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Impact of gamma-glutamyl carboxylase gene polymorphisms on warfarin dose requirement: A systematic review and meta-analysis

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ABSTRACT

Background: The Gamma-glutamyl carboxylase (GGCX) gene, as with Vitamin K Epoxide Reductase Complex Subunit 1(VKORC1), CytochromeP450 Complex Subunit 14 F2 (CYP4F2) and CytochromeP450 Complex Subunit2C9 (CYP2C9), is a candidate predictor for appropriate maintenance warfarin dose. However, the association between GGCX gene polymorphisms and warfarin dose requirement is still controversial. To quantify the influence of GGCX polymorphisms on warfarin dose requirements, we performed a systematic review and meta-analysis.
Methods: According to PRISRM statement (Preferred reporting items for systematic reviews and meta-analyses), a comprehensive literature search was undertaken through August 2014 looking for eligible studies in Embase, Pubmed,Web of Science and the Cochrane Library. The impact of GGCX polymorphisms on mean daily warfarin dose (MDWD) was counted by means of Z test. RevMan 5.2.7 software (developed by the Cochrane Collaboration) was applied to analyze the relationship between GGCX gene polymorphisms and warfarin dose requirements.
Results: Nineteen articles including 21 studies with a total of 6957 patients were included in the meta-analysis. Among three investigated single nucleotide polymorphisms (SNPs), rs11676382 showed higher CC genotype frequencies in Asian than those in Caucasian(97.7% vs. 86.9%); patients who were "G carriers" (that is, carried the GGCX rs11676382 CG or GG genotypes) required 27% lower warfarin dose than CC genotype[95%Confidence Interval(CI) = 17%-37%, $P = 0.000$, $I^2\% = 82.0$ and $P_Q = 0.000$], moreover, stratified analysis by ethnicity showed similar results in Caucasian(23% lower, 95%CI = 12%-33%), but not in Asian. With respect to genetic variation of rs699664 and rs121714145 SNPs, no significant impact on warfarin dose requirements were demonstrated.
Conclusions: This meta-analysis suggested that GGCX rs11676382 polymorphism may be one of factors affecting the dose of warfarin requirement, and the effects are different in different ethnicities. Further studies about this topic in different ethnicities with larger samples are expected to be conducted to validate our results.

Introduction

Warfarin is commonly used in clinical treatment of various disorders, especially thromboembolic disease. The narrow therapeutic range of warfarin dosage may increase the risk of recurrence of thromboembolism and bleeding [1], so it is difficult to establish the appropriate dose of warfarin to achieve anticoagulation. Over time, many factors have proven to be associated with therapeutic warfarin dose requirements, including basic elements such as gender, age, weight, and body surface area [2–4]; however, these elements do not explain all of the differences that currently exist among warfarin dose requirements among different individuals and different ethnicities.

In recent years, it has been shown that the genetic variability of the pharmacokinetics and pharmacodynamics of warfarin may be important in determining the individual and interethnic differences in appropriate warfarin dosage [5–9]. VKORC1, CYP4F2, and CYP2C9 polymorphisms contribute to inter-population difference in warfarin doses among diverse geographic regions [5,7,8]. GGCX, as a key cofactor for the activation of clotting factors (VII, IX, X) to reduce Vitamin K [10], is also a possible candidate, given that recent studies have found a significant relationship between GGCX genotype and warfarin dose [11–18].

The association between GGCX SNPs polymorphisms and warfarin dose requirements is biologically plausible. The GGCX gene, located on chromosome 2p12 in humans and consisting of 15 exons, plays the critical pharmacodynamic role in the generation of vitamin K-dependent

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proteins. GGCX can active the clotting factors VII, IX, X, and prothrombin by reducing vitamin K to vitamin K-2, 3-epoxide [19]. In addition, GGCX-knockout mice die at birth from massive hemorrhage [20]. Crosier et al. [21] found that GGCX SNPs showed significant associations with percent undercarbosylated osteocalcin, a measure of vitamin K-dependent carboxylation in extra-hepatic proteins, which would influence warfarin dose requirements in order to achieve stable oral anticoagulation.

For the past decades, a number of studies on the relationship between GGCX gene and warfarin dose requirement had been reported [11–18, 22–30]. These studies provide certain evidences that the genotype-based dose predictions may in future enable personalised drug treatment from the start of warfarin therapy. In those studies, the rs699664 SNP in exon 8, rs12714145 SNP in intron 2, and rs11676382 SNP in intron 14 were the most researched SNPs. However, variant GGCX alleles have been associated with warfarin dosing in some studies with different ethnicities [12,23,31], but have not been consistently replicated in other studies [32,33]. Therefore, it is necessary to substantiate or validate the impact of GGCX polymorphisms in patients taking warfarin. The aim of this study was to quantify the effects of individual GGCX genotype on warfarin dose requirements, by means of a systematic literature review and meta-analysis.

Materials and Methods

This meta-analysis was conducted according to PRISMA statement (Preferred reporting items for systematic reviews and meta-analyses), including search strategy, selection criteria,data extraction and data analysis [34].

Identification of Eligible Studies

We used the following search terms: "Gamma-glutamyl carboxylase" or "GGCX" in combination with "polymorphism," "mutation," or "variant" in combination with "warfarin," in Embase, PubMed, Web of Science and the Cochrane Library up to Aug, 2014. Two investigators (YFS and LS) conducted an extensive literature search independently for all publications. There was no language restriction. Articles in reference lists were also hand-searched. Only human studies were searched.

Inclusion and Exclusion Criteria

The following criteria were used to choose suitable studies:

(1) The following GGCX gene SNPs was studied: rs699664 (G8016A, c.974G>A), rs12714145 (c.214+597G>A, 3261G>A), rs11676382 (12970C>G);
(2) Studies offering information regarding the number of genotypes, the maintenance dose of warfarin (mean with either standard deviation or 95% confidence interval) separately for GGCX genotype groups.

Publications were excluded from the meta-analysis if:

(1) Review articles, letters, case reports, editorials, and conference abstracts.
(2) The articles did not include genotype frequencies or warfarin dose, and this information was not available from the authors contacted.

Data Extraction

Two investigators (YFS and LS) independently extracted data from the included studies. The recorded data mainly included genotype frequencies of GGCX and warfarin dose (mean, SD) for each genotype. Also extracted from the eligible studies was basic information including the first author's name, publication date, country, ethnicity, indication of warfarin, total sample size (male, female), mean age of sample, target value of international normalized ratio (INR), and genotyping method. Accuracy of the extracted information was verified by ensuring that the data recorded by the 2 investigators matches; if it did not, the investigators rechecked the data extracted. If the two investigators could not reach an agreement, the dispute was submitted to a third reviewer (QX) to decide.

Quality Score Assessment

The quality of the selected studies was independently assessed by two investigators (CL and YFS) independently following the criteria predefined by Little et al. [35]. These quality criteria were based on: (1) analytic validity of genotyping (genotyping method, quality control measures, timing of sample collection and analysis, and types of samples used); (2) selection of study subjects (geographic area recruitment period, exclusion criteria for cases and controls, mean age and standard deviation or age range of study subjects, and distribution by sex); (3) confounding factors, including population stratification (potential correlation of the genotype identified and taken into consideration in design or analysis); and (4) statistical issues (method of analysis used in reference, and software used to perform the analysis). If most of the above criteria were satisfied, a study was graded as "++". A study that fulfilled some of the criteria would be graded as "+"; few or no criteria fulfilled resulted in a grade of "-" [35].

Statistical Analysis

To remove any heterogeneity caused by deviation in warfarin pharmacokinetics and pharmacodynamics sensitivity among different study populations, in our meta-analysis, the maintenance of warfarin dose for each GGCX genotype group was normalized using the homozygous wild-type group as a reference. The normalization procedure was performed by dividing the mean dose and associated standard deviations in each group by the mean maintenance dose in the GGCX reference group [36].

For the GGCX rs699664 and rs12714145, we defined carriers of AG or AA genotype as "A carriers", A carriers (AG+AA), AA and AG were compared with GG genotype (reference group).With respect to GGCX rs11676382, CG or GG genotype were defined as "G carriers", because GG frequencies were too small, only G carriers (CG+GG) were compared with CC genotype (reference group). The calculated mean differences represent relative rather than absolute differences in maintenance dose; in other words, a mean difference of 0.5 would indicate a 50% increase in warfarin dose requirement [36].

The weight is the inverse of its standard differences of warfarin dose in each study, and the effect of each GGCX genotype on warfarin dose was defined as mean difference (MD), which was calculated by subtracting the normalized mean warfarin dose for the respective genotypes from the reference. The Weight Mean Difference (WMD) was used as indicator of effect, which was calculated by multiplying the MD and related weight of each study. Each study's WMD was summed to arrive at the total WMD [31]. The impact of GGCX SNPs on mean daily warfarin dose (MDWD) was counted by means of Z test; a P value <0.05 was considered statistically significant. Heterogeneity was assessed by a chi-squared Q test and I-squared statistics, as has been previously described in the literature [37, 38]. If $P_Q < 0.1$ or $I^2 > 50\%$, we considered the heterogeneity significant, and a random-effects model was conducted using the DerSimonian and Laird method. Otherwise, a fixed-effects model (the Mantel-Haenszel method) was used. Stratified analysis was carried out by ethnicity and the mean target INR. We defined the mean target INR < 2.5 and INR ⩾ 2.5 as two separated subgroups.

Sensitivity analyses were carried out by excluding studies one by one, especially excluded study with low quality or small sample size. Begg's funnel plot and Egger's test were used to investigate the publication

bias in the meta-analysis; $P < 0.05$ indicated that the result was statistically significant.

All the tests in this meta-analysis were conducted with RevMan 5.2.7 (Cochrane Collaboration) and STATA software (version 12.0; Stata Corporation, College Station, Texas, USA).

Results

Literature Selection and Study Characteristics

Fig. 1 shows the flow of selecting studies. According to the inclusion and exclusion criteria, 19 articles including 21 studies in 143 candidate publications were included in the meta-analysis. A total of 6957 patients were included between 2005 and 2014, of which 3552 patients for rs6996694, 1842 patients for rs11676382 and 1771 patients for rs12714145. Eleven eligible studies referred to the presence of the GGCX rs6996694 polymorphism [11–16,22,23,25], five for rs11676382 [18,23,26–28] and five for rs12714145 [3,17,18,22,29]. The articles by Schelleman H et al. [23] and Cavallari L et al. [3] were separated as different studies because they included two ethnicities and two SNPs respectively. Most of the studies are from Asian [12–17,22,25–27]; two studies from USA [18,23]. The main indications of warfarin are for use during heart valve replacement (HVR), deep vein thrombosis (DVT), atrial fibrillation (AF), pulmonary embolism (PE), and ischemic stroke. All studies except Schelleman H et al. [23] were graded as "++" during the quality score assessment; one article in Chinese [25]

and the others were published in English. The characteristics of the included studies are shown in Table 1.

Allele Frequencies in Different Ethnicities

GGCX gene frequencies and warfarin dose requirements are shown in Table 1. On average, the *GGCX* rs699664 frequencies of GG, GA and AA were 46.0%, 44.7%, and 9.3% for Asian, and 45.6%, 46.3% and 8.1% for Caucasian. No statistically significant difference was shown between the two ethnicities ($\chi^2 = 0.296$, $P = 0.863$). Similar results were showed in G allele vs. A allele model ($\chi^2 = 0.021$, $P = 0.884$). However, the frequencies of GG, GA and AA in African American were 20.9%, 44.3% and 34.8% respectively, which was significant difference with the other two ethnicities ($\chi^2 = 158.98$, $P = 0.000$).

For the *GGCX* rs11676382, genotype and allele frequencies of this site showed significant difference among Asian and Caucasian population ($P = 0.000$). The Asian population have a higher CC frequency than that of Caucasian group (97.7% vs. 86.9%). There was no GG genotype reported in Asian population.

With respect to GGCX rs12714145, significant difference was showed in genotype frequencies comparison ($\chi^2 = 17.770$, $P = 0.000$) in Caucasian and Asian, but not in allele frequencies comparison, and the Caucasian population have a higher GG frequency than that of Asian population (46.5% vs. 36.7%). However, the frequencies of GG, GA and AA in African American were similar with Asian population
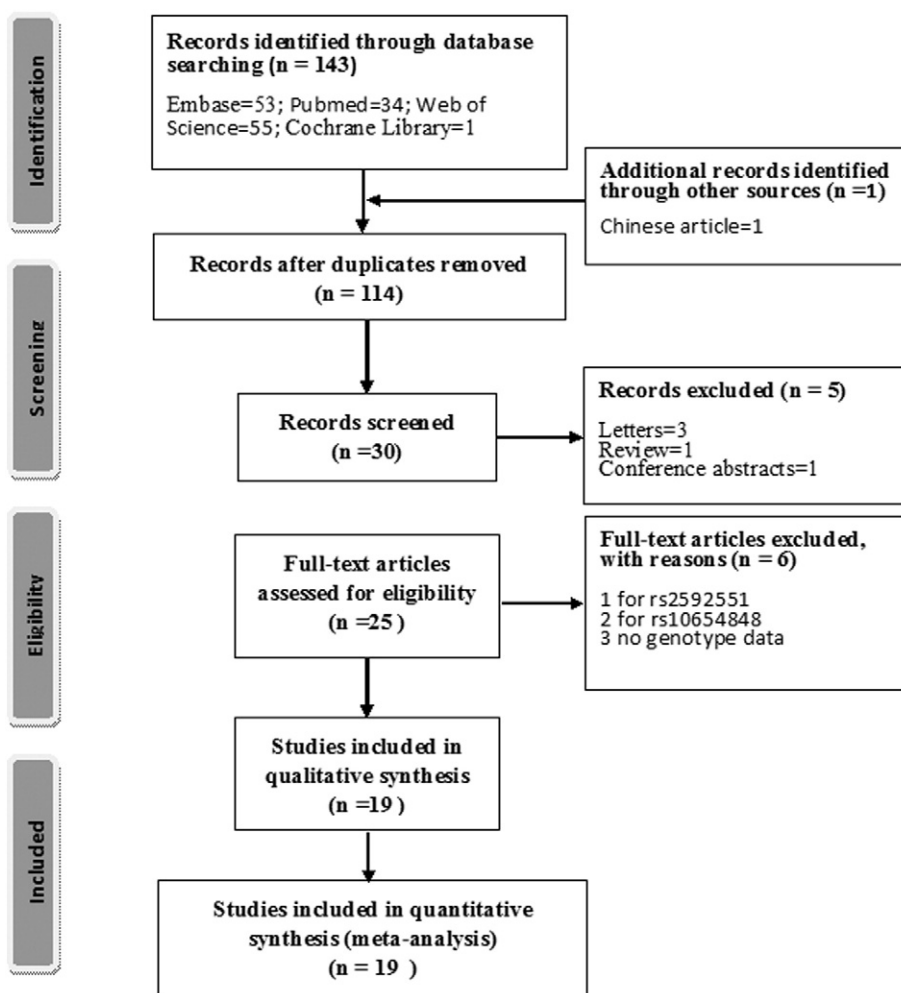

Fig. 1. Flow diagram of included studies for this meta-analysis.

Table 1
The characteristics of the included studies.

Study	Country	Ethnicity	Number (men %)	Age	Indication of warfarin	INR target range	Gene frequencies%			Warfarin dose (mean ± SD, md/day)			Quality score
rs699664							**GG**	**GA**	**AA**	**GG**	**GA**	**AA**	
Loebstein,R 2005	Israel	Caucasian	100(52)	62.0	HV,AF, DVT,PT	2.7 ± 0.5	50.0	41.0	9.0	5.50 ± 1.80	5.8 ± 1.90	5.14 ± 2.00	++
Kimura R 2007	Japan	Asian	93(71)	68.1 ± 10.0	Stoke	1.6-2.6	51.6	41.9	6.5	3.25 ± 1.19	2.63 ± 0.77	2.79 ± 1.07	++
Ohno M 2009	Japan	Asian	125(60)	73.1 ± 11.6	AF,DVT, PE	1.5-3.0	54.4	37.6	8.0	2.86 ± 1.26	2.49 ± 1.24	2.15 ± 1.11	++
Liu Y 2010	China	Asian	794(43)	46.8 ± 11.9	HV	1.8-3.0	45.3	45.7	8.9	1.54 ± 1.18	1.55 ± 1.05	1.61 ± 1.11	++
Choi JR 2011	Korean	Asian	564(54)	63.2 ± 11.7	HVR,AF,DVT,PT	2.1 ± 0.8	47.0	44.3	8.7	3.50 ± 2.00	3.00 ± 1.80	3.00 ± 2.00	++
SL Zhong 2012	China	Asian	841(42)	51.3 ± 7.5	HVR,AF,DVT	1.8-3.0	45.5	45.5	8.9	3.00 ± 1.67	2.90 ± 1.67	2.90 ± 1.67	++
Schelleman 2010	USA	African American	22	N/A	N/A	2.0-3.0	9.1	45.5	45.5	6.96 ± 1.13	6.61 ± 2.49	5.71 ± 2.38	+
		Caucasian	36	N/A	N/A	2.0-3.0	33.3	61.1	5.6	5.80 ± 2.57	5.25 ± 2.17	5.19 ± 1.29	+
Lou Y 2012	China	Asian	488(44.7)	56.7 ± 12.1	HVR,AF,DVT,PT	1.8-2.5	45.5	42.0	12.5	3.65 ± 1.52	3.37 ± 1.36	3.63 ± 1.24	++
Liang Y 2013	China	Asian	280(46.0)	47.9 ± 12.5	N/A	1.5-3.0	42.5	48.9	8.5	3.09 ± 1.04	3.46 ± 1.24	3.51 ± 1.44	++
Cavallari L 2013	USA	African American	208	58 ± 16	N/A	3.0	22.1	44.2	33.7	5.88 ± 0.74	5.98 ± 0.79	6.20 ± 1.22	++
rs11676382							**CC**	**CG**	**GG**	**CC**	**CG**	**GG**	
Krishna 2014	Indian	Asian	240(36.7)	43.3 ± 11.2	HVR,AF,DVT	3.0–3.5	97.9	2.1	-	4.8 ± 2.20	2.6 ± 0.50	N/A	++
King CR 2010	USA	Caucasian	985(50.7)	60 (15)	N/A	2.3 (0.4)	88.6	11.2	0.2	4.9 ± 1.60	4.1 ± 1.60	3.8 ± 2.20	++
Rathore SS 2014	Indian	Asian	225(67.1)	37.7 ± 1.3	VT	20.-3.5	94.2	4.9	0.9	0.056 (0.023)*	0.053 (0.024)*	N/A	++
Wypasek E 2014	Poland	Caucasian	479(53)	48.8 ± 14.7	N/A	2.0-3.0	84.3	14.8	0.9	47.8 ± 8.79#	33.87 ± 3.67#	N/A	++
Schelleman 2010	USA	Caucasian	36	N/A	N/A	2.0-3.0	88.9	11.1	N/A	46.5 ± 23.06#	38.3 ± 4.73#	N/A	+
rs121714145							**GG**	**GA**	**AA**	**GG**	**GA**	**AA**	
Liang Y 2013	China	Asian	283(46.0)	47.9 ± 12.5	N/A	1.5-3.0	36.7	51.6	11.7	3.20 ± 1.02	3.39 ± 1.25	3.35 ± 1.35	++
Huang SW 2010	China	Asian	217(41.5)	51.3 (15.0)	HVR,AF,DVT	1.8-3.0	38.7	53.9	7.3	2.69 ± 1.07	2.91 ± 1.16	3.39 ± 1.40	++
Geisen C 2011	Germany	Caucasian	75(49.3)	64 (19–92)	HVR,AF,VT	2.0-3.0	34.7	45.3	20.0	2.14 ± 0.28	2.16 ± 0.39	2.33 ± 0.46	++
King CR 2010	USA	Caucasian	985(50.7)	60 (15)	N/A	2.3 (0.4)	47.7	41.1	11.7	5.1 ± 1.60	4.7 ± 1.60	4.7 ± 1.60	++
Cavallari L 2013	USA	African American	211	58 ± 16	—	3.0	33.6	50.7	15.6	5.88 ± 0.84	5.93 ± 1.02	5.90 ± 0.92	

AF, Atrial Fibrillation; HVR, Heart Valve Replacement; MI, Myocardial Infarction; DVT, Deep Vein Thrombosis; PVR, Prosthetic Valve Replace; PE, Pulmonary Embolism; N/A, no data.
++, High score; +, Low score.
* mean daily dose, mg/kg body weight (SD).
# Warfarin dose (mean ± SD, md/week).

(37.6%, 52.6% and 9.8% for Asian, 33.6%, 50.7% and 15.6% for African American, respectively)

Meta Analyses Results

Impact of GGCX rs699664 on MDWD

The influence of GGCX rs699664 polymorphism on warfarin dose requirement is shown in Fig. 2. The total number of patients carrying AA, AG and GG were 367, 1476 and 1511, respectively. When compared to individuals with the homozygous GG genotype, all comparison model showed no significant impact on warfarin dose requirements. Stratified analysis by ethnicity and mean target INR showed similar results with the overall analysis (Table 2).

Impact of GGCX rs12714145 on MDWD

The influence of GGCX rs12714145 on warfarin dose requirement is shown in Fig. 3. The total number of patients carrying AA, AG and GG were 252, 807 and 752, respectively. The impact of rs12714145 on MDWD were similar to rs699664 and no significant difference was found in overall analysis. Only the analysis of AA vs. AG shows homogeneity ($I^2\% = 0.0$ and $P_Q = 0.500$ for heterogeneity) and a fixed-effects model was used, however, the other comparison models were all used random-effects model. Stratified analysis by ethnicity and mean target INR showed similar results with the overall analysis, however, the AA+AG vs. GG comparison model showed an decreased effect in Caucasian population, but opposite effect for Asian (Table 2).

Impact of GGCX rs11676382 on MDWD

The influence of GGCX rs11676382 on warfarin dose requirement is shown in Fig. 4. The total number of patients carrying CC, CG and GG

were 1638, 1930 and 11, respectively. However, because the GG genotype frequencies of the rs11676382 variant was below 5%, this meta-analysis only performed a CG+GG vs. GG comparison model. Analysis showed statistical heterogeneity in this comparison model ($I^2 = 82\%$ and $P_Q = 0.000$), hence a random-effects model was used. The result provided an evidence that G allele carriers required 27% ($P = 0.000$, 95%CI = 22%-33%) lower MDWD than that of CC genotype. Stratified analysis by ethnicity and mean target INR showed showed similar results in Caucasian group (23% lower, $P = 0.000$, 95%CI = 12%-33%, $I^2 = 84\%$ and $P_Q = 0.002$ for heterogeneity), INR < 2.5 group (17% lower, $P = 0.000$, 95%CI = 11%-23%, only one study) and INR ⩾ 2.5 group (31% lower, $P = 0.000$, 95%CI = 18%-44%, $I^2 = 75\%$ and $P_Q = 0.008$ for heterogeneity) using random-effects model, but not in Asian subgroup ($P = 0.270$) (Table 2).

Subgroup Analysis

Considering the influence of ethnicity on warfarin dosage requirement and the main determinant of warfarin dose adjustment is the value of INR, we also performed a stratified analysis by ethnicity and the mean target INR (Table 2). No statistically significant results happened in Asian group in all analysis, yet, with respect to rs11676382, G allele carriers showed 23% ($P = 0.000$, 95%CI = 12%-33%) lower warfarin dose requirement than CC genotype in Caucasian group. The results of stratified analysis according to the mean target INR were similar with the overall analysis and stratified analysis by ethnicity.

Sensitivity Analysis and Publication Bias

Sensitivity analysis was carried out by excluding each study one at a time. Further sensitivity analysis was performed by excluding the study of Schelleman H et al. [23], in which the sample is obviously smaller than that in other studies, moreover, the study was
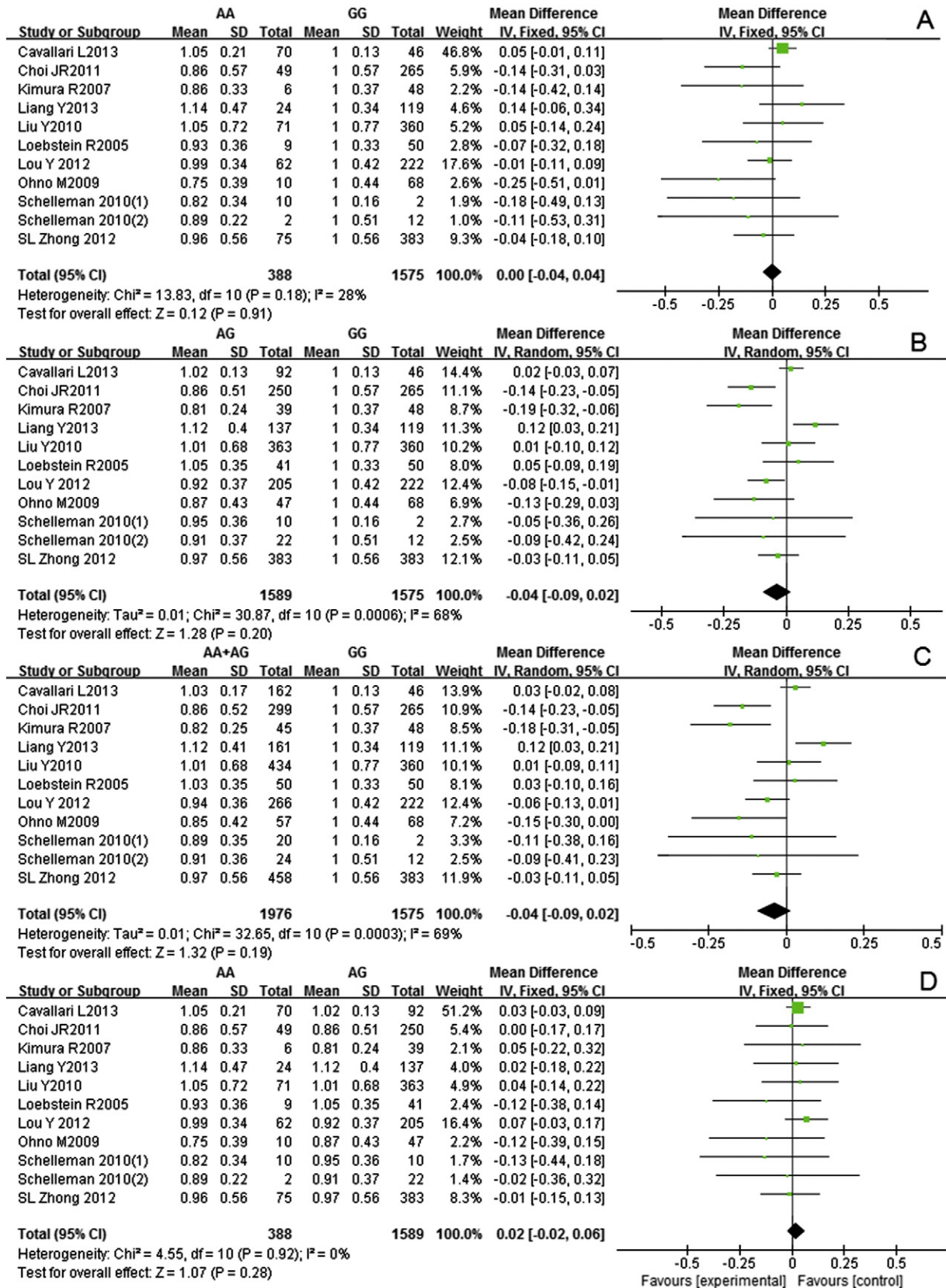
A

Study or Subgroup	AA Mean	AA SD	AA Total	GG Mean	GG SD	GG Total	Weight	Mean Difference IV, Fixed, 95% CI
Cavallari L2013	1.05	0.21	70	1	0.13	46	46.8%	0.05 [-0.01, 0.11]
Choi JR2011	0.86	0.57	49	1	0.57	265	5.9%	-0.14 [-0.31, 0.03]
Kimura R2007	0.86	0.33	6	1	0.37	48	2.2%	-0.14 [-0.42, 0.14]
Liang Y2013	1.14	0.47	24	1	0.34	119	4.6%	0.14 [-0.06, 0.34]
Liu Y2010	1.05	0.72	71	1	0.77	360	5.2%	0.05 [-0.14, 0.24]
Loebstein R2005	0.93	0.36	9	1	0.33	50	2.8%	-0.07 [-0.32, 0.18]
Lou Y 2012	0.99	0.34	62	1	0.42	222	17.6%	-0.01 [-0.11, 0.09]
Ohno M2009	0.75	0.39	10	1	0.44	68	2.6%	-0.25 [-0.51, 0.01]
Schelleman 2010(1)	0.82	0.34	10	1	0.16	2	1.9%	-0.18 [-0.49, 0.13]
Schelleman 2010(2)	0.89	0.22	2	1	0.51	12	1.0%	-0.11 [-0.53, 0.31]
SL Zhong 2012	0.96	0.56	75	1	0.56	383	9.3%	-0.04 [-0.18, 0.10]
Total (95% CI)			**388**			**1575**	**100.0%**	**0.00 [-0.04, 0.04]**

Heterogeneity: Chi² = 13.83, df = 10 (P = 0.18); I² = 28%
Test for overall effect: Z = 0.12 (P = 0.91)

B

Study or Subgroup	AG Mean	AG SD	AG Total	GG Mean	GG SD	GG Total	Weight	Mean Difference IV, Random, 95% CI
Cavallari L2013	1.02	0.13	92	1	0.13	46	14.4%	0.02 [-0.03, 0.07]
Choi JR2011	0.86	0.51	250	1	0.57	265	11.1%	-0.14 [-0.23, -0.05]
Kimura R2007	0.81	0.24	39	1	0.37	48	8.7%	-0.19 [-0.32, -0.06]
Liang Y2013	1.12	0.4	137	1	0.34	119	11.3%	0.12 [0.03, 0.21]
Liu Y2010	1.01	0.68	363	1	0.77	360	10.2%	0.01 [-0.10, 0.12]
Loebstein R2005	1.05	0.35	41	1	0.33	50	8.0%	0.05 [-0.09, 0.19]
Lou Y 2012	0.92	0.37	205	1	0.42	222	12.4%	-0.08 [-0.15, -0.01]
Ohno M2009	0.87	0.43	47	1	0.44	68	6.9%	-0.13 [-0.29, 0.03]
Schelleman 2010(1)	0.95	0.36	10	1	0.16	2	2.7%	-0.05 [-0.36, 0.26]
Schelleman 2010(2)	0.91	0.37	22	1	0.51	12	2.5%	-0.09 [-0.42, 0.24]
SL Zhong 2012	0.97	0.56	383	1	0.56	383	12.1%	-0.03 [-0.11, 0.05]
Total (95% CI)			**1589**			**1575**	**100.0%**	**-0.04 [-0.09, 0.02]**

Heterogeneity: Tau² = 0.01; Chi² = 30.87, df = 10 (P = 0.0006); I² = 68%
Test for overall effect: Z = 1.28 (P = 0.20)

C

Study or Subgroup	AA+AG Mean	AA+AG SD	AA+AG Total	GG Mean	GG SD	GG Total	Weight	Mean Difference IV, Random, 95% CI
Cavallari L2013	1.03	0.17	162	1	0.13	46	13.9%	0.03 [-0.02, 0.08]
Choi JR2011	0.86	0.52	299	1	0.57	265	10.9%	-0.14 [-0.23, -0.05]
Kimura R2007	0.82	0.25	45	1	0.37	48	8.5%	-0.18 [-0.31, -0.05]
Liang Y2013	1.12	0.41	161	1	0.34	119	11.1%	0.12 [0.03, 0.21]
Liu Y2010	1.01	0.68	434	1	0.77	360	10.1%	0.01 [-0.09, 0.11]
Loebstein R2005	1.03	0.35	50	1	0.33	50	8.1%	0.03 [-0.10, 0.16]
Lou Y 2012	0.94	0.36	266	1	0.42	222	12.4%	-0.06 [-0.13, 0.01]
Ohno M2009	0.85	0.42	57	1	0.44	68	7.2%	-0.15 [-0.30, 0.00]
Schelleman 2010(1)	0.89	0.35	20	1	0.16	2	3.3%	-0.11 [-0.38, 0.16]
Schelleman 2010(2)	0.91	0.36	24	1	0.51	12	2.5%	-0.09 [-0.41, 0.23]
SL Zhong 2012	0.97	0.56	458	1	0.56	383	11.9%	-0.03 [-0.11, 0.05]
Total (95% CI)			**1976**			**1575**	**100.0%**	**-0.04 [-0.09, 0.02]**

Heterogeneity: Tau² = 0.01; Chi² = 32.65, df = 10 (P = 0.0003); I² = 69%
Test for overall effect: Z = 1.32 (P = 0.19)

D

Study or Subgroup	AA Mean	AA SD	AA Total	AG Mean	AG SD	AG Total	Weight	Mean Difference IV, Fixed, 95% CI
Cavallari L2013	1.05	0.21	70	1.02	0.13	92	51.2%	0.03 [-0.03, 0.09]
Choi JR2011	0.86	0.57	49	0.86	0.51	250	5.4%	0.00 [-0.17, 0.17]
Kimura R2007	0.86	0.33	6	0.81	0.24	39	2.1%	0.05 [-0.22, 0.32]
Liang Y2013	1.14	0.47	24	1.12	0.4	137	4.0%	0.02 [-0.18, 0.22]
Liu Y2010	1.05	0.72	71	1.01	0.68	363	4.9%	0.04 [-0.14, 0.22]
Loebstein R2005	0.93	0.36	9	1.05	0.35	41	2.4%	-0.12 [-0.38, 0.14]
Lou Y 2012	0.99	0.34	62	0.92	0.37	205	16.4%	0.07 [-0.03, 0.17]
Ohno M2009	0.75	0.39	10	0.87	0.43	47	2.2%	-0.12 [-0.39, 0.15]
Schelleman 2010(1)	0.82	0.34	10	0.95	0.36	10	1.7%	-0.13 [-0.44, 0.18]
Schelleman 2010(2)	0.89	0.22	2	0.91	0.37	22	1.4%	-0.02 [-0.36, 0.32]
SL Zhong 2012	0.96	0.56	75	0.97	0.56	383	8.3%	-0.01 [-0.15, 0.13]
Total (95% CI)			**388**			**1589**	**100.0%**	**0.02 [-0.02, 0.06]**

Heterogeneity: Chi² = 4.55, df = 10 (P = 0.92); I² = 0%
Test for overall effect: Z = 1.07 (P = 0.28)

Favours [experimental]   Favours [control]

Fig. 2. Forest plots of impact of *GGCX* gene rs699664 polymorphism on warfarin dose requirement. **A**: AA vs. GG carriers, **B**: AG vs. GG carriers, **C**: A carriers (AA+AG) vs. GG carriers, **D**: AA vs. AG carriers. Mean (SD): mean and standard deviation of normalized warfarin doses associated with each genotype. CI: confidence interval.

defined as "+" according to quality score assessment. However, we did not find that this analysis revealed any significant change or revision in findings.

We also performed a stratified analysis by ethnicity (Table 2). No statistically significant results happened in Asian group in all analysis, yet, with respect to rs11676382, G allele carriers showed 23% (*P* =

Table 2
Stratified analysis by ethnicity and mean target INR.

Comparison models	overall	Ethnicities			Mean INR	
		Caucasian	Asian	African-American	INR < 2.5	INR ⩾ 2.5
rs699664						
AA vs. GG	0[-4,4]	-8[-3,14]	-3[-9,3]	-1[-22,19]	-3[-9,3]	3[-3,9]
AG vs. GG	-4[-9,2]	3[-10,16]	-6[-13,2]	2[-3,6]	-6[-13,2]	2[-2,6]
AA vs. AG	2[-2,6]	-8[-29,12]	3[-3,9]	2[-3,8]	3[-3,9]	2[-4,7]
AA+AG vs. GG	-4[-9,2]	1[-11,14]	-5[-13,2]	3[-2,7]	-5[-13,2]	2[-2,8]
rs12714145						
AA vs. GG	2[-6,10]	0[-17,16]	10[-3,24]	0[-6,6]	4[-13,21]	2[-4,8]
AG vs. GG	1[-5,7]	-4[-13,5]	7[0,14]	1[-4,6]	1[-10,13]	1[-3,5]
AA vs. AG	1[-3,5]	2[-4,7]	4[-10,17]	-1[-7,5]	1[-5,7]	1[-5,7]
AA+AG vs. GG	1[-6,8]	**-7[-11,-4]**	**7[1,14]**	1[-3,5]	2[-10,14]	1[-3,5]
rs11676382						
CG+GG vs. CC	**-27[-37,-17]**	**-23[-33,-12]**	-33[-70,5]	N/A	**-17[-23,-11]**	**-31[-44,-18]**

Data were showed as weight mean difference [95% Confidence Intervals].
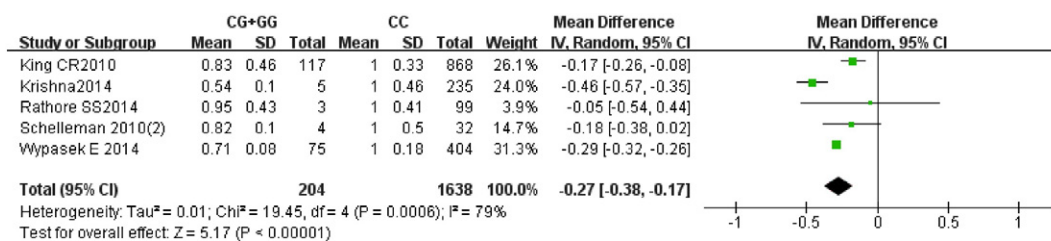-, reduction of warfarin dose requirement.
N/A, no data.

0.000, 95%CI = 13%-33%) lower warfarin dose requirement than CC genotype in Caucasian group.

Begg's funnel plot and Egger's linear regression test were also conducted to investigate the publication bias; the results of each study's genotype with respect to effect on warfarin dose reductions against the inverse standard error showed that no significant publication bias was detected in each compared model (All *P* > 0.05, data not shown).
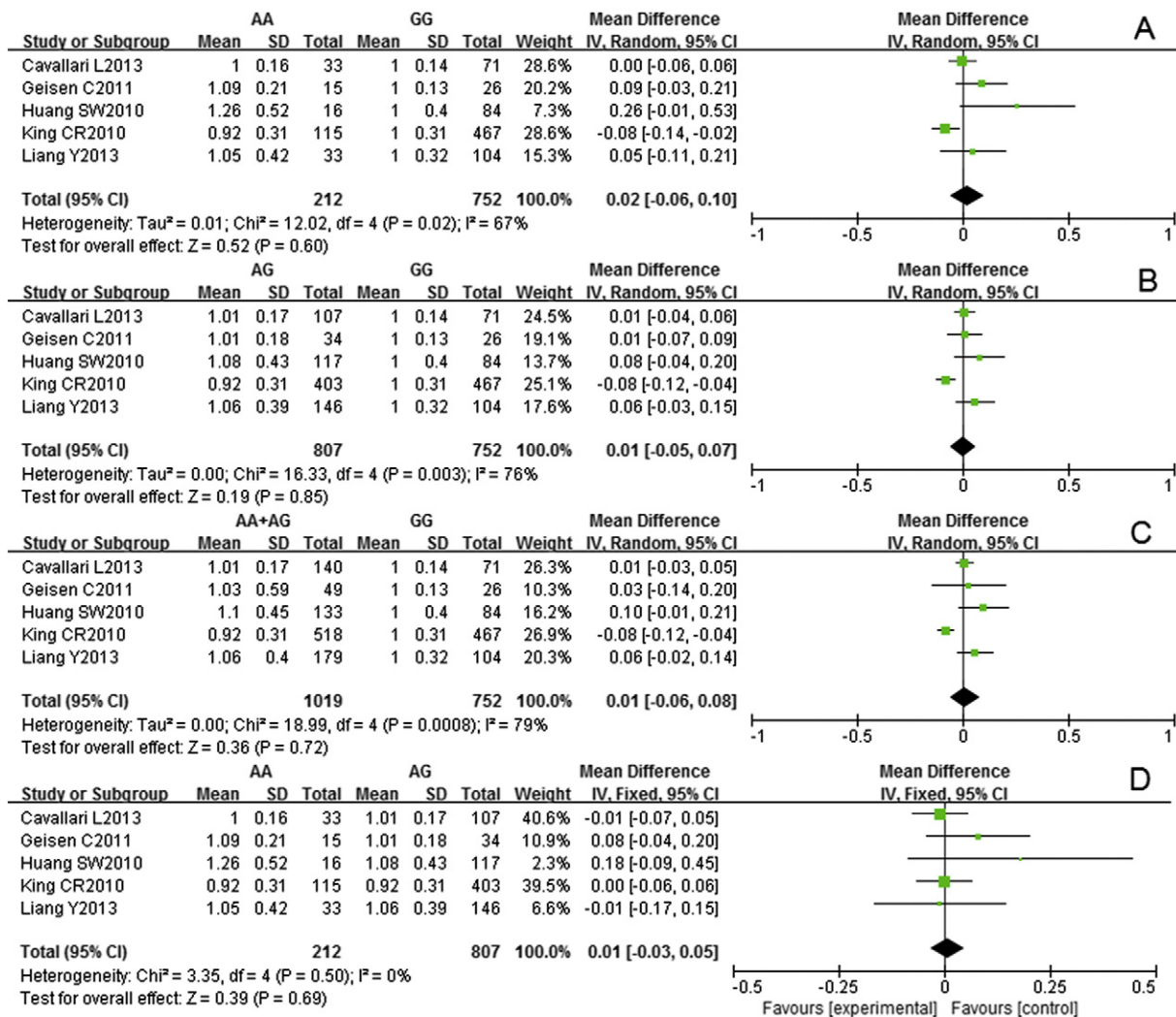
Fig. 3. Forest plots of impact of *GGCX* gene rs12714145 polymorphism on warfarin dose requirement. **A**: AA vs. GG carriers, **B**: AG vs. GG carriers, **C**: A carriers (AA+AG) vs. GG carriers, **D**: AA vs. AG carriers. Mean (SD): mean and standard deviation of normalized warfarin doses associated with each genotype. CI: confidence interval.

Study or Subgroup	CG+GG Mean	SD	Total	CC Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI	Mean Difference IV, Random, 95% CI
King CR2010	0.83	0.46	117	1	0.33	868	26.1%	-0.17 [-0.26, -0.08]	
Krishna2014	0.54	0.1	5	1	0.46	235	24.0%	-0.46 [-0.57, -0.35]	
Rathore SS2014	0.95	0.43	3	1	0.41	99	3.9%	-0.05 [-0.54, 0.44]	
Schelleman 2010(2)	0.82	0.1	4	1	0.5	32	14.7%	-0.18 [-0.38, 0.02]	
Wypasek E 2014	0.71	0.08	75	1	0.18	404	31.3%	-0.29 [-0.32, -0.26]	
Total (95% CI)			204			1638	100.0%	-0.27 [-0.38, -0.17]	

Heterogeneity: Tau² = 0.01; Chi² = 19.45, df = 4 (P = 0.0006); I² = 79%
Test for overall effect: Z = 5.17 (P < 0.00001)

Fig. 4. Forest plots of impact of *GGCX* gene rs11676382 polymorphism on warfarin dose requirement. Only CG+GG vs. CC model was carried out in this study because of few GG genotype. Mean (SD): mean and standard deviation of normalized warfarin doses associated with each genotype. CI: confidence interval.

Discussion

In the current systematic review and meta-analysis, our results indicated that *GGCX* polymorphisms influence warfarin dose requirements. The G carriers of the *GGCX* rs11676382 require warfarin dose that are 27% lower than that in carriers of CC genotype. However, no effects were found in the genetic variants of the *GGCX* rs699664 and rs12714145 SNPs. These results are based on data from almost 6957 patients, and the estimates are likely to be the most comprehensive published to date.

Genetic variation has a clinically important impact on stable warfarin dose. The association of *CYP2C9* genotype and warfarin dose requirement has previously been investigated in a meta-analysis, which included approximately 8000 patients, and demonstrated that 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 [36]. Yang et al. [31] performed a meta-analysis based on 19 studies to investigate the impact of *VKORC1* gene polymophism on warfarin dosage requirement, and their results indicates that the *VKORC1* 1173CT and −1639GA heterozygosities carriers required approximately 50% higher warfarin doses than 1173TT and −1639AA homozygosities carriers. Caldwell et al. [7] also found that patients with 2 TT alleles of *CYP4F2* require approximately 1 mg/day more warfarin than patients with 2 CC alleles. However, it has been found that polymorphisms of the above three genes, together with clinical characters such as gender, age, weight and body surface area, can only explain about 50% of the effect on warfarin dose requirement [7,39,40]. Therefore, other genetic variants, as this study suggested, the *GGCX* polymorphism may be considered in warfarin-dosing algorithms in predicting warfarin dose among different patients, although our pooled analysis indicated that effect of *GGCX* polymorphisms was lower than that of *CYP2C9* and *VKORC1* in terms of influencing warfarin maintenance dose.

Over the past decades, the rs699664 loci is the most studied SNP of *GGCX*, however, the importance of genetic polymorphisms of this SNP on warfarin dose requirement is still controversial. Our meta-analysis expanded the sample size and increasing the efficiency of statistical tests, and found no significant association between *GGCX* rs699664 polymorphism and warfarin dose requirement. Therefore, *GGCX* rs699664 polymorphism was not considered to affect individual warfarin dose requirements. Actually, Only three studies examined the effect of a subset of *GGCX* rs699664 polymorphism on warfarin dose in our systematic review, interestingly, this studies are all performed in Asia [12,15, 22]. Selection bias and recall bias of the study population should be considered when explained these inconsistent results.

Of note, a study shows that rs699664 and rs12714145 are within a region of strong linkage disequilibrium (LD) [9], however, rs11676382 is an unaffiliated tagSNP with rs699664 [41], therefore, it is not surprised that rs699664 and rs12714145 polymorphism showed the same impact on warfarin dose requirement in overall analysis, and the genetic variations of rs11676382 loci has a different impact on warfarin dose requirement. In addition, this study found rs12714145 A carriers showed different impact on warfarin dose compared to GG genotype in Caucasian and Asian group, but not in rs699664. In fact, for the

rs12714145 SNP, only two studies for Caucasian and Asian populations respectively, rendering the analysis a lower statistical power, therefore, the inconsistent results may be due to insufficient studies for rs12714145 SNP.

Considering racial background influenced warfarin dosage, we carried out a stratified analysis by ethnicity. The results indicated the impact of rs11676382 polymorphism on warfarin dose requirement mainly existed in Caucasian. This difference, however, was not statistically significance, although there was similar trend, in Asian group, which indicates that the effects of rs11676382 polymorphism on warfarin dose requirement are different in ethnicities. In fact, the genotype and allele frequencies of *GGCX* rs11676382 showed significant difference among Asian and Caucasian population in our systematic review, therefore, different genetic backgrounds may contribute to the discrepancy. However, we should notice that there are only two Asian studies in the analysis of *GGCX* rs11676382, and limited studies and small sample sizes may lead to insufficient statistical power, therefore, we should carefully interpreted the negative results. No significant difference in the allele frequency of rs699664 and rs12714145 SNPs between Asian and Caucasian, thus, rs699664 and rs12714145 may not be the genetic factor attributing to ethnic differences in warfarin dose.

The main determinant of warfarin dose adjustment is the value of INR, while the racial background, such as genetic, cultural (diet related), or both, is a determinant of warfarin dosage to maintain the target INR [42]. After adjusted for confounding factors, Dang et al. [43] found that all of the mean weekly warfarin doses were significantly different in Asians, Hispanics, Whites and African Americans, with a INR goal of 2 to 3.Therefore, the target INR is mainly influenced by ethnicity. Our subgroup analysis further confirmed this point, and it is possible to understand the results of subgroup analysis according to the mean target INR were similar with stratified analysis by ethnicity. In addition, target INR is also affected by age. The guidelines of the Japanese Circulation Society for the treatment of nonvalvular atrial fibrillation suggested that target INR 2.0-3.0 is suitable for patients <70 years of age and lower intensity with INR 1.6-2.6 for ≥70 years of age [44]. However, our systematic review found only one study with patients ≥70 years of age [13], therefore, we did not further performed a stratified analysis by different age.

Due to different warfarin dose in different study populations, it is understandable that significant heterogeneity were observed in some compared models, however, any significant change or revision in findings was also not found in sensitivity analysis, especially excluded the study with low quality scores and small sample numbers. Taken together, we believe our pooled analysis of the impact of GGCX genotype on warfarin dose requirements are reliable.

Limitations and Perspectives

There are several limitations in our meta-analysis that need to be considered when interpreting the results. First, as we know now, VKORC1, CYP2C9, and CYP4F2 have been confirmed as predictors of warfarin dose requirement. Theoretically, the normalization procedure used in our meta-analysis should remove any influences of VKORC1, CYP2C9 and CYP4F2 genotype on the GGCX–warfarin dose association. However, due to lack of original data from authors or studies that

combine two or more of the above genes (i.e., the *GGCX* polymorphism with one of the other 3 genes), this remains to be shown in future studies. Second, although the warfarin dose effects of rs11676382 is demonstrated, we should notice that the number of studies in patients of rs11676382 SNP was small (n = 5). It is possible that the lack of association in the Asian group in rs11676382 SNP is related to insufficient studies. Therefore, the results need to be further confirmed in larger sample and different ethnicities. At last, previous studies most focus on Asian and Caucasian populations, other ethnicity are need to further investigate because our study indicates that the effects are different in ethnicities.

In the past few years, the novel oral anticoagulants (NOACs), such as Xa inhibitors rivaroxaban, apixaban, and edoxaban, have been approved for clinical use. An advantage of the NOACs over warfarin is their stable and consistent pharmacokinetics. However, despite being significantly more costly than warfarin (approximately $6–7 per day),it is difficult to find an suitable antidote in the event of major bleeding using NOACS [45]. For now, warfarin is still the most important anticoagulants, and need for modeling more precise warfarin-dosing algorithms based on pharmacogenomics.

Conclusions

This is the first meta-analysis about the impact of GGCX gene polymorphisms on warfarin dose requirements and the results showed that GGCX rs11676382 polymorphism have a significant impact on warfarin dose requirements, especially in Caucasian populations. More and larger studies in multiethnic population are expected to be published on this topic in the future, as it remains a topic of concern and interest.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

YS, SL and XQ conducted the literature search and quality assessment and contributed to the drafting of the manuscript. YS and TL performed data extraction and statistical analysis. XL consulted on statistical analysis. YD and ZW critically revised drafts of the manuscript. JC conceived and designed the review, assisted with the inclusion/exclusion criteria, acted as independent assessors and critically revised drafts of the manuscript. All of the authors contributed to the interpretation of data and all of the authors critically revised the manuscript. All of the authors are guarantors for the study. All of the authors read and approved the final manuscript.

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Appendix A. Supplementary Data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.thromres.2015.01.029.

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