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Influence of *CYP2C9* genotype on warfarin dose requirements—a systematic review and meta-analysis

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Abstract

Purpose To quantify the influence of common cytochrome P450 2C9 (*CYP2C9*) polymorphisms on warfarin dose requirements.

Methods A systematic review and a meta-analysis, calculating the warfarin dose reduction associated with the five most common variant *CYP2C9* genotypes.

Results Thirty-nine studies (7,907 patients) were included in the meta-analysis. Compared to the *CYP2C9**1/*1 genotype, the *CYP2C9**1/*2, *CYP2C9**1/*3, *CYP2C9**2/*2, *CYP2C9**2/*3, and *CYP2C9**3/*3 required warfarin doses that were 19.6 (95% confidence interval 17.4, 21.9), 33.7 (29.4, 38.1), 36.0 (29.9, 42.0), 56.7 (49.1, 64.3), and 78.1% (72.0, 84.3) lower, respectively. The impact of *CYP2C9* genotype tended to be larger in patients without interacting drugs.

Conclusions Previous studies have rarely been powered to determine the quantitative influence of specific *CYP2C9* genotypes on warfarin dose requirements. The results from our pooled analysis are likely to be the most accurate to date and the methodology could serve as a model for future pharmacogenetic meta-analyses.

Keywords Warfarin · *CYP2C9* · Pharmacogenetics · Genetic polymorphism · Meta-analysis · Drug interactions

Introduction

Warfarin is an oral anticoagulant widely used for thrombosis prevention in patients with predisposing conditions, e.g. atrial fibrillation, heart valve implants, or a confirmed episode of thrombosis or thromboembolism. Its use is limited by a narrow therapeutic interval combined with a large interindividual variability in the dose required for adequate anticoagulant effect. S-warfarin, the more potent enantiomer of racemic warfarin, is almost exclusively metabolized by the hepatic cytochrome P450 2C9 enzyme [1]. In 1999, Aithal et al. [2] published the first evidence that polymorphisms in the gene coding for this enzyme, *CYP2C9*, had a significant impact on the warfarin dose requirement in affected individuals. Since then, a large number of studies in various patient populations have confirmed that carriers of one or two low-active *CYP2C9* alleles require lower warfarin doses, with *CYP2C9**2 and *CYP2C9**3 being the most well-documented of these variant alleles [1].

However, translation of *CYP2C9* genotype information into specific warfarin dose recommendations requires precise quantitative information about the impact of individual *CYP2C9* genotype combinations on the expected dose requirement, as well as an understanding of how the magnitude of this gene-dose interaction differs among different patient populations. In vitro, *CYP2C9**2 and *CYP2C9**3 metabolize S-warfarin with a capacity of approximately 12 and <5% compared to the wild-type *CYP2C9**1 allele, respectively [3–5]. Unfortunately, these in vitro estimates are not easily translated into clinical recommendations, since the pharmacologic properties of warfarin are complicated with both enantiomers contributing to the anticoagulant effect, but with different potencies and completely disparate pharmacokinetics. Pharmacoge-

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netic recommendations should therefore preferably be based on reliable in vivo estimates that could also prove to be very useful in the development and evaluation of pharmacokinetic/pharmacodynamic (PK/PD) computer simulation models. Although a large number of studies have investigated the association between *CYP2C9* genotype and warfarin dose requirements in vivo, these studies have seldom been powered to give useful quantitative estimates of the genotype effect, and the estimated dose adjustments required in individuals with specific *CYP2C9* genotypes differ widely among studies.

The aim of our study was to quantify the effects of individual *CYP2C9* genotype on warfarin dose requirements, by means of a systematic literature review and meta-analysis.

Methods

Search strategy

The literature search was conducted in PubMed and EMBASE (finished December 19, 2007). Searches were not restricted in time or language. Text words and keywords included in the search strategy are presented in the “Appendix.” In cases of missing data or unclarities, authors were contacted for additional information or unpublished data.

Study selection criteria

One reviewer (JL) initially evaluated articles for eligibility. The selected articles were re-evaluated by LH and MA, and the final inclusion decision was based on consensus among the three reviewers. Included studies had to meet the following inclusion criteria: (1) clinical cohort or cross-sectional study in warfarin-treated patients, (2) *CYP2C9* genotyping performed in all patients or in a random selection of patients (selection based, e.g., on warfarin dose, bleeding tendency etc. was not allowed), (3) warfarin maintenance dose (mean and standard deviation) presented separately for the *CYP2C9* *1/*1 genotype group and at least one of the following groups: *CYP2C9* *1/*2, *1/*3, *2/*2, *2/*3, *3/*3.

There were no restrictions of inclusion on the basis of patient characteristics, publication type (journal article, abstract, conference proceedings or unpublished data), or publication language.

Data extraction

Data from all eligible studies were extracted and summarized independently by two reviewers (LH and MA).

Recorded data included characteristics of the studies, demographic data, and warfarin maintenance doses for each of the six *CYP2C9* genotypes mentioned above. Any discrepancies were resolved by consensus. When applicable, the authors of the studies were contacted for additional information.

Statistical methods

In each of the included studies, the maintenance doses in the six *CYP2C9* genotype groups were normalized using the *CYP2C9* *1/*1 group as reference (by dividing the dose and associated standard deviations in each group by the mean maintenance dose in the *CYP2C9* *1/*1 group). This normalization procedure was performed to remove any heterogeneity caused by differences in pharmacodynamic warfarin sensitivity among study populations, since the absolute reduction in warfarin dose requirement associated with variant *CYP2C9* genotypes was clearly correlated to the mean dose in the reference groups in the populations studied (data not shown).

The normalized dose in each of the five genotype groups *CYP2C9* *1/*2, *1/*3, *2/*2, *2/*3, and *3/*3 were compared to that in the *CYP2C9* *1/*1 group (reference group) by means of five separate meta-analyses. Each meta-analysis included those selected studies that presented warfarin dose data for at least two individuals with the *CYP2C9* *1/*1 group and two individuals with the comparator genotype. The studies were weighted using the inverse variance method, and the effect of *CYP2C9* genotype on warfarin maintenance dose was expressed as mean difference (MD). However, due to the normalization procedure, the calculated mean differences represent relative rather than absolute differences in maintenance dose, e.g., a mean difference of -0.5 would indicate a 50% reduction in warfarin dose requirement. Some studies analyzed variant *CYP2C9* alleles other than *2 and *3, while most did not. To achieve a uniform definition of the reference *CYP2C9**1 allele across studies, any *CYP2C9* allele other than *2 or *3 was considered wild-type (*1) in the analyses, if possible.

Homogeneity among studies was tested by means of Cochran's Q test and calculation of the variation across studies attributable to heterogeneity rather than chance (I^2). When substantial heterogeneity was demonstrated (defined as a Cochran Q test P value <0.1 or a I^2 value >25%) a random-effects model was used to calculate the overall MD, otherwise a fixed-effect model was used.

Sensitivity analyses by means of meta-regressions were performed to assess the impact of various study characteristics on the association between *CYP2C9* genotype and warfarin dose requirement. For each of the five meta-analyses, the influence of potential sources of heterogeneity

on the genotype-dose association was investigated by means of study-level weighted univariate linear regressions. The dependent variable in these analyses was the normalized dose reduction associated with one specific variant *CYP2C9* genotype, and each study was weighted by the inverse sum of the within-trial variance and the residual between-trial variance. The following independent variables were examined: age, African ethnicity, Asian ethnicity, use of interacting drugs, and target INR. African and Asian ethnicities were coded as binary variables, set to 0 or 1 if $\leq 10\%$ or $\geq 90\%$ of the included patients belonged to that specific ethnic group, respectively. Studies in ethnically heterogeneous patient groups were excluded from this analysis. Use of interacting drugs was set to 1 in studies where use of drugs potentially interacting with warfarin was allowed and to 0 in those where patients using such drugs had been excluded. Target INR was coded as 0, 1, and 2 in studies with low (2.0), mid-range (2.5–2.6), and high target INRs (2.7–3.0), respectively.

Subgroup analyses were performed to eliminate sources of heterogeneity identified in the meta-regressions. After dividing the studies according to these study characteristics, the main analysis was repeated separately in each subgroup. Only subgroups large enough to provide useful estimates were included in these analyses.

The influence of individual studies on the outcome of meta-analyses was examined by means of exclusion sensitivity forest plots and the possible influence of publication bias was graphically evaluated by means of funnel plots where normalized dose reductions were plotted versus inverse standard error.

The weighted linear regressions were performed using StatsDirect statistical software version 2.7.2 (StatsDirect, Sale, Cheshire, UK). All other statistical analyses were performed using MIX version 1.61 for Windows, October 2007 [6].

Results

Identification of studies

Figure 1 shows the flow of studies, from identification to final inclusion. A total of 1,171 citations were identified in the literature search. Of these, 39 met the inclusion criteria and were included in the meta-analysis [2, 7–44]. Thirty-six studies [2, 7–30, 32–34, 36, 38–44] were published in English, one in French [35], one in Russian [37], and one in Spanish [31]. Three studies [13, 20, 43] presented data separately for different ethnic groups. Data from these studies were subdivided according to ethnicity and treated as separate studies in the analysis.

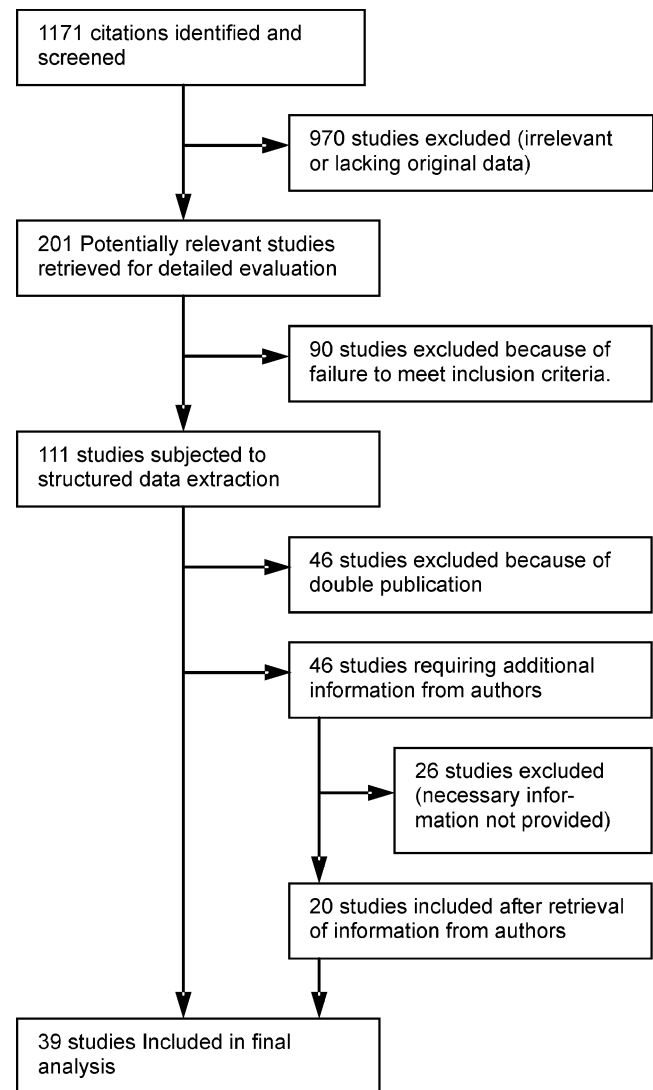


Fig. 1 Flow diagram showing the number of citations identified, retrieved, extracted, and included in the final analysis

Study characteristics

Table 1 presents the characteristics of the 39 studies included in the meta-analysis. All studies were published between 1999 and 2007. Twelve studies were performed in Asia, thirteen in the Americas, and fourteen in Europe. A total of 8,048 patients were included, and data from 7,907 patients (5,749 *CYP2C9**1/*1 carriers, 1,216 *1/*2, 759 *1/*3, 78 *2/*2, 81 *2/*3, and 24 *3/*3) could be evaluated in the meta-analysis. The mean age (weighted according to study size) of the patients was 64 years (range 57–66.9) and 59% were men (range 44–67%).

Meta-analysis

The outcome of the individual meta-analyses in each *CYP2C9* genotype group is presented in Figs. 2, 4, 5, and

Table 1 Characteristics of included studies

Reference	Country	Number	Men	Age (years) ^a	Weight (kg) ^a	Predominant ethnicity	Interacting drugs allowed	Target INR (mean)	Dose (mg/week)	CYP2C9 allele frequencies ^b			Included patients per CYP2C9 genotype group ^c					
										*1	*2	*3	*1/*1	*1/*2	*2/*2	*2/*3	*3/*3	
Aithal 1999 [2]	UK	37	41%	68.8	-	Caucasian	No	2.5	31.5	82%	9%	8%	24	7	6	0	0	0
Anderson 2007 [7]	USA	175	53%	61.1	94.9	Caucasian 94%	Yes ^d	2.5	36.5	85%	11%	5%	125	34	13	0	3	0
Aquilante 2006 [8]	USA	350	87%	69	92.7	Caucasian 91%	Yes	2.6	37.1	83%	12%	5%	248	58	29	10	2	0
Borgiani 2007 [9]	Italy	146	53%	68.2	78	Caucasian >90%	-	2.7	25.2	74%	17%	9%	76	43	20	0	5	0
Carlquist 2006 [10]	USA	225	49%	71	84	Caucasian >90%	Yes	2.5	30.8	86%	9%	5%	165	38	20	0	2	0
Chern 2006 [11]	Taiwan	37	54%	-	-	Asian 100%	-	-	19.6	97%	0%	3%	35	0	2	0	0	0
Cho 2007 [12]	Korea	108	64%	67.4	66	Asian 100%	Yes	2.5	28.7	94%	0%	6%	96	0	12	0	0	0
Gan 2004 (Indians) [13]	Malaysia	27	52%	54	-	Indian 100%	-	2.8	44.2	85%	4%	11%	20	0	5	0	0	0
Gan 2004 (Malay) [13]	Malaysia	27	67%	49	-	Asian 100%	-	2.8	22.8	89%	0%	11%	22	0	4	0	0	0
Gredic 2003 [14]	Croatia	59	58%	57.3	-	Caucasian >90%	-	-	35	83%	10%	7%	40	11	7	0	0	0
Higashi 2002 [15]	USA	185	64%	59.9	-	Caucasian 96%	Yes	2.5	35.7	81%	11%	8%	127	28	18	4	3	5
Hill 2006 [16]	USA	84	51%	58.2	-	Caucasian	Yes	-	38.3	83%	11%	7%	57	16	9	0	0	0
Hillman 2004 [17]	USA	453	57%	70.6	-	Caucasian 95%	Yes	-	32.9	81%	12%	7%	295	86	55	7	8	2
Khan 2004 [18]	UK	53	64%	67	-	Caucasian	No	-	28	81%	11%	8%	34	12	6	0	0	0
Kimura 2006 [19]	Japan	92	71%	68.1	59.8	Asian 100%	-	2.1	20.7	95%	0%	5%	83	0	9	0	0	0
Lindi 2007 (Afric.) [20]	USA	226	47%	57.5	89.1	African 100%	Yes	2.5	42.3	97%	1%	2%	204	5	7	0	0	0
Lindi 2007 (Cauc.) [20]	USA	218	55%	63.5	87.8	Caucasian 100%	Yes	2.5	34.6	84%	11%	5%	153	40	19	3	3	0
Linder 2002 [21]	USA	56	55%	68.9	84.40	Caucasian 96%	Yes	2.5	35.9	78%	16%	7%	34	13	4	0	0	0
Loebstein 2001 [22]	Israel	174	56%	63	-	-	Yes	-	41.3	84%	10%	6%	108	28	0	0	0	0
Margaglione 2000 [23]	Italy	180	56%	42.3	-	Caucasian >90%	Yes	-	39.7	73%	18%	9%	88	62	0	0	2	0
Miao 2007 [24]	China	178	42%	54.6	58.4	Asian 100%	No	2.0	14.1	96%	0%	4%	162	0	16	0	0	0
Millican 2007 [25]	USA	92	52%	58.2	-	Caucasian 86%	Yes	-	37.8	78%	14%	8%	58	17	11	2	4	0
Momary 2007 [26]	USA	115	28%	57	-	Asian 100%	No	-	45	96%	3%	1%	92	6	3	0	0	0
Moridani 2006 [27]	Canada	189	56%	69.0	-	Caucasian 94%	-	2.6	34.7	81%	11%	8%	126	32	20	3	4	3
Mushiroda 2006 [28]	Japan	828	67%	68	-	Asian 100%	-	-	17.5	98%	0%	2%	790	0	37	0	0	0
Muszkat 2007 [29]	Israel	119	48%	65.8	74.9	-	Yes	2.5	29.9	72%	17%	11%	64	25	18	0	0	0
Pehelina 2005 [30]	Russia	62	56%	47.6	-	Caucasian 100%	No	2.8	39.7	86%	9%	5%	46	10	5	0	0	0
Raggio 2006 [31]	Uruguay	53	43%	59.4	-	Hispanic 100%	Yes	2.8	40.5	75%	13%	11%	27	14	12	0	0	0
Sconce 2005 [32]	UK	297	54%	67	-	Caucasian >90%	No	2.5	25.8	74%	17%	10%	165	66	42	10	0	0
Scordo 2002 [33]	Italy	93	61%	68	-	Caucasian >90%	No	2.5	32	75%	12%	13%	54	15	16	2	4	2
Shikata 2004 [34]	Japan	45	60%	63.1	56.9	Asian 100%	No	3.0	26.6	98%	0%	2%	43	0	2	0	0	0
Sigaret 2003 [35]	France	126	23%	87	-	Caucasian >90%	Yes	2.5	21	79%	12%	9%	80	20	18	2	6	0
Spreafico 2003 [36]	Italy	125	67%	49.7	76	Caucasian >90%	Yes****	2.5	30.4	78%	13%	9%	75	26	16	3	4	0
Sychev 2007 [37]	Russian	82	57%	59.6	88.2	Caucasian >90%	No	2.5	36.1	80%	10%	10%	55	12	10	0	2	2
Takahashi 2006 [38]	Japan	64	59%	61	56.5	Asian 100%	Yes	2.0	24.5	98%	0%	2%	62	0	2	0	0	0

Tanira 2007 [39]	Oman	142	57%	42.8	-	-	-	3.0	40.7	89%	8%	4%	112	18	10	2	0	0
Taube 2000 [40]	UK	561	-	-	Caucasian	Yes	-	-	33.3	84%	11%	5%	392	107	53	3	6	0
Topic 2004 [41]	Croatia	181	44%	60	-	No	2.5	2.5	28.5	76%	17%	7%	104	49	18	5	4	0
Wadelius 2004 [42]	Sweden	201	67%	66.9	80.2	Yes	-	-	33.2	82%	11%	7%	134	42	21	0	0	2
Wadelius 2008 [44]	Sweden	1487	63%	66	-	Yes	2.5	2.5	35.8	82%	11%	7%	986	276	176	22	19	8
Zhao 2004 (Asians) [43]	Singapore	59	59%	58	60	No	-	-	25.9	97%	0%	3%	55	0	4	0	0	0
Zhao 2004 (Malay.) [43]	Singapore	37	46%	57	66.6	No	-	-	23.8	95%	0%	5%	33	0	4	0	0	0

^a Mean or median
^b Values refer to allele frequencies, where *CYP2C9**1 also includes any alleles other than *CYP2C9**1, *2 or *3. In some studies, the sum of the three allele frequencies differs somewhat from 100%, due to rounding of the values
^c The number of patients included in the meta-analysis may differ from the number presented in the source publication if presented data precluded analysis or additional data was supplied by the authors
^d Some interacting drugs were allowed, while others were not

6, and in Table 2. The forest plots in Figs. 2, 3, 4, 5, and 6 show the relative dose reduction associated with specific *CYP2C9* genotypes in each included study along with the overall result of the meta-analysis. The brackets denote 95% confidence intervals and the size of each box is proportional to the number of included patients. When compared to individuals with the homozygous *CYP2C9**1 genotype, carriers of the *CYP2C9**1/*2, *CYP2C9**1/*3, *CYP2C9**2/*2, *CYP2C9**2/*3, and *CYP2C9**3/*3 genotypes required warfarin doses that were 19.6 (95% confidence interval 17.4, 21.9), 33.7 (29.4, 38.1), 36.0 (29.9, 42.0), 56.7 (49.1, 64.3), and 78.1% (72.0, 84.3) lower, respectively.

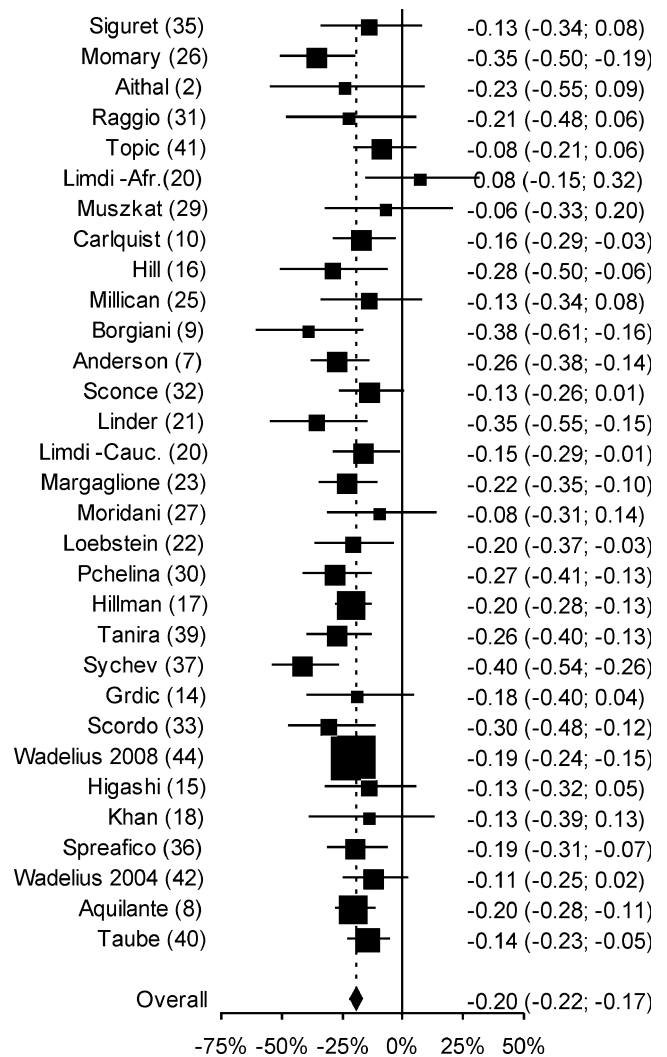


Fig. 2 Forest plot of the reduction in warfarin dose requirement in carriers of the *CYP2C9* *1/*2 genotype, as compared to individuals with the wild type *CYP2C9* *1/*1 genotype. Brackets denote 95% confidence intervals

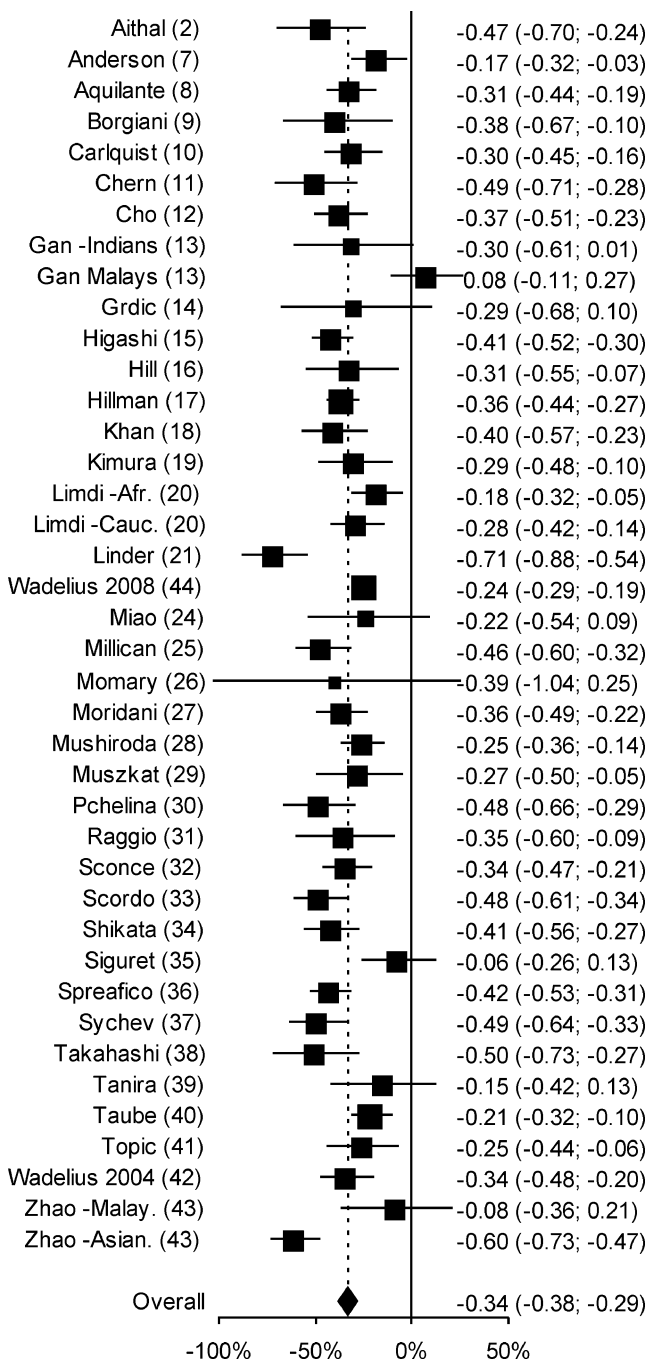


Fig. 3 Forest plot of the reduction in warfarin dose requirement in carriers of the *CYP2C9* *1/*3 genotype, as compared to individuals with the wild type *CYP2C9* *1/*1 genotype. Brackets denote 95% confidence intervals

Heterogeneity

All meta-analyses except those comparing *CYP2C9**1/*2 and *CYP2C9**2/*2 with *CYP2C9**1/*1 showed evidence of statistical heterogeneity across studies (Cochran's Q test P values <0.0001 to 0.035, I^2 values 47 to 81%), and hence a random-effects model was used in these analyses.

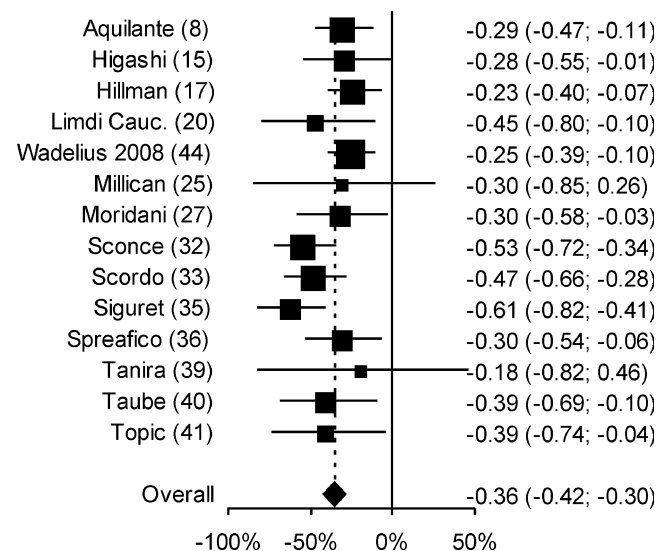


Fig. 4 Forest plot of the reduction in warfarin dose requirement in carriers of the *CYP2C9* *2/*2 genotype, as compared to individuals with the wild type *CYP2C9* *1/*1 genotype. Brackets denote 95% confidence intervals

None of the meta-regressions demonstrated a significant ($P < 0.05$) association between the examined predictors and study outcome. Studies including predominantly patients of African descent indicated a smaller impact of the *CYP2C9**1/*3 genotype, as compared to studies including predominantly Caucasian or Asian patients (-20.5 vs -35.2%). However, this difference had a low level of significance ($P = 0.29$) and no similar effect was seen for

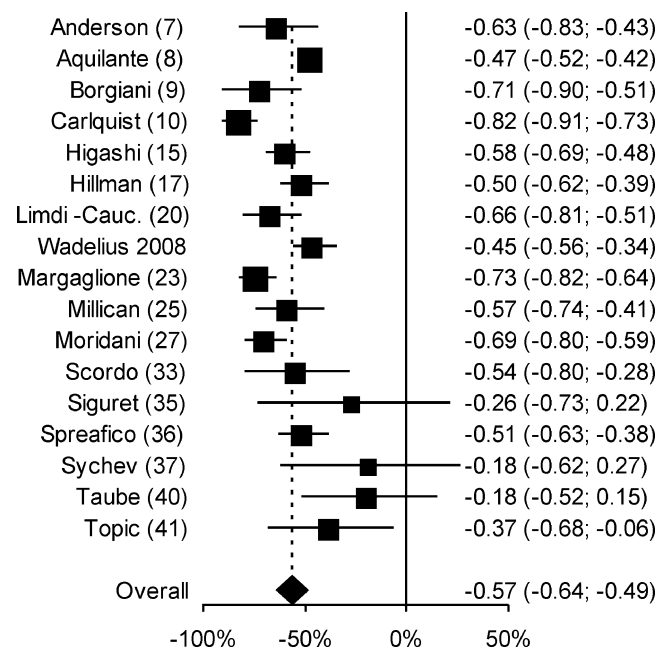


Fig. 5 Forest plot of the reduction in warfarin dose requirement in carriers of the *CYP2C9* *2/*3 genotype, as compared to individuals with the wild type *CYP2C9* *1/*1 genotype. Brackets denote 95% confidence intervals

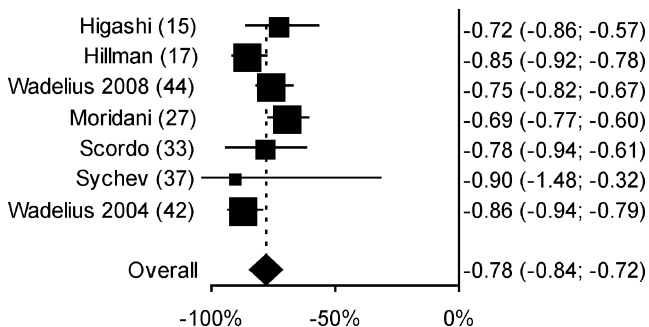


Fig. 6 Forest plot of the reduction in warfarin dose requirement in carriers of the *CYP2C9* *3/*3 genotype, as compared to individuals with the wild type *CYP2C9* *1/*1 genotype. Brackets denote 95% confidence intervals

the *CYP2C9**1/*2 genotype (-21.0 vs -20.1%, $P=0.92$). The effect of African ethnicity could not be evaluated in the double-mutated genotype groups (*CYP2C9**2/*2, *2/*3, and *3/*3) due to lack of data. Asian ethnicity had no impact on the *CYP2C9**1/*3 effect (-34.9 vs -34.3%, $P=0.93$) and could not be evaluated in the other genotype groups.

Three *CYP2C9* genotypes (*CYP2C9**1/*2, *1/*3, and *2/*2) were associated with substantially smaller dose reductions in studies allowing the use of potentially interacting drugs, compared to studies where such drugs were not allowed (-17.6 vs -23.7%, -32.9 vs -40.6%, and -33.4 vs -48.4%, respectively). The P values of these analyses ranged from 0.11 to 0.15. No similar effect of interacting drugs was observed in the *CYP2C9**2/*3 or *3/*3 groups, but these results had a high level of uncertainty ($p=0.25$ and $p=0.87$, respectively).

In the *CYP2C9**2/*2 group, the genotype effect tended to be larger in studies with older patients (slope -0.75 percent units per year of age, $P=0.07$) but results from the remaining genotype groups were conflicting and highly nonsignificant (P values 0.27–0.95). Similarly, higher target INRs were associated with a larger *CYP2C9**1/*2 effect (-9.7, -18.8, and -27.9% in the low-, medium-, and high-INR group, respectively, $P=0.13$), but results in the other genotype groups were diverging and highly nonsignificant (P values 0.43–0.57).

Table 2 Effect of variant *CYP2C9* genotypes on warfarin dose requirements

<i>CYP2C9</i> genotype	Reduction in warfarin dose requirement ^a	P value
*1/*1	Reference	Reference
*1/*2	19.6% (17.4, 21.9)	$<10^{-15}$ ($z=17.2$)
*1/*3	33.7% (29.4, 38.1)	$<10^{-15}$ ($z=15.1$)
*2/*2	36.0% (29.9, 42.0)	$<10^{-15}$ ($z=11.6$)
*2/*3	56.7% (49.1, 64.3)	$<10^{-15}$ ($z=14.7$)
*3/*3	78.1% (72.0, 84.3)	$<10^{-15}$ ($z=24.8$)

^a Mean (95% confidence interval)

The results of the subgroup analyses are presented in Table 3. Restricting the analysis to studies in Caucasian patients or to studies in Caucasian patients where use of potentially interacting drugs was explicitly allowed had very little effect on the overall results. Studies in Caucasians not using any interacting drugs demonstrated approximately 20% larger effects of the *CYP2C9**1/*2 and *1/*3 genotypes, as compared to the main analysis. In the less powered *CYP2C9**2/*2 and *2/*3 groups, these studies showed a 35% increased and 26% decreased gene effect, but with wide confidence intervals.

Publication bias

Funnel plots of each study’s genotype-associated dose reductions against the inverse standard error did not indicate any asymmetries suggestive of publication bias (data not shown).

Discussion

In this systematic review and meta-analysis, we demonstrate that carriers of the *CYP2C9* *1/*2, *1/*3, *2/*2, *2/*3, and *3/*3 genotypes require warfarin doses that are 19.6, 33.7, 36.0, 56.7, and 78.1% lower than in carriers of the wild-type *CYP2C9* *1/*1 genotype, respectively. These results are based on data from almost 8,000 genotyped individuals, and the estimates are likely to be the most accurate published to date.

The main limitation of the study is its dependence on aggregated data, making it less easy to evaluate the influence of individual patient characteristics on warfarin dose requirements. The sensitivity analyses did not unambiguously link any of the examined study characteristics to the study outcome. However, use of interacting drugs was associated with attenuated gene-dose effects in a majority of the genotypes, with P values ≤ 0.15 . This is by no means evidence of interacting drugs being an important predictor of the *CYP2C9* effect in warfarin-treated patients, but since the sensitivity analyses have comparatively low statistical power and are hypothesis-generating rather than hypothesis-testing, the finding was further explored in the subgroup analysis. The results from this analysis indicate that the dose reductions observed in the main analysis should be valid in patient populations where interacting drugs may be used, while the effect of *CYP2C9* genotype may be even larger in patients where the gene-dose association is not partially obscured by interacting drugs.

In addition to *CYP2C9*, the polymorphic vitamin K epoxide reductase subcomplex 1 (*VKORC1*) gene is an important determinant of warfarin dose requirement, as it alters the pharmacodynamic sensitivity (i.e., the concentra-

Table 3 Effect of variant *CYP2C9* genotypes on warfarin dose requirements in selected subgroups

<i>CYP2C9</i> genotype	Caucasians ^a	Caucasians ^a , no interacting drugs	Caucasians ^a , interacting drugs ^b
*1/*1	Reference	Reference	Reference
*1/*2	19.9% (17.4, 22.4)	23.1% (11.0, 35.1)	19.0% (16.1, 21.9)
*1/*3	35.1% (29.6, 40.7)	40.9% (32.2, 49.5)	33.4% (25.1, 41.7)
*2/*2	36.8% (28.5, 45.1)	48.7% (36.3, 61.2)	33.7% (21.6, 45.8)
*2/*3	58.0% (50.0, 66.0)	42.0% (23.9, 60.0)	58.8% (47.5, 70.1)
*3/*3	78.1% (72.0, 84.3)	NA ^c	80.6% (73.8, 87.3)

Reductions in warfarin dose requirements are presented as mean (95% confidence interval)

^a Analyses includes only studies where $\geq 90\%$ of the patients were Caucasian

^b Studies where use of drugs potentially interacting with warfarin was not an exclusion criterion

^c Meta-analysis could not be performed due to insufficient data

tion-effect relationship) to warfarin [45–47]. Being located on chromosome 16, *VKORC1* is not linked to *CYP2C9* (chromosome 10) and therefore it contributes to the warfarin dose independently of the *CYP2C9* genotype [45, 47, 48]. Warfarin-sensitive *VKORC1* alleles (haplotype A) are less common in Africans than in Caucasians or Asians, contributing to higher warfarin dose requirements in the former ethnic group [20]. Theoretically, the normalization procedure used in our meta-analysis should remove any influences of *VKORC1* genotype on the *CYP2C9*-dose association, since a reduced pharmacodynamic sensitivity to warfarin would be expected to increase the dose requirements by an equal percentage in all *CYP2C9* genotype groups. However, this remains to be demonstrated in vivo and the lack of *VKORC1* data is a potential weakness of our meta-analysis. The number of publications presenting warfarin dose data in patient cohorts characterized with regard to *VKORC1* genotype is still too limited to allow for a large meta-analysis of potential interactions between *VKORC1* and *CYP2C9* as predictors of warfarin dose requirements, but this should be addressed when sufficient data have accumulated.

The sensitivity analysis revealed a large albeit nonsignificant interethnic difference in *CYP2C9**1/*3-effect between Africans and other ethnic groups. Although no such effect was seen in the *CYP2C9**1/*2 analysis, the number of studies in patients of African descent was small ($n=2$), rendering the analysis a low statistical power. In addition to the interethnic differences in *VKORC1* allele frequencies discussed above, *CYP2C9* alleles other than *1, *2, and *3 could theoretically attenuate the *CYP2C9* effect in Africans, by being allocated to the *CYP2C9**1/*1 group and altering the dose requirement in this group. Several such allelic variants confined to African populations have been described [20] and others may remain unidentified. Due to these uncertainties regarding interethnic differences in *CYP2C9* dose effect, the subgroup analyses were restricted to studies in Caucasian patients. However, this restriction had little, if any, effect on the outcome of the meta-analysis.

In this meta-analysis, only the two most well-investigated variant *CYP2C9* alleles, *2 and *3, were taken into account, and any other alleles identified in the studies were treated as being the wild-type *1. This could theoretically dilute the apparent effect of the *2 and *3 alleles by introducing “hidden” slow metabolizers into the reference group. The choice to restrict the analysis to three alleles had two reasons: In most of the available studies (79%) only the *CYP2C9* *1, *2, and *3 alleles had been analyzed and to pool data in the meta-analysis, it was necessary to apply a uniform definition of the *1 allele (i.e., any *CYP2C9* allele other than *2 and *3). In addition, the protocol chosen should give a valid estimate of the predictive properties of a *CYP2C9* analysis that includes specifically the *CYP2C9* *2 and *3 alleles—the analysis proposed in most published genotype-based dosing algorithms.

Taken together, we believe our pooled estimates of the impact of *CYP2C9* genotype on the warfarin dose requirements are reliable, with the potential to substantially improve the interpretation of clinical genotyping results and the precision of PK/PD models based on published gene-dose-effect associations and estimates of warfarin’s pharmacokinetic and pharmacodynamic properties. An even more important application of the data would be to validate the predictive properties of regression-based dose prediction algorithms. These algorithms are usually based on patient samples too small to address the dose requirements in specific genotype groups and may, for example, treat *CYP2C9**2 and *3 equally or pool heterozygous and homozygous carriers of variant alleles. Since homozygous and compound variant genotypes are rare, this practice has little effect on the overall predictive value of the algorithms, but it is likely to cause substantial overestimations of the dose in the vulnerable individuals carrying these genotypes. By comparing the predicted dose reductions associated with specific *CYP2C9* genotypes to those observed in our meta-analysis and critically evaluating any substantial discrepancies, dangerous prediction errors could be avoided. In addition, combining our genotype-dose association esti-

mates with high-quality enantiospecific dose-concentration data could give valuable new information on the relative in vivo potencies of the two warfarin enantiomers.

The association of *CYP2C9* genotype and adverse outcomes of warfarin treatment has previously been investigated in a meta-analysis [49], but to our knowledge the current study is the first meta-analysis based on a systematic review that addresses the association between a drug-metabolizing cytochrome P450 enzyme and the dose requirements of its substrates. We believe further use of this methodology could prove to be very fruitful in the field of pharmacogenetics, which is often characterized by a body of small studies insufficiently powered to provide reliable quantitative estimates of the drug-gene interaction size.

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Appendix

PubMed

Warfarin as MeSH or text word was combined with each of the following search phrases:

- **MeSH**
- Cytochromes
- Cytochrome p-450 enzyme system
- Genotype
- Genes
- Genetics
- Alleles
- Polymorphism, genetic
- Pharmacogenetics
- **Text words**
- Cytochrome
- Cytochromes
- Cytochrome-p-450
- Cytochrome p-450
- p-450
- Cytochrome P450
- Cytochrome P 450
- cyp2c9
- 2c9
- cyp2c
- Genotype
- Genotypes
- Gene
- Genes
- Genetic
- Genetics
- Allele
- Alleles
- Polymorphism
- Polymorph
- Polymorphisms
- Polymorphic
- Pharmacogenetics
- **EMBASE**
- Warfarin/exp AND cytochrome p450 2c9/exp

References

1. Wadelius M, Pirmohamed M (2007) Pharmacogenetics of warfarin: current status and future challenges. *Pharmacogenomics* 7:99–111
2. Aithal GP, Day CP, Kesteven PJ, Daly AK (1999) Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *Lancet* 353:717–719
3. Haining RL, Hunter AP, Veronese ME, Trager WF, Rettie AE (1996) Allelic variants of human cytochrome P450 2C9: baculovirus-mediated expression, purification, structural characterization, substrate stereoselectivity, and prochiral selectivity of the wild-type and I359L mutant forms. *Arch Biochem Biophys* 333:447–458
4. Rettie AE, Wienkers LC, Gonzalez FJ, Trager WF, Korzekwa KR (1994) Impaired (S)-warfarin metabolism catalysed by the R144C allelic variant of CYP2C9. *Pharmacogenetics* 4:39–42
5. Sullivan-Klose TH, Ghanayem BI, Bell DA, Zhang ZY, Kaminsky LS, Shenfield GM, Miners JO, Birkett DJ, Goldstein JA (1996) The role of the CYP2C9-Leu359 allelic variant in the tolbutamide polymorphism. *Pharmacogenetics* 6:341–349
6. Bax L, Yu LM, Ikeda N, Tsuruta H, Moons KG (2006) Development and validation of MIX: comprehensive free software for meta-analysis of causal research data. *BMC Med Res Methodol* 6:50
7. Anderson JL, Horne BD, Stevens SM, Grove AS, Barton S, Nicholas ZP, Kahn SF, May HT, Samuelson KM, Muhlestein JB, Carlquist JF (2007) Randomized trial of genotype-guided versus standard warfarin dosing in patients initiating oral anticoagulation. *Circulation* 116:2563–2570
8. Aquilante CL, Langae TY, Lopez LM, Yarandi HN, Tromberg JS, Mohuczy D, Gaston KL, Waddell CD, Chirico MJ, Johnson JA (2006) Influence of coagulation factor, vitamin K epoxide reductase complex subunit 1, and cytochrome P450 2C9 gene polymorphisms on warfarin dose requirements. *Clin Pharmacol Ther* 79:291–302
9. Borgiani P, Ciccacci C, Forte V, Romano S, Federici G, Novelli G (2007) Allelic variants in the CYP2C9 and VKORC1 loci and interindividual variability in the anticoagulant dose effect of warfarin in Italians. *Pharmacogenomics* 8:1545–1550
10. Carlquist JF, Horne BD, Muhlestein JB, Lappe DL, Whiting BM, Kolek MJ, Clarke JL, James BC, Anderson JL (2006) Genotypes of the cytochrome p450 isoform, CYP2C9, and the vitamin K epoxide reductase complex subunit 1 conjointly determine stable warfarin dose: a prospective study. *J Thromb Thrombolysis* 22:191–197
11. Chern HD, Ueng TH, Fu YP, Cheng CW (2006) CYP2C9 polymorphism and warfarin sensitivity in Taiwan Chinese. *Clin Chim Acta* 367:108–113
12. Cho HJ, Sohn KH, Park HM, Lee KH, Choi B, Kim S, Kim JS, On YK, Chun MR, Kim HJ, Kim JW, Lee SY (2007) Factors affecting the interindividual variability of warfarin dose requirement in adult Korean patients. *Pharmacogenomics* 8:329–337

13. Gan GG, Phipps ME, Ku CS, Teh A, Sangkar V (2004) Genetic polymorphism of the CYP2C9 subfamily of 3 different races in warfarin maintenance dose. *Int J Hematol* 80:295–296
14. Grdic M, Coen D, Zadro R (2003) Effect of cytochrome P450 CYP2C9 genotype on warfarin sensitivity. In: 4th Croatian Congress of Medical Biochemists, 24–28 September 2003, Zadar
15. Higashi MK, Veenstra DL, Kondo LM, Wittkowsky AK, Srinouanprachanh SL, Farin FM, Rettie AE (2002) Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. *JAMA* 287:1690–1698
16. Hill CE, Duncan A, Wirth D, Nolte FS (2006) Detection and identification of cytochrome P-450 2C9 alleles *1, *2, and *3 by high-resolution melting curve analysis of PCR amplicons. *Am J Clin Pathol* 125:584–591
17. Hillman MA, Wilke RA, Caldwell MD, Berg RL, Glurich I, Burmester JK (2004) Relative impact of covariates in prescribing warfarin according to CYP2C9 genotype. *Pharmacogenetics* 14:539–547
18. Khan T, Wynne H, Wood P, Torrance A, Hankey C, Avery P, Kesteven P, Kamali F (2004) Dietary vitamin K influences intra-individual variability in anticoagulant response to warfarin. *Br J Haematol* 124:348–354
19. Kimura R, Miyashita K, Kokubo Y, Akaiwa Y, Otsubo R, Nagatsuka K, Otsuki T, Okayama A, Minematsu K, Naritomi H, Honda S, Tomoike H, Miyata T (2007) Genotypes of vitamin K epoxide reductase, gamma-glutamyl carboxylase, and cytochrome P450 2C9 as determinants of daily warfarin dose in Japanese patients. *Thromb Res* 120:181–186
20. Limdi NA, McGwin G, Goldstein JA, Beasley TM, Arnett DK, Adler BK, Baird MF, Acton RT (2008) Influence of CYP2C9 and VKORC1 1173C/T genotype on the risk of hemorrhagic complications in African-American and European-American patients on warfarin. *Clin Pharmacol Ther* 83:312–321
21. Linder MW, Looney S, Adams JE III, Johnson N, Antonino-Green D, Lacefield N, Bukaveckas BL, Valdes R Jr (2002) Warfarin dose adjustments based on CYP2C9 genetic polymorphisms. *J Thromb Thrombolysis* 14:227–232
22. Loebstein R, Yonath H, Peleg D, Almog S, Rotenberg M, Lubetsky A, Roitelman J, Harats D, Halkin H, Ezra D (2001) Interindividual variability in sensitivity to warfarin—nature or nurture? *Clin Pharmacol Ther* 70:159–164
23. Margaglione M, Colaizzo D, D'Andrea G, Brancaccio V, Ciampa A, Grandone E, Di MG (2000) Genetic modulation of oral anticoagulation with warfarin. *Thromb Haemost* 84:775–778
24. Miao L, Yang J, Huang C, Shen Z (2007) Contribution of age, body weight, and CYP2C9 and VKORC1 genotype to the anticoagulant response to warfarin: proposal for a new dosing regimen in Chinese patients. *Eur J Clin Pharmacol* 63:1135–1141
25. Millican EA, Lenzi PA, Milligan PE, Grosso L, Eby C, Deych E, Grice G, Clohisey JC, Barrack RL, Burnett RS, Voora D, Gatchel S, Tiemeier A, Gage BF (2007) Genetic-based dosing in orthopedic patients beginning warfarin therapy. *Blood* 110:1511–1515
26. Momary KM, Shapiro NL, Viana MA, Nutescu EA, Helgason CM, Cavallari LH (2007) Factors influencing warfarin dose requirements in African-Americans. *Pharmacogenomics* 8:1535–1544
27. Moridani M, Fu L, Selby R, Yun F, Sukovic T, Wong B, Cole DE (2006) Frequency of CYP2C9 polymorphisms affecting warfarin metabolism in a large anticoagulant clinic cohort. *Clin Biochem* 39:606–612
28. Mushiroda T, Ohnishi Y, Saito S, Takahashi A, Kikuchi Y, Saito S, Shimomura H, Wanibuchi Y, Suzuki T, Kamatani N, Nakamura Y (2006) Association of VKORC1 and CYP2C9 polymorphisms with warfarin dose requirements in Japanese patients. *J Hum Genet* 51:249–253
29. Muszkat M, Blotnik S, Elami A, Krasilnikov I, Caraco Y (2007) Warfarin metabolism and anticoagulant effect: a prospective, observational study of the impact of CYP2C9 genetic polymorphism in the presence of drug-disease and drug-drug interactions. *Clin Ther* 29:427–437
30. Pchelina SN, Sirotkina OV, Taraskina AE, Vavilova TV, Shwarzman AL, Schwartz EI (2005) The frequency of cytochrome P450 2C9 genetic variants in the Russian population and their associations with individual sensitivity to warfarin therapy. *Thromb Res* 115:199–203
31. Raggio V, Esperon P, Lorenzo M, Taub I, Cuesta A, Rodriguez A, Ortiz V, Kuster F, Lluberas R, Stoll M (2006) Variantes de los genes CYP2C9 y apolipoproteína E en la respuesta individual a la warfarina. *Rev Urug Cardiol* 21:104–116
32. Sconce EA, Khan TI, Wynne HA, Avery P, Monkhouse L, King BP, Wood P, Kesteven P, Daly AK, Kamali F (2005) The impact of CYP2C9 and VKORC1 genetic polymorphism and patient characteristics upon warfarin dose requirements: proposal for a new dosing regimen. *Blood* 106:2329–2333
33. Scordo MG, Pengo V, Spina E, Dahl ML, Gusella M, Padriani R (2002) Influence of CYP2C9 and CYP2C19 genetic polymorphisms on warfarin maintenance dose and metabolic clearance. *Clin Pharmacol Ther* 72:702–710
34. Shikata E, Ieiri I, Ishiguro S, Aono H, Inoue K, Koide T, Ohgi S, Otsubo K (2004) Association of pharmacokinetic (CYP2C9) and pharmacodynamic (factors II, VII, IX, and X; proteins S and C; and gamma-glutamyl carboxylase) gene variants with warfarin sensitivity. *Blood* 103:2630–2635
35. Siguret V, Gouin I, Golmard JL, Geoffroy S, Andreux JP, Pautas E (2004) [Cytochrome P450 2C9 polymorphisms (CYP2C9) and warfarin maintenance dose in elderly patients]. *Rev Med Interne* 25:271–274
36. Spreafico M, Peyvandi F, Pizzotti D, Moia M, Mannucci PM (2003) Warfarin and acenocoumarol dose requirements according to CYP2C9 genotyping in North-Italian patients. *J Thromb Haemost* 1:2252–2253
37. Sychev DA, Mikheeva I, Kropacheva ES, Ignat'ev IV, Bulytova I, Ramenskaia GV, Dobrovol'skii AB, Panchenko EP, Kukes VG (2007) [The influence of CYP2C9 genetic polymorphism on the pharmacokinetics and pharmacodynamics of warfarin in patients with constant atrial fibrillation]. *Klin Med (Mosk)* 85:57–60
38. Takahashi H, Wilkinson GR, Nutescu EA, Morita T, Ritchie MD, Scordo MG, Pengo V, Barban M, Padriani R, Ieiri I, Otsubo K, Kashima T, Kimura S, Kijima S, Echizen H (2006) Different contributions of polymorphisms in VKORC1 and CYP2C9 to intra- and inter-population differences in maintenance dose of warfarin in Japanese, Caucasians and African-Americans. *Pharmacogenet Genomics* 16:101–110
39. Tanira MO, Al-Mukhaini MK, Al-Hinai AT, Al Balushi KA, Ahmed IS (2007) Frequency of CYP2C9 genotypes among Omani patients receiving warfarin and its correlation with warfarin dose. *Community Genet* 10:32–37
40. Taube J, Halsall D, Baglin T (2000) Influence of cytochrome P-450 CYP2C9 polymorphisms on warfarin sensitivity and risk of over-anticoagulation in patients on long-term treatment. *Blood* 96:1816–1819
41. Topic E, Stefanovic M, Samardzija M (2004) Association between the CYP2C9 polymorphism and the drug metabolism phenotype. *Clin Chem Lab Med* 42:72–78
42. Wadelius M, Sorlin K, Wallerman O, Karlsson J, Yue QY, Magnusson PK, Wadelius C, Melhus H (2004) Warfarin sensitivity related to CYP2C9, CYP3A5, ABCB1 (MDR1) and other factors. *Pharmacogenomics* 4:40–48
43. Zhao F, Loke C, Rankin SC, Guo JY, Lee HS, Wu TS, Tan T, Liu TC, Lu WL, Lim YT, Zhang Q, Goh BC, Lee SC (2004) Novel CYP2C9 genetic variants in Asian subjects and their influence on maintenance warfarin dose. *Clin Pharmacol Ther* 76:210–219

44. Wadelius M, Chen LY, Lindh JD, Eriksson N, Ghori MJ, Bumpstead S, Holm L, McGinnis R, Rane A, Deloukas P (2008) The largest prospective warfarin-treated cohort supports genetic forecasting. *Blood*. doi:10.1182/blood-2008-04-149070
45. Li T, Chang CY, Jin DY, Lin PJ, Khvorova A, Stafford DW (2004) Identification of the gene for vitamin K epoxide reductase. *Nature* 427:541–544
46. Rieder MJ, Reiner AP, Gage BF, Nickerson DA, Eby CS, McLeod HL, Blough DK, Thummel KE, Veenstra DL, Rettie AE (2005) Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose. *N Engl J Med* 352:2285–2293
47. Rost S, Fregin A, Ivaskevicius V, Conzelmann E, Hortnagel K, Pelz HJ, Lappégard K, Seifried E, Scharrer I, Tuddenham EG, Muller CR, Strom TM, Oldenburg J (2004) Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2. *Nature* 427:537–541
48. Gray IC, Nobile C, Muresu R, Ford S, Spurr NK (1995) A 2.4-megabase physical map spanning the CYP2C gene cluster on chromosome 10q24. *Genomics* 28:328–332
49. Sanderson S, Emery J, Higgins J (2005) CYP2C9 gene variants, drug dose, and bleeding risk in warfarin-treated patients: a HuGENet systematic review and meta-analysis. *Genet Med* 7:97–104