

# Effect of Apolipoprotein E $\epsilon$ 4 Allele on the Progression of Carotid Atherosclerosis Through Apolipoprotein Levels

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**Background:** The apolipoprotein E (ApoE) genetic variation may be involved in the development of Carotid Atherosclerosis (CAS) disease. So far, few data are available on the role of ApoE isoforms in CAS. The association between this ApoE genotype and CAS remains controversial. The aim of this study was to investigate ApoE gene polymorphism in relation to CAS and the relationships between ApoE gene polymorphism and plasma lipid levels in the ShaanXi Han populations.

**Patients and methods:** The study group enrolled 399 CAS participants and 399 non-CAS controls. ApoE gene polymorphisms were determined by Polymerase chain reaction and hybridization.

**Results:** The  $\epsilon$ 3/ $\epsilon$ 4 genotype and  $\epsilon$ 4 allele in patients with CAS were significantly higher than control participants. In stratified analyses by age and sex, the elevated risk conferred by  $\epsilon$ 4 allele was evident in adults under 60 years old, but not in adults over 60 years old, females and males.  $\epsilon$ 4 carriers had significantly elevated ApoB and ApoB/ApoA and decreased ApoE levels than  $\epsilon$ 2 carriers in CAS patients. After adjusting for confounding factors, hypertension, ApoA-I, low density lipoprotein cholesterol (LDL-C), triglycerides (TG) and  $\epsilon$ 4 allele were significant independent risk factor for CAS. ApoE- $\epsilon$ 4 allele was associated with a nearly 1.5-fold increased risk of CAS.

**Conclusion:** This study provides convincing evidence that  $\epsilon$ 4 allele, hypertension, ApoA-I, LDL-C and TG levels are independent risk factor for CAS in the ShaanXi Han populations. ApoE polymorphism was associated with CAS and this association was partly mediated through blood lipids. Also, the clinical use of genomic data may become useful in optimizing individual preventative and therapeutic strategies.

**Keywords:** apolipoprotein E, carotid atherosclerosis, gene polymorphism, blood lipids

## Introduction

CAS remains a widely recognised risk factor for stroke and transient ischemic attack.<sup>1</sup> About 15% of ischemic strokes are caused by atherosclerotic stenosis of the internal carotid artery.<sup>2</sup> Atherosclerotic disease is a complex, multifactorial process characterized by deposition and accumulation of cholesterol along the arterial wall.<sup>3</sup>

The ApoE gene has been implicated in both triglyceride and cholesterol metabolism plays a significant role in the development and progression of atherosclerosis.<sup>4</sup> Genetic variation at the ApoE locus significantly affects lipoprotein concentrations.<sup>5</sup> The ApoE genotypes were classified into three different alleles ( $\epsilon$ 2,  $\epsilon$ 3 and  $\epsilon$ 4) and six different

genotypes ( $\epsilon 2/\epsilon 2$ ,  $\epsilon 2/\epsilon 3$ ,  $\epsilon 2/\epsilon 4$ ,  $\epsilon 3/\epsilon 3$ ,  $\epsilon 3/\epsilon 4$  and  $\epsilon 4/\epsilon 4$ ). Among three alleles,  $\epsilon 4$  allele is associated with increased levels of total plasma cholesterol and LDL cholesterol levels, while  $\epsilon 2$  allele was associated with the reverse effect.<sup>6</sup>

The ApoE gene polymorphism has been associated with increased risk of coronary artery disease.<sup>7,8</sup> However, the association between this ApoE genotype and CAS remains controversial.<sup>9,10</sup> The  $\epsilon 2$  allele was associated with a reduced risk for CAS, compared with participants with  $\epsilon 3$  allele.<sup>9</sup> In the other study the same was suggested for the carriers of  $\epsilon 4$  allele.<sup>11</sup> Many studies did not identify adequately powered to assess whether ApoE genotypes was associated with CAS. To date, few studies have been conducted in China. Therefore, the aim of this study was to investigate the role of ApoE genotypes in carotid artery atherosclerosis risk and the interrelationship between lipid profiles and ApoE alleles and genotypes among the population of northwest China.

## Methods

### Subjects

A total of 399 Chinese CAS patients and 399 controls were collected in the First Affiliated Hospital of Xi'an Jiao Tong University between May 2018 and May 2019. The gender and age were matched between the two groups. Most of the study participants are ethnically Han Chinese and residents living in Shaanxi Province, northwest China. All the study participants underwent the same carotid ultrasound examination in the ultrasound department of our hospital by two experienced sonographers. Cardiovascular or cerebrovascular disease was excluded. This study was conducted in accordance with the Declaration of Helsinki, approved by the Ethical Committee of the First Affiliated Hospital of Xi'an Jiao Tong University, and informed consent was got from every patient.

### DNA Extraction and Genotyping

Peripheral blood (2 mL) was drawn from the cubital vein into ethylene diamine tetra-acetic acid (EDTA) containing blood collection tubes. Genomic DNA was extracted from peripheral blood using a commercial isolation kit (Sinochips Bioscience Co., Ltd., Zhuhai, Guangdong, China) and stored at  $-20^{\circ}\text{C}$ . Three ApoE alleles ( $\epsilon 2$ ,  $\epsilon 3$  and  $\epsilon 4$ ) were detected by an ApoE Genotyping Kit (Sinochips Bioscience Co., Ltd., Zhuhai, Guangdong, China). PCR was performed according to the following protocol:  $50^{\circ}\text{C}$  for 2 minutes, pre-denaturation at  $95^{\circ}\text{C}$  for 15 minutes, followed by 45 cycles at  $94^{\circ}\text{C}$  for 30 seconds and  $65^{\circ}\text{C}$  for 45 seconds. The details of the experimental procedure were reported in our previous paper.<sup>7</sup>

### Statistical Analyses

The statistical analysis was performed using SPSS statistical software (Version 16.0).

Continuous variables data are presented as mean, standard deviation and categorical variables using the frequency and percentages. The chi-square test and one-way ANOVA were used to determine the statistical significance of differences between specific ApoE genotypes and clinical characteristics. The ApoE genotype and allele frequencies for Hardy-Weinberg equilibrium proportions were tested by Pearson's Chi-square test. Logistic regression analysis was used to assess the role of the ApoE genotype and other coexisting factors in CAS. In all tests, differences with two-tailed alpha-probability  $p < 0.05$  were considered significant.

## Results

The clinical characteristics of the study population are described in Table 1.

Participants consisted of 399 control participants [236 males and 163 females aged  $59.16 \pm 13.15$  years, mean  $\pm$  standard deviation (SD)], 399 patients with CAS (250 males and 149 females, age  $60.39 \pm 12.75$  years). The age, sex distribution and smoking did not significantly differ between the two groups ( $P=0.178$ ,  $0.310$  and  $0.825$ , respectively). Baseline characteristics and CAS risk factors, such as hypertension, diabetes mellitus and drinking were significantly different between the 2 groups. TG, TC, ApoA-I, ApoB and ApoE levels were significantly higher in the CAS group compared with the control group ( $P<0.05$  for all). There were no significant differences in the levels of high-density lipoproteins (HDL), LDL-C, Cr, ApoB/ApoA-I, lipoprotein(a)[Lp(a)], direct bilirubin (DB) and indirect bilirubin (IB) (all  $P > 0.05$ ).

**Table 1** Characteristics of the Study Population

Factors	Controls (n=399)	CAS (n=399)	P value
Age (years)	59.16±13.15	60.39±12.75	0.178 <sup>a</sup>
Males/females	236/163	250/149	0.310 <sup>b</sup>
Smoking	143(35.84%)	146(36.59%)	0.825 <sup>b</sup>
Drinking	75(18.80%)	104(26.07%)	0.014 <sup>b</sup>
Diabetes	79(19.80%)	108(27.07%)	0.015 <sup>b</sup>
Hypertension	185(46.37%)	247(61.90%)	0.000 <sup>b</sup>
TC	3.88±0.95	4.04±0.95	0.025 <sup>a</sup>
HDL	1.03±0.28	1.03±0.36	0.983 <sup>a</sup>
LDL-C	2.30±0.81	2.41±0.85	0.059 <sup>a</sup>
TG	1.49±1.21	1.77±1.05	0.001 <sup>a</sup>
Cr	64.43±28.00	64.78±22.71	0.853 <sup>a</sup>
ApoA-I	1.15±0.22	1.21±0.49	0.039 <sup>a</sup>
ApoB	0.77±0.22	0.80±0.21	0.047 <sup>a</sup>
ApoB/ApoA-I	0.69±0.33	0.69±0.21	0.746 <sup>a</sup>
ApoE	37.78±15.01	39.66±17.05	0.017 <sup>a</sup>
Lipoprotein	191.79±188.73	195.35±200.12	0.805 <sup>a</sup>
DB	4.54±3.30	4.11±1.91	0.030 <sup>a</sup>
IB	9.06±5.25	8.82±4.30	0.501 <sup>a</sup>

**Note:** aP values were calculated by Student's t-tests. bP values were calculated from two-sided chi-square test.

**Abbreviations:** CAS, carotid atherosclerosis; TC, total cholesterol; HDL, high-density lipoproteins; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; Cr, creatinine; DB, direct bilirubin; IB, indirect bilirubin; ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; ApoE, apolipoprotein E.

**Table 2** The Distributions of Genotypes and Alleles of the ApoE Gene in the CAS Patients and Controls

Genotype, n (%)	CAS Group(n=399)	Control Group(n=399)	OR (95% CI)	P <sup>a</sup>	χ <sup>2</sup>	P <sup>b</sup>
E2/E2	6(1.5%)	1(0.3%)	6.072 (0.728,50.703)	0.129	14.754	0.011
E2/E3	47(11.8%)	53(13.3%)	0.872 (0.573,1.326)	0.512		
E2/E4	8(2.0%)	13(3.3%)	0.608 (0.249,1.482)	0.269		
E3/E3	245(61.4%)	272(68.2%)	0.743 (0.555,0.994)	0.045		
E3/E4	86(21.6%)	58(14.5%)	1.615 (1.120,2.331)	0.010		
E4/E4	7(1.8%)	2(0.5%)	3.545 (0.732,17.169)	0.18	6.759	0.034
HWE	χ <sup>2</sup> = 4.336, P = 0.362	χ <sup>2</sup> = 9.122, P = 0.058				
Alleles, n (%)						
E2	67(8.4%)	68(8.5%)	0.984 (0.692,1.400)	0.928		
E3	623(78.1%)	655(82.1%)	0.777 (0.607,0.995)	0.045		
E4	108(13.5%)	75(9.4%)	1.509 (1.104,2.062)	0.010		

**Notes:** aP and OR (95% CI) values were calculated by logistic regression adjusted for age, gender, and traditional cardiovascular risk factors. bP values were calculated from two-sided chi-square tests or Fisher's exact tests.

**Abbreviations:** CAS, carotid atherosclerosis; HWE, Hardy-Weinberg equilibrium.

The results of frequency of ApoE alleles and genotypes in CAS cases and control subjects are shown in Table 2. The genotype frequency of the ApoE polymorphism was consistent with Hardy-Weinberg equilibrium (HWE) in both cases and controls groups (both  $P>0.05$ ). ApoE  $\epsilon 3/\epsilon 3$  genotype (61.4% and 68.2%) and  $\epsilon 3$  allele (78.1% and 82.1%) were predominant in CAS patients and controls. The distribution of ApoE genotypes and alleles between cases and controls of our study was significantly different ( $\chi^2=14.754$  and  $P<0.05$ ,  $\chi^2=6.759$  and  $P<0.05$ , respectively). Patients with CAS had a significantly higher frequency of  $\epsilon 3/\epsilon 4$  genotype (OR =1.615, 95% CI = 1.120–2.331,  $P<0.05$ ) and  $\epsilon 4$  allele (OR =1.509, 95% CI = 1.104–2.062,  $P<0.05$ ) than healthy controls. Further, patients with CAS had a significantly lower  $\epsilon 3/\epsilon 3$  genotype (OR =0.743, 95% CI = 0.555–0.994,  $P<0.05$ ) and  $\epsilon 3$  allele (OR =0.777, 95% CI = 0.607–0.995,  $P<0.05$ ) frequencies than did the control participants.

**Table 3** Stratified Analyses Between ApoE Polymorphism and Risk of CAS

	Age≤60				Age>60				Males				Females			
Genotype, n (%)	CAS	Control	OR (95% CI)	P	CAS	Control	OR (95% CI)	P	CAS	Control	OR (95% CI)	P	CAS	Control	OR (95% CI)	P
E2/E2	2(1.0%)	1(0.5%)	2.107 (0.190,23.417)	0.964	4(2.1%)	0(0.0%)	0.979 (0.959,1.000)	0.124	5(2.0%)	0(0.0%)	0.980 (0.963,0.998)	0.062	1(0.7%)	1(0.6%)	1.095 (0.068,17.657)	0.949
E2/E3	25 (12.1%)	30 (13.8%)	0.856 (0.485,1.512)	0.665	22 (11.5%)	23 (12.6%)	0.895 (0.480,1.668)	0.726	27 (10.8%)	29 (12.3%)	0.864 (0.495,1.509)	0.608	20 (13.4%)	24 (14.7%)	0.898 (0.473,1.703)	0.742
E2/E4	3(1.4%)	6(2.8%)	0.517 (0.128,2.096)	0.547	5(2.6%)	7(3.8%)	0.668 (0.208,2.145)	0.496	5(2.0%)	6(2.5%)	0.782 (0.236,2.598)	0.688	3(2.0%)	7(4.3%)	0.458 (0.016,1.804)	0.412
E3/E3	127 (61.4%)	157 (72.4%)	0.607 (0.403,0.913)	0.016	118 (561.5%)	115 (63.2%)	0.929 (0.611,1.412)	0.73	155 (62.0%)	163 (69.1%)	0.731 (0.502,1.064)	0.102	90 (60.4%)	109 (66.9%)	0.756 (0.476,1.200)	0.235
E3/E4	46 (22.2%)	22 (10.1%)	2.532 (1.462,4.386)	0.001	40 (120.8%)	36 (19.8%)	1.067 (0.645,1.767)	0.8	55 (22.0%)	38 (16.1%)	1.470 (0.929,2.324)	0.099	31 (20.8%)	20 (12.3%)	1.878 (1.018,3.466)	0.042
E4/E4	4(1.9%)	1(0.5%)	4.256 (0.472,38.399)	0.341	3(1.6%)	1(0.5%)	2.873 (0.296,27.874)	0.653	3(1.2%)	0(0.0%)	0.988 (0.975,1.002)	0.249	4(1.9%)	2(1.2%)	2.221 (0.401,12.304)	0.600
Alleles, n (%)																
E2	32(7.7%)	38(8.8%)	0.873 (0.534,1.426)	0.587	35(9.1%)	30(8.2%)	1.117 (0.670,1.860)	0.672	42 (8.4%)	35(7.4%)	1.145 (0.718,1.0827)	0.570	25 (8.4%)	33 (10.1%)	0.813 (0.471,1.403)	0.456
E3	325 (78.5%)	366 (84.3%)	0.678 (0.478,0.962)	0.029	298 (77.6%)	289 (79.4%)	0.899 (0.634,1.275)	0.551	392 (78.4%)	393 (83.3%)	0.730 (0.529,1.007)	0.055	231 (77.5%)	262 (80.4%)	0.842 (0.573,1.238)	0.382
E4	57 (13.8%)	30(6.9%)	2.150 (1.351,3.421)	0.001	51 (13.3%)	45 (12.4%)	1.086 (0.707,1.668)	0.707	66 (13.2%)	44(9.3%)	1.479 (0.987,2.216)	0.056	42 (14.1%)	31(9.5%)	1.561 (0.953,2.557)	0.075

**Note:** P and OR (95% CI) values were calculated by logistic regression adjusted for age, gender, and traditional cardiovascular risk factors.

**Abbreviation:** CAS, carotid atherosclerosis.

**Table 4** Relationships Between Serum Lipid Profile and ApoE Allele in CAS Patients and Control Participants

CAS Patient								Control Participants						
Factors	$\epsilon 2(\epsilon 2\epsilon 2+\epsilon 2\epsilon 3)$	$\epsilon 3(\epsilon 3)$	$\epsilon 4(\epsilon 3\epsilon 4+\epsilon 4\epsilon 4)$	$P^a$	$P^b$	$P^c$	$P^d$	$\epsilon 2(\epsilon 2\epsilon 2+\epsilon 2\epsilon 3)$	$\epsilon 3(\epsilon 3)$	$\epsilon 4(\epsilon 3\epsilon 4+\epsilon 4\epsilon 4)$	$P^a$	$P^b$	$P^c$	$P^d$
TC	3.94±0.86	4.02±0.96	4.19±0.99	0.223	0.604	0.140	0.127	3.78±1.10	3.88±0.92	4.08±1.00	0.245	0.490	0.165	0.108
HDL	1.01±0.21	1.05±0.42	1.02±0.25	0.774	0.564	0.588	0.901	1.00±0.21	1.03±0.28	1.07±0.31	0.243	0.470	0.366	0.206
LDL-C	2.34±0.73	2.40±0.85	2.50±0.92	0.468	0.613	0.326	0.253	2.18±1.04	2.29±0.76	2.50±0.84	0.131	0.375	0.104	0.051
TG	1.95±1.21	1.70±1.03	1.86±1.05	0.195	0.117	0.219	0.610	1.75±1.07	1.47±1.32	1.43±0.84	0.315	0.146	0.859	0.198
Cr	62.87±16.64	64.10±16.94	68.08±36.08	1.196	0.726	0.165	0.194	62.04±20.23	64.93±30.78	65.71±22.78	0.799	0.520	0.858	0.520
ApoA-I	1.21±0.19	1.22±0.61	1.17±0.20	0.585	0.840	0.301	0.578	1.16±0.19	1.14±0.22	1.15±0.23	0.867	0.599	0.855	0.784
ApoB	0.72±0.18	0.80±0.21	0.85±0.23	0.002	0.011	0.059	0.000	0.71±0.25	0.77±0.21	0.82±0.22	0.065	0.089	0.020	0.198
ApoB/ApoA-I	0.60±0.16	0.68±0.20	0.75±0.23	0.000	0.013	0.009	0.000	0.62±0.27	0.71±0.37	0.72±0.20	0.171	0.075	0.784	0.106
ApoE	59.12±28.90	36.54±11.28	36.10±13.09	0.000	0.000	0.813	0.000	51.93±19.61	35.39±12.52	34.69±13.70	0.000	0.009	0.747	0.058
Lipoprotein	193.74±210.20	198.50±203.57	191.00±190.73	0.951	0.876	0.761	0.937	126.67±1091.79	204.54±206.33	198.19±152.65	0.032	0.008	0.827	0.002
DB	3.82±2.06	4.24±1.89	3.94±1.86	0.223	0.149	0.209	0.712	4.52±2.38	4.43±3.21	5.03±4.44	0.488	0.851	0.437	0.232
IB	8.31±3.20	9.12±4.45	8.48±4.53	0.298	0.216	0.230	0.820	8.92±4.28	8.85±5.31	10.18±5.27	0.227	0.929	0.088	0.211

**Notes:** aP value shows the differences compared between groups ( $\epsilon 2$ ,  $\epsilon 3$ ,  $\epsilon 4$ ). bP values obtained when comparing  $\epsilon 2$  subjects with  $\epsilon 3$  subjects. cP values obtained when comparing  $\epsilon 4$  subjects with  $\epsilon 3$  subjects. dP values obtained when comparing  $\epsilon 2$  subjects with  $\epsilon 4$  subjects.

**Abbreviations:** CAS, carotid atherosclerosis; TC, total cholesterol; HDL, high-density lipoproteins; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; Cr, creatinine; DB, direct bilirubin; IB, indirect bilirubin; ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; ApoE, apolipoprotein E.

**Table 5** Logistic Regression Analysis of the Risk of CAS in Northwest of China Population

Variables	P- value	OR (95% CI)
Hypertension	0.002	1.646(1.201–2.256)
ApoA-I	0.003	5.431(1.744–16.910)
LDL-C	0.025	2.682(1.131–6.361)
TG	0.010	1.341(1.072–1.677)
ε4	0.026	1.536(1.053–2.240)

**Abbreviations:** CAS, carotid atherosclerosis; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride.

**Table 6** Multiple Logistic Regression Analysis for CAS Patients and Control Subjects

ε4		
	OR (95% CI)	P-value
Age≤60	2.136(1.241–3.676)	0.006
Age>60	1.146(0.654–2.006)	0.635
Males	1.673(1.038–2.696)	0.035
Females	1.288(0.672–2.471)	0.446

**Table 3** to assess the relationship between ApoE genotype and CAS, we conducted stratified analysis by age (dichotomized into ≤60 years and >60 years) and sex. Results showed that compared with control participants, CAS patients had a significantly higher frequency ε3/ε4 in adults over 60 years old (OR =2.532, 95% CI = 1.462–4.386,  $P=0.001$ ) and females (OR =1.878, 95% CI = 1.018–3.466,  $P=0.042$ ), but not in adults over 60 years old (OR =1.067, 95% CI = 0.645–1.767,  $P=0.800$ ) and males (OR =1.470, 95% CI = 0.929–2.324,  $P=0.099$ ). CAS patients also had a significantly higher frequency ε4 allele than healthy controls in adults under 60 years old (OR =2.150, 95% CI = 1.351–3.421,  $P=0.001$ ), but not in adults over 60 years old (OR =1.086, 95% CI = 0.707–1.668,  $P=0.707$ ), females (OR =1.561, 95% CI = 0.953–2.557,  $P=0.075$ ) and males (OR =1.470, 95% CI = 0.929–2.324,  $P=0.099$ ).

In **Table 4**, consistent with previous studies,<sup>7</sup> ApoE genotypes were grouped into three categories according to ε2 and ε4 carrier status: ε2 carriers (ε2/ε2, ε2/ε3), ε4 carriers (ε4/ε4, ε4/ε3), and reference (ε3/ε3). In the patients with CAS, ε2 carriers had significantly lower ApoB and ApoB/ApoA-I levels and higher ApoE level than ε3 and ε4 carriers. Additionally, control participants with ε2 carriers had a significantly lower level of lipoprotein than ε3 and ε4 carriers, and ε2 carriers had significantly higher ApoE level than ε4 carriers.

In **Table 5**, multivariate logistic regression analysis was used to determine the independent predictors of CAS, and the results indicated significantly higher risks of CAS in the presence of hypertension (OR =1.646, 95% CI = 1.201–2.256,  $P=0.002$ ), high ApoA-I (OR =5.431, 95% CI = 1.744–16.910,  $P=0.003$ ), high LDL-C (OR =2.682, 95% CI = 1.131–6.361,  $P=0.025$ ), high TG (OR =1.341, 95% CI = 1.072–1.677,  $P=0.010$ ) and the ε4 allele (OR =1.536, 95% CI = 1.053–2.240,  $P=0.026$ ). In addition, to exclude the potentially confounding effects of age and sex, multivariate logistic regression analysis stratified according to age and sex was performed. ε4 allele was a significant and independent risk factor for male (OR = 1.673, 95% CI = 1.038–2.696,  $P=0.035$ ) and adults under 60 years old (OR = 2.136, 95% CI = 1.241–3.676,  $P=0.006$ ), but not for female (OR = 1.288, 95% CI = 0.672–2.471,  $P=0.446$ ) and adults over 60 years old (OR =1.146, 95% CI = 0.654–2.006,  $P=0.635$ ) (**Table 6**).

## Discussion

CAS is one of the primary causes of stroke, which accounts for over 80% of all stroke cases.<sup>12</sup> The timely detection and intervention of CAS are important for the prevention of stroke.

ApoE is a 34 kDa proteins consisting of 299 amino acids, encoded by the ApoE gene located on chromosome 19 q13.2.<sup>13</sup> Previous studies have demonstrated ApoE genotypes and allele frequencies vary between different races and ethnicities.<sup>14</sup> The present study investigated that in Northwest China, ApoE  $\epsilon 3/\epsilon 3$  genotype and  $\epsilon 3$  allele were predominant in majority of populations. These results were consistent with previous findings.<sup>15</sup> In this study, we investigated the association between CAS and ApoE in a case-control study. Logistic regression analysis clearly showed a significant tendency of  $\epsilon 4$  allele towards an increased risk for the progression of CAS. Similar to our study, previous studies suggested that individuals with  $\epsilon 4/\epsilon 4$  genotype had higher risk of carotid plaque, whereas carriers of  $\epsilon 2$  had lower risk compared with those with  $\epsilon 3/\epsilon 3$  genotype.<sup>16,17</sup> However, in the Rotterdam study and Luc et al study demonstrated that the  $\epsilon 2/\epsilon 3$  genotype has a limited protective effect against CAS due to its low frequency of occurrence, while ApoE4 is not a major risk factor for carotid artery atherosclerosis.<sup>9,18</sup> Since age and sex seem to be an important disease factor contributing to relationship between ApoE and CAS, we conducted stratified analysis by age and sex. Our results showed that  $\epsilon 4$  increased the CAS risk nearly 1.5-fold overall in males and 2.1-fold in young patients with CAS but not in females and elderly patients with CAS. Hou et al found that ApoE4 genotype may be a risk factor for CAS in elderly Han males.<sup>19</sup> Makaruk et al also reported  $\epsilon 4$  allele were more liable to develop CAS in patients under 50 years of age.<sup>20</sup> These results were similar to our results. However, in the Framingham study, an association between ApoE genotype and CAS was only observed in females but not in males. The modifying effect of sex on the association between CAS and ApoE is still unclear.

CAS is a complex disease caused by genetic and environmental factors, and abnormalities of lipid metabolism such as elevated serum levels of TC, TG, LDL-C are considered as major risk factors for CAS.<sup>21</sup> In our study, we also found that the patients with CAS had higher TG and TC levels. Furthermore, logistic regression analysis indicated that LDL-C and TG levels were strong independent risk factors. However, traditional risk factors fail to explain all cardiovascular morbidity and mortality, investigators have sought to identify new risk indices.<sup>22</sup> The exact mechanisms underlying the association between ApoE polymorphism and CAS are not fully understood. The common ApoE polymorphism explains about 7% of variation in plasma cholesterol levels.<sup>23</sup> However, in our study, we did not find the  $\epsilon 4$  carriers are associated with higher TC, HDL, LDL-C and TG levels compared with  $\epsilon 2$  and  $\epsilon 3$  carriers, respectively, in all subjects and healthy controls. This were consistent with those published by Lee et al.<sup>24</sup> Shin et al reported that ApoE polymorphism was associated with CAS and this association was partly mediated through blood lipids and may also be mediated through non-lipid pathways.<sup>16</sup> The absence of ApoE in macrophage promotes atherosclerosis without changing plasma cholesterol.<sup>25</sup> Raffai et al found that ApoE may promote the development of CAS through inflammatory response.<sup>26</sup> Conversely, recent animal experiments studies showed that ApoE enhanced miR-146a levels in monocytes and macrophages, suppressing NF-kappaB-mediated inflammation and atherosclerosis.<sup>27</sup> These contradictory findings may have been due to differences in participant ethnicities and sample size.

ApoA-I the primary protein component of HDL, plays an important role in reducing the CAS process, and one major atheroprotective mechanism is their role in reverse cholesterol transport, a process that removes excess cholesterol from peripheral tissues to the liver for excretion.<sup>28</sup> By contrast, ApoB is the main apolipoprotein of chylomicrons and LDL, and are associated with the development of atherosclerosis.<sup>7</sup> Therefore, the ApoB/ApoA-I ratio may be better indicators of the risk of subclinical atherosclerosis and better therapeutic targets for reducing the risk of plaque formation and cerebrovascular disease.<sup>29,30</sup> However, our study found that ApoA-I levels were significantly higher in patients with CAS than control participants and associated with a 5.4 fold increase risk of CAS. Besides, ApoA-I plasma levels were not affected by the ApoE polymorphism. These findings are in contrast to previously reported data, which showed that ApoA-I were also lower in subjects with CAS.<sup>30</sup> We also found that CAS patients had significantly higher ApoB levels compared to control participants, while ApoB/ApoA-I ratios were not differ significantly between the two groups. In addition, ApoB levels and ApoB/ApoA-I ratios were significantly lower in  $\epsilon 2$  carriers than in  $\epsilon 3$  or  $\epsilon 4$  carriers in CAS patients, but not in control participants. The relationship between ApoA-I, ApoB level and CAS had been rarely studied. Zivanovic et al and Jun et al showed that high levels of ApoB and ApoB/ApoA-I ratio were significantly associated with CAS.<sup>29,30</sup> ApoE protein is synthesized in the liver and macrophages, and it plays a crucial role in antiatherogenic properties by regulating regulating lipid and lipoprotein metabolism.<sup>31</sup> The present study showed that ApoE levels were significantly depleted in the CAS group compared with the control group. We also observed a significantly trend towards higher levels of ApoE in  $\epsilon 2$  carriers than in  $\epsilon 3$  and  $\epsilon 4$  carriers in CAS patients.



## Study Strength and Limitations

The strength of this study is the first to find a correlation between ApoE polymorphism and CAS risk in a Northwest Han Chinese population. The clinical characteristics, lipid levels and ApoE gene polymorphisms indicators into the final analysis to exclude the influence of related confounding factors on the results. Despite the strengths of our study, there were some inherent limitations. (1) As a retrospective analysis, original data shortage constrained assessment of potential gene–environment interactions. (2) The small sample size of this study, which may have influenced the accuracy of the results to some extent. (3) Most of the present participants may receive were receiving lipid-lowering treatment at the time of inclusion, which may have potential impacts on association between blood lipid profiles and CAS risk.

## Conclusions

In conclusion, this study suggested that the  $\epsilon 4$  allele was associated with CAS in the population of Northwest region of Shaanxi in China. In stratified analyses by age and sex, this elevated risk in individuals aged under 60 years old, but not in males, females and aged over 60 years old. In addition,  $\epsilon 4$  allele was associated with elevated ApoB and ApoB/ApoA and decreased ApoE levels. ApoE polymorphism was associated with CAS and this association was partly mediated through blood lipids and may also be mediated through non-lipid pathways. Since the limited sample size, and thus further studies with larger sample population are needed to clear our findings.

## Ethics Approval and Consent to Participate

This study was approved by the Ethical Committee of the First Affiliated Hospital of Xi'an Jiao Tong University and we got the informed consent of each patients.

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## 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.

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## Disclosure

The authors declare that they have no competing interests.

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