Significant interaction of *APOE* rs4420638 polymorphism with HDL-C and APOA-I levels in coronary heart disease in Han Chinese men

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ABSTRACT. Apolipoprotein E (*APOE*) is recognized for its importance in lipoprotein metabolism and cardiovascular disease. We evaluated the association between *APOE* rs4420638 genotypes and circulating lipid concentrations along with the risk of coronary heart disease (CHD). We conducted a case-control study involving 1508 individuals to investigate the contribution of rs4420638 to the risk of CHD in Han Chinese. In addition, we performed a meta-analysis to evaluate the association between rs4420638 and CHD in Europeans and Asians. The results show that rs4420638 is significantly correlated with increased CHD risk in male Han Chinese [P = 0.040, odds ratio (OR) = 1.34, 95% confidential interval (95%CI) = 1.01-1.78] and is likely to increase the risk of CHD under the dominant model in males (P =

0.036, OR = 1.38, 95%CI = 1.02-1.88). A further subgroup analysis by rs4420638 genotype found a significant association of rs4420638 AA with high-density lipoprotein cholesterol (HDL-C) (P = 0.012) and APOA-I levels (P = 0.0001) in males. The meta-analysis suggests that rs4420638 significantly increases the risk of CHD (OR = 1.18, 95%CI = 1.14-1.22, P < 0.0001, fixed-effect method). Our case-control study shows that rs4420638 genotype AA has a significant association with the concentrations of circulating HDL-C and APOA-I in CHD in Han Chinese males. The meta-analysis suggests that rs4420638 is associated with CHD risk in Europeans and Asians.

Key words: Coronary heart disease; High-density lipoprotein cholesterol; HDL-C; *APOE*; APOA-I; Polymorphism

INTRODUCTION

It is widely accepted that coronary heart disease (CHD) is a major public health problem worldwide (Graff-Iversen et al., 2014). CHD is a complex disease and is influenced by a combination of genetic and environmental factors (Puddu et al., 2014). Despite extensive efforts in the identification of genetic factors that affect susceptibility to CHD (Roberts and Stewart, 2012), the underlying mechanisms remain unclear.

Circulating blood lipid concentration is a strong independent risk factor for CHD (Rached et al., 2014). Total cholesterol (TC) and lipoproteins, including apolipoproteins A, B, and E (APOA, APOB, and APOE), may play an important role in the developmental and physiological processes of CHD. Research has shown that high levels of low-density lipoprotein cholesterol (LDL-C) and TC lead to atherosclerosis and CHD (Isley, 2006), and that high-density lipoprotein cholesterol (HDL-C) levels are negatively correlated to CHD (Subedi et al., 2014).

APOE [Online Mendelian Inheritance in Man (OMIM) No. 107741], which encodes APOE protein, has the chromosomal locus 19q13.32 and consists of four exons and three introns. It is a member of the LDL receptor gene family and exists in a cluster with apolipoprotein C-I and C-II genes (*APOC1* and *APOC2*) (Onat et al., 2013). Defects in APOE can cause an increase in the levels of plasma cholesterol and triglycerides (TGs) due to impaired clearance of chylomicron, very low-density lipoprotein, and LDL-C remnants (Mendivil et al., 2013). APOE is recognized for its importance in lipoprotein metabolism and cardiovascular disease (Onat et al., 2013). *APOE* polymorphisms (rs429358 and rs7412) have shown an association with serum lipids in certain populations (Smalinskiene et al., 2013). These variants are associated with differences in lipid risk factors and atherosclerosis (Heidari et al., 2013). *APOE* polymorphisms have been extensively studied as predictors of cardiovascular disease (Smalinskiene et al., 2013). The single nucleotide polymorphism (SNP) rs4420638 is located in the *APOE-C1-C2* cluster on the 19q13 chromosome. It has been shown to be in high linkage disequilibrium with rs429358 ($r^2 > 0.7$) and to be significantly associated with the increased risk of CHD (Elliott et al., 2009). SNP rs4420638 is associated with the modulation of many lipids in humans (Grallert et al., 2012).

In this study, we recruited 776 CHD patients and 732 controls from Zhejiang in Eastern China and performed a case-control study to investigate the contribution of rs4420638

polymorphism to the risk of CHD in the Han Chinese population. We also examined the association of the genotypes with circulating lipid concentrations. In addition, we performed a meta-analysis to evaluate the association between rs4420638 and CHD risk in Europeans and Asians.

MATERIAL AND METHODS

Sample collection

The study was approved by the Ethics Committee of the School of Medicine, Ningbo University. All 1508 unrelated inpatients were recruited from the Affiliated Hospitals of Ningbo University (Ningbo Lihuili Hospital, Ningbo No. 3 Hospital and Ningbo Yinzhou Hospital) and the Second Affiliated Hospital of Zhejiang University between May 2008 and April 2013. All volunteers were defined through the standardized coronary angiography protocol (Higgs et al., 2005) and the results were assessed by two or three individual physicians. Among these, 776 CHD patients (533 males and 243 females, mean age: 62.4 ± 0.3 years) were diagnosed by angiographic evidence, including coronary arteries with 50% or greater stenosis, or a history of prior angioplasty or coronary artery bypass surgery. The 732 controls (419 males and 313 females, mean age: 59.7 ± 0.4 years) were patients that had less than 50% occlusion of any coronary vessel in the angiographic results and no history of atherosclerotic vascular disease. All individuals were Han Chinese, originating from Zhejiang Province and without congenital heart disease, cardiomyopathy, or severe liver or kidney disease.

Biochemical variables

Blood samples were collected in 3.2% sodium citrate-treated tubes and stored at 4°C for short periods or at -80°C for long periods. Levels of plasma TG, serum TC, HDL-C, and LDL-C were measured using an enzymatic end point assay (Lopes-Virella et al., 1977). The APOA-I, APOB, and APOE levels were measured using the transmission turbidimetry method (Jiang et al., 2013).

Genotyping

Genomic DNA was isolated from peripheral blood samples using a nucleic acid extraction automatic analyzer (Lab-Aid 820, Xiamen, China) and all the DNA samples were stored in TE buffer (TE is Tris and ethylenediaminetetraacetic acid), at -80°C. The polymerase chain reaction (PCR) primers for rs4420638 were as follows: forward, 5'-ACGTTGGATGATTG AACCCTCAGCCTAGCA-3'; reverse, 5'-ACGTTGGATGAAAAACCTCAGCCCCTCATC -3'; extension primer, 5'-GCAGCTCGGGTAGACCACAC-3'. The PCR amplification was performed on an ABI GeneAmp® PCR System 9700 (dual 384-well blocks) (Applied Biosystems, Foster City, CA, USA). Primer extension for genotyping was performed on a MassARRAY iPLEX® assay platform (Sequenom, San Diego, CA, USA) according to the manufacturer instructions. The PCR process included an initial denaturation at 94°C for 15 s, followed by 45 cycles at 94°C for 20 s, 56°C for 30 s, 72°C for 1 min, and then a final extension at 72°C for 1 min. Quality controls were described in our previous study (Huang et al., 2013).

Retrieval of published studies and data extraction

A literature search was performed for the meta-analysis up to April 17, 2014, in electronic databases including PubMed, Web of Science, and China National Knowledge Infrastructure (CNKI). We used the following Medical Subject heading (MeSH) terms for searching: "polymorphism"; "ApoE"; "ApoC1"; and "rs4420638" paired with "coronary heart disease" or "coronary artery disease". We also read the full text to find relevant information. References listed in the retrieved articles, review articles, or previous meta-analyses were searched to appraise other studies for potential relevance. Data were extracted by two authors and divergence was resolved by consensus. The information collected from each study comprised the author, publication year, number of cases and controls, OR, and 95%CI.

Statistical analyses

In the biochemical analysis between CHD cases and controls, continuous variables were described by mean and standard deviation (SD) using the Student t-test. For the non-parametric indicators, the Kruskal-Wallis test was applied. All P values were adjusted for age and gender in the comparison of lipid levels. Hardy-Weinberg equilibrium was analyzed by the Arlequin program (version 3.5; Bern, Switzerland). Comparison of the genotype distribution and allele frequencies between cases and controls was calculated using the CULMP16 software according to the method described previously (Huang et al., 2013). Power analysis of the case-control study was performed using the Power and Sample Size Calculation software (version 3.0.43; Nashville, TN, USA). ORs and 95%CIs were all estimated by chi-squared analyses using the SPSS 18.0 statistic software. A two-tailed P value <0.05 was considered to be statistically significant.

The meta-analysis was performed using the Stata statistical software (Version 11; StataCorp. LP, College Station, TX, USA). Heterogeneity of the studies was calculated by the inconsistency index (I^2) test at a significance level of 50%. The random-effect model was used to calculate the combined ORs for the studies with strong heterogeneity ($I^2 > 50\%$). Otherwise, the fixed-effect model was used (Lian et al., 2013). Publication bias was determined by funnel plots.

RESULTS

The genotypes and allele frequencies of the rs4420638 polymorphism in the case and control groups are shown in Table 1. The distributions of genotypes were all within Hardy-Weinberg equilibrium (P > 0.05). No association was found between rs4420638 and CHD (χ^2 = 2.82, P = 0.931, OR = 1.21, 95%CI = 0.97-1.50). However, a breakdown analysis by gender indicated a significant association in males (χ^2 = 4.20, P = 0.040, OR = 1.34, 95%CI = 1.01-1.78), in contrast with a lack of association in females (χ^2 = 0.01, P = 0.920, OR = 1.02, 95%CI = 0.71-1.46).

Significantly increased risk was observed between rs4420638 and CHD under the dominant model in males (Table 2; AA versus AG+GG, χ^2 = 4.37, P = 0.036, OR = 1.38, 95%CI = 1.02-1.88). No significant results were found in the alternative genetic models and subgroup analyses by gender and age (Tables 2 and 3; P > 0.05).

Table 1. Genotype and allele distributions of rs4420638.

Gender	Groups (N)	Genotype (N)			χ^2	P (d.f. = 2)	Protective allele	Risk allele	χ^2	P (d.f. = 1)	OR (95%CI)
		AA (%)	AG (%)	GG (%)			A (%)	G (%)			
All	Cases (776)	582 (75.0)	183 (23.6)	11 (1.4)	3.10	0.21	1347 (86.8)	205 (13.2)	2.82	0.931	1.21
	Controls (732)	577 (78.8)	146 (19.9)	9 (1.2)			1300 (88.8)	164 (11.2)			(0.97-1.50)
Male	Cases (533)	394 (73.9)	133 (25.0)	6 (1.1)	4.45	0.11	921 (86.4)	145 (13.6)	**4.20**	**0.040**	**1.34**
	Controls (419)	334 (79.7)	82 (19.6)	3 (0.7)			750 (89.5)	88 (10.5)			**(1.01-1.78)**
Female	Cases (243)	188 (77.4)	50 (20.6)	5 (2.1)	0.02	0.99	426 (87.7)	60 (12.4)	0.01	0.920	1.02
	Controls (313)	243 (77.6)	64 (20.4)	6 (1.9)			550 (87.9)	76 (12.1)			(0.71-1.46)

Results with P values less than 0.05 are in bold font.

Table 2. Genetic testing under the dominant and recessive models.

Gender	Groups (N)	Dominant		χ^2	P (d.f. = 1)	OR (95%CI)	Recessive		χ^2	P (d.f. = 1)	OR (95%CI)
	rs4420638	AA	AG+GG				AA+AG	GG			
All	Cases (776)	582	194	3.10	0.078	1.24	765	11	0.10	0.750	1.16
	Controls (732)	577	155			(0.98-1.58)	723	9			(0.48-2.80)
Male	Cases (533)	394	139	**4.37**	**0.036**	**1.38**	527	6	NA	NA	1.58
	Controls (419)	334	85			**(1.02-1.88)**	416	3			(0.39-6.35)
Female	Cases (243)	188	55	0.01	0.920	1.02	238	5	NA	NA	1.07
	Controls (313)	243	70			(0.68-1.52)	307	6			(0.32-3.56)

Results with P values less than 0.05 are in bold font.

Table 3. Breakdown association test in different age subgroups.

Gender	Age (years)	Groups (N) rs4420638	Genotype (N)			χ^2	P (d.f. = 2)	Allele (N)		χ^2	P (d.f. = 1)	OR (95%CI)
			AA	AG	GG			A	G			
All	<65	Cases (448)	343	100	5	1.37	0.51	786	110	1.32	0.250	1.18 (0.89-1.57)
		Controls (500)	398	98	4			894	106			
	≥65	Cases (328)	239	83	6	1.65	0.44	561	95	0.90	0.343	1.18 (0.83-1.68)
		Controls (232)	179	48	5			406	58			
Male	<65	Cases (320)	246	72	2	1.50	0.47	564	76	1.37	0.242	1.24 (0.86-1.78)
		Controls (296)	239	56	1			534	58			
	≥65	Cases (213)	148	61	4	2.37	0.31	357	69	1.99	0.162	1.39 (0.88-2.21)
		Controls (123)	95	26	2			216	30			
Female	<65	Cases (128)	97	28	3	0.44	0.80	222	34	0.33	0.574	1.15 (0.72-1.84)
		Controls (204)	159	42	3			360	48			
	≥65	Cases (115)	91	22	2	0.32	0.85	204	26	0.25	0.623	0.86 (0.49-1.53)
		Controls (109)	84	22	3			190	28			

It is well known that the *APOE* variant is a predictor of plasma lipid levels in cardio-vascular disease (Yin et al., 2013). Therefore, we further stratified the data analysis into different lipids by rs4420638 genotype. As shown in Table 4 and Figure 1, there were significantly higher levels of HDL-C (P = 0.014) and APOA-I (P < 0.0001) in controls than in cases in the rs4420638-AA carriers. In a further breakdown analysis by gender, we observed similar significant associations between HDL-C (P = 0.012; Table 5) and APOA-I levels (P = 0.0001; Figure 1) with CHD in the male rs4420638-AA carriers. However, no evidence of association was found in the female subgroup analysis (data not shown).

Table 4. Comparison of lipid levels between cases and controls under the dominant model.

Character	AA			AG+GG		
	Case	Control	P	Case	Control	P value
Age (years)	62.48 ± 9.19	59.62 ± 10.95	<0.0001	62.16 ± 10.04	60.06 ± 9.35	0.046
Gender (male)	394	334	0.098	139	85	0.126
LDL-C (mM)	2.55 ± 0.93	2.58 ± 0.85	0.670	2.63 ± 1.01	2.62 ± 0.89	0.458
TC (mM)	4.32 ± 1.15	4.40 ± 1.06	0.944	4.44 ± 1.20	4.44 ± 0.99	0.377
HDL-C (mM)	1.09 ± 0.27	1.14 ± 0.27	**0.014**	1.08 ± 0.28	1.15 ± 0.33	0.204
TG (mM)	1.67 ± 1.04	1.62 ± 0.99	0.246	1.78 ± 1.25	1.57 ± 0.89	0.071
APOA-I (mg/dL)	0.99 ± 0.22	1.05 ± 0.21	**<0.0001**	1.01 ± 0.24	1.01 ± 0.23	0.646
APOB (mg/dL)	0.78 ± 0.27	0.81 ± 0.26	0.138	0.84 ± 0.29	0.81 ± 0.26	0.195
APOE (mg/dL)	4.37 ± 1.72	4.43 ± 1.85	0.890	4.29 ± 1.62	4.13 ± 1.67	0.175

Results with P values less than 0.05 are in bold font. P values were adjusted for age and gender. LDL-C = low-density lipoprotein cholesterol; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; TG = triglycerides.
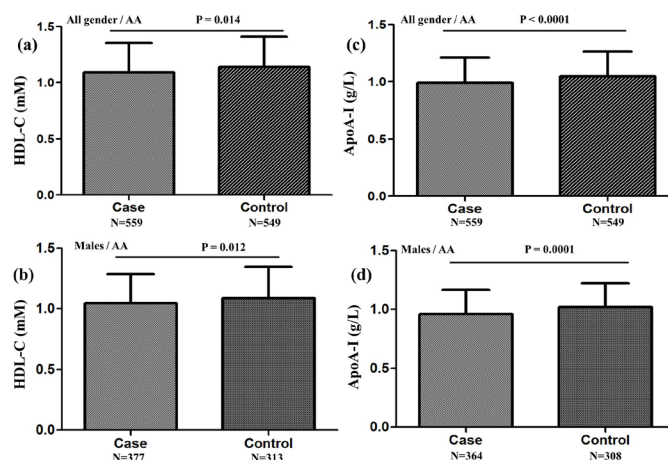

Figure 1. rs4420638 genotype AA shows significant association with HDL-C and APOA-I levels in CHD patients. (**a**) Comparison of HDL-C levels between cases and controls according to the genotype AA; (**b**) Comparison of HDL-C level in males between cases and controls according to the genotype AA; (**c**) Comparison of APOA-I levels in males between cases and controls according to the genotype AA; (**d**) Comparison of APOA-I levels between cases and controls according to the genotype AA.

Table 5. Comparison of lipid levels in gender subgroups under the dominant model.

Character	AA			AG+GG		
	Case	Control	P value	Case	Control	P value
Male						
Age (years)	61.65 ± 9.34	58.12 ± 11.25	<0.0001	61.90 ± 10.60	58.98 ± 9.60	0.039
LDL-C (mM)	2.46 ± 0.91	2.46 ± 0.83	0.592	2.55 ± 1.02	2.53 ± 0.86	0.669
TC (mM)	4.15 ± 1.10	4.25 ± 1.03	0.631	4.30 ± 1.21	4.30 ± 0.90	0.722
HDL-C (mM)	1.05 ± 0.24	1.09 ± 0.26	**0.012**	1.05 ± 0.29	1.10 ± 0.33	0.362
TG (mM)	1.65 ± 1.03	1.70 ± 1.11	0.833	1.75 ± 1.24	1.63 ± 0.96	0.307
ApoA-I (mg/dL)	0.96 ± 0.21	1.02 ± 0.20	**0.0001**	0.98 ± 0.24	0.99 ± 0.23	0.865
ApoB (mg/dL)	0.76 ± 0.26	0.79 ± 0.26	0.218	0.81 ± 0.29	0.82 ± 0.27	0.908
ApoE (mg/dL)	4.10 ± 1.55	4.36 ± 2.09	0.232	4.08 ± 1.54	3.83 ± 1.72	0.264

Results with P values less than 0.05 are in bold font. P values were adjusted for age. LDL-C = low-density lipoprotein cholesterol; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; TG = triglycerides.

As shown in Figure 2, our meta-analysis involved 18 study stages (Clarke et al., 2006; Farrall et al., 2006; Helgadottir et al., 2007; Hofman et al., 2007; Samani et al., 2007; Schunkert et al., 2008; Stirnadel et al., 2008; Elliott et al., 2009; Erdmann et al., 2009; Myocardial Infarction Genetics Consortium et al., 2009; Marques-Vidal et al., 2009; Stewart et al., 2009; Waterworth et al., 2010; Ken-Dror et al., 2010) among 20,498 cases and 52,405 controls from European and Asian ethnic backgrounds. No significant heterogeneity was found among the enrolled studies (I^2 = 30.6%, P = 0.107). The pooled ORs suggest a strong protective effect of rs4420638 against CHD risk (OR = 1.18, 95%CI = 1.14-1.22, P < 0.0001, fixed-effect method). Funnel plots were used to assess publication bias in the studies. As shown in Figure 3, the results do not show any evidence of publication bias (P_{Begg} = 0.13, P_{Egger} = 0.36). A further subgroup analysis by ethnicity showed that rs4420638 was significantly associated with CHD risk in both Europeans (OR = 1.18, 95%CI = 1.13-1.22, P < 0.0001) and Asians (OR = 1.19, 95%CI = 1.09-1.30, P < 0.0001) (Table 6).

Study	Populations	Study stage	Case	Control	OR, Fixed, 95% CI	OR (95%CI)	Weight%
Farrall (2006)	European	PROCARDIS	4070	4258		1.22(1.12-1.34)	16.05
Clarke (2006)	European	ISIS	2073	1493		1.27(1.12-1.44)	8.17
Samani (2007)	European	WTCCC	1926	2937		1.07(0.97-1.18)	13.44
Samani (2007)	European	GerMIFS-I	995	1644		1.09(0.92-1.29)	4.52
Helgadottir (2007)	European	PENN CATH	933	468		1.25(0.98-1.60)	2.15
Hofman (2007)	European	Rotterdam	779	5195		1.05(0.89-1.25)	4.47
Schunkert (2008)	European	Inter Heart EW	796	895		1.02(0.85-1.24)	3.62
Schunkert (2008)	Asian Indian	Inter Heart IA	1137	1215		1.13(0.96-1.34)	4.64
Stirnadel (2008)	European	GEMS	262	1580		1.27(1.00-1.61)	2.28
Elliott (2009)	Asian Indian	LOLIPOP IA	1250	12568		1.22(1.08-1.38)	8.59
Elliott (2009)	European	LOLIPOP EW	896	5761		1.16(1.02-1.32)	7.77
Erdmann (2009)	European	GerMIFS-II	1222	1298		1.27(1.10-1.47)	6.14
Stewart (2009)	European	OHS	1542	1455		1.43(1.25-1.64)	7.00
Kathiresan (2009)	European	MEDSTAR	943	483		1.02(0.78-1.32)	1.87
Marques (2009)	European	CoLaus	202	5233		0.95(0.72-1.26)	1.65
Waterworth (2010)	European	EPIC-Norfolk1	421	2698		1.22(0.99-1.50)	2.99
Ken-Dror (2010)	European		275	2492		1.09(0.84-1.41)	1.92
Our study (2014)	Asian Chinese		776	732		1.21(0.97-1.50)	2.72
Overall			20498	52405		1.18(1.14-1.22)	100.00

Heterogeneity chi-squared = 24.48 (d.f. = 17) P = 0.107
I-squared (variation in ES attributable to heterogeneity) = 30.6%
Test of ES = 1: Z = 9.37 P < 0.0001

0.64   1.00   1.64
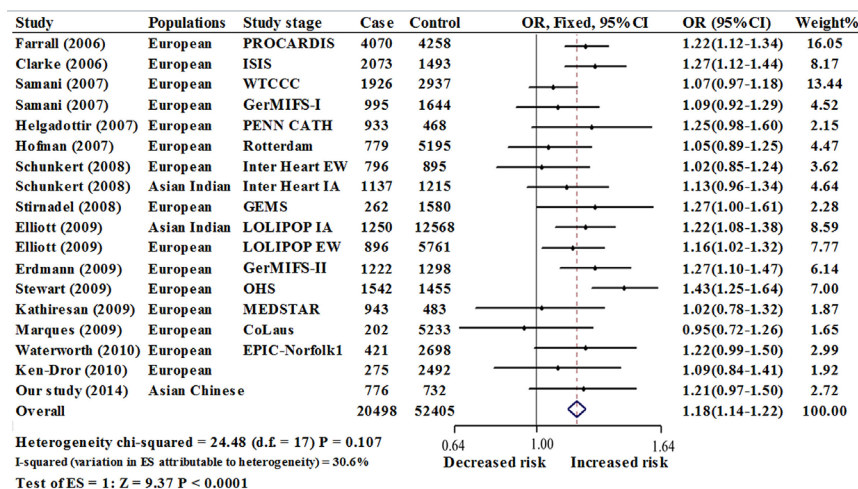**Decreased risk**   **Increased risk**

Figure 2. Meta-analysis of the studies between rs4420638 of *APOE-C1-C4-C2* gene and CHD risk. WTCCC: Wellcome Trust Case Control Consortium; GerMIFS1: German MI Family Studies I; LOLIPOP EW: London Life Sciences Population European White; PROCARDIS: Precocious Coronary Artery Disease study; LOLIPOP IA: London Life Sciences Population Indian Asian; GerMIFS2: German MI Family Studies I; ISIS: International Studies of Infarct Survival; OHS: Ottawa Heart Study.
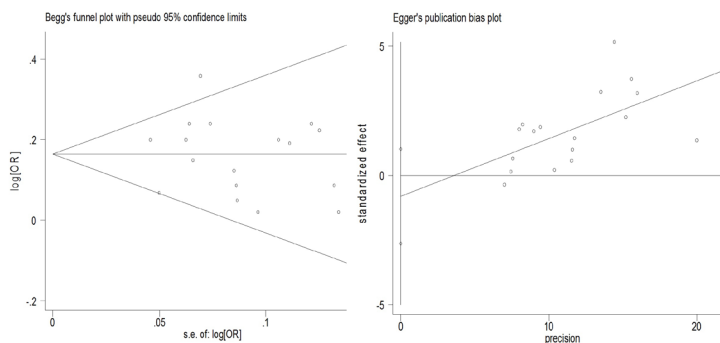

Figure 3. Funnel plot of the association between rs4420638 and CHD risk. $P_{(Begg)}$ = 0.13; $P_{(Egger)}$ = 0.36.

Table 6. Summary estimates for the ORs of rs4420638 polymorphism in subgroup analyses.

Study population	Studies/stages (N)	OR (95%CI)	Weight (%)	Z	P (z)	I^2	P	Heterogeneity statistics
European	15	1.18 (1.13-1.22)	84.05	8.09	<0.0001	41.30%	0.048	23.86
Asian	3	1.19 (1.09-1.30)	15.95	3.82	<0.0001	0.00%	0.760	0.55
Overall	18	1.18 (1.14-1.22)	100.00	9.37	<0.0001	30.60%	0.107	24.48

DISCUSSION

The goal of our study was to explore the association between *APOE* rs4420638 and circulating lipid concentrations, along with the risk of CHD in Han Chinese. Our results show that rs4420638 is significantly correlated with increased CHD risk in Han Chinese males (P = 0.040, OR = 1.34, 95%CI = 1.01-1.78). This variant is likely to increase the risk of CHD under the dominant model in males (P = 0.036, OR = 1.38, 95%CI = 1.02-1.88). Gender-specific difference has been shown in many human diseases (Weiss et al., 2006). Epidemiologic evidence shows that men have a higher cardiovascular disease risk than women worldwide (Ginter and Simko, 2013). In previous studies, we have shown that CHD risk is gender-dependent in Han Chinese (Zhou et al., 2012). This observation can possibly be explained by the hormonal profiles and behaviors that differ between the two genders (Barrett-Connor, 2007).

HDL-C, the smallest of the lipoprotein molecules, has been regarded as an antiatherogenic lipoprotein in humans (Toth, 2005). Epidemiological studies have proved that high HDL-C concentration protects against cardiovascular diseases, and low HDL-C concentrations may increase the risk of atherosclerosis (Hamrefors et al., 2010). APOA-I, the major protein component of HDL in plasma, can help clear cholesterol and contributes to atheroma regression (Asztalos and HDL Atherosclerosis Treatment Study, 2004). APOA-I protein activity is associated with high HDL-C concentration and a reduced risk of CHD (Asztalos and HDL Atherosclerosis Treatment Study, 2004). Our results also indicate that the rs4420638-AA genotype is significantly associated with the reduction of HDL-C and APOA-I levels in CHD cases.

Circulating levels of blood lipids are consistently associated with the risk of CHD (Genoux et al., 2013). The lipid levels are affected by different genetic and acquired factors. Several genes are associated with lipid levels and the risk of cardiovascular diseases (Gungor et al., 2012). APOE is a glycoprotein that plays a fundamental role in lipid metabolism. *APOE* polymorphisms are related to lipid levels in CHD patients (Smalinskiene et al., 2013). SNP rs4420638, located in the *APOE-C1-C2* cluster, is significantly associated with plasma LDL-C and APOB concentration (Ken-Dror et al., 2010). In our study, significant interaction is found between the rs4420638 genotype and HDL-C and APOA-I concentrations in Han Chinese males. These findings could be partly explained by the particular genetic backgrounds and diet habits of the Chinese.

Several lines of study have confirmed that *APOE* polymorphisms contribute to the risk of CHD. A previous meta-analysis combining nine studies was performed and suggested a significant association between *APOE* polymorphism rs4420638 and CHD risk (Elliott et al., 2009). However, their results in Asian subgroup studies were controversial, which might have been due to the low number of studies or genetic heterogeneity. In this meta-analysis, a total of 18 studies including 72,903 individuals were examined to confirm the significant association. Our results establish that rs4420638 results in an 18% increase in the risk of CHD in European and Asian populations.

There were several limitations to our study. Firstly, the sample size of the case-control study was moderate and may not have been sufficient to identify susceptible genes with moderate or minor effects. Secondly, only one SNP was checked for its association with CHD. Other genetic polymorphisms of *APOE* may also contribute to the risk of CHD. Further investigation in large samples is necessary for other polymorphisms that may be related to CHD in various populations. Thirdly, a power calculation for rs4420638 in the male group analysis showed that our case-control study had a 53.2% power to detect a relative risk at a significance level of 0.05. The P value was 0.040 between cases and controls in the male group, which may not have been retained after multiple test corrections. We can exclude the chance of a random positive finding of this SNP. Our sample size was comparatively small, but should have been sufficient to describe a tendency that may guide clinical practice, since our meta-analysis confirmed that rs4420638 presents a significantly increased risk of CHD in both Europeans and Asians.

In conclusion, our case-control study showed that rs4420638 in the *APOE-C1-C2* cluster displays a strong relationship with CHD risk in Han Chinese males. The rs4420638 genotype AA showed significant association with the circulating HDL-C and APOA-I concentrations in males. Meta-analysis supports the association between rs4420638 and CHD risk in European and Asian populations.

Conflicts of interest

The authors declare no conflict of interest.

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