Effect of Apolipoprotein E ε4 Allele on the Progression of Carotid Atherosclerosis Through Apolipoprotein Levels

Wenbing Ma¹,², *, Liting Zhang¹, *, Lei Luo¹, Suya Zhang¹, *, Shuang Yang¹, Hongping Yao¹, Lei Zhang¹, Xiaoyun Lu², Weiyi Feng¹

¹Department of Pharmacology, The First Affiliated Hospital of Xi’an Jiaotong University, Xi’an, Shaanxi, 710061, People’s Republic of China; ²Department of Biological Science and Bioengineering, Key Laboratory of Biomedical Information Engineering of the Ministry of Education, School of Life Science and Technology, Xi’an Jiaotong University, Xi’an, Shaanxi, 710049, People’s Republic of China

*These authors contributed equally to this work

Correspondence: Weiyi Feng, Department of Pharmacology, the First Affiliated Hospital of Xi’an Jiaotong University, Xi’an, Shaanxi, 710061, People’s Republic of China, Tel/Fax +86-029-85323242, Email fengweiyi@mail.xjtu.edu.cn; Xiaoyun Lu, Department of Biological Science and Bioengineering, Key Laboratory of Biomedical Information Engineering of the Ministry of Education, School of Life Science and Technology, Xi’an Jiaotong University, Xi’an, Shaanxi, 710049, People’s Republic of China, Tel/Fax +86-029-82668463, Email luxy05@xjtu.edu.cn

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 ε3/ε4 genotype and ε4 allele in patients with CAS were significantly higher than control participants. In stratified analyses by age and sex, the elevated risk conferred by ɛ4 allele was evident in adults under 60 years old, but not in adults over 60 years old, females and males. ε4 carriers had significantly elevated ApoB and ApoB/ApoA and decreased ApoE levels than ε2 carriers in CAS patients. After adjusting for confounding factors, hypertension, ApoA-I, low density lipoprotein cholesterol (LDL-C), triglycerides (TG) and ε4 allele were significant independent risk factor for CAS. ApoE-ε4 allele was associated with a nearly 1.5-fold increased risk of CAS.

Conclusion: This study provides convincing evidence that ε4 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.¹ About 15% of ischemic strokes are caused by atherosclerotic stenosis of the internal carotid artery.² Atherosclerotic disease is a complex, multifactorial process characterized by deposition and accumulation of cholesterol along the arterial wall.³

The ApoE gene has been implicated in both triglyceride and cholesterol metabolism plays a significant role in the development and progression of atherosclerosis.⁴ Genetic variation at the ApoE locus significantly affects lipoprotein concentrations.⁵ The ApoE genotypes were classified into three different alleles (ε2, ε3 and ε4) and six different
genotypes (ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4 and ε4/ε4). Among three alleles, ε4 allele is associated with increased levels of total plasma cholesterol and LDL cholesterol levels, while ε2 allele was associated with the reverse effect. Among three alleles, ε4 allele is associated with increased levels of total plasma cholesterol and LDL cholesterol levels, while ε2 allele was associated with the reverse effect.

The ApoE gene polymorphism has been associated with increased risk of coronary artery disease. However, the association between this ApoE genotype and CAS remains controversial. The ε2 allele was associated with a reduced risk for CAS, compared with participants with ε3 allele. In the other study the same was suggested for the carriers of ε4 allele. 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°C. Three ApoE alleles (ε2, ε3 and ε4) were detected by an ApoE Genotyping Kit (Sinochips Bioscience Co., Ltd., Zhuhai, Guangdong, China). PCR was performed according to the following protocol: 50°C for 2 minutes, pre-denaturation at 95°C for 15 minutes, followed by 45 cycles at 94°C for 30 seconds and 65°C for 45 seconds. The details of the experimental procedure were reported in our previous paper.

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±13.15 years, mean ±standard deviation (SD)), 399 patients with CAS (250 males and 149 females, age 60.39± 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 \( P > 0.05 \)).
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 ε3/ε3 genotype (61.4% and 68.2%) and ε3 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 ε3/ε4 genotype (OR =1.615, 95% CI = 1.120–2.331, $P<0.05$) and ε4 allele (OR =1.509, 95% CI = 1.104–2.062, $P<0.05$) than healthy controls. Further, patients with CAS had a significantly lower ε3/ε3 genotype (OR =0.743, 95% CI = 0.555–0.994, $P<0.05$) and ε3 allele (OR =0.777, 95% CI = 0.607–0.995, $P<0.05$) frequencies than did the control participants.

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 $^a$ |
| Males/females | 236/163 | 250/149 | 0.310 $^b$ |
| Smoking | 143(35.84%) | 146(36.59%) | 0.825 $^b$ |
| Drinking | 75(18.80%) | 104(26.07%) | 0.014 $^b$ |
| Diabetes | 79(19.80%) | 108(27.07%) | 0.015 $^b$ |
| Hypertension | 185(46.37%) | 247(61.90%) | 0.000 $^b$ |
| TC | 3.88±0.95 | 4.04±0.95 | 0.925 $^a$ |
| HDL | 1.03±0.28 | 1.03±0.36 | 0.983 $^a$ |
| LDL-C | 2.30±0.81 | 2.41±0.85 | 0.059 $^a$ |
| TG | 1.49±1.21 | 1.77±1.05 | 0.001 $^a$ |
| Cr | 64.43±28.00 | 64.78±22.71 | 0.853 $^a$ |
| ApoA-I | 1.15±0.22 | 1.21±0.49 | 0.039 $^a$ |
| ApoB | 0.77±0.22 | 0.80±0.21 | 0.047 $^a$ |
| ApoB/ApoA-I | 0.69±0.33 | 0.69±0.21 | 0.746 $^a$ |
| ApoE | 37.78±15.01 | 39.66±17.05 | 0.017 $^a$ |
| Lipoprotein | 191.79±188.73 | 195.35±200.12 | 0.805 $^a$ |
| DB | 4.54±3.30 | 4.11±1.91 | 0.030 $^a$ |
| IB | 9.06±5.25 | 8.82±4.30 | 0.501 $^a$ |

Note: $^a$ Values were calculated by Student’s t-tests. $^b$ 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) | $P$ value | $\chi^2$ | $P$ value |
|----------------|------------------|----------------------|-------------|------------|----------|------------|
| 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 | 9.122 | 0.058 |
| E2/E4 | 8(2.0%) | 13(3.3%) | 0.608 (0.249,1.482) | 0.269 | 6.759 | 0.034 |
| E3/E3 | 245(61.4%) | 272(68.2%) | 0.743 (0.555,0.994) | 0.045 | 0.861 | 0.355 |
| E3/E4 | 86(21.6%) | 58(14.5%) | 1.615 (1.120,2.331) | 0.010 | 0.354 | 0.554 |
| E4/E4 | 7(1.8%) | 2(0.5%) | 3.545 (0.732,17.169) | 0.18 | 0.354 | 0.554 |
| HWE | $\chi^2 = 4.336, P = 0.362$ | $\chi^2 = 9.122, P =0.058$ | | | | |

Notes: $^a$ and OR (95% CI) values were calculated by logistic regression adjusted for age, gender, and traditional cardiovascular risk factors. $^b$ Values were calculated from two-sided chi-square tests or Fisher’s exact tests.

Abbreviations: CAS, carotid atherosclerosis; HWE, Hardy–Weinberg equilibrium.
Table 3 Stratified Analyses Between ApoE Polymorphism and Risk of CAS

| 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       | 0.064     | 4(2.1%)  | 0(0.0%) | 0.979       | 0.032     | 5(2.0%)  | 0(0.0%) | 0.980       | 0.062     | 1(0.7%)  | 1(0.6%) | 1.095       | 0.949     |
|                | (0.190,23.417) | (1.190,23.417) | (0.959,1.000) | (0.959,1.000) | (0.963,0.998) | (0.963,0.998) | (0.968,17.657) | (0.968,17.657) | (0.973,1.003) | (0.973,1.003) | (0.977,17.657) | (0.977,17.657) | (0.982,17.657) | (0.982,17.657) |
| E2/E3          | 25       | 30      | 0.856       | 0.665     | 22       | 23      | 0.895       | 0.072     | 27       | 29      | 0.864       | 0.068     | 20       | 24      | 0.898       | 0.742     |
|                | (12.1%)  | (13.8%) | (11.5%)     | (12.6%)   | (11.5%)  | (12.6%) | (11.5%)     | (12.6%)   | (11.5%)  | (12.6%) | (11.5%)     | (12.6%)   | (11.5%)  | (12.6%) | (11.5%)     | (12.6%)   |
| E2/E4          | 3(1.4%)  | 6(2.8%) | 0.517       | 0.547     | 5(2.6%)  | 7(3.8%) | 0.668       | 0.496     | 5(2.0%)  | 6(2.5%) | 0.782       | 0.688     | 3(2.0%)  | 7(4.3%) | 0.458       | 0.412     |
|                | (0.128,2.096) | (0.128,2.096) | (0.208,2.145) | (0.208,2.145) | (0.236,2.598) | (0.236,2.598) | (0.261,1.004) | (0.261,1.004) | (0.273,1.004) | (0.273,1.004) | (0.276,1.004) | (0.276,1.004) | (0.279,1.004) | (0.279,1.004) |
| E3/E3          | 127      | 157     | 0.607       | 0.016     | 118      | 115     | 0.929       | 0.73      | 155      | 163     | 0.731       | 0.102     | 90       | 109     | 0.756       | 0.235     |
|                | (61.4%)  | (72.4%) | (561.5%)    | (63.2%)   | (611.5%) | (63.2%) | (611.5%)    | (63.2%)   | (611.5%) | (63.2%) | (611.5%)    | (63.2%)   | (611.5%) | (63.2%) | (611.5%)    | (63.2%)   |
| E3/E4          | 46       | 22      | 2.532       | 0.001     | 40       | 36      | 1.067       | 0.8       | 55       | 38      | 1.470       | 0.099     | 31       | 20      | 1.878       | 0.042     |
|                | (22.2%)  | (10.1%) | (1462,4.386) | (20.8%)   | (1462,4.386) | (20.8%) | (1462,4.386) | (20.8%)   | (1462,4.386) | (20.8%) | (1462,4.386) | (20.8%)   | (1462,4.386) | (20.8%) | (1462,4.386) | (20.8%)   |
| E4/E4          | 4(1.9%)  | 0(0.5%) | 4.256       | 0.341     | 3(1.6%)  | 1.05%   | 2.873       | 0.653     | 3(1.2%)  | 0(0.0%) | 0.988       | 0.249     | 4(1.9%)  | 2(1.2%) | 2.221       | 0.600     |
|                | (0.472,3.399) | (0.472,3.399) | (0.296,2.874) | (0.296,2.874) | (0.296,2.874) | (0.296,2.874) | (0.296,2.874) | (0.296,2.874) | (0.296,2.874) | (0.296,2.874) | (0.296,2.874) | (0.296,2.874) | (0.296,2.874) | (0.296,2.874) |
| Alleles, n (%) | E2       | 32(7.7%) | 38(8.8%)    | 0.873     | 0.587    | 35(9.1%) | 0(0.0%)     | 1.117     | 0.672    | 42       | 35(7.4%)   | 1.145     | 0.570    | 25       | 33       | 0.813      | 0.456     |
|                |          | (0.534,1.426) | (0.534,1.426) | (0.670,1.860) | (0.670,1.860) | (0.670,1.860) | (0.670,1.860) | (0.670,1.860) | (0.670,1.860) | (0.670,1.860) | (0.670,1.860) | (0.670,1.860) | (0.670,1.860) | (0.670,1.860) |
|                | E3       | 325      | 366        | 0.678     | 0.029    | 298      | 289        | 0.899     | 0.551    | 392      | 393       | 0.730     | 0.055    | 231      | 262      | 0.842      | 0.382     |
|                |          | (78.5%)  | (84.3%)    | (0.478,0.962) | (0.478,0.962) | (0.478,0.962) | (0.478,0.962) | (0.478,0.962) | (0.478,0.962) | (0.478,0.962) | (0.478,0.962) | (0.478,0.962) | (0.478,0.962) | (0.478,0.962) |
|                | E4       | 57       | 30(6.9%)   | 2.150     | 0.001    | 51       | 45         | 1.086     | 0.707    | 66       | 44(9.3%)  | 1.479     | 0.056    | 42       | 31(9.5%) | 1.561      | 0.075     |
|                |          | (13.8%)  | (13.3%)    | (13.3%)   | (13.3%)  | (13.3%)  | (13.3%)    | (13.3%)   | (13.3%)  | (13.3%)  | (13.3%)   | (13.3%)   | (13.3%)  | (13.3%)  | (13.3%)  | (13.3%)  | (13.3%) |

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

| Factors       | CAS Patient |         |         | CAS Patient |         |         | Control Participants |         |         |         |         |         |         |
|---------------|-------------|---------|---------|-------------|---------|---------|----------------------|---------|---------|---------|---------|---------|---------|
|               | ε2(ε2+ε3)  | ε3(ε3) | ε4(ε3+ε4+ε4) | P a  | P b  | P c  | ε2(ε2+ε3)  | ε3(ε3) | ε4(ε3+ε4+ε4) | 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          | 39.12±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±203.37 | 191.00±190.73 | 0.951 | 0.876 | 0.761 | 0.937 | 126.67±109.17 | 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 (ε2, ε3, ε4). bP values obtained when comparing ε2 subjects with ε3 subjects. cP values obtained when comparing ε4 subjects with ε3 subjects. dP values obtained when comparing ε2 subjects with ε4 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 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, 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. The timely detection and intervention of CAS are important for the prevention of stroke.
ApoE is a 34 kDa protein consisting of 299 amino acids, encoded by the ApoE gene located on chromosome 19 q13.2.13 Previous studies have demonstrated ApoE genotypes and allele frequencies vary between different races and ethnicities.14 The present study investigated that in Northwest China, ApoE ε3/ε3 genotype and ε3 allele were predominant in majority of populations. These results were consistent with previous findings. 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 ε4 allele towards an increased risk for the progression of CAS. Similar to our study, previous studies suggested that individuals with ε4/ε4 genotype had higher risk of carotid plaque, whereas carriers of ε2 had lower risk compared with those with ε3/ε3 genotype.16,17 However, in the Rotterdam study and Luc et al study demonstrated that the ε2/ε3 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.9,18 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 ε4 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.19 Makaruk et al also reported ε4 allele were more liable to develop CAS in patients under 50 years of age.20 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.21 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.22 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.23 However, in our study, we did not find the ε4 carriers are associated with higher TC, HDL, LDL-C and TG levels compared with ε2 and ε3 carriers, respectively, in all subjects and healthy controls. This were consistent with those published by Lee et al.24 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.16 The absence of ApoE in macrophage promotes atherosclerosis without changing plasma cholesterol.25 Raffai et al found that ApoE may promote the development of CAS through inflammatory response.26 Conversely, recent animal experiments studies showed that ApoE enhanced miR-146a levels in monocytes and macrophages, suppressing NF-kappaB-mediated inflammation and atherosclerosis.27 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.28 By contrast, ApoB is the main apolipoprotein of chylomicrons and LDL, and are associated with the development of atherosclerosis.7 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.29,30 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.30 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 ε2 carriers than in ε3 or ε4 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.29,30 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.31 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 ε2 carriers than in ε3 and ε4 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 e4 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, e4 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.

Acknowledgments
We gratefully acknowledge all sample donors who participated in this study.

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 work was supported by the Natural Science Basic Research Program of Shaanxi (grant number 2021-SF-130) and the Foundation of the First Affiliated Hospital of Xi’an Jiaotong University (grant number 2020ZYTS-08).

Disclosure
The authors declare that they have no competing interests.

References
1. Grbic E, Gorkic N, Pleskovic A, et al. Association between rs2107595 HDAC9 gene polymorphism and advanced carotid atherosclerosis in the Slovenian cohort. Lipids Health Dis. 2020;19(1):1. doi:10.1186/s12944-020-01255-1
2. Volkers EJ, Algra A, Kappelle LJ, et al. Safety of carotid revascularization in patients with a history of coronary heart disease. Stroke. 2019;50(2):413–418. doi:10.1161/STROKEAHA.118.023085
3. Wu H, Fang F, Wang C, Hong X, Chen D, Huang X. Selective molecular recognition of low density lipoprotein based on beta-cyclodextrin coated electrochemical biosensor. Biosensors-Basel. 2021;11:7.
4. Ben Hassen C, Gutierrez-Pajas JL, Guimaraes C, et al. Apolipoprotein-mediated regulation of lipid metabolism induces distinctive effects in different types of breast cancer cells. Breast Cancer Res. 2020;22(1):1. doi:10.1186/s13058-020-01276-9
5. Hou J, Deng Q, Guo X, Deng X, Zhong W, Zhong Z. Association between apolipoprotein E gene polymorphism and the risk of coronary artery disease in Hakka postmenopausal women in southern China. Lipids Health Dis. 2020;19(1):1. doi:10.1186/s12944-020-01323-6
6. Galvez AS, Ramirez H, Placencia P, et al. Single nucleotide polymorphisms in apolipoprotein b, apolipoprotein e, and methylenetetrahydrofolate reductase are associated with serum lipid levels in Northern Chilean subjects. A pilot study. Front Genet. 2021;12:640956. doi:10.3389/fgene.2021.640956

7. Ma W, Ren X, Zhang L, Dong H, Lu X, Feng W. Apolipoprotein E gene polymorphism and coronary artery disease risk among patients in Northwest China. Pharmacogenomics Pers Med. 2021;14:1591–1599. doi:10.2174/PGPM.S338285

8. Karjalainen J-P, Mononen N, Huttunen-Kahonen N, et al. New evidence from plasma ceramides links ApoE polymorphism to greater risk of coronary artery disease in Finnish adults. J Lipid Res. 2019;60(9):1622–1629. doi:10.1194/jlr.M092809

9. Slooter AJC, Bots ML, Havekes LM, et al. Association of ApoE E4 and carotid artery atherosclerosis - The Rotterdam study. Stroke. 2001;32(9):1947–1952. doi:10.1161/hs001.095377

10. Hanon O, Girerd X, Luong V, Jeunemaitre X, Laurent S, Safar ME. Association between the apolipoprotein E polymorphism and arterial wall thickness in asymptomatic adults. J Hypertens. 2000;18(4):431–436. doi:10.1097/00044872-200004040-00012

11. Karvonen J, Kauma H, Kervinen K, et al. Apolipoprotein E polymorphism affects carotid artery atherosclerosis in smoking hypertensive men. J Hypertens. 2002;20(12):2371–2378. doi:10.1097/00044872-200212000-00015

12. Zhao J, Huangfu C, Chang Z, et al. Inflammation and enhanced atherogenesis in the carotid artery with altered blood flow in an atherosclerosis-resistant mouse strain. Physiol Rep. 2021;9(11):e14829. doi:10.14814/phy2.14829

13. Piccarducci R, Daniele S, Polini B, et al. Apolipoprotein E polymorphism and oxidative stress in human peripheral blood cells: can physical activity reactivate the proaerogenesis system through epigenetic mechanisms? Oxid Med Cell Longev. 2021;2021:8869849. doi:10.1155/2021/8869849

14. Iacono D, Feltis GC. Impact of Apolipoprotein E gene polymorphism during normal and pathological conditions of the brain across the lifespan. Aging. Us. 2019;11:787–816.

15. Zhao L-L, Su G, Chen L-X, et al. Apolipoprotein E polymorphisms are associated with ischemic stroke susceptibility in a Northwest China Han population. Biosci Rep. 2017;37(6):BSR201711088. doi:10.1042/BSR20171088

16. Shin M-H, Choi J-S, Rhee J-A, et al. ApoE polymorphism and carotid atherosclerosis in Korean population: the Dong-gu Study and the Namwon Study. Atherosclerosis. 2014;232(1):180–185. doi:10.1016/j.atherosclerosis.2013.11.027

17. Davignon J, Gregg RE, Sing CF. Apolipoprotein-E Polymorphism and Atherosclerosis. Arteriosclerosis. 1998;8:1–21. doi:10.1161/01.ATV.8.1.1

18. Djousse L, Myers RH, Province MA, et al. Influence of apolipoprotein E, smoking, and alcohol intake on carotid atherosclerosis - National heart, lung, and blood institute family heart study. Stroke. 2002;33(5):1357–1361. doi:10.1161/01.STR.0000014325.54063.1A

19. Hou J, Wu H. The effects of ApoE gene polymorphisms on lumbar spine bone mineral density and carotid atherosclerosis. Clin Biochem. 2012;45(3):219–222. doi:10.1016/j.clinbiochem.2011.11.014

20. Lugowska A, Bydakova G, Ilyushkina A, et al. Elevated dipeptidyl peptidase iv (dpp-iv) activity in plasma from patients with various lysosomal diseases. Diagnostics. 2021;11(2):320. doi:10.3390/diagnostics11020320

21. Kazemi T, Hajjhosseini M, Moossavi M, Hemmati M, Ziaee M. Cardiovascular risk factors and atherogenic indices in an Iranian population: a cohort study. Clin Med Insights Cardiol. 2018;12:117954681875923. doi:10.1177/117954681875923

22. Goloba M, Raghuram R, Botros N, et al. Early life microcirculatory blood pressure changes in low birth weight infants born to normotensive mothers: a cohort study. Am J Hypertens. 2019;32(6):570–578. doi:10.1093/ajh/hpz034

23. Griffin BA, Walker CG, Jabb SA, et al. ApoE4 genotype exerts greater benefit in lowering plasma cholesterol and apolipoprotein B than Wild Type (E3/E3), after replacement of dietary saturated fats with low glycaemic index carbohydrates. Nutrients. 2018;10(10):1524. doi:10.3390/nu10101524

24. Lee J-H, Hong S-M, Shin Y-A. Effects of exercise training on stroke risk factors, homocysteine concentration, and cognitive function according the ApoE genotype in stroke patients. J Exerc Rehabil. 2018;14(2):267–274. doi:10.3390/jer.1836108.054

25. Brampton C, Pomozi V, Chen L-H, et al. ABCG6 deficiency promotes dyslipidemia and atherosclerosis. Sci Rep. 2021;11(1):3881. doi:10.1038/s41598-021-82966-y

26. Raffai RL, Leob SM, Weisgraber KH. Apolipoprotein E promotes the regression of atherosclerosis independently of lowering plasma cholesterol levels. Arterioscler Thromb Vasc Biol. 2005;25:436–441. doi:10.1161/01.ATV.0105.103811

27. Pereira-da-Silva T, Napoleao P, Costa MC, et al. Circulating miRNAs are associated with the systemic extent of atherosclerosis: novel observations for miR-27b and miR-146. Diagnostics. 2021;11(2):318. doi:10.3390/diagnostics11020318

28. Rivas-Urbina A, Rull A, Aldana-Ramos J, et al. Subcutaneous administration of apolipoprotein J-Derived Mimetic Peptide-D-113-122 apoJ improves LDL and HDL function and prevents atherosclerosis in LDLR-KO Mice. Biomolecules. 2020;10:829. doi:10.3390/biom10060829

29. Zivanovic Z, Divjak I, Jovicevic M, et al. Association between apolipoproteins AI and B and ultrasound indicators carotid atherosclerosis. Curr Vasc Pharmacol. 2018;16:376–384. doi:10.2174/1570161115666171010123157

30. Jun JE, Choi YJ, Lee Y-H, et al. ApoB/ApoA-I ratio is independently associated with carotid atherosclerosis in type 2 diabetes mellitus with well-controlled LDL cholesterol levels. Korean J Intern Med. 2018;33(1):138. doi:10.3904/kjim.2017.396

31. Lozano S, Padilla V, Avila ML, et al. ApoE gene associated with cholesterol-related traits in the Hispanic population. Genes. 2021;12(11):1768. doi:10.3390/genes12111768