The SNPs rs429358 and rs7412 of APOE gene are Association With Dyslipidemia but Not Cerebral Infarction in Northern Chinese Han population

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Research Article

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Abstract

Background

To analyze the relationship between apolipoprotein E(APOE) (including the SNPs rs429358 and rs7412) gene and cerebral infarction(CI) in Northern Chinese Han population.

Methods

457 CI patients and 416 controls (without CI) in Chinese Han population were recruited for our study to test the SNPs by polymerase chain reaction (PCR)-fluorescence probe technique.

Results

The distribution of APOE genotypes were in Hardy-Weinberg equilibrium, and the frequencies of $\epsilon 4$ allele or $\epsilon 4$ containing were significant difference between CI and control groups. Compared to $\epsilon 2$ or $\epsilon 3$ carriers, $\epsilon 4$ carriers had a high level of total cholesterol (TC) and low-density lipoprotein cholesterol(LDL-C) and high presence of dyslipidemia in recruited peoples. And we found APOE $\epsilon 4$ allele was associated with an increased risk of dyslipidemia in the control and CI groups (OR = 2.232, 95% CI= 1.143-4.361, $P < 0.000$; OR = 3.442, 95% CI= 1.885-6.284, $P = 0.019$). However, further logistic regression showed that $\epsilon 4$ allele or carriers were not the independent risk factors of CI (OR = 2.232, 95% CI= 1.143-4.361, $P = 0.144$; OR = 3.442, 95% CI= 1.885-6.284, $P = 0.162$).

Conclusion

APOE $\epsilon 4$ allele was associated with an increased risk of dyslipidemia (OR = 2.232, 95% CI= 1.143-4.361; OR = 3.442, 95% CI= 1.885-6.284), but $\epsilon 4$ allele or carriers were not the independent risk factors of CI in Northern Chinese Han population (OR = 2.232, 95% CI= 1.143-4.361, $P = 0.144$; OR = 3.442, 95% CI= 1.885-6.284, $P = 0.162$).

1. Introduction

Stroke is still one of the main causes of morbidity and mortality in China and around the world^{1,2}. The incidence of stroke presents a gradient from North to south, among incident and prevalent strokes, CI accounts for 69.6% and 77.8% in China¹¹. CI is a complex disease affected by genetic and other factors, which not only has high morbidity and mortality, but also has a high risk of recurrence and disability³. Studies showed that APOE polymorphisms are related to the occurrence and prognosis of CI by affecting the occurrence of atherosclerosis and cerebral infarction by regulating the level of blood lipids^{4,17}, but the conclusion are inconsistent.

Apolipoprotein E is a glycoprotein encoded by APOE gene located on chromosome 19, which synthesized principally in the liver. It plays an important role in the transport of cholesterol, and is also involved in the occurrence and prognosis of many diseases^{5,6}. The APOE has two common, nonsynonymous polymorphism, 526C > T (rs7412) and 388T > C (rs429358), which produce three alleles or isoforms, including $\epsilon 2$ (388T-526T)/ $\epsilon 3$ (388T-526C)/ $\epsilon 4$ (388C-526C) that give rise to 6 possible genotypes ($\epsilon 3/\epsilon 4$, $\epsilon 3/\epsilon 3$, $\epsilon 4/\epsilon 4$, $\epsilon 4/\epsilon 3$, $\epsilon 2/\epsilon 3$, $\epsilon 3/\epsilon 2$)⁶. APOE polymorphisms may affect the incidence of atherosclerosis and CI by the influence on serum lipid, so as to increase the risks of CI^{7,8}. Meanwhile, compared with the Caucasian population, $\epsilon 4$ carrier in Asian population has a higher risk of CI⁹. And in China, there are some regions have studied the association between APOE and CI (including the risk factors), and the conclusions are inconsistent due to different region and population. What's more, there are few studies focused on the Northern in Chinese Han population.

Therefore, it is necessary to explore the relationship between APOE polymorphisms and CI in Northern Chinese Han population, so as to provide a theoretical grounding for the prevention of CI.

2. Materials And Methods

2.1 Study Design and Participants

To investigate the relationship of APOE polymorphisms and CI, we recruited 416 CI patients and 457 controls which visited the Department of Neurology in the First Hospital of Hebei Medical University between July 2019 to December 2019. Inclusion criteria for CI: (1) Age ≥ 45 years old, Han population; (2) The diagnosis of CI refers to the 2018 Chinese Guidelines of CI, the CT or MRI examination of the brain confirms that there are ischemic lesions in the brain. Patients were excluded if they were also diagnosed with Alzheimer's Disease(AD), cardiovascular-heart diseases(CHD) or they were taken lipid-lowering drugs within one month (through confirming the patient's case record), and have serious infection, tumor, severe liver or kidney dysfunction, and other disease that effect lipid. This study was approved by the Ethics Committee of the first Hospital of Hebei Medical University [approval number: 20190418]. The patients and their families knew about the research project and signed the informed consent form.

2.2 APOE Genotype Testing

Genomic DNA was extracted from blood which was taken from the antecubital vein, using the DNA Blood Midi Kit (Xiamen amply Co., Ltd., Xiamen, China). SNP genotype was performed using Human SLC01B1 and APOE Gene Detection Kit (Youzhiyou Medical Technology Co., Ltd. Wuhan, China). The PCR reaction conditions were as follows: UNG(Uracil-N-Glycosylase) treatment: circulating at 37 °C for 10 min; Predenaturation stage: circulating once at 95 °C for 5min; In the PCR stage, the cycle was repeated for 40 times at 95°C 15s and 60°C 45s (the fluorescence signal was set to be collected at the end of this stage). We excluded the $\epsilon 2/\epsilon 4$ genotype on the count of $\epsilon 2$ and $\epsilon 4$ have opposite effects on serum lipid(2).

2.3 Plasma lipid measurements

After fasting overnight for 12 hours, blood samples were obtained by venipuncture. The Beckman LX20 automatic blood biochemical analyzer was used to detect triacylglycerol(TG), TC, high-density lipoprotein cholesterol (HDL-C) and LDL-C. Dyslipidemia was defined as a level of TG ≥ 1.70 mmol/L, hypercholesterolemia TC ≥ 5.72 mmol/L or HDL ≤ 0.90 mmol/L or LDL ≥ 3.64 mmol/L; Hyperhomocysteinemia was defined as a level of ≥ 20 mmol/L¹¹.

2.4 Statistical analysis

The χ^2 test is used to assess the equilibrium of Hardy-Weinberg; General information were compared with ANOVA or Wilcoxon rank sum test; Associations between APOE polymorphisms and the CI were determined by univariable and multivariable logistic regression to generate Odds ratios. Statistical analysis was performed using SPSS version 22.0. $P < 0.05$ was considered to demonstrate statistically significant differences.

3 Results

3.1 APOE genotypic and allelic frequencies in recruited subjects

The distribution of APOE genotypes and alleles in recruited subjects are given in **Table 1**. The genotypic distribution of APOE gene were in Hardy-Weinberg equilibrium. Among the six genotypes of APOE gene in Northern Chinese Han population, $\epsilon 3/\epsilon 3$ accounted for 69.68%, followed by $\epsilon 2/\epsilon 3$ (13.69%), $\epsilon 3/\epsilon 4$ (13.8%), $\epsilon 2/\epsilon 4$ (1.24%), $\epsilon 2/\epsilon 2$ (0.79%) and $\epsilon 4/\epsilon 4$ (0.79%). The frequency of $\epsilon 4$ allele was significantly higher in CI patients compared with the controls.

3.2 The baseline characteristics of the study population

The clinical characteristics of the CI and control groups were shown in **Table 2**. There were no significant difference in age, BMI, UA, LP(a) and the presence of diabetes, family history of stroke between the two groups. However, in CI group the level of LDL-C and proportion of hypertension, dyslipidemia, drinking history, smoking history and hyperhomocysteinemia was significantly higher than that in the control group ($P < 0.05$).

3.3 The relationship of APOE polymorphisms and CI

Logistic regression analysis was performed to determine independent risk factors for CI, and results indicated the high level of TC and high presence of hypertension and hyperhomocysteinemia are the risk factors of CI, but the $\epsilon 4$ allele or $\epsilon 4$ containing were not the independent risk factors in model 1, 2 (**Table 3**) (OR = 2.232, 95% CI = 1.143-4.361, $P = 0.144$; OR = 3.442, 95% CI = 1.885-6.284, $P = 0.162$).

3.4 The relationship between APOE polymorphisms and CI's risk factors

To analyze the relationship between APOE polymorphisms and CI's risk factors, we divided CI and control groups into three groups: E2 group ($\epsilon 3/\epsilon 4 + \epsilon 4/\epsilon 4$), E3 group ($\epsilon 3/\epsilon 3$), E4 group ($\epsilon 3/\epsilon 4 + \epsilon 4/\epsilon 4$) according to the APOE alleles. We excluded the $\epsilon 2/\epsilon 4$ genotype because the opposite effect in lipid metabolism ($n = 11$). And we found that the level of TC and LDL-C and the presence of dyslipidemia were significantly different among the three groups. Compared to $\epsilon 2$ or $\epsilon 3$ carrier, $\epsilon 4$ carrier had a high level of TC and LDL-C and high incidence of dyslipidemia (**Table 4**). After further multivariate analysis brought APOE alleles into the equation adjusting for age, BMI, gender, Type II diabetes, hypertension, drinking history, smoking history to explore the correlation between APOE alleles and dyslipidemia. We found that APOE $\epsilon 4$ allele was associated with an increased risk of dyslipidemia in the control and CI groups (OR = 2.232, 95% CI = 1.143-4.361; OR = 3.442, 95% CI = 1.885-6.284) (Table 5).

Discussion

In China, the morbidity and mortality of stroke have a gradient from North to south, and the burden of stroke is heaviest in the Northern and central regions due to the difference of diet and lifestyle. CI is most common type of stroke, which is a group of syndromes causing local cerebral ischemia, hypoxic necrosis, and a loss of corresponding neurological functions. As one of genetic factors of CI, APOE is not only related to CI, but also have a close connection with CI's risk factors, such as hypertension¹⁴, diabetes¹⁵, hyperlipidemia^{16, 17} and atherosclerosis¹⁸.

Studies have shown that there is a significant association between CI and $\epsilon 4$ allele^{19, 20}, and it has been found that the frequency of $\epsilon 4$ allele in Asian with CI is higher than that in Caucasian population²⁰, and $\epsilon 4$ allele carrier is more likely to develop CI in Asian population²¹. In Chinese population, many studies indicated that $\epsilon 4$ allele were the risk for the presence of CI²²⁻²⁴. In our study we found that the frequency of $\epsilon 4$ allele in CI group was significantly higher than that in control group ($P < 0.01$) in North China, which was similar to the results of previous studies on Chinese population, suggesting that the frequency of $\epsilon 4$ allele is more higher in CI patients^{8, 23, 24}. However, after further study we didn't found $\epsilon 4$ allele was an independent risk factor for CI in North China, which was consistent with the results of Guangdong (China)²⁵, Guangxi (Han population of China)³ and Belgians²⁶, but different from those in Northwest China (Han nationality)²⁷, Guangdong (Hakka population)²³, South Indian²⁸ and Koreans²⁹ which indicated that APOE $\epsilon 4$ was associated with susceptibility to CI. In our opinion, there are may be two reasons for the difference; First, there are regional and ethnic differences in the distribution of APOE polymorphisms, that cause the opposite results; Second, in our study we excluding patients with coronary heart disease³⁰, dementia³¹, cardiovascular disease and neurological disorders³² that closely related to APOE polymorphisms, which may affect the association between APOE and CI.

Although we did not find the direct relationship between APOE polymorphisms and CI, we found that the levels of TC, LDL-C and presence of hyperlipidemia in E4 group were significantly higher compared with $\epsilon 3/\epsilon 3$ genotype, which were familiar with previous studies^{17, 33}. And after further study we found APOE $\epsilon 4$ was associated with an increased risk of dyslipidemia in the control and CI groups. Therefore, we speculate that APOE polymorphisms may affect the incidence of CI by influencing the level of lipid, but not a independent risk factors. Therefore, for those patients who carry the $\epsilon 4$ allele, because of its high

incidence of dyslipidemia and weaken fat metabolism, they should pay close attention to the level of lipid level. If the cholesterol level rises, statin should be actively taken, so as to achieve the goal of reduction the lipid levels. What's more, patients are encouraged to control cholesterol intake, eat less and eat more, and avoid too much fish and meat, advocates a good lifestyle, smoking cessation and alcohol restriction, and proper activities.

There are some advantages and limitations in our study. This is the first study focus on the relationship between APOE polymorphisms and CI(including the risk factors) in North China Han population. What's more, we exclude the patients with CHD, AD that have a strong relationship with APOE polymorphisms, which make the result more reliable. There are also some limitations. One hand, the sample is not very large, which may affect the results; on the other hand, CI is also closely associated with lifestyle, diet, activity et al, but we didn't consider it. Further studies are required to consider as many influence factors as possible to determine the relationship of APOE polymorphisms and CI.

Conclusions

APOE ϵ 4 allele was associated with an increased risk of dyslipidemia, but ϵ 4 allele or carriers were not the independent risk factors of CI in Northern Chinese Han population. That indicated us that APOE polymorphisms may affect the incidence of CI by influencing the level of lipid, but not a independent risk factors. APOE gene detection are also suggested as part of prevention of dyslipidemia, thus reducing the risk of related diseases.

Abbreviations

APOE: Apolipoprotein E; CI: cerebral infarction; PCR: polymerase chain reaction; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; AD: Alzheimer's Disease; CHD: cardiovascular-heart diseases; TG: triacylglycerol; HDL-C: high-density lipoprotein cholesterol.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the principles of the Declaration of Helsinki and Institutional Review Board of Hebei Medical University First Affiliated Hospital [approval number: 20190418]. Informed contents were obtained from all enrolled patients.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

XW Ma was the main coordinator of the project and was responsible for the study design. DY Gou and SHe drafted the manuscript of the present paper. MW Yuan and J Yang were involved in the supervising of data collection. ZQLi performed the statistical analyses. XW Ma and YY Wang contributed with manuscript revision. All authors contributed intellectually to this manuscript and approved this final version.

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References

1. Wang W, Jiang B, Sun H, Ru X, Sun D, Wang L, Wang L, Jiang Y, Li Y, Wang Y, Chen Z, Wu S, Zhang Y, Wang D, Wang Y, Feigin VL. Prevalence, Incidence, and Mortality of Stroke in China: Results from a Nationwide Population-Based Survey of 480 687 Adults. *Circulation*. 2017;135:759-771

2. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet*. 2017;390:1151-1210
3. Gu L, Su L, Chen Q, Liang B, Qin Y, Xie J, Wu G, Yan Y, Long J, Wu H, Tan J, Dou W, Chen W, Wu P, Wang J. Association between the apolipoprotein E gene polymorphism and ischemic stroke in Chinese populations: New data and meta-analysis. *Exp Ther Med*. 2013;5:853-859
4. Yan H, Yuan Y, Zhang P, Huang Z, Chang L, Gui Y. Association of the ApoE gene polymorphism and dietary factors with cerebral infarction and circulating lipid concentrations. 2015;14:665-670
5. Utermann G. Apolipoprotein E polymorphism in health and disease. *Am Heart J*. 1987;113:433-440
6. Utermann G, Hees M, Steinmetz A. Polymorphism of apolipoprotein E and occurrence of dysbetalipoproteinaemia in man. *Nature*. 1977;269:604-607
7. Satizabal CL, Samieri C, Davis-Plourde KL, Voetsch B, Aparicio HJ, Pase MP, Romero JR, Helmer C, Vasani RS, Kase CS, Debette S, Beiser AS, Seshadri S. APOE and the association of fatty acids with the risk of stroke, coronary heart disease, and mortality. *Stroke*. 2018;49:2822-2829
8. Yan HQ, Yuan Y, Zhang P, Huang Z, Chang L, Gui YK. Association of the ApoE gene polymorphism and dietary factors with cerebral infarction and circulating lipid concentrations. *Genet Mol Res*. 2015;14:665-670
9. Wei LK, Au A, Menon S, Griffiths LR, Kooi CW, Irene L, Zhao J, Lee C, Alekseevna AM, Hassan MRA, Aziz ZA. Polymorphisms of MTHFR, eNOS, ACE, AGT, ApoE, PON1, PDE4D, and ischemic stroke: Meta-Analysis. 2017;26:2482-2493
10. [2016 Chinese guideline for the management of dyslipidemia in adults]. *Zhonghua Xin Xue Guan Bing Za Zhi*. 2016;44:833-853
11. Yi X, Han Z, Zhou Q, Lin J, Liu P. 20-Hydroxyeicosatetraenoic acid as a predictor of neurological deterioration in acute minor ischemic stroke. 2016;47:3045-3047
12. Kukumbeg M, Zaw AM, Wong DHC, Toh CM, Chan BPL, Seet RCS, Wong PTH, Yim EKF. Characterization and functional assessment of endothelial progenitor cells in ischemic stroke patients. 2021;17:952-967
13. Shi J, Liu Y, Liu Y, Li Y, Qiu S, Bai Y, Gu Y, Luo J, Cui H, Li Y, Zhao Q, Zhang K, Cheng Y. Association between ApoE polymorphism and hypertension: A meta-analysis of 28 studies including 5898 cases and 7518 controls. *Gene*. 2018;675:197-207
14. Liu S, Liu J, Weng R, Gu X, Zhong Z. Apolipoprotein E gene polymorphism and the risk of cardiovascular disease and type 2 diabetes. *BMC Cardiovasc Disord*. 2019;19:213
15. Doliner B, Dong C, Blanton SH, Gardener H, Elkind M, Sacco RL, Demmer RT, Desvarieux M, Rundek T. Apolipoprotein e gene polymorphism and subclinical carotid atherosclerosis: The northern manhattan study. *J Stroke Cerebrovasc Dis*. 2018;27:645-652
16. Martinez-Magana JJ, Genis-Mendoza AD, Tovilla-Zarate CA, Gonzalez-Castro TB, Juarez-Rojop IE, Hernandez-Diaz Y, Martinez-Hernandez AG, Garcia-Ortiz H, Orozco L, Lopez-Narvaez ML, Nicolini H. Association between APOE polymorphisms and lipid profile in Mexican Amerindian population. *Mol Genet Genomic Med*. 2019;7:e958
17. Zhu H, Xue H, Wang H, Ma Y, Liu J, Chen Y. The association of apolipoprotein E (APOE) gene polymorphisms with atherosclerosis susceptibility: A meta-analysis. *Minerva Cardioangiol*. 2016;64:47-54
18. Wang C, Yan W, Wang H, Zhu J, Chen H. APOE polymorphism is associated with blood lipid and serum uric acid metabolism in hypertension or coronary heart disease in a Chinese population. 2019;20:1021-1031
19. Kumar A, Kumar P, Prasad M, Misra S, Kishor PA, Chakravarty K. Association between Apolipoprotein epsilon4 Gene Polymorphism and Risk of Ischemic Stroke: A Meta-Analysis. *Ann Neurosci*. 2016;23:113-121
20. Kumar A, Kumar P, Prasad M, Misra S, Kishor PA, Chakravarty K. Association between Apolipoprotein epsilon4 Gene Polymorphism and Risk of Ischemic Stroke: A Meta-Analysis. *Ann Neurosci*. 2016;23:113-121
21. Wang B, Zhao H, Zhou L, Dai X, Wang D, Cao J, Niu W. Association of genetic variation in apolipoprotein E and low density lipoprotein receptor with ischemic stroke in Northern Han Chinese. 2009;276:118-122
22. Wu H, Huang Q, Yu Z, Wu H, Zhong Z. The SNPs rs429358 and rs7412 of APOE gene are association with cerebral infarction but not SNPs rs2306283 and rs4149056 of SLC01B1 gene in southern Chinese Hakka population. *Lipids Health Dis*. 2020;19:202
23. Chen C, Hu Z. ApoE polymorphisms and the risk of different subtypes of stroke in the chinese population: A comprehensive Meta-Analysis. *Cerebrovasc Dis*. 2016;41:119-138
24. Zhong Z, Wu H, Ye M, Yang Y, Luo W, Wu Y, Wu H, Zhong M, Zhao P. Association of APOE gene polymorphisms with cerebral infarction in the chinese population. 2018;24:1171-1177

25. Abboud S, Viiri LE, Lütjohann D, Goebeler S, Luoto T, Friedrichs S, Desfontaines P, Gazagnes M, Laloux P, Peeters A, Seelldrayers P, Lehtimäki T, Karhunen P, Pandolfo M, Laaksonen R. Associations of apolipoprotein E gene with ischemic stroke and intracranial atherosclerosis. 2008;16:955-960
26. Zhao L, Su G, Chen L, Yan Q, Wang X, Yuan W, Wang L, Zhang Z. Apolipoprotein E polymorphisms are associated with ischemic stroke susceptibility in a Northwest China Han population. 2017;37
27. Das S, Kaul S, Jyothy A, Munshi A. Association of APOE (E2, E3 and E4) gene variants and lipid levels in ischemic stroke, its subtypes and hemorrhagic stroke in a South Indian population. *Neurosci Lett*. 2016;628:136-141
28. Um JY, Kim HM, Park HS, Joo JC, Kim KY, Kim YK, Hong SH. Candidate genes of cerebral infarction and traditional classification in Koreans with cerebral infarction. *Int J Neurosci*. 2005;115:743-756
29. Graner M, Kahri J, Varpula M, Salonen RM, Nyyssönen K, Jauhiainen M, Nieminen MS, Syvanne M, Taskinen MR. Apolipoprotein E polymorphism is associated with both carotid and coronary atherosclerosis in patients with coronary artery disease. *Nutr Metab Cardiovasc Dis*. 2008;18:271-277
30. 2021 Alzheimer's disease facts and figures. *Alzheimers Dement*. 2021;17:327-406
31. Ribalta J, Vallve JC, Girona J, Masana L. Apolipoprotein and apolipoprotein receptor genes, blood lipids and disease. *Curr Opin Clin Nutr Metab Care*. 2003;6:177-187
32. de Knijff P, van den Maagdenberg AM, Frants RR, Havekes LM. Genetic heterogeneity of apolipoprotein E and its influence on plasma lipid and lipoprotein levels. *Hum Mutat*. 1994;4:178-194

Tables

Table.1 Distribution of APOE alleles and genotypes n,%

Alleles/Genotypes	IS Cases (N=464)	Controls (N=420)	All(N=884)	P
Alleles				
ε2	74(8.7)	72(8.57)	147(8.35)	0.694
ε3	762(83.35)	713(84.88)	1474(83.75)	0.118
ε4	92(7.95)	55(6.55)	147(8.35)	0.010*
Genotypes				
ε2/ε2	2(0.4)	5(1.19)	7(0.79)	0.203
ε2/ε3	63(15.1)	58(13.81)	121(13.69)	0.92
ε2/ε4	7(1.5)	4(0.95)	11(1.24)	0.456
ε3/ε3	313(69)	303(72.14)	616(69.68)	0.13
ε3/ε4	73(15.73)	49(11.67)	122(13.80)	0.08
ε4/ε4	6(1.29)	1(0.24)	7(0.79)	0.127
ε4 containing	86(18.53)	54(12.86)	129(15.84)	0.021*

*P<0.05

Table.2 Clinical characteristics of CI patients and control participants

characteristics	Controls (N=416)	CI (N=457)	P
Age(̄years)	59.94±3.63	60.68±3.57	0.303
BMI(̄Kg/m2)	25.46±10.76	25.1±10.49	0.132
Male(n,%)	202(48.6%)	294(64.3%)	0.000*
Hypertension(n,%)	222(53.4%)	307(67.2%)	0.000*
Diabetes(n,%)	74(17.8%)	101(22.1%)	0.112
Hyperhomocysteinemia	37(8.9%)	121(26.5%)	0.000*
Drinking history(n,%)	71(17.1%)	162(35.4%)	0.000*
Smoking history(n,%)	67(16.1%)	157(34.4%)	0.000*
Stroke history	30(7.2%)	35(7.7%)	0.802
TG(mmol/L)	1.47±0.81	1.58±0.95	0.074
TC(mmol/L)	4.76±1.15	4.86±1.1	0.153
HDL(mmol/L)	1.17±0.28	1.07±0.26	0.000*
LDL(mmol/L)	2.94±0.88	3.06±0.80	0.037*
UA(̄mol/L)	322.17±88.86	323.74±85.04	0.799
Lp(a)(mg/L)	252.02±299.13	240.56±236.47	0.548

BMI=body mass index. Values expressed as median (range). * $P<0.05$

Table.3 Multivariable logistic regression analysis for risk factors of ischemic stroke

Characteristics	Model 1		Model 2	
	P value	OR (95%CI)	P value	OR (95%CI)
Age	0.105	1.012 (0.997–1.027)	0.101	1.101 (0.998–1.027)
Gender	0.878	0.972 (0.68–1.39)	0.865	0.97 (0.678–1.386)
BMI	0.259	0.805 (0.553–1.173)	0.260	0.805 (0.552–1.174)
TG	0.265	0.888 (0.722–1.094)	0.258	0.887 (0.72–1.092)
TC	0.029	1.617 (1.051–2.488)	0.028	1.62 (1.053–2.493)
HDL	0.000	0.189 (0.087–0.411)	0.000	0.187 (0.086–0.407)
LDL	0.361	0.784 (0.465–1.321)	0.380	0.791 (0.469–1.334)
Hypertension(n,%)	0.000	1.745 (1.291–2.358)	0.000	1.741 (1.288–2.354)
Diabetes(n,%)	0.325	1.206 (0.830–1.751)	0.316	1.211 (0.833–1.758)
Hyperhomocysteinemia(%)	0.000	3.023 (1.973–4.634)	0.000	3.004 (1.959–4.606)
dyslipidemia(n,%)	0.267	0.817 (0.571–1.168)	0.255	0.812 (0.567–1.163)
Stroke history(n,%)	0.519	0.832 (0.475–1.456)	0.498	0.824 (0.47–1.443)
Smoking history(n,%)	0.160	1.433 (0.868–2.367)	0.162	1.413 (0.866–2.363)
Drinking history(n,%)	0.026	1.767 (1.071–2.917)	0.025	1.774 (1.075–2.929)
ε4-containing	0.162	1.349(0.887-2.051)		
APOE allele				
ε3			1.000	
ε2			0.583	1.125 (0.739–1.715)
ε4			0.144	1.371 (0.898–2.049)

Model 1 includes ε4-containing gene; **Model 2** includes three different *APOE* alleles.

* $P<0.05$

Table.4 Clinical characteristics of CI and control groups

characteristics	CI group				control group			
	E2	E3	E4	<i>P</i>	E2	E3	E4	<i>P</i>
Age(̳years)	61(10.37)	59.42(10.87)	60.43(10.69)	0.53	59.65±10.15	60.13±10.85	59.12±11.13	0.808
Male(n,%)	204(65.2%)	42(64.6%)	48(60.8%)	0.76	31(49.2%)	145(47.9%)	26(52%)	0.857
BMI(Kg/m2)	24.96(3.59)	25.74(3.4)	25.09(3.59)	0.28	24.53±2.93	25.6±3.62	25.79±4.31	0.083
TG(mmol/L)	1.53(0.86)	1.57(0.63)	1.79(1.41)	0.10	1.36±0.66	1.43±0.79	1.88±1	0.001*
TC(mmol/L)	4.77(0.99)	4.54(1.01)	5.5(1.34)	0.000*	4.14±0.82	4.83±1.17	5.08±1.06	0.000*
HDL(mmol/L)	1.07(0.25)	1.08(0.3)	1.08(0.25)	0.97	1.2±0.27	1.17±0.28	1.12±0.32	0.268
LDL(mmol/L)	2.99(0.76)	2.81(0.77)	3.57(0.8)	0.000*	2.36±0.72	3.02±0.88	3.21±0.72	0.000*
UA(̳mol/L)	334.34±105.11	317.56±83.73	335.03±95.85	0.335	334.34±105.11	317.56±83.73	335.034±95.85	0.335
Lp(a)(mg/L)	208.89±322.18	255.67±274.75	289.23±403.41	0.882	208.89±322.18	255.67±274.75	289.23±403.41	0.882
Hypertension(n,%)	45(69.2%)	210(67.1%)	52(65.8%)	0.909	36(57.1%)	155(51.2%)	31(62%)	0.293
Diabetes(n,%)	13(20%)	72(23%)	16(20.3%)	0.709	10(15.9%)	51(16.8%)	13(26%)	0.265
Hyperhomocysteinemia	21(32.3%)	81(25.9%)	19(24.1%)	0.489	9(14.3%)	7(23.1%)	9(18%)	0.526
dyslipidemia(n,%)	40(61.5%)	170(54.3%)	63(79.7%)	0.000*	24(38.1%)	160(52.8%)	36(72%)	0.002*
Stroke history	10(15.4%)	16(5.1%)	9(11.4%)	0.007*	4(6.3%)	22(7.3%)	4(8%)	0.943
Drinking history(n,%)	20(30.8%)	112(35.8%)	30(38.0%)	0.651	10(15.9%)	51(16.8%)	10(20%)	0.827
Smoking history(n,%)	20(30.8%)	110(35.1%)	27(34.2%)	0.000*	11(17.5%)	50(16.5%)	6(12%)	0.689

Table.5 Logistic regression analysis of risks of dyslipidemia in CI and control groups

Characteristics	CI group		controls	
	P value	OR (95%CI)	P value	OR (95%CI)
BMI	0.021	1.072(1.01-1.137)	-	-
Age	0.016	0.976(0.957-0.996)	-	-
Diabetes	0.024	1.754(1.078-2.855)	0.001	2.687(1.534-4.705)
Smoking history	-	-	0.017	1.975(1.131-3.448)
APOE allele	-	-		
̵3	1		1	
̵2	0.41	1.265(0.723-2.215)	0.032	0.536(0.303-0.948)
̵4	0.000	3.442(1.885-6.284)	0.019	2.232(1.143-4.361)