The 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 *CYP4F2* gene is known to influence mean coumarin dose. The aim of the present study was to undertake a meta-analysis at 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 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%CI 7-10%), with a higher effect in females, in patients taking acenocoumarol and in Whites. 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.

INTRODUCTION

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 inter-individual variability in therapeutic dose make coumarin therapy difficult to manage. Many studies have showed two genes, *CYP2C9* and *VKORC1,* that 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 Food and Drug Administration (FDA) has updated the label for warfarin twice, advising that two variants in the *CYP2C9* gene (C144R and I359L) and one in the *VKORC1* gene (G-1639A) might be taken into consideration when initiating warfarin therapy (Warfarin (Coumadin) product labeling, FDA. http://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 undergoing 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 endpoints 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 was detected.[13] 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.[14,15] The incorporation of *CYP4F2* into existing models might improve the accuracy of dose prediction with coumarins.[16,17] 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 non-synonymous 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 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 till August 2011), individual patient data were obtained from 19 studies.[12,18-35] From one co-author we obtained an additional dataset related to an article not previously included 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.[16,17,37-52]

Thus, 38 articles were included in the present work from authors who agreed to share individual patient data: 20 from the first systematic search, 18 from the second systematic search. Data from one study were divided into two distinct cohorts according with the main author subdivision of sample into discovery and validation cohorts.[46] Moreover, data from two studies had been collected in one cohort.[16,43] 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 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, 7 cohorts evaluated this association for acenocoumarol and one for phenprocoumon. Information on *CYP4F2, VKORC1* and *CYP2C9*3* genotyping were available for all 39 cohorts, while *CYP2C9*2* genotype was recorded for 35 out of the 39 (89.7%) cohorts. All studies but one[19] included both male and female participants with a minimum of 24% males. One study selected very elderly patients (mean age 86.7 years).[35] Data on 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 1 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%CI 0.07 to 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 to 0.08), corresponding to a 7% increase in mg/week; while for TT vs CC subjects it is 0.17 (95%CI 0.15 to 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 females) 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 INR, adjustment for other drugs, consistency of genotype frequencies with the HW equilibrium, quality score and other polymorphisms was found (**Table 2**). The figures for the different meta-analyses in subgroups are presented in **Figure S3 and S4**.

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; 0.27 and 0.27 for Blacks). For cohorts that included Black patients, addition of the *CYP2C9**5 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 Whites 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 R2 of our model with those calculated for two previously published models are reported in **Table S3** and are basically comparable, ranging from 0.41 to 0.47 for Whites, 0.44 for Asians and from 0.23 to 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 so identified such that males had a lower effect of the T-allele when compared to females. 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, 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.

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*, while *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 out 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 being the study performed by Borgiani with a +0.26 estimate and the one by Isaza with a -0.05 which however have a 95%CI which is around to +0.07, not far from our total effect size (slightly less than 10%).[18,48]

However, the funnel plot shows a certain asymmetry, almost significant when analysed using Egger's. 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 add also drug 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-HETE 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 sex is not unexpected: also in other studies exploring other cardiovascular actions, some CYP polymorphisms have shown a differential effect in males and females 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 pharmacogenetics equations estimating coumarin dose have been developed using large samples sizes,[14,15] 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 since multiple cohorts from Europe were also included. In fact, Gage's equation is derived from a more homogeneous group of patients collected in 3 centers in the US (St. Louis, San Antonio and Gainesville) with a 4th trial included in the validation cohort.[14] By contrast, the *International Warfarin Pharmacogenetics Consortium* (IWPC) collected 21 research groups from 9 different countries and finally include only patients with a target INR between 2-3 (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 2 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. in the importance of genotype has been further shown in the ENGAGE AF-TIMI 48 trial, which compared the clinical efficacy of edoxaban, a direct oral anticoagulant, with warfarin, in a pre-specified genetic sub-analysis. Stratification of patients according to *CYP2C9* and *VKORC1* polymorphisms revealed that the three groups identified, normal responders, sensitive responders, and highly sensitive responders, the last group were 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* polymorphism but also the *CYP4F2* polymorphism have recently been performed. In non-valvular 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 6 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 patient' 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 to 7 (median: 5), but this did not affect *CYP4F2*3*–coumarin dose association, since 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 polymorphism, probably because we could not include more variants in *CYP2C9* that were demonstrated to be especially important in this ethnic group.[2] Since 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.

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 that can guide physicians to estimate the stable dose of warfarin according to genotypes, anthropometric and demographic factors, ethnicity, and the use of other drugs.

Anyhow, since RCTs, that tested genetic prediction models with the *CYP4F2*3* SNP, showed contradictory results[6, 56], the utility of these models in clinical practice need to be established in further RTCs 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.[13] 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) and found 38 additional studies that could potentially be included (see flow diagram) according to the inclusion criteria (see Supplementary Material). 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 out 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*); 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 (LD) with the reference polymorphism and rs10871454 (-1168C>T) located in the Syntaxin 4 A–placental (*STX4A*) gene, flanking the *VKORC1* gene, which showed a LD of 0.99 with the reference polymorphism.

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

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: sex, 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 (ED and MM) 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 Chi Square test in controls. We applied a scale with a maximum score of 7 points (see Supplementary Material 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% Confidence Intervals (CI), 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, sex, 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). 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). We performed meta-regression analysis to assess the influence on Summary Estimates (SE) of different study features: type of drugs (acenocoumarol/warfarin), sex, 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 (HW) equilibrium, quality score (<5/≥5), *CYP2C9*2/*3* (wild-type/any polymorphism) and *VKORC1* (wild-type/any polymorphism). When significant differences according to specific study factors were suggested by meta-regression, stratified analyses were performed for *CYP4F2*3*-coumarin dose association on subgroups of significant factors.

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

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 (version 9.4) and STATA (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, while 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 which were available in the majority of studies (Table 1): age, BMI, sex, 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 data set 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[14,15] 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).

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 polymorphisms were modified by concomitant drugs.

The assumption of exchangeability for this analysis was briefly discussed in the Supplementary Methods. 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.

Study Highlights:

- **What is the current knowledge on the topic?**

  Coumarin drugs have a narrow therapeutic index but single nucleotide polymorphisms in the *CYP2C9* 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 knowledge?**

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

- **How might this change clinical pharmacology or translational science?**

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

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.

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Figures legends

Figure 1. Forest plot for the difference in logarithm of stable coumarin dose* for subjects with CYP4F2 polymorphism (CT+TT) compared to subjects with CYP4F2 wild-type (CC), according to dominant model.

Figure 2. Flow diagram.

List of supplemental file titles:

- **Supplementary Methods**
- **Supplementary Material: Discussion and SAS Code**
- **Supplementary Tables**
- **Supplementary Figures**