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Association of genetic variation in apolipoprotein E and low density lipoprotein receptor with ischemic stroke in Northern Han Chinese☆

Bin Wang [a,1], Hongye Zhao [a,1], Li Zhou [b], Xiaoxia Dai [c], Dayu Wang [d], Jun Cao [e], Wenquan Niu [f,*]

[a] Department of Physiology, Qiqihar Medical College, Heping Street 249, Fularji District, 161042 Qiqihar, Heilongjiang, China
[b] Central Laboratory, Qiqihar Medical College, Heping Street 249, Fularji District, 161042 Qiqihar, Heilongjiang, China
[c] Department of Nephrology, The Third Hospital of Qiqihar, 161042 Qiqihar, Heilongjiang, China
[d] Occupational Disease Department, The Yizhong Group Hospital for Staff and Workers, 161042 Qiqihar, Heilongjiang, China
[e] Department of Property Theory and Complication Science of Materia Medica, Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Dongzhimen Inner Nanxiao Street 16, Dongcheng District, 100700 Beijing, China
[f] Ruijin Hospital, Shanghai Institute of Hypertension, Ruijin Second Road 197, 200025 Shanghai, China

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ABSTRACT

Background: Ischemic stroke is the end phenotype of a complex interaction between various genetic and environmental factors.
Objective: We aimed to explore association of two lipid-relevant genetic variants and conventional risk factors with risk of having ischemic stroke in Northern Han Chinese.
Methods: Genotyping was performed in 396 ischemic stroke patients and 396 controls that were all recruited from the four hospitals of Qiqihar city. Data were analyzed using χ^2 test, logistic regression and haplotype analyses.
Results: Significant differences were observed for genotype and allele distributions of *APOE* ε2/ε3/ε4 polymorphism ($P<0.001$) with ε4 allele conferring a 2.19-fold risky effect ($P<0.001$), while no statistical differences were found for *LDLR* C1773T distributions. Haplotype analysis indicated the remarkable differences for haplotypes harboring *APOE* "ε2" or "ε4" alleles between cases and controls after the stringent Bonferroni correction. Moreover, all multiple-testing associations remained significant using false discovery rate (FDR) method. Further our multiple logistic regression analysis showed significant associations of hypertension status (OR=5.37, $P<0.001$) and *APOE* ε2 (OR=0.45, $P<0.001$) and ε4 (OR=1.50, $P=0.003$) alleles with ischemic stroke after controlling confounders, and their correlations with plasma lipid profiles were strengthened by stratification of alleles and hypertension status combined.
Conclusions: Our results not only demonstrated potential interactions of *APOE* ε2/ε3/ε4 and *LDLR* C1773T polymorphisms with risk of having ischemic stroke, but also added the evidence of independent role of hypertension and *APOE* ε2/ε3/ε4 polymorphism in the development of this disorder in Northern Han Chinese.

1. Introduction

Ischemic stroke, accounting for 80% of the stroke population, prevails globally and appends a major burden on health and healthcare costs. Although stroke may be largely preventable, an understanding of the associated risks is of scientific interest [1]. It has been proposed that ischemic stroke is the end phenotype of a complex interaction between an individual's genetic background and various environmental factors [2,3]. Much is known about the conventional risk factors that attribute to the development of stroke, such as lipid profiles [4,5] and hypertension [6,7], while less is known about the genetic factors that could predispose or modify the types and consequences of stroke.

Awareness is mounting indicating that patients with ischemic stroke tend to have a high prevalence of associated-dyslipidemias, such as the elevated levels of plasma total cholesterol [8]. Results from several large randomized trials showed that lipid-lowering therapy can result in the reduced risk of ischemic stroke [9,10]. Furthermore, some studies have suggested that associations of lipid levels with stroke risk vary in stroke subtypes and patient subgroups [4]. Given the complex nature of ischemic stroke, it is likely that a number of genes rather than a single gene account for the heritability of this disorder. It has been speculated that variants in lipid-relevant genes might explain most of the heritability in the general population [9–11]. A favorite example in this

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* Corresponding author. Tel.: +86 021 64370045x610905.
E-mail address: niuwenquan@yahoo.cn (W. Niu).
[1] These authors contributed equally to this work.

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respect is that apolipoprotein E (apoE for protein, *APOE* for gene) genetic variation has been estimated to account for 2% to 11% of the total variation in serum or plasma cholesterol levels in apparently healthy white population [11]. It is thus reasonable to conceive that multiple genetic and/or physiological safeguards have been developed to maintain lipoprotein levels within a range of physiologically acceptable levels. To address this issue, we explored the association of two lipid-relevant genetic polymorphisms (*APOE*: ε2/ε3/ε4, rs7412/429358 and low density lipoprotein receptor gene (*LDLR*): C1773T, rs688) and conventional risk factors with risk of having ischemic stroke in Northern Han Chinese.

The genes encoding human apoE and LDLR are mapped to chromosome 19q13.2 and 19p13.2, respectively. The candidate genes were selected from biochemical pathways that have been implicated in the transport and metabolism of lipids and lipoproteins [12–14]. In addition, the genes and polymorphisms were chosen based on prior evidence of potential functionality and significant association in cerebrovascular diseases [11,15–20].

2. Methods

2.1. Study population

All study subjects were of Northern Han Chinese descent and absent of consanguinity at enrollment. Stroke patients and healthy controls were recruited simultaneously from four hospitals, The Rongjun Farm Hospital for Staff and Workers, The Third Hospital of Qiqihar, The Yizhong Group Hospital for Staff and Workers, and The First Affiliated Hospital Qiqihar Medical College, from 2000 to 2003 in Qiqihar city, which is the second largest city in Heilongjiang province. All patients with a clinical diagnosis of acute ischemic stroke admitted to these hospitals were eligible for the present study. However, only those patients confirmed by a computed tomography (CT) scan and/or magnetic resonance imaging (MRI) were included. All the patients (n=396) were categorized into 1 of the 3 subtypes: atherosclerotic cerebral thrombosis (n=153), lacunar infarction (n=156), and cerebral embolism (n=87) and were free of hemorrhagic stroke and anti-platelet and statin usage. Moreover, patients with autoimmune disease or chronic inflammation were also excluded from this study. A team of professional doctors reviewed the eligibility of the study patients. Healthy controls (n=396), which were collected from the aforementioned hospitals, were without a clinical history of cerebrovascular diseases, diabetes (with the threshold of fasting plasma glucose 6.99 mmol/L) and renal insufficiency through extensive clinical examination. In addition, controls were matched with patients for area, gender and age.

2.2. Measurement of anthropometric index and lipid-relevant profiles

Data on body weight, height, history of hypertension and diabetes, as well as anti-platelet and statin usage were obtained using a self-designed questionnaire. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). All patients or their families and controls gave written informed consent to the extraction and subsequent use of their blood. This study was reviewed and approved by the Ethics Committee of the Qiqihar Medical College.

Venous blood (5 mL) was collected from each subject and heparinized. The serum was simultaneously isolated and frozen for biochemical assay. Genomic DNA was extracted from peripheral leukocytes using proteinase K/phenol/chloroform purification, followed by ethanol precipitation, and stored in 10 mM Tris–HCl, 1 mM Na_2-EDTA, pH 8.0.

Fasting plasma triglyceride (TG), total cholesterol (TC), and high density lipoprotein cholesterol (HDLC) concentrations were determined enzymatically using commercially available kits and auto analyzer, and plasma low density lipoprotein cholesterol (LDLC) concentration was estimated using Friedewald's formula. The diagnosis of hypertension was based on blood pressure above a defined cutoff value (140/90 mmHg for systolic/diastolic blood pressure) according to the subjects' records before the stroke event or being on antihypertensive therapies.

2.3. Genotyping

Genotypes of the two studied polymorphisms were determined using polymerase chain reaction (PCR), with further restriction analysis. The amplification of *APOE* ε2/ε3/ε4 (rs7412/429358) polymorphism was performed with the primers 5'-AAC AAC TGA CCC CGG TGG CG-3' (sense) and 5'-ATG GCG CTG AGG CCG CGC TC-3' (antisense). PCR reactions began with an initial denaturation at 94 °C (5 min), then 30 cycles of 94 °C (45 s), 65 °C (45 s) and 72 °C (45 s), followed by a final extension at 72 °C (5 min). PCR products were restricted with *Hha*I, and separated on 8% polyacrylamide gels. The *LDLR* C1773T (rs688) polymorphism was genotyped according to a previous report [21].

2.4. Statistical analysis

Statistical analysis was performed using SAS version 9.1.3 (Institute Inc., Cary, NC, USA). Means of continuous variables were logarithmically normalized and compared by the Student's t-test. The χ^2 test was used to assess the goodness-of-fit between the observed allele frequencies and the expected counterparts by Hardy–Weinberg (H–W) equilibrium and to evaluate the differences in genotype/allele distributions between cases and controls. Multiple logistic regression analyses were employed to assess the extent to which the studied polymorphisms and risk factors were associated with the presence of ischemic stroke using a full model. For each odds ratio (OR), we calculated 2-tailed probability value and 95% confidence interval (CI). Continuous data were expressed as mean ± SD, and two-sided $P<0.05$ was considered statistically significant unless indicated otherwise.

The linkage disequilibrium degree was identified by the Haploview software version 4.1 available at www.broad.mit.edu/mpg/haploview. The linkage disequilibrium coefficients were shown as D'. Haplotype frequencies were estimated using EH/EH+ program [22]. We compared haplotype frequencies between cases and controls by χ^2 test from a series of 2 × 2 contingency tables by combining the other haplotypes. The haplotype-derived probability value was corrected by the Bonferroni method according to the number of tests performed. In addition, to adjust for multiple-testing association, we employed the false discovery rate (FDR) method with an FDR q value threshold of 0.20 as suggested by Smith et al. [23].

3. Results

The basic characteristics of the study population were shown in Table 1. The percent of hypertension status and plasma triglyceride

Table 1
The basic characteristics of the study population

Characteristics	Cases	Controls	P[a]
Number	396	396	
Age (years)	57.33 ± 8.21	57.26 ± 8.09	0.636
Male (%)	52.78	51.01	0.619[b]
BMI (kg/m^2)	25.48 ± 4.01	25.16 ± 3.95	0.244
Hypertension (%)	46.21	8.59	<0.001[b]
TG (mg/dL)	131.26 ± 55.08	114.37 ± 51.64	<0.001
TC (mg/dL)	201.48 ± 37.34	202.05 ± 48.55	0.510
HDLC (mg/dL)	52.06 ± 20.99	58.94 ± 19.86	0.001
LDLC (mg/dL)	119.32 ± 31.08	113.98 ± 30.22	0.352

Abbreviations: BMI: body mass index; TG: triglyceride; TC: total cholesterol; HDLC: high density lipoprotein cholesterol; LDLC: low density lipoprotein cholesterol.
Continuous variables are expressed as mean ± SD before normalization.
[a] P was calculated using Student's t-test after the logarithmical normalization.
[b] P was calculated using χ^2 test.