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Effect of *CYP4F2*, *VKORC1*, and *CYP2C9* in Influencing Coumarin Dose: A Single-Patient Data Meta-Analysis in More Than 15,000 Individuals

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Abstract

The cytochrome P450 *(CYP)4F2* gene is known to influence mean coumarin dose. The aim of the present study was to undertake a meta-analysis at the individual patients level to capture the possible effect of ethnicity, gene—gene interaction, or other drugs on the association and to verify if inclusion of *CYP4F2*3* variant into dosing algorithms improves the prediction of mean coumarin dose. We asked the authors of our previous meta-analysis (30 articles) and of 38 new articles retrieved by a systematic review to send us individual patients' data. The final collection consists of 15,754 patients split into a derivation and validation cohort. The *CYP4F2*3* polymorphism was consistently associated with an increase in mean coumarin dose (+9% (95% confidence interval (CI) 7–10%), with a higher effect in women, in patients taking acenocoumarol, and in white patients. The inclusion of the *CYP4F2*3* in dosing algorithms slightly improved the prediction of stable coumarin dose. New pharmacogenetic equations potentially useful for clinical practice were derived.

Coumarins have proved to be effective in the treatment of thromboembolic disease and despite the introduction of direct oral anticoagulants, they remain one of the most widely prescribed family of drugs worldwide.[1]

The narrow therapeutic index and high interindividual variability in therapeutic dose make coumarin therapy difficult to manage. Many studies have showed two genes, cytochrome P450 *(CYP)2C9* and *VKORC1*, which are associated with variation in warfarin, phenprocoumon, and acenocoumarol maintenance doses requirement and have suggested some clinical benefits from genotype-guided dosing.[2] On the basis of such data, the US Food and Drug Administration has updated the label for warfarin twice, advising that two

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AUTHOR CONTRIBUTIONS

E.D., S.R., C.F., and J.A.J. wrote the manuscript. E.D., S.R., M.M., J.A.J., A.T., and C.F. designed the research. A.T., A.G., M.M., E.D., T.L., P.B., C.C., A.J.C., A.M.B., H.Y.T., C.D.-F., M.R.B., S.B., P.D., M.D.C., J.K.B., R.L.B., L.H.C., K.D., M.H., L.-Z.Z., H.-J.C., R.G.-C., V.R., Y.N., T.M., I.Y.G., R.B.K., K.H., K.I., C.I., L.B., E.J.-V., M.C.-G., M.K.K., K.B.F.H., H.S.G., K.E.L., M.T.M.L., S.A.L., S.S., C.M., L.S., E.G., M.Ö., A.P., R.K., A.P., V.S., M.-A.L., V.K.K., G.S.-K., J.P., J.C.D., A.H.R., B.M., S.S.R., H.S., R.A., M.H.A.S., S.I.K., N.A.L., C.R., A.S., C.D., I.M.S., H.-H.Z., S.-L.T., V.T., V.L., Y.Z., A.H.M.-vdZ., T.I.V., A.dB., M.T., C.F.Z., V.P., J.E.Z., M.P., and J.A.J. performed the research. E.D., S.R., A.G., C.F., and P.M. analyzed the data.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

CONFLICT OF INTEREST

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variants in the *CYP2C9* gene (C144R and I359L) and one in the Vitamin K epOxide Reductase Complex subunit 1 (*VKORC1*) gene (G-1639A) might be taken into consideration when initiating warfarin therapy (warfarin product labeling, US Food and Drug Administration https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/009218s107lbl.pdf).

Although there have been contradictory results in randomized clinical trials (RCTs) about the utility of genotype-guided dosing of coumarin drugs when compared with either standard clinical care or clinical algorithms,[3–5] a recent RCT in patients under-going elective hip or knee arthroplasty[6] showed superiority of genetic dosing compared with clinical dosing. Some, but not all, meta-analyses have also shown an improvement in clinical end points, such as bleeding events.[3,7–11] Moreover, none of the trials included in the meta-analyses included *CYP4F2*3* polymorphism (1347C>T; c.1297G>A; p.Val433Met; rs2108622), whose effect on coumarin dose was discovered later when compared with *CYP2C9* and *VKORC1*.

Our previous meta-analysis performed on aggregate data from 30 studies showed that *CYP4F2* variation was associated with nearly 8% higher coumarin doses in T allele carriers. Indeed, a possible gene–gene interaction and an effect of race on the genetic effect were detected.[12] Despite the low effect size, *CYP4F2* is currently regarded as the third most influential genetic locus with respect to coumarin drug maintenance dose. Older studies, which compared pharmacogenetic algorithms with either clinical-based algorithms or fixed-dose approach, did show a possible improvement in prediction only in selected subgroups.[13,14] The incorporation of *CYP4F2* into existing models might improve the accuracy of dose prediction with coumarins.[15,16] Recently, the Clinical Pharmacogenetics Implementation Consortium updated the guidelines for *CYP2C9* and *VKORC1* genotypes and warfarin dosing, including evidence from the published literature for the nonsynonymous variant *CYP4F2*3* (1347C>T; c.1297G>A; p.Val433Met; rs2108622), which was found to be significantly associated with altered dose requirements for coumarin anticoagulants.[2] In order to clarify the actual clinical utility of including the *CYP4F2* polymorphism into pharmacogenetic dosing algorithms, some essential information is needed. Thus, we performed a meta-analysis at the individual patients level to understand the real effect size of this polymorphism and to test how much either a possible gene–gene interaction or the effect of ethnicity or other covariates could modify the pharmacogenetic association and prove to be useful in creating new pharmacogenetic equations. We hereby provide the largest meta-analysis of *CYP2C9*, *VKORC1*, and *CYP4F2* polymorphisms affecting the dose of warfarin and acenocoumarol in samples collected from 25 different countries, including more than 15,000 participants treated with coumarin drugs. New pharmacogenetic equations potentially useful for clinical practice have been derived for different ethnic groups.

RESULTS

Characteristics of included studies

Starting from the 30 articles included in our previous meta-analysis (search from inception until August 2011), individual patient data were obtained from 19 studies.[17–35] From one coauthor we obtained an additional dataset related to an article not previously included

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because no data about the *CYP4F2* polymorphism were present in the original publication.[36] From the group of 38 articles retrieved from the new search (from September 1, 2011, to September 14, 2016), individual patient data were obtained from 18 studies (Figure 1).[15,16,37–52]

Thus, 38 articles were included in the present work from authors who agreed to share individual patient data: 20 from the first systematic search and 18 from the second systematic search. Data from one study were divided into two distinct cohorts according to the main author's subdivision of sample into discovery and validation cohorts.[46] Moreover, data from two studies had been collected in one cohort.[15,43] Finally, data from one study was divided into two cohorts: one cohort treated with acenocoumarol and the other with phenprocoumon treatment.[44] This resulted in 39 cohorts that were considered for the meta-analysis, including a total of 15,754 patients. Characteristics of the individual studies are summarized in Table 1. Thirty-one cohorts examined the association between *CYP4F2* polymorphism and the maintenance dose of warfarin; seven cohorts evaluated this association for acenocoumarol and one for phenprocoumon. Information on *CYP4F2*, *VKORC1*, and *CYP2C9*3* genotyping were available for all 39 cohorts, whereas *CYP2C9*2* genotype was recorded for 35 of the 39 cohorts (89.7%). All studies but one[19] included both male and female participants with a minimum of 24% men. One study selected very elderly patients (mean age 86.7 years).[35] Data on body mass index (BMI) and drugs known to potentially interfere with warfarin were available for 31 and 27 cohorts, respectively. All studies were published between 2006 and 2016.

Association between CYP4F2*3 polymorphism and stable coumarin dose

Figure 2 shows the forest plot for the difference in log dose of warfarin for subjects with at least one T allele (CT + TT) *CYP4F2* as compared to wild-type (CC) subjects, according to a dominant model. The estimated effect size was 0.09 (95% confidence interval (CI) 0.07–0.10), corresponding to a 9% increase in mg/week (95% CI 7–10%). The funnel plot (see Figure S1) is compatible with no effect of bias on publication.

Separate estimates for CT and TT *CYP4F2* genotypes are reported in Figure S2: the estimated effect size for CT vs. CC subjects is 0.07 (95% CI 0.06–0.08), corresponding to a 7% increase in mg/week; whereas for TT vs. CC subjects it is 0.17 (95% CI 0.15–0.19), corresponding to a 19% increase in mg/week. In Table 2, the analysis of the available subgroups highlights that the effect of the *CYP4F2*3* polymorphism is significant in whites and Asians but not in blacks and other ethnic groups. Moreover, there was a significant difference by gender for the effect of the *CYP4F2* polymorphism on coumarin dose (the effect is significantly higher in women) and by type of coumarin drugs (the effect was lower for warfarin as compared to acenocoumarol). No significant difference in the effects of smoking, target International Normalized Ratio (INR), adjustment for other drugs, consistency of genotype frequencies with the Hardy-Weinberg equilibrium, quality score, and other polymorphisms was found (Table 2). The figures for the different meta-analyses in subgroups are presented in Figures S3 and S4.

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Stable coumarin dose predictive model

Table 3 presents the predictive model for logarithm of stable coumarin dose according to patients' clinical and genetics characteristics. As statistical test for model fit (R^2) is reported for both the test and validation cohorts. Looking at our calculated model on the whole dataset, adjusted R^2 was slightly higher for models including *CYP4F2*3* polymorphisms than for models without *CYP4F2*3* for all the ethnic groups except blacks (for warfarin dose, adjusted R^2 for models with and without *CYP4F2*3* polymorphism were, respectively, 0.51 and 0.50 for whites, 0.43 and 0.42 for Asians, and 0.27 and 0.27 for blacks). For cohorts that included black patients, addition of the *CYP2C9**5 single-nucleotide polymorphism (SNP) to the models did not result in substantial improvement of the adjusted R^2 (Table 3). Further prediction models also including concomitant drugs (amiodarone, etc.) and smoking habits are presented in Table S1.

Beta coefficients for single-gene and gene–gene interaction are presented in Table 4 for each ethnicity and drug subgroups.

The effect of potentially interacting drugs could be evaluated only on a subsample of the cohorts and is presented in Table S2. Patients taking amiodarone or drugs classified as CYP inhibitors required a lower dose, whereas patients taking CYP inducers required a higher dose of coumarin drugs. If the effect of the drugs was considered, the beta estimate for *CYP4F2* and the other SNPs varied slightly but remained significant for most analyses. No significant interaction between SNPs and drugs were detectable apart from *CYP2C9*2* and rifampin and all CYP inhibitors and all CYP inducers in white patients consuming acenocoumarol. Another weak but nominally significant interaction was present between *CYP2C9*2* and statin or aspirin in black patients on chronic warfarin therapy (Table S2).

The comparison of R^2 of our model with those calculated for two previously published models are reported in Table S3 and are basically comparable, ranging from 0.41−0.47 for whites, 0.44 for Asians, and from 0.23−0.33 for blacks.

DISCUSSION

In our previous meta-analysis on the effect of the *CYP4F2* rs2108622 (1347C>T; c. 1297G>A; p.Val433Met; *CYP4F2*3*), we found that the estimated effect size was nearly 10%. In this individual patient data meta-analysis, we have not only confirmed this finding in a larger cohort of primary studies that include all the available study-specific covariates but can add other important findings. Contrary to what was found in the first meta-analysis, a slight but significant effect of gender was identified such that men had a lower effect of the T allele when compared to women.

Indeed, a higher dose of coumarin drugs was needed in carriers of the T allele if they were whites or Asians but not in blacks or in other ethnic groups (Indians, browns from Brazil, and Egyptians), but the latter is probably a reflection of the lower sample size. We also identified differences between different coumarin drugs: patients taking acenocoumarol and carrying the T allele needed a higher dose of the drug when compared with patients taking warfarin and carrying the same polymorphism.

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There was no effect of other possible important covariates, such as smoking, age, and indication for coumarin, and no interactions with the other relevant polymorphisms were found.

Evaluation of the beta estimate of the tested SNPs confirmed that the larger effect is due to the *VKORC1* followed by *CYP2C9*, whereas *CYP4F2* had a limited effect size.

Looking at primary studies, the large majority of them are in line with the results of the meta-analysis, and only 4 of the 39 have a central point of the estimate below the 0 line. Even the point estimate for the effect of *CYP4F2* is not so different between primary studies. The extremes are the study performed by Borgiani *et al.*[18] with a + 0.26 estimate and the one by Isaza *et al.*[48] with a −0.05, which have a 95% CI that is around + 0.07, not far from our total effect size (slightly < 10%).

However, the funnel plot shows a certain asymmetry, almost significant when analyzed using Egger's test. It is therefore possible that unpublished negative studies could affect the real estimate of the effect of the *CYP4F2*3* polymorphism.

Differently from our previous meta-analysis, we could also add drugs as moderating parameters at least in some subgroups, and as expected, this evaluation decreased heterogeneity.

The functionality of the *CYP4F2* polymorphism has been shown in relation to the production of 20-hydroxyeicosatetraenoic acid derived by arachidonic acid and in differences in mRNA production by liver cells in carriers of different alleles.[53]

The interaction of the *CYP4F2* polymorphism with gender is not unexpected: also in other studies exploring other cardiovascular actions, some CYP polymorphisms have shown a differential effect in men and women probably due to an interaction with either androgens or estrogens.[54] Even in animal models these differences are evident, at least for blood pressure determination.[54]

Due to our large sample size, we could calculate and subsequently validate different prediction models that included the effect of the *CYP4F2*3*, the other well-known polymorphisms of *CYP2C9* and *VKORC1*, and the other covariates differentiating the effect of gender and ethnicity and obtaining discrete coefficient of determinations that indicate a good fit of the models. Other predictive pharmacogenetic equations estimating coumarin dose have been developed using large samples sizes,[13,14] but both the International Warfarin Pharmacogenetics Consortium and the "Warfarin dosing" equations used only *CYP2C9* and *VKORC1* genetic variation to estimate warfarin dose and the R^2 estimate for the final model (which also included amiodarone), obtaining values of 0.47 and 0.53, respectively. These results are in line with our data for white subjects, but our results are more generalizable because multiple cohorts from Europe were also included. In fact, Gage's equation is derived from a more homogeneous group of patients collected in three centers in the United States (St. Louis, San Antonio, and Gainesville) with a fourth trial included in the validation cohort.[13] By contrast, the International Warfarin Pharmacogenetics Consortium collected 21 research groups from 9 different countries and finally included only patients with a target

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INR between 2 and 3 (n = 5,052). Their final model was not divided according to ethnicity but instead the ethnicity variable was added in the model. Indeed, outlier patients were excluded from the final analysis. It is worth mentioning that the final sample size of our study is more than two times the previous studies for warfarin, and we have also calculated predictive models for acenocoumarol.

Even if newer anticoagulants have substantially changed clinical practice, especially in developed countries, the use of coumarin drugs is still widespread in the world, so that equations like the one derived from our study will be clinically useful for many years. The importance of genotype has been further shown in the Effective aNticoaGulation with factor xA next GEneration in Atrial Fibrillation-Thrombolysis In Myocardial Infarction (ENGAGE AF-TIMI) 48 trial, which compared the clinical efficacy of edox-aban, a direct oral anticoagulant, with warfarin in a prespecified genetic subanalysis. Stratification of patients according to *CYP2C9* and *VKORC1* polymorphisms revealed that of the three groups identified, normal responders, sensitive responders, and highly sensitive responders, the last group was found to spend a greater proportion of time over-anticoagulated compared with normal responders but only for the first 90 days of treatment.[55]

RCTs using not only the *CYP2C9* and *VKORC1* poly-morphism but also the *CYP4F2* polymorphism have recently been performed. In nonvalvular atrial fibrillation, no apparent advantage was found for the group randomized to genotype base dose,[56] but in a recent trial in patients aged 65 years or older initiating warfarin for elective hip or knee arthroplasty conducted at six US medical centers, genotyping reduced the combined risk of major bleeding, INR of 4 or greater, venous thromboembolism, or death.[6]

In another trial that compared a genotype-guided algorithm vs. physician management for the initiation of acenocoumarol, a higher proportion of patients in the genetic group reached and maintained a steady dose than patients randomized to routine practice when starting oral anticoagulation.[57]

Limitations and strengths of the study

Our individual data meta-analysis has limitations. First, although we applied a sensitive search strategy for the retrieval of potentially eligible studies, we cannot rule out the possibility that some relevant studies might not have been included. Indeed, not all the potentially eligible studies were added to the meta-analysis because the authors did not share individual patients' data. Second, adjustment for certain covariates, such as amiodarone, was possible in only a limited sample of patients. The quality score of the included studies was heterogeneous, ranging from 3−7 (median: 5), but this did not affect *CYP4F2*3*–coumarin dose association, because we found no statistically significant difference in the estimates for studies with lower and higher quality score. Finally, our genotyping-based algorithms in blacks have low predictivity even including the *CYP2C9**5 poly-morphism, probably because we could not include more variants in *CYP2C9* that were demonstrated to be especially important in this ethnic group.[2] Because the exclusion of specific *CYP2C9* variants from the dosing algorithm in blacks can lead to overdosing, we would recommend against the use of the specific dosing algorithms in patients of African ancestry[2] until more specific algorithms have been developed.

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Strengths of our collaborative study are the large sample size with several ethnic groups allowing for generalizability of the results and the possibility to have equations not only for warfarin but also for acenocoumarol based on a quite large sample size. The heterogeneity was low possibly because most of the variables associated with mean coumarin dose have been considered in our models.

CONCLUSION

In conclusion, we have undertaken the largest individual patient data meta-analysis, including the *CYP4F2* polymorphism, in patients taking warfarin or other coumarin drugs. Our data show that the *CYP4F2* rs2108622 polymorphism affects the dose requirements of these drugs in whites and Asians but not in blacks or other ethnic groups. We also provide reliable prediction models, which can guide physicians to estimate the stable dose of warfarin according to genotypes, anthropometric and demographic factors, ethnicity, and the use of other drugs.

Regardless, because RCTs that tested genetic prediction models with the *CYP4F2*3* SNP showed contradictory results,[6,56] the utility of these models in clinical practice needs to be established in further RCTs before their widespread utilization in clinical settings.

METHODS

Search strategy and eligibility criteria

The 30 articles included in our previous meta-analysis were considered all potentially eligible for the present study.[12] To expand our search to articles published after the date fixed for final inclusion in the previous meta-analysis, we searched Medline and Web of Science from September 1, 2011, to September 14, 2016, by applying the same search algorithm used previously (see Supplementary Material S1) and found 38 additional studies that could potentially be included (see Figure 1) according to the inclusion criteria (see Supplementary Material S1). All 68 studies evaluated for inclusion were clinical cohort or cross-sectional studies that have performed genotyping of *CYP4F2* in combination with *CYP2C9* (at least one of the two variants of interest) and/or *VKORC1* in coumarin-treated patients. As per our previous study, we considered the following polymorphisms: rs2108622 (1347C>T; 1297G>A; p.Val433Met; *CYP4F2*3*) for *CYP4F2*, rs1799853 (430C>T) and rs1057910 (1075A>C) for *CYP2C9* (also known as *CYP2C9*2* and *CYP2C9*3*), and rs9923231 (−1639 G>A) for *VKORC1*. In relation to the latter variant, we also included data from studies that used the two alternative polymorphisms: rs9934438 (1173C>T) in the *VKORC1* gene, which is in complete linkage disequilibrium with the reference polymorphism and rs10871454 (−1168C>T) located in the Syntaxin 4 A-(*STX4A*) gene, flanking the *VKORC1* gene, which showed a linkage disequilibrium of 0.99 with the reference polymorphism.

In our previous analysis, consistent with published studies, the performance of our regression was low, especially in black subjects, in which an effect of other SNPs especially in *CYP2C9* is considered important. Thus, in the five cohorts in which at least the *CYP2C9*5* variant was available we repeated the analysis by adding this polymorphism.

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Data collection

We asked the first/last or corresponding authors of the retrieved primary studies to participate in a collaborative meta-analysis on individual patient data. Authors who were willing to collaborate were finally included if their original database contained the following mandatory data for single patients: gender, age, race, genotypes, indication for coumarin therapy, INR target, type of coumarin used, and maintenance dose. Additional information on body weight, height, and use of interacting drugs were also recorded when available. Each cohort has been assigned to one single study unless otherwise specified. For studies containing overlapping samples we considered the first published study or the one that enrolled the largest number of patients. Data were harmonized into a pooled database. Two researchers (E.D. and M.M.) cross-checked trial details provided by the authors against published articles. Any inconsistencies were discussed with the original trialists, and corrections were made when appropriate. As for our previous meta-analysis, we graded the quality of epidemiologic studies in general, applying items taken from the Newcastle–Ottawa Quality Assessment Scale for Cohort Studies indicators specific to the quality of genetic association studies, and indicators specific for coumarin (e.g., stable anticoagulation). Quality assessment also included departure from Hardy-Weinberg equilibrium that was calculated by the χ^2 test in controls. We applied a scale with a maximum score of seven points (see Supplementary Material S1 for details).

Statistical analysis

Two-stage analysis for the association between *CYP4F2*3* polymorphism and stable coumarin dose.—We calculated study-specific estimates, with 95% CIs, for the difference in log dose of coumarin for subjects with at least one *CYP4F2* T allele (CT + TT) compared to wild-type (CC) subjects, according to a dominant model. Separate estimates for CT and TT genotypes were also calculated as a sensitivity analysis. These study-specific estimates were obtained by fitting general linear models with log dose of coumarin as the dependent variable and *CYP4F2*3* polymorphism as the independent variable. All the models were adjusted for available study-specific covariates, including age, gender, race, BMI, smoking status, indication for coumarin treatment, INR target, concomitant drugs, *CYP2C9*2* and **3* polymorphisms, and *VKORC1* polymorphism.

Following the two-stage analysis approach, we pooled study-specific estimates with random-effects models, using the DerSimonian and Laird method (see Supplementary Methods S1). We evaluated homogeneity among study-specific estimates by the Q statistic and I^2, which represents the percentage of total variation across studies that is attributable to heterogeneity rather than to chance (see Supplementary Methods S1). We performed metaregression analysis to assess the influence on Summary Estimates of different study features: type of drugs (acenocoumarol/warfarin), gender, ethnicity (whites/Asians/blacks/others), INR target (< 2.5/2.5/> 2.5), current smoking status, study adjustment for concomitant drugs (yes/no), deviation from Hardy-Weinberg equilibrium, quality score (< 5/≥ 5), *CYP2C9*2/*3* (wild-type/any polymorphism), and *VKORC1* (wild-type/any poly-morphism). When significant differences according to specific study factors were suggested by metaregression, stratified analyses were performed for *CYP4F2*3*–coumarin dose association on subgroups of significant factors.

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We assessed possible participation bias by drawing funnel plots and by Egger's test (see Supplementary Methods S1).

The *P* values < 0.05 were considered statistically significant for all the tests apart from the *Q* statistic, where *P* values < 0.10 were considered statistically significant. The analysis was carried out using the SAS (SAS Institute Inc, Cary, NC) version 9.4 and STATA (StataCorp, College Station, TX) version 13 software.

Stable coumarin dose predictive model.—Due to significant differences in coumarin dose and *CYP4F2*3* association for different drugs and ethnic groups, the individual data analysis on the pooled dataset was always reported for each type of drug (acenocoumarol/warfarin) and for each ethnic group.

For each ethnic and drug subgroup, we randomly chose 2/3 of patients as the "derivation cohort" for developing dose-prediction models, whereas the remaining 1/3 of the patients constituted the "validation cohort," which was used for testing the final selected model. In order to keep a large sample size for prediction model construction, we included covariates that were available in the majority of studies (Table 1): age, BMI, gender, indication for treatment, *CYP4F2*3*, *CYP2C9*2*, *3*, and *5* (for blacks), and *VKORC1* polymorphisms, by using general linear models with log dose of coumarin as dependent variable. To use an additive genetic model, we coded the number of variant alleles at each locus as 0, 1, or 2. Sensitivity analyses were also conducted on the whole cohort of subjects by including further available covariates collected in a smaller number of studies (concomitant drugs, especially amiodarone, and smoking status) to assess their role in stable coumarin dose prediction. The coefficient of determination (R^2) was calculated both for the main prediction model on the "derivation cohort" and for models included in sensitivity analyses. We applied the scores obtained from the main prediction model to the validation dataset and also calculated the R^2.

For the sake of comparison, we also applied scores obtained from two previously published models for warfarin dose prediction[13,14] to our validation cohort and converted the scores to units of mg/week. In order to correctly compare our proposed model with each of the two previously published models, R^2 was calculated on the subset of subjects for whom both scores could be calculated on the basis of available data. In order to assess the importance of *CYP4F2*3* on warfarin dose prediction in our data, we also compared dose predictions from our pharmacogenetic model, including *CYP4F2*3* in the whole dataset with that from our model excluding *CYP4F2*3* by using the adjusted R^2 as defined by Darlington (see Supplementary Methods S1).

Gene–gene and gene–drug interactions were investigated by adding an interaction term to the main prediction model fitted on the whole cohort of subjects (for each drug/ethnicity subgroup) in order to have the largest sample size to test for interaction. Moreover, we performed subgroup analyses according to the use or not of specific concomitant drugs, to evaluate whether the change in coumarin dose associated with specific gene poly-morphisms were modified by concomitant drugs.

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The assumption of exchangeability for this analysis was briefly discussed in the Supplementary Methods S1. The *P* values < 0.05 were considered statistically significant. The analyses were carried out using SAS version 9.4 software. The SAS code is available as Supplementary Material S1.

Supplementary Material

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

- Coumarin drugs have a narrow therapeutic index, but single-nucleotide polymorphisms (SNPs) in the cytochrome P450 (*CYP2)C9* and *VKORC1* genes may help in predicting the dose.

WHAT QUESTION DID THIS STUDY ADDRESS?

- Do genetic algorithms, including the *CYP4F2*3* SNP, perform better than old ones in predicting mean coumarin dose?

WHAT DOES THIS STUDY ADD TO OUR KNOW LEDGE?

- In this single-patient meta-analysis, we confirm that *CYP4F2*3* influences mean coumarin dose especially in women, in patients taking acenocoumarol, and in white patients but with a low effect size.

HOW MIGHT THIS CHANGE CLINICAL PHA RMACOLOGY OR TRANSLATIONAL SCIENCE?

- New pharmacogenetic equations potentially useful for clinical practice have been derived for different ethnic groups.

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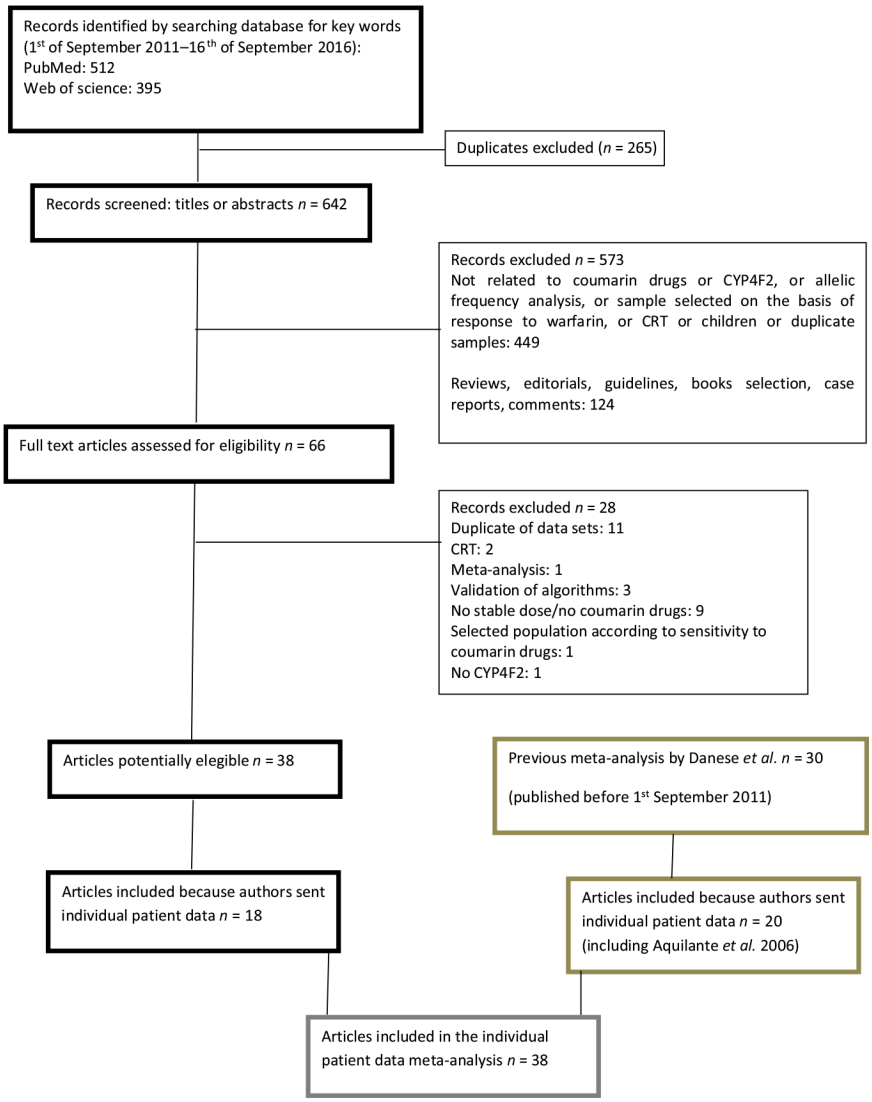

Figure 1.
Flow diagram. CRT, controlled randomized trial; *CYP4F2*, cytochrome P450 F2.

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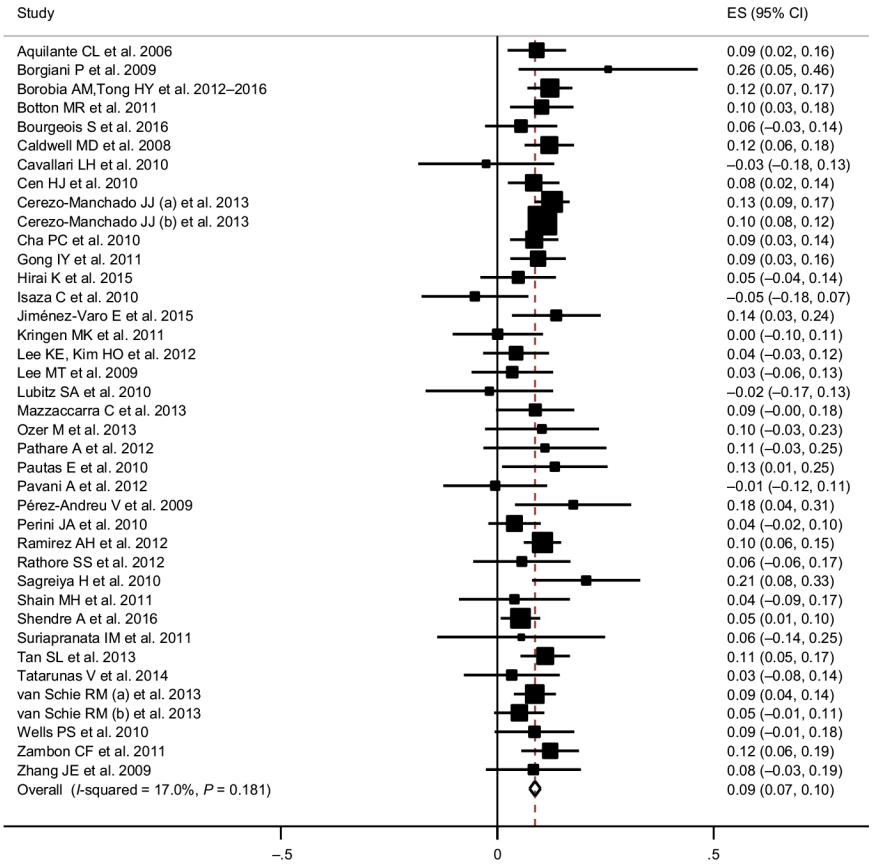

Figure 2.

Forest plot for the difference in logarithm of stable coumarin dose* for subjects with cytochrome P450 (*CYP*)*4F2* polymorphism (CT + TT) compared with subjects with (*CYP*)*4F2* wild-type (CC), according to dominant model.

CI=Confidence Intervals; ES=Estimate

* exp(ES) gives the relative percentage difference as weekly dose in mg

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Table 1

Descriptive characteristics of studies included in the analysis

First author (ref)	PY	Country	Ethnicity	Subjects, *n*	Men (%)	Age, years (mean ± SD)	Drug	INR target	Gene polymorphisms	Available confounders[a]
Aquilante[36]	2006	Florida (USA)	Whites (93%) Blacks (7%)	344	300 (87%)	69 ± 11	Warfarin	2.5–3.5	*CYP2C9**2, *CYP2C9**3, *CYP2C9**5, *VKORC1, CYP4F2*	Age, BMI, gender, smoking, indication for treatment
Borgiani[18]	2009	Italy	Whites	141	75 (53%)	69 ± 12	Warfarin	2.0–4.0	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, gender, indication for treatment
Borobia[15] Tong[43]	2012–2016	Spain	Whites (Spanish)	679	345 (51%)	68 ± 13	Acenocoumarol	2.0–3.0	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender, smoking, indication for treatment, other drugs
Botton[28]	2011	Brazil	Whites	279	155 (57%)	63 ±14	Warfarin	1.8–3.5	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*[b]	Age, BMI, gender, smoking, other drugs
Bourgeois[45]	2016	UK	Whites	217	119 (55%)	71 ± 11	Warfarin	2.0–4.0	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender, smoking, indication for treatment, other drugs
Caldwell[17]	2008	Wisconsin (USA)	Whites	429	252 (59%)	69 ± 11	Warfarin	2.0–3.5	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender, indication for treatment, other drugs
Cavallari[29]	2010	Illinois (USA)	Blacks	208	57 (27%)	56 ± 16	Warfarin	2.0–4.0	*CYP2C9**2, *CYP2C9**3, *CYP2C9**5, *VKORC1, CYP4F2*	Age, BMI, gender, smoking, indication for treatment, other drugs
Cen[30]	2010	China	Asians	221	103 (47%)	45 ±12	Warfarin	1.5–3.0	*CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender, other drugs
Cerezo-Manchado (a)[46]	2013	Spain	Whites	943	459 (49%)	75 ± 9	Acenocoumarol	2.0–3.5	*CYP2C9**2, *CYP2C9**3, *VKORC1,CYP4F2*	Age, gender, indication for treatment, other drugs
Cerezo-Manchado (b)[46]	2013	Spain	Whites	3,882	1,916 (49%)	74 ± 10	Acenocoumarol	2.0–3.5	*CYP2C9**2, *CYP2C9**3, *VK0RC1, CYP4F2*[b]	Age, gender, indication for treatment, other drugs
Cha[31]	2010	Japan	Asians	440	293 (77%)	68 ± 11	Warfarin	1.5–3.0	*CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender, indication for treatment, other drugs
Gong[32]	2011	UK	Whites (95%) Blacks (3%) Asians (2%)	167	96 (57%)	60 ± 18	Warfarin	2.0–3.0	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, gender, indication for treatment
Hirai[47]	2015	Japan	Asians	217	143 (66%)	68 ±10	Warfarin	1.5–3.0	*CYP2C9**2, *CYP2C9**3, *VK0RC1, CYP4F2*	Age, gender, indication for treatment
Isaza[48]	2010	Colombia	Hispanic	145	72 (50%)	55 ± 15	Warfarin	2.0–3.0	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*[b]	Age, BMI, gender, indication for treatment
Jimenez-Varo[49]	2014–2015	Spain	Whites	162	89 (55%)	73 ± 9	Acenocoumarol	2.0–3.0	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*[b]	Age, BMI, gender, smoking, indication for treatment, other drugs
Kringen[33]	2011	Norway	Whites	105	87 (83%)	60 ± 9	Warfarin	1.9–3.6	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, gender, other drugs
Lee[50]	2012	Korea	Asians	188	62 (33%)	59 ± 10	Warfarin	2.0–3.0	*CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender, smoking, other drugs
Lee[27]	2009	China	Asians	233	130 (56%)	63 ± 13	Warfarin	1.7–3.0	*CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender, indication for treatment, other drugs
Lubitz[34]	2010	New York (USA)	Whites (68%) Blacks (20%) Asians (12%)	155	97 (63%)	69 ± 14	Warfarin	2.0–3.0	*CYP2C9**2, *CYP2C9**3, *CYP2C9**5, *VKORC1, CYP4F2*	Age, BMI, gender, smoking, indication for treatment, other drugs
Mazzaccara[51]	2013	Italy	Whites	256	142 (55%)	67 ± 11	Warfarin	1.6–3.9	*CYP2C9**2, *CYP2C9*3, VKORC1, CYP4F2*	Age, BMI, gender, indication for treatment, other drugs
Ozer[52]	2013	Turchia	Whites	107	53 (50%)	54 ± 14	Warfarin	1.5–3.0	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender, smoking, indication for treatment
Pathare[37]	2012	Oman	Asians	188	88 (47%)	51 ± 17	Warfarin	2.0–3.0	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender, smoking, indication for treatment, other drugs
Pautas[35]	2010	France	Whites	272	65 (24%)	87 ±6	Warfarin	2.5	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, gender, other drugs
Pavani[38]	2012	India	Indians	207	108 (52%)	40 ± 13	Warfarin	2.0–3.5	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender
Perez-Andreu[19]	2009	Spain	Whites	100	100 (100%)	65 ±6	Acenocoumarol	2.0–3.0	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age

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First author (ref)	PY	Country	Ethnicity	Subjects, *n*	Men (%)	Age, years (mean ± SD)	Drug	INR target	Gene polymorphisms	Available confounders[a]
Perini[20]	2010	Brazil	Whites (50%) Brown (30%) Blacks (20%)	390	186 (48%)	54 ± 15	Warfarin	2.0–3.5	*CYP2C9**2, *CYP2C9**3, *CYP2C9**5, *VKORC1, CYP4F2*[b]	Age, BMI, gender, smoking, indication for treatment, other drugs
Ramirez[39]	2012	Tennessee (USA)	Whites	1,029	586 (57%)	65 ± 15	Warfarin	1.6–3.5	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender, smoking, indication for treatment, other drugs
Rathore[16]	2012	India	Indians	217	145 (67%)	39 ± 12	Acenocoumarol	2.0–3.5	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*[b]	Age, BMI, gender, smoking
Sagreiya[21]	2010	California (USA)	Whites (75%) Asians (17%) Blacks (8%)	101	58 (57%)	64 ±15	Warfarin	1.8–3.5	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender, smoking, indication for treatment, other drugs
Shain[22]	2011	Egypt	Egyptians	188	84 (44%)	48 ± 15	Warfarin	1.5–3.0	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender, smoking, indication for treatment
Shendre[40]	2016	Alabama (USA)	Whites (58%) Blacks (41%) Asians (1%)	1,169	610 (52%)	61 ± 16	Warfarin	1.8–3.2	*CYP2C9**2, *CYP2C9**3, *CYP2C9**5, *VKORC1, CYP4F2*	Age, BMI, gender, smoking, indication for treatment, other drugs
Suriapranata[23]	2011	Indonesia	Asians	85	48 (56%)	57 ± 11	Warfarin	1.5–2.5	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender, smoking, indication for treatment, other drugs
Tan[41]	2013	China	Asians	317	95 (30%)	45 ± 10	Warfarin	1.8–3.0	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender
Tatarunas[42]	2014	Lithuania	Whites	189	118 (62%)	65 ± 11	Warfarin	2.0–3.5	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender, smoking, other drugs
van Schie (a)[44]	2013	Netherlands	Whites	568	328 (58%)	70 ± 11	Other (phenprocoumon)	2.0–3.5	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender, indication for treatment, other drugs
van Schie (b)[44]	2013	Netherlands	Whites	397	217 (55%)	73 ±9	Acenocoumarol	2.0–3.5	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender, indication for treatment, other drugs
Wells[24]	2010	Canada	Whites	246	136 (55%)	61 ± 14	Warfarin	2.0–3.0	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender, smoking, indication for treatment, other drugs
Zambon[25]	2011	Italy	Whites	371	231 (62%)	73 ± 9	Warfarin	2.5	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender, smoking, indication for treatment
Zhang[26]	2009	UK	Whites	202	120 (59%)	66 ± 14	Warfarin	2.0–3.0	*CYP2C9**2, *CYP2C9**3, *VKORC1, CYP4F2*	Age, BMI, gender, smoking, indication for treatment, other drugs

BMI, body mass index; CYP, cytochrome P450; INR, International Normalized Ratio; PY, publication year.

[a]Covariates with < 20% of missing data are here indicated and used in the multivariate analysis.

[b]CYP2F4 not in Hardy-Weinberg equilibrium.

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Table 2

Subgroup analyses of association studies of the CYP4F2 polymorphism on coumarin dose requirements

Variable	Subgroup (*N* studies)	Difference[a] (95% CI)	I^2 (*Q* test *F* value)	Meta regression *P* value
Ethnicity	Whites (26)	0.10 (0.08–0.11)	15% (0.25)	**0.002**
	Asians (10)	0.08 (0.05–0.11)	0% (0.85)	**0.02**
	Blacks (5)	0.05 (−0.04; 0.14)	21% (0.28)	0.36
	Others (5)[b]	0.01 (−0.05; 0.06)	0% (0.72)	Reference
Drug	Acenocoumarol (7)	0.11 (0.09–0.13)	13% (0.33)	**0.03**
	Warfarin (31)	0.08 (0.06–0.09)	9% (0.33)	
Sex	Males (39)	0.07 (0.06–0.09)	16% (0.20)	**0.03**
	Females (38)	0.10 (0.08–0.12)	19% (0.16)	
INR target	< 2.5 (11)	0.08 (0.05–0.11)	0% (0.79)	Reference
	2.5 (23)	0.09 (0.07–0.11)	22% (0.17)	0.42
	> 2.5 (18)	0.08 (0.06–0.10)	0% (0.49)	0.93
Smoking	No (21)	0.09 (0.07–0.11)	0% (0.68)	0.74
	Yes (12)	0.07 (−0.02; 0.15)	33% (0.12)	
Other drugs considered	No (7)	0.08 (0.03–0.12)	47% (0.08)	0.72
	Yes (32)	0.09 (0.08–0.10)	10% (0.31)	
Hardy-Weinberg equilibrium	No (6)	0.08 (0.04–0.12)	53% (0.06)	0.92
	Yes (33)	0.09 (0.07–0.10)	9% (0.33)	
Quality score	< 5	0.08 (0.06–0.10)	29% (0.11)	0.46
	≥ 5	0.09 (0.08–0.11)	6% (0.39)	
CYP2C9	*CYP2C9 *1*1*	0.08 (0.07–0.10)	18% (0.18)	0.73
	*CYP2C9 *1*2/*1*3/*2*2/*2*3/*3*3*	0.09 (0.06–0.12)	25% (0.10)	
VK0RC11	*VK0RC1* GG	0.08 (0.06–0.10)	4% (0.40)	0.13
	VKORC 1 AA/AG	0.10 (0.08–0.11)	10% (0.30)	

Data in bold are significant at $P < 0.05$.

CI, confidence interval; CYP, cytochrome P450; INR, International Normalized Ratio.

[a]Difference in logarithm of stable coumarin dose of subjects with *CYP4F2* polymorphism (CT + TT) compared with subjects with *CYP4F2* wild-type (CC), according to dominant model.

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[b] Includes Indian, Egyptian, brown, and Hispanic.

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Table 3

Predictive model for logarithm of stable coumarin dose according to patients' clinical and genetic characteristics

WHITES								
	Acenocoumarol				Warfarin			
Variable	**Parameter estimate (95% CI)**	***P* value**	**R^2 test (*N* = 2,744)**	**R^2 validation (*N* = 1,410)**	**Parameter estimate (95% CI)**	***P* value**	**R^2latest (*N* = 3,016)**	**R^2 validation (*N* = 1,532)**
Intercept	4.069 (3.883; 4.256)	< 0.0001	0.33	0.28	3.981 (3.887; 4.075)	< 0.0001	0.51	0.52
Age[a]	−0.014 (−0.015; −0.012)	< 0.0001			−0.009 (−0.010; −0.008)	< 0.0001		
BMI[a]	−0.002 (−0.006; 0.002)	0.28			0.010 (0.008; 0.012)	< 0.0001		
Male gender	0.014 (−0.024; 0.052)	0.47			0.123 (0.098; 0.148)	< 0.0001		
Indication for treatment[b]	0.000 (−0.042; 0.042)	0.98			−0.043 (−0.069; −0.017)	0.001		
CYP2C9 *2 1-allele	−0.190 (−0.232; −0.147)	< 0.0001			−0.231 (−0.261; −0.202)	< 0.0001		
CYP2C9 *2 2-alleles	−0.359 (−0.484; −0.234)	< 0.0001			−0.513 (−0.600; −0.426)	< 0.0001		
CYP2C9 *3 1-allele	−0.394 (−0.446; −0.342)	< 0.0001			−0.387 (−0.425; −0.350)	< 0.0001		
CYP2C9 *3 2-alleles	−1.214 (−1.522; −0.907)	< 0.0001			−1.316 (−1.502; −1.131)	< 0.0001		
VK0RC1 AG	−0.291 (−0.332; −0.249)	< 0.0001			−0.266 (−0.292; −0.240)	< 0.0001		
VK0RC1 AA	−0.762 (−0.816; −0.708)	< 0.0001			−0.666 (−0.704; −0.629)	< 0.0001		
CYP4F2 CT	0.018 (−0.022; 0.058)	0.39			0.073 (0.047; 0.098)	< 0.0001		
CYP4F2 TT	0.100 (0.041; 0.159)	0.0009			0.191 (0.147; 0.235)	< 0.0001		
ASIANS								
	Acenocoumarol				Warfarin			
Variable	**Parameter estimate**	***P* value**	**R^2 test (*N*=0)**	**R^2 validation (*N*=0)**	**Parameter estimate**	***P* value**	**R^2 test (N = 292)**	**R^2 validation (N = 146)**
Intercept	-	-	-	-	3.484 (3.112; 3.855)	< 0.0001	0.45	0.42
Age[a]	-	-			-0.005 (−0.008; −0.001)	0.02		
BMI[a]	-	-			0.014 (0.004; 0.023)	0.004		
Male gender	-	-			0.058 (−0.050; 0.167)	0.29		
Indication for treatment[b]	-	-			−0.027 (−0.139; 0.084)	0.63		
*CYP2C9**2 1-allele	-	-			−0.114 (−0.351; 0.124)	0.35		

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WHITES								
	Acenocoumarol				Warfarin			
Variable	**Parameter estimate (95% CI)**	***P* value**	**R^2 test (*N* = 2,744)**	**R^2 validation (*N* = 1,410)**	**Parameter estimate (95% CI)**	***P* value**	**R^2latest (*N* = 3,016)**	**R^2 validation (*N* = 1,532)**
*CYP2C9**2 2-alleles	-	-			-	-		
*CYP2C9**3 1-allele	-	-			−0.224 (−0.428; −0.020)	0.03		
*CYP2C9**3 2-alleles	-	-			−1.065 (−1.717; −0.412)	0.002		
VK0RC1 AG	-	-			−0.422 (−0.574; −0.271)	< .0001		
VK0RC1 AA	-	-			−0.827 (−0.975; −0.679)	< .0001		
CYP4F2 CT	-	-			0.117 (0.003; 0.231)	0.04		
CYP4F2 TT	-	-			0.124 (−0.075; 0.324)	0.22		
BLACKS								
	Acenocoumarol				Warfarin			
Variable	**Parameter estimate**	***P* value**	**R^2test (/V=0)**	**R^2 validation (*N* = 0)**	**Parameter estimate**	***P* value**	**R^2 test (*N* = 534)**	**R^2 validation (*N* = 288)**
Intercept	-	-	-	-	3.875 (3.692; 4.061)	< 0.0001	0.30	0.22
Age[a]	-	-			−0.009 (−0.011; −0.006)	< 0.0001		
BMI[a]	-	-			0.010 (0.007; 0.015)	< 0.0001		
Male gender	-	-			0.152 (0.086; 0.219)	< 0.0001		
Indication for treatment[b]	-	-			−0.090 (−0.160; −.0183)	0.01		
*CYP2C9**2 1-allele	-	-			−0.007 (−0.149;0.133)	0.93		
*CYP2C9*2* 2-alleles	-	-			-	-		
CYP2C9 *3 1-allele	-	-			−0.469 (−0.666; −0.270)	< 0.0001		
CYP2C9 *3 2-alleles	-	-			-	-		
CYP2C9 *5 1-allele	-	-			−0.436 (−0.736; −0.137)	0.005		
CYP2C9 *5 2-alleles	-	-			-	-		
VK0RC1 AG	-	-			−0.284 (−0.585; −0.020)	0.07		
VK0RC1 AA	-	-			−0.281 (−0.588; −0.020)	< 0.0001		
CYP4F2 CT	-	-			−0.0382 (−0.124; 0.050)	0.40		
CYP4F2 TT	-	-			0.300 (−0.068; 0.664)	0.11		

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Statistical test for model fit ($R2$) is reported both for the test and validation cohorts. Due to significant heterogeneity, separate models are reported for different ethnic groups and drugs. BMI, body mass index; CI, confidence interval; CYP, cytochrome P450.

[a] Estimate for 1 unit increase.

[b] Estimate for the following indication for treatment: fibrillation/flutter, cardiomyopathy/left ventricular dilation, postorthopedic.

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Table 4

Beta coefficients (P values) for single genes and gene–gene interaction

Ethnicity	Drug	*N* subjects (*N* studies)	*CYP4F2*	*CYP2C9*	*VKORC1*	*CYP4F2*CYP2C9*	*CYP4F2*VKORC1*	*CYP2C9*VKORC1*
Whites	Acenocoumarol	4,154 (5)	**0.08 (0.0002)**	**−0.22 (< 0.0001)**	**−0.40 (< 0.0001)**	−0.02 (0.51)	−0.03 (0.21)	−0.01 (0.79)
	Warfarin	4,548 (15)	**0.08 (0.0001)**	**−0.30 (< 0.0001)**	**−0.38 (< 0.0001)**	−0.001 (0.96)	0.02 (0.37)	−0.01 (0.55)
Asians	Acenocoumarol	0(0)	NE	NE	NE	NE	NE	NE
	Warfarin	438 (8)	0.10 (0.34)	**−0.26 (0.05)**	**−0.46 (< 0.0001)**	0.12 (0.36)	−0.08 (0.48)	−0.004 (0.98)
Blacks	Acenocoumarol	0(0)	NE	NE	NE	NE	NE	NE
	Warfarin	815 (5)	0.04 (0.30)	**−0.20 (0.0004)**	**−0.27 (< 0.0001)**	0.004 (0.97)	−0.02 (0.82)	0.02 (0.83)
Others	Acenocoumarol	0(0)	NE	NE	NE	NE	NE	NE
	Warfarin	701 (7)	−0.08 (0.13)	**−0.19 (0.003)**	**−0.27 (< 0.0001)**	0.07 (0.31)	0.09 (0.13)	−0.05 (0.48)
All	All	11,435 (29)	**0.07 (< 0.0001)**	**−0.24 (< 0.0001)**	**−0.37 (< 0.0001)**	0.02 (0.21)	0.02 (0.23)	−0.02 (0.12)

Data in bold are significant at $P < 0.05$.

Ethnicity-specific and drug-specific models are adjusted by study, age, gender, body mass index, and indication for treatment. The final model is also adjusted by ethnicity and drug. For each gene, the reference category is the gene polymorphism according to the dominant model (heterozygous + variant homozygous vs. wt). For the analysis on blacks, *CYP2C9* included, beyond *2 and *3, also *5 polymorphism. CYP, cytochrome P450; NE, not estimated.

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