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Association of liver X receptor α (*LXRα*) gene polymorphism and ischemic stroke

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ABSTRACT. We examined the relationship between the liver X receptor α gene (*LXRα*) rsl2221497 polymorphism and the susceptibility to ischemic stroke in a Chinese population. The polymerase chain reaction-restriction fragment length polymorphism technique was used to detect the genotype of rsl2221497 in the *LXRα* gene of 300 stroke patients and 300 healthy control subjects. The chi-square test was used to analyze the genotype distribution between the 2 groups. We found that the risk of stroke in carriers with the AA + GA genotype was 2.12-fold higher than that in GG genotype carriers (odds ratio = 2.12, 95% confidence interval: 1.58-2.99, $P < 0.05$). The risk of stroke in carriers of the A allele increased by 1.03-fold compared to that in G allele carriers (odds ratio = 2.03, 95% confidence interval: 1.44-3.01, $P < 0.01$). After adjusting for other confounding factors such smoking, hypertension, and diabetes, the A allele was found to be an independent risk factor for stroke. Therefore, the rsl2221497 polymorphism in the *LXRα* gene

was associated with the susceptibility to stroke in a Chinese population.

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

INTRODUCITON

Ischemic stroke is a multifactorial disease resulting from interactions between genetic and environmental factors (Hamzi et al., 2013; Markov, 2013; Egashira et al., 2013). Currently, platelet reactivity, lipid infiltration, and vascular endothelial cell injury are thought to be the main pathological processes involved in ischemic stroke (Jansen et al., 2013; Orozco et al., 2013). Similarly to ischemic heart disease, lipid metabolism disorder is an independent risk factor for stroke (Jakobsson et al., 2012; Zhang et al., 2013). Liver X receptor α (LXRα) is a class of nuclear receptor family members (Sharma et al. 2013) that are mainly distributed in the liver, adipose tissue, kidney, small intestine, and macrophages and control the stable internal environment of cholesterol level, lipoprotein metabolism, and fat synthesis (A-González and Castrillo, 2011). The pathophysiological role of LXRα in humans is not currently thoroughly understood. Recent studies have indicated that *LXRα* is a candidate gene for metabolic syndrome and diabetes (Faulds et al., 2010; Hazra et al., 2012). Diabetes is the main risk factor for stroke. However, the relationship between genetic polymorphisms of *LXRα* and stroke remains unclear. Therefore, we performed a case-control study to analyze the association of *LXRα* gene polymorphisms with stroke in a Chinese population.

MATERIAL AND METHODS

Subjects

The present study was approved by the Ethics Committee of The Affiliated Fourth Centre Hospital of Tianjin Medical University, and informed consent was obtained from all participants.

We selected 300 patients who had been diagnosed with stroke in The Affiliated Fourth Centre Hospital of Tianjin Medical University from March 2009 to March 2013. The average age of the stroke patients was 58.33 ± 10.13 years. Stroke was diagnosed according to the World Health Organization ischemic stroke diagnostic criteria of 1979. We also selected 300 sex- and age-matched healthy subjects as the control group. Disease history, physical examination, laboratory tests (serum, urine, stool routine tests, serum lipids, and glucose), and other preliminary tests were performed. The history of smoking, hypertension, and diabetes were determined. All subjects were of Han Chinese ethnicity.

Clinical data collection

We collected clinical data, including past medical history, family disease history, smoking and drinking history, weight, height, blood pressure, and body mass index (BMI) in the present study. We also collected 2 mL of 12-h-fasting venous blood to determine the serum concentration of triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting blood glucose (FBG), and other biochemical parameters.

Preparation of DNA from peripheral blood leukocytes

Genomic DNA was extracted from whole blood according to the protocol provided with the DNA extraction kit (Beijing Bioteck, Beijing, China). We dissolved the DNA in Tris-ethylenediaminetetraacetic acid buffer and stored the samples at -20°C.

Genotyping

We performed genotyping analysis using the polymerase chain reaction-restriction fragment length polymorphism method. The primer sequences were as follows: upstream primer: 5'-GGCTTACTC CAA TAA TCC CCA CAC TT-3', downstream primer: 5'-AAGGAAGAAGGCAGGTAATGATGAAGGAG-3'. The method used for genotype identification has been described previously (Legry et al., 2011).

Statistical analysis

We performed data analysis using SPSS l7.0 (SPSS, Inc., Chicago, IL, USA). 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 using the direct counting method. Hardy-Weinberg equilibrium was analyzed using the chi-square test, and genotype and allele frequency distribution between the 2 groups were compared using the chi-square test or Fisher's exact test. A multivariate logistic regression model was used to analyze risk factors of stroke. The odds ratios (ORs) and 95% confidence intervals (95%CIs) were used to express the relative risk.

RESULTS

Characteristics of the 2 groups

As shown in Table 1, there was no significant difference in the mean age, gender, and BMI between the 2 groups (all $P > 0.05$). However, there were significant differences between the 2 groups in TG, TC, HDL-C, LDL-C, and FBG (all $P < 0.05$).

Genotype and allele frequency distribution

Genotypes in the stroke and the control groups were found to be in Hardy-Weinberg equilibrium ($P > 0.05$). Genotype analysis showed that stroke risk in AA + GA genotype carriers was 2.12-fold higher than that in GG genotype carriers (OR = 2.12, 95%CI = 1.58-2.99, $P < 0.05$). The frequency of the A allele in the CHD group was significantly higher than that in the control group ($P < 0.01$), with an OR of 2.03 (95%CI = 1.44-3.01) (Table 2).

Logistic regression analysis

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

Risk factors	Control (N = 300)	Stroke (N = 300)	P values
Age (years)	58.33 ± 10.13	58.54 ± 10.36	0.656
Female [N (%)]	86 (28.7)	89 (29.7)	0.212
BMI (kg/m^2)	24.66 ± 4.55	25.10 ± 4.22	0.144
GLU (mM)	4.25 ± 0.56	5.77 ± 0.72	<0.01
TG (mM)	1.46 ± 0.67	2.33 ± 0.48	<0.01
TC (mM)	4.33 ± 0.59	5.21 ± 0.82	<0.01
HDL-C (mM)	1.31± 0.32	1.01 ±0.53	0.023
LDL-C (mM)	2.22 ± 0.83	2.81 ±0.80	0.031

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

In multivariate logistic regression analysis, stroke was considered to be the dependent variable. Age, gender, BMI, TC, TG, LDL-C, HDL-C, FBG, the A allele, smoking history, hypertension history, and diabetes mellitus history were considered to be independent variables in logistic regression analysis. The results showed that HDL-C, TC, TG, history of hypertension, smoking history, age, BMI, and the A allele were independent risk factors for stroke, and the OR for the A allele was 2.03 (95%CI: 1.44-3.01, $P < 0.01$) after adjusting for other confounding factors.

Table 2. Distributions of genotypes and alleles in the case and control groups.

rsl2221497	Genotypes	Group		P value
		Control [N (%)]	Stroke [N (%)]	
Genotype	GG	255 (85.0)	231 (77.0)	0.011
	GA	42 (14.0)	60 (20.0)	
	AA	3 (1.0)	9 (3.0)	
Allele	G	552 (92.0)	522 (87.0)	0.010
	A	48 (8.0)	78 (13.0)	

DISCUSSION

In the present study, we found that the A allele in *LXRα* increased the risk of stroke in the Chinese Han population. This is the first study to identify the relationship between the *LXRα* polymorphism and stroke.

LXRα regulates various target genes involved in lipid uptake, spillover, and lipid metabolism (Jia et al., 2013) in the following manner: 1) LXRα can mediate binding and transporting factor Al (ABCAl) and other factors such as ABCGl, ABCG5, ABCG4, and ABCG8; 2) LXRα can activate human macrophages Niemann-Pick Cl protein and C2 protein to promote cholesterol transport from the endosome chamber to the cytoplasmic membrane; 3) LXRα can promote ApoE, ApoC-I, C-II, C-IV receptor expression, which regulate cholesterol outflow in adipocytes and macrophages; 4) LXRα can control the liver- and macrophage-regulating enzymes such as phospholipid transfer protein and lipoprotein lipase remodeling lipoproteins. In addition, LXRα can inhibit many inflammatory cytokines and the expression of chemokines (Zhang et al., 2001; Watanabe et al., 2013; Jin et al., 2013; Chen et al., 2013; Zhao et al., 2014). All of these functions indicate that the LXRα signaling pathway plays an

important role in the development of atherosclerosis and ischemic stroke.

In the present study, we found that the *LXRα* A allele frequency was significantly higher in stroke patients than in the healthy population. After adjusting for confounding factors such as age, gender, cholesterol, fasting glucose, hypertension, diabetes, and smoking history, the A allele remained an independent risk factor of stroke. This indicates that this locus variant significantly increases the risk of stroke.

In conclusion, we found an association between genetic variations in the *LXRα* gene and stroke in a Han Chinese population. Our results increase the understanding of the mechanism of stroke development.

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