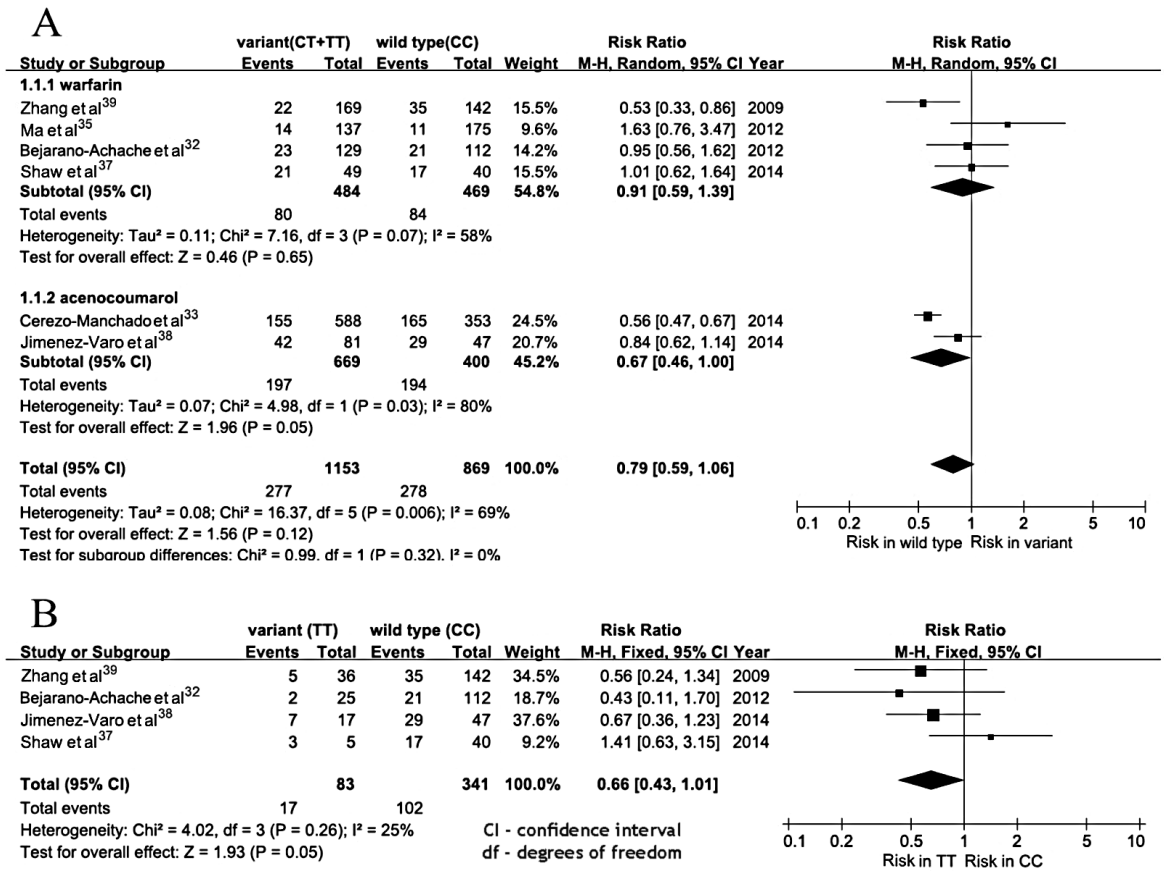

Figure 3 - Forest plots of over-anticoagulation in the CYP4F2*3 variant as compared with CYP4F2*1*1 in: **A**) showed the relative over-anticoagulation of carriers of CYP4F2*3 compared with CYP4F2*1*1, and subgroup analysis according to coumarin drugs, and **B**) showed the relative over-anticoagulation of homozygotes for CYP4F2*3 compared with CYP4F2*1*1. Events/total - the numbers of patients with events/the numbers of total patients.

and major hemorrhagic complications.[34-37,39] Similarly, we found a 20% lower risk of major bleeding in patients carrying the CYP4F2*3 variant relative to the wild-type, but the pooled effect estimate did not reach a level of statistical significance (OR: 0.80; 95% CI: 0.64-1.01; p=0.06).

Impact of the CYP4F2 gene on over-anticoagulation. Six studies evaluated the impact of CYP4F2 polymorphism on anticoagulation quality of coumarin using the excessive anticoagulation (INR>4) as the outcome.[32-35,38,39] In comparison with the wild-type, the CYP4F2*3 variant had no significant effect on reduced over-anticoagulation events (RR: 079; 95% CI: 0.59-1.06; p=0.12). We then analyzed the contribution of CYP4F2*3 allelic status to over-anticoagulation events, a lower risk for over-anticoagulation was found in homozygotes for CYP4F2*3 allele relative to wild-type homozygotes, but there are no statistical significance (RR: 0.66; 95% CI: 0.43-1.01; p=0.05). Subgroup analysis was performed by coumarin drug.[40] As shown in **Figure 3**, 4 studies using warfarin, and 2 studies using acenocoumarol were recruited in the subgroup analysis, but no statistical significant results was observed.

Heterogeneity and sensitive analysis. Meta-analysis of relationship between the CYP4F2*3 variant and over-anticoagulation showed statistical heterogeneity among studies (I^2=69%, p=0.006), and used a random-effects model. To reduce the heterogeneity, we carried out coumarin drugs stratification for over-anticoagulation (**Figure 3**). For the hemorrhage related meta-analysis, low heterogeneity across studies was observed in the association between the CYP4F2 genotype and total bleeding complications (I^2=21%, p=0.27), or CYP4F2 and major hemorrhage (I^2=30%, p=0.22). Sensitivity analysis was conducted by deselecting studies one by one. When we excluded the study by Ma et al,[35] which was defined as a score of 6 points according to Newcastle-Ottawa Scale, significant changes were

Table 2 - Pooled results excluded in the study of Ma et al[35] in sensitivity analysis.

Event	Genetic model	I^2	*P*-value for heterogeneity	Effect model	Studies	Events/total		Pooled ratio	*P*-value for effect
						CYP4F2*3	CYP4F2*1		
Total hemorrhage	TT+CT versus CC	0%	0.72	Fixed	5	278/812	328/791	0.80 (0.64 - 0.99)	0.04
Major hemorrhage	TT+CT versus CC	0%	0.61	Fixed	4	240/732	298/744	0.77 (0.61 - 0.97)	0.03
Over-anticoagulation (INR>4)	TT+CT versus CC	64%	0.03	Random	5	263/1016	267/694	0.73 (0.55 - 0.95)	0.02

INR - international normalized ratio

observed. In results of this sensitivity analysis (**Table 2**), the CYP4F2*3 variant was shown as a protective factor for total bleeding complications, with low heterogeneity (I^2=0%, p=0.72). The CYP4F2*3 variant was also a statistically significant factor that reduced major hemorrhage complications. In addition, the CYP4F2*3 variant significantly correlated with decreased over-anticoagulation events compared with the CYP4F2*1*1 (**Table 2**).

The funnel plot and fail-safe number were not conducted to estimate publication bias because of the limitation of included studies number.

Discussion. Even though direct oral anticoagulants (dabigatran, rivaroxaban, and apixaban)[41-44] and non-pharmaceutical technique (left atrial appendage closure)[45] as available alternatives to the vitamin K antagonists are effective and safe, coumarin will remain to be major anticoagulant agents over the next few years in account of costs, indications, contraindications, and specific antidotes.[46-48] Therefore, it remains important to study coumarin pharmacogenetics associations, and apply key discoveries to further improve the benefit-risk balance of the drugs. These factors that influence coumarin pharmacokinetics and pharmacodynamics not only contribute to therapeutic dose variability, but also to safe and effective outcomes. If our findings is confirmed, it will help to inform therapy choices, and thereby bringing potential benefits to patients with the CYP4F2*1/*1 genotype. In consideration of higher hemorrhagic risk of wild-type patients, clinicians would prescribe direct oral anticoagulants (DOACs), or tailor monitoring intensity to patients taking coumarin drugs.

In this systematic review and meta-analysis, our results indicate that the CYP4F2*3 variant shows a nonsignificant influence on the extent of bleeding complications and over-anticoagulation. But significant influences were found in the sensitivity analysis excluding the study of Ma et al.[35] The unstable results might be explained by the following reasons: firstly, samples from 7 included studies were mainly derived from Caucasians, and the population of the excluded study was Asian. Thus, ethnicity is likely to be a major factor to the significant changes. Secondly, lower quality scores of the excluded study may generate the inconsistent and unreliable pooled results in the sensitivity analysis.

The meta-analysis should be regarded as preliminary exploration that should be further estimated using a larger sample, and powered to detect subtle influence of the CYP4F2 polymorphism. The CYP2C9 and VKORC1 associated with coumarin dose requirement have previously been proven to correlate with risks of hemorrhage. A meta-analysis based on 22 studies,[49] indicated that both CYP2C9*2/*3 and VKORC1(1173) variants were associated with increased over-anticoagulation risk, and the CYP2C9*2/*3 variant was relevant to significantly higher risk for warfarin bleeding complications. Jimenez-Varo et al[38] suggested that VKORC1, CYP2C9, and other SNPs should be considered in prevention of over-anticoagulation and bleeding events in the initiation of acenocoumarol therapy. Our meta-analysis eventually included the relatively small number of eligible studies. Thus, despite the lack of statistical significance, we cannot exclude the possibility that the subtle impact of the CYP4F2 polymorphism on coumarin dose requirement also existing on safe outcomes.

Limitation and prospects. Our systematic meta-analysis has several limitations. First, out results were based on small numbers of included studies, and most of the participants were Caucasian. It was disappointing that some meaningful studies were not included in our meta-analysis because the available data and useful information were not acquired.[50-52] In addition, we did not conduct a stratification to investigate the impact of genotypes on response of coumarin during diverse periods of oral anticoagulation treatment.[32,38] Finally, data related to risks of bleeding were not adjusted for other genetic factor and nongenetic predictors.

We expect that safety and effectiveness of coumarin received more attention in future pharmacogenomics research, such as the association between the CYP4F2 polymorphism and thromboembolic events,

hemorrhagic complications, time within therapeutic INR range, and time to therapeutic INR. There are potential modifications of the benefit-risk balance of coumarin relative to DOACs through identifying for CYP4F2*3 status. Thus, we suggest that subgroup analysis comparing the efficacy and safety in coumarin-treated patients with CYP4F2*3 variant and subjects taking DOACs should be conducted in studies of VKAs versus DOACs. We also suggest designing some high-quality pharmacogenetics studies and collecting reliable data to develop a rating scale, which would be used to evaluate bleeding and thromboembolism risks during oral anticoagulation treatment based on patients' relevant genotypes. This type of supplemental reference could facilitate to inform treatment decisions, dosage, or monitoring in routine clinical practice.

Carriers of the CYP4F2*3 variant have a diminished capacity of the enzyme to metabolize vitamin K, which is likely to lead to relatively higher levels of vitamin K in the liver. The abundant hepatic vitamin K might provide some kind of a buffer against fluctuated concentration of vitamin K that can trigger over-anticoagulation events, and even hemorrhagic complications. There were studies reporting that the VKORC1 polymorphism could bring different anticoagulation quality during different phases of anticoagulation.[53,54] It has been proven that CYP4F2 is a primary vitamin K1 oxidase that mediates the metabolism of vitamin K1, which cooperates with VKORC1 to limit accumulation of vitamin K1.[19] Therefore, the effect of the CYP4F2 polymorphism may not be the same during the induction and maintenance phases. Furthermore, it is meaningful to evaluate the interactive effect of VKORC1 and CYP4F2 polymorphisms on the risk of bleeding.[8]

In conclusion, we found that the effect of the CYP4F2 polymorphism on the risk of bleeding, or over-anticoagulation to coumarin failed to reach a level of statistical significance. The subtle influence of the CYP4F2 polymorphism could not be excluded and should be further investigated.

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