

# Apolipoprotein E Gene $\epsilon$ 4 Allele is Associated with Atherosclerosis in Multiple Vascular Beds

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**Background:** Atherosclerosis is a systemic disease that can involve multiple vascular beds. The risk factors for atherosclerosis in multiple vascular beds remain unclear. Apolipoprotein E (APOE) is involved in inflammation and lipid deposition in the process of atherosclerosis. The objective of this study was to investigate whether *APOE* polymorphisms are associated with atherosclerosis in multiple vascular beds.

**Methods:** A total of 416 patients with atherosclerosis in single vascular bed and 658 patients with atherosclerosis in multiple vascular beds were included. *APOE* genotypes were detected and the differences of *APOE* genotypes between the groups were compared. Logistic regression analysis was performed to analyze the relationship between *APOE* genotypes and atherosclerosis in multiple vascular beds.

**Results:** *APOE* E3/E4 genotype frequency was lower in the patients with atherosclerosis in multiple vascular beds than that of patients with atherosclerosis in single vascular bed (11.4% vs 17.8%,  $P=0.004$ ). There was no significant difference in age and gender distribution, proportion of history of smoking, alcohol consumption, hypertension, and diabetes mellitus between the two groups (all  $P>0.05$ ), and among patients with different *APOE* alleles (all  $P>0.05$ ). Logistic regression analysis indicated that *APOE* E3/E4 genotype (E3/E4 vs E3/E3: odds ratio (OR) 0.598, 95% confidence interval (CI): 0.419–0.854,  $P=0.005$ ), and *APOE*  $\epsilon$ 4 allele ( $\epsilon$ 4 vs  $\epsilon$ 3: OR 0.630, 95% CI: 0.444–0.895,  $P=0.010$ ) associated with atherosclerosis in multiple vascular beds.

**Conclusion:** *APOE*  $\epsilon$ 4 allele is associated with atherosclerosis in multiple vascular beds.

**Keywords:** *APOE*, polymorphism, atherosclerosis, multiple vascular beds

## Introduction

Atherosclerosis refers to the occurrence of lipidoses in the intima of the artery, thickening of the intima, and gradually forming plaque, which reduces the elasticity of the artery, causes the formation of artery stenosis and thrombosis, and causes the obstruction of the artery blood supply,<sup>1</sup> and ischemic changes.<sup>2,3</sup> As a systemic disease, atherosclerosis can cause damage to multiple vascular beds throughout the body, mainly involving large and medium elastic arteries of systemic circulation, including carotid arteries, intracranial arteries, coronary arteries, aorta, renal arteries and peripheral arteries.<sup>4</sup> Atherosclerosis lesions with two or more vascular beds throughout the body known as atherosclerosis in multiple vascular beds.<sup>5</sup> About 20% of patients with atherosclerosis have lesions in multivessel beds.<sup>6</sup> Major adverse cardiac events (MACE) outcomes are associated with the number and location of plaques, and studies of high-risk plaque characteristics can help assess the risk of MACE.<sup>7</sup> Therefore, it is of great significance to predict the population at risk of atherosclerosis in multiple vascular beds for the prevention and treatment of cardiovascular diseases.<sup>8</sup>

It is believed that the formation process of atherosclerosis is related to inflammation, lipid deposition, and fibroplasia regulated by immune cells.<sup>9</sup> And studies have shown that a functional deficiency or decrease in the overall level of apolipoprotein E (ApoE) can produce an imbalance in the number and function of immune cells.<sup>10,11</sup> At the same time, lipid-enriched spleen macrophages release interleukin-23 (IL-23), inducing a large amount of hematopoietic stem and progenitor cells (HSPCs) to be released from the bone marrow niche.<sup>12</sup> When HSPCs are located in the extramedullary

position, they can encounter granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3),<sup>13</sup> and the net effect leads to the proliferation of HSPCs, the production of extramedullary leukocytes, and atherosclerosis is accelerated.<sup>14</sup> As is an important plasma lipoprotein involved in lipid metabolism, ApoE deficiency in human may result in an abnormal ability to process cholesterol.<sup>15,16</sup>

The development of atherosclerosis is influenced by many factors, including eating habit, lifestyle, and environmental factors.<sup>17,18</sup> Genetic predisposition is also an unavoidable factor in atherosclerosis.<sup>19</sup> The *APOE* gene contains 4 exons and 3 introns.<sup>20</sup> Three major alleles ( $\epsilon$ 2(rs429358 T-rs7412 T),  $\epsilon$ 3(rs429358 T-rs7412 C), and  $\epsilon$ 4(rs429358 C-rs7412 C)) were formed based on two non-synonymous SNPs: rs7412 (526 C>T) and rs429358 (388 C>T).<sup>21</sup> Each allele can encode one protein isoform and form six different gene phenotypes: E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, and E4/E4.<sup>22</sup> Differences in ethnicity, and genetic background can affect the onset and progression of atherosclerosis.<sup>23–25</sup> Several studies suggested that *APOE* gene polymorphisms have been widely recognized as a risk factor for atherosclerosis.<sup>26–28</sup> However, the difference of *APOE* polymorphisms between atherosclerosis in multiple vascular beds and atherosclerosis in single vascular bed, and the relationship of *APOE* polymorphisms and atherosclerosis in multiple vascular beds remain unclear. In this study, we compared the differences between atherosclerosis in multiple vascular beds and atherosclerosis in single vascular bed, and analyzed risk factors for atherosclerosis in multiple vascular beds.

## Materials and Methods

### Study Population

Atherosclerosis in single vascular bed means that only one vascular bed has obvious atherosclerosis clinically. Atherosclerosis in multiple vascular beds refers to clinically significant atherosclerosis in two or more arterial vascular beds ( $\geq 2$  arterial beds co-existing).<sup>5,29</sup> Patients with atherosclerosis in single vascular bed and multiple vascular beds treated in Meizhou People's Hospital were recruited from January 2016 to June 2020. The clinical data of these patients (age, gender, history of smoking, history of alcohol consumption, hypertension, and diabetes mellitus) were collected from the medical records system of our hospital. The study was performed under the guidance of the Declaration of Helsinki and approved by the Ethics Committee of Medicine, Meizhou People's Hospital (Clearance No.: 2024-C-17). All participants were informed on the study procedures and goals and informed consent was obtained from all the participants.

Atherosclerotic plaque is the direct sign of atherosclerosis, and the clinical diagnosis of atherosclerosis is generally through imaging detection technology to identify plaque.<sup>30,31</sup> Plaque was defined as a focal structure that invaded the lumen of the artery by at least 0.5 mm, or by 50% of the surrounding endo-media thickness, or by a thickness greater than 1.5 mm measured from the outer membrane to the endo-lumen interface.<sup>32</sup> Atherosclerosis is determined by examining arterial plaque using techniques such as angiography, magnetic resonance imaging (MRI), computed tomography, or color Doppler ultrasonography, assessed by two senior radiologists in a double-blind evaluation. Potential subjects were included in any radiograph-based test showing arterial plaque. The diagnostic criteria for atherosclerosis were in accordance with the relevant diagnostic criteria formulated by the Cardiovascular Branch and the Neurology Branch of the Chinese Medical Association. Inclusion criteria are as follows: (1) Clinically diagnosed atherosclerosis, including coronary artery, carotid artery, cerebral artery, limb artery, and other vascular lesions; (2) The patient's medical records are complete; (3) Age  $\geq 18$  years old. The exclusion criteria were: (1) Patients with incomplete clinical data; (2) Patients with severe infectious diseases, autoimmune diseases, organ dysfunction, and other diseases; (3) Age  $< 18$  years old. The diagnostic criteria for hypertension are systolic blood pressure of  $\geq 140$  mm Hg, diastolic blood pressure of  $\geq 90$  mm Hg.<sup>33</sup> The diagnostic criteria for diabetes mellitus were fasting blood glucose  $\geq 126.1$  mg/dL, blood glucose  $\geq 200$  mg/dL 2 hours after loading.<sup>34</sup>

### Determination of Serum Lipids and APOE Genotyping

Fasting blood was collected and serum was isolated. Triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), Apolipoprotein A1 (Apo-A1), and Apolipoprotein B (ApoB) levels in serum samples were assessed using automatic biochemical analysis system (Olympus AU5400

system, Tokyo, Japan) and corresponding kits. Whole blood samples were collected and genomic DNA was extracted. *APOE* polymorphisms (rs7412 and rs429358) were detected by *APOE* genotyping kit (Sinochips Bioscience Co., Ltd., Zhuhai, Guangdong, China) based on a gene chip method.<sup>35,36</sup>

## Statistical Analysis

Categorical variables are expressed as percentages. The differences of genotype composition ratios and allele frequencies among patients with atherosclerosis in single vascular bed and atherosclerosis in multiple vascular beds were analyzed by the  $\chi^2$  test. To measure the relative risk of *APOE* genotypes and alleles, logistic regression analysis was performed after adjusting for the factors of demographic characteristics, personal history, and disease history.  $P < 0.05$  was set as statistically significant.

## Results

### Characteristics of Subjects

A total number of 1074 patients with atherosclerosis were recruited. In this study, there were 736 males (68.5%) and 338 females (31.5%). There were 353 (32.9%) patients aged <65 years and 721 (67.1%) patients aged  $\geq 65$  years. There were 233 (21.7%) cases have a history of smoking, 37 (3.4%) cases have a history of alcoholism, 707 (65.8%) cases with hypertension history, 337 (31.4%) cases with diabetes mellitus history. This study included 416 patients with atherosclerosis in single vascular bed and 658 patients with atherosclerosis in multiple vascular beds. There was no significant difference in distributions of age and gender, proportion of smoking, alcohol consumption, hypertension, and diabetes between patients with single vascular bed and atherosclerosis in multiple vascular beds (all  $P > 0.05$ ). The difference of serum lipid-lipoprotein levels was not statistically significant (Table 1).

**Table 1** Clinical Characteristics of Patients with Atherosclerosis in Multiple Vascular Beds and Patients with Atherosclerosis in Single Vascular Bed

	Total (n=1074)	Patients with Atherosclerosis in Single Vascular Bed (n=416)	Patients with Atherosclerosis in Multiple Vascular Beds (n=658)	P values
Age, years				
<65, n(%)	353 (32.9%)	139 (33.4%)	214 (32.5%)	0.790
$\geq 65$ , n(%)	721 (67.1%)	277 (66.6%)	444 (67.5%)	
Gender				
Male, n(%)	736 (68.5%)	284 (68.3%)	452 (68.7%)	0.893
Female, n(%)	338 (31.5%)	132 (31.7%)	206 (31.3%)	
History of smoking, n(%)	233 (21.7%)	87 (20.9%)	146 (22.2%)	0.649
History of alcohol consumption, n(%)	37 (3.4%)	14 (3.4%)	23 (3.5%)	1.000
Hypertension, n(%)	707 (65.8%)	269 (64.7%)	438 (66.6%)	0.552
Diabetes mellitus, n(%)	337 (31.4%)	137 (32.9%)	200 (30.4%)	0.418
Serum lipid-lipoprotein levels				
TG, mmol/L	1.71 $\pm$ 1.41	1.78 $\pm$ 1.45	1.67 $\pm$ 1.39	0.190
TC, mmol/L	4.92 $\pm$ 1.39	4.86 $\pm$ 1.48	4.96 $\pm$ 1.33	0.276
HDL-C, mmol/L	1.24 $\pm$ 0.37	1.23 $\pm$ 0.37	1.25 $\pm$ 0.37	0.305
LDL-C, mmol/L	2.83 $\pm$ 1.02	2.76 $\pm$ 1.04	2.87 $\pm$ 1.01	0.110
Apo-A1, g/L	1.07 $\pm$ 0.29	1.05 $\pm$ 0.29	1.08 $\pm$ 0.30	0.112
Apo-B, g/L	0.91 $\pm$ 0.32	0.90 $\pm$ 0.33	0.92 $\pm$ 0.31	0.290

**Abbreviations:** TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; Apo-A1, apolipoprotein A1; Apo-B, apolipoprotein B.

## Distribution Frequencies of APOE Genotypes and Alleles in Patients with Atherosclerosis in Multiple Vascular Beds and Patients with Atherosclerosis in Single Vascular Bed

The *APOE* genotypes in the patients with atherosclerosis in single vascular bed ( $\chi^2=4.782$ ,  $P=0.310$ ) and patients with atherosclerosis in multiple vascular beds ( $\chi^2=7.284$ ,  $P=0.122$ ) conformed to the Hardy-Weinberg equilibrium. Compared to the patients with atherosclerosis in single vascular bed, *APOE* E3/E4 genotype frequency was lower in the patients with atherosclerosis in multiple vascular beds (11.4% vs 17.8%,  $P=0.004$ ) (Table 2).

## Comparison of Characteristics and Lipid Levels Among Patients Stratified by APOE Alleles

Patients with the E2/E4 genotype ( $n=17$ ) were excluded from this analysis because of the  $\epsilon 2$  and  $\epsilon 4$  alleles have opposite effects on lipid metabolism.<sup>35,36</sup> The patients carried  $\epsilon 4$  allele had higher level in TG ( $1.99\pm 1.66$  mmol/L vs  $1.64\pm 1.34$  mmol/L), TC ( $5.33\pm 1.34$  mmol/L vs  $4.86\pm 1.38$  mmol/L), LDL-C ( $3.18\pm 1.02$  mmol/L vs  $2.80\pm 1.00$  mmol/L), Apo-B ( $1.03\pm 0.33$  g/L vs  $0.90\pm 0.31$  g/L) than those with  $\epsilon 3$  allele; patients with  $\epsilon 4$  allele had higher TC ( $5.33\pm 1.34$  mmol/L vs  $4.75\pm 1.44$  mmol/L), LDL-C ( $3.18\pm 1.02$  mmol/L vs  $2.58\pm 1.05$  mmol/L), Apo-B ( $1.03\pm 0.33$  g/L vs  $0.85\pm 0.31$  g/L) than those with  $\epsilon 2$  allele (all  $P<0.05$ ) (Table 3).

## Association of the Risk Factors with Atherosclerosis in Multiple Vascular Beds

*APOE* E3/E4 genotype (E3/E4 vs E3/E3: odds ratio (OR) 0.599, 95% confidence interval (CI): 0.421–0.852,  $P=0.004$ ) and  $\epsilon 4$  allele ( $\epsilon 4$  vs  $\epsilon 3$ : OR 0.630, 95% CI: 0.445–0.892,  $P=0.009$ ) were associated with atherosclerosis in multiple vascular beds in univariate logistic regression analysis. In multivariate logistic regression analysis, *APOE* E3/E4 genotype (E3/E4 vs E3/E3: OR 0.598, 95% CI: 0.419–0.854,  $P=0.005$ ), and  $\epsilon 4$  allele ( $\epsilon 4$  vs  $\epsilon 3$ : OR 0.630, 95% CI: 0.444–0.895,  $P=0.010$ ) were associated with atherosclerosis in multiple vascular beds after adjusting for gender, age, smoking, drinking, hypertension, and diabetes mellitus (Table 4).

## Discussion

Atherosclerosis is a systemic disease that can involve multiple vascular beds.<sup>37,38</sup> Atherosclerotic patients with multi-vessel beds have significantly higher morbidity and mortality than patients with single-vessel bed.<sup>39</sup> Currently, most

**Table 2** Distribution Frequencies of APOE Genotypes and Alleles in Patients with Atherosclerosis in Multiple Vascular Beds and Patients with Atherosclerosis in Single Vascular Bed

Variable	Genotype/allele	Total (n=1074)	Patients with atherosclerosis in single vascular bed (n=416)	Patients with atherosclerosis in multiple vascular beds (n=658)	$\chi^2$	P values
APOE genotypes	E2/E2	8(0.7%)	3(0.7%)	5(0.8%)	0.005	1.000
	E2/E3	132(12.3%)	52(12.5%)	80(12.2%)	0.028	0.924
	E2/E4	17(1.6%)	3(0.7%)	14(2.1%)	3.237	0.082
	E3/E3	762(70.9%)	283(68.0%)	479(72.8%)	2.811	0.098
	E3/E4	149(13.9%)	74(17.8%)	75(11.4%)	8.710	0.004
	E4/E4	6(0.6%)	1(0.2%)	5(0.8%)	1.238	0.414
APOE allele	$\epsilon 2$	165(7.7%)	61(7.3%)	104(7.9%)	0.234	0.678
	$\epsilon 3$	1805(84.0%)	692(83.2%)	1113(84.6%)	0.746	0.398
	$\epsilon 4$	178(8.3%)	79(9.5%)	99(7.5%)	2.609	0.109
	HWE ( $\chi^2$ , P)	$\chi^2=1.841$ , $P=0.765$	$\chi^2=4.782$ , $P=0.310$	$\chi^2=7.284$ , $P=0.122$		

**Abbreviations:** APOE, apolipoprotein E; HWE, Hardy Weinberg Equilibrium.

**Table 3** Clinical Characteristics of Subjects Stratified by APOE Alleles

Clinical Characteristics	$\epsilon 2$ (E2/E2 + E2/E3) (n=140)	$\epsilon 3$ (E3/E3) (n=762)	$\epsilon 4$ (E3/E4 + E4/E4) (n=155)	P values
Age, years				
<65, n(%)	44(31.4%)	242(31.8%)	62(40.0%)	0.130
$\geq 65$ , n(%)	96(68.6%)	520(68.2%)	93(60.0%)	
Gender				
Male, n(%)	96(68.6%)	527(69.2%)	99(63.9%)	0.427
Female, n(%)	44(31.4%)	235(30.8%)	56(36.1%)	
History of smoking, n(%)	26(18.6%)	167(21.9%)	38(24.5%)	0.465
History of alcohol consumption, n(%)	4(2.9%)	31(4.1%)	2(1.3)	0.227
Hypertension, n(%)	94(67.1%)	508(66.7%)	93(60.0%)	0.263
Diabetes mellitus, n(%)	53(37.9%)	232(30.4%)	47(30.3%)	0.216
Serum lipid-lipoprotein levels				
TG, mmol/L	1.78 $\pm$ 1.53	1.64 $\pm$ 1.34 <sup>§</sup>	1.99 $\pm$ 1.66*	0.017
TC, mmol/L	4.75 $\pm$ 1.44 <sup>§</sup>	4.86 $\pm$ 1.38 <sup>§</sup>	5.33 $\pm$ 1.34 <sup>#*</sup>	<0.001
HDL-C, mmol/L	1.26 $\pm$ 0.40	1.25 $\pm$ 0.37	1.22 $\pm$ 0.30	0.661
LDL-C, mmol/L	2.58 $\pm$ 1.05 <sup>§</sup>	2.80 $\pm$ 1.00 <sup>§</sup>	3.18 $\pm$ 1.02 <sup>#*</sup>	<0.001
Apo-A1, g/L	1.07 $\pm$ 0.26	1.06 $\pm$ 0.31	1.08 $\pm$ 0.25	0.694
Apo-B, g/L	0.85 $\pm$ 0.31 <sup>§</sup>	0.90 $\pm$ 0.31 <sup>§</sup>	1.03 $\pm$ 0.33 <sup>#*</sup>	<0.001

**Notes:** # compared with  $\epsilon 2$ ,  $p < 0.05$ ; \* compared with  $\epsilon 3$ ,  $p < 0.05$ ; <sup>§</sup> compared with  $\epsilon 4$ ,  $p < 0.05$ .

**Abbreviations:** APOE, apolipoprotein E; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; Apo-A1, apolipoprotein A1; Apo-B, apolipoprotein B.

**Table 4** Multivariate Logistic Regression of Variables Related to Atherosclerosis in Multiple Vascular Beds

Variables		Univariate OR (95% CI)	P values	Multivariate OR (95% CI)	P values
Age ( $\geq 65$ / $< 65$ )		1.041(0.802–1.352)	0.762	1.027(0.782–1.348)	0.848
Gender (Male/ Female)		0.981(0.753–1.277)	0.884	1.047(0.787–1.395)	0.751
History of smoking (Yes/No)		1.078(0.799–1.455)	0.621	1.113(0.798–1.554)	0.528
History of alcohol consumption (Yes/No)		1.040(0.529–2.045)	0.909	0.987(0.493–1.975)	0.970
Hypertension (Yes/No)		1.088(0.840–1.408)	0.522	1.072(0.822–1.398)	0.607
Diabetes mellitus (Yes/No)		0.889(0.683–1.157)	0.383	0.871(0.665–1.141)	0.317
APOE genotypes					
	E3/E3	1.000(reference)		1.000(reference)	
	E2/E2	0.985(0.234–4.151)	0.983	1.002(0.237–4.233)	0.998
	E2/E3	0.909(0.622–1.328)	0.621	0.920(0.630–1.346)	0.669
	E3/E4	0.599(0.421–0.852)	0.004	0.598(0.419–0.854)	0.005
	E4/E4	2.954(0.343–25.412)	0.324	2.946(0.342–25.396)	0.325
APOE alleles					
	$\epsilon 3$	1.000(reference)		1.000(reference)	
	$\epsilon 2$	0.913(0.631–1.321)	0.630	0.925(0.638–1.340)	0.679
	$\epsilon 4$	0.630(0.445–0.892)	0.009	0.630(0.444–0.895)	0.010

**Abbreviations:** APOE, apolipoprotein E; OR, odds ratio; CI, confidence interval.

scholars believe that atherosclerosis is caused by the joint action of many risk factors. However, there are different degrees of arterial lesions in different parts of the same patient, which indicates that the risk factors for atherosclerosis may have different degrees of effect on arteries in different parts. A study in mouse models has found that the location specificity of the formation of atherosclerosis in different vascular beds is genetically controlled.<sup>40</sup>

In the current study, the relationship between APOE gene rs7412 and rs429358 polymorphisms and atherosclerosis in multiple vascular beds was analyzed. The results showed that the frequency of the APOE E3/E4 genotype was lower in the patients with atherosclerosis in multiple vascular beds than that in the patients with atherosclerosis in single vascular

bed (11.4% vs 17.8%,  $P=0.004$ ). Multivariate logistic regression indicated that *APOE* E3/E4 genotype and *APOE*  $\epsilon 4$  allele were associated with atherosclerosis in multiple vascular beds. At present, there have been some reports on the relationship between *APOE* genotypes and cardiovascular and cerebrovascular diseases. *APOE* subtypes, especially *APOE2* and *APOE4*, have been confirmed as markers of arteriosclerotic cardiovascular disease.<sup>41</sup> *APOE*  $\epsilon 4$  allele is closely associated with dyslipidemia and coronary heart disease.<sup>42</sup> *APOE*  $\epsilon 4$  and  $\epsilon 2$  alleles are risk factors for peripheral artery disease (PAD).<sup>43</sup> *APOE* gene variant is associated with intracranial atherosclerosis (ICAS).<sup>26</sup> *APOE*  $\epsilon 4$  allele was a risk factor for carotid atherosclerosis (CAS).<sup>44</sup> *APOE*  $\epsilon 2/\epsilon 2$  genotype was significantly associated with a higher risk of PAD, but not with other vascular diseases.<sup>45</sup> Of course, there are some opposite results. *APOE*  $\epsilon 4$  allele was not associated with carotid atherosclerosis.<sup>46</sup> There was no relationship between *APOE* polymorphism and PAD.<sup>47</sup> *APOE* genotype is not associated with cerebral atherosclerosis.<sup>48</sup>

*APOE* is an important component of plasma lipoprotein, a major ligand of lipid transporter and low-density lipoprotein receptor, through which it clears triglyceride-rich lipoproteins from circulation.<sup>27,49</sup> Therefore, the main function of *APOE* is to resist arteriosclerosis and maintain the balance of plasma lipid metabolism.<sup>50,51</sup> The mechanism of *APOE* involved in atherosclerosis may be related to the role of *APOE* in lipid accumulation and the regulation of inflammation in vascular endothelium.<sup>52,53</sup> Atherosclerosis is the result of lipid infiltration, oxidative stress and inflammation in the intima cells. *APOE* is a glycoprotein that helps stabilize and dissolve lipoproteins in the blood circulation.<sup>20</sup> Although  $\epsilon 3$  is the dominant allele, the *APOE* alleles frequency varies widely across populations.<sup>35,54</sup> In human evolution, the  $\epsilon 4$  allele is considered an ancestral *APOE* allele.<sup>55,56</sup> The activity of *APOE4* is poor, and its function is not enough to maintain the balance of lipid metabolism and inflammatory response in blood vessels, so *APOE*  $\epsilon 4$  allele carriers are more prone to multiple atherosclerosis. Atherosclerosis is a systemic disease process, most commonly coronary and cerebral atherosclerosis, and recent attention has focused on the combination of atherosclerosis in other vascular beds and their risk factors.<sup>5</sup> It is the first report of the relationship of *APOE* genotypes and atherosclerosis in multiple vascular beds.

In addition, there is a relationship between *APOE* genotypes and lipid levels differences. *APOE* is the ligand for the LDL-C receptor family of proteins and itself assimilates and transfers lipids, and the binding ability of *APOE2* to LDL-C was significantly reduced.<sup>20</sup> The *APOE*  $\epsilon 4$  allele was associated with elevated levels of LDL-C.<sup>21,57-60</sup> Some studies suggested that *APOE*  $\epsilon 4$  allele was related to elevated TG,<sup>61-63</sup> TC,<sup>59,64</sup> and Apo-B.<sup>60</sup> However, another study has found no statistically significant difference in lipid levels in patients with different *APOE* alleles.<sup>65</sup> In present study, the patients with  $\epsilon 4$  allele had higher levels in TC, LDL-C, and Apo-B than those with  $\epsilon 3$  allele and  $\epsilon 2$  allele, respectively. To sum up, more studies are needed to reveal the effects of different *APOE* alleles on lipid levels. In addition, the influence of *APOE* alleles on the regulation of different lipid profiles may be caused by the combined action of many factors.<sup>66-68</sup> The effects of these factors add complexity to the regulation of lipid levels.

Several recognized cardiovascular risk factors were associated with the development of multi-site atherosclerotic disease.<sup>69</sup> However, there was no significant relationship between gender, age, smoking, alcohol consumption, hypertension, and diabetes mellitus and the risk of atherosclerosis in multiple vascular beds in this study. Smoking, heavy drinking, and a history of hypertension, and diabetes mellitus are all recognized factors that increase the risk of cardiovascular disease.<sup>70</sup> Study has found that the incidence of arterial disease may vary by gender.<sup>71</sup> A study found that smoking was associated with subclinical atherosclerosis in multi-vessel beds in a Japanese male population.<sup>72</sup> Old age, smoking, and hypertension were associated with atherosclerosis in multiple vascular beds.<sup>73</sup> The prevalence of hypertension and type 2 diabetes mellitus in patients with multi-vessel atherosclerotic disease is significantly higher than that in patients with single-vessel disease.<sup>74</sup> To sum up, the occurrence of atherosclerosis is the result of a variety of factors.

This study found that *APOE*  $\epsilon 4$  allele is associated with atherosclerosis in multiple vascular beds. However, there are some shortcomings. First, no imaging data was collected during the data collection process owing to a retrospective study, the relationship between *APOE* polymorphisms and the degree of embolization of sclerotic vessels was not investigated. Second, the subjects of this study come from a single medical institution, and selection bias is inevitable because the population is not completely representative. Third, this study only studied the relationship between *APOE* genotypes and the risk of atherosclerosis in multiple vascular beds, and did not analyze the expression of *APOE*. Future researches need to conduct targeted research on the above deficiencies.

## Conclusion

An association between *APOE* genotypes and atherosclerosis in multiple vascular beds was found in this cohort study. *APOE*  $\epsilon 4$  allele is associated with atherosclerosis in multiple vascular beds. It can provide reference for the identification and management of patients with atherosclerosis in multiple vascular beds.

## Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Ethics Approval

All participants were informed on the study procedures and goals and informed consent was obtained from all the participants. The study was performed under the guidance of the Declaration of Helsinki and approved by the Ethics Committee of Medicine, Meizhou People's Hospital.

## Acknowledgments

The authors thank their colleagues, who were not listed in the authorship of the Meizhou People's Hospital, for their helpful comments on the manuscript.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Funding

This study was supported by the Science and Technology Program for Social Development of Meizhou (Grant No.: 2023B09).

## Disclosure

The authors declare that they have no competing interests.

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