The effect of *CYP4F2, VKORC1 and CYP2C9* polymorphisms in influencing mean coumarin dose. A single patient data meta-analysis in more than 15,000 individuals.

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Supplementary Methods

In addition to the primary studies already included in our previously published meta-analysis, we performed a new literature search scrutinizing Medline and Web of Science from September 1, 2011 to September 14, 2016, without language restrictions. The search algorithm combined the

categories for “drug,” “cytochrome,” and “gene” by the Boolean operator “AND.” The search terms (medical subject headings and text words) in each category were combined with the operator “OR.” The following search strategy was applied: (warfarin OR coumarin OR coumadin OR acenocoumarol OR phenprocoumon) AND (CYP4F2 OR 4F2 OR cytochrome) AND (gene OR genetic* OR genomic* OR pharmacogenet* OR pharmacogenom* OR polymorph*).

In addition, in order to identify advance online publications, we searched the online databases of the 10 journals with the highest frequency of eligible publications as indexed by ISI Web of Science. We manually searched the tables of contents of the issues of these journals for 2005–2011, along with the bibliographies of relevant articles to retrieve further potential publications.

Study selection and inclusion/exclusion criteria.

We considered as potentially eligible observational studies published in full text where the *CYP4F2* rs2108622 were genotyped along with the *CYP2C9* (at least one out of the two variants of interest rs1799853 and rs1057910) and/or *VKORC1* (rs9923231) in coumarin treated patients (see also main manuscript). The studies selected had to meet the following inclusion criteria: (i) clinical cohort or cross-sectional study in coumarin-treated patients, (ii) CYP4F2 genotyping performed in all patients or in a random selection of patients. There were no restrictions in the inclusion criteria with respect to patient demographic information, including age, body weight, height, use of interacting drugs, indication for coumarin use, and target INR range.

We excluded studies that were published only as abstracts, conference reports, case reports, reviews, and notes. Other exclusion criteria were: randomized clinical trials where specific algorithms were applied, studies in children, studies that selected participants on the basis of coumarin dose, case reports.

Quality of the primary studies

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). We also checked for departure from Hardy–Weinberg equilibrium by Chi Square test in controls.

We applied a scale with a maximum score of 7 points. One point was assigned for each of the follow indicators: absence of large population stratification (ethnic homogeneity ≥90%), assessment of compliance to coumarin drug therapy, consideration of Vitamin K intake, consistency of observed genotype frequencies with the Hardy-Weinberg equilibrium (defined as $p>0.05$), exclusion of critical patients (e.g. patients with overt liver or renal disease, malignant disease, hospitalization within the earlier 4 weeks, congestive heart failure, thyroid disease or chronic gastrointestinal conditions), stable anticoagulation (defined as INR values within the therapeutic range occurring in three consecutive measurements or for a minimum period of three weeks), exclusion or consideration of medications potentially interacting with coumarin drugs.

Two investigators independently scored quality (ED, MM), and disagreements were resolved by consensus. In subgroup analyses, studies with a median scores <5 were compared against studies with median scores ≥5.

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,[1] we pooled study-specific estimates with random-effects models, using the DerSimonian and Laird method. 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.[2] 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.[3]

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[4,5] 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.[6]

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.

P-values <0.05 were considered statistically significant. The analyses were carried out using SAS (version 9.4) software.

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