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Impact of CYP2C9, VKORC1 and CYP4F2 genetic polymorphisms on maintenance warfarin dosage in Han-Chinese patients: A systematic review and meta-analysis

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ABSTRACT

Introduction: Warfarin is the most commonly used antithrombotic drug. Single nucleotide polymorphisms (SNPs) of CYP2C9, CYP4F2, VKORC1 1173 and VKORC1-1639 influence warfarin maintenance dosage. We aimed to determine the impact of SNPs of these genes on mean daily warfarin dosage (MDWD) in Han-Chinese patients.

Methods: Strict literature inclusion criteria were established, and literature searching was performed on PubMed, Embase and Cochrane Library for English articles and CNKI, CBM and Wanfang database for Chinese articles before September 2, 2014. Revman 5.3 was used to analyze the relationship between gene SNPs and MDWD in Han-Chinese subjects.

Results: We included 33 studies researching the impact of gene SNPs on MDWD in Han-Chinese subjects. CYP2C9 *3/*3, *1/*3 and *3 carriers needed a 72% (95% confidence interval [CI]: 62.0%–81.0%), 28% (22.0%–33.0%) and 26% (21.0%–32.0%) lower MDWD, respectively, than CYP2C9 *1/*1 carriers. CYP4F2 TT, CT and T carriers required a 18% (7.0%–30.0%), 7% (7.0%–7.0%) and 11% (7.0%–14.0%) higher MDWD, respectively, than CYP4F2 CC carriers. VKORC1 1173 CC, CT and C carriers required a 98% (78.0%–118.0%), 49% (37.0%–62.0%) and 56% (44.0%–67.0%) higher MDWD, respectively, than VKORC1 1173 TT carriers. VKORC1-1639 GG, GA and G carriers needed a 101% (53.0%–149.0%), 40% (36.0%–45.0%) and 38% (35.0%–42.0%) higher MDWD, respectively, than VKORC1-1639 AA carriers.

Conclusions: This meta-analysis is the first to report the relationship between genotypes and MDWD among Han-Chinese patients. The results showed that SNPs of CYP2C9, CYP4F2, VKORC1 1173 and VKORC1-1639 significantly influenced the MDWD in Han-Chinese patients.

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1. Introduction

Warfarin is the most commonly used oral anticoagulant. Its therapeutic window is rather narrow, and its dose must be adjusted according to the international normalized ratio (INR). A high target INR leads to a high risk of bleeding, while embolism events will occur if the target INR is too low. Many clinical and environmental factors, including age, sex, race, body size, co-morbidities and co-medications, as well as gene mutations affect warfarin dose requirements (Xie et al., 2001; Cavallari et al., 2010; Carlquist et al., 2006; Cheng et al., 2009; Klein et al., 2009; Yoshizawa et al., 2009). Cytochrome P450 2C9 (CYP2C9, rs1057910), cytochrome P450 4F2 (CYP4F2, also knew as V433M, rs2108622) and vitamin K epoxide reductase complex subunit 1 (VKORC1, include VKORC1 1173, rs9934438 and VKORC1-1639 also known as 3673, rs9923231) gene polymorphisms are widely considered to be associated with interindividual variations in warfarin dosage.

CYP2C9, VKORC1 and CYP4F2 gene polymorphisms can explain 40%–60% of the variation in interindividual warfarin doses (Klein et al., 2009; Rieder et al., 2005; Krishna Kumar et al., 2014; Lee et al., 2006; Veenstra et al., 2005; Wadelius et al., 2005), while non-genetic factors such as age and sex are considered to account for <15% of this variation (Visscher et al., 2009). The US Food and Drug Administration recommends that genotyping be carried out before the prescription of warfarin in order to improve its therapeutic effect (FDA, 2007).

Several meta-analyses (Sanderson et al., 2005; Lindh et al., 2009a; Yang et al., 2010; Jorgensen et al., 2012; Liang et al., 2012a) have explored the impact of CYP2C9, CYP4F2, VKORC1 1173 and VKORC1-1639 gene polymorphisms on mean daily warfarin dosage (MDWD) in Caucasian, African and Asian subjects. As the first meta-analysis of the impact of gene polymorphism warfarin dosage requirement, Sanderson et al. (2005) included 19 studies in their research and found that patients with CYP2C9*2 and CYP2C9*3 alleles need lower MDWD than wild-type homozygotes CYP2C9*1*1. Subsequently, Lindh et al., (2009a) and Jorgensen et al., (2012) also the drew similar conclusions by conducting meta-analysis separately. Yang et al. (2010) found that the impacts of gene polymorphism on warfarin dosage requirement were significantly different between Caucasian and Asian population. Patients with VKORC1 1173 CTand 1173 CC required 44% (95% CI, 32%, 56%) and 97% (95% CI, 73%, 122%) higher MDWD than 1173 TT carriers. VKORC1-1639GA and -1639 GG carriers required 52% (95% CI, 41%, 64%) and 102% (95% CI, 85%, 118%) higher MDWD than -1639AA carriers. Liang et al. (2012a) reported that carriers of CYP4F2 CT, TT genotypes required 10.0% (95% CI, 4.0–15.0) and 21.0% (95% CI, 9.0–33.0) higher warfarin doses than homozygous CC respectively. Although genetic associations with warfarin response vary between ethnicities, but most of the previous meta-analyses were conducted by including Caucasian, African and Asian subjects.

However, no such meta-analysis has been conducted of studies involving only Han-Chinese subjects. Such an analysis is required because the size of the Han-Chinese population is up to 1.37 billion (M-S Wen.pdf). We therefore conducted a systematic review and meta-analysis to clarify the relationship between gene polymorphisms and MDWD in the Han-Chinese, and to determine which genotype must be tested for before prescribing warfarin. Our study includes 33 papers published in recent years about CYP2C9, CYP4F2, VKORC1 1173 and VKORC1-1639 gene polymorphisms in the Han-Chinese.

2. Medthods

2.1. Search strategy

We searched the PubMed, Embase, Cochrane Library, Chinese National Knowledge Infrastructure (CNKI), China Biology Medicine (CBM) and Wanfang databases for articles published before September 2, 2014. Print periodicals were also searched. The literature search was limited to studies published in English and Chinese. Studies written in English were searched for on the Cochrane Library, PubMed and Embase databases, and studies written in Chinese were searched for on the CNKI, CBM and Wanfang database or identified through the print periodical search. The keywords were warfarin (MeSH Terms) and Chinese (MeSH Terms) plus any of the following terms: genes (MeSH Terms, mutation (MeSH Terms), polymorphisms (MeSH Terms), Genetic Polymorphism (MeSH Terms), pharmacogenetics (MeSH Terms), CYP2C9 (MeSH Terms), CYP4F2 (MeSH Terms), VKORC1 (MeSH Terms), VKORC1 1173 (Free Terms) and VKORC1-1639 (Free Terms). Corresponding Chinese medical terms were used when we searched on CNKI databases for literatures written in Chinese.

2.2. Study selection

To be included, studies had to meet the following criteria: (1) patients received warfarin treatment, (2) patients were Han-Chinese, (3) at least one of the four target genes was tested and (4) warfarin maintenance dosage was mentioned along with the target gene(s). There were no special limits on INR range, patient characteristics (diseases, age, weight and height) and use of other drugs.

2.3. Data extraction

The research data were extracted and sorted by two reviewers (Chen CM and Chen ZJ) independently. Information such as year of publication, location (province or city), disease types, target INR, gene frequencies (wild type and variant type) and average warfarin maintenance dosage was extracted from the selected studies. Then, the two reviewers checked the integrity and accuracy of the extracted data, and resolved any differences or common points of confusion by discussion. In case of disagreements, other researchers read the literature and decided whether or not to include the study. If there was unclear or missing information in any of the studies, we were to contact the authors via phone or e-mail to obtain additional information.

2.4. Study quality assessment

We applied the checklist recommended by the Cochrane handbook (Julian and Green, 2011) as well as other methods recommended in related literature (Little et al., 2002; Jüni et al., 1999) to assess the quality of the selected papers: (1) study purpose, (2) validity of genetic analysis, including type of study sample, time of sample collection, definition of each genotype and genotyping methods used, (3) subject selection, including geographic area from which the subjects were recruited, subjects' age (mean age and standard deviation or age range), sex ratio, and (4) statistical issues, e.g., number of subjects included and analysis method.

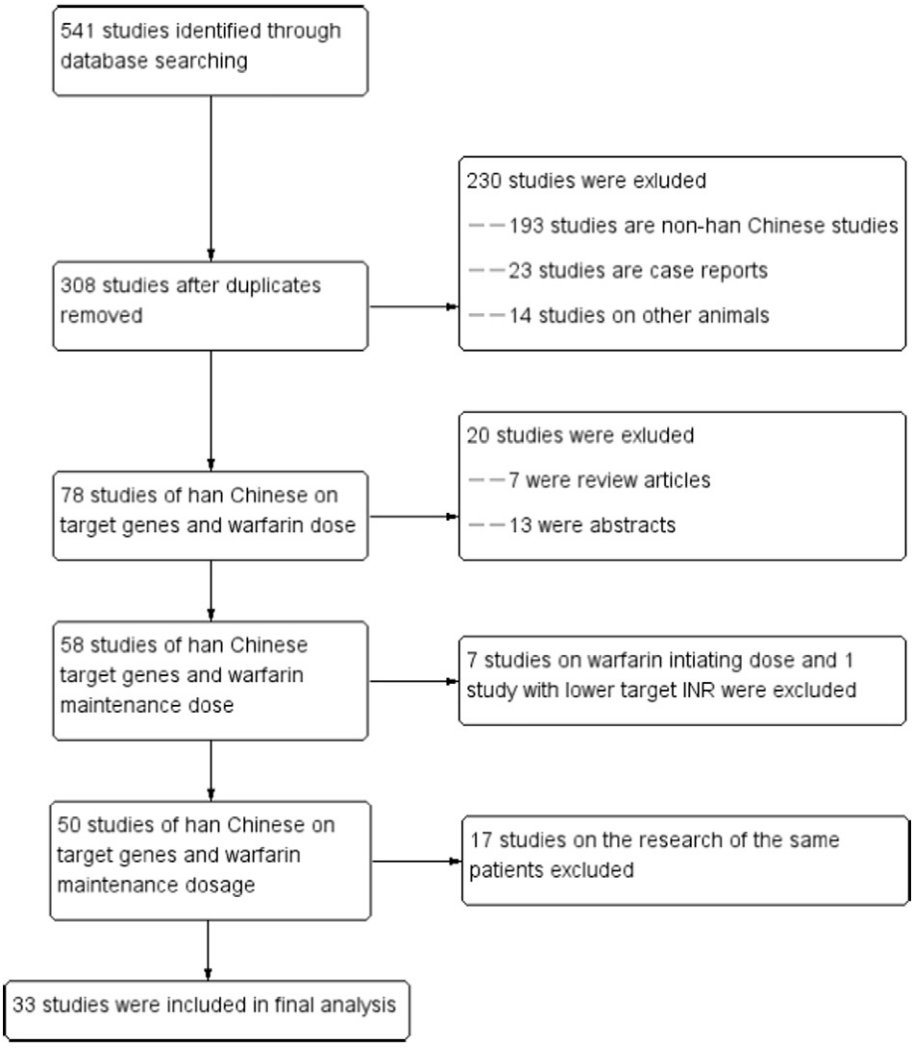

Fig. 1. Flow diagram showing the number of citations identified, retrieved, extracted and included in the final analysis.

2.5. Statistics

The data extracted from each eligible study were inputted into a computer. Revman 5.3 (Cochrane Collaboration) was used to analyze the relationship between MDWD and gene single nucleotide polymorphisms (SNPs) in the Han-Chinese. Four independent analyses were carried out for the four target genes (CYP2C9, CYP4F2, VKORC1 1173 and VKORC1-1639). Each analysis contained at least three studies. We referred to previously described methods (Lindh et al., 2009a; Liang et al., 2012a) to normalize the maintenance dose by using the homozygous wild-type group as a reference. In each study, the mean dose and standard deviation for each gene type was divided by the dose for the homozygous wild type. For clearer expression, we defined CYP2C9 *1/*3 or *3/*3 patients as "CYP2C9 *3 carriers," CYP4F2 CT or TT patients as "CYP4F2 T carriers," VKORC1 1173 CT or CC patients as "VKORC1 1173 C carriers" and VKORC1-1639 GA or GG patients as "VKORC1-1639 G carriers".

In the analysis of the impact of the CYP2C9 gene, the normalized dose for the genotypes CYP2C9 *1/*3, *3/*3 and *3 were compared to those for the genotype CYP2C9 *1/*1. Furthermore, the data for the CYP2C9 *1/*3 group were compared to that for the CYP2C9 *3/*3 group. Thus, there were four independent analyses for the gene CYP2C9. Similarly, we carried out four analyses for the other genes as well: CYP4F2, CT, TT and T carriers vs. CC carriers and CT vs. TT carriers; VKORC1 1173, CT, CC and C carriers vs. TT carriers and CT vs. CC carriers; and VKORC1-1639, GA, GG and G carriers vs. AA carriers and GA vs. GG carriers. Studies were weighted using the inverse variance method, and the effect of each genotype on MDWD was presented as the mean difference.

Since the data were normalized, the calculated mean difference represented the relative difference rather than the actual difference in maintenance dose. For example, a mean difference of 0.5 indicates a 50% increase in warfarin dose. In each analysis, the sum of the mean differences in every study was equal to the total weighted mean difference. We used the Z test to examine the impact of SNPs on MDWD, and the level of statistical significance was set to $P < 0.05$. Heterogeneity among studies was tested using Cochran's Q test (Mantel–Haenszel chi-square test), and measured using the variation across studies attributable to heterogeneity rather than due to chance (I^2). A P-value ≥ 0.1 or an I^2 value ≤ 25% indicated that no or low heterogeneity existed among the studies. In this case, the fixed-effects model was used; otherwise, the random-effects model was selected.

We performed sensitivity analyses by deselecting the studies one by one in chronological order. We also conducted the funnel plots by using Revman 5.3 to check for publication bias. Software STATA 12.0 was used to explore the source of heterogeneity in the analysis results via meta-regression analysis. Software Comprehensive Meta Analysis V2 was used to conduct a cumulative meta analysis of gene CYP 4F2.

Table 1
Characteristics of included studies.

	Studies	Study location	Indication of warfarin	Number of sample (ALL/M)	Age	INR target range	Genotype frequencies											
							CYP2C9			CYP4F2			VKORC1 1173			VKORC1-1639		
							*1/*1	*1/*3	*3/*3	CC	CT	TT	TT	TC	CC	AA	GA	GG
1	Cen et al. (2010)	Guangdong	MHVR	222/104	45 ± 12	1.8–2.4	91.0	8.0	1.0	52.0	41.0	7.0	–	–	–	–	–	–
2	Chen et al. (2014)	Beijing	HVR,AF	551/308	51 (43–60)	1.6–2.5	91.2	8	0.2	52.3	38.8	8.9	–	–	–	–	–	–
3	Cheng et al. (2009)	Fujian	AF, HVR	248/132	68.86 ± 6.09	1.5–3.0	86.7	12.1	1.2	–	–	–	–	–	–	65.3	26.6	8.1
4	Du et al. (2010)	Beijing	AF,DVT,HVR,PE	190/109	60 (18–89)	1.5–3.0	91.58	8.42	0	–	–	–	–	–	–	85.79	13.16	1.05
5	Gao et al. (2010)	Fujian	MHVR	119/50	44.58	1.5–2.5	92.44	7.56	0	–	–	–	–	–	–	84.03	15.97	0
6	Gu et al. (2010)	Chongqing	MHVR	127/59	44.3 ± 17.6	1.5–2.0	85	12.6	2.4	–	–	–	–	–	–	–	–	–
7	Huang et al. (2009a)	Guangdong	HVR,AF,DVT	266/123	51.5 ± 15.0	1.8–3.0	90.2	9.4	0.4	–	–	–	77.4	21.1	1.5	–	–	–
8	Huo et al. (2008)	Guangdong	HVR	93/45	21–62	1.5–2.0	84.95	15.05	0	–	–	–	–	–	–	–	–	–
9	Jiang et al. (2007)	Jiangsu	/	102/51	53.6 ± 16	1.5–2.5	–	–	–	–	–	–	81.4	15.7	2.9	–	–	–
10	Li et al. (2012)	Jiangxi	MVR,AVR,DVR	352/123	61.8 ± 6.6	1.8–2.5	–	–	–	58	34.9	7.1	–	–	–	–	–	–
11	Li and Sheng (2010)	Jiangsu	HVR,AF	73/41	54.98 ± 14.10	1.5–3.0	80.82	16.44	2.74	–	–	–	–	–	–	76.71	19.18	4.11
12	Liang et al. (2012b)	Beijing	AF,DVT,PE,HVR	115/71	64.9 ± 13.0	2.0–3.0	92.2	7.8	0	41.7	47.8	10.4	–	–	–	85.2	14.8	0
13	Liang et al. (2013)	Yunnan	–	300/138	47.9 ± 12.5	1.5–3.0	92	7.3	0.7	57.3	40	2.7	–	–	–	–	–	–
14	Liu and Zhang (2010)	Beijing	PE	108/46	59.02	2.0–3.0	–	–	–	–	–	–	–	–	–	77.78	21.3	0.92
15	Lou et al. (2012)	Beijing	HVR,AF	161/89	64.53	1.5–3.0	87.58	12.42	0	–	–	–	–	–	–	–	–	–
16	Lu et al. (2013)	Jiangsu	MHVR	197/82	47.0 (18–76)	1.5–2.8	94.4	5.6	0	–	–	–	–	–	–	80.7	18.8	0.5
17	Ma et al. (2012)	Beijing	AF,DVT,PE,HVR	312/119	56.6 ± 16.0	1.6–3.0	87.8	12.2		56.1	43.9		–	–	–	84.9	15.1	
18	Meng et al. (2011)	Jiangsu	RHD, AF, DVT	125/48	51.16	1.8–3.0	–	–	–	–	–	–	87.2	12	0.8	87.2	12	0.8
19	Miao et al. (2007)	Jiangsu	AF,DVT,PE,HVR	178/74	54.7	1.5–3.0	91	9	0	–	–	–	–	–	–	83.7	15.7	0.6
20	Tan et al. (2013)	Hunan	MHVR.	317/95	45.2 ± 10.5	2.1–2.8	91.5	7.9	0.6	63.4	33.75	2.84	–	–	–	80.7	18	1.3
21	Tang et al. (2009)	Beijing	VTE	205/108	60.1 ± 13.8	2.0–3.0	–	–	–	–	–	–	86.8	12.2	1	–	–	–
22	Veenstra et al. (2005)	Hongkong	AF, DVT, RHD	69/32	58.0 ± 10.0	1.8–3.2	94.2	5.8	0	–	–	–	–	–	–	76.8	20.3	2.9
23	Wang and Zhong (2013)	Sichuan	Orthopedic Surgery	214/114	51.6 ± 7.5	2.0–3.0	90.7	8.4	0.9	–	–	–	–	–	–	79.9	19.2	0.9
24	Wang et al. (2011)	Liaoning	/	196/80	61.89	1.8–3.0	–	–	–	50.51	42.86	6.63	–	–	–	–	–	–
25	Wei et al. (2012)	Jiangsu	NVAF,DVT,MHVR	325/153	66.5 ± 12.9	1.5–3.0	90.8	9.2	0	56	33.3	10.8	86.8	12.9	0.3			
26	Yang et al. (2011)	Jiangsu	/	178/74	55.3	1.5–3.0	–	–	–	–	–	–	86.5	12.9	0.56	–	–	–
27	Yuan et al. (2005)	Taiwan	/	104/56	58.6 ± 14.4	1.58–2.55	–	–	–	–	–	–	–	–	–	79.8	18.3	1.9
28	Zhang et al. (2012b)	Jiangsu	MHVR	197/82	52.92 ± 11.76	1.5–2.8	–	–	–	58.38	37.06	4.57	–	–	–	–	–	–
29	Zhang et al. (2007a)	Xinjiang	HVR	88/41	45.1	1.5–2.0	77.27	14.77	0	–	–	–	–	–	–	–	–	–
30	Zhang et al. (2012a)	Beijing	VTE,PE	297/148	64	2.0–3.0	91.2	8.8	0	–	–	–	85.5	13.8	0.7	–	–	–
31	Zhang et al. (2007b)	Fujian	RHD,AF	129/52	45.79 ± 12.06	1.5–3.0	90.7	8.15	0.8	–	–	–	–	–	–	74.42	23.26	2.3
32	Zheng et al. (2008)	Beijing	/	123	/	/	92.68	6.5	0	–	–	–	–	–	–	–	–	–
33	Zhuang et al. (2014)	Shanghai	AF,DVT,HVR	214/110	65.72 ± 10.59	1.5–3.0	92.52	7.48	0	57.94	42.06		82.71	17.29	0	–	–	–

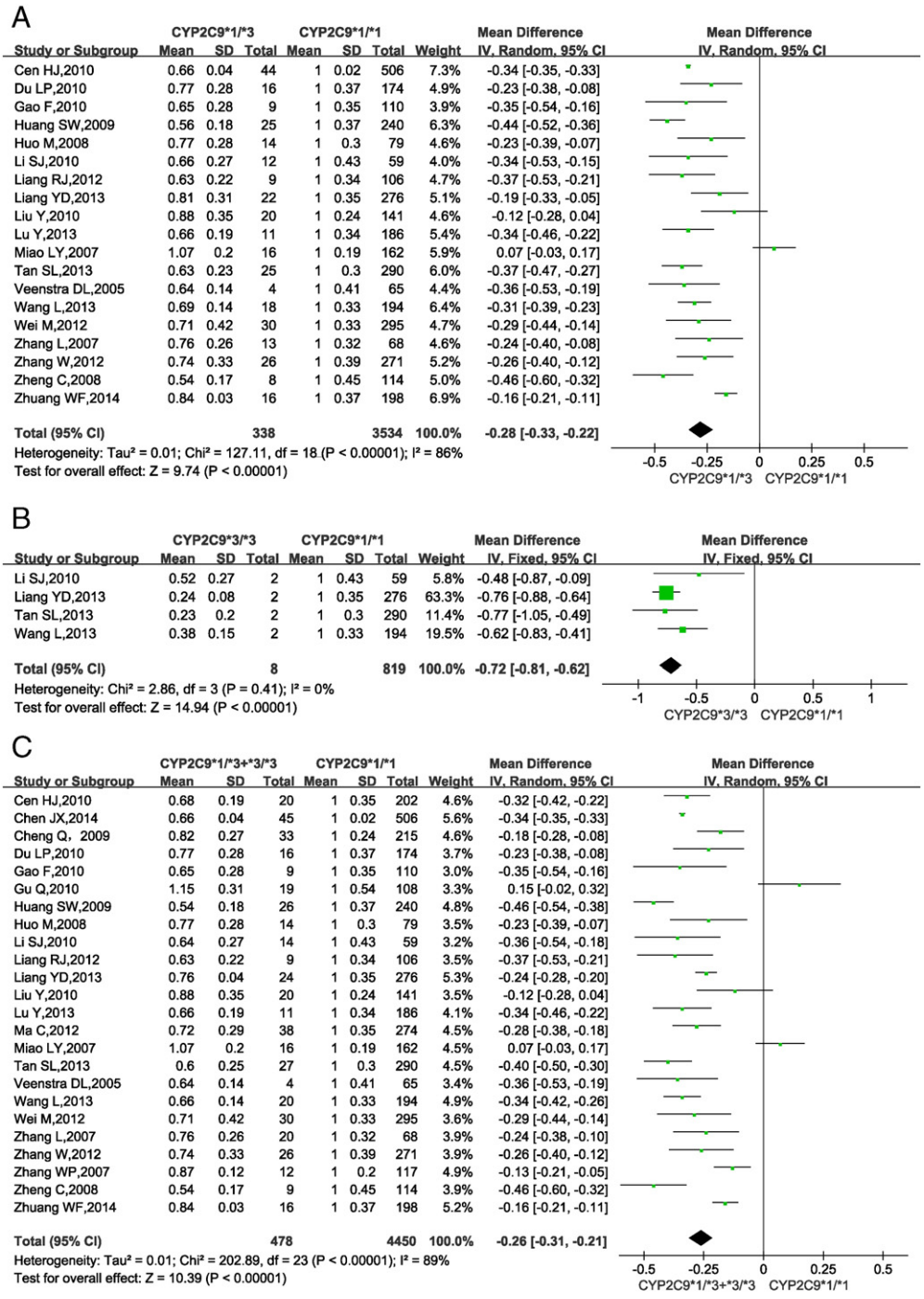

Fig. 2. Forest plots of impact of CYP2C9 SNPs on warfarin dosage requirements. (A) Relative warfarin dosage requirements of CYP2C9 *1/*3 carriers compared to those of wild-type CYP2C9 *1/*1 carriers. (B) CYP2C9 *3/*3 vs. *1/*1 carriers. (C) CYP2C9 *3 carriers (*1/*3 or *3/*3) vs. *1/*1 carriers. SD: standard deviation of normalized warfarin doses associated with each genotype. CI: confidence interval.

3. Results

3.1. Study selection

The literature screening process is illustrated in Fig. 1. Initially, 541 studies were retrieved, of which only 33 eligible studies were included in this systematic review and meta-analysis. Of the eligible studies, 16 were in English (Veenstra et al., 2005; Cen et al., 2010; Chen et al., 2014; Gu et al., 2010; Huang et al., 2009a; Li et al., 2012; Liang et al., 2012b; Liang et al., 2013; Lu et al., 2013; Ma et al., 2012; Miao et al., 2007; Tan et al., 2013; Wei et al., 2012; Yang et al., 2011; Yuan et al., 2005; Zhang et al., 2012a), the another 17 were in Chinese (Cheng et al., 2009; Du et al., 2010; Gao et al., 2010; Huo et al., 2008; Jiang et al., 2007; Li and Sheng, 2010; Liu and Zhang, 2010; Lou et al., 2012; Meng et al., 2011; Tang et al., 2009; Wang and Zhong, 2013; Wang et al., 2011; Zhang et al., 2012b; Zhang et al., 2007a; Zhang et al., 2007b; Zheng et al., 2008; Zhuang et al., 2014).

3.2. Study characteristics

All patients included in this meta-analysis were Han-Chinese from mainland China, Hong Kong and Taiwan. A total of 6495 patients were included in the current meta-analysis. The indications for warfarin in these

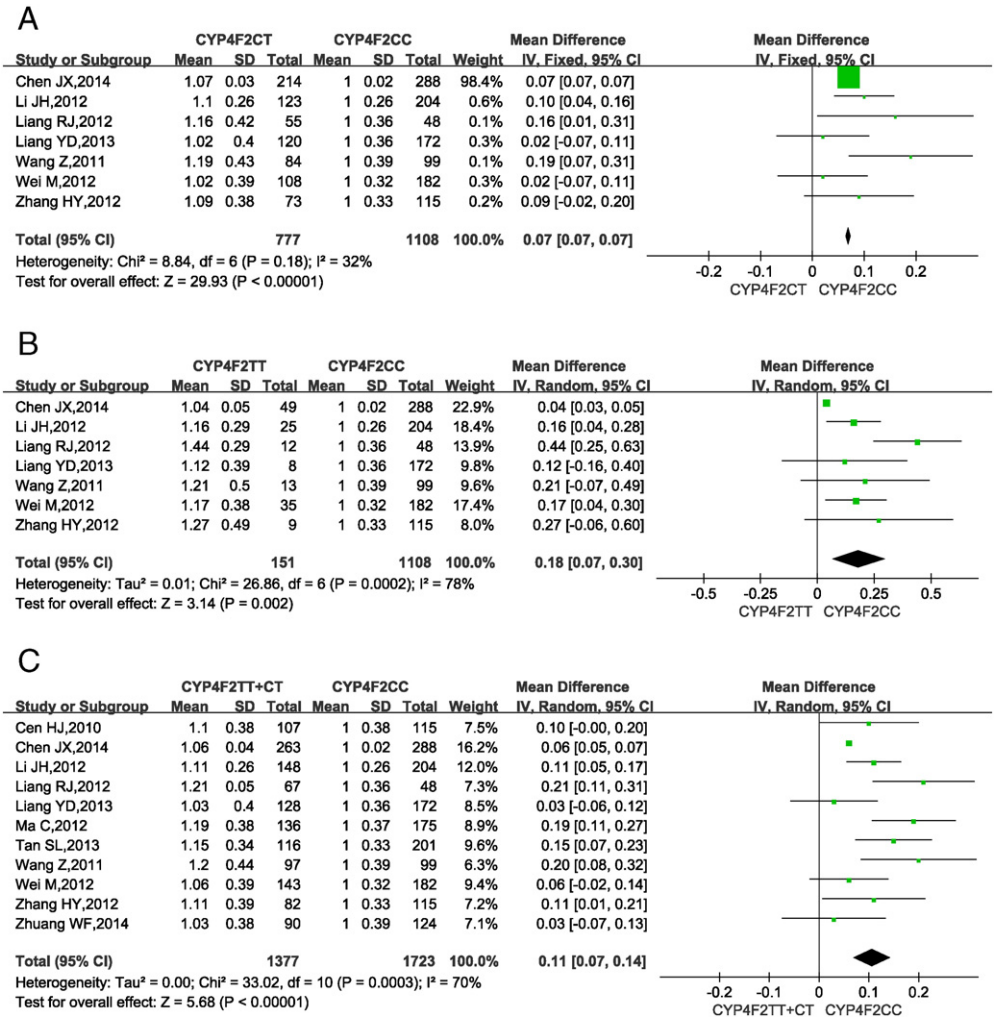

Fig. 3. Forest plots of impact of CYP4F2 T > C SNPs on warfarin dosage requirements. (A) Relative warfarin dosage requirements of CYP4F2 CT carriers compared to those of homozygous wild-type CYP4F2 CC carriers. (B) CYP4F2 TT vs. CC carriers. (C) CYP4F2 T carriers (CT or TT) vs. CC carriers. SD: standard deviation of normalized warfarin doses associated with each genotype. CI: confidence interval.

patients were venous thromboembolism, deep vein thrombosis, atrial fibrillation, rheumatic heart disease, atrial valve replacement, mitral valve replacement, mechanical heart valve replacement and pulmonary embolism. Some eligible studies analyzed two or more genotypes. Of the 33 studies, 24 (Cheng et al., 2009; Veenstra et al., 2005; Cen et al., 2010; Chen et al., 2014; Gu et al., 2010; Huang et al., 2009a; Liang et al., 2012b; Liang et al., 2013; Lu et al., 2013; Ma et al., 2012; Miao et al., 2007; Tan et al., 2013; Wei et al., 2012; Zhang et al., 2012a; Du et al., 2010; Gao et al., 2010; Huo et al., 2008; Li and Sheng, 2010; Liu and Zhang, 2010; Zhang et al., 2007a; Zhang et al., 2007b; Zheng et al., 2008; Zhuang et al., 2014; Yang and Han, 2012) investigated CYP2C9 gene polymorphisms, 11 (Cen et al., 2010; Chen et al., 2014; Li et al., 2012; Liang et al., 2012b; Liang et al., 2013; Ma et al., 2012; Tan et al., 2013; Wei et al., 2012; Wang et al., 2011; Zhang et al., 2012b; Zhuang et al., 2014) assessed CYP4F2 gene polymorphisms, 8 (Huang et al., 2009a; Wei et al., 2012; Yang et al., 2011; Zhang et al., 2012a; Jiang et al., 2007; Meng et al., 2011; Tang et al., 2009; Zhuang et al., 2014) examined VKORC1 1173 gene polymorphisms and 16 (Cheng et al., 2009; Veenstra et al., 2005; Gu et al., 2010; Liang et al., 2012b; Lu et al., 2013; Ma et al., 2012; Miao et al., 2007; Tan et al., 2013; Yuan et al., 2005; Du et al., 2010; Gao et al., 2010; Li and Sheng, 2010; Lou et al., 2012; Meng et al., 2011; Wang and Zhong, 2013; Zhang et al., 2007b) analyzed VKORC1-1639 gene polymorphisms. The characteristics of the studies are shown in Table 1.

3.3. CYP2C9 gene polymorphisms and warfarin dosage requirement

Fig. 2 shows the impact of CYP2C9 gene SNPs on warfarin dosage requirements in Han-Chinese patients. 5 studies (Cheng et al., 2009; Cen et al., 2010; Gu et al., 2010; Ma et al., 2012; Zhang et al., 2007b) only provided pooled data for genotypes CYP2C9 *1/*3 and *3/*3 rather than separate data for each genotype. So, the data from these studies were included in only one analysis (CYP2C9 *1/*3 + *3/*3 vs. *1/*1). Genotype CYP2C9 *3/*3 could not be found in 12 studies (Veenstra et al., 2005; Liang et al., 2012b; Lu et al., 2013; Miao et al., 2007; Wei et al., 2012; Zhang et al., 2012a; Du et al., 2010; Gao et al., 2010; Huo et al., 2008; Liu and Zhang, 2010; Zhuang et al., 2014) or only 1 patient was found in 3 studies (Chen et al., 2014; Huang et al., 2009a; Zheng et al., 2008), So the data from these studies were excluded from the analysis of CYP2C9 *3/*3 vs. *1/*1.

Statistical homogeneity was found among studies comparing CYP2C9 *3/*3 vs. *1/*1 ($P = 0.41$, $I^2 = 0\%$; Fig. 2B), so the fixed-effects model was applied. In contrast, significant heterogeneity was found among studies comparing CYP2C9 *1/*3 vs. *1/*1 ($P < 0.00001$, $I^2 = 86\%$, Fig. 2A) and among those comparing CYP2C9 *1/*3 + *3/*3 vs. *1/*1 ($P < 0.00001$, $I^2 = 89\%$; Fig. 2C), so the random-effects model was applied. The results showed that compared with CYP2C9 *1/*1 carriers, CYP2C9 *1/*3 and *3/*3 carriers required a 28% (95% confidence interval: 22.0%–33.0%) and 72% (62.0%–81.0%) lower MDWD. CYP2C9 *3 carriers required a

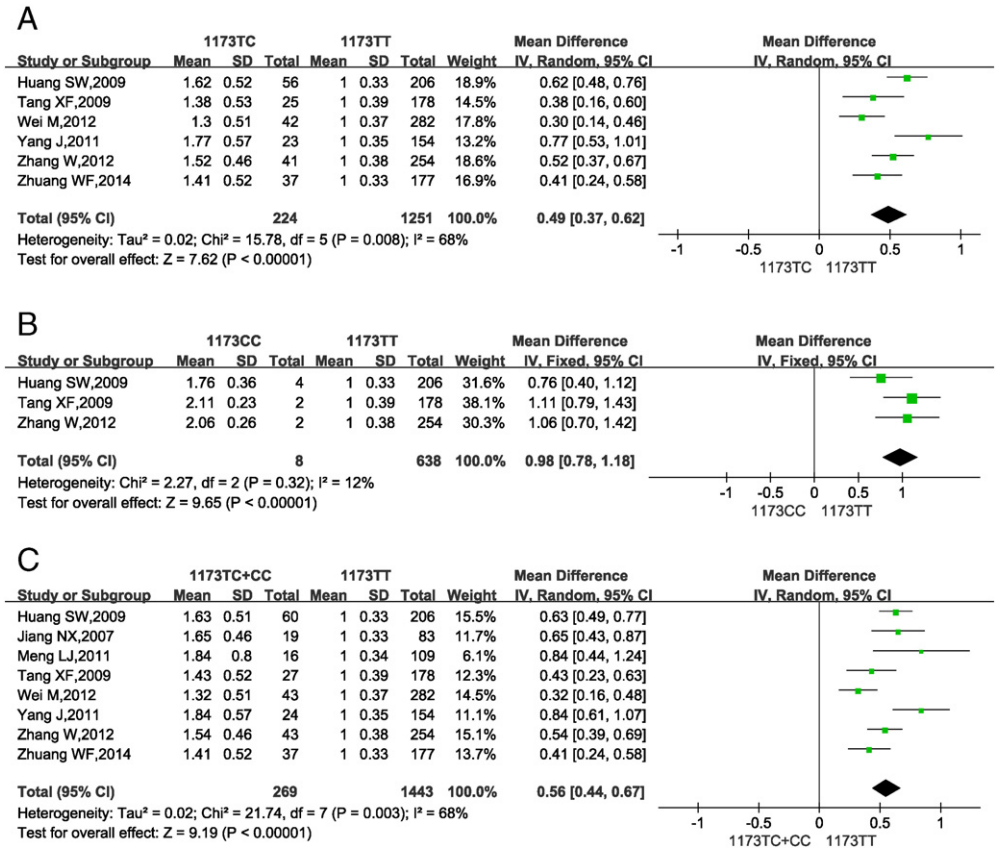

Fig. 4. Forest plots of impact of VKORC1 1173 C > T SNPs on warfarin dosage requirements. (A) Relative warfarin dosage requirements of CT carriers compared to those of wild-type VKORC1 1173 TT carriers. (B) VKORC1 1173 CC vs. TT carriers. (C) VKORC1 1173 C carriers (TC or CC) vs. TT carriers.SD: standard deviation of normalized warfarin doses associated with each genotype. CI: confidence interval.

26% (21.0%–31.0%) lower MDWD than CYP2C9 *1/*1 carriers. All *P*-values from the test for overall effect were <0.05.

3.4. CYP4F2 gene polymorphisms and warfarin dosage requirement

Fig. 3 shows the impact of CYP4F2 gene SNPs on warfarin dosage requirements in Han-Chinese patients. 4 studies (Cen et al., 2010; Ma et al., 2012; Tan et al., 2013; Zhuang et al., 2014) only provided pooled data for the genotypes CYP4F2 CT and TT, so the data from these studies were used in a single analysis, i.e., CYP4F2 CT + TT vs. CC.

Statistical homogeneity was found among studies comparing CYP4F2 CT vs. CC ($P = 0.18$, $I^2 = 32\%$; Fig. 3A), so the fixed-effects model was applied. In contrast, significant heterogeneity was found among studies comparing CYP4F2 TT vs. CC ($P < 0.0002$, $I^2 = 78\%$, Fig. 3B) and among those comparing CYP4F2 TT + CT vs. CC ($P < 0.0003$, $I^2 = 70\%$; Fig. 3C), so the random-effects model was applied. The results showed that the MDWD was 7% (7.0%–7.0%) and 18% (7.0%–30.0%) higher in CYP4F2 CT and TT carriers, respectively, than in CYP4F2 CC carriers. CYP4F2 T carriers (CT or TT) needed a 11% (7.0%–14.0%) higher MDWD than CC carriers. All *P*-values from the test for overall effect were <0.05.

3.5. VKORC1 1173 gene polymorphisms and warfarin dosage requirement

Fig. 4 shows the impact of VKORC1 1173 gene SNPs on warfarin dosage requirements in Han-Chinese subjects. 2 studies (Jiang et al., 2007; Meng et al., 2011) reported the pooled MDWD for VKORC1 1173 CT and CC carriers, so the data from these studies were used in a single analysis (VKORC1 1173 CT + CC vs. TT). Genotype VKORC1 1173 CC was not found in 1 study and only 1 patient was found in 1 studies (Wei et al., 2012; Yang et al., 2011). So the data from these studies were excluded from the analysis of VKORC1 1173 CC vs. TT.

Statistical homogeneity was found among studies comparing VKORC1 1173 CC vs. TT ($P = 0.32$, $I^2 = 12\%$; Fig. 4B), so the fixed-effects model was applied. Significant heterogeneity was seen among studies assessing VKORC1 1173 TC vs. TT ($P = 0.008$, $I^2 = 68\%$; Fig. 4A) and VKORC1 1173 TC + CC vs. TT ($P = 0.003$, $I^2 = 68\%$; Fig. 4C), so the random-effects model was used. The results showed that VKORC1 1173 TC and CC carriers required a 49% (37.0%–62.0%) and 98% (78.0%–118.0%) higher MDWD, respectively, than TT carriers. VKORC1 1173 C carriers (CT or CC) needed a 56% (44.0%–67.0%) higher MDWD than TT carriers. All *P*-values from the test for overall effect were <0.05.

3.6. VKORC1-1639 gene polymorphisms and warfarin dosage requirement

Fig. 5 shows the impact of VKORC1-1639 gene SNPs on warfarin dosage requirements in Han-Chinese subjects. 2 (Liang et al., 2012b; Gao et al., 2010) studies did not report the genotype VKORC1-1639 GG only were excluded from the analysis of VKORC1-1639 GG vs. AA. 6 studies (Cheng et al., 2009; Ma et al., 2012; Yuan et al., 2005; Du et al., 2010; Lou et al., 2012; Meng et al., 2011) provided pooled data for VKORC1-1639 GA and GG carriers, so the data from these studies were included in a single analysis of VKORC1-1639 GA + GG vs. AA.

Significant heterogeneity was found among studies assessing VKORC1-1639 gene polymorphisms (Fig. 5A–5C; $P < 0.00001$, $I^2 = 93\%$; $P < 0.00001$, $I^2 = 97\%$; $P < 0.00001$, $I^2 = 90\%$), so the random-effects model was selected. The results showed that compared to VKORC1-1639 AA carriers, GA and GG carriers required a 40% (36.0%–45.0%) and 101% (53.0%–149.0%) higher MDWD, respectively. VKORC1-1639 G carriers (GA or GG) needed a 38% (35.0%–42.0%)

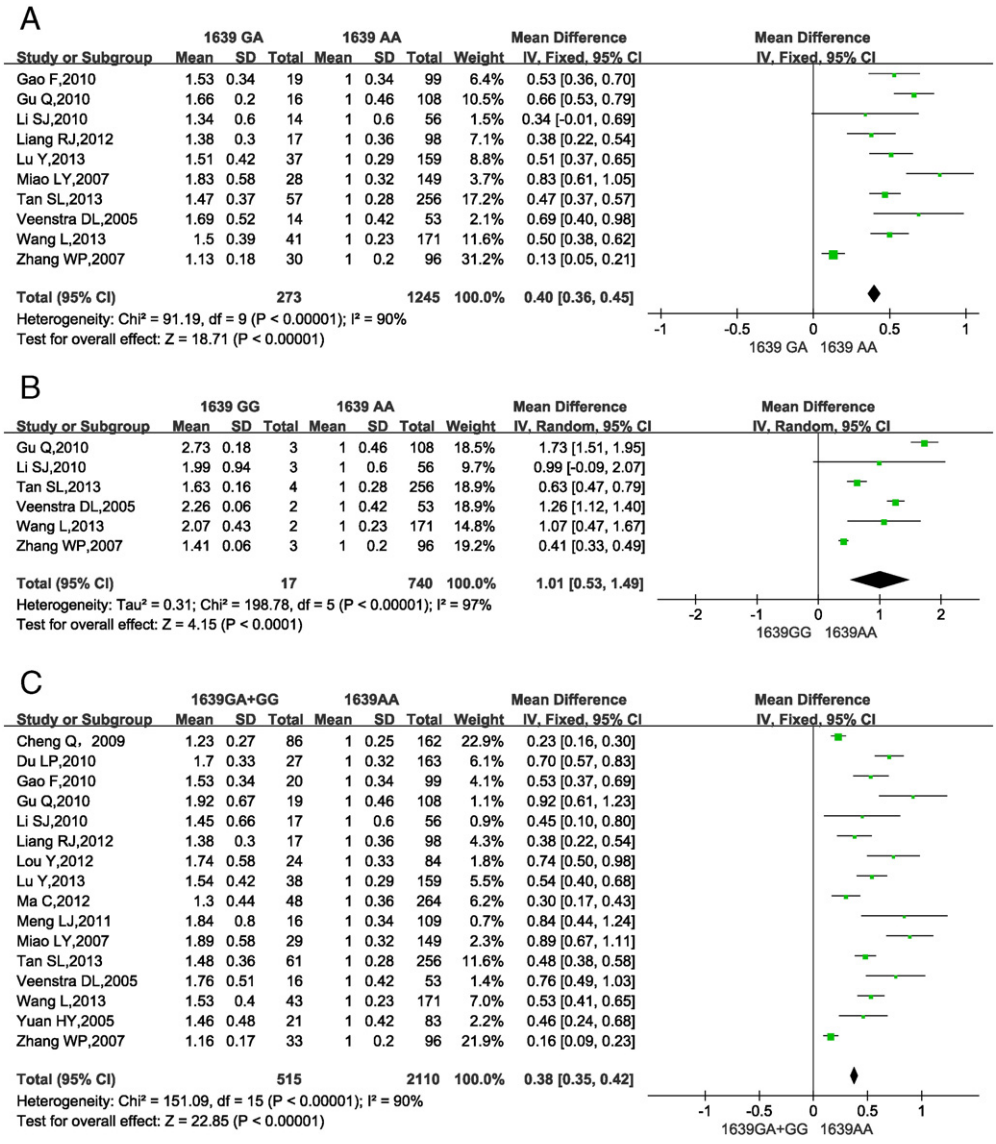

Fig. 5. Forest plots of impact of VKORC1-1639 G > A SNPs on warfarin dosage requirements. (A) Relative warfarin dosage requirements of VKORC1-1639 GA carriers compared to VKORC1-1639 AA carriers. (B) VKORC1-1639 GG vs. AA carriers. (C) VKORC1-1639 G (GA or GG) vs. AA carriers.SD: standard deviation of normalized warfarin doses associated with each genotype. CI: confidence interval.

higher MDWD than AA carriers. All *P*-values from the test for overall effect were <0.05.

3.7. Heterogeneity and sensitivity analysis

Sensitivity analysis was performed by deselecting the studies one by one in chronological order. The results were not changed greatly when any study was deselected, and no study was found to be significantly associated with statistical heterogeneity, which indicated that the results of the analysis were stable and reliable.

Since high statistical heterogeneity was found among some analyses of genes CYP2C9, CYP4F2, VKORC1 1173 C > T and VKORC1-1639 G > A, the software STATA 12.0 was used to explore the source of heterogeneity via meta-regression analysis (Liang et al., 2012a). The year of publication, language of publication, location of patients, mean age of patients, number of patients, proportion of men and median INR were used as covariates of the mean difference in MDWD in each meta-

Table 2
Results of meta-regression analysis of various covariates from studies on CYP2C9.

Covarirate	Coef.	Sta. Err	t	p > |t|	I-squared_res (%)	Adj R-squared (%)
Published year	−0.015594	0.0117369	−1.33	0.198	85.82	5.70
Language	0.0412391	0.0603107	−0.68	0.502	85.60	−1.74
Location	0.0352089	0.0292356	1.20	0.242	86.68	−0.42
Age	0.0005659	0.0036818	0.15	0.879	88.31	−5.53
Number of patients	−0.0003186	0.000255	−1.25	0.225	82.73	4.85
Male ratio	−0.2032788	0.4329343	−0.47	0.644	86.50	−4.94
Median INR	−0.205372	0.127046	−1.62	0.121	88.83	7.13

None of the covariates was significantly correlated with heterogeneity.

Table 3
Results of meta-regression analysis of various covariates from studies on CYP4F2.

Covarirate	Coef.	Sta. err	t	p > |t|	I-squared_res (%)	Adj R-squared (%)
Published year	−0. 0264162	0.0144864	−1.82	0.102	42.13	46.43
Language	0. 0020607	0.0493276	0.04	0.968	70.96	−16.98
Location	−0. 0381552	0.0229246	−1.66	0.130	71.90	−5.00
Age	0. 0009953	0.0025719	0.39	0.708	67.34	−16.15
Number of patients	−0.0002609	0.0002848	−0.92	0.387	49.99	−10.66
Male ratio	−0.1809945	0.2084318	−0.87	0.408	53.38	7.61
Median INR	0.0554843	0.1109978	0.50	0.629	60.80	−3.88

None of the covariates was significantly correlated with heterogeneity.

Table 4
Results of meta-regression analysis of various covariates from studies on VKORC1 1173.

Covarirate	Coef.	Sta. err	t	p > |t|	I-squared_res (%)	Adj R-squared (%)
Published year	−0.0300199	0. 0305746	−0.98	0.364	64.70	6.64
Language	−0.0247233	0.1403689	−0.18	0.866	71.93	−26.42
Location	0.0950507	0.15246227	0.62	0.556	71.89	−24.05
Age	−0.0162225	0.0071395	−2.27	0.063	51.09	49.93
Number of patients	−0.0013617	0.0007849	−1.73	0.133	62.14	26.79
Male ratio	−2.884117	1.343607	−2.15	0.075	57.16	42.96
Median INR	−0.1214239	0.4457683	−0.27	0.794	72.39	−26.28

None of the covariates was significantly correlated with heterogeneity.

regression analysis. In order to improve this process, we set certain values for the variables. For language, English was set as 0 and Chinese as 1. For location, we divided the whole country into five regions: the region of Taiwan and Hong Kong, southeast China, southwest China, northeast China and northwest China, which were set as 0, 1, 2, 3 and 4, respectively. The meta-regression results for each genotype are shown in Tables 2–5.

3.8. Publication bias

Initially, publication bias between the studies was checked via Revman 5.3, and the results did not indicate significant publication bias (data not shown).

4. Discussion

Polymorphisms of genes CYP2C9, VKORC1 1173 T > C and VKORC1-1639 G > A account for 40%–60% of the interindividual variation in warfarin dosage (Klein et al., 2009; Rieder et al., 2005; Krishna Kumar et al., 2014; Lee et al., 2006; Veenstra et al., 2005; Wadelius et al., 2005). Recently, another gene, CYP4F2, was found to be moderately associated with the interindividual variation in warfarin dosage requirements (Liang et al., 2012a; Cen et al., 2010). The frequencies of these four genes differ among people of different ethnicities (Table 6).

Some meta-analyses (Sanderson et al., 2005; Lindh et al., 2009a; Yang et al., 2010; Jorgensen et al., 2012; Liang et al., 2012a) have researched the impact of these four genes on the MDWD in Caucasian, African and Asian patients. Thus far, however, no meta-analysis has specifically analyzed the impact of these genes in the Han-Chinese. Our study is the first to explore the impact of these four genes on MDWD in Han-Chinese patients.

We found that among Han-Chinese subjects, CYP2C9 *3*3, *1/*3 and *3 carriers require 72% (62.0%–81.0%), 28% (22.0%–33.0%) and 26% (21.0%–31.0%) less warfarin maintenance dosage, respectively, than CYP2C9 *1/*1 carriers. Although the homozygous variant CYP2C9 *3*3 was very rare among the Han-Chinese (0.39%), the warfarin maintenance dosage was much lower in these subjects than in homozygous wild-type carriers. Thus, if given the same loading dose, patients with the CYP2C9 *3*3 genotype will have a higher risk of bleeding than CYP2C9 *1/*1 carriers (Ma et al., 2012). Lindh et al. (2009b) reported that among Caucasians, the warfarin maintenance dosage was 78.1% (72.0%–84.3%) and 35.1% (29.4%–38.1%) lower in CYP2C9 *3/*3 and *1/*3 patients, respectively, than in *1/*1 patients. It appears that the influence of CYP2C9 gene polymorphisms on warfarin maintenance dosage is greater in Caucasian patients than in Han-Chinese patients. However, the distribution of the CYP2C9 *3 genotype in the Han-Chinese significantly differs from that in Caucasians, Africans (Table 5) and South and West Asians (Gaikwad et al., 2014). In this meta-analysis, among 4928 patients, the allele frequencies of CYP2C9 *1/*1, *1/*3 and *3/*3 were 91.0%, 8.44% and 0.55% respectively. The frequency of mutant *3 is lower in the Han-Chinese than in Caucasians and Indians. Furthermore, mutant *2 is common in Caucasians and South and West

Table 5
Results of meta-regression analysis of various covariates from studies on VKORC1–1639.

Covarirate	Coef.	Sta. err	t	p > |t|	I-squared_res (%)	Adj R-squared (%)
Published year	−0.1861517	0.1581693	−1.18	0.259	95.83	3.99
Language	−0.0325182	0.0307319	−1.06	0.308	96.67	1.30
Location	−0.0849311	0.0726921	−1.17	0.262	92.10	6.16
Age	−0.0011833	0.0108254	−0.11	0.915	96.93	−7.83
Number of patients	−0.0012891	0.0010766	−1.20	0.251	96.93	2.46
Male ratio	−0.4980054	0.9576941	−0.52	0.611	96.67	−5.93
Median INR	−0.6058801	0.4032263	−1.50	0.155	95.51	9.90

None of the covariates was significantly correlated with heterogeneity.

Table 6
Frequencies of the target genotypes in different regions.

Population	CYP2C9 genotype frequencies(%)						No. of subjects	Reference
	*1/*1	*1/*3	*3/*3	*1/*2	*2/*2	*2/*3		
Han-Chinese	90.41	8.94	0.58	0.06	–	–	4928	Current review
Japanese	95.26	4.41	0.09	–	–	–	2277	Gaikwad et al. (2014)
Korean	90.82	9.10	0.08	–	–	–	1151	Gaikwad et al. (2014)
Indian	76.84	13.48	1.65	6.80	0.39	0.84	3510	Gaikwad et al. (2014)
Caucasian	66	12	0.5	19	1.4	1.3	1490	Wadelius et al. (2009); Daneshjou et al. (2013)
African	93.71	2.32	–	3.97	–	–	302	Daneshjou et al. (2013); Mushiroda et al. (2006)

Population	VKORC1 1173 genotype frequencies (%)			No. of subjects	Reference
	TT	TC	CC		
Han-Chinese	84.29	14.89	0.82	1712	Current review
Japanese	83.33	15.94	0.72	828	Mushiroda et al. (2006); Choi et al. (2011)
Korean	87.41	11.7	0.89	564	Choi et al. (2011); Kumar et al. (2013)
Indian	77.47	21.26	1.26	470	Kumar et al. (2013); Krajciova et al. (2014)
Caucasian	17.38	46.67	35.95	420	Krajciova et al. (2014); Limdi et al. (2008)
African	0.9	18.7	80.4	225	Limdi et al. (2008); Chin et al. (2013)

Population	VKORC1-1639 genotype frequencies (%)			No. of subjects	Reference
	AA	GA	GG		
Han-Chinese	79.87	17.77	2.36	2524	Current review
Japanese	83.21	16.06	0.72	800	Mushiroda et al. (2006); Choi et al. (2011)
Korean	86.91	13.09	–	298	Chin et al. (2013); Scott et al. (2008)
Indian	77.45	37.3	1.35	470	Kumar et al. (2013); Krajciova et al. (2014)
Caucasian	15	49	36	1461	Wadelius et al. (2009); Daneshjou et al. (2013)
African	2.0	17.7	80.3	300	Scott et al. (2008); Cha et al. (2010)

Puopulation	CYP4F2 genotype frequencies (%)			No. of subjects	Reference
	CC	CT	TT		
Han-Chinese	55.30	37.86	6.83	3100	Current review
Japanese	52.95	39.77	7.27	440	Cha et al. (2010); Wypasek et al. (2014)
Korean	41.69	46.65	11.66	403	Choi et al. (2011); Kumar et al. (2013)
Indian	33.5	49.4	17.1	445	Kumar et al. (2013); Krajciova et al. (2014)
Caucasian	7.31	41.75	50.94	479	Wypasek et al. (2014); Bress et al. (2012)
African	88.76	10.85	0.39	258	Bress et al. (2012); Ye et al. (2014)

Asians, but it is very rare in East Asians, including the Han-Chinese, Koreans and Japanese. Genome-wide association studies concerning the Han-Chinese seldom provide data on CYP2C9 *2 patients. We therefore had no occasion to conduct an analysis to determine the influence of CYP2C9 *2 on MDWD in Han-Chinese subjects.

Although Kringen et al. (2011) and Lee et al. (2009) have found that the CYP4F2 gene has little effect on warfarin maintenance dosage in Caucasians and the Han-Chinese, many other researchers consider that this impact is not negligible and that CYP4F2 SNPs should be taken into account before prescribing warfarin (Kringen et al., 2011). In the included studies, there were significant differences in MDWD among subjects with different CYP4F2 genotypes. We found that in Han-Chinese subjects, the MDWD was 18% (7.0%–30.0%), 7% (7.0%–7.0%) and 11% (7.0%–14.0%) higher in CYP4F2 TT, CT and T carriers, respectively, than in CYP4F2 CC patients. The corresponding values in Caucasians were 23.0% (7.0%–39.0%), 10.0% (4.0%–15.0%) and 11.0% (7.0%–15.0%) (Liang et al., 2012a). The Forest plots for CYP 4F2 showed some studies that were not statistically significant, yet the aggregate meta analysis data showed statistical difference. So we performed a cumulative meta analysis of CYP 4F2 on software Comprehensive Meta Analysis V2. But the results showed that the studies were not statistically significant (Fig. 6A–C).

We also found that in Han-Chinese subjects, the warfarin maintenance dosage was 98% (78.0%–118.0%), 49% (37.0%–62.0%) and 56% (44.0%–67.0%) higher in VKORC1 1173 CC, TC and C carriers (TC or CC), respectively, than in TT carriers. The warfarin maintenance dosage was 101% (53.0%–149.0%), 45% (41.0%–49.0%) and 38% (35.0%–42.0%) higher in Han-Chinese VKORC1-1639 GG, GA and G carriers (GA or GG), respectively, than in AA carriers. In 1712 subjects, the frequencies of VKORC1 1173 TT, CT and CC were 84.29%, 14.89% and 0.82% respectively, while in 2625 subjects, the frequencies of VKORC1-1639 AA, GA and GG were 79.87%, 17.77% and 2.36%, respectively. Not only VKORC1 1173 C mutations and VKORC1-1639 G mutations have similar frequencies in the Han-Chinese, but also both mutations had a similar impact on MDWD in Han-Chinese patients. Physicians can test for any one of these two genotypes to ensure rational drug usage and save hospitalization costs.

It is well-known that Caucasians and Africans require a higher MDWD than the Han-Chinese, who require a nearly 40% lower MDWD than Caucasian patients (Xie et al., 2001). One black male patient was reported to require a 60-mg daily dose of warfarin to elicit a therapeutic anticoagulant response (Hallak et al., 1993). Caucasian or African patients who need >15 mg/day warfarin are considered warfarin resistant (Osinbowale et al., 2009), whereas for the Han-Chinese, the resistance level is 6 mg (Yuan et al., 2005). The frequencies of the VKORC1 1173 C and VKORC1-1639 G alleles, which increase the expression of VKORC1 mRNA and render patients insensitive to warfarin (Rieder et al., 2005), in the Han-Chinese are quite different from those in Caucasians and Africans. Approximately 16% Han-Chinese patients are VKORC1 1173 C or VKORC1-1639 G carriers, while most Caucasian and African patients are VKORC1 1173 C or VKORC1-1639 G carriers (Table 6). This may explain why Caucasians and Africans require a higher MDWD than the Han-Chinese to achieve the same target INR range, despite all other factors being equal. Huang SW's study have shown that the pharmacogenetics-based dosing algorithm improve the time to reach the stable dosing of warfarin in Han-Chinese patients (Huang et al., 2009b). And pharmacogenetics-based dosing algorithm may be useful in helping the clinicians to prescribe warfarin with greater safety and efficiency (Huang et al., 2009b). But it still need to constuct a pharmacogenetics-based dosing algorithm for Han-Chinese patients by muti-center.

It is a stern reality that gene sequencing is very expensive and is not covered by medical insurance providers in many countries, and the average patient cannot bear the expenses of testing all four genes. After the warfarin loading dose and the subsequent INR test, physicians can estimate whether or not a patient is sensitive to warfarin. From the results of this meta-analysis, we deduced that testing for the CYP2C9 gene can reveal warfarin-sensitive patients, while testing for the VKORC1 gene can reveal warfarin-insensitive patients. Therefore,

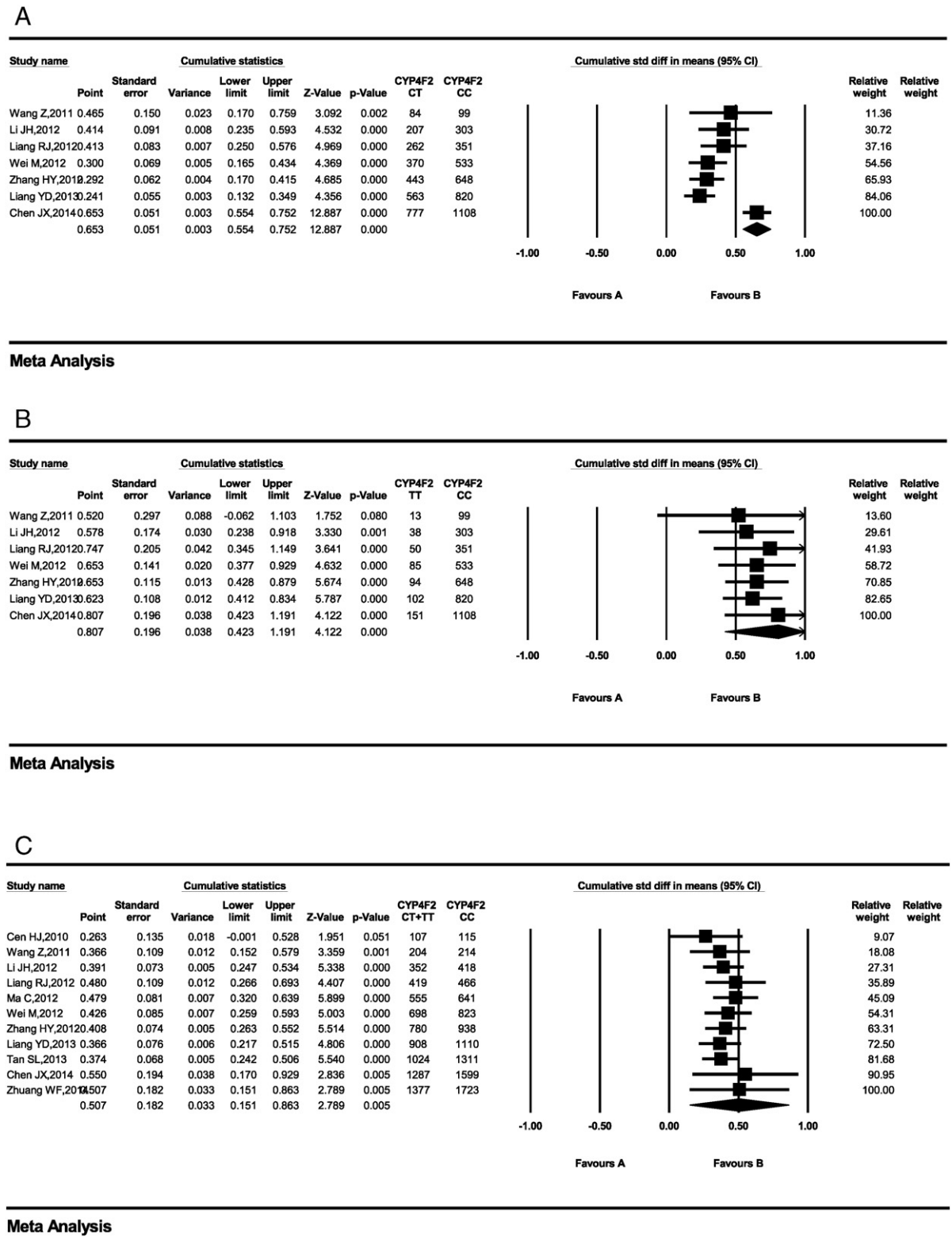

Fig. 6. Cumulative meta-analysis of CYP 4F2 on warfarin dosage requirements in chrononologic order. (A) CYP4F2 CT vs. CC carriers. (B) CYP4F2 TT vs. CC carriers. (C) CYP4F2 T carriers (CT or TT) vs. CC carriers. CI: confidence interval.

conversely, doctors can select warfarin-sensitive patients for CYP2C9 testing, and warfarin-insensitive patients for VKORC1 testing. Thus, long-term warfarin users will save on medical costs while still receiving appropriate treatment.

4.1. Limitations and perspectives

We found that during warfarin treatment to prevent embolization, the target INR set by doctors varies greatly among different regions.

For example, during warfarin therapy to prevent thrombosis in heart valve replacement patients, Gu et al. (2010), Huo et al. (2008) and Zhang et al. set the target INR to 1.5–2.0, but Tan et al. (2013) set this to 2.1–2.8. Most of the included studies were from southeast China and northeast China, and more samples from other regions should be analyzed to confirm the results of our meta-analysis.

5. Conclusions

Polymorphisms of CYP2C9, VKORC1 1173 and VKORC1-1639 significantly affect warfarin maintenance dosage in Han-Chinese patients. CYP4F2 gene polymorphisms explain part of the dosage difference, though the effect of this gene is lower than that of the other three. The distribution of these four genes in the Han-Chinese is different from that in other major populations, which could explain the differences in the required warfarin dosage between Han-Chinese and other populations.

Conflicts of interest

The authors declared no conflict of interest.

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