





Genetic Factors Influencing Warfarin Dose in Black-African Patients: A Systematic Review and Meta-Analysis

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Warfarin is the most commonly used oral anticoagulant in sub-Saharan Africa. Dosing is challenging due to a narrow therapeutic index and high interindividual variability in dose requirements. To evaluate the genetic factors affecting warfarin dosing in black-Africans, we performed a meta-analysis of 48 studies (2,336 patients). Significant predictors for *CYP2C9* and stable dose included rs1799853 (*CYP2C9*2*), rs1057910 (*CYP2C9*3*), rs28371686 (*CYP2C9*5*), rs9332131 (*CYP2C9*6*), and rs28371685 (*CYP2C9*11*) reducing dose by 6.8, 12.5, 13.4, 8.1, and 5.3 mg/week, respectively. *VKORC1* variants rs9923231 (-1639G>A), rs9934438 (1173C>T), rs2359612 (2255C>T), rs8050894 (1542G>C), and rs2884737 (497T>G) decreased dose by 18.1, 21.6, 17.3, 11.7, and 19.6 mg/week, respectively, whereas rs7294 (3730G>A) increased dose by 6.9 mg/week. Finally, rs12777823 (*CYP2C* gene cluster) was associated with a dose reduction of 12.7 mg/week. Few studies were conducted in Africa, and patient numbers were small, highlighting the need for further work in black-Africans to evaluate genetic factors determining warfarin response.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Warfarin dosing requirements vary due to clinical and genetic factors.

WHAT QUESTION DID THIS STUDY ADDRESS?

What are the genetic factors affecting warfarin dosing in black-African patients?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

This paper provides a quantitative estimate of the effect of different genetic variants on weekly warfarin dose requirements in black-African patients.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Most of the work in genomics, including pharmacogenomics, has been undertaken in white patients. This paper, therefore, provides valuable insights into what has been done in black-African patients, and where further work needs to be undertaken. Understanding important ethnicity-specific genetic factors and incorporating them in warfarin dosing algorithms should ultimately improve anticoagulation quality for an underrepresented patient group.

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Cardiovascular disease is a major public health burden worldwide, sub-Saharan Africa (SSA) included. In 2016, ~ 1.2 million deaths (12.9% of all deaths) in SSA were attributed to cardiovascular disease.¹ Warfarin, an oral anticoagulant, is important for management of venous thromboembolism, valvular heart disease, and prevention of stroke in patients with atrial fibrillation. Despite the advent of new oral anticoagulants, warfarin remains the anticoagulant of choice in SSA, and other low-income and emerging countries, mainly because of its significantly lower cost. Treatment with warfarin is difficult due to its narrow therapeutic window, large interpatient variability in dose requirements, and International Normalized Ratio (INR) monitoring requirements. In SSA, the problems are compounded by high HIV and tuberculosis (TB) prevalence, lack of clinical expertise and infrastructure, and lack of validated dosing algorithms. Poor anticoagulation can lead to thrombotic or bleeding events: Warfarin is among the top five drugs leading to hospitalization from adverse reactions in South Africa.²

To improve accuracy of warfarin dosing, several dose-prediction algorithms based on both clinical and genetic factors have been developed.³ Studies in white patients have revealed that genetic polymorphisms in *CYP2C9* (encodes a warfarin-metabolizing enzyme) and *VKORC1* (encodes warfarin's molecular target) together with age, height, weight, and interacting drugs account for ~ 50% of the required individual daily dose variability.³ However, these algorithms have largely been developed in white patients, and may not be applicable to other populations, including black-Africans.⁴ This was demonstrated by the Clarification of Optimal Anticoagulation through Genetics (COAG) trial, in which a genotype-guided dosing algorithm performed worse for African Americans when compared with a clinical algorithm.⁵ This has been partly explained by the different allele frequencies in *CYP2C9* and *VKORC1* across the ethnicities. For instance, whereas the *VKORC1* rs9923231 allele alone explains ~ 20–25% of the variance in warfarin maintenance dose in white and Asian populations (respective allele frequencies 0.39 and 0.89), it only accounts for ~ 6% of dose variability in African populations (allele frequency 0.05).^{6,7} Similarly, the *CYP2C9* alleles *CYP2C9*2* (rs1799853) and *CYP2C9*3* (rs1057910) are more prevalent in white patients (respective allele frequencies of 0.12 and 0.07) when compared with Asians (< 0.01 and 0.03) and Africans (both < 0.01).⁷ In black-Africans, additional *CYP2C9* alleles (*CYP2C9*5*, **6*, **8*, and **11*) may be more important than *CYP2C9*2* and *CYP2C9*3*.^{4,8}

It is important that all relevant ethnicity-specific variants affecting warfarin dose requirements are identified, characterized, and accounted for to improve effectiveness of algorithms and to ensure that health inequities are not worsened. Previous reviews evaluating genetic factors modulating warfarin response in black-African patients have had several limitations, including a lack of structured search strategy and focus on a limited number of genetic factors. This systematic review and meta-analysis has, therefore, critically evaluated the current evidence on black-African specific genetic factors affecting warfarin dose requirements, and other outcomes representing warfarin response.

METHODS

Search strategy and selection criteria

A predefined protocol (PROSPERO: CRD42018110485) based on the principles of the Cochrane Handbook for Systematic Reviews of Interventions⁹ and the Human Genome Epidemiology Network (HuGENet) HuGE Review Handbook¹⁰ was followed. This report adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Table S1). On October 30, 2018, the University of Liverpool's DISCOVER platform was used to search > 480 online databases. Studies were identified using medical subject headings and text words related to "African" AND "warfarin" AND "genetic factors" (Table S2). To determine the completeness of the DISCOVER search, a separate search was conducted in the MEDLINE database (Table S2). One hundred percent consistency was observed. Next, lists of references from the identified studies were examined to identify additional eligible articles. To identify unpublished trials, trial registries, including ClinicalTrials.gov and the International Clinical Trials Registry Platform, were searched. Experts in the field were also contacted to identify further eligible studies. Unless a translated text was available, studies reported in a non-English language were excluded. There was neither restriction by year of publication nor by publication status.

Observational and interventional studies where at least 5% of recruited warfarin-treated patients were black-Africans, and which investigated the effect of at least one genetic factor on warfarin dose requirements and/or treatment response were included. For randomized controlled trials, only data from patients in the genotyped arm(s) were considered. The primary outcome was stable maintenance dose, and coprimaries outcomes were time to stable dose and bleeding events. Secondary outcomes were: INR above range in week 1 of treatment, time to achieving therapeutic INR, proportion of time spent within therapeutic INR range, warfarin sensitivity (≤ 1.5 mg/day on 3 successive clinic visits), and warfarin resistance (> 10 mg/day on 3 successive clinic visits). However, there was no restriction of inclusion criteria to studies that only investigated one or more of these outcomes; rather, studies investigating any other outcomes of warfarin response were also included.

Data extraction and quality assessment

Two reviewers (I.G.A. and R.O.) independently screened titles and abstracts of the retrieved bibliographic records for eligibility and assessed full texts of potentially eligible studies for inclusion. A data extraction tool was developed to extract relevant information related to study and patient characteristics, study quality, outcomes, and results. If key information could not be extracted from the published report, the study's authors were contacted, and the data requested. Studies using the same or overlapping datasets (identified with reference to geographic regions, authors and their affiliations, and recruitment sites) were flagged as such by identifying them as being part of a cluster of studies, to ensure that effect estimates from the same dataset were not included in the same meta-analysis more than once. To assess the methodological quality of each included study, two reviewers (I.G.A. and R.O.) used criteria previously developed to assess the methodological quality of pharmacogenetic studies.¹¹ Disagreements were resolved by consensus.

Data synthesis and analysis

Data synthesis. If ≥ 2 studies were present, a pooled estimate of effect for each gene variant and outcome combination was obtained by undertaking a meta-analysis. Which genotype groups to compare was dependent on what comparisons had been made in the primary papers. For *CYP2C9* and where only summary genotype data was provided, the three genotype groups (wild-type homozygote, heterozygote, and mutant-type homozygote) for each variant were obtained using a strategy provided in Text S1. A genetic model-free approach¹² was used to calculate the pooled effect estimates, such that a particular genetic model

did not have to be assumed. The genetic model-free approach was implemented in Stata version 14¹³ using code provided in **Text S1**. Where there were no variant-type homozygotes, standard meta-analyses using R version 3.5.1¹⁴ (R meta package)¹⁵ were performed. Pooled mean differences with 95% confidence intervals (CIs) were generated and Forest plots prepared for each genetic variant-outcome combination analyzed, using R version 3.5.1.¹⁴

Heterogeneity measures. The magnitude of inconsistency in the study results was assessed by visually examining Forest plots and considering the I^2 statistic.⁹ Arbitrarily chosen categories of heterogeneity were defined as follows: $I^2 < 30\%$, low; $I^2 30\text{--}70\%$, moderate; and $I^2 > 70\%$, high.

Selective reporting. Selective reporting was investigated as part of the methodological quality assessment.

Publication bias. Where > 10 studies were available, publication bias was assessed using the linear regression test of funnel plot asymmetry (implemented using the metabias function in the R meta package).¹⁵ A P value < 0.1 was considered to show publication bias. Where asymmetry was suggested by a visual assessment, we performed exploratory analyses to investigate and adjust it (trim and fill analysis) using the trimfill function (R metafor package).¹⁶

Sensitivity and subgroup analyses. Sensitivity analyses were performed to assess the impact of the analysis approach used (the genetic-model free approach vs. the commonly used bivariate pairwise approach)¹² and the strategy used to infer summary data for each *CYP2C9* genotype group (**Text S1**) on the pooled effect estimates while subgroup analyses were performed stratified by subpopulation (country used as proxy for ethnicity), to try and address moderate and high heterogeneity.

Secondary meta-analyses. One of the largest warfarin-related studies to date is the International Warfarin Pharmacogenetics Consortium (IWPC) study in which 21 research groups from 9 countries contributed individual patient data for a total of 5,700 warfarin-treated patients.¹⁷ As IWPC was a secondary study, it did not fit our eligibility criteria for inclusion, but we felt it important to include data from IWPC where it had not been reported in any of the included papers. However, as it was not possible to identify which of the 21 datasets in IWPC corresponded to which included study report, the IWPC data was ignored from the primary meta-analyses but secondary meta-analyses in which eligible IWPC sites were included (while excluding all studies whose population came from a site that was part of IWPC to avoid duplication) were conducted. It was not possible to assess the methodological quality of the IWPC datasets.

Confidence in cumulative evidence. The strength of the body of evidence and the quality and strength of recommendations was assessed according to the Venice interim criteria.¹⁸

RESULTS

Study selection and characteristics

Figure 1 depicts the literature search and selection process. Over 150 single nucleotide polymorphisms across > 18 genes and > 25 outcomes were investigated by 77 studies. **Table S3** provides details of the studies; studies including similar populations are clustered together. Most studies ($n = 42$; 55%) had a retrospective cohort design, whereas others were prospective ($n = 25$; 33%), both retrospective and prospective ($n = 4$;

5%), randomized controlled trials ($n = 4$; 5%), and case-control studies ($n = 2$; 3%). The median number of black-African patients in the included studies was 115 (interquartile range 31–269). Variant-specific details and the associations investigated for each of the primary, secondary, and other outcomes in the different studies are provided in **Tables S4** (*CYP2C9*), **S5** (*VKORC1*), **S6** (*CYP4F2*), **S7** (other genes), and **S8** (other outcomes).

Methodological quality and risk of bias

Qualitative methods were used to assess the methodological rigor of included studies¹¹ (**Table S9** and **Spreadsheet S1**). Most did not report using genotype quality control procedures ($n = 39$; 51%) and had not reported whether genotyping personnel were blinded to outcome status ($n = 56$; 73%). The reporting of missing genotype data was low across studies, with none of the studies reporting missing data ($n = 38$, 49%) conducting checks for missingness at random. Only 15 (20%) studies undertook tests for cryptic population stratification, with 27 (35%) studies not reporting testing for Hardy-Weinberg equilibrium. Only 2 (2.6%) studies provided details and justification of the modes of inheritance utilized. There was also a large variability in outcome definitions. For instance, 40 different “stable dose” definitions were observed in 56 studies (**Table S10**). The definitions of time to stable dose (5 different definitions in 5 studies), bleeding events (16 in 17 studies), time to therapeutic INR (6 in 6 studies), warfarin sensitivity, and resistance (1 in 1 study) in the included papers are shown in **Table S11**. Last, only 14 (18%) of the trials reported measuring adherence to treatment. Although many issues of concern were raised in terms of the methodological quality, no studies stood out in terms of being of particularly low quality overall, and, therefore, sensitivity analyses based on methodological rigor were not performed.

Meta-analyses

Forty-eight studies representing 2,336 patients were included in the primary meta-analyses. For the remaining studies, even after contacting authors, data were insufficient to allow their inclusion. Summary results for all included studies are provided in **Table 1** and **Figures 2–4** (stable dose), **Figure S1** (time to stable dose), and **Figure S2** (proportion of time in therapeutic range). **Tables S4–S8** show which studies were excluded and why. Results, if available, for studies that could not be included in the meta-analyses are also summarized (**Tables S4–S7** and **S12–S14**).

Stable dose. Regarding *CYP2C9* and stable dose (**Table 1**, **Figure 2**), significant predictors included rs1799853 (*2), rs1057910 (*3), rs28371686 (*5), rs9332131 (*6), and rs28371685 (*11) with heterozygotes respectively requiring 6.75 (95% CI 4.59 to 8.91), 12.51 (95% CI 6.83 to 18.18), 13.38 (95% CI 10.07 to 16.68), 8.10 (95% CI 0.83 to 15.36), and 5.31 (95% CI 0.43 to 10.18) mg/week less warfarin compared to wild-type homozygotes. The rs2256871 (*CYP2C9**9) mutant-type homozygotes on the other hand required 17.15 (95% CI 9.14 to 15.16) mg/week more compared to wild-type homozygotes, although the strength of evidence for this association was considered weak (only three mutant-type homozygotes). Only the association between rs1799853 (*CYP2C9**2) and stable dose met

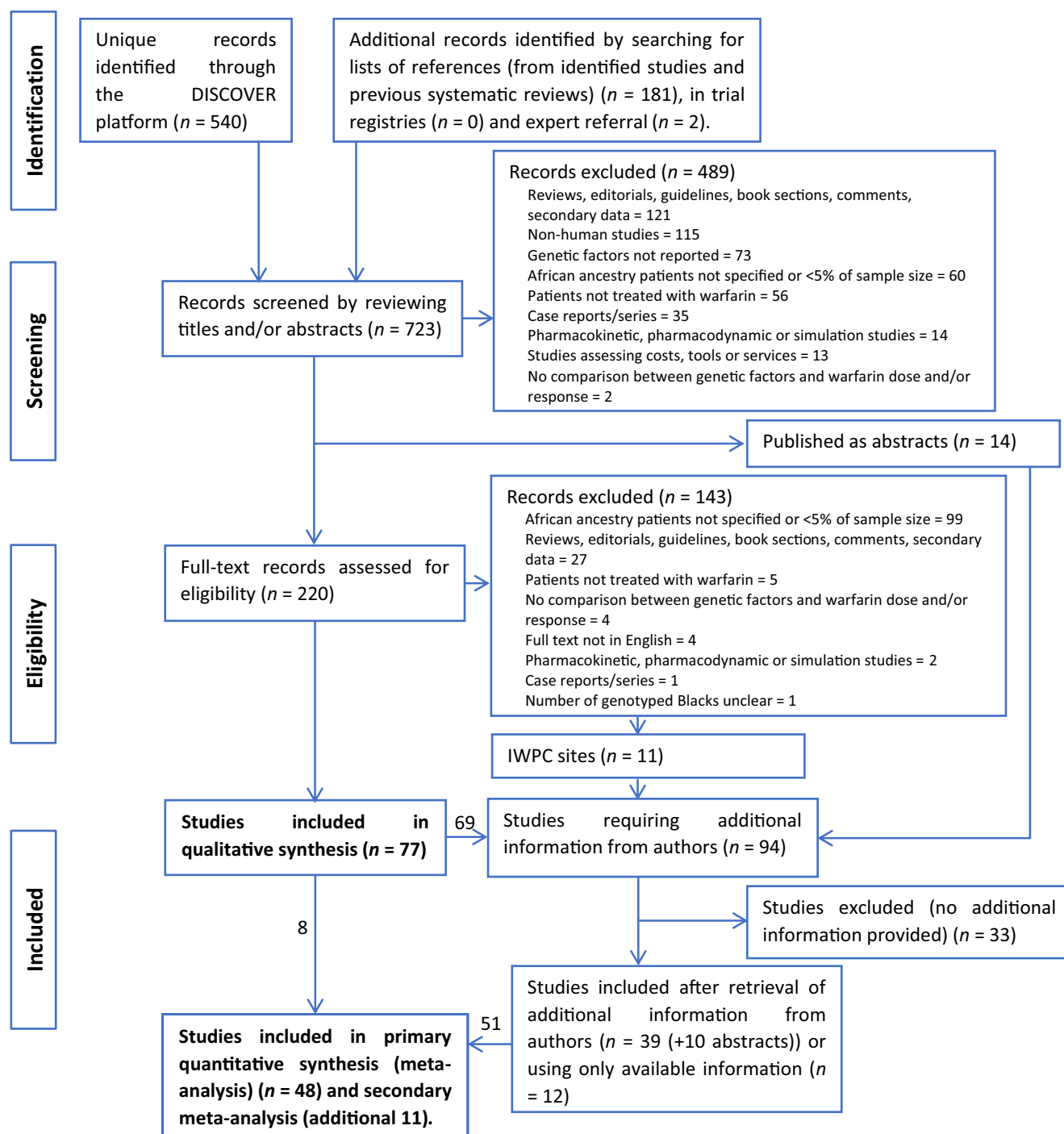


Figure 1 Evidence search and selection. IWPC, International Warfarin Pharmacogenetics Consortium. [Colour figure can be viewed at wileyonlinelibrary.com]

our predefined criteria for assessing publication bias (> 10 included studies), and for this we did not find any evidence to support it (linear regression test of funnel plot asymmetry P value = 0.85).

The *VKORC1* variants rs9923231 ($-1639G>A$), rs9934438 ($1173C>T$), rs2359612 ($2255C>T$), rs8050894 ($1542G>C$), and rs2884737 ($497T>G$) also led to reductions in weekly dose requirements: homozygotes for the variant alleles required 18.13 (95% CI 13.92 to 22.33), 21.56 (95% CI 17.20 to 25.92), 17.30 (95% CI 12.74 to 21.86), 11.66 (95% CI 4.42 to 18.91),

and 19.61 (95% CI 14.32 to 24.90) mg/week less warfarin, respectively, when compared with wild-type homozygotes. By contrast, heterozygotes required 10.28 (95% CI 7.31 to 13.25), 11.14 (95% CI 7.53 to 14.76), 6.40 (95% CI 2.76 to 10.05), 3.77 (95% CI 0.05 to 7.49), and 8.16 (95% CI 3.46 to 12.87) mg/week less, respectively, compared with wild-type homozygotes (Table 1, Figure 3). Conversely, mutant-type homozygotes and heterozygotes for the rs7294 ($3730G>A$) variant required modest warfarin weekly dose increments of 6.93 (95% CI

Table 1 Summary results for associations between genetic variants and stable warfarin dose (mg/week)

Gene	Variants			Included studies	Total N	Genotype counts			Pooled estimates (mean differences (95% CI), heterogeneity)			Strength of evidence ^a
	rs ID ^a	Common name	Function ^a			WT/WT	WT/V	V/V	WT/V vs. WT/WT	V/V vs. WT/WT	V/V vs. WT/WT	
CYP2C9	rs1799853	*2	Missense	13 ^{5,21,37-47}	2,193	2,054	139	0	-6.75 (-8.91; -4.59), <i>I</i> ² = 15%	NA	NA	Moderate
	rs1057910	*3	Missense	9 ^{5,21,38,39,42-44,46,47}	1,754	1,706	48	0	-12.51 (-18.18; -6.83), <i>I</i> ² = 62%	NA	NA	Moderate
	rs28371686	*5	Missense	6 ^{39-41,43,46,47}	1,297	1,277	20	0	-13.38 (-16.68; -10.07), <i>I</i> ² = 0%	NA	NA	Moderate
	rs9332131	*6	Frame-shift	3 ^{40,43,47}	1,044	1,027	17	0	-8.10 (-15.36; -0.83), <i>I</i> ² = 0%	NA	NA	Moderate
	rs7900194	*8	Missense	7 ^{39-41,43,45,46,48}	929	821	97	11	-4.54 (-126.77; 117.70), <i>I</i> ² = 0%	-0.01 (-0.05; 0.04), <i>I</i> ² = 0%	NA	Moderate
	rs2256871	*9	Missense	2 ^{40,48}	299	242	54	3	0.06 (-4.29; 4.41), <i>I</i> ² = 0%	17.15 (9.14; 25.16), <i>I</i> ² = 0%	NA	Weak
rs28371685	*11	Missense	7 ^{38,40,43,45-48}	1,509	1,474	35	0	-5.31 (-10.18; -0.43), <i>I</i> ² = 0%	NA	NA	Moderate	
VKORC1	rs9923231	-1639G>A	Promoter region	12 ^{5,21,38,39,41-47,49}	2,019	1,601	389	29	-10.28 (-13.25; -7.31), <i>I</i> ² = 23%	-18.13 (-22.33; -13.92), <i>I</i> ² = 64%	NA	Moderate
	rs9934438	1173C>T	Intronic	5 ^{39,40,50-52}	713	506	177	30	-11.14 (-14.76; -7.53), <i>I</i> ² = 0%	-21.56 (-25.92; -17.20), <i>I</i> ² = 0%	NA	Moderate
	rs7294	3730G>A	3'UTR	4 ^{40,48,51,53}	666	222	319	125	4.83 (1.11; 8.55), <i>I</i> ² = 0%	6.93 (3.48; 10.38), <i>I</i> ² = 2%	NA	Moderate
	rs2359612	2255C>T	Intronic	3 ^{40,51,53}	535	298	182	55	-6.40 (-10.05; -2.76), <i>I</i> ² = 0%	-17.30 (-21.86; -12.74), <i>I</i> ² = 47%	NA	Moderate
	rs8050894	1542G>C	Intronic	4 ^{40,50,51,53}	663	313	284	66	-3.77 (-7.49; -0.05), <i>I</i> ² = 30%	-11.66 (-18.91; -4.42), <i>I</i> ² = 60%	NA	Moderate
	rs2884737	497T>G	Intronic	2 ^{40,53}	282	185	77	20	-8.16 (-12.87; -3.46), <i>I</i> ² = 0%	-19.61 (-24.90; -14.32), <i>I</i> ² = 0%	NA	Moderate ^a
CYP4F2	rs2108622	*3	Missense	5 ^{21,39,47,54,55}	990	824	160	6	2.64 (-292.45; 297.72), <i>I</i> ² = 0%	2.46 (-32.97; 37.89), <i>I</i> ² = 0%	NA	Moderate
CYP2C cluster	rs12777823	-	Intergenic	4 ^{43,45,47,56}	970	556	344	70	-4.40 (-7.38; -1.42), <i>I</i> ² = 23%	-12.74 (-17.58; -7.91), <i>I</i> ² = 34%;	NA ^a	Moderate
CALU1	rs2290228	11G>A	Missense	2 ^{39,47}	293	254	34	5	-1.53 (-22.47; 25.53), <i>I</i> ² = 52%	NA ^a	NA ^a	Weak
NQO1	rs1800566	*2	Missense	2 ^{39,55}	303	208	85	10	-0.18 (-7.22; 6.87), <i>I</i> ² = 0%	7.63 (-7.33; 22.60), <i>I</i> ² = 0%	NA	Weak

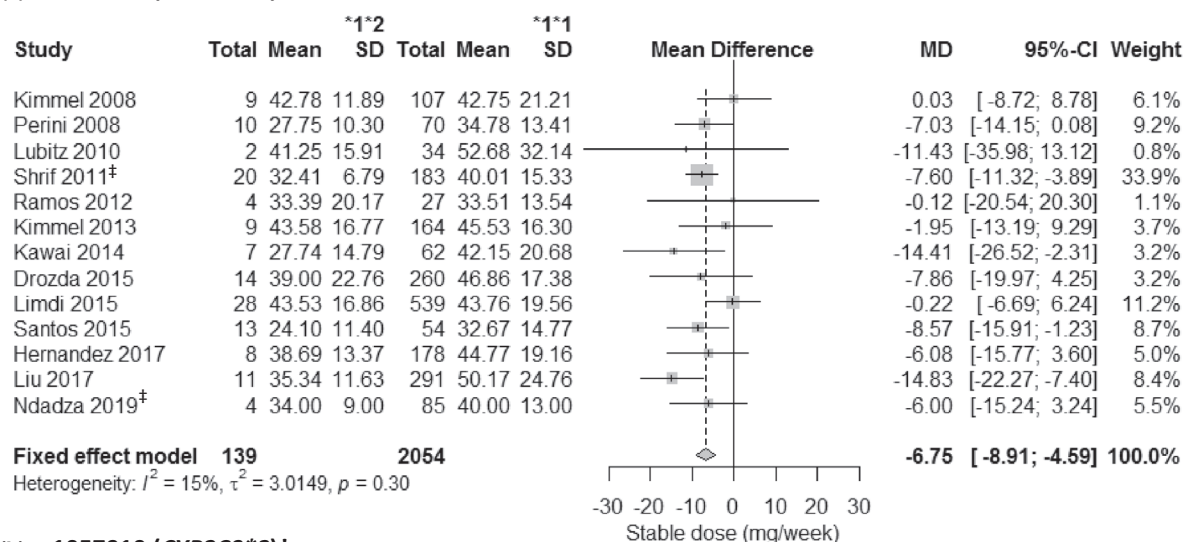
CI, confidence interval; NA, not applicable; UTR, untranslated region.

*CYP2C9 star allele.

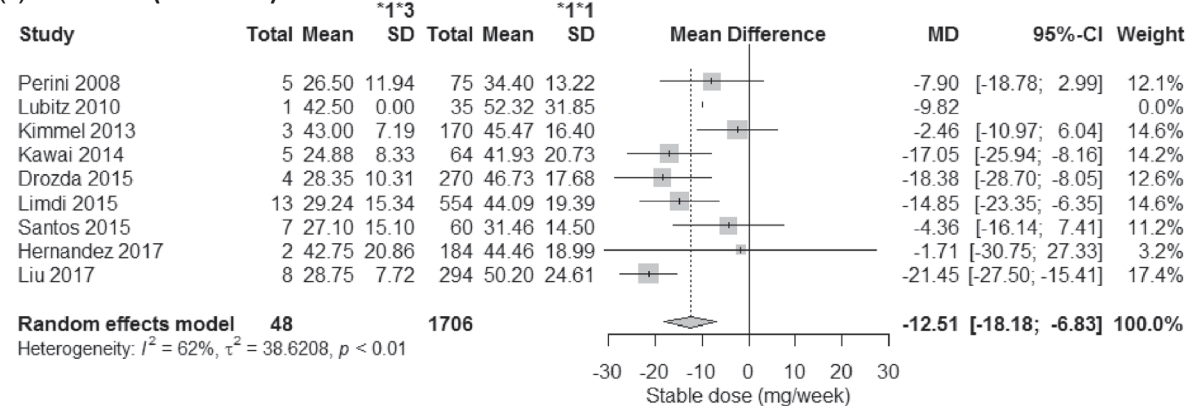
^ars ID, reference SNP (single nucleotide polymorphism) identifier; V, variant-type allele; WT, wild-type allele. ^bFor CYP2C9, except for *6 (no function) and *9 (normal function), variants have decreased function.

^cBased on the Venice interim criteria. ^dGenetic model-free approach could not run ("missing values encountered" error). One study³⁹ had one variant-type homozygote taking 77 mg/week whereas another⁴⁷ had four variant-type homozygotes taking 26.3 ± 17.5 mg/week. ^eSecondary analysis (n = 675) also considered.

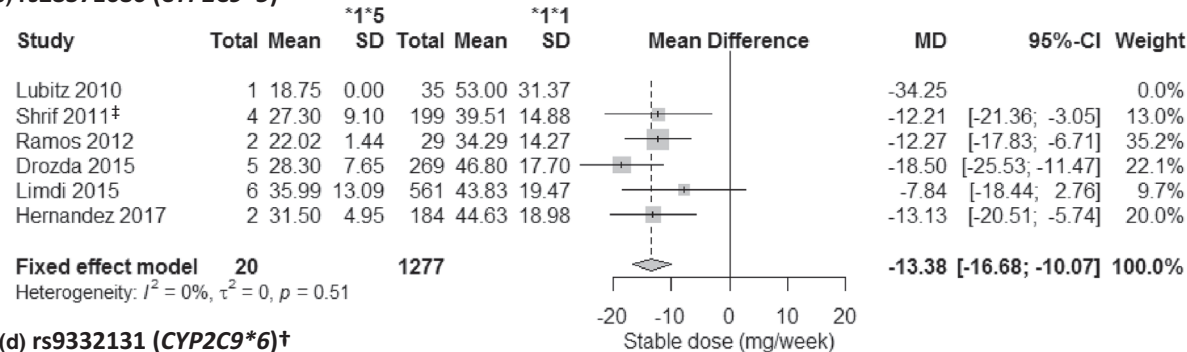
(a) rs1799853 (CYP2C9*2)†



(b) rs1057910 (CYP2C9*3)†



(c) rs28371686 (CYP2C9*5)†



(d) rs9332131 (CYP2C9*6)†

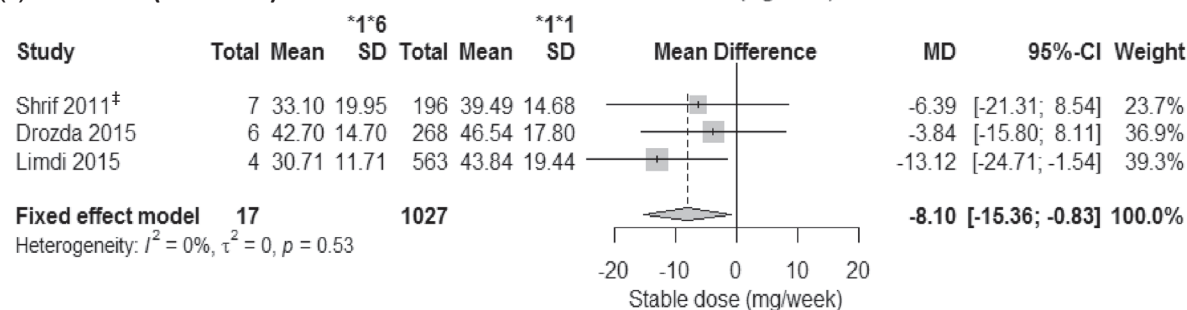
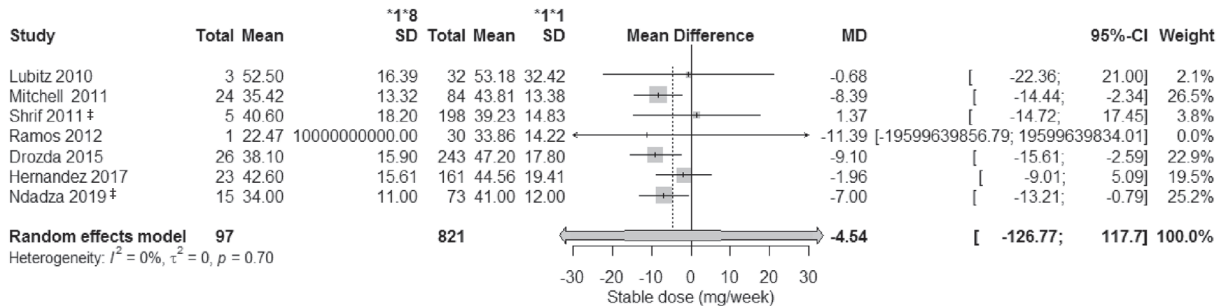
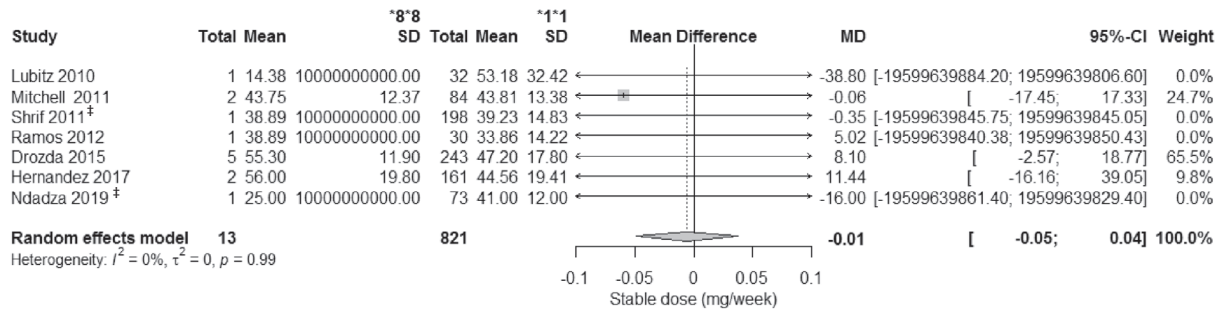
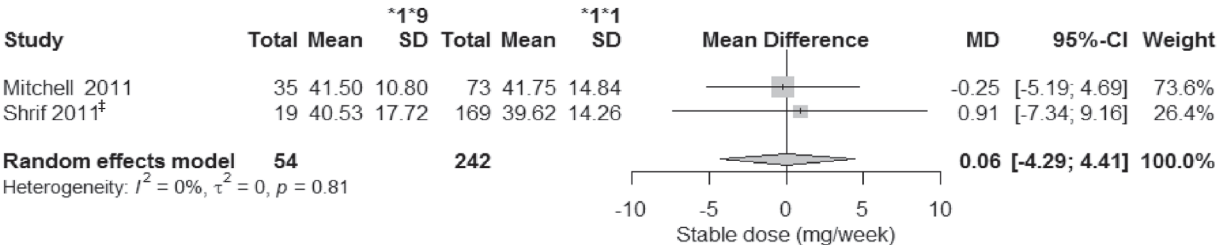
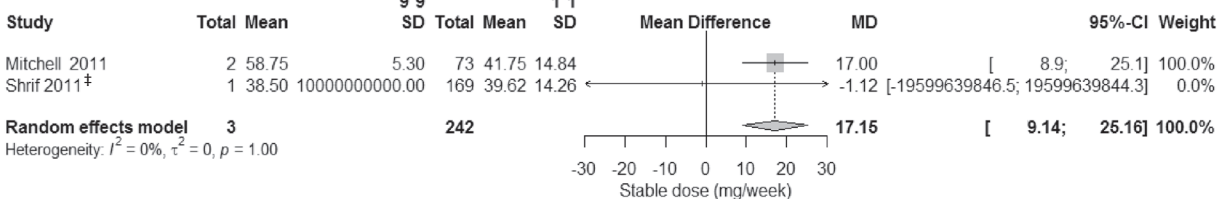


Figure 2 Forest plots for associations between CYP2C9 and stable warfarin dose. *CYP2C9 star allele, †standard meta-analysis (fixed effects assumed with low heterogeneity ($I^2 < 30\%$), else random effects), ‡article as data source (otherwise author-provided). CI, confidence intervals; CYP2C9, cytochrome P450 family 2 subfamily C member 9; MD, mean difference.

(e) rs7900194 (CYP2C9*8)



(f) rs2256871 (CYP2C9*9)



(g) rs28371685 (CYP2C9*11)[†]

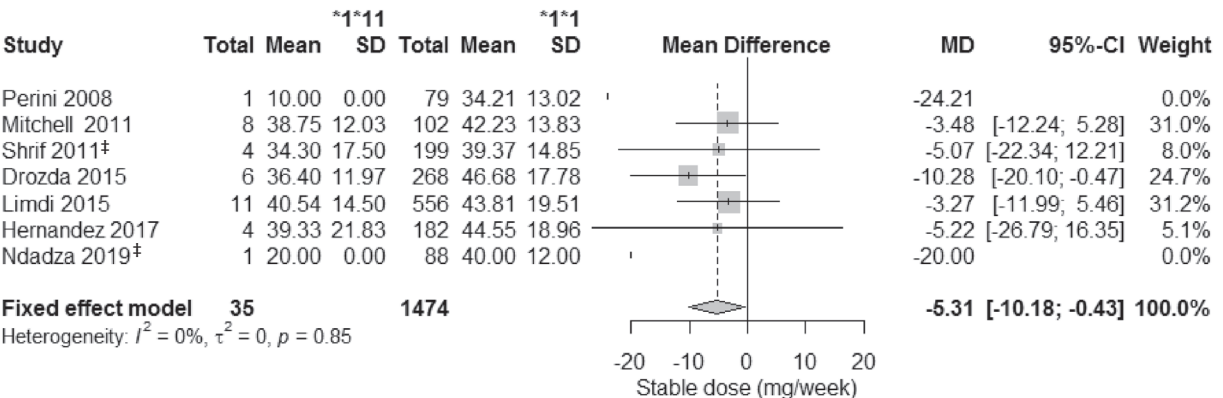


Figure 2 (Continued)

3.48 to 10.38) and 4.83 (95% CI 1.11 to 8.55) mg, respectively. Regarding publication bias, only the first genotype-contrast (wild-type homozygotes vs. heterozygotes) for the rs9923231 (-1639G>A) allele met our predefined criteria (four studies

had zero weight in the second genotype contrast), and for this we found some evidence of publication bias (linear regression test of funnel plot asymmetry P value = 0.05). The trim and fill random effects analysis method estimated that the number

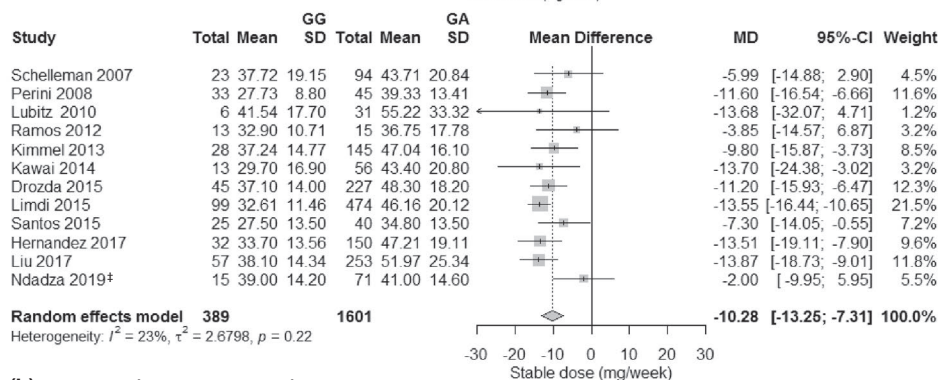
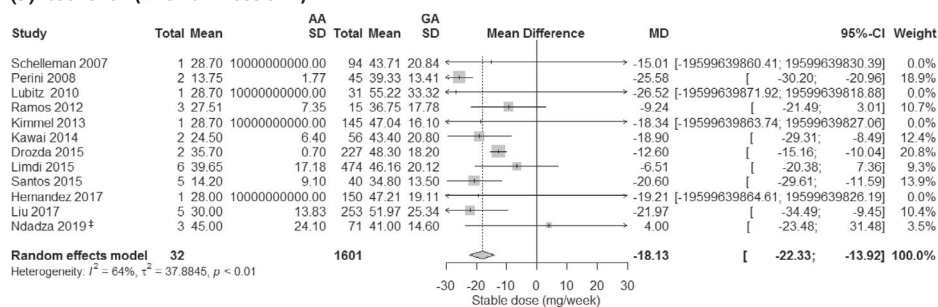
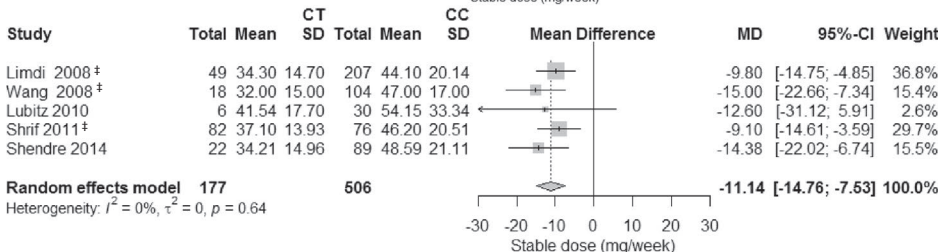
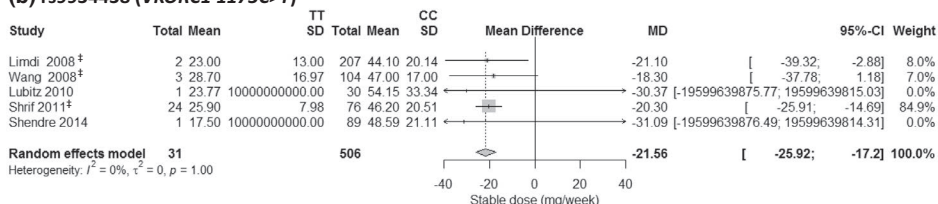
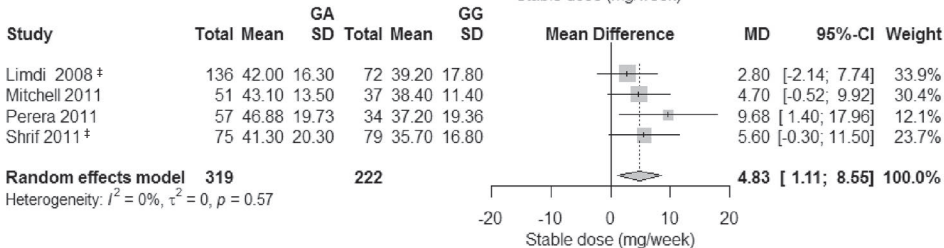
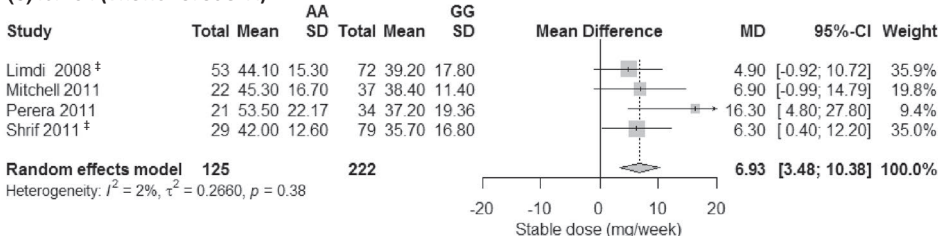
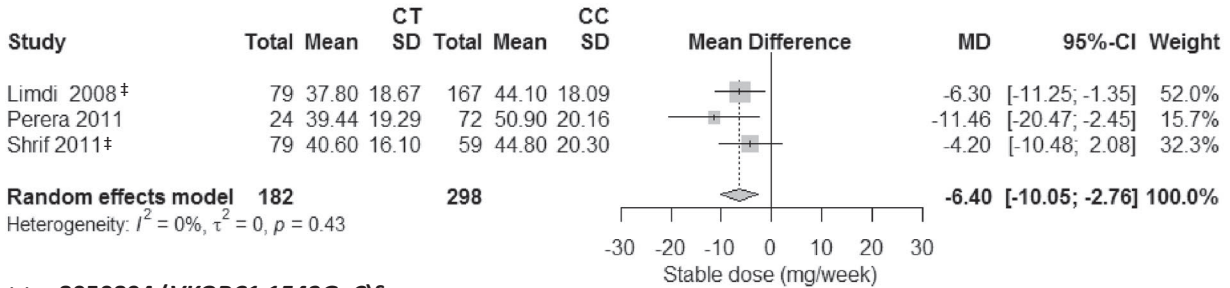
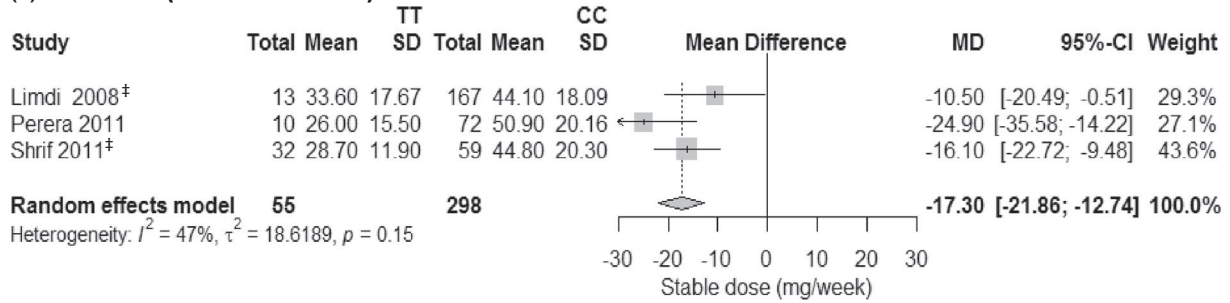
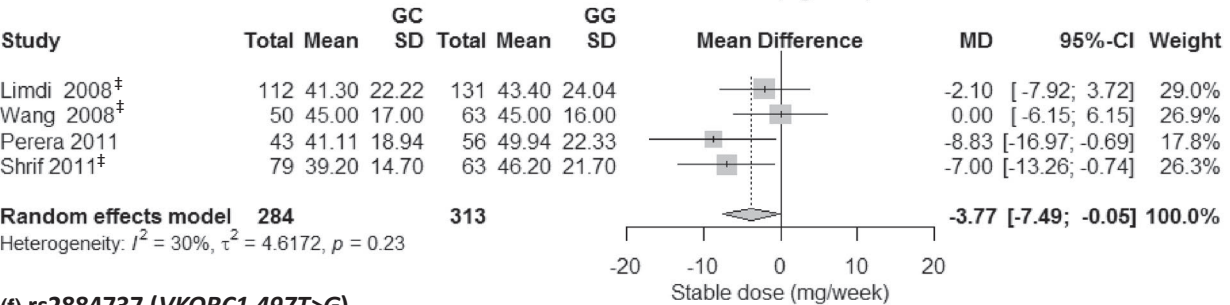
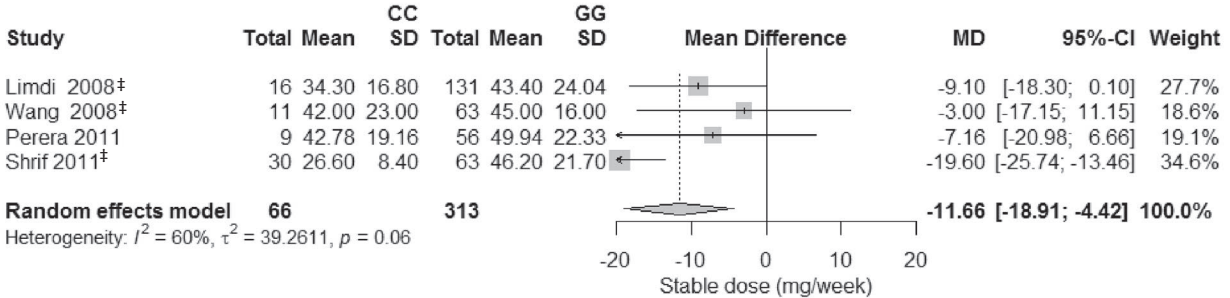
(a) rs9923231 (*VKORC1* -1639G>A)(b) rs9934438 (*VKORC1* 1173C>T)(c) rs7294 (*VKORC1* 3730G>A)

Figure 3 Forest plots for associations between *VKORC1* and stable warfarin dose. †Article as data source (otherwise author-provided), ‡Shrif study estimates flipped, first genotype contrast with high heterogeneity so requires cautious interpretation. CI, confidence intervals; MD, mean difference; *VKORC1*, vitamin K epoxide reductase complex subunit 1.

(d) rs2359612 (VKORC1 2255C>T)



(e) rs8050894 (VKORC1 1542G>C)§



(f) rs2884737 (VKORC1 497T>G)

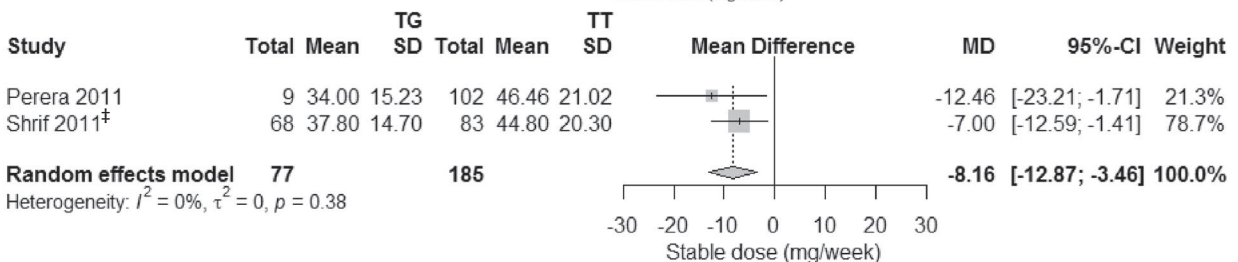
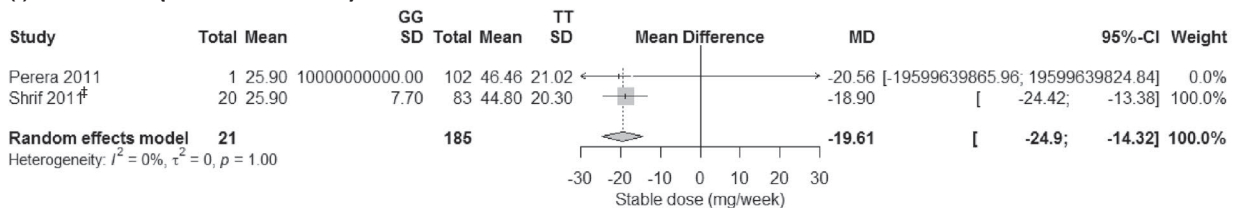


Figure 3 (Continued)

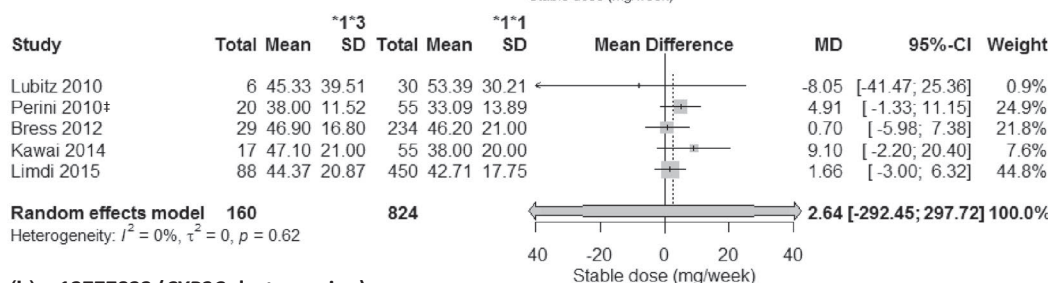
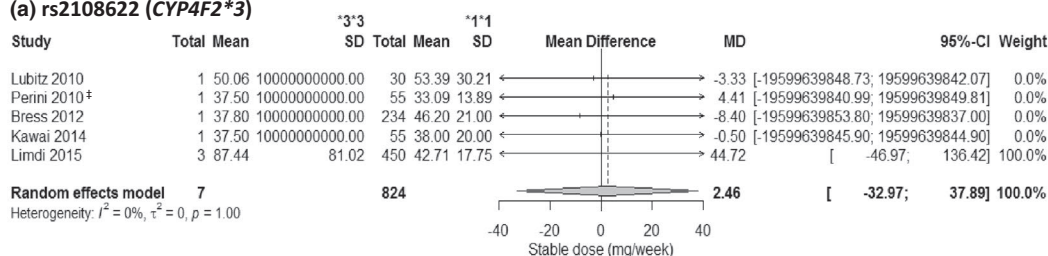
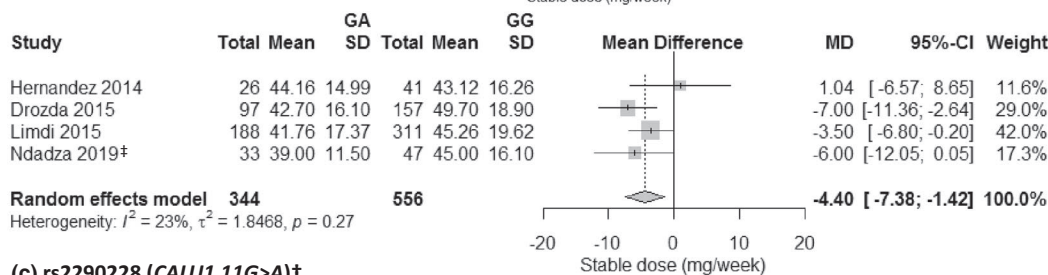
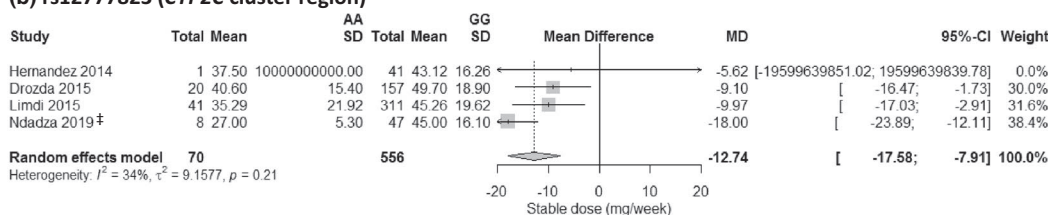
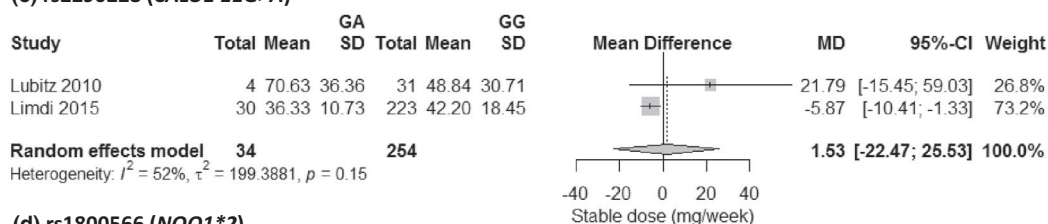
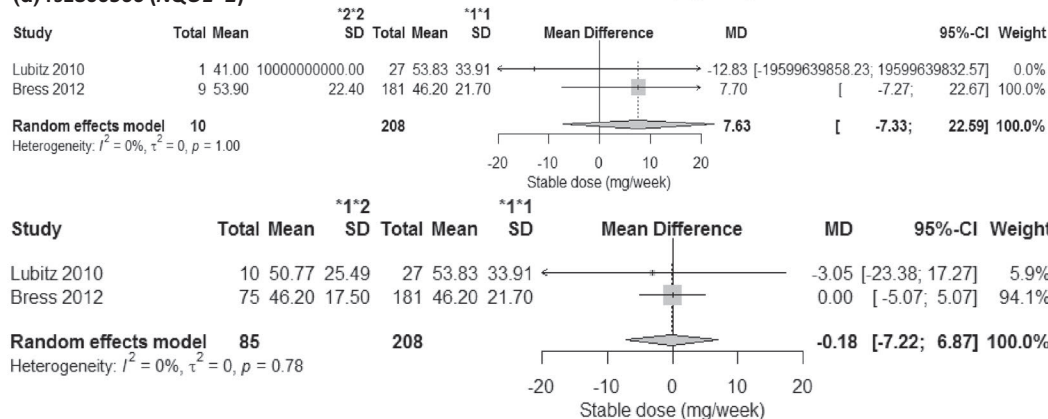
(a) rs2108622 (CYP4F2*3)**(b) rs1277823 (CYP2C cluster region)****(c) rs2290228 (CALU1 11G>A)†****(d) rs1800566 (NQO1*2)**

Figure 4 Forest plots for associations between other genes and stable warfarin dose. **CYP4F2* and *NQO1* star alleles, †standard meta-analysis (fixed effects assumed with low heterogeneity ($I^2 < 30\%$), else random effects), ‡article as data source (otherwise author-provided). CI, confidence intervals; *CYP2C*, cytochrome P450 family 2 subfamily C; *CYP4F2*, cytochrome P450 family 4 subfamily F member 2; MD, mean difference; *NQO1*, NAD(P)H quinone dehydrogenase 1.

of missing studies was two and that these missing trials did not affect the statistical significance of the pooled effect estimate (Figure S3).

For other gene regions (Table 1, Figure 4), only the mutant-type homozygotes and heterozygotes for the rs12777823 (*CYP2C* gene cluster) variant required weekly doses that were significantly different from those of the corresponding wild-type homozygotes (respectively, 12.74 (95% CI 7.91 to 17.58) and 4.40 (95% CI 1.42 to 7.38) mg/less).

The results of the secondary analyses including IWPC sites (coded site_1, site_2, site_5, site_11, site_14, site_16, site_17, site_19, site_20, site_21, and site_22 in the IWPC data and ethnicity datasets)¹⁹ (Figure S4) were similar to those obtained during the primary analyses except for *CYP2C9*6*, *CYP2C9*11*, *VKORC1 2255C>T*, and *VKORC1 1542G>C*, which were no longer statistically significant (estimates for heterozygotes vs. wild-type homozygotes respectively being -0.45 (95% CI -11.87 to 10.96), -4.05 (95% CI -10.44 to 2.35), -3.25 (95% CI -6.75 to 0.25), and -3.11 (95% CI -6.31 to 0.10) mg/week). On the other hand, *CYP2C9*8* produced statistically significant estimates for heterozygotes vs. wild-type homozygotes (-6.42 (95% CI -9.44 to -3.31) mg/week) in the pairwise meta-analysis (Figure S5). Interestingly, the nonsignificant estimate for variant-type homozygotes vs. wild-type homozygotes was in the opposite direction (6.41 (95% CI -2.22 to 15.05) mg/week). Regarding the strategy used to infer summary data for each *CYP2C9* genotype group and except for *11 which was no longer statistically significant (heterozygotes vs. wild-type homozygotes estimate -3.36 (95% CI -9.24 to 2.53) mg/week), the results mirrored those of the primary meta-analyses (Figure S6 and Tables S15–S16).

Finally, where it was possible to conduct country-specific analyses (≥ 2 studies included from the same country), we carried out subgroup analyses based on the country from which participants were recruited. The countries where studies were conducted included the United States ($n = 40$ studies), Brazil ($n = 5$), South Africa ($n = 2$), and Sudan ($n = 1$; Table S17). Population-specific analyses produced nonsignificant estimates for only rs1057910 (*CYP2C9*3*; Brazil-only studies, wild-type homozygotes vs. heterozygotes estimate -6.27 (95% CI -14.26 to 1.72) mg/week, $I^2 = 0\%$), which differed from the overall pooled estimates that were statistically significant.

Bleeding events and other outcomes. We could not conduct meta-analyses for this outcome because follow-up time differed across the three studies (28 days⁵ vs. 2 years²⁰ vs. 5 years²¹). In the individual studies, the comparisons between genetic variants and bleeding events were not statistically significant (Tables S4–S7). Other outcomes are shown in Figures S1–S2.

DISCUSSION

We have comprehensively evaluated the effect of genetic factors that determine warfarin stable dose requirements and other end points in black-African patients. We have largely focused on genes involved in warfarin's pharmacokinetics (*CYP2C9*) and pharmacodynamics (*VKORC1*), all of which have been implicated in determining warfarin response in white patients.

CYP2C9 is the main metabolizing enzyme for the more potent S-enantiomer. The most commonly studied polymorphisms, rs1799853 (*CYP2C9*2*) and rs1057910 (*CYP2C9*3*), produce protein isoforms with only about 12% and < 5% of wild-type enzyme activity.^{22,23} Interestingly, although these polymorphisms are less common in black-Africans,⁷ the effect on the reduction in weekly warfarin dose requirement (6.8 and 12.5 mg, respectively), was similar to that observed in white patients (3.9 and 12.5 mg/week less),²⁴ indicating that these polymorphisms should not be excluded from dosing algorithms.

Other *CYP2C9* polymorphisms (rs28371686 (*CYP2C9*5*), rs9332131 (*CYP2C9*6*), and rs28371685 (*CYP2C9*11*)), which are more prevalent in black-Africans⁷ led to reductions in warfarin weekly dose by 13.4, 8.1, and 5.3 mg, respectively. These polymorphisms also lead to reduced,²⁵ null²⁶, and reduced²⁷ catalytic function, respectively. *CYP2C9*8* (rs7900194) heterozygotes also required decreased weekly warfarin dose (4.5 mg) as predicted by the functional effects of the allele,²⁸ but this did not reach statistical significance. This could be attributed to the mutant-type homozygotes ($n = 7$) who required higher warfarin doses, as shown in the bivariate sensitivity analysis. *CYP2C9*9* (rs2256871), despite the fact that it results in a change from histidine to arginine at position 251, has minimal effect on protein function.⁸ Thus, the higher warfarin dose requirements (17.2 mg/week) for the three mutant-type heterozygotes should be interpreted with caution, given the small sample size.

The *VKORC1* variants rs9923231 (-1639G>A), rs9934438 (1173C>T), rs2359612 (2255C>T), rs8050894 (1542G>C), and rs2884737 (497T>G) also led to reductions in weekly dose requirements by up to 18.1, 21.6, 17.3, 11.7, and 19.6 mg, respectively. Some of these results are similar to those previously observed in white patients.²⁴ For instance, homozygotes for the rs9923231 and rs9934438 variant alleles required 20.0 and 22.0 mg/week less warfarin, respectively, compared with wild-type homozygotes (comparable to 18.1 and 21.6 mg/week in black-African patients). This is biologically plausible for rs9923231 (-1639G>A), which is part of an enhancer box (E-box) consensus sequence CANNTG that may function as a repressor binding site.²⁹ The G>A polymorphism leads to reduced transcription and decreased warfarin requirements.^{29,30} However, this mechanism is yet to be confirmed, a process complicated by the fact that this variant is in near perfect linkage disequilibrium (LD) with several other variants, including the intronic rs9934438 (1173C>T) variant, which were also investigated in this study. The functions of the intronic rs2359612 (2255C>T), rs8050894 (1542G>C), and rs2884737 (497T>G) variants are also unknown. The 1,000 genomes population frequencies of these *VKORC1* variants (rs9923231, rs9934438, rs2359612, rs8050894, and rs2884737) are, respectively, 0.05, 0.05, 0.18, 0.26, and 0.01 in black-Africans compared with 0.39, 0.39, 0.39, 0.40, and 0.26 in individuals of European ancestry.⁷ Two (rs9923231 and rs9934438) are in LD ($r^2 > 0.9$) in the black-Africans, whereas four (rs9923231, rs9934438, rs8050894, and rs2359612) are in LD in Europeans.

The rs7294 (*VKORC1 3730G>A*) variant (population frequency 0.45 in black-Africans, 0.37 in Europeans⁷) increased weekly warfarin requirements by up to 6.9 mg. It is located in the

3'-untranslated region, which can be targeted by microRNAs resulting in gene silencing either by translational repression or by mRNA degradation. For instance, *miR-133a*, which targets this region, has been previously implicated in *VKORC1* regulation.³¹ Specifically, the G>A mutation decreases the binding capacity of *miR-133a* leading to decreased translational repression and more copies of *VKORC1* mRNA, which would lead to higher warfarin dose requirements, as we observed.

In addition, rs12777823 (*CYP2C* cluster) was also associated with stable dose. Although its role is currently unknown, it is associated with warfarin clearance in black patients.³² Despite being common in other populations (respective allelic frequencies of 0.15 and 0.31 in European and East Asian 1,000 genomes⁷ populations), this effect is observed in Africans only (allelic frequency of 0.25), suggesting that it may be in LD with an unknown causal variant.³²

Even though *CALU1* and *NQO1* are thought to be involved in warfarin's mechanism of action (respectively binding to the vitamin K epoxide reductase complex and potentially reducing the quinone form of vitamin K),³³ the missense variants that we included in the meta-analyses (respectively, rs2290228 and rs1800566) were not significantly associated with stable dose requirements. However, the strength of evidence for these associations was considered weak given the small sample sizes (only two studies included for each meta-analysis).

We did not conduct meta-analyses for bleeding events. Although the individual studies did not show an effect of genetic factors on the risk of bleeding, this may merely reflect a lack of power. Indeed, Limdi *et al.*²⁰ found that rs1057910 (*CYP2C9*3*) increased the risk of bleeding (hazard ratio 1.85) when African American data were combined with European American data.

Several limitations of this study should be acknowledged. First, despite our comprehensive search strategy, we could not undertake some meta-analyses because of inadequate reporting. This was confounded by a low response rate (30.3%; 10 of 33 authors) when we requested additional information. However, the information obtained from the 10 individual authors was extensive, representing 39 studies and 10 abstracts. Consequently, only 33 (35.1%) of 94 studies could not be included in the quantitative syntheses. Although no studies stood out in terms of being of particularly low quality overall, there were many methodological issues of concern, including heterogeneity in study populations and outcomes. Most studies included African Americans and Brazilians who are of West African ancestry, and so generalization to other sub-Saharan African populations should be done with caution. Another issue that needs to be taken into account is the degree of admixture with European and Amerindian populations³⁴ in the black populations studied so far, which was not evaluated in most of the individual studies. Despite these concerns, the subgroup analyses showed that most pooled estimates were not significantly affected by the subpopulations involved. Finally, we excluded studies that did not report "black" participants and we could have, therefore, missed important data. For instance, we excluded nine Egyptian studies during full-text screening. Our decision to exclude Egyptians could be justified by a genomewide association study that revealed

that North African populations share more genomic ancestry with some Asian populations compared with those from sub-Saharan Africa.³⁵

In conclusion, our systematic review provides a quantitative estimate of the effect of different genetic variants on warfarin weekly dose requirements in black-African patients. By showing that some variants that are more prevalent in black-Africans may be important determinants of warfarin weekly dose requirements, this review has further demonstrated the importance of population-specific dosing algorithms. Moreover, the total number of black patients studied ($n = 2,336$) is much lower compared to white patients ($n > 5400$ as of December 19, 2007³⁶), and many of the studies were conducted in the United States and Brazil, where there is a significant degree of admixture. This further emphasizes the fact that the number of studies conducted in Africa is small, which is worrisome given that warfarin is the most commonly used anticoagulant on this continent. In response to the poor quality of anticoagulation in sub-Saharan Africa, we have recently embarked on a collaborative project in Uganda and South Africa (War-PATH: WARfarin anticoagulation in PATients in Sub-SaHaran Africa; <http://warpath.info/>) with the main aim of identifying clinical and genetic factors determining warfarin dose variability and ultimately develop better clinical and genetic dosing algorithms to improve anticoagulation quality.

SUPPORTING INFORMATION

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

Supplemental Methods, Figures and Tables. docx. Spreadsheet S1. Xlsx.

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CONFLICT OF INTEREST

S.A.L. receives sponsored research support from Bristol Myers Squibb/Pfizer, Bayer AG, and Boehringer Ingelheim and has consulted for Bristol Myers Squibb/Pfizer and Bayer AG. All other authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

I.G.A., A.L.J., and M.P. wrote the manuscript. I.G.A., E.J.Z., A.L.J., and M.P. designed the research. I.G.A. and R.O. performed the research. I.G.A. and A.L.J. analyzed the data. A.K., C.D., G.S., H.Z., J.A.P., J.Y.R., J.D., L.H.C., L.R.M., M.T.B., M.A.P., N.A.L., P.C.J.L.S., S.E.K., S.A.L., S.A.S., and V.K.K. contributed new reagents/analytical tools.

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