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The association between apolipoprotein A1-C3-A5 gene cluster promoter polymorphisms and risk of ischemic stroke in the northern Chinese Han population

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Abstract

Objective: Given its effects on lipid metabolism, the apolipoprotein A1-C3-A5 (*APOA1-C3-A5*) gene cluster is thought to play an important role in ischemic stroke pathogenesis. Here, we evaluated whether the *APOA1-C3-A5* cluster is associated with ischemic stroke in the northern Chinese Han population.

Methods: This case–control study analyzed 812 patients with ischemic stroke and 844 healthy controls with regard to four *APOA1-C3-A5* cluster promoter single nucleotide polymorphisms (SNPs), rs670, rs2854116, rs2854117, and rs662799, using the SNaPshot Multiplex sequencing assay. Potential associations among ischemic stroke, genotyping, and allele frequencies were assessed.

Results: *APOA1* rs670 CT/TT genotypes, *APOA5* rs662799 AG/GG genotypes, and the *APOC3* rs2854116 CC genotype were associated with an increased risk of ischemic stroke according to multivariate logistic analysis after adjusting for confounding factors. A significantly increased risk for ischemic stroke was also identified among high-risk haplotypes (C-C-T-A and T-T-C-A) for rs670–rs2854116–rs2854117–rs662799.

Conclusion: This study showed that rs670, rs2854116, and rs662799 SNPs of the *APOA1-C3-A5* cluster are associated with ischemic stroke in the northern Chinese Han population.

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Introduction

Stroke is a leading cause of death worldwide.[1] Ischemic stroke, which is the most common form, occurs as a result of local cerebral ischemia caused by an obstruction within a brain-supplying blood vessel. Ischemic stroke is a complex disease that involves many risk factors, including genetic variants and environmental factors. The search for the genetic factors that increase ischemic stroke risk may facilitate the development of novel and effective approaches to identify high-risk groups. However, we have identified only a few single nucleotide polymorphisms (SNPs), such as rs5128 in Apolipoprotein C3 (*APOC3*),[2] rs266729 in adiponectin (*ADIPOQ*),[3] and rs2108622 in cytochrome P450 family 4 subfamily F member 2 (*CYP4F2*),[4] that are associated with susceptibility to ischemic stroke in large-scale genome-wide association studies.[5–7]

Dyslipidemia often occurs in patients with ischemic stroke. Lipids and lipoproteins have been traditionally associated with atherosclerosis in carotid arteries, which is the leading cause of ischemic stroke. Atherosclerosis often begins with an endothelial injury that makes the endothelium susceptible to the accumulation of lipids and the deposition of thrombus. The apolipoprotein A1-C3-A5 (*APOA1-C3-A5*) gene cluster on chromosome 11q23 (Apo11q) is among the most well-characterized regions of the human genome because of its dynamic association with plasma lipids and lipoproteins. Moreover, its associated haplotypes are highly informative genetic markers in diseases related to hyperlipidemia. Four promoter SNPs, rs670, rs2854116, rs2854117, and rs662799 in the *APOA1-C3-A5* cluster are strongly associated with dyslipidemia through their influence of triglyceride (TG) and very low-density lipoprotein levels.[8–10] Because dyslipidemia is an important risk factor for ischemic stroke, the genes involved in dyslipidemia might be candidates for assessing ischemic stroke susceptibility. Although individual genetic variants within the Apo11q gene cluster have been independently associated with ischemic stroke in some studies,[11–13] researchers drew different conclusions and no studies have been able to establish a direct association between haplotypes and the risk of ischemic stroke. Owing to the functional and positional relevance, it is necessary to examine the entire *APOA1-C3-A5* cluster as a whole.

We performed a hospital-based case–control study of 812 patients with ischemic stroke and 844 control participants. We analyzed the relationship between ischemic stroke and four SNPs (rs670, rs2854116, rs2854117, and rs662799) in the promoter of the *APOA1-C3-A5* cluster in the northern Chinese Han population.

Discussion

The present study evaluated the association between ischemic stroke risk and four functional polymorphisms within the promoter region of the *APOA1-C3-A5* cluster among a northern Chinese Han sample of 812 ischemic stroke cases and 844 controls. Our findings suggest that the rs670 CT/TT genotypes in *APOA1*, the rs662799 AG/GG genotypes in *APOA5*, and the rs2854116 CC genotype in *APOC3* are associated with an increased risk of ischemic stroke. Furthermore, we identified a significantly

Table 1. Characteristics and risk factors for stroke.

Variable	Cases n (%)	Controls n (%)	P-value
Age (years)	64.34 ± 8.44	63.70 ± 6.68	0.086
Age (≤60/>60 years)	292 (36.0)/520 (64.0)	276 (32.7)/568 (67.3)	0.163
Gender (male/female)	447 (55.0)/365 (45.0)	448 (53.1)/396 (46.9)	0.422
BMI (≤22.9/>22.9)	414 (51.0)/398 (49.0)	548 (64.9)/296 (35.1)	<0.001
Diabetes mellitus	218 (26.8)	62 (7.3)	<0.001
Hypertension	494 (60.8)	170 (20.1)	<0.001
History of smoking	282 (34.7)	130 (15.4)	<0.001
History of alcohol use	146 (18.0)	94 (11.1)	0.005
TG (≤1.7/>1.7)	520 (64.0)/292 (36.0)	574 (68.0)/270 (32.0)	0.088
TC (≤5.72/>5.72)	624 (76.8)/188 (23.3)	721 (86.8)/112 (13.3)	<0.001
LDL (≤3.64/>3.64)	658 (81.0)/154 (19.0)	746 (88.4)/98 (11.6)	<0.001

Abbreviations: BMI, body mass index; TG, triglyceride; TC, total cholesterol; LDL, low-density lipoprotein

increased risk of ischemic stroke among the high-risk haplotypes (C-C-C-A and T-T-C-A) for rs670–rs2854116–rs2854117–rs662799. To the best of our knowledge, this study is the first to describe the role of the *APOA1-C3-A5* cluster promoter SNPs with regard to the prevalence of ischemic stroke. Moreover, it is also the first to conduct a haplotype analysis of the northern Chinese Han population.

Previous studies have demonstrated that the SNPs located in the *APOA1-C3-A5* cluster are associated with metabolic syndrome, insulin resistance, and cardiovascular disease in several ethnic populations. The *APOA1-C3-A5* cluster has also been associated with dyslipidemia in many studies.[9,14–16] In a Taiwanese population, the haplotype containing the minor alleles of *APOA5* rs662799 and *APOA1* rs670 was more prevalent among cases than controls and was significantly associated with the risk of hypertriglyceridemia,[17] whereas *APOA5* rs662799 had a significant and independent effect on serum TG concentrations in a Japanese population.[18]

Regarding the association of the *APOA1-C3-A4-A5* cluster with metabolic syndrome,[19] a previous study showed that participants who carry the minor allele of *APOA1* rs670 had an increased risk of developing metabolic syndrome compared with participants with the GG genotype.[8] A meta-analysis found that European and Chinese individuals with the C allele of *APOA5* rs662799 were at a 33% and 40% increased risk of developing metabolic syndrome, respectively. Furthermore, *APOA5* rs662799 was reported to interact with the environmental factors associated with metabolic syndrome.[19–21]

The relationship between *APOC3* promoter polymorphisms and a predisposition to insulin resistance has been underscored in a number of studies that identified variants rs2854116 and rs2854117 as having a greater likelihood of insulin resistance compared with wild-type.[22–25] *APOC3* promoter polymorphisms were also associated with coronary artery disease,[26] while *APOA1* rs670 is a risk marker for coronary artery disease and myocardial infarction among the Indian[27] and Japanese populations,[28] respectively. Interestingly, the C allele of *APOA5* rs662799 was previously associated with higher levels of TG and TG/high-density lipoprotein (HDL) cholesterol in coronary heart disease and with higher Gensini scores in women only, indicating some degree of gender specificity in the effect of genetic variation.[29] However, little was known about the interactions between *APOA1-*

Table 2. Allele and genotype frequencies of SNPs among cases and controls and their main effects on stroke risk.

SNP	Cases	Percentage	Controls	Percentage	OR (95% CI)*	P-value
rs670						
CC (ref)	431	53.10%	468	55.50%	1.00 (ref)	–
CT	306	37.30%	340	40.30%	1.02 (0.76–1.36)	0.890
TT	75	9.20%	36	4.30%	0.83 (0.52–1.30)	0.410
Dominant model CT + TT vs CC					1.10 (0.91–1.33)	0.33
Recessive model TT vs CC + CT					2.28 (1.52–3.44)	<0.001
rs2854116						
TT (ref)	263	32.4%	248	29.4%	1.00 (ref)	–
TC	376	46.3%	426	50.5%	0.83 (0.67–1.04)	0.105
CC	173	21.3%	170	20.1%	0.96 (0.73–1.26)	0.768
Dominant model TC + CC vs TT					0.86 (0.70–1.06)	0.169
Recessive model CC vs TT + TC					1.07 (0.84–1.35)	0.601
rs2854117						
CC (ref)	268	33.00%	256	30.30%	1.00 (ref)	–
CT	378	46.60%	418	49.50%	0.86 (0.63–1.18)	0.358
TT	170	20.40%	170	20.10%	0.93 (0.63–1.37)	0.725
Dominant model CT + TT vs CC					0.88 (0.72–1.09)	0.242
Recessive model TT vs CC + CT					1.02 (0.80–1.30)	0.882
rs662799						
AA (ref)	393	48.40%	448	53.10%	1.00 (ref)	–
AG	322	39.70%	340	40.30%	0.98 (0.73–1.33)	0.904
GG	97	11.90%	56	6.60%	0.87 (0.58–1.31)	0.507
Dominant model AG + GG vs AA					1.21 (0.99–1.46)	0.057
Recessive model GG vs AA + AG					1.91 (1.35–2.69)	<0.001

Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval
*ORs and 95% CIs were calculated via a logistic regression.

C3-A5 polymorphisms and ischemic stroke. Consistent with previous results regarding these functional SNPs, our findings suggest that T allele carriers of *APOA1* (rs670), C allele carriers of *APOC3* (rs2854116), and G allele carriers of *APOA5* (rs662799) are associated with an increased risk of ischemic stroke

The mechanism that underlies the associations between these genetic variants and ischemic stroke is unknown. Functional *APOA1-C3-A5* SNPs mediate the widely replicated associations with hypertriglyceridemia and low HDL cholesterol; these SNPs have been studied extensively because they can alter the nuclear transcription factors that mediate apoprotein formation.[9,10,17,30] However, previous reports found that rs662799 had no effect on relative luciferase expression, and no transcriptional factors mapped to this site.[31] Cui et al.[32] conducted experiments using human lung fibroblasts with a heterozygous genotype (rs2266788 CT) and confirmed the effects of microRNA 3201 binding to the *APOA5* 3′ untranslated region and subsequent APOA5 expression.

Given previous knowledge about high linkage disequilibrium between rs2266788 and rs662799, we suppose that the effect of rs662799 variation on ischemic stroke is likely attributable to rs2266788 as a causal variant. Moreover, an increasing number of studies have demonstrated that *APOA1-C3-*

Table 3. Risk factors according to multiple logistic regression.

Factor	B	SE	Wald	OR (95% CI)*	P-value*
Age	0.033	0.125	0.072	1.034 (0.810–1.320)	0.789
Gender	0.041	0.119	0.120	1.042 (0.826–1.316)	0.729
BMI	0.638	0.120	28.135	1.893 (1.495–2.396)	<0.001
Diabetes mellitus	1.263	0.178	50.202	3.538 (2.494–5.018)	<0.001
Hypertension	1.721	0.123	195.251	5.589 (4.390–7.114)	<0.001
History of smoking	0.691	0.151	21.036	1.995 (1.485–2.680)	<0.001
History of alcohol use	0.172	0.183	0.886	1.188 (0.830–1.701)	0.347
TG	0.500	0.196	6.494	1.648 (1.122–2.421)	0.011
TC	0.691	0.174	15.770	1.995 (1.419–2.806)	<0.001
LDL	0.585	0.166	12.358	1.995 (1.295–2.486)	<0.001
rs670					
CT vs CC	0.452	0.180	6.273	1.571 (1.103–2.236)	0.003
TT vs CC	1.644	0.271	36.740	5.175 (3.042–8.807)	<0.001
rs2854116					
TC vs TT	0.104	0.274	0.145	1.110 (0.649–1.898)	0.660
CC vs TT	0.887	0.436	4.138	2.428 (1.033–5.709)	0.031
rs2854117					
CT vs CC	−0.067	0.272	0.061	0.935 (0.549–1.593)	0.614
TT vs CC	−0.744	0.436	2.914	0.475 (0.202–1.117)	0.059
rs662799					
AG vs AA	0.361	0.152	5.640	1.435 (1.065–1.934)	0.004
GG vs AA	0.732	0.235	9.697	2.079 (1.311–3.294)	0.001

Abbreviations: BMI, body mass index; TG, triglyceride; TC, total cholesterol; LDL, low-density lipoprotein OR, odds ratio; CIs, confidence intervals

*ORs and 95% CIs were calculated based on logistic regression and adjusted for age, gender, BMI, diabetes mellitus, hypertension, history of smoking, history of alcohol use, TG, TC, and LDL.

Wald is a chi-square value equal to B divided by the square error of the standard error (S.E.). Wald is used to test the B value. It was calculated by SPSS.

Table 4. Haplotype frequencies among cases and controls and their relationship with stroke risk.

Haplotype	Cases (%)	Controls (%)	OR (95% CIs)	P-value
C-T-C-A	464 (27.5)	459 (28.3)	0.943 (0.809–1.099)	0.453
C-T-C-G	178 (10.6)	179 (11.1)	0.936 (0.751–1.166)	0.554
C-C-T-A	432 (25.6)	328 (20.2)	1.340 (1.138–1.579)	<0.001
C-C-T-G	160 (9.5)	145 (8.9)	1.056 (0.833–1.337)	0.654
T-T-C-A	209 (12.4)	134 (8.3)	1.547 (1.230–1.945)	<0.001
T-C-T-A	91 (5.4)	127 (7.8)	0.664 (0.503–0.877)	0.004

Abbreviations: OR, odds ratio; CIs, confidence intervals

A5 haplotypes affect TG and HDL cholesterol because they are in linkage disequilibrium.[8–10,16,20,33] These findings suggest that some serum lipid parameters are partially influenced by interactions between *ApoA1-C3-A5* haplotypes, and that these features together contribute to increased individual ischemic stroke susceptibility. *APOA1-C3-A5* polymorphisms might enhance the conformation or function of the apoprotein

related to hypertriglyceridemia, which might in turn explain why our participants were at a high risk of ischemic stroke if they were carriers of the T allele of *APOA1* (rs670), the T allele of *APOA3* (rs2854116), or the G allele of *APOA5* (rs662799). Ding et al previously analyzed the influence of rs662799 on the serum lipid profile, and reported that it was correlated with TG levels, but not independently related with ischemic stroke risk in the studied Turkish subjects.[34] This discrepancy could reflect differences in geographical ethnic groups, genetic backgrounds, diseases, age, sample sizes, study design, or the selection pattern of samples between the two studies.

Our study has some shortcomings that should be recognized. First, its sample size was small, and in the absence of large-scale investigations into the role of *APOA1-C3-A5* in ischemic stroke, it remains unclear whether our findings apply to other ethnic populations. Second, ischemic stroke is a complex disease, and the effects of multiple genes and environmental factors were not evaluated in the present study. Finally, our study should be considered preliminary and the results need to be confirmed in more detailed expression and translational studies.

In summary, we demonstrated that common variants of *APOA1*, *APOC3*, and *APOA5* are associated with ischemic stroke, indicating that *APOA1-C3-A5* variants significantly predict a high ischemic stroke risk. Furthermore, the *APOA1-C3-A5* haplotypes significantly affect the risk of ischemic stroke among the northern Chinese Han population. Variations of these genes may serve as useful genetic markers to diagnose ischemic stroke.

Materials and methods

Samples

We recruited 812 patients with ischemic stroke and 844 healthy controls matched for age and gender. Patient data were collected from the Department of Neurology at the First Affiliated Hospital of China Medical University between June 2014 and December 2015. Individuals ranged in age from 40 to 80 years old. Patients were identified as eligible if they were receiving their first diagnosis of acute ischemic stroke based on a neurological examination and radiological imaging. These diagnoses included the sudden onset of a focal neurological deficit that persisted for more than 24 h with a corresponding infarction on brain imaging (computed tomography [CT] or magnetic resonance imaging [MRI]). Patients with transient ischemic attack, cerebral embolism, cerebral trauma, cerebrovascular malformations, coagulation disorders, autoimmune diseases, tumors, or chronic infectious diseases were excluded. Cases with renal or liver diseases, hematopathy, occlusive arterial disease, or phlebothrombosis of the limbs were also excluded. The controls were recruited from the Physical Examination Department of the First Affiliated Hospital of China Medical University. None of the controls showed evidence of stroke or other neurological diseases. Control participants with tumors, autoimmune diseases, liver ailments, nephrosis, or hematological diseases were excluded. All participants were of Han ethnicity and lived in Liaoning Province, northern China. Clinical records, including general condition (gender, age, BMI, ethnicity, history of smoking, and alcohol use), past history of diabetes and hypertension, serum TG, total cholesterol, low-density lipoprotein, and HDL, were collected from patients and controls. Brain CT or MRI, echocardiography, carotid ultrasound, transcranial Doppler, and electrocardiogram examinations were also required for all patients. BMI was categorized based on the Asian classification of obesity, such that a BMI > 22.9 was considered overweight. Hyperlipidemia was

defined as a total plasma cholesterol level of 5.72 mmol/L, a plasma TG level of 1.7 mmol/L, or the current use of lipid-lowering drugs. The Institutional Ethical Committee of the First Affiliated Hospital of China Medical University approved this study, and it was conducted according to the Code of Ethics of the World Medical Association (Declaration of Helsinki). Written informed consent was obtained from all participants.

SNP selection

Well-studied or functional SNPs were selected based on previous studies that documented associations between SNPs in the Apolipoprotein *APOA1-C3-A5* cluster and dyslipidemia or its components. These SNPs included *APOA1* rs670,[8,35] *APOC3* rs2854116,[11,36–38] *APOC3* rs2854117,[36–38] and *APOA5* rs662799.[8,37,39] The minor allele frequency (MAF) of these four SNPs was >0.05 in the Chinese Han population.

DNA extraction and genotyping

Genomic DNA was extracted from 200 μL of EDTA-anticoagulated peripheral blood using a DNA Purification Kit (Promega, Madison, WI, USA). DNA purity was measured via spectrophotometry, and DNA samples were stored at −80°C. Genotypes were analyzed using the SNaPshot Multiplex Kit (Applied Biosystems Co., Ltd., Foster City, CA, USA). The sequences of PCR primers are shown in Table 5. SNaPshot reactions were performed in a final volume of 10 μL, including 5 μL of the SNaPshot Multiplex Kit (ABI), 1 μL of primer mix, 2 μL of water, and 2 μL of templates, which consisted of the multiplex PCR products for the different genes. The SNaPshot reaction procedures were as follows: initial denaturation at 96°C for 1 min, then 28 cycles of denaturation at 96°C for 10 s, annealing at 55°C for 5 s, and extension at 60°C for 30 s. Amplified samples were stored at 4°C. Extension products were purified over a 1-h incubation period with 1 U of shrimp alkaline phosphatase (Takara, Otsu, Shiga, Japan) at 37°C, followed by incubation at 75°C for 15 min to inactivate the enzyme. Purified products (0.5 μL) were mixed with 9 μL of Hi–Di and 0.5 μL of the Liz120 size standard (Applied Biosystems Co., Ltd.). Samples were incubated at 95°C for 5 min and then loaded onto an ABI 3130XL DNA sequence detector for capillary electrophoresis. Experimental results were analyzed using GeneMapper 4.0 (Applied Biosystems Co., Ltd.).

Table 5. Primer sequences (5′–3′).

SNP	Primer sequence
rs670	Forward: ACTCATTGCA GCCAGGTGAGGA
	Reverse: AGGACCAGTG AGCAGCAACAGG
rs2854116_2854117	Forward: ACTCGCCTGC CTGGATTGAAAC
	Reverse: GCCCTGAACAC AGCCTGGAGTA
rs662799	Forward: GCAGCCCCTGA AAGCTTCACTA
	Reverse: CTGAGCATTTGG GCTTGCTCTC

Statistical analyses

All statistical analyses were performed using Statistical Product and Service Solutions (SPSS) v20.0 if not otherwise specified. All tests were two-tailed, and significance was defined as $P<0.05$. Pearson's χ^2-test was used to compare the distribution of demographic variables and to examine the differences in risk factors and genotypes with regard to alleles and haplotypes between cases and controls. For each genotype,

HWE was tested with a goodness-of-fit χ^2-test. An unconditional logistic regression was performed to calculate the ORs and 95% CIs to estimate the association between certain genotypes and ischemic stroke. Given the sample size of 812 cases and 844 controls, assuming a genotypic relative risk for a recessive model of 2, an MAF of 0.2, a 1.88% population prevalence of ischemic stroke, and a Type I error probability of 0.05, we would be unable to reject the null hypothesis that OR = 1 with a power of 88.62%. Based on the observed frequencies of the four SNPs, we used the SHEsis analysis platform to calculate linkage disequilibrium indices (D′ and r^2) and infer haplotype frequencies.[40,41]

Results

We identified 812 patients with ischemic stroke and 844 controls. The general characteristics of these participants are summarized in Table 1. No significant difference was found between controls and patients with regard to age or gender. However, a higher prevalence of conventional risk factors for ischemic stroke, such as body mass index (BMI), diabetes mellitus, hypertension, smoking history, and alcohol use, was observed among patients with ischemic stroke compared with the control group.

Genotype and allele frequencies of the four SNPs among patients and controls are shown in Table 2. All allele distributions were consistent with Hardy–Weinberg equilibrium (HWE).

As shown in Table 2, neither the genotype nor the allele frequencies of *APOA1* rs670 differed significantly between patients and controls. However, compared with combined variant genotypes (CT + TT), the rs670 TT genotype significantly increased susceptibility to ischemic stroke under a recessive genetic model (odds ratio [OR] = 2.28, 95% confidence interval [CI] = 1.52–3.44, $P < 0.001$; Table 2). Similar results were also found for the genotypes of *APOA5* rs662799. Under a recessive model, the *APOA5* rs662799 GG genotype was associated with a higher ischemic stroke risk (OR = 1.91 (95% CI = 1.35–2.69, $P < 0.001$; Table 2). Analyses of *APOC3* rs2854117 and rs2854117 polymorphisms found no significant associations with ischemic stroke under any of the genetic models (Table 2).

Additional analyses were performed using multiple logistic regression, and more significant results were found after adjusting for confounding factors. Using the lowest-risk genotype (*APOA1* rs670 CC) as a reference, we identified a significantly increased risk for ischemic stroke among the high-risk rs670 genotypes CT and TT (adjusted OR = 1.571, 95% CI = 1.103–2.236, P = 0.003 and adjusted OR = 5.175, 95% CI = 3.042–8.807, $P < 0.001$, respectively) (Table 3). The association between an increased risk of ischemic stroke and the homozygous variant *APOA5* rs662799 GG genotype and the heterozygous variant AG was also significant (adjusted OR = 1.435, 95% CI = 1.065–1.934, P = 0.004 for AG and OR = 2.079, 95% CI = 1.311–3.294, P = 0.001 for GG) (Table 3). Compared with the *APOC3* rs2854116 TT homozygote, the CC homozygote was associated with a significantly increased risk of ischemic stroke (adjusted OR = 2.428, 95% CI = 1.033–5.709, P = 0.031) (Table 3)

The four SNPs were in linkage disequilibrium in this sample (i.e., $D' > 0.8$ or $r2 > 0.4$). Of all possible haplotypes, only six showed a frequency of >0.03 among both cases and controls, and were included in the haplotype analysis. These six haplotypes represented 96.5% of the chromosomes of the cases and 95.2% of those of the controls. No difference was observed in the overall haplotype distribution between cases and controls. Haplotype analysis revealed that the frequencies of the C-C-T-A and T-T-C-A haplotypes of rs670–rs2854116–rs2854117–rs662799 were significantly higher

among patients than controls (OR = 1.340, 95% CI = 1.138–1.579, P < 0.001 and OR = 1.547, 95% CI = 1.230–1.945, P < 0.001) (Table 4).

Author contributions

Yanzhe Wang (yanzhewangcmu@126.com): Conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analytical tools, drafted the article and revised it critically, and approved the final version to be published.
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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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References

1. Heart disease and stroke statistics–2015 update: a report from the American Heart Association. *Circulation* 2015; 131: e535..
2. Wang Y, Yin X, Li L, et al. Association of Apolipoprotein C3 genetic polymorphisms with the risk of ischemic stroke in the Northern Chinese Han Population. *PloS One* 2016; 11: e0163910.
3. Liu F, He Z, Deng S, et al. Association of adiponectin gene polymorphisms with the risk of ischemic stroke in a Chinese Han population. *Mol Bio Rep* 2011; 38: 1983–1988.
4. Deng S, Zhu G, Liu F, et al. CYP4F2 gene V433M polymorphism is associated with ischemic stroke in the male Northern Chinese Han population. *Prog Neuropsychopharmacol Biol Psychiatry* 2010; 34: 664–668.
5. Ranta A and Lichtman JH. Health economics of cerebrovascular disease: can we do better? *Neurology* 2015; 84: 2204–2205.
6. Powers WJ, Derdeyn CP, Biller J, et al. 2015 AHA/ASA focused update of the 2013 guidelines for the early management of patients with acute ischemic stroke regarding endovascular treatment: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2015; 6: 345–354.
7. Feigin VL, Krishnamurthi R, Bhattacharjee R, et al. New strategy to reduce the global burden of stroke. *Stroke* 2015; 46: 1740–1747.
8. Wu Y, Yu Y, Zhao T, et al. Interactions of environmental factors and APOA1-APOC3-APOA4-APOA5 gene cluster gene polymorphisms with metabolic syndrome. *PloS One* 2016; 11: e0147946.
9. Ou HJ, Huang G, Liu W, et al. Relationship of the APOA5/A4/C3/A1 gene cluster and APOB gene polymorphisms with dyslipidemia. *Genet Mol Res* 2015; 14: 9277–9290.
10. Liu Y, Ordovas JM, Gao G, et al. Pharmacogenetic association of the APOA1/C3/A4/A5 gene cluster and lipid responses to fenofibrate: the genetics of lipid-lowering drugs and diet network study. *Pharmacogenet Genomics* 2009; 19: 161–169.
11. Lantz M, Sjostrand C and Kostulas K. Ischemic stroke and patent foramen ovale: risk factors and genetic profile. *J Stroke Cerebrovasc Dis* 2013; 22: 841–845.
12. Pi Y, Zhang L, Yang Q, et al. Apolipoprotein A5 gene promoter region-1131T/C polymorphism is associated with risk of ischemic stroke and elevated triglyceride levels: a meta-analysis. *Cerebrovasc Dis* 2012; 33: 558–565.

13. Li X, Su D, Zhang X, et al. Association of apolipoprotein A5 gene promoter region -1131T>C with risk of stroke in Han Chinese. *Eur J Intern Med* 2011; 22: 99–102.
14. Yin RX, Li YY, Wu JZ, et al. Interactions between the apolipoprotein a1/c3/a5 haplotypes and alcohol consumption on serum lipid levels. *Alcohol Clin Exp Res* 2013; 37: 234–243.
15. Hsu MC, Lee KT, Hsiao WC, et al. The dyslipidemia-associated SNP on the APOA1/C3/A5 gene cluster predicts post-surgery poor outcome in Taiwanese breast cancer patients: a 10-year follow-up study. *BMC cancer* 2013; 13: 330.
16. Yin RX, Li YY and Lai CQ. Apolipoprotein A1/C3/A5 haplotypes and serum lipid levels. *Lipids Health Dis* 2011; 10: 140.
17. Chien KL, Fang WH, Wen HC, et al. APOA1/C3/A5 haplotype and risk of hypertriglyceridemia in Taiwanese. *Clin Chim Acta* 2008; 390: 56–62.
18. Nabika T, Nasreen S, Kobayashi S, et al. The genetic effect of the apoprotein AV gene on the serum triglyceride level in Japanese. *Atherosclerosis* 2002; 165: 201–204.
19. Song KH, Cha S, Yu SG, et al. Association of apolipoprotein A5 gene -1131T>C polymorphism with the risk of metabolic syndrome in Korean subjects. *Biomed Res Int* 2013; 2013: 585134.
20. Hosseini-Esfahani F, Mirmiran P, Daneshpour MS, et al. Dietary patterns interact with APOA1/APOC3 polymorphisms to alter the risk of the metabolic syndrome: the Tehran Lipid and Glucose Study. *Br J Nutr* 2015; 113: 644–653.
21. Son KY, Son HY, Chae J, et al. Genetic association of APOA5 and APOE with metabolic syndrome and their interaction with health-related behavior in Korean men. *Lipids Health Dis* 2015; 14: 105.
22. Miller M, Rhyne J, Chen H, et al. APOC3 promoter polymorphisms C-482T and T-455C are associated with the metabolic syndrome. *Arch Med Res* 2007; 38: 444–451.
23. Waterworth DM, Talmud PJ, Luan J, et al. Variants in the APOC3 promoter insulin responsive element modulate insulin secretion and lipids in middle-aged men. *Biochim Biophys Acta* 2003; 1637: 200–206.
24. Waterworth DM, Talmud PJ, Humphries SE, et al. Variable effects of the APOC3-482C > T variant on insulin, glucose and triglyceride concentrations in different ethnic groups. *Diabetologia* 2001; 44: 245–248.
25. Waterworth DM, Ribalta J, Nicaud V, et al. ApoCIII gene variants modulate postprandial response to both glucose and fat tolerance tests. *Circulation* 1999; 99: 1872–1877.
26. Shanker J, Perumal G, Rao VS, et al. Genetic studies on the APOA1-C3-A5 gene cluster in Asian Indians with premature coronary artery disease. *Lipids Health Dis* 2008; 7: 33.
27. Chhabra S, Narang R, Lakshmy R, et al. APOA1-75 G to A substitution associated with severe forms of CAD, lower levels of HDL and apoA-I among northern Indians. *Dis Markers* 2005; 21: 169–174.
28. Shioji K, Mannami T, Kokubo Y, et al. An association analysis between ApoA1 polymorphisms and the high-density lipoprotein (HDL) cholesterol level and myocardial infarction (MI) in Japanese. *J Hum Genet* 2004; 49: 433–439.
29. Wang Y, Lu Z, Zhang J, et al. The APOA5 rs662799 polymorphism is associated with dyslipidemia and the severity of coronary heart disease in Chinese women. *Lipids Health Dis* 2016; 15: 170.
30. Song Y, Zhu L, Richa M, et al. Associations of the APOC3 rs5128 polymorphism with plasma APOC3 and lipid levels: a meta-analysis. *Lipids Health Dis* 2015; 14: 32.
31. Charriere S, Bernard S, Aqallal M, et al. Association of APOA5 -1131T>C and S19W gene polymorphisms with both mild hypertriglyceridemia and hyperchylomicronemia in type 2 diabetic patients. *Clin Chim Acta* 2008; 394: 99–103.
32. Cui G, Li Z, Li R, et al. A functional variant in APOA5/A4/C3/A1 gene cluster contributes to elevated triglycerides and severity of CAD by interfering with microRNA 3201 binding efficiency. *J Am Coll Cardiol* 2014; 64: 267–277.
33. Ding Y, Zhu MA, Wang ZX, et al. Associations of polymorphisms in the apolipoprotein APOA1-C3-A5 gene cluster with

acute coronary syndrome. *J Biomed Biotechnol* 2012; 2012: 509420.
34. Can Demirdogen B, Sahin E, Turkanoglu Ozcelik A, et al. Apolipoprotein A5 polymorphisms in Turkish population: association with serum lipid profile and risk of ischemic stroke. *Mol Biol Rep* 2012; 39: 10459–10468.
35. Al-Bustan SA, Al-Serri AE, Annice BG, et al. Re-sequencing of the APOAI promoter region and the genetic association of the -75G > A polymorphism with increased cholesterol and low density lipoprotein levels among a sample of the Kuwaiti population. *BMC Med Genet* 2013; 14: 90.
36. Petersen KF, Dufour S, Hariri A, et al. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. *N Engl J Med* 2010; 362: 1082–1089.
37. Lin B, Huang Y, Zhang M, et al. Association between apolipoprotein C3 Sst I, T-455C, C-482T and C1100T polymorphisms and risk of coronary heart disease. *BMJ Open* 2014; 4: e004156.
38. Kozlitina J, Boerwinkle E, Cohen JC, et al. Dissociation between APOC3 variants, hepatic triglyceride content and insulin resistance. *Hepatology* 2011; 53: 467–474.
39. Jiang Y, Ma J, Li H, et al. Effect of apolipoprotein C3 genetic polymorphisms on serum lipid levels and the risk of intracerebral hemorrhage. *Lipids Health Dis* 2015; 14: 48.
40. Li Z, Zhang Z, He Z, et al. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn). *Cell Res* 2009; 19: 519–523.
41. Shi YY and He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res* 2005; 15: 97–98.