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Association between the *LXRα* polymorphism and stroke in a Chinese population

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ABSTRACT. We examined the relationship between the liver X receptor α gene (*LXRα*) polymorphism and the susceptibility to stroke. We utilized the single fluorescent-labeled probe technique to detect the genotype of rs12221497 in the *LXRα* gene in 400 stroke patients and 400 healthy control subjects. The difference in genotype distribution between the 2 groups was analyzed using the chi-square test. Serum lipids and glucose levels between the different genotypes were also compared. We found that the risk of stroke in carriers with the AA + GA genotype was 2.02-fold higher than that in GG genotype carriers (odds ratio = 2.02, 95% confidence interval = 1.18-2.87, $P < 0.05$), and that the risk of stroke in carriers with the A allele increased by 0.606-fold compared to that in G allele carriers (odds ratio = 1.606, 95% confidence interval = 1.158-2.228). Logistic regression analysis showed that after adjusting for other confounding factors, the A allele

was an independent risk for stroke. However, there were no differences in serum lipids and glucose levels between each genotype. We conclude that the rs12221497 polymorphism in the *LXR*α gene was associated with the susceptibility to stroke in a Han Chinese population.

Key words: Gene; Liver X receptor α; Polymorphism; Stroke

INTRODUCTION

Ischemic stroke is a multifactorial disease resulting from the interaction between genetic factors and environmental factors (Boshuisen et al., 2013; Zende et al., 2013). Recently, several mechanisms, including vascular endothelial cell injury, platelet reactivity, lipid infiltration, and vascular smooth muscle cell proliferation, were suggested to be the main pathological processes involved in stroke (Orozco et al., 2013; Sharma et al., 2013). A previous study indicated that elevated serum low-density lipoprotein (LDL)-cholesterol (C) is an independent risk factor for ischemic stroke (Igase et al., 2012). A number of studies suggested that genetic polymorphisms in several lipid-related genes, such as *ABCB1* (Hindorff et al., 2008), *APOE* (Kumar et al., 2013), and *APOA5* (Can Demirdöğen et al., 2012), were associated with ischemic stroke risk. Liver X receptor α (LXRα) is a class of nuclear receptor family members (Jakobsson et al., 2012) that are mainly distributed in the liver, adipose tissue, kidney, small intestine, and macrophages. Their main physiological functions include controlling cholesterol level, lipoprotein metabolism, and fat synthesis (Faulds et al., 2010; González and Castrillo, 2011). Several studies indicated that the *LXR*α gene polymorphism was associated with metabolic syndrome, coronary artery disease, and diabetes (Hazra et al., 2012; Jia et al., 2013). However, the relationship between *LXR*α gene polymorphisms and stroke remains unclear.

In the present study, we performed a case-control study to explore the association between *LXR*α gene polymorphisms and stroke in a Chinese Han population.

MATERIAL AND METHODS

Subjects

The present study included 400 unrelated adult Chinese patients with acute hemispheric ischemic stroke and 400 symptom-free Chinese controls. Cases were selected among patients suffering from atherothrombotic ischemic stroke admitted to neurology services within 24 h after symptom onset. Patient recruitment was performed consecutively. Stroke was defined as clinical designation for a rapidly developing loss of brain function that lasted for at least 24 h and had no apparent cause other than that of vascular origin. The cerebral infarction was initially diagnosed based on neurological examination and brain computed tomography. In order to be considered eligible, the patients had to meet the following criteria: anterior circulation stroke, no other major illnesses, including autoimmune diseases, neoplasms, coagulopathies, hepatic, or renal failure, no known embolic source (aortic arch, cardiac, or carotid), no family history of hematological, autoimmune, or chronic inflammatory diseases, and no history of myocardial infarction within 3 weeks.

Control subjects were selected randomly from the neurology outpatient clinics who

did not have stroke or transient ischemic attack at any time. All exclusion criteria were applied to the controls as well as not having ischemic heart disease, carotid stenosis (lumen narrowing), or ulcerated carotid plaque.

Clinical data and blood collection

Clinical data, including past medical history, family disease history, smoking and drinking history, weight, height, blood pressure, and body mass index (BMI), were collected in the present study.

Next, 2 mL 12-h fasting venous blood was collected, and the triglyceride, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), LDL-C, fasting blood glucose, and other biochemical parameters were detected using a biochemical analyzer (Dimension AR/AVL Clinical Chemistry System, Newark, NJ, USA).

Preparation of DNA from peripheral blood leukocytes

Blood genomic DNA was extracted from whole blood according to the protocol of the DNA extraction kit (Beijing Bioteck, Beijing, China), dissolved in TE buffer, and stored at -20°C.

Genotyping

The single fluorescent-labeled probe technique was utilized for genotyping. The primer sequence was as follows: upstream primer: 5'-GGC TTA CTC CAA TAA TCC CCA CAC TT-3', downstream primer: 5'-AAG GAA GAA GGC AGG TAA TGA TGA AGG AG-3'.

Statistical analysis

SPSS l3.0 (SPSS, Inc., Chicago, IL, USA) was utilized for statistical analysis. Continuous data are reported as means ± standard deviation. Differences between the 2 groups were compared using the Student *t*-test. Genotype and allele frequency distribution in the case group and control group were calculated by direct counting. Hardy-Weinberg equilibrium was analyzed using the chi-square test, while genotype and allele distributions between the 2 groups were compared using the chi-square test or the Fisher exact test. A multivariate logistic regression model was used to analyze risk factors of stroke, and the odds ratios (ORs) and 95% confidence intervals (95%CIs) were utilized to describe relative risk.

RESULTS

Characteristics of study participants

The clinical and metabolic characteristics of the study population are shown in Table 1. The mean age, gender, and BMI were not significantly different between the 2 groups ($P > 0.05$). However, there were significant differences between the 2 groups in triglyceride, TC, HDL-C, LDL-C, and fasting blood glucose levels (all $P < 0.05$).

Table 1. Demographic and risk profile of the study population.

Risk factors	N (%) or mean ± SD		P values
	Control (N = 400)	Stroke (N = 400)	
Age (years)	58.22 ± 11.28	58.10 ± 10.48	0.665
Female (%)	101 (25.3)	112 (28.0)	0.332
BMI (kg/m^2)	25.5 ± 3.40	24.76 ± 3.91	0.132
GLU (mM)	4.65 ± 1.21	5.24 ± 1.02	<0.001
TG (mM)	1.44 ± 0.62	1.95 ± 0.46	0.022
TC (mM)	4.22 ± 0.74	5.33 ± 0.76	0.013
HDL-C (mM)	1.30 ± 0.41	1.21 ± 0.54	0.232
LDL-C (mM)	2.24 ± 0.81	2.90 ± 0.88	0.019
Smoking (N)	194 (0.485)	233 (0.582)	0.011
Hypertension (N)	182 (0.455)	243 (0.607)	0.002
Diabetes (N)	143 (0.357)	188 (0.470)	0.013
Obesity (N)	168 (0.420)	198 (0.495)	0.087

BMI = body mass index; GLU = glucose; TG = triglycerides; TC = cholesterol; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

Genotype and allele frequencies

All genotyped single nucleotide polymorphisms were in Hardy-Weinberg equilibrium in the control group (P > 0.05; data not shown). Table 2 shows detailed information for genotypes as well as allele frequencies.

Table 2. Distributions of single nucleotide polymorphisms of the *LXRα* gene in case and control groups.

rs12221497		Group		P value
		Control [N (%)]	Stroke [N (%)]	
Genotype	GG	341 (85.2)	309 (77.3)	0.015
	GA	52 (13.0)	81 (20.3)	
	AA	7 (1.8)	10 (2.4)	
Allele	G	734 (91.75)	699 (87.37)	0.004
	A	66 (8.25)	101 (12.63)	

We found that the risk of stroke in carriers with the AA + GA genotype was 2.02-fold higher than that in GG genotype carriers (OR = 2.02, 95%CI = 1.18-2.87, P < 0.05), and the risk of stroke in carriers with the A allele increased by 0.606 times compared to that for G allele carriers (OR = 1.606, 95%CI = 1.158-2.228).

Serum lipids and fasting glucose in different genotypes

There were a total of 800 cases in the stroke group and the control group; 650 cases had the GG genotype and 150 cases had the GA + AA genotype. There were significant differences between carriers with the GG genotype and carriers with the GA or AA genotype regarding LDL-C and TC levels (Table 3).

Logistic regression analysis

In multivariate logistic regression analysis, we found that LDL-C, TC, history of hypertension, smoking history, age, BMI, and A allele were independent risk factors for stroke; the OR for the A allele was 1.88 (P < 0.05, 95%CI = 1.09-3.55) after adjusting for other confounders.

Table 3. Serum lipids and glucose levels for each genotype.

Lipids and glucose	N (%) or mean ± SD		P values
	GG (N = 650)	GA+AA (N = 150)	
GLU (mM)	4.86 ± 0.71	4.94 ± 0.68	0.544
TG (mM)	1.74 ± 0.63	1.79 ± 0.51	0.642
TC (mM)	4.61 ± 0.85	5.47 ± 0.75	0.036
HDL-C (mM)	1.15 ± 0.32	1.17 ± 0.45	0.186
LDL-C (mM)	2.32 ± 0.81	2.71 ± 0.73	0.031

GLU = glucose; TG = triglycerides; TC = total cholesterol; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

DISSCUSSION

In the present study, we found that *LXR*α A allele carriers had a higher risk of stroke in the Chinese Han population.

LXRα can regulate various target genes involved in lipid uptake, spillover, and lipid metabolism (Zhao et al., 2014). The main function of activated LXRα includes: 1) mediating the binding and transporting factor Al (ABCAl), ABCGl, ABCG5, ABCG4, and ABCG8 located in the human macrophages and small intestine target genes ATP to promote endogenous lipid membrane transport; 2) activating human macrophages Niemann-Pick C1 protein (NPC1) and C2 protein (NPC2) to promote cholesterol transport from the endosome chamber to the cytoplasmic membrane; 3) promoting receptor ApoE, ApoC-I, C-II, and C-IV expression, which regulate cholesterol outflow in adipocytes and macrophages; 4) controlling liver and macrophages regulating enzymes such as the phospholipid transfer protein and lipoprotein lipase remodeling lipoproteins.

In addition, LXRα can inhibit various inflammatory cytokines and chemokines (Watanabe et al., 2013; Jin et al., 2013), indicating that the LXRα signaling pathway plays an important role in atherosclerosis development. Previous studies also confirmed this view. The synthesis of the LXR agonist can inhibit the development of atherosclerosis, which may result from expression regulation of the underlying metabolic and inflammatory genes (Legry et al., 2011; Fan et al., 2012).

In the present study, we found that in Chinese Han patients, the A allele frequency in the *LXR*α gene was significantly higher than that in healthy controls. A allele carriers showed a 0.88-fold increased risk of stroke (OR = 1.88). After adjusting for age, gender, cholesterol, fasting glucose, hypertension, diabetes, smoking history, and other confounding factors, the A allele was still an independent risk factor of stroke ($P < 0.05$). A recent study suggested that *LXR*α polymorphisms were significantly correlated with BMI and HDL-C concentration (Liu et al., 2013). In a French-Canadian population, serum cholesterol levels in A allele carriers were higher than those in GG homozygotes (Robitaille et al., 2007). In the present study, we found significant differences in TC and LDL-C between A allele carriers and GG homozygotes.

In conclusion, we found an association between genetic variations in the *LXR*α gene and stroke in a Han population. Our results increase the understanding of the mechanism of stroke development. The study also confirmed that lipid metabolism is a risk factor of stroke at the genetic level.

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