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ARTICLE

CYP2C9, *VKORC1*, and *CYP4F2* polymorphisms and pediatric warfarin maintenance dose: a systematic review and meta-analysis

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Abstract

Studies on the effect of cytochrome P450 2C9 (*CYP2C9*), vitamin K epoxide reductase complex subunit 1 (*VKORC1*), and cytochrome P450 4F2 (*CYP4F2*) polymorphisms on warfarin maintenance dose in children are conflicting. We conducted a systematic review and meta-analysis to evaluate the effect of these polymorphisms on warfarin maintenance dose in children. We searched relevant literature using the MEDLINE, EMBASE, and Cochrane Central Register of Controlled Trial libraries without any language restrictions from their inception to 23 July 2017. Dose differences are expressed as standardized mean difference (SMD) or mean difference (MD) with 95% confidence intervals (CI). This review was registered in the PROSPERO prospective register of systematic reviews (CRD42015016172). We included a total of nine studies (745 participants) in the meta-analysis. Patients with *CYP2C9* *1/*2, *1/*3, *2/*2, *2/*3, or *3/*3 required a lower warfarin maintenance dose compared with patients with *CYP2C9* *1/*1 (SMD = −0.610, 95% CI: −0.802 to −0.419, $I^2 = 0\%$). Patients with *VKORC1*-1639GA or AA required a lower warfarin maintenance dose compared with patients with *VKORC1*-1639GG (SMD = −0.666, 95% CI: −0.887 to −0.445, $I^2 = 33\%$). However, no associations were observed between *CYP4F2* polymorphisms and warfarin maintenance dose (MD = 0.005 mg/kg/day, 95% CI: −0.006 to 0.015, $I^2 = 0\%$). These results were not affected by a sensitivity analysis. Our meta-analysis provides evidence that *CYP2C9* and *VKORC1* variant statuses affect warfarin maintenance dose in children, but not CYP4F2.

Introduction

Warfarin is the most commonly prescribed oral anticoagulant for the treatment and prevention of thromboembolic events. Challenges related to the use of warfarin pertain to its low therapeutic index and the large interindividual variability in maintenance dose requirement [1]. Although variation in the initial and maintenance dose is known to depend on body weight, age, and dietary vitamin K intake as well as concomitant medications, there is growing evidence for a strong hereditary component [2–4]. Over the last decade, a large body of evidence has accumulated showing that polymorphisms in three genes influence warfarin maintenance dose in adults [5–7]: cytochrome P450 2C9 (*CYP2C9*) responsible for the metabolism of S warfarin, vitamin K epoxide reductase complex subunit 1 (*VKORC1*) as the molecular target of warfarin, and cytochrome P450 4F2 (*CYP4F2*) as the primary vitamin K1 oxidase in the liver. Several meta-analyses have already shown the impact of *CYP2C9* [7], *VKORC1* [8], and *CYP4F2* [9] on warfarin maintenance dose in adults. The Clinical Pharmacogenetics Implementation Consortium published dosing guidelines [10] in 2017, and recommended the use of pharmacogenetic testing to determine initial warfarin doses in adults.

The expression and function of proteins, including drug-metabolizing enzymes, develop during infancy and childhood,

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although the speed and patterns of development vary among different proteins. As a result, the phenotype–genotype relationship is often different between adults and children, making it difficult to extrapolate adult findings to children. Several pharmacogenetic studies have reported on the effects of genotypes on optimal warfarin maintenance doses in children [11–19]. However, the findings are not consistent. Recently, Zhang et al. [20, 21] conducted systematic reviews and meta-analyses exploring the effect of *CYP2C9* and *VKORC1* variants on warfarin maintenance dose in children indicating that the two gene polymorphisms significantly correlated with warfarin maintenance dose. However, given the heterogeneity of warfarin therapy, the additional contribution of other important factors, such as age, indication, or target international normalized ratio (INR), to warfarin maintenance dose remains to be elucidated. In addition, the effect of *CYP4F2* variants on warfarin maintenance dose in children has not been systematically analyzed to date.

We therefore conducted a systematic review and meta-analysis to evaluate the effects of *CYP2C9*, *VKORC1,* and *CYP4F2* genotypes on warfarin maintenance dose in children. We analyzed the original data of the included studies to estimate variant-specific warfarin doses for clinical purposes.

Material and methods

We followed the principles of the Cochrane Handbook for Systematic Reviews of Interventions [22], the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocol 2015 (PRISMA-P) [23], and the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (Supplementary Table 1) [24]. Our systematic review protocol [25] (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4917995/pdf/13643_2016_Article_280.pdf) was registered with the PROSPERO on 27 February 2015 (registration number: CRD42015016172). Two investigators (MT and TK) independently reviewed the articles for eligibility, risk of bias, and data extraction. Disagreements were resolved through discussion or adjudication by a third reviewer (SI).

Search strategy and sources

A comprehensive literature search without language restrictions was conducted by a specialized librarian from their inception to 23 July 2017, including MEDLINE, EMBASE, and the Cochrane Central Register of Controlled Trial libraries, as well as gray literature. The following search strategy was applied: “warfarin” AND “target SNPs” AND “children”. Details of the search strategy are provided in Supplementary Materials 1–3. In addition, we manually searched the reference lists of each eligible study included in the present and previous systematic reviews to identify additional relevant articles. We also searched the International Clinical Trials Registry Platform Search Portal and ClinicalTrials.gov for ongoing or recently completed trials and PROSPERO for ongoing or recently completed systematic reviews.

Study selection

We included original studies which met the following criteria:

(1) Observational (cohort or cross-sectional) studies or randomized controlled trials in warfarin-treated pediatric patients;
(2) Genotypes of *CYP2C9*, *VKORC1*, or *CYP4F2* reported;
(3) Children aged 0–18 years of age receiving warfarin for at least 1 month;
(4) Maintenance doses of warfarin presented according to each genotype.

There were no restrictions in the inclusion criteria regarding patient demographic information including body weight, height, use of concurrent drugs, indication for warfarin use, and target INR ranges. We excluded case–control studies, case reports, case series, reviews, editorials, news, and original studies which did not report the outcome of interest in both the children and adults, and duplicated studies. Pharmacogenetic studies involving only adults aged 18 or older were excluded. When a study involved both adult and pediatric patients, we excluded the study if the mean or median age of the study population exceeded 18 years old and a subgroup analysis regarding children was unavailable.

Data extraction

In accordance with the protocol [25], we extracted information pertaining to study identification (first author, year of publication, and country where patient recruitment took place), study design, patient population (number of enrolled patients, age, gender, predominant ethnicity, concomitant drug use, diet, indication for warfarin treatment, target INR range), and primary outcome measurements (allele frequencies and warfarin maintenance dose for each genotype of *CYP2C9*, *VKORC1*, or *CYP4F2*). We defined warfarin maintenance dose as the dose required to achieve three consecutive INR measurements within the target therapeutic range over a minimum of 4 weeks. The two reviewers independently extracted the data of all eligible studies and summarized them. Disagreements between the reviewers were resolved by discussion. We also contacted the original authors for missing or additional data to standardize the unit of warfarin dose to mg/kg/day.

Assessment of the risk of bias

We assessed the risk of bias of all eligible studies in accordance with the Newcastle–Ottawa Scale (NOS) (the full score is "9 stars", and high quality is defined as "7 stars" or more) [26]. In addition, we applied the Strengthening the Reporting of Genetic Association studies (STREGA) statement [27] containing 12 items associated with valid data reported in the study. For each item, there are three categories; "yes" is scored 2, "cannot tell" is scored 1, or "no" is scored 0. A total score over 16 (maximum score: 24) was arbitrarily defined as high quality.

Outcomes

Primary outcomes were differences in warfarin maintenance doses in children to achieve therapeutic targets between children with the wild type or the variants for each gene as follows:

1. Between the *CYP2C9* variants (*CYP2C9* *1/*2, *CYP2C9* *1/*3, *CYP2C9* *2/*2, *CYP2C9* *2/*3, or *CYP2C9* *3/*3) and the wild type (*CYP2C9* *1/*1).
2. Between the *VKORC1* variants (*VKORC1*-1639GA, *VKORC*-1639AA, *VKORC1*-1173CT, or *VKORC1*-1173CT) and the wild type (*VKORC1*-1639GG or *VKORC1*-1173TT).
3. Between the *CYP4F2* variants (*CYP4F2*-CT or *CYP4F2*-TT) and the wild type (*CYP4F2*-CC).

In the analyses, we combined the data regarding haplotypes of *VKORC1*-1173C>T and *VKORC1*-1639G>A, because these two single-nucleotide polymorphisms (SNPs) have a strong linkage disequilibrium [28]. We retrieved the warfarin maintenance doses from the eligible studies as mg/kg/day. If needed, we contacted the authors for information. Mean dose differences (MD: mg/kg/day) between the variants and wild type with 95% confidence interval (CI) were estimated. If weight-adjusted doses in mg/kg/day were unavailable for data synthesis, we calculated the standardized mean difference (SMD) of the warfarin doses, which is the mean differences (MD) of the doses (mg/day) divided by the pooled standard deviation. The SMD expresses the size of the effect in each study relative to the variability observed in that study, allowing a direct comparison of the effect of the gene polymorphisms across trials that used different scales.

Statistical analysis

We weighed the studies using the inverse variance method, and expressed the effect of each genetic variant on warfarin maintenance doses as absolute MD and/or SMD with 95% CI between the respective variant and the wild type, as defined in the section on primary outcomes above. The magnitude of the effect size represented by the SMD was interpreted as follows: small, if the absolute value of the SMD was <0.4; moderate, if it was between 0.4 and 0.7; and large, if >0.7 [22].

Anticipating heterogeneity between studies, we combined the data using the DerSimonian and Laird random effect model [29]. A two-sided *P* value of less than 0.05 indicates a nominally significant overall association. We assessed the magnitude of inconsistency in the study results using the I^2 statistic. We defined the three categories of heterogeneity as follows: $I^2 < 30\%$, low; I^2 30–70%, moderate; $I^2 > 70\%$, high. If significant heterogeneity was observed, further sensitivity analyses were performed to detect potential confounding variables. Publication bias was checked visually using a funnel plot. All statistical analyses were performed using the Review Manager Version 5.3 (The Nordic Cochrane Center, Copenhagen, Denmark) or EZR (Saitama Medical Center, Jichi Medical University), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [30].

Sensitivity analysis and subgroup analysis

We conducted sensitivity analyses by excluding low-quality studies (i.e., NOS < 7) to assess sources of heterogeneity. We also performed prespecified subgroup analyses in a variety of subsets found in the studies: studies including patients under 1 year of age and those without children under 1 year of age; studies with a patient median age <6 years and those with a patient median age ≥6 years; studies with a median target INR < 2.5 and those with a median target INR ≥ 2.5; studies with participants with a similar ancestral background; and studies with children who underwent the Fontan procedure, as an indication of warfarin therapy, and those with heart valve problems.

GRADE framework

We used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) guidelines to determine the quality and strength of recommendations [31]. Quality was adjudicated as high (further research is very unlikely to change our confidence in the estimate of effect), moderate (further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate), low (further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate), or very low (very uncertain about the estimate of effect) for each of the gene variants.

Results

Study identification and selection

The study selection process is shown in Fig. 1. We identified a total of 523 potentially relevant articles at the first screening. A total of 22 articles met the eligibility criteria, of which two articles [32, 33] were excluded because of overlapping samples, and no articles were identified from reference lists. Therefore, 20 articles [11–19, 34–44] were included in the systematic review. We contacted corresponding authors of the articles to obtain additional information; eight authors [17, 34–36, 38–40, 43] did not provide us with additional information. Eventually, 12 studies involving 745 participants were included in the quantitative analyses. Nine studies [11, 12, 14–16, 18, 19, 37, 44] provided information on both MD and SMD, and the remaining three [13, 41, 42] provided SMD data only.

Study characteristics

The characteristics of the individual studies included in the systematic review are summarized in Table 1 and Table 2. All the studies were published between 2010 and 2016. In the 12 included studies, [11–16, 18, 19, 37, 41, 42, 44] the predominant ancestral background was Caucasian (six studies), Japanese (three), Egyptian (one), Turkish (one), and Iranian (one). Five studies included patients less than 1 year of age. The main indication for warfarin was the Fontan procedure in six studies. The median target INR was less

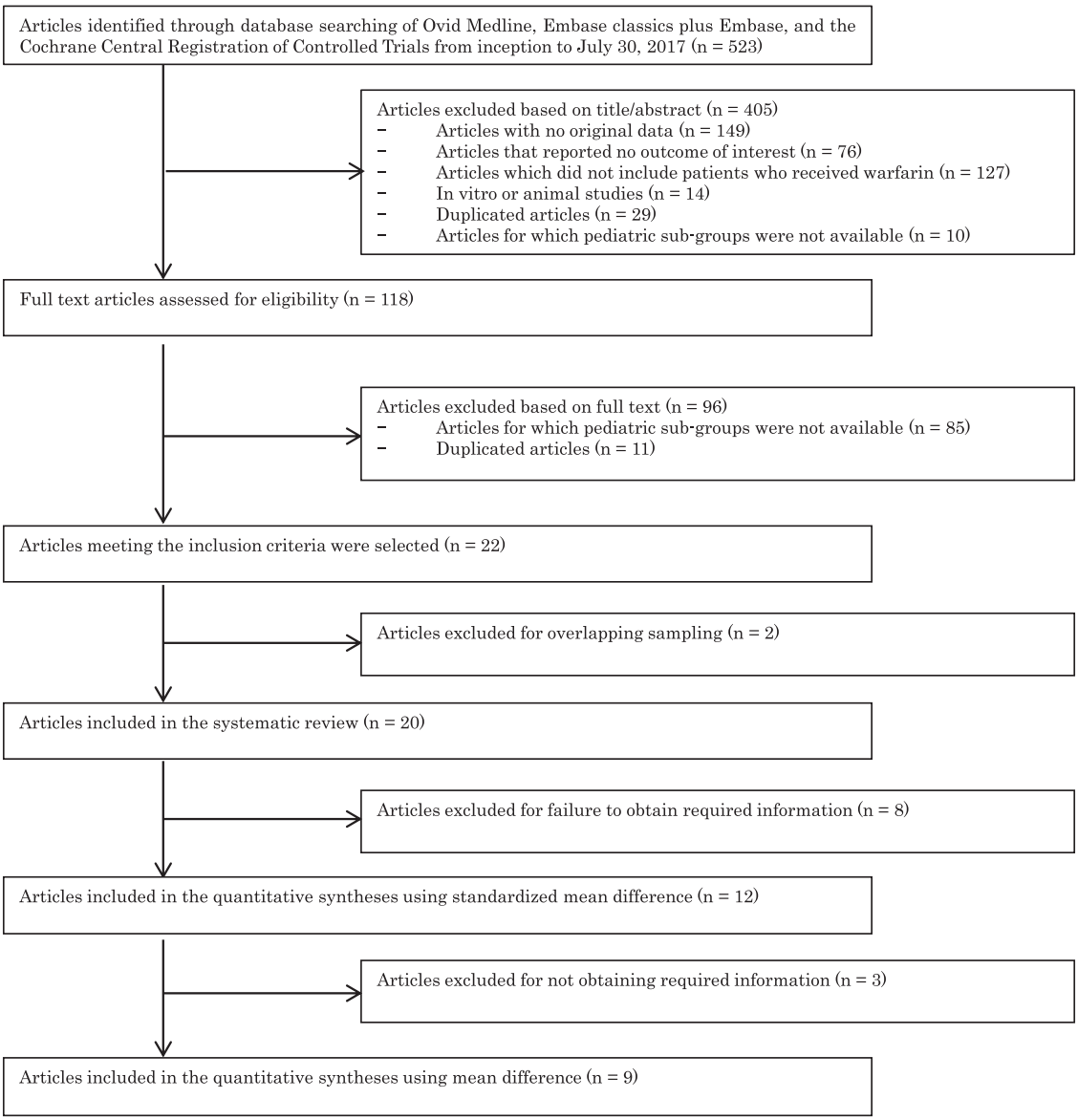

Fig. 1 Flow diagram showing the number of citations identified, retrieved, extracted, and included in the final analysis

Table 1 Characteristics of included studies in the systematic reviews

First author's name (published year)	Study location	Publication type	Number of participants	Number of male/female	Age of years median with range or mean ± SD	Predominant ethnicity, number	Indication for warfarin, number	Median target INR	Total score of NOS	Total score of STREGA
Nowak-Gottl (2010) [17]	German	Full text	34	NA	NA	Germany (Unknown frequency)	NA	NA	6	16
Lojo (2010) [34]	USA	Abstract	20	NA	NA (2–18)	NA	NA	NA	3	8
Mitchell (2011) [35]	Canada, German	Abstract	NA	NA	NA (1–19)	NA	NA	NA	4	10
Bomgaars (2011) [36]	USA	Abstract	84	43/31	11* (1–20)	NA	NA	NA	4	8
Kato (2011) [14]	Japan	Full text	48	33/15	9.1 (0.4–19)	Japanese, 48	Fontan, 44 Others, 4	1.84*	6	16
Biss (2012) [11]	UK	Full text	120	82/38	11 (1–18)	Caucasian, 91 Indian/Pakistani, 10 Chinese, 4 Black Caribbean, 3 Black African, 3 South-East Asian/Filipino, 2 Other, 7	Fontan, 64 Heart valve replacement, 18 Coronary aneurysm, 11 Dilated cardiomyopathy, 6 Deep vein thrombosis, 6 Pulmonary hypertension, 5 Stroke, 2 Others, 8	2.6	9	20
Moreau (2012) [15]	France	Full text	83	46/37	7.4 (0.3–18)	Caucasian (Unknown frequency)	Fontan(main) Heart valve replacement Cardiomyopathy Kawasaki disease Arrythmias Pulmonary arterial hypertension Lupus Antiphospholipid syndrome (Unknown frequency)	2.5	8	18
Hamberg (2013) [33]	Sweden	Full text	49	26/23	7.2 (0.3–17)	Caucasian, 40 Asian, 6 African, 2 Other, 1	Heart valve replacement, 21 Fontan, 13 Cardiomyopathy, 7 Others, 8	2.7	9	20
Hirai (2013) [12]	Japan	Full text	37	22/15	10.2 (2–17)	Japanese, 37	Heart valve replacement Fontan (Unknown frequency)	1.6	7	17
Nguyen (2013) [16]	USA	Full text	37	26/11	9.6 (1.8–18.6)	Caucasian, 27 African American, 7 Asian, 3	Fontan, 20 Heart valve replacement, 5 Complex congenital heart disease, 4 Kawasaki disease, 3 Endocarditis, 2 Marfan syndrome, 2 Rheumatic heart disease, 1	2.7	8	17
Beaune (2013) [38]	France	Abstract	120	NA	NA	NA	NA	NA	3	4
Lala (2013) [39]	USA	Full text	26	16/10	4.4* (0.3–18)	Hispanic, 16 Caucasian, 7 Arican American, 2 Other, 1	Fontan, 46 Heart valve replacement, 8 Kawasaki disease, 5 Cardiomyopathy, 1	2.4	8	17
Hawcutt (2014) [40]	UK	Full text	97	NA	2.3* (NA)	Caucasian, 97	Fontan, 64 Other cardiac disease, 23 Noncardiac disease, 12	2.2	8	17
Shaw (2014) [18]	Canada	Full text	77	42/35	4.7 (0.7–18)	Caucasian Asian Other (Unknown frequency)	Fontan, 34 Heart valve replacement, 21 Deep venous thromobosis, 9 Cardiomyopathy, 3 Coronary aneurysms, 3 Pulmonary hypertension, 3 Stroke, 2	2.5	9	21
Vear (2014) [19]	USA	Full text	100	46/54	12 (1.0–19.9)	Caucasian, 85 African American, 8 Hispanic, 3 Other, 3 Missing, 1	Deep venous thromobosis, 46 Heart valve replacement, 26 Prophyxis, 8 Fontan, 6 Stroke, 6 Pulmonrary Hypertension, 6	2.5	7	17

Table 1 (continued)

First author's name (published year)	Study location	Publication type	Number of participants	Number of male/female	Age of years median with range or mean ± SD	Predominant ethnicity, number	Indication for warfarin, number	Median target INR	Total score of NOS	Total score of STREGA
							Arrhythmia, 2 Others, 8			
Kamal El-Din (2014) [13]	Egypt	Full text	41	23/18	6.6 ± 3.0[†]	Egyptian, 41	Heart valve replacement, 26 Other disease, 15	2.4*	5	16
Tabib (2015) [41]	Iran	Full text	50	35/15	11.4 ± 3.4[†]	Unknown, 50 (Maybe Iranian, 50)	Heart valve replacement, 41 Fontan, 9	2.4	7	13
Dilge Taşkın (2016) [42]	Turkey	Full text	58	31/27	13.4 ± 4.7	Unknown, 58 (Maybe Turk, 58)	Heart valve replacement, 38 Thrombosis, 11 Cardiomyopathy, 5	2.5	8	20
Marek (2016) [43]	USA	Full text	32	22/10	4.8 (1.1–17)	Hispanic, 10 Not Hispanic, 21 Unknown, 1	Congenital heart disease, 11 Heart valve replacement, 11 Thrombosis, 6 Other, 4	NA	7	18
Wakamiya (2016) [44]	Japan	Full text	45	38/7	8.1 (0.3–18)	Japanese, 45	Fontan physiology, 19 Valve replacement, 13 Kawasaki disease, 10 Loeys-Dietz syndrome, 1 Thrombosis, 1 Stent placement,1	1.9	8	17

Shaded cell indicates the studies not included in the meta-analysis

DVT Deep Venous Thrombosis, *NOS* Newcastle Ottawa Scale, *STREGA* STrengthening the REporting of Genetic Association Studies, *NA* not available

* Mean

[†] mean ± SD

than 2.5 in five studies. The NOS scores were over 7 in all studies (Supplementary Table 2), except one study [17] and STREGA scores were over 16 in all studies (Supplementary Table 3), indicating that the quality of these studies met the general requirements for the meta-analysis.

Meta-analysis of the effects of the gene variants on warfarin maintenance dose

CYP2C9

We included eight studies [11, 12, 15, 16, 18, 19, 37, 41] (four studies were excluded because of insufficient dose information [13, 42] or low numbers of variants [14, 44]) in the meta-analysis of SMD, involving 540 patients (Fig. 2). Warfarin maintenance doses were lower with variants than with wild type (SMD = −0.610, 95% CI: −0.802 to −0.419) with low heterogeneity ($I^2 = 0\%$). Specifically, heterozygous variants showed a moderate decrease in warfarin maintenance dose compared with the wild type (SMD = −0.512, 95% CI: −0.713 to −0.311, $I^2 = 0\%$), while homozygous variants showed an even lower warfarin maintenance dose compared with the wild type (SMD = −1.099, 95% CI: −1.528 to −0.670, $I^2 = 0\%$) although the number of homozygous patients was small Table 3.

Of the eight studies mentioned above, seven studies [11, 12, 15, 16, 18, 19, 37] expressed warfarin maintenance doses as mg/kg/day. Therefore, we analyzed these seven studies using MD (mg/kg/day), which involved 490 patients (Supplementary Fig. 1). Patients with any of the *CYP2C9* variants required significantly lower warfarin maintenance doses than the wild type patients, with low heterogeneity (MD = −0.041 mg/kg/day, 95% CI: −0.054 to −0.028, $I^2 = 9\%$). Patients with heterozygous and homozygous variants also showed a significantly lower warfarin maintenance dose than wild-type patients (Supplementary Table 4). Of note, there was no indication of publication bias in the respective funnel plot (Supplementary Fig. 8A). Our findings indicate that children with the *CYP2C9* heterozygous variant and homozygous variant required 0.17 and 0.12 mg/kg/day on average as an initial dose, respectively, instead of a standard initial maintenance dose of 0.2 mg/kg/day to maintain a target INR of 2.5 [45].

VKORC1

Nine studies [11, 13, 15, 16, 18, 19, 37, 41, 42] involving 611 patients were included in the meta-analysis of SMD (three studies [12, 14, 44] were excluded because of low numbers of the variants). Warfarin maintenance doses were lower in the variant groups than in the wild-type group, with moderate heterogeneity (SMD = −0.666, 95% CI: −0.887

Table 2 Genotype frequencies of included studies in the systematic reviews

First author's name (published year)	Unit of warfarin maintenance dose	Genotype frequencies, number (warfarin maintenance dose: mean ± SD)											
		CYP2C9				VKORC1					CYP4F2		
		Wild type (*1/*1)	Heterozygous type (*1/*2 or *3)	Homozygous type (*2 or *3/*2 or *3)	Any variant type (*1, *2, or *3/*2 or *3))	Wild type (1639GG or 1173CC)	Heterozygous type (1639GA or 1173CT)	Homozygous type (1639AA or 1173TT)	Any variant type (1639GA, 1639AA, 1173 CT, or 1173TT)	Wild type (1347CC)	Heterozygous type (1347CT)	Homozygous type (1347TT)	Any variant type (1347CT or TT)
Nowak-Gottl (2010) [17]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Lojo (2010) [34]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Mitchell (2011) [35]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Bomgaars (2011) [36]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Kato (2011) [14]	mg/kg/day	47 (0.104 ± 0.038)	1 (0.077)	0	1 (0.077)	1 (0.135)	9 (0.137 ± 0.040)	38 (0.095 ± 0.033)	47 (0.103 ± 0.038)	No measurement	No measurement	No measurement	No measurement
Biss (2012) [11]	mg/kg/day	84 (0.124 ± 0.064)	34 (0.083 ± 0.034)	2 (0.044 ± 0.009)	36 (0.081 ± 0.034)	43 (0.148 ± 0.069)	55 (0.102 ± 0.042)	22 (0.063 ± 0.028)	77 (0.091 ± 0.042)	61 (0.108 ± 0.049)	49 (0.109 ± 0.056)	10 (0.141 ± 0.118)	59 (0.114 ± 0.070)
Moreau (2012) [15]	mg/kg/day	53 (0.14 ± 0.08)	25 (0.11 ± 0.05)	5 (0.10 ± 0.06)	30 (0.11 ± 0.05)	25 (0.16 ± 0.07)	43 (0.13 ± 0.08)	15 (0.08 ± 0.03)	58 (0.12 ± 0.07)	46 (0.13 ± 0.08)	26 (0.12 ± 0.07)	11 (0.16 ± 0.07)	37 (0.13 ± 0.07)
Hamberg (2013) [33]	mg/kg/day	34 (0.166 ± 0.071)	13 (0.152 ± 0.068)	2 (0.075 ± 0.021)	15 (0.123 ± 0.063)	19 (0.180 ± 0.069)	23 (0.148 ± 0.069)	7 (0.088 ± 0.039)	30 (0.134 ± 0.068)	29 (0.157 ± 0.078)	19 (0.137 ± 0.052)	1 (0.290)	20 (0.148 ± 0.059)
Hirai (2013) [12]	mg/kg/day	29 (0.086 ± 0.026)	2 (0.091 ± 0.030)	0	2 (0.091 ± 0.030)	0	4 (0.114 ± 0.011)	33 (0.082 ± 0.026)	37 (0.086 ± 0.026)	20 (0.081 ± 0.026)	16 (0.090 ± 0.026)	1 (0.12)	37 (0.091 ± 0.026)
Nguyen (2013) [16]	mg/kg/day	27 (0.158 ± 0.085)	10 (0.136 ± 0.083)	0	10 (0.136 ± 0.083)	10 (0.223 ± 0.066)	17 (0.153 ± 0.082)	10 (0.078 ± 0.026)	27 (0.120 ± 0.073)	No measurement	No measurement	No measurement	No measurement
Beaune (2013) [38]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Lala (2013) [39]	NA	22 (NA)	4 (NA)	0	4 (NA)	7 (NA)	8 (NA)	11 (NA)	19 (NA)	No measurement	No measurement	No measurement	No measurement
Hawcutt (2014) [40]	NA	61 (NA)	31 (NA)	5 (NA)	36 (NA)	40 (NA)	45 (NA)	12 (NA)	57 (NA)	No measurement	No measurement	No measurement	No measurement
Shaw (2014) [18]	mg/kg/day	54 (0.170 ± 0.103)	22 (0.106 ± 0.060)	1 (0.102)	23 (0.106 ± 0.058)	31 (0.177 ± 0.117)	32 (0.144 ± 0.067)	14 (0.108 ± 0.088)	46 (0.133 ± 0.075)	36 (0.146 ± 0.119)	36 (0.157 ± 0.074)	5 (0.139 ± 0.068)	41 (0.155 ± 0.072)
Vear (2014) [19]	mg/kg/day	67 (0.139 ± 0.136)	25 (0.090 ± 0.049)	1 (0.078)	26 (0.090 ± 0.048)	32 (0.163 ± 0.177)	54 (0.106 ± 0.062)	10 (0.077 ± 0.041)	64 (0.102 ± 0.060)	57 (0.126 ± 0.136)	32 (0.115 ± 0.080)	11 (0.146 ± 0.104)	43 (0.123 ± 0.086)
Kamal El-Din (2014) [13]	mg/day	27 (NA)	11 (NA)	3 (NA)	14 (NA)	7 (5.00 ± 1.53)	24 (NA)	10 (NA)	34 (4.85 ± 1.31)	No measurement	No measurement	No measurement	No measurement
Tabib (2015) [41]	mg/day	22 (4.2 ± 1.6)	8 (2.6 ± 1.0)	20 (3 ± 1.1)	28 (2.9 ± 1.1)	24 (3.9 ± 1.5)	24 (2.4 ± 1.1)	2 (5.6 ± 0.9)	3 (2.6 ± 1.4)	No measurement	No measurement	No measurement	No measurement
Dilge Taşkın (2016) [42]	mg/day	22 (4.35 ± 1.25)	30 (NA)	6 (NA)	36 (NA)	11 (4.80 ± 1.55)	36 (4.26 ± 1.24)	11 (3.58 ± 1.09)	47 (4.16 ± 1.24)	No measurement	No measurement	No measurement	No measurement
Marek (2016) [43]	mg/kg/day	25	7	0	7	15	11	6	17	No measurement	No measurement	No measurement	No measurement
Wakamiya (2016) [44]	mg/kg/day	45 (0.075 ± 0.055)	0	0	0	0	8 (0.112 ± 0.037)	37 (0.069 ± 0.032)	45 (0.076 ± 0.036)	17 (0.078 ± 0.049)	24 (0.074 ± 0.068)	4 (0.072 ± 0.037)	28 (0.074 ± 0.064)

Shaded cell indicates the studies not included in the meta-analysis

NA not available

* Mean

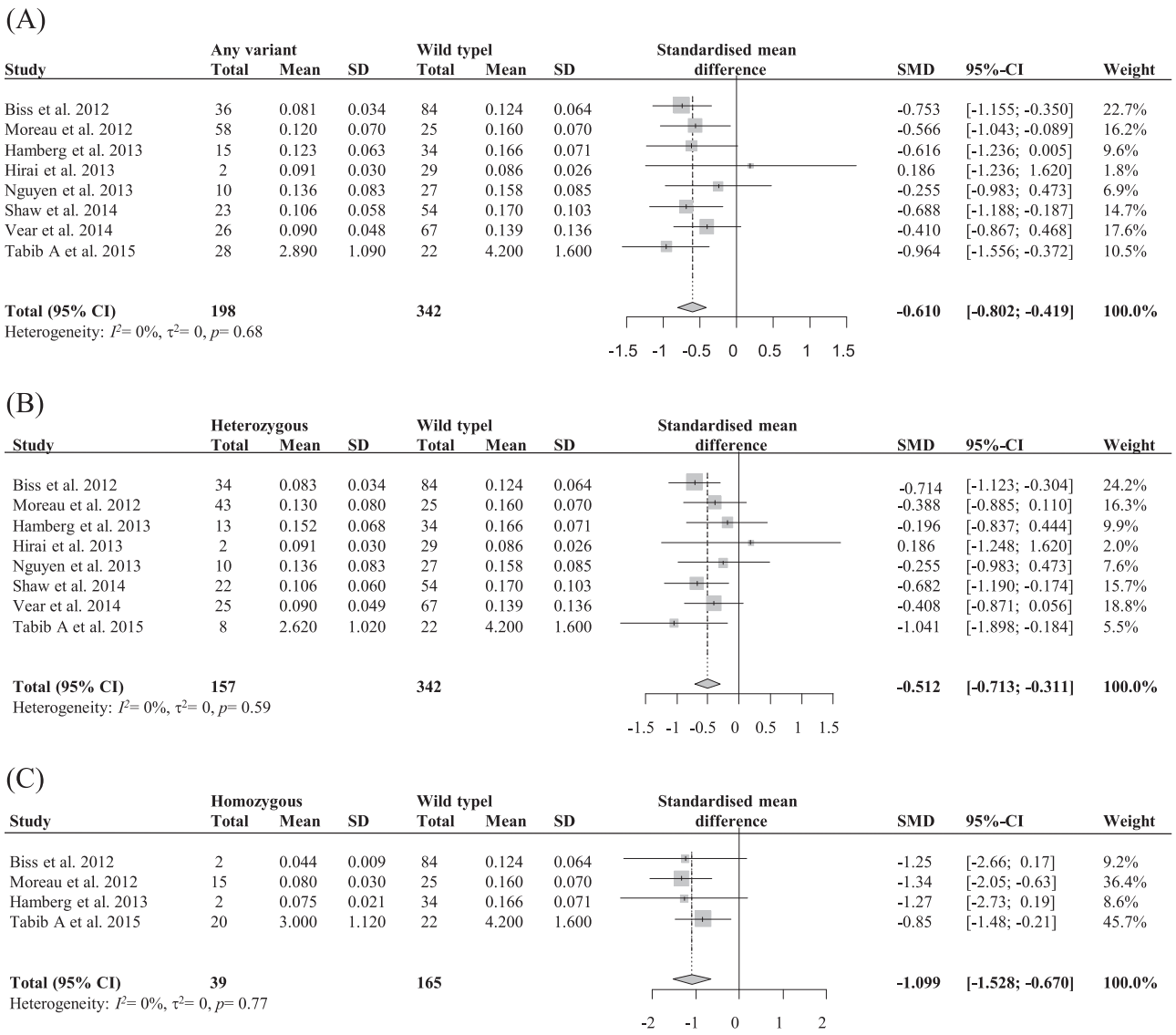

Fig. 2 Forest plots of the standardized mean difference (SMD) of the effect of *CYP2C9* single-nucleotide polymorphisms on warfarin maintenance dose. **a** Any *CYP2C9* variant vs. wild type carriers. **b** *CYP2C9* heterozygous vs. wild type carriers. **c** *CYP2C9* homozygous vs. wild type carriers. Unit of effect size; mg/kg/day. SD standard deviation, CI confidence interval. Wild type; *CYP2C9* *1/*1 carriers. Heterozygous type; *CYP2C9* *1/*2 or *1/*3 carriers. Homozygous type; *CYP2C9* *2/*2, *2/*3, or *3/*3 carriers

to −0.445, $I^2 = 33\%$), as shown in Fig. 3a. Similar to the findings for *CYP2C9*, the heterozygous variants of *VKORC1* showed moderately reduced warfarin maintenance doses with low heterogeneity, compared with the wild type (SMD = −0.594, 95% CI: −0.782 to −0.410, $I^2 = 0\%$), while homozygous variants showed markedly reduced doses with high heterogeneity (SMD = −0.912, 95% CI: −1.450 to −0.374, $I^2 = 68\%$), as shown in Fig. 3b, c.

Of the nine studies analyzed, six studies (462 patients) [11, 15, 16, 18, 19, 37] expressed warfarin maintenance doses as mg/kg/day. Patients with any of the *VKORC1* variants required lower warfarin maintenance doses than the wild-type patients, with low heterogeneity (MD = −0.052 mg/kg/day, 95% CI: −0.070 to −0.035, $I^2 = 28\%$) (Supplementary Fig. 2A). The dose difference was similarly significant between children with a heterozygous variant and those with the wild type (MD = −0.041 mg/kg/day, 95% CI: −0.056 to −0.026, $I^2 = 0\%$), and between patients with the homozygous variant and those with the wild type (MD = −0.089 mg/kg/day, 95% CI: −0.115 to −0.064, $I^2 = 48\%$), respectively (Supplementary Fig. 2B, C and Supplementary Table 5). The study funnel plots were symmetrically distributed, indicating that there was no publication bias (Supplementary Fig. 8B). Although the recommended initial dose of warfarin is 0.2 mg/kg/day in children, those with the *VKORC1* heterozygous and homozygous variants may require only 0.16 and 0.11 mg/kg/day as an initial maintenance dose, respectively.

Table 3 Effect of CYP2C9, VKORC1, and CYP4F2 polymorphism on warfarin maintenance dose in meta-analysis

Factors	No. of studies	No. of any variant	No. of wild type	SMD (95% CI)	MD(95% CI), mg/kg/day	Heterogeneity, I^2
CYP2C9 any variant vs. wild type	8	198	342	−0.610 (−0.802, −0.419)	–	0%
	7	170	320	–	−0.041 (−0.054, −0.028)	9%
CYP2C9 heterozygous variant vs. wild type	8	157	342	−0.512 (−0.713, −0.311)	–	0%
	7	149	320	–	−0.033 (−0.047, −0.019)	18%
CYP2C9 homozygous variant vs. wild type	4	39	165	−1.099 (−1.528, −0.670)	–	0%
	3	19	143	–	−0.082 (−0.096, −0.067)	0%
VKORC1 any variant vs. wild type	9	381	230	−0.666 (−0.887, −0.445)	–	33%
	6	274	188	–	−0.052 (−0.070, −0.035)	28%
VKORC1 heterozygous variant vs. wild type	8	226	223	−0.594 (−0.782, −0.410)	–	0%
	6	206	188	–	−0.041 (−0.056, −0.026)	0%
VKORC1 homozygous variant vs. wild type	8	81	223	−0.912 (−1.450, −0.374)	–	72%
	6	68	188	–	−0.089 (−0.115, −0.064)	68%
CYP4F2 any variant vs. wild type	7	245	266	–	−0.005 (−0.006, 0.015)	0%
CYP4F2 heterozygous variant vs. wild type	7	202	266	–	−0.000 (−0.010, 0.011)	0%
CYP4F2 homozygous variant vs. wild type	5	39	217	–	0.015 (−0.012, 0.042)	0%

SMD standardized mean difference, *MD* mean difference

CYP4F2

We analyzed seven studies (511 patients) [11, 12, 15, 18, 19, 37, 44], which provided a warfarin maintenance dose in mg/kg/day (Fig. 4 and Supplementary Table 6). No significant difference was found in the warfarin maintenance doses between the *CYP4F2* variants and wild-type *CYP4F2* without heterogeneity (any variant vs. wild type; MD = 0.005 mg/kg/day, CI: −0.006 to 0.015, $I^2 = 0\%$, $p = 0.97$; heterozygous vs. wild type; MD = 0.000 mg/kg/day, 95% CI: −0.010 to 0.011, $I^2 = 0\%$, $p = 0.81$; homozygous vs. wild type; MD = 0.015 mg/kg/day, 95% CI: −0.012 to 0.042, $I^2 = 0\%$, $p = 0.82$). No sign of publication bias was seen in the funnel plot (Supplementary Fig. 8C).

Subgroup analysis

Subgroup analyses based on main ancestral background (Caucasians and non-Caucasians) or age (<1 and ≥1 year of age, or <6 and ≥6 years of age) showed significant differences, similar to those found in the main analyses, in terms of warfarin maintenance dose between the *CYP2C9* and *VKORC1* variants and wild type, with the exception of *CYP2C9* in those with a non-Caucasian ancestral background (Supplementary Figs. 3–5).

While the effect of *VKORC1* polymorphisms was not statistically significant in the subset of studies with a median target INR < 2.5 (any variant vs. wild type; SMD = −0.54, CI: −1.28 to 0.19, $I^2 = 54\%$), a significant effect with mild heterogeneity was observed in those with a median target INR ≥ 2.5 (any variants vs. wild type; SMD = −0.71, CI: −0.99 to −0.43, $I^2 = 46\%$). The impact of *CYP2C9* and *CYP4F2* variants on the warfarin dose was not changed in the subgroup analysis based on the target INR (Supplementary Fig. 6).

The effect of the *CYP2C9* polymorphisms was detected in the post-Fontan procedure subgroup (any variant vs. wild type; SMD = −0.63, CI: −0.88 to −0.38, $I^2 = 0\%$), but this was not the case for valve replacement and deep vein thrombosis subgroups (any variant vs. wild type; SMD = −0.43, CI: −0.95 to 0.09, $I^2 = 59\%$). The effect of *VKORC1* and *CYP4F2* polymorphisms in both subgroups did not change in the subgroup analysis based on the indication for warfarin (Supplementary Fig. 7).

Sensitivity analyses

Exclusion of a low-quality study did not change the findings described above, which indicates that the results of our analysis were stable.

GRADE framework

We used the GRADE approach and classified the quality of the evidence. The evidence quality for the effect of *VKORC1* on warfarin maintenance dose was "moderate", and "high" for the effect of *CYP2C9* and *CYP4F2*. There is substantial statistical heterogeneity for the *VKORC1* findings (Table 4).

Discussion

We performed the largest meta-analysis in children published to date through international collaboration with investigators, providing solid evidence of the effect of *CYP2C9*, *VKORC1*, and *CYP4F2* on warfarin maintenance

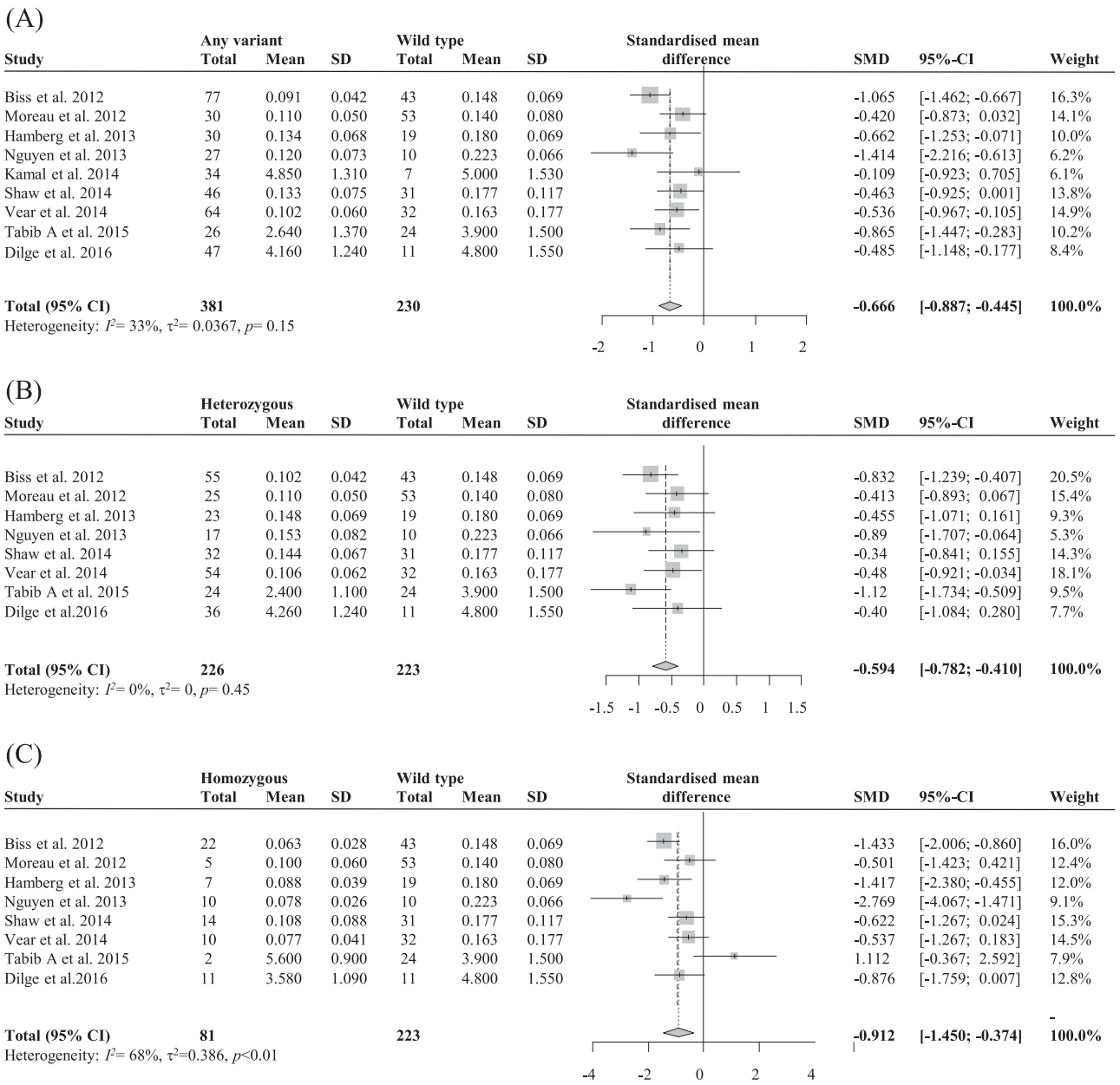

Fig. 3 Forest plots of the standardized mean difference (SMD) of the effect of *VKORC1*-1639 or -1173 single-nucleotide polymorphisms on warfarin maintenance dose. **a** Any *VKORC1* variant vs. wild-type carriers. **b** *VKORC1* heterozygous vs. wild-type carriers. **c** *VKORC1* homozygous vs. wild-type carriers. Unit of effect size; mg/kg/day. SD standard deviation, CI confidence interval. Wild type; *VKORC1*-1639GG or -1173CC carriers. Heterozygous type; *VKORC1*-1639GA or -1173CT carriers. Homozygous type; *VKORC1*-1639AA or -1173TT carriers

dose in children. *CYP2C9* and *VKORC1* polymorphisms significantly impact warfarin maintenance dose, whereas the *CYP4F2* polymorphism does not show such an effect. Furthermore, we were able to estimate the effect size using not only the SMD but also the absolute MD of the dose. This enabled us to translate the impact of the gene variants to absolute warfarin doses in mg/kg.

Children with *CYP2C9* variants were shown to require significantly lower doses than *CYP2C9* wild-type carriers, and the dose reduction was estimated to be 0.041 mg/kg/day on average (equivalent to a 20.5% reduction from an initial maintenance dose of 0.2 mg/kg/day). We also found that *VKORC1* variant carriers require a dose reduction of 0.052 mg/kg/day on average compared with *VKORC1* wild-type carriers (26% reduction from 0.2 mg/kg/day). Given that the effects range from 19.6 to 78.1% for *CYP2C9* and from 32 to 63% for *VKORC1* variants in adults, our findings suggest smaller effects in children than in adults [7, 8]. Our findings justify the inclusion of *CYP2C9* and *VKORC1* pharmacogenetic status in the dosing algorithms for warfarin in children.

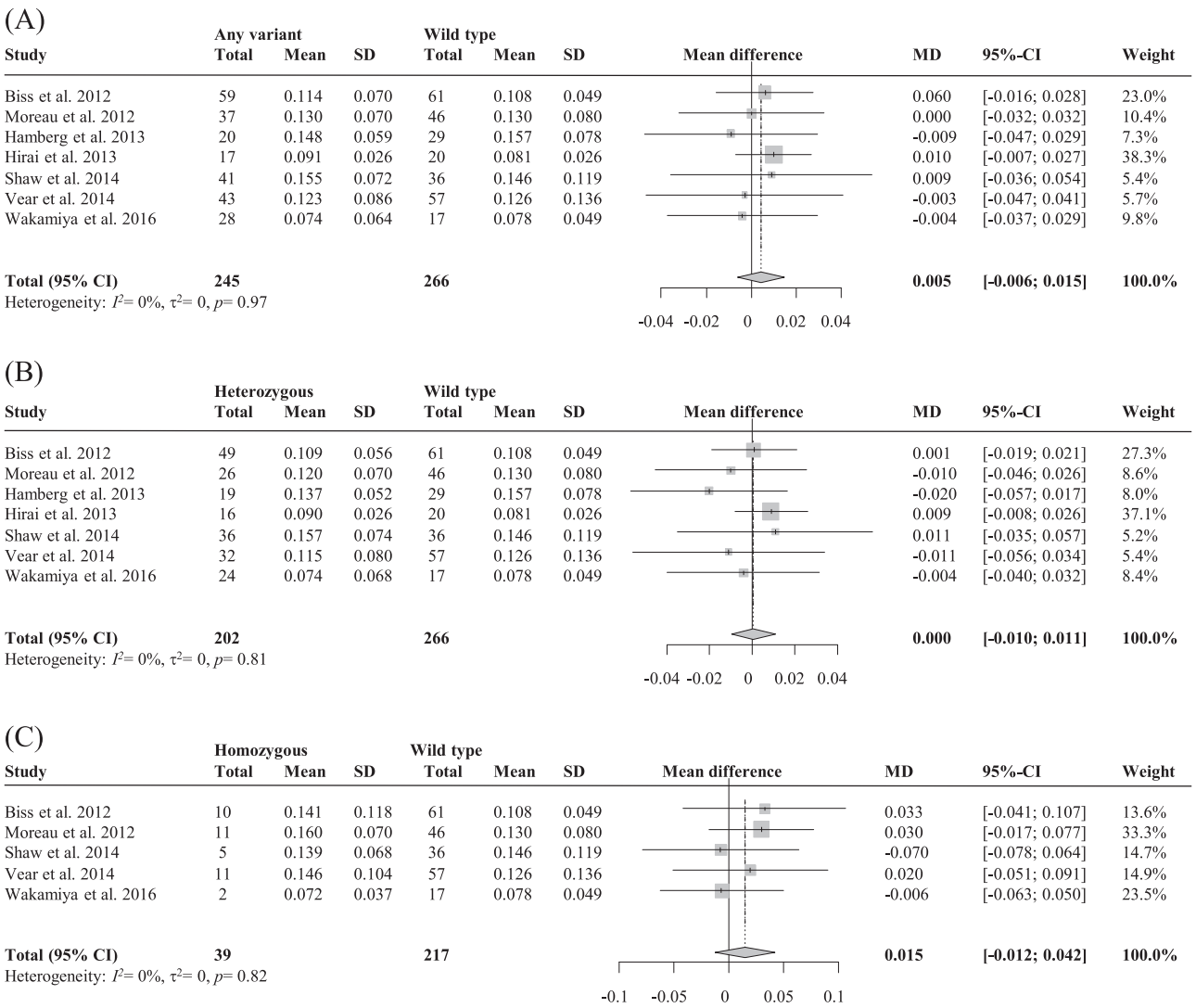

Fig. 4 Forest plots of the mean difference (MD) of the effect of *CYP4F2* single-nucleotide polymorphisms on warfarin maintenance dose. **a** Any *CYP4F2* variant vs. wild-type carriers. **b** *CYP4F2* heterozygous vs. wild-type carriers. **c** *CYP4F2* homozygous vs. wild-type carriers. Unit of effect size; mg/kg/day. SD standard deviation, CI confidence interval. Wild type; *CYP4F2*-CC carriers. Heterozygous type; *CYP4F2*-CT carriers. Homozygous type; *CYP4F2*-TT carriers

The *CYP4F2* variants we investigated may predispose individuals to an increased warfarin requirement because of the reduced removal of vitamin K from the body. However, in the present analyses, these variants did not have a significant impact on warfarin maintenance doses, although the latest meta-analysis in an adult population showed otherwise [46]. Importantly, we observed that the point estimate of the effect size was extremely small in children, compared with the reported adult data [46], and therefore, this cannot be explained solely by small sample sizes in the pediatric studies. Children and adults show highly discrepant profiles of circulating Gla-proteins including osteocalcin [47, 48]. Although speculative, vitamin K disposition maybe fundamentally different in rapidly growing children, compared with adults, influencing the impact of *CYP4F2* function on the warfarin stable dose.

We conducted subgroup analyses to assess whether clinical and demographic factors modify the effect of gene polymorphisms on warfarin maintenance dose. Overall, the findings were similar to those of the main analyses. For example, significant effects of *CYP2C9* and *VKORC1* variants on warfarin dose were also observed in the subgroup of studies with subjects of Caucasian background, although interpretation of the findings in the non-Caucasian groups requires caution because of small sample sizes. Similarly, the results of the subgroup analysis based on age did not differ from the main findings. However, our analyses are not based on individual patient data, and therefore, the

Table 4 GRADE evidence profile

Quality assessment							No. of patients		Effects		
No. of studies	Design	Risk of bias	Inconsistency (I^2)	Indirectness	Imprecision	Publication bias	Variant type	Wild type	SMD (95% CI)[a]	MD (95% CI) mg/kg/day	Quality
Effect of CYP2C9 gene polymorphism on warfarin maintenance dose											
8	Observational studies	Not serious[a]	Not serious[†] ($I^2 = 0\%$)	Not serious	Not serious	Undetected	198	342	−0.610 (−0.802, −0.419)	–	High
7	Observational studies	Not serious	Not serious ($I^2 = 9\%$)	Not serious	Not serious	Undetected	170	320	–	−0.041 (−0.054, −0.028)	High
Effect of VKORC1-1639 or 1173 gene polymorphism on warfarin maintenance dose											
9	observational studies	Not serious	Serious ($I^2 = 33\%$)	Not serious	Not serious	Undetected	381	230	−0.666 (−0.887, −0.445)	–	Moderate
6	Observational studies	Not serious	Not serious ($I^2 = 28\%$)	Not serious	Not serious	Undetected	274	188	–	−0.052 (−0.070, −0.035)	High
Effect of CYP4F2 gene polymorphism on warfarin maintenance dose											
7	Observational studies	Not serious	Not serious ($I^2 = 0\%$)	Not serious	Not serious	Undetected	245	266	–	0.005 (−0.005, 0.015)	High

GRADE Working Group grades of evidence: high quality, further research is very unlikely to change our confidence in the estimate of effect; moderate quality, further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate; low quality, further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate; very-low quality, we are very uncertain about the estimate

[a]Concerning the common cutoff values considered for SMD, the effect of the gene polymorphism on warfarin maintenance dose was regarded as small when the SMD was less than −0.4, moderate when it was between −0.4 and −0.7, and large when it was less than −0.7

[†] mean ± SD

influence of age, target INR, and indication for warfarin requires further study.

Our study has some limitations. First, most of the analyzed studies consist of Caucasian subjects. Although the relative homogeneity of our study population probably reduced variation in our findings, generalization to other populations requires caution. Second, in our analyses, warfarin stable doses per day were expressed as weight-adjusted values (i.e., mg/kg/day). Weight-adjusted doses of most drugs change with age, including warfarin. Except for neonates, young children tend to have higher clearance per body weight than adults [48], which results in higher dosing rates per body weight to achieve the same average concentrations in serum at steady state. The age dependency of the weight-adjusted stable dose of warfarin is likely to have introduced variability to our findings compared with those obtained using age-insensitive allometrically scaled doses. However, this factor does not systematically influence the point estimate of the between-genotype stable warfarin dose differences. Third, we did not consider gene–gene interactions. The findings may change depending on combinations of *CYP2C9* and *VKORC1* variants.

Our meta-analysis provides robust evidence that CYP2C9 and VKORC1 polymorphisms affect warfarin maintenance dose in children, indicating the clinical utility of pharmacogenetics-guided warfarin therapy in children.

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Author contribution SI and LRB initiated the study planning and all contributed to the study design. MT collected data, performed statistical analysis, interpreted the results, and drafted the paper. TK designed the study and collected data. TB, FK, SV, RH, KS, BC, AH, MW, HK, MT, TW, MY, KH, and KI provided data for the study. TK, LRB, and SI critically revised the paper. All authors read, provided feedback, and approved the final paper.

Compliance with ethical standards

Conflict of interest SI received grants from Novartis Pharma AG, grants from UCB Pharma Gmbh, personal fees from AbbVie, outside the submitted work; and he is a member of the American Society of Clinical Pharmacology & Therapeutics, and the Canadian Society of Pharmacology and Therapeutics. He is also one of the Associate Editors of "Clinical Pharmacology & Therapeutics", which conducts peer-review and publishes articles, some of which include CPIC guidelines and original articles on pharmacogenomics/genetics. The remaining authors declare that they have no conflict of interest.

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