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ORIGINAL ARTICLE

Association of an apolipoprotein E polymorphism with circulating cholesterols and hypertension: a meta-based Mendelian randomization analysis

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Prospective studies have reported that circulating cholesterol abnormalities are predictive of hypertension. As a test of the causal influence of circulating cholesterols on hypertension, we used a meta-based Mendelian randomization analysis to determine whether the apolipoprotein E (*ApoE*) gene polymorphism ε2/ε3/ε4 related to cholesterol changes is associated with hypertension. Data were available from 17 study populations, encompassing 2896 hypertensive patients and 2898 controls. A random effects model was applied irrespective of a between-study heterogeneity, and publication bias was examined using a funnel plot and Egger's test. An overall comparison of the *ApoE* gene alleles ε4 with ε3 yielded a significant 81% increased risk for hypertension (95% confidence interval (95% CI): 1.41–2.32; $P<0.0005$). Restricting the analysis to populations of Asian descent resulted in a 1.87-times higher likelihood of developing hypertension (95% CI: 1.41–2.32; $P<0.0005$). Compared with ε3 carriers, ε4 allele carriers had significantly higher levels of total cholesterol (standardized mean difference (SMD)=0.39; 95% CI: 0.2–0.57; $P<0.0005$) and low-density lipoprotein cholesterol (SMD=0.43; 95% CI: 0.23–0.64; $P<0.0005$). The predicted odds ratio (OR) for a 1 mmol l^{-1} increase in total cholesterol was 4.58 (95% CI: 1.83–67.21) when all qualified studies were included and 4.98 (95% CI: 1.94–76.36) for Asian-descent populations. Similarly, the predicted OR for a 1-mmol l^{-1} increase of low-density lipoprotein cholesterol was 3.97 (95% CI: 1.71–43.48) in all populations and 4.29 (95% CI: 1.81–43.38) in Asian-descent populations. Taken together, our findings suggest a casual influence of high circulating total cholesterol and low-density lipoprotein cholesterol levels on the development of hypertension.
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Keywords: apolipoprotein E; causality; Mendelian randomization; polymorphism

INTRODUCTION

Prospective studies have generally reported that circulating lipid abnormalities are predictive of the subsequent development of hypertension.[1–3] For example, a top-to-bottom fifth comparison of total cholesterol distribution yielded a relative multivariable-adjusted hypertension risk of 1.24 (95% confidence interval (95% CI): 1.01–1.53) in men,[3] and age- and multivariable-adjusted risks of 1.21 (95% CI: 1.11–1.33) and 1.12 (95% CI: 1.02–1.23) in women.[2] However, doubts about this claim have been raised by the observation that in women, the risk is considerably lower after multivariable adjustment, suggesting that the value is susceptible to confounding. Moreover, in prospective studies, the association between an exposure and disease is subject to Berkson's bias if the subject selection is based on an exposure-disease risk combination.[4,5] Furthermore, reverse causation might obscure this relationship, because obesity, an established risk factor for hypertension, may exert a less favorable influence on lipid levels. Consequently, understanding the influence of circulating lipids on hypertension requires additional studies to provide robust insights into the causal association between the intermediate phenotype and disease.

To minimize residual confounding factors and reverse causation, and to make causal inferences from observational epidemiology, an advisable approach is to apply Mendelian randomization with genetic variation as a proxy for a directly measured risk factor, because genotype distributions are believed to be independent of confounders and to be unmodified by disease processes.[6] The rationale of the Mendelian randomization is to establish causality between the factor influenced by a polymorphism and a disease outcome via the joint polymorphism-disease and polymorphism-phenotype associations.[7] Therefore, the different sustained levels of circulating lipids, such as total cholesterol

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and high- and low-density lipoprotein cholesterol, should translate into a differential risk of hypertension, if a causal link exists.

Polymorphisms of the apolipoprotein E (*ApoE*) gene have been studied extensively in the context of a variety of metabolic dyslipidemias and clinical endpoints, such as hypercholesterolemia,[8,9] Alzheimer's disease,[10] stroke[11] and hypertension.[12] In particular, the *ApoE* gene polymorphism ε2/ε3/ε4 is of significant interest, because two recent meta-analyses consistently suggested that ε4 allele carriers were at increased risk for hypertension, and that this effect was more pronounced in Chinese populations.[13,14] Additionally, this polymorphism was reportedly linked to changes in cholesterol levels[15–18] and therefore should be associated with hypertension if a causality exists between cholesterol levels and hypertension.

Given the insufficient sample sizes of the individual studies that associated the *ApoE* gene ε2/ε3/ε4 polymorphism with circulating cholesterol, hypertension, or both, we decided to evaluate this association using a meta-analysis that aimed to test the causal influence of the circulating total cholesterol and high- and low-density lipoprotein cholesterol levels on hypertension.

METHODS

Search strategy for the identification of studies

We identified the published articles through the PubMed and EMBASE search engines, as well as through the China Biological Medicine (http://sinomed.imicams.ac.cn/index.jsp) and Wanfang (http://www.wanfangdata.com.cn) databases before 18 January 2011, using the Boolean combination of subject terms (apolipoprotein E or ApoE or Apo E) and (hypertension or blood pressure) and (polymorphism or allele or genotype or variant or variation). The articles selected were restricted to human populations (other than families) and articles written in either English or Chinese. The full text of the retrieved articles was scrutinized to determine whether information on the topic of interest was included. The reference lists of the retrieved articles and systematic reviews were also checked for articles that had not been identified in the initial search. For studies involving more than one geographic or ethnic group, each group was treated separately.

Inclusion/exclusion criteria

Articles were considered qualified for this meta-analysis if they examined the hypothesis that the *ApoE* gene polymorphism ε2/ε3/ε4 is associated with hypertension, circulating cholesterols, or both; if they followed a case–control, nested case–control, or cross-sectional study design; and if they provided sufficient information on the ε2/ε3/ε4 genotype counts in hypertensive patients and controls for determining an estimate of odds ratio (OR) and the corresponding 95% CI, or sufficient information on circulating total cholesterol and high- and low-density lipoprotein cholesterol levels across the ε2/ε3/ε4 alleles for determining the standardized mean difference (SMD) and its 95% CI.

Hypertension was defined as systolic blood pressure equal to or greater than 140 mm Hg, diastolic blood pressure equal to or greater than 90 mm Hg, or previous treatment with antihypertensive drugs. Circulating cholesterols were measured using standard methods. Studies evaluating secondary hypertension or other types of monogenic hypertension were excluded. In cases in which there were multiple articles from the same study population (in whole or in part), the most complete and recent results were used.

Extracted information

The following data were extracted from each qualified study: the first author's last name, the publication date, the population's ethnicity, the baseline characteristics of the study population (including age, gender and body mass index), the ApoE ε2/ε3/ε4 genotype counts in patients and controls, and the circulating total cholesterol and high- and low-density lipoprotein cholesterol levels across the ε2 (ε2/2+ε2/3), ε3 (ε3/3) and ε4 (ε3/4+ε4/4) allele carriers. All cholesterol units were standardized to millimole per liter. For consistency, quantitative variables expressed as the mean ± s.e. were converted to the mean ± s.d. Data extraction and study quality were assessed in duplicate.

Statistical analysis

We used the random effects model and the DerSimonian and Laird method to combine the individual effect size estimates, and the estimate of heterogeneity was determined using the Mantel–Haenszel model.[19] The unadjusted OR and SMD, and the corresponding 95% CI were used to compare genetic contrasts between patients and controls, as well as to compare the circulating cholesterol levels between ε3 allele carriers and carriers of the other ApoE alleles.

The between-study heterogeneity was assessed using the inconsistency index I^2 statistic (ranging from 0 to 100%), which is defined as the percentage of the observed between-study variability, which is due to heterogeneity rather than chance. In this method, higher values suggest the existence of heterogeneity.[20,21] The funnel plots and Egger regression asymmetry test were used to assess publication bias. Egger's test can detect funnel plot asymmetry by determining whether the intercept deviates significantly from zero in a regression of the standardized effect estimates against their precision.

It is widely accepted that genetic association studies are more closely relevant to randomized trials than other types of epidemiological studies owing to Mendel's second law of independent assortment of alleles, which states that assortment of alleles should be independent of environmental or behavioral factors.[22,23] In the Mendelian randomization analysis, the risk estimate was calculated from the ratio of the coefficient of the association between the polymorphism and disease to that of the association between the polymorphism and the assessed circulating cholesterol levels as a reflection of the potential causal effect of high circulating cholesterol levels on the hypertension risk.

A probability less than 0.05 was judged to be significant except for the I^2 and Egger's statistics, for which a significance level of less than 0.1 was chosen. The data management and statistical analyses were performed using STATA software, version 11.0 (StataCorp LP, College Station, TX, USA) for Windows.

RESULTS

Search results and study characteristics

After our two recently published meta-analyses, which associated the *ApoE* gene polymorphism ε2/ε3/ε4 with hypertension using the results from English-language literature[13] and from Chinese populations,[14] we focused on whether circulating cholesterol levels related to this polymorphism are causal factors in hypertension, drawing from published studies in English and Chinese. In this study, we only assessed ε2/ε3/ε4 allelic association, so one study that provided combined genotype counts was removed.[12] The detailed selection process is presented in Figure 1. A total of 17 articles,[17,24–39] involving 19 study populations with 2896 hypertensive patients and 2898 healthy controls, were analyzed. The baseline characteristics of all the qualified studies was mirrored in the above two meta-analyses.[13,14]

A total of 7 of the 19 studies provided information regarding the association between the polymorphism and cholesterol. The distribution of total cholesterol and high- and low-density lipoprotein cholesterol levels across the ε2 (ε2/2+ε2/3), ε3 (ε3/3) and ε4 (ε3/4+ε4/4) alleles is summarized in Table 1. We must emphasize that, as suggested by Kesäniemi *et al.*,[40] because of the opposite net effect between the ApoE ε2 and ε4 alleles, genotype ε2/4 should be excluded from the genotype–phenotype analysis.

Association of the *ApoE* gene alleles with hypertension

As shown in Figure 2a, a comparison of the ε2 *vs.* the ε3 ApoE allele indicated only a nonsignificantly (2%) increased risk for hypertension (OR=1.02; 95% CI: 0.83–1.26; P=0.834). This observation was accompanied by moderate evidence of between-study heterogeneity (I^2=44.2%; P=0.02), but had a low probability of publication bias as reflected by the suggestive symmetry of the funnel plot (Figure 3a) and by Egger's test (P=0.45). Contrastingly, a comparison of allele ε4 with

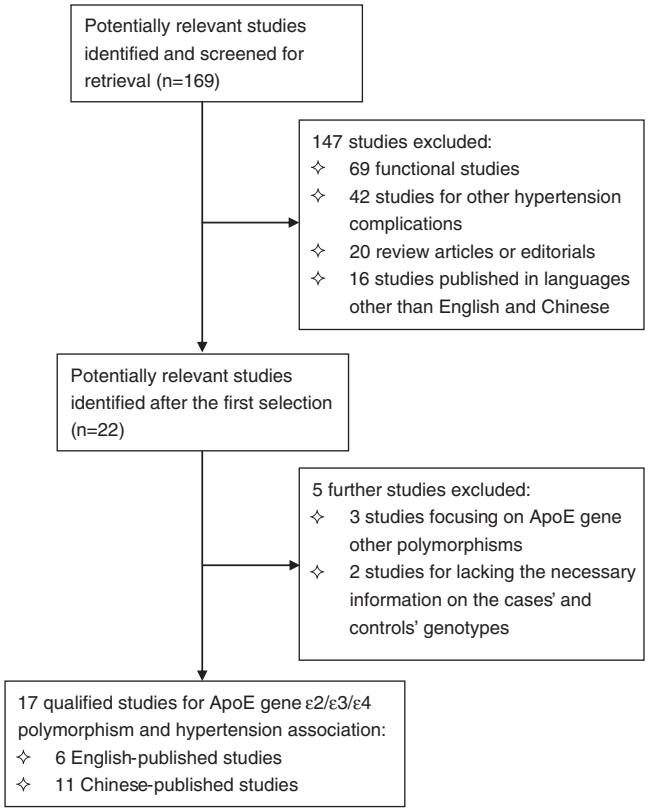

Figure 1 Flow diagram of the search strategy and the selection of studies used in this meta-analysis.

ε3 yielded a significant 81% increased risk for hypertension (95% CI: 1.41–2.32; $P<0.0005$; Figure 2b). However, significant between-study heterogeneity ($I^2=44.2\%$; $P=0.02$; Figure 3b) and publication bias (Egger's test: $P=0.002$) obscured the strength of this association. Given that, all but two study populations (one of Turks and one of Brazilians) were of Asian descent; we repeated the assessment of this association in Asian-descent populations and observed a slightly higher risk (OR=1.87; 95% CI: 1.41–2.32; $P<0.0005$; Supplementary Figure S1A). We found that heterogeneity was somewhat alleviated, and there was no apparent publication bias (Egger's test: $P=0.108$; Supplementary Figure S1B).

Association of the *ApoE* gene alleles with circulating cholesterols

Because seven studies, all of the subjects recruited from China, measured total cholesterol and high- and low-density lipoprotein cholesterol levels across the ε2 (ε2/2+ε2/3), ε3 (ε3/3) and ε4 (ε3/4+ε4/4) alleles, we performed an overall allele–phenotype association. Compared with subjects with the ε3 allele, those with the ε2 allele had significantly lower levels of total cholesterol (SMD=−0.69; 95% CI: −0.99 to −0.39; $P<0.0005$; Figure 4a) and low-density lipoprotein cholesterol (SMD=−0.44; 95% CI: −0.59 to −0.29; $P<0.0005$; Figure 5a), but had increased, albeit non-significant, high-density lipoprotein cholesterol levels (SMD=0.08; 95% CI: −0.06 to 0.21; $P=0.258$; Supplementary Figure S2A). By contrast, compared with subjects with the ε3 allele, those with the ε4 allele had significantly higher levels of total cholesterol (SMD=0.39; 95% CI: 0.2–0.57; $P<0.0005$; Figure 4b) and low-density lipoprotein cholesterol (SMD=0.43; 95% CI: 0.23–0.64; $P<0.0005$; Figure 5b), but no association was observed between the ε4 allele and high-density lipoprotein cholesterol levels (SMD=0.02; 95% CI: −0.16 to 0.19; $P=0.852$; Supplementary Figure S2B).

Table 1 Distributions of total cholesterol, high- and low-density lipoprotein cholesterol levels across ε2 (ε2/2+ε2/3), ε3 (ε3/3) and ε4 (ε3/4+ε4/4) alleles

	ε2/2+ε2/3 (n=291)		*ε3/3 (n=1421)*		*ε3/4+ε4/4 (n=430)*	
First author and reference	*Mean*	*s.d.*	*Mean*	*s.d.*	*Mean*	*s.d.*
Total cholesterol, mmol l^{-1}						
Zhang *et al.*[30]	4.39	0.85	5.03	0.52	5.46	0.64
Zhang *et al.*[32]	4.56	0.44	4.78	0.28	4.92	0.36
Zhang *et al.*[32]	4.76	0.38	5.2	0.36	5.32	0.28
Zhang *et al.*[32]	4.65	0.43	4.91	0.33	4.9	0.4
Liu *et al.*[34]	3.99	1.03	4.34	1.02	4.63	1.04
Li *et al.*[26]	4.85	0.98	5.62	1.03	6.46	1.05
Niu *et al.*[17]	4.8	1	5	0.8	5.3	1
High-density lipoprotein cholesterol, mmol l^{-1}						
Zhang *et al.*[30]	1.36	0.41	1.48	0.42	1.5	0.57
Zhang *et al.*[32]	1.52	0.25	1.52	0.16	1.56	0.27
Zhang *et al.*[32]	1.45	0.32	1.47	0.23	1.5	0.18
Zhang *et al.*[32]	1.54	0.18	1.5	0.15	1.49	0.32
Liu *et al.*[34]	1.18	0.36	1.19	0.35	1.26	0.33
Li *et al.*[26]	1.48	0.33	1.45	0.36	1.44	0.29
Niu *et al.*[17]	1.5	1	1.4	0.3	1.3	0.3
Low-density lipoprotein cholesterol, mmol l^{-1}						
Zhang *et al.*[30]	2.93	0.6	3.3	0.53	3.71	0.66
Zhang *et al.*[32]	2.23	0.43	2.47	0.32	2.58	0.36
Zhang *et al.*[32]	2.42	0.37	2.57	0.38	2.67	0.44
Zhang *et al.*[32]	2.29	0.34	2.47	0.37	2.54	0.43
Liu *et al.*[34]	2.23	0.77	2.57	0.82	2.7	0.81
Li *et al.*[26]	3.81	0.77	4.52	0.89	5.35	0.67
Niu *et al.*[17]	3	0.8	3.2	0.7	3.6	0.3

Predicted association of circulating cholesterols with hypertension from Mendelian randomization in the mixed populations

Under the assumption of a linear-logistic relationship between circulating cholesterol levels and the odds of hypertension, statistical significance was reached only for total cholesterol and low-density lipoprotein cholesterol. Specifically, in comparing allele ε4 with ε3, the predicted OR for a 1 mmol l^{-1} increase in total cholesterol was 4.58 (95% CI: 1.83–67.21) when all qualified studies were included and 4.98 (95% CI: 1.94–76.36) in mixed populations of only Asian descent. With regard to low-density lipoprotein cholesterol, the predicted OR for a 1-mmol l^{-1} increase between allele ε4 *vs.* ε3 was 3.97 (95% CI: 1.71–43.48) in all populations and 4.29 (95% CI: 1.81–43.38) in Asian-descent populations. Because the 95% CIs of the predicted estimates above excluded the null hypothesis value of 1, we could safely reject the null hypothesis at the 5% level. Therefore, our data provided strong evidence for a causal influence of high circulating total cholesterol and low-density lipoprotein cholesterol levels on hypertension. Moreover, the direction of these predicted estimates was consistent with the association of the *ApoE* gene ε2/ε3/ε4 polymorphism with hypertension risk and circulating cholesterol levels.

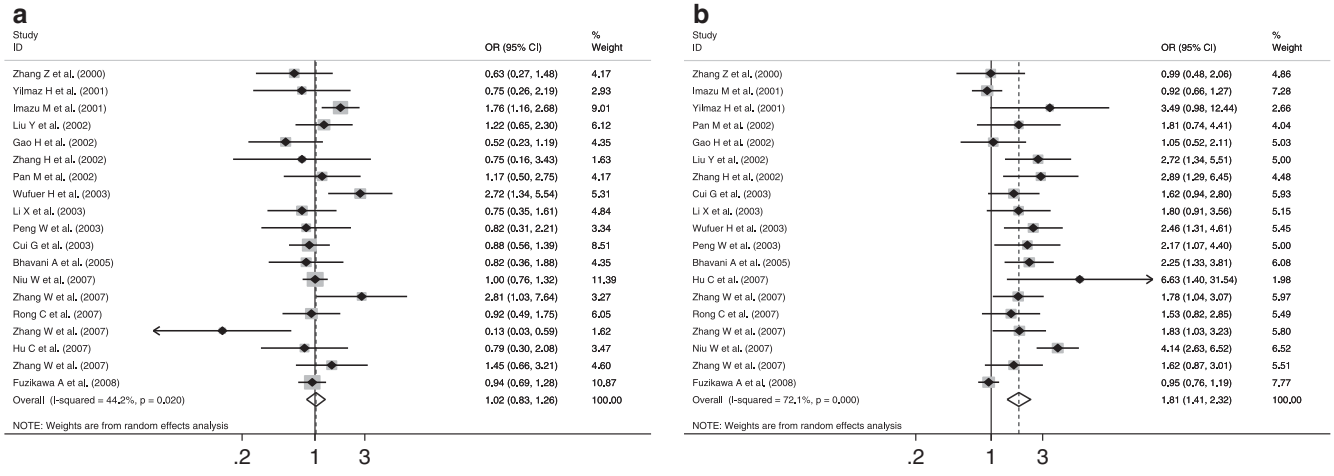

Figure 2 Forest plots (random effects model) of the hypertension risk associated with the *ApoE* gene ε2/ε3/ε4 alleles for ε2 *vs.* ε3 (**a**) and for ε4 *vs.* ε3 (**b**) in all the study populations. The summary odds ratio (OR) estimate is shown by the center of a solid diamond of which the left and right extremes represent the corresponding 95% confidence interval (95% CI). The area of the diamond is proportional to the weight of the study. The hollow diamond (and broken line) represents the overall risk estimate. The solid vertical line is set at the null value (OR=1.0). A full color version of this figure is available at the *Hypertension Research* journal online.

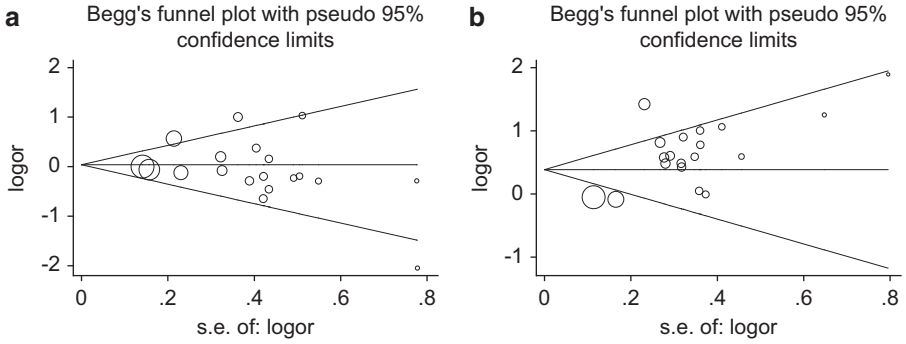

Figure 3 Funnel plots of the studies on the effect of the ε2/ε3/ε4 *ApoE* gene polymorphisms on hypertension risk for allele ε2 *vs.* ε3 contrast (**a**) and for allele ε4 *vs.* ε3 contrast (**b**). The vertical axis represents the log of the odds ratio (OR); the horizontal axis represents the s.e. of log (OR). The funnel plots are drawn with the 95% confidence limits. The symbols representing the data in the plot are sized proportionally to the inverse variance.

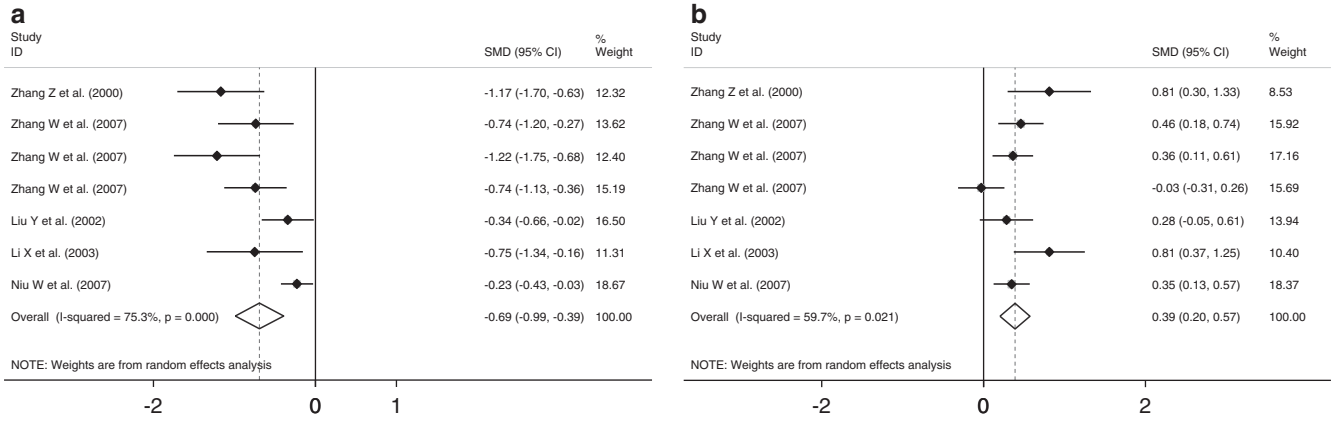

Figure 4 Forest plots (random effects model) of circulating cholesterol levels associated with the *ApoE* gene ε2/ε3/ε4 alleles for ε2 *vs.* ε3 (**a**) and for ε4 *vs.* ε3 (**b**) from the available studies. The vertical axis represents the standardized mean difference (SMD) of the circulating cholesterol levels. Each box represents the SMD estimate (a solid diamond), and the left and right extremes represent the corresponding 95% confidence interval (95% CI). The area is proportional to the weight of the study. The hollow diamond (and broken line) represents the overall SMD estimate. The solid vertical line is set at the null value (SMD=0). A full color version of this figure is available at the *Hypertension Research* journal online.

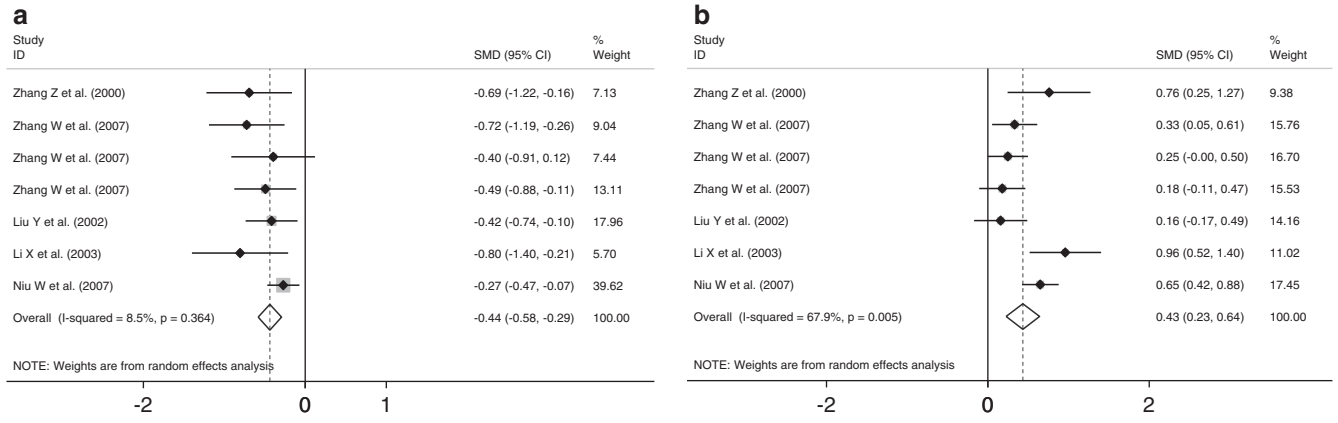

Figure 5 Forest plots (random effects model) of circulating low-density lipoprotein cholesterol levels associated with the *ApoE* gene ε2/ε3/ε4 alleles for ε2 *vs.* ε3 (**a**) and for ε4 *vs.* ε3 (**b**) from the available studies. The vertical axis represents the standardized mean difference (SMD) of circulating low-density lipoprotein cholesterol levels. Each box represents the SMD estimate (a solid diamond), and the left and right extremes represent the corresponding 95% confidence interval (95% CI). The area is proportional to the weight of the study. The hollow diamond (and broken line) represents the overall SMD estimate. The solid vertical line is set at the null value (SMD=0). A full color version of this figure is available at the *Hypertension Research* journal online.

DISCUSSION

Using a comprehensive meta-analysis, we determined that the *ApoE* gene polymorphism ε2/ε3/ε4 is significantly associated with hypertension, which is in agreement with the results from two recent meta-analyses,[13,14] and is associated with large changes in circulating cholesterol levels. Under the rationale of the Mendelian randomization, we utilized this polymorphism as an instrumental variable, which is associated with circulating cholesterol levels, and we demonstrated the causal influence of high total cholesterol and low-density lipoprotein cholesterol levels on hypertension. To our knowledge, this is the first study to report the causality between high circulating cholesterol levels and hypertension, using the Mendelian randomization approach.

Hypertension has been strongly linked to a variety of lipoprotein metabolic abnormalities.[41] The selection of the ε2/ε3/ε4 *ApoE* gene polymorphism as a tool to decipher cholesterol–hypertension causality is strongly supported by biological data, because it has been estimated that this polymorphism accounts for up to 11% of the total variance in circulating cholesterol levels in apparently healthy Caucasians.[42] This is consistent with our pooled results, which indicated that a 1-mmol l^{-1} increase in total cholesterol corresponded to a 4.58-times higher likelihood of developing hypertension, and the same increase in low-density lipoprotein cholesterol corresponded to a 3.97-times higher likelihood. Because genotypes are invariant over time and can exert an effect on circulating cholesterol levels over a lifetime, the convincing ORs that related circulating total cholesterol and low-density lipoprotein cholesterol levels to hypertension risk in our Mendelian randomization analysis could represent the average lifetime risk attributable to these cholesterols.

The differences in the bioavailability of the ε2/ε3/ε4 polymorphism proteins could be attributed to the fact that, because of the lack of a supradyl group, the under-oxidized ε4 lipoproteins are more easily cleared by scavenger receptors than are ε3 lipoproteins.[43] Furthermore, according to the functional explorations, structural defects in the *ApoE* gene product might result in an impaired interaction between the ApoE-containing lipoproteins with their receptors and thus, induce the development of atherogenic dyslipidemias and premature cardiovascular diseases.[44] *ApoE* gene knockout mice exhibited hypertension and endothelial dysfunction.[45] Additionally, an *ApoE* gene polymorphism was found to significantly affect physiological activity by determining high-density lipoprotein cholesterol levels, indicating that environmental factors are important modulators of the effect of this polymorphism on circulating lipid levels.[46] Therefore, one of this study's concerns, which has been regarded as a potential limitation of the Mendelian randomization, is related to the possible existence of pleiotropic effects of the ε2/ε3/ε4 polymorphism on other lipid or metabolic pathways that influence hypertension risk. However, we accepted the risk of introducing an error in the causal effect inferred from the instrumental variable analysis. Because very few studies have provided additional information concerning the distribution of other hypertension risk factors (such as body mass index, smoking and drinking) across the ApoE ε2/ε3/ε4 alleles, we cannot address the question of pleiotropy in the present study. If the data were available, we expect that they would show that the ε2/ε3/ε4 polymorphism is related to other risk factors.

We cannot exclude the possible linkage disequilibrium of the ε2/ε3/ε4 *ApoE* gene polymorphism with another genetic variant that is actually responsible for lipid variation, but there is no evidence that this is the case. However, as suggested by Sheehan *et al.*,[5] it is somewhat irrelevant whether ε2/ε3/ε4 or another polymorphism is the causal variant for the phenotype when the two polymorphisms are in linkage disequilibrium, because either one could be considered an instrumental variable.

Other possible limitations to the use of the Mendelian randomization here include the possibility of population stratification, power deficiency, canalization, a lack of knowledge about the confounding factors and an inability to detect the effect of acute changes in the circulating cholesterol levels on hypertension.[47] However, given the large sample sizes (n=5797) and the lack of publication bias in ethnically homogeneous subgroups, population stratification and power deficiency are unlikely to have a considerable impact on the results. Because canalization—a developmental compensation that can compensate for disruptive environmental or genetic forces[5]—is difficult to test outside of animal models, we cannot determine its effect in this meta-analysis. Although the effects of acute changes in circulating cholesterol levels on hypertension are not our major concern, several large-scale cohort studies have consistently reported the independent predictive value of some lipid fractions, such as total cholesterol, on hypertension risk in both adolescents and adults,[1–3] indicating a smaller effect of acute cholesterol changes.

Even though our funnel plots and statistical tests showed low probability of publication bias, it remains a possibility, because we screened out the 'gray literature' (articles published in languages other than English and Chinese). However, the influence of publication bias on gene–disease association is expected to result in overestimation, not underestimation.[48] Although publication bias might not affect the conclusions of this study, more studies are warranted to quantify the effect given the wide CIs obtained for the risk estimates. Moreover, with respect to the early onset of hypertension in youth, because most of the studies in this meta-analysis recruited subjects aged $\geqslant$45 years (for whom environmental factors are likely to contribute more prominently than a genetic component to the development of hypertension),[49] large association studies in a younger population of hypertensive subjects will be of added interest.

Despite the aforementioned limitations inherent in the Mendelian randomization, our results provide convincing evidence regarding the casual influence of high total cholesterol and low-density lipoprotein cholesterol levels on the development of hypertension, adding weight to the results from previous large-scale cohort studies. Although further analyses are warranted to evaluate the clinical utility of total cholesterol and low-density lipoprotein cholesterol levels in the treatment and control of hypertension, future studies should concentrate on elucidating the exact mechanisms by which these cholesterol levels can predict future hypertension.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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