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Association between apolipoprotein E polymorphisms and premature coronary artery disease: a meta-analysis

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Abstract

Background: Although several studies have explored the genetic polymorphisms of apolipoprotein E (*APOE*) and their impact on premature coronary artery disease (PCAD), there is still some controversy regarding the significance of their association. Our aim is to estimate the association between *APOE* polymorphisms and PCAD via meta-analysis.
Methods: All relevant case-control studies and cohort studies published in Chinese or English prior to March 2016 were searched for in electronic databases. Detailed information concerning each piece of literature was independently extracted by two researchers. We used STATA11.0 to process all data and to determine the pooled odds ratio (OR). Altogether, four genetic models were applied to calculate OR and 95% confidence interval (CI): (1) *ε2* allele vs. *ε3* allele; (2) *ε2* carriers vs. *ε3/3*; (3) *ε4* allele vs. *ε3* allele; (4) *ε4* carriers vs. *ε3/3*.
Results: Eighteen studies concerning *APOE* polymorphisms and their impact on PCAD were included in the final analysis. The pooled analysis displayed that the *ε2* allele and *ε2* carriers increased the risk of PCAD significantly among Asians (OR 1.54; 95% CI, 1.09–2.17; OR 1.65; 1.10–2.47), while they showed protective effects on PCAD in Caucasians (OR 0.77; 95% CI, 0.62–0.95; OR 0.69; 0.54–0.89). Subjects with the *ε4* allele and *ε4* carriers showed significant associations with PCAD (OR 1.62; 95% CI, 1.27–2.06; OR 1.65; 1.27–2.15).
Conclusions: Our investigation supported the fact that the *ε2* allele in *APOE* may appear as a risk factor for PCAD in Asians while a protective factor in Caucasians and that the *ε4* allele acted as a genetic risk factor for PCAD.

Keywords: apolipoprotein E; genetic risk; polymorphism; premature coronary artery disease.

Introduction

Coronary artery disease (CAD), a multifactorial heart disorder resulting from all kinds of predisposing environmental and genetic factors [1], is still one of the leading causes of disability and death around the world, accounting for 14.8% of global death [2]. Premature coronary artery disease (PCAD) is defined as CAD that occurs in males <55 years old or females <65 years old [3]. Even though PCAD constitutes only about 30% of all CAD subjects [4, 5], it is highly stressful for the patients' families and generates a heavy social burden because of the longer period that the patient lives with the disease in comparison to older patients with CAD [6]. Large epidemiological studies have revealed that genetic factors have a strong influence on early onset CAD [7]. One known factor is the apolipoprotein E (*APOE*) gene [8].

Apolipoprotein E (apoE) is a multifunction glycoprotein containing 299 amino acids, which are encoded by the *APOE* gene [9]. It acts as cholesterol carrier and plays a major role in mediating the transportation and metabolism of lipids [10]. The *APOE* gene is situated at 19q13.2 of the human chromosome and has four exons and three introns [11]. As the genetic polymorphisms which are most widely studied in *APOE*, rs429358 and rs7412 together define the *ε2*, *ε3*, and *ε4* alleles, which encode three major protein isoforms (E2, E3, E4) and generate six different genotypes (*ε2/4*, *ε2/3*, *ε3/4*, *ε3/3*, *ε2/2*, *ε4/4*) [12]. There is significant

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discrepancy in structure and function among the three protein isoforms [12]. *ε3* is the wild type, and its amino acids at positions 112 and 158 are cysteine and arginine, respectively [10]. It has normal affinity for low-density lipoprotein receptors (LDLR) and modulates the clearance of lipoprotein remnants from the plasma [12]. When the 112th amino acid is replaced by arginine, *ε3* mutates into *ε4*. Similarly, *ε3* mutates into *ε2* when the 158th amino acid is replaced by cysteine [13]. *ε4* has similar high affinity for LDLR as *ε3* and enhanced binding capacity for lipids, so it impairs the process of lipolysis and is associated with high levels of very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and total cholesterol [12]. As for *ε2*, it has defective affinity ability for LDLR, which would delay the clearance of VLDL. *ε2* is associated with low levels of LDL and total cholesterol [12].

Several meta-analyses have been conducted to explore the impact of *APOE* polymorphisms on CAD. The analysis conducted by Hong Xu found that the *ε2* allele in *APOE* decreased the prevalence of myocardial infarction while the *ε4* allele increased the incidence [14]. Two other meta-analyses conducted in the Chinese population also found a high risk of the *ε4* allele for CAD [15, 16]. However, no meta-analysis explored the genetic risk of *APOE* in PCAD. Due to strong evidence that genetic factors have an influence on PCAD and that *APOE ε4* has an indirect impact on CAD [17], examining the impact of *APOE* polymorphisms on PCAD could eliminate possible confounding factors which are related to advanced age and could allow for more precise evaluation of the risk of inherited mutations. Although several studies have explored the genetic polymorphisms of *APOE* and their impact on PCAD [8, 18–34], the significance of their association is yet to be determined. Our aim is to estimate the association between *APOE* polymorphisms and PCAD by conducting this meta-analysis.

Materials and methods

Search strategy

All relevant case control studies and cohort studies exploring *APOE* polymorphisms and their relationship with PCAD published in Chinese or English prior to March 28th, 2016 were searched for in electronic databases, including PubMed, Embase, Web of Science, Wanfang Database, Chinese National Knowledge Infrastructure, VIP Database, and China Biological Medicine using all feasible combinations of the following key words: ('CAD' OR 'coronary heart disease' OR 'ischemic heart disease' OR 'myocardial infarction' OR 'angina pectoris' OR 'acute heart failure' OR 'cardiovascular disease') AND ('gene polymorphisms' OR 'allele' OR 'genotype' OR 'single nucleotide polymorphism') AND ('apolipoprotein E' OR 'apoE') AND ('premature' OR 'young'). The references of all retrieved literature were also screened. In case of duplicated data in publications studying the same group of people, we kept the more recent publication or the one that had the greatest sample size.

Inclusion and exclusion criteria

To identify all eligible publications, the inclusion criteria were listed as follows: (1) studies explored *APOE* polymorphisms and their association with PCAD; (2) case control studies or cohort studies; (3) case groups needed to be clearly diagnosed with PCAD, which was defined as CAD that occurs in males <55 or females <65 [3]; (4) control groups should be without CAD; (5) the distribution of both sample sizes and alleles should be reported in the studies; and (6) the study is of high quality.

The exclusion criteria listed as follows were used: (1) reviews; (2) case reports; (3) letters; and (4) duplicated researches.

Data extraction

Two authors independently screened the literature to minimize selection bias. For potential relevant literature whose full-texts could not be acquired online, emails were sent to corresponding authors to obtain full text. Detailed information concerning each piece of eligible literature was extracted by two researchers independently. Information included first author's name, year of publication, ethnicity, clinical subtype, source of control, male percentage, mean age, mean BMI, sample size of controls and cases, genotyping method, Hardy-Weinberg equilibrium (HWE), the distribution of genotypes and frequencies of allele in cases and controls. Any disputes were settled by a third researcher.

Quality assessment of included studies

The quality of all eligible publications was assessed by two authors based on the Newcastle-Ottawa assessment scale [35]. Asterisks (*) were used as quality indicators, and better quality studies would be awarded more stars for a maximum of up to nine stars. Studies with more than six stars were perceived as high quality. Moreover, two authors assessed the risk of bias in each literature based on the Quality In Prognosis Studies (QUIPS) tool [36], which contains six bias domains. The bias of each domain is rated as having high, moderate, or low risk of bias. Any disputes were settled by a third researcher.

Statistical analysis

We applied pooled odds ratio (OR) and its corresponding 95% CI to assess the intensity of the influence of *APOE* polymorphisms on PCAD, and Z test was used to examine the statistical significance of the OR. As *ε3/3* is the most common genotype among humans [37], we designated the *ε3* allele or genotype *ε3/3* as the reference category in our meta-analysis. Genotypes *ε2/2* and *ε4/4* were detected

in only a few studies. Altogether, four genetic models were applied to calculate OR and 95% CI: (1) *ε2* allele vs. *ε3* allele; (2) *ε2* carriers vs. *ε3/3*; (3) *ε4* allele vs. *ε3* allele; (4) *ε4* carriers vs. *ε3/3*. (Patients with genotypes *ε2/3* and *ε2/2* were included in *ε2* carriers. Similarly, patients with genotypes *ε4/3* and *ε4/4* were included in *ε4* carriers) [16]. The distribution of genotypes was examined by χ^2-test to test if it fulfilled the HWE criteria. We used the I^2 test to examine the heterogeneity among all publications (I^2<25%, low heterogeneity; 25%≤I^2<50%, moderate heterogeneity; I^2≥50%, high heterogeneity) [38]. For publications with moderate or high heterogeneity, we chose random effects models to merge OR; otherwise, fixed effects models were selected. Different features concerning studied populations resulted in discrepancies in results, thus we conducted stratified analyses by ethnicity (Caucasian, Asian, or African), clinical subtype (CAD or MI), source of control [population based (PB) or hospital based (HB)], male percentage (≥65% or <65%), and sample size (≥250 or <250). Furthermore, we utilized univariate meta-regression analyses to identify the source of heterogeneity among studies and investigate the effects of different moderators (ethnicity, mean age, HWE, source of control, clinical subtype, male percentage, and mean BMI). Sensitivity analysis was conducted to test the stabilization of the results by omitting each research. Begg's test [39] and Egger's test [40] were performed to detect the publication bias. All the data were processed by STATA11.0 for windows (Stata, College Station, TX, USA). A p-value < 0.05 indicated statistically significant.

Results

Study characteristics

The detailed screening process for all relevant literature is displayed in a flow diagram shown in Figure 1. Initially, 472 relevant publications were identified, while only 18 research reports covering a total of 2361 PCAD and 2811 controls fulfilled the inclusion criteria and were included in our analysis. Detailed information concerning each piece of eligible publication was listed in Table 1. The distribution of genotypes and frequencies of *APOE* allele is shown in Table 2. Results of quality assessment based on the Newcastle-Ottawa assessment scale are displayed in Table 3, while the results of risk of bias assessment are shown in Table 4.

Association between *ε2* allele and PCAD

The results of the *ε2* allele and its carriers are shown in Figures 2 and 3. We chose random effects models to merge all data based on its moderate heterogeneity (for *ε2* allele vs. *ε3* allele, I^2=31.2%, τ^2=0.067; for *ε2* carriers vs. *ε3/3*, I^2=29.7%, τ^2=0.085). Overall, the *ε2* allele and its carriers had a protective effect on PCAD although the difference was not statistically significant (OR 0.90; 95% CI, 0.72–1.13; OR 0.87; 0.67–1.14). When stratified by ethnicity, Asian *ε2* allele carriers significantly had an increased risk of PCAD (OR 1.54; 95% CI, 1.09–2.17; OR 1.65; 1.10–2.47) while protective effects were shown for PCAD in Caucasians (OR 0.77; 95% CI, 0.62–0.95; OR 0.69; 0.54–0.89). Forest plots of subgroup analyses by ethnicity were displayed in Figure 4. We performed subgroup analyses by clinical subtype and found decreased susceptibility of MI in the *ε2* allele vs. *ε3* allele model (OR 0.70; 95%CI, 0.49–0.98). Significant protective effects of the *ε2* allele and its carriers on PCAD also existed in population-based studies (OR 0.78; 95% CI, 0.64–0.95; OR 0.73; 0.57–0.93) and in the male ≥65% subgroup (OR 0.77; 95% CI, 0.63–0.95; OR 0.69; 0.54–0.89), and the increased risk of PCAD was only present in *ε2* carriers in the male <65% subgroup (OR 1.76; 95% CI, 1.15–2.71), while other subgroups did not reveal any significant associations. The detailed results of all subgroup analyses were shown in Table 5.

Association between *ε4* allele and PCAD

Due to the heterogeneity among studies exploring the associations between the *ε4* allele and *ε4* carriers with

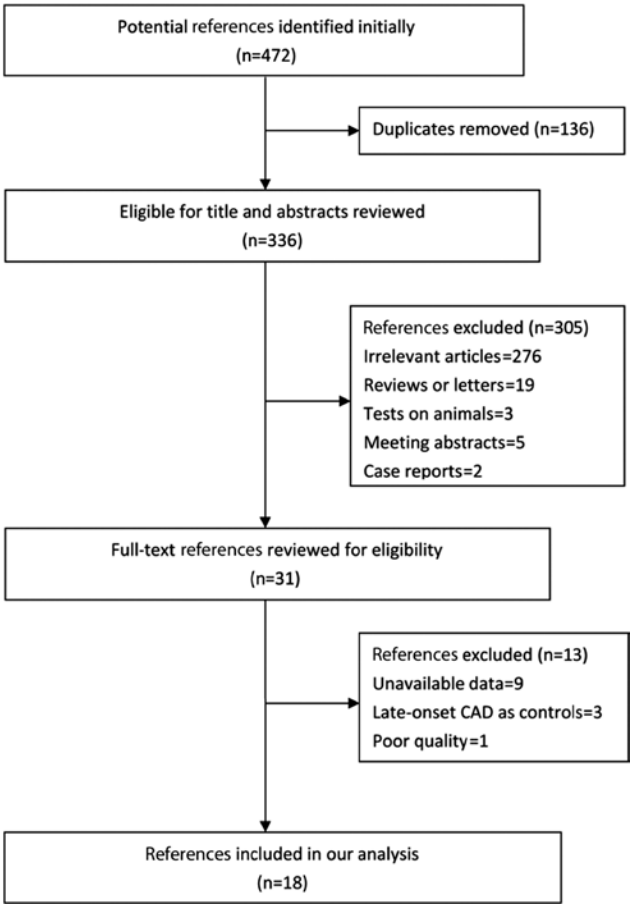

Figure 1: Flow diagram of the study selection.

Table 1: Characteristics of all studies included in the meta-analysis.

First author	Year	Ethnicity	Clinical subtype	Source of control	% of male	Mean age	Mean BMI	Sample size		Genotyping method	HWE
								Case	Control		
van Bockxmeer [8]	1992	Caucasian	CAD	PB	100	NA	NA	91	172	RFLP	N
Peacock [18]	1992	Caucasian	MI	PB	100	40.4	25.5	87	91	RFLP	NA
Miettinen [19]	1994	Caucasian	CAD	HB	90.9	40.0	NA	80	50	DNA-Seq	Y
Zhao [20]	2000	Asian	MI	HB	100	45.8	24.4	50	49	RFLP	Y
Petrovic [21]	2000	Caucasian	CAD	PB	86.5	51.4	27.0	166	130	RFLP	Y
Batalla [22]	2000	Caucasian	MI	HB	100	42.5	NA	220	200	RFLP	Y
Viitanen [23]	2001	Caucasian	CAD	PB	66.7	53.6	27.1	118	110	RFLP	Y
Peng [24]	2001	Asian	CAD	HB	53.4	51.8	NA	52	180	RFLP	Y
Yang [25]	2001	Asian	CAD	PB	NA	50.1	30.7	68	136	RFLP	Y
Mamotte [26]	2002	Caucasian	CAD	PB	66.9	41.6	26.6	564	639	RFLP	Y
Kumar [27]	2003	Asian	MI	PB	92.5	36.3	22.9	35	45	RFLP	N
Letonja [28]	2004	Caucasian	CAD	PB	0	55.1	27.2	147	114	RFLP	Y
Ranjith [29]	2004	African	MI	PB	NA	NA	NA	195	300	RFLP	N
Kolovou [30]	2005	Caucasian	CAD	PB	100	NA	NA	73	103	RFLP	Y
Aasvee [31]	2006	Caucasian	MI	PB	100	49.0	28.2	71	85	RFLP	Y
Balcerzyk [32]	2007	Caucasian	CAD	PB	67.2	42.4	26.2	140	107	RFLP	Y
Chu [33]	2007	Asian	CAD	HB	54.8	59.0	NA	92	220	RFLP	N
Djan [34]	2011	Caucasian	CAD	PB	100	NA	28.1	30	30	RFLP	N

HB, hospital-based; PB, population-based; HWE, Hardy-Weinberg equilibrium; RFLP, restriction fragment length polymorphism; DNA-Seq, DNA sequence testing.

Table 2: The distribution of genotypes and frequencies of *APOE* allele in each eligible literature.

First author	Year	Case						Control						Case			Control		
		ε2/2	ε2/3	ε2/4	ε3/3	ε3/4	ε4/4	ε2/2	ε2/3	ε2/4	ε3/3	ε3/4	ε4/4	ε2	ε3	ε4	ε2	ε3	ε4
van Bockxmeer [8]	1992	1	7	4	42	29	7	0	18	3	110	41	0	13	120	47	21	279	44
Peacock [18]	1992	–	–	–	–	–	–	–	–	–	–	–	–	11	124	39	17	132	33
Miettinen [19]	1994	0	5	1	42	30	2	0	3	0	33	11	3	6	119	35	3	80	17
Zhao [20]	2000	0	4	0	40	6	0	0	5	0	41	3	0	4	90	6	5	90	3
Petrovic [21]	2000	0	16	5	119	26	0	0	20	0	91	18	1	21	280	31	20	220	20
Batalla [22]	2000	0	9	1	174	32	4	0	18	1	151	28	2	10	389	41	19	348	33
Viitanen [23]	2001	0	4	3	65	37	9	0	8	2	70	27	3	7	171	58	10	175	35
Peng [24]	2001	0	11	2	24	13	2	0	27	3	126	24	0	13	72	19	30	303	27
Yang [25]	2001	0	5	0	45	12	6	1	12	0	106	15	1	5	107	24	14	239	17
Mamotte [26]	2002	5	36	12	340	156	15	4	68	16	383	149	19	58	872	198	92	983	203
Kumar [27]	2003	0	6	1	12	6	10	2	9	0	32	0	2	7	36	27	13	73	4
Letonja [28]	2004	0	14	1	105	27	0	0	10	3	79	22	0	15	251	28	13	190	25
Ranjith [29]	2004	0	7	3	139	45	1	3	18	3	228	43	5	10	330	50	27	517	56
Kolovou [30]	2005	0	3	0	61	8	1	0	9	3	67	23	1	3	133	10	12	166	28
Aasvee [31]	2006	2	7	2	41	16	3	1	13	3	52	16	0	13	105	24	18	133	19
Balcerzyk [32]	2007	0	10	1	98	29	2	0	10	1	82	14	0	11	235	34	11	188	15
Chu [33]	2007	4	19	4	38	27	0	0	41	5	140	34	0	31	122	31	46	355	39
Djan [34]	2011	0	0	1	16	13	0	0	1	0	19	4	1	1	45	14	1	43	6

PCAD (for *ε4* allele vs. *ε3* allele, I^2=69.9%, τ^2=0.168; for *ε4* carriers vs. *ε3/3*, I^2=63.4%, τ^2=0.171), we applied random effects models to calculate the pooled OR. The results showed that the *ε4* allele and its carriers had significantly increased prevalence of PCAD (OR 1.62; 95% CI, 1.27–2.06; OR 1.65; 1.27–2.15), as shown in Figures 5 and 6. Furthermore, our subgroup analyses found a higher susceptibility of PCAD in the *ε4* allele and in *ε4* carriers among Caucasians (OR 1.31; 95% CI, 1.05–1.64; OR 1.31; 1.02–1.68), Asians (OR 3.31; 95% CI, 2.00–5.49; OR 3.34; 2.02–5.52), CAD (OR 1.58; 95% CI, 1.18–2.12; OR 1.62; 1.19–2.20), MI (OR 1.77; 95% CI, 1.08–2.88; OR 1.89; 1.01–3.54),

Table 3: The results of quality assessment according to the Newcastle-Ottawa quality assessment scale.

First author	Year	Total stars	Adequate case definition	Representativeness of the cases	Selection of controls	Definition of controls	Comparability of cases and controls on the basis of the design or analysis	Ascertainment of exposure	Same ascertainment exposure method for both cases and controls	Non-response rate
van Bockxmeer [8]	1992	7		*	*	*	*	*	*	*
Peacock [18]	1992	7	*		*	*	**	*	*	
Miettinen [19]	1994	7	*	*		*	*	*	*	*
Zhao [20]	2000	7	*			*	**	*	*	*
Petrovic [21]	2000	7			*	*	**	*	*	*
Batalla [22]	2000	7		*		*	**	*	*	*
Viitanen [23]	2001	7	*	*	*	*		*	*	*
Peng [24]	2001	7	*			*	**	*	*	*
Yang [25]	2001	8	*	*	*	*	*	*	*	*
Mamotte [26]	2002	7	*	*	*	*		*	*	*
Kumar [27]	2003	9	*	*	*	*	**	*	*	*
Letonja [28]	2004	8	*		*	*	**	*	*	*
Ranjith [29]	2004	7		*	*	*	*	*	*	*
Kolovou [30]	2005	7	*	*	*	*		*	*	*
Aasvee [31]	2006	6			*		**	*	*	*
Balcerzyk [32]	2007	8	*		*	*	**	*	*	*
Chu [33]	2007	6		*		*	*	*	*	*
Djan [34]	2011	6	*		*	*		*	*	*

*A study can be awarded a maximum of one star for each item within the selection and exposure categories. **A maximum of two stars can be given for comparability.

Table 4: The results of risk of bias assessment based on the QUIPS tool.

First author	Year	Study participation	Study attrition	Prognostic factor measurement	Outcome measurement	Study confounding	Statistical analysis and reporting
van Bockxmeer [8]	1992	Low	Low	Low	Moderate	Low	Low
Peacock [18]	1992	Moderate	Moderate	Low	Low	Low	Low
Miettinen [19]	1994	Low	Low	Low	Low	Low	Low
Zhao [20]	2000	Moderate	Low	Low	Low	Low	Low
Petrovic [21]	2000	Moderate	Low	Low	Moderate	Low	Low
Batalla [22]	2000	Low	Low	Low	Moderate	Low	Low
Viitanen [23]	2001	Low	Low	Low	Low	Moderate	Low
Peng [24]	2001	Moderate	Low	Low	Moderate	Low	Low
Yang [25]	2001	Low	Low	Low	Low	Low	Low
Mamotte [26]	2002	Low	Low	Low	Moderate	Moderate	Low
Kumar [27]	2003	Low	Low	Low	Moderate	Low	Low
Letonja [28]	2004	Moderate	Low	Low	Low	Low	Low
Ranjith [29]	2004	Low	Low	Low	Moderate	Low	Low
Kolovou [30]	2005	Low	Low	Low	Moderate	Moderate	Low
Aasvee [31]	2006	Moderate	Low	Low	Moderate	Low	Low
Balcerzyk [32]	2007	Moderate	Low	Low	Moderate	Low	Low
Chu [33]	2007	Low	Low	Low	Moderate	Low	Low
Djan [34]	2011	Moderate	Low	Low	Low	Moderate	Low

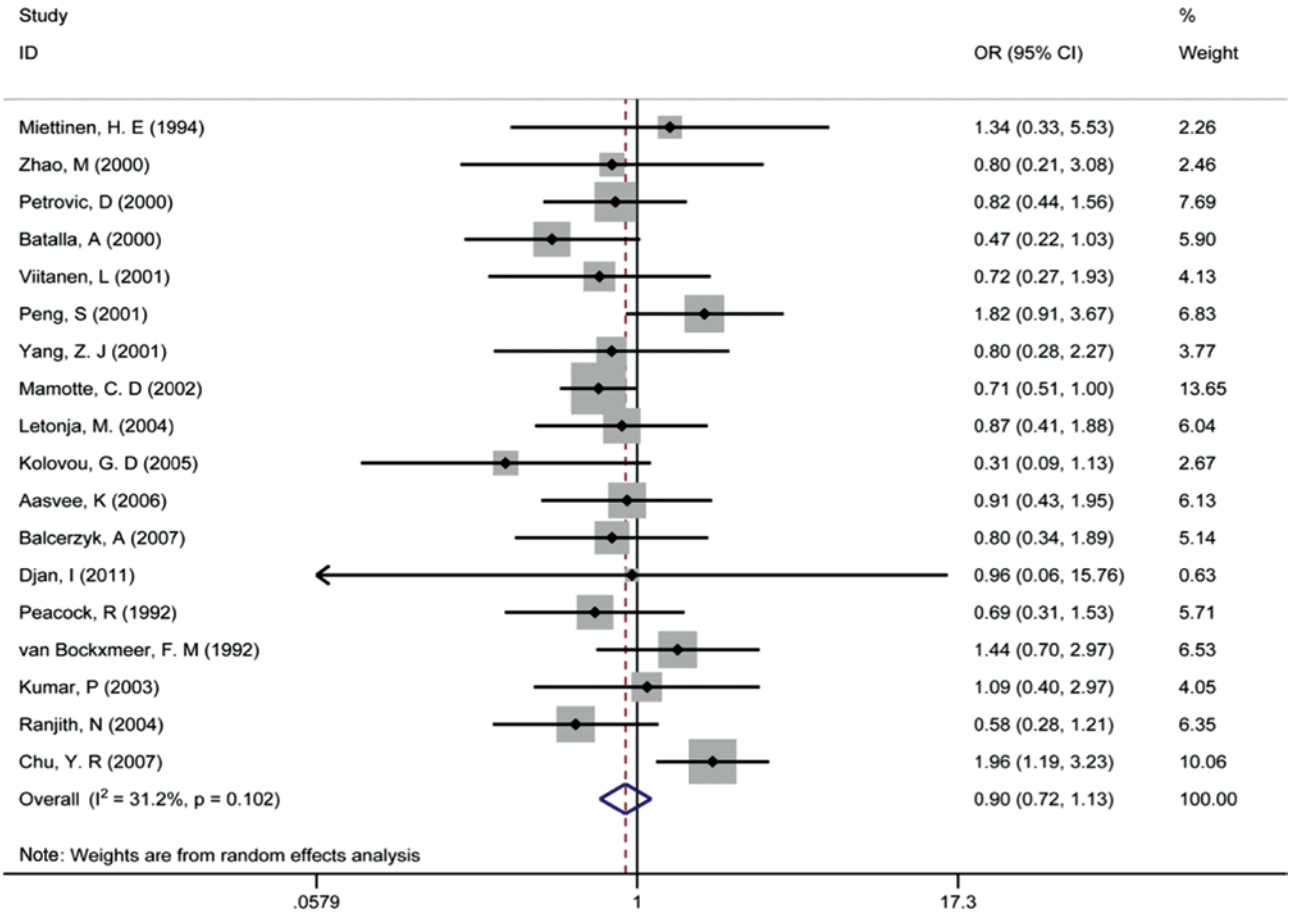

Figure 2: Pooled calculated OR of the association between the *ε2* allele and premature coronary artery disease.

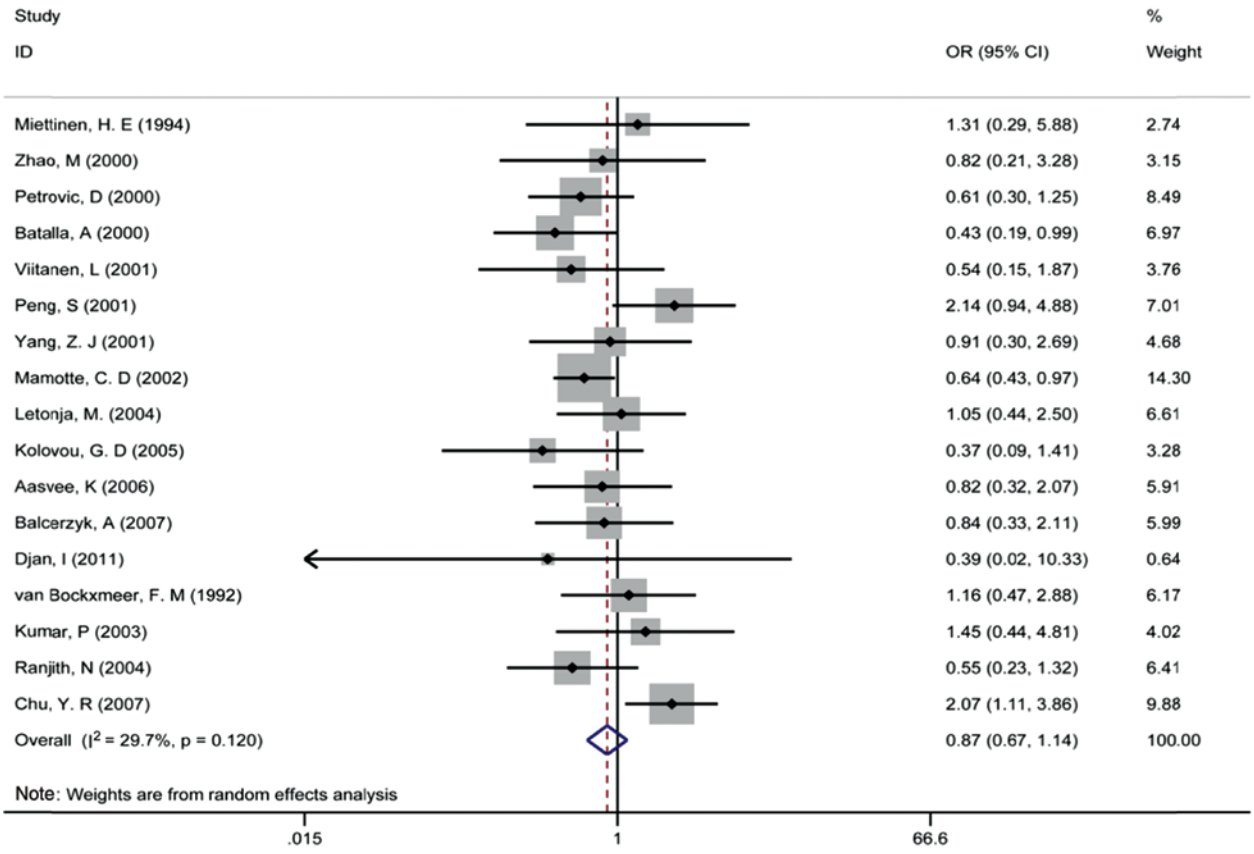

Figure 3: Pooled calculated OR of the association between *ε2* carriers and premature coronary artery disease.

population-based studies (OR 1.57; 95% CI, 1.17–2.10; OR 1.54; 1.13–2.11), hospital-based studies (OR 1.78; 95% CI, 1.19–2.66; OR 1.99; 1.21–3.27), and male ≥65% studies (OR 1.52; 95% CI, 1.15–2.03; OR 1.51; 1.10–2.09). Sample size did not affect this significant association. The detailed information was shown in Table 5. Forest plots of subgroup analyses by ethnicity were displayed in Figure 7.

Meta-regression

Univariate meta-regression analyses revealed that ethnicity accounted for 55.2% of the between-study heterogeneity for the *ε2* allele vs. *ε3* allele model (adjusted R^2=63.47%, p=0.05), 100% of the between-study heterogeneity for the *ε2* carriers vs. *ε3/3* model (adjusted R^2=100%, p=0.00), 28.6% of the between-study heterogeneity for the *ε4* allele vs. *ε3* allele model (adjusted R^2=40.55%, p=0.02), and 24.0% of the between-study heterogeneity for the *ε4* carriers vs. *ε3/3* model (adjusted R^2=30.21%, p=0.04). Age accounted for 100% of the between-study heterogeneity (adjusted R^2=100%, p=0.01) and source of control 55.2% of the between-study heterogeneity (adjusted R^2=67.24%, p=0.04) for the *ε2* allele vs. *ε3* allele model, while the other factors did not show significant results, which suggested that ethnicity was the main source of heterogeneity. Detailed results of meta-regression analyses were listed in Table 6.

Sensitivity analysis

Sensitivity analyses showed that the overall calculated OR did not change significantly after excluding each literature, which confirmed the stability and reliability of our analysis.

Publication bias

No obvious asymmetry appeared in funnel plots shown in Figure 8. At the same time, Begg's correlation (*ε2* vs. *ε3*: p=1.00; *ε2* carriers vs. *ε3/3*: p=0.97; *ε4* vs. *ε3*: p=0.23; *ε4* carriers vs. *ε3/3*: p=0.17) and Egger's regression (*ε2* vs. *ε3*: p=0.75; *ε2* carriers vs. *ε3/3*: p=0.97; *ε4* vs. *ε3*: p=0.06; *ε4* carriers vs. *ε3/3*: p=0.05) did not reveal any obvious publication bias among our eligible publications.

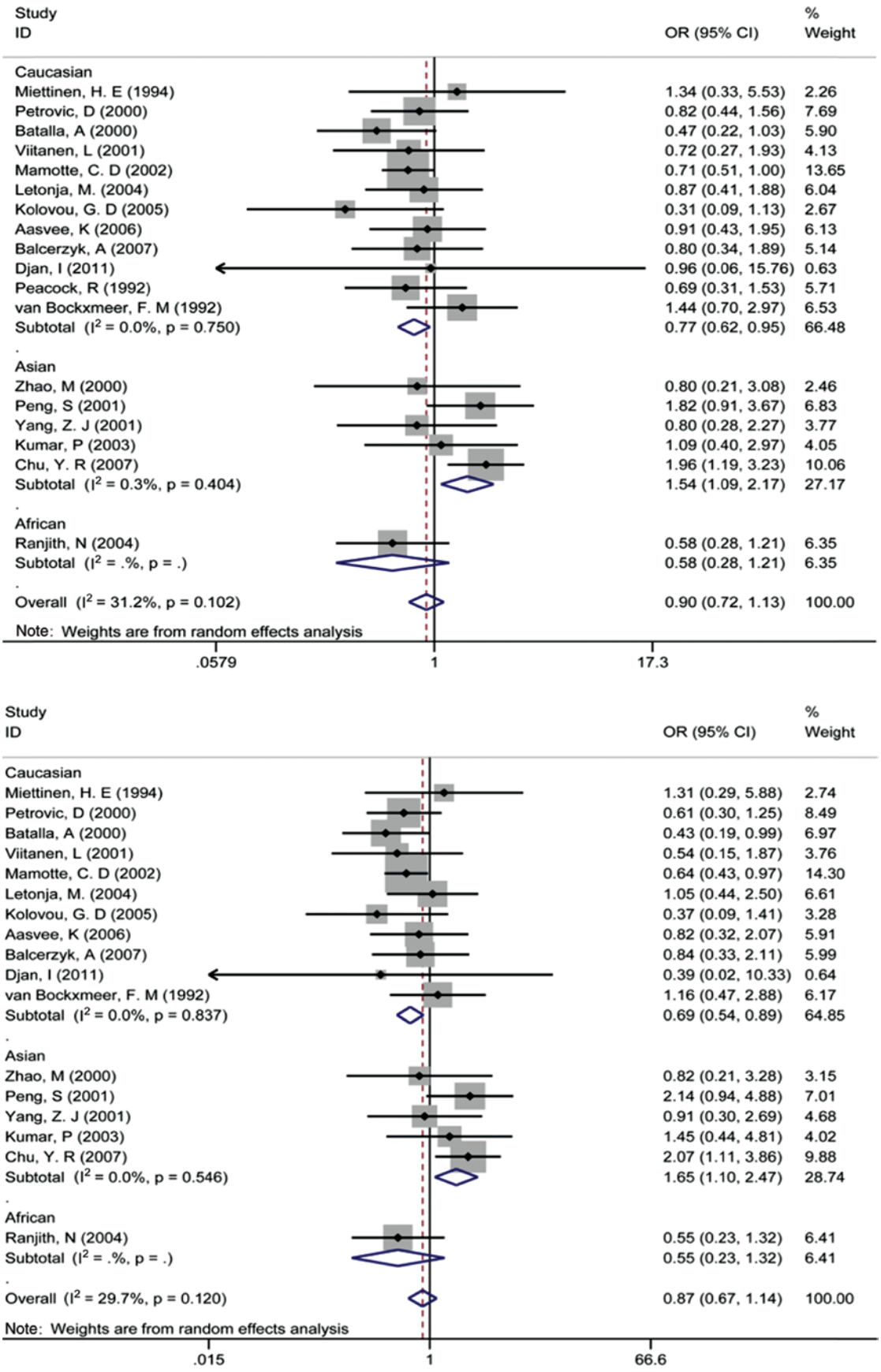

Figure 4: Forest plots of subgroup analyses by ethnicity in the *ε2* analyses.

Table 5: The results of subgroup analyses.

Subgroup	No. of studies	*ε2* vs. *ε3*			*ε4* vs. *ε3*			No. of studies	*ε2* carrier vs. *ε3/3*			*ε4* carrier vs. *ε3/3*		
		OR	P_{OR}	I^2 (%)	OR	P_{OR}	I^2 (%)		OR	P_{OR}	I^2 (%)	OR	P_{OR}	I^2 (%)
Ethnicity														
Caucasian	12	0.77 (0.62, 0.95)	0.01	0.0	1.31 (1.05, 1.64)	0.02	53.5	11	0.69 (0.54, 0.89)	0.00	0.0	1.31 (1.02, 1.68)	0.04	45.6
Asian	5	1.54 (1.09, 2.17)	0.01	0.3	3.31 (2.00, 5.49)	0.00	52.6	5	1.65 (1.10, 2.47)	0.02	0.0	3.34 (2.02, 5.52)	0.00	33.9
African	1	0.58 (0.28, 1.21)	0.15	–	1.40 (0.93, 2.10)	0.11	–	1	0.55 (0.23, 1.32)	0.18	–	1.57 (1.00, 2.48)	0.05	–
Clinical subtype														
CAD	12	1.00 (0.74, 1.35)	0.99	43.1	1.58 (1.18, 2.12)	0.00	72.0	12	0.95 (0.68, 1.33)	0.77	39.2	1.62 (1.19, 2.20)	0.00	64.8
MI	6	0.70 (0.49, 0.98)	0.04	0.0	1.77 (1.08, 2.88)	0.02	71.0	5	0.67 (0.43, 1.04)	0.07	0.0	1.89 (1.01, 3.54)	0.05	68.1
Source of control														
PB	13	0.78 (0.64, 0.95)	0.02	0.0	1.57 (1.17, 2.10)	0.00	74.2	12	0.73 (0.57, 0.93)	0.01	0.0	1.54 (1.13, 2.11)	0.01	65.9
HB	5	1.19 (0.65, 2.20)	0.58	61.9	1.78 (1.19, 2.66)	0.01	48.1	5	1.20 (0.60, 2.41)	0.61	62.7	1.99 (1.21, 3.27)	0.01	54.1
% of male														
≥65	13	0.77 (0.63, 0.95)	0.02	0.0	1.52 (1.15, 2.03)	0.00	69.0	12	0.69 (0.54, 0.89)	0.00	0.0	1.51 (1.10, 2.09)	0.01	62.4
<65	3	1.55 (0.97, 2.48)	0.07	37.0	1.79 (0.85, 3.74)	0.12	79.9	3	1.76 (1.15, 2.71)	0.01	0.0	2.04 (0.91, 4.60)	0.09	77.1
Sample size														
≥250	7	0.90 (0.61, 1.32)	0.58	64.5	1.39 (1.05, 1.83)	0.02	65.4	7	0.82 (0.54, 1.24)	0.35	58.4	1.41 (1.05, 1.88)	0.02	57.6
<250	11	0.92 (0.68, 1.24)	0.58	0.0	1.88 (1.28, 2.78)	0.00	70.1	10	0.98 (0.68, 1.41)	0.90	0.0	2.01 (1.27, 3.17)	0.00	64.2

P_{OR}, p-value for OR.

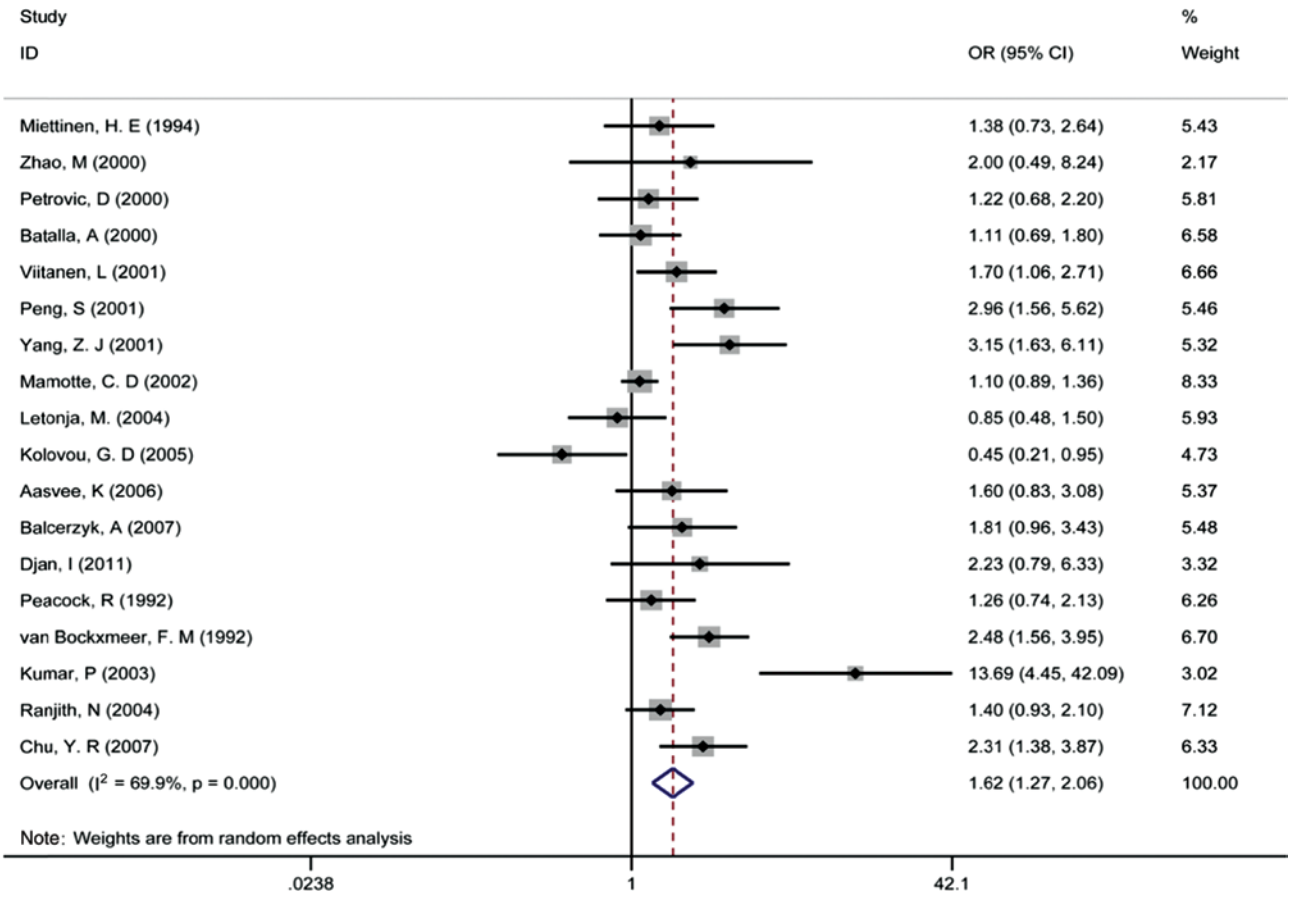

Figure 5: Pooled calculated OR of the association between the *ε4* allele and premature coronary artery disease.

Discussion

Although several recent meta-analyses explored the genetic risk of *APOE* polymorphisms in CAD [14–16], our analysis was the first comprehensive assessment in PCAD. Merging the data of all eligible publications revealed that subjects with the *ε4* allele conferred 62% more predisposition to PCAD, while *ε4* carriers had 65% higher risk for PCAD. People with the *ε2* allele and *ε2* carriers showed moderately protective effects on PCAD when merging all data. The results indicated that mutation of *ε3–ε4* in *APOE* may be a genetic risk factor for PCAD.

Meta-regression analyses showed that ethnicity could explain most of the heterogeneity among studies exploring the associations between the *ε2* allele or *ε2* carriers and PCAD. Increased risk for PCAD was found in Asians with the *ε2* allele, and we proposed that the *ε2* allele may be a genetic risk factor for PCAD in Asians. This was in contrast to the decreased susceptibility of PCAD occurring in Caucasian subjects with the *ε2* allele or *ε2* carriers, which provided evidence that the *ε2* allele was a genetic protective factor for PCAD in Caucasians. It was assumed that the association between the *ε2* allele and type III hyperlipoproteinemia which is associated with PCAD [41], contributed to this geographical variability. Instead of high prevalence of the *ε2/2* genotype in type III hyperlipoproteinemia seen in most other geographical areas, Asians are characterized by high prevalence of *ε2/3* and *ε2/2* genotypes [42, 43], which may be responsible for the genetic risk of the *ε2* allele for PCAD in Asians. As the number of *ε2/2* genotype in the population is very low, the *ε2* allele could still act as a protective factor for Caucasians based on its association with low levels of LDL. Besides, differences in dietary habits exist between Caucasians and Asians [44], which could partially explain this discrepancy. Limited by the relatively small sample size of Asians in the included literature, further larger sample studies are still needed to confirm this result. As for the *ε4* allele and *ε4* carriers, Asian people showed much higher risk for PCAD compared with Caucasians, which may due to the differences in dietary habits between Caucasians and Asians [44]. When categorized by source of control, the *ε2* allele and *ε2* carriers showed significant associations with

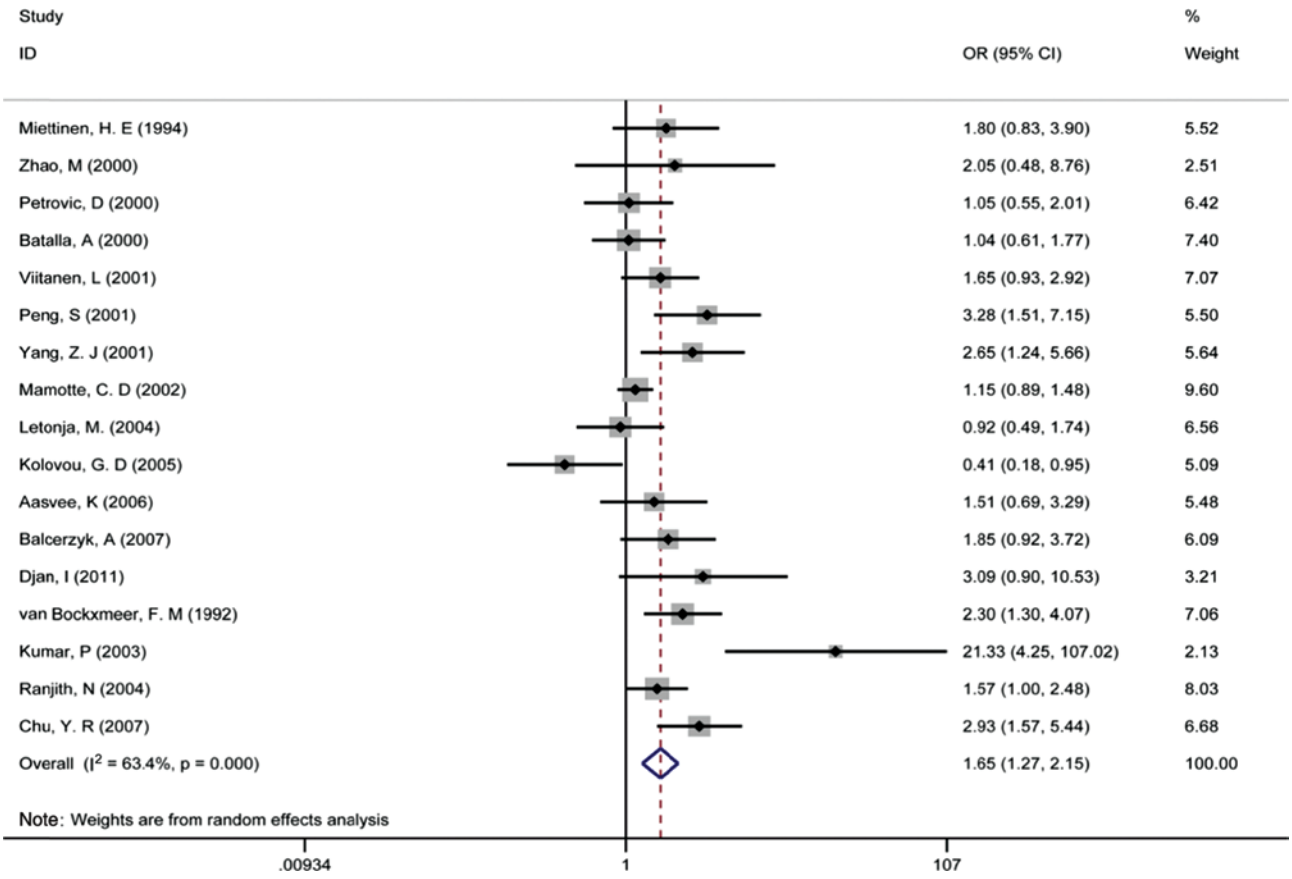

Figure 6: Pooled calculated OR of the association between *ε4* carriers and premature coronary artery disease.

PCAD in population-based studies while no significant associations were found in hospital-based studies. Controls from hospital-based cases may have had diseases associated with *APOE* polymorphisms, which would confound the impact of *APOE* polymorphisms on PCAD and which may have been responsible for this discrepancy. In subgroup analysis by male percentage, the four models showed significant association with PCAD in studies where males percentage were ≥65%, while only *ε2* carriers showed significant effects in the males percentage <65% subgroup. A number of epidemiological investigations indicated that lifestyle factors, such as tobacco smoking, alcohol consumption, and physical activity, could interact with the *APOE* gene to influence plasma lipid concentration [45, 46]. Compared to men, the proportion of smokers or drinkers is relative lower in females. Thus, the effect of *APOE* polymorphisms on PCAD may display discrepancy in sex. When stratified by sample size, no significant associations were found in the *ε2* allele and *ε2* carriers in sample sizes <250 or ≥250, indicating that heterogeneity did not originate from sample size. Even though possession of the *ε4* allele was associated with elevated risk of PCAD in both overall and subgroup analyses, heterogeneity among studies remained high.

The mechanisms for the influence of *APOE* polymorphisms on PCAD are partially understood. A number of studies investigated the associations of *APOE* polymorphisms with lipid level [47], metabolic syndrome [48], and arteriostenosis [49]. Evidence suggested that apoE participated in the metabolism of lipids in the liver, and different isoforms of apoE differed in affinity to corresponding receptors and performed different functions [50]. E4 is associated with high levels of LDL, total cholesterol and risk of carotid artery stenosis, while E2 is associated with reduction of LDL and total cholesterol [17]. Thus, *APOE* polymorphisms affect the prevalence of PCAD indirectly through mediating the concentration of plasma lipid. Additionally, *ε4* in *APOE* has an intimate association with increased apoB and is known as a risk factor for hypertension [51], which may be a contributing factor to its negative effect on PCAD.

From the results, we confirmed that people with the *ε4* allele are at high risk of PCAD and need to be detected early and to receive appropriate counseling in case contracting PCAD. Asians with the *ε2* allele need to be paid more attention as well.

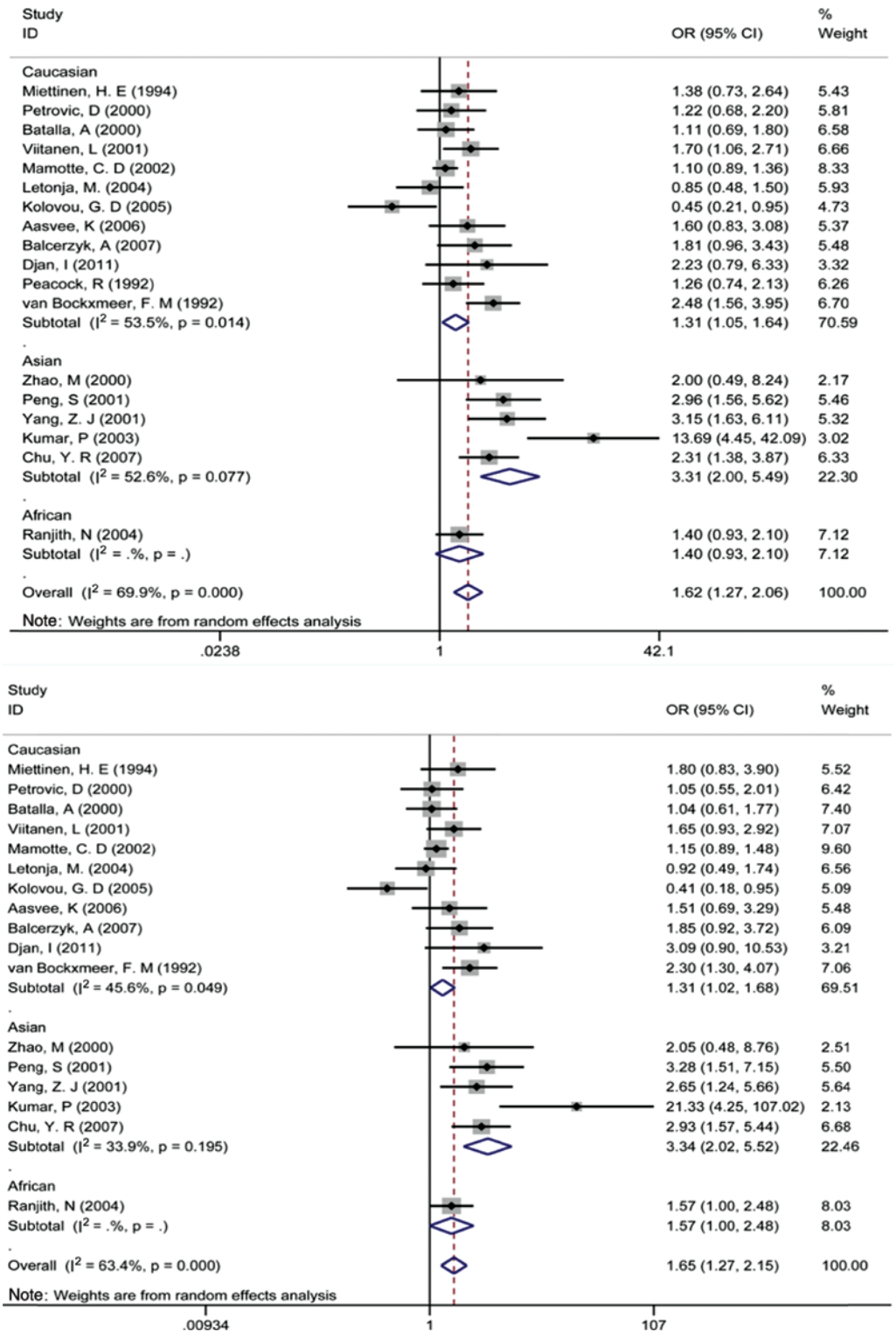

Figure 7: Forest plots of subgroup analyses by ethnicity in the *ε4* analyses.

Limitations

Although the obvious effect of *APOE* on PCAD was detected, several limitations remind us to interpret the results with caution. Firstly, several authors did not respond to our request to obtain original data, which could affect the integrity of the information included, so we did not include their studies in our meta-analysis.

Table 6: The results of meta-regressions.

Indicator	Ethnicity	Mean age	Hardy-Weinberg Equilibrium	Source of control	Clinical subtype	% of male	Mean BMI
ε2 allele vs. *ε3* allele							
R^2 (%)	63.47	100	41.99	67.24	16.54	2.95	–
τ^2	0.03	0	0.05	0.03	0.07	0.09	0
p-Value	0.05	0.01	0.10	0.04	0.18	0.32	0.91
ε2 carriers vs. *ε3/3*							
R^2 (%)	100	68.48	38.50	55.84	5.84	16.87	–
τ^2	0	0.04	0.07	0.05	0.11	0.11	0
p-Value	0.00	0.08	0.12	0.07	0.33	0.19	0.71
ε4 allele vs. *ε3* allele							
R^2 (%)	40.55	−24.51	15.19	−9.56	−15.84	−14.39	−26.76
τ^2	0.12	0.22	0.20	0.23	0.24	0.26	0.29
p-Value	0.02	0.83	0.09	0.70	0.74	0.73	0.49
ε4 carriers vs. *ε3/3*							
R^2 (%)	30.21	−29.44	22.95	−1.84	−21.06	−21.45	−86.31
τ^2	0.13	0.18	0.14	0.19	0.22	0.22	0.22
p-Value	0.04	0.91	0.09	0.48	0.81	0.77	0.49

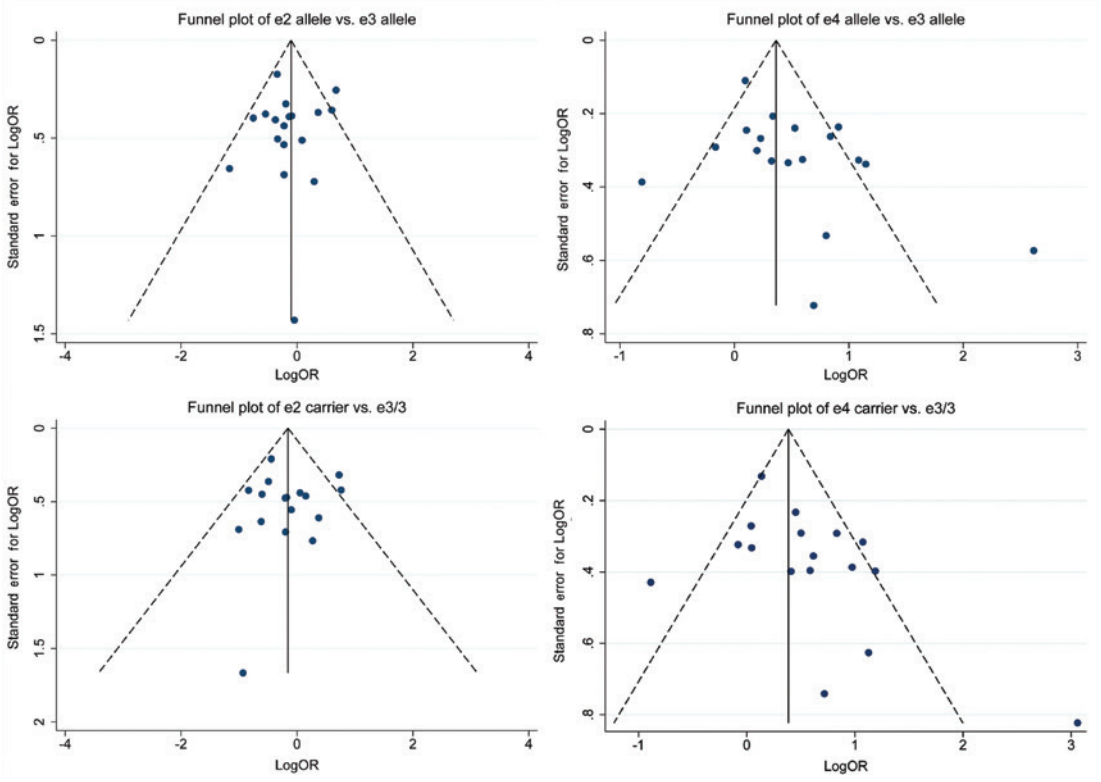

Figure 8: Funnel plots for the four models.

Secondly, the heterogeneity among studies exploring the association between the *ε4* allele or *ε4* carriers and PCAD remained a very high level even though we applied subgroup analysis. The last, the number of *ε2/2* is too low in our research.

Conclusions

In conclusion, our study described the distribution of *APOE* polymorphisms in PCAD and detected that the *ε2* allele appeared as a risk factor for PCAD in Asians while

remaining a protective factor in Caucasians, and confirmed that the *ε4* allele in *APOE* was associated with a high risk for PCAD in general. Furthermore, larger sample investigations of different subgroups are still needed to obtain a more definite conclusion.

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