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ORIGINAL ARTICLE

Association Between the Gene Polymorphisms of HDAC9 and the Risk of Atherosclerosis and Ischemic Stroke

Guo qingxu[1] · **Zhang Yan**[2] · **Xu Jiannan**[3] · **Liu Yunlong**[1]

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Abstract Genome-wide association studies have demonstrated various polymorphisms of histone deacetylase 9 (HDAC9) gene was strong risk locus for large-vessel stroke, but the results were controversial. This study aims to replicate the association between the previous detected SNPs of HDAC9 and the susceptibility of ischemic stroke. The study population consisted of 262 consecutive patients diagnosed with ischemic stroke and 300 age and gender matched unrelated controls between October 2012 and October 2014. Rs11984041, rs2389995, and rs2240419 of HDAC9 were genotyped and compared between the cases and controls. The SNP rs11984041 of HDAC9 was found nonpolymorphic in the population involved. The G allele of rs2389995 was found significantly associated with decreased risk of ischemic stroke, no matter with the codominant (AG v.s AA, 0.53 (0.36–0.77), $P < 0.001$; GG v.s AA, 0.63 (0.27–1.43), $P < 0.001$), dominant (AG + GG v.s AA, 0.54 (0.38–0.78), $P < 0.001$), or the recessive model (GG vs AA + AG, 0.75 (0.33–1.71), $P < 0.001$). On the other hand, The T allele of rs2240419 was found significantly associated with increased risk of ischemic stroke, no matter with the codominant (CT v.s CC, 1.75 (1.22–2.51), $P < 0.001$; TT v.s CC, 2.67 (1.55–4.61), $P < 0.001$), dominant (CT + TT v.s CC, 1.93 (1.38–2.71), $P < 0.001$), or the recessive model (TT vs CC + CT, 2.07 (1.23–3.47), $P < 0.001$). No linkage disequilibrium was found between rs2389995 and rs2240419 of HDAC9. In conclusion, the present study demonstrated the SNP rs11984041 of HDAC9 was nonpolymorphic in Chinese Han population. The minor G allele of rs2389995 significantly decreased and the minor T allele of rs2240419 significantly increased the risk of ischemic stroke.

Keywords Histone deacetylase 9 · Atherosclerosis · Ischemic stroke · Polymorphism

Introduction

Stroke and coronary artery disease (CAD) are one of the most common causes of loss of disability and premature death years worldwide [1]. About a quarter of all strokes are classified as large-vessel stroke most of which are attributed to atherosclerosis. Atherosclerosis is an important pathological mechanism in the incidence of ischemic stroke. Atherosclerosis is a chronic inflammatory disease of the arterial vessel wall, with complications such as acute coronary syndrome or myocardial infarction, and cerebrovascular disease such as stroke [2, 3].

Large-vessel stroke shares many risk factors with CAD and both conditions have a strong heritable component. Several clinical parameters are associated with common diseases and are helpful for predicting and preventing these common diseases. In a genome-wide association study, the rs11984041 of histone deacetylase 9 (HDAC9) gene was identified as the strongest risk locus for large-vessel stroke to date [4]. Variants at this locus were subsequently shown to be also associated with CAD and common carotid intima media thickness [5, 6], suggesting that the effects of the 7p21.1 region are mediated through atherosclerosis. However, a recent study depended on the Chinese Han population demonstrated the rs11984041 of HDAC9 was not polymorphic at al. Rs2389995 and

✉ Liu Yunlong
liuyunlong_dragon@163.com

[1] Department of Vascular Surgery, The General Hospital of Beijing Military Region, No.5 Nancangmen, Beijing, China

[2] Ophthalmology Department, The General Hospital of Beijing Military Region, Beijing, China

[3] Department of Cardiology, Yanjiao People's Hospital, Hebei, China

rs2240419, the two other SNPs of chromosome 7q21.1, were significantly associated with large-vessel stroke risk (*P* values of 0.035 and 0.042, respectively) [7].

The HDAC9 gene encodes histone deacetylase 9, which is known to be involved in heart and muscle tissue development. HDAC9 is a part of the HDAC gene family, which is able to regulate gene expression patterns via deacetylate histones, resulting in chromatin condensation and transcriptional repression. The present study aims to detect whether the SNPs rs11984041, rs2389995, and rs2240419 of HDAC9 is associated with the risk of large vessel atherosclerosis.

Methods

Study Population

The study population consisted of 262 consecutive patients diagnosed with ischemic stroke and 300 age and gender matched unrelated controls between October 2012 and October 2014 at the the general hospital of beijing Militmary region. The study was approved by the Bioethics Committee of the the general hospital of beijing military region. The diagnosis of ischemic stroke was confirmed by brain magnetic resonance imaging and/or head computed tomography. Patients with cerebral embolism, arterial inflammation, malignancy, trauma, drugs, blood disease, cerebral infarction caused by vascular malformation or aneurysm, liver and kidney disease, or thyroid disease were excluded from the study. The subjects with cerebrovascular disease, neurological diseases, kidney disease, blood disorders, cancer, peripheral vascular disease, and autoimmune diseases were excluded from the control group. All subjects were of Chinese Han population and informed consent was provided. Population characteristics are listed in Table 1.

Table 1 The summary of the basic characteristics of the groups

	Ischemic stroke	Control
No. of patients	262	300
Age (year)	69.9 ± 8.1	68.8 ± 6.8
Gender (male)	167	180
Smoking (n)	124	/
Alcoholics (n)	33	/
Hypertension (n)	117	/
Diabetes mellitus (n)	46	/
Total cholesterol, mg/dL	193.9 ± 38.9	/
Triglyceride, mg/dL	140.0 ± 78.2	/
HDL-Cholesterol, mg/dL	55.0 ± 15.2	/
LDL-Cholesterol, mg/dL	120.0 ± 38.9	/
Uric acid, mg/dL	6.5 ± 1.5	/
Homocysteine, μmol/L	11.2 ± 3.1	/

Blood Collection and DNA Extraction

2 mL fasting venous blood was taken from the antecubital vein and placed in ethylenediaminetetraacetic acid-containing tubes. A Wizard® Genomic DNA Purification kit (Promega Corporation, Madison, WI, USA) was used for DNA extraction.

Genotyping

Primers were designed according to the reported sequences on the GenBank database and were synthesized by Shanghai GeneCore Biotechnologies Co., Ltd. (Shanghai, China). Polymerase chain reaction (PCR) amplification was carried out in a total volume of 25 ml containing 100 ng of genomic DNA and 10 pmol of each primer using a 9700 Thermal Cycler System (Applied Biosystems Inc., Foster City, CA) for 35 cycles at 95 °C for 40 s, 58 °C for 35 s, 72 °C for 40 s, and a final extension step at 72 °C for 5 min. PCR products exhibiting abnormally migrating bands were sequenced using an ABI 3500 Genetic Analyzer (Applied Biosystems Inc). A sequenced normal control and a negative control (with no DNA sample) were included in every experiment.

Statistical Analysis

The SPSS 19.0 software (SPSS, Inc.; Chicago, IL, USA) was used to analyze the data. A gene counting method was used to calculate the frequencies of genotypes and alleles in each group and the Hardy-Weinberg equilibrium was analyzed using the $\chi 2$ test. Genotype and allele frequencies in these groups were analyzed using the $\chi 2$ test. The odds ratio (OR) and 95 % confidence interval (CI) were calculated. Continuous results are expressed as the mean ± SD and compared with the Student t-test. *P* value <0.05 was considered to be a significant difference..

The linkage disequilibrium (LD) mapping and the associations between haplotypes of selected SNPs and risk of ischemic stroke were estimated by the HaploView software. The $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Patient Characteristics

A total of 262 patients diagnosed with ischemic stroke and 300 age and sex matched unrelated controls were enrolled in this study. Demographic data of the population studied and the number of individuals in each group were shown in Table 1. The cases have a mean age of 69.9 ± 8.1, and the cases of68.8 ± 6.8. There are 167 males (63.7 %) among the cases

and 180 males (60.0 %) among the controls. There were no significant differences between groups in terms of age and gender. The smoking rate of the cases is 47.3 %, and the alcoholics rate is 12.6 %. Hypertension happened in 117 cases and diabetes mellitus happened in 46 cases that developed ischemic stroke. The average total cholesterol of the cases was 193.9 ± 38.9 mg/dL, triglyceride was 140.0 ± 78.2 mg/dL, HDL-Cholesterol was 55.0 ± 15.2 mg/dL, LDL-Cholesterol was 120.0 ± 38.9 mg/dL, uric acid was 6.5 ± 1.5 mg/dL, and homocysteine was 11.2 ± 3.1 μmol/L.

Association of HDAC9 Polymorphisms with Ischemic Stroke Susceptibility

As expected, the distribution of the genotypes of rs2389995 and rs2240419 on HDAC9 conformed to the Hardy–Weinberg equilibrium and the genotyping success rate was 100 %. However, similar with the previously mentioned study [7], the present study also found the SNP rs11984041 of HDAC9 was nonpolymorphic in Chinese Han population.

The G allele of rs2389995 was found significantly associated with decreased risk of ischemic stroke, no matter with the codominant (AG v.s AA, 0.53 (0.36–0.77), $P < 0.001$; GG v.s AA, 0.63 (0.27–1.43), $P < 0.001$), dominant (AG + GG v.s AA, 0.54 (0.38–0.78), $P < 0.001$), or the recessive model (GG vs AA + AG, 0.75 (0.33–1.71), $P < 0.001$). On the other hand, The T allele of rs2240419 was found significantly associated with increased risk of ischemic stroke, no matter with the codominant (CT v.s CC, 1.75 (1.22–2.51), $P < 0.001$; TT v.s CC, 2.67 (1.55–4.61), $P < 0.001$), dominant (CT + TT v.s CC, 1.93 (1.38–2.71), $P < 0.001$), or the recessive model (TT vs CC + CT, 2.07 (1.23–3.47), $P < 0.001$). Tables 2 listed the genotyped and allele distributions of the SNPs for the cases and controls.

The linkage disequilibrium (LD) analysis between rs2389995 and rs2240419 showed that these two polymorphisms had no linkage disequilibrium (Fig. 1).

Discussion

Stroke is the major cause of adult chronic disability and the third leading cause of death after cardiovascular disease and the malignant tumor [8]. Approximately 85 % of all strokes are ischemic and there are 3 common subtypes of ischemic stroke: small vessel stroke, cardioembolic, and large artery atherosclerosis, with the last one happened most commonly [9]. Age, diabetes, hypertension, and hypercholesterolemia have been identified to be the risk factors of stroke. Genetic factors have also been found to be particularly important in the pathogenesis of ischemic stroke, but few associations between genetic factors and ischemic stroke have been replicated.

The HDAC9 encodes proteins responsible for deacetylation of histones and therefore regulation of chromatin structure and gene transcription, and various studies have replicated the role of the HDAC9 gene in stroke [6, 7, 10–12]. SNP rs11984041, which is within the final intron of the gene HDAC9, was firstly identified by GWAS as a novel risk factor for atherosclerosis and ischemic stroke, with an OR of 1.38(95 % CI, 1.22–1.57) [4]. Another European GWAS study also demonstrated the HDAC9 significantly increased the risk of large-vessel ischaemic stroke, with an OR of 1.42, 95 % CI of 1.28–1.57 [13]. Additionally, previous studies demonstrated the polymorphism rs11984041 of HDAC9 gene was not only associated with CAD and common carotid intima media thickness, but also identified as the strongest risk locus for large-vessel stroke to date [4–6]. However, this SNP seems to be nonpolymorphic at all among the Chinese Han

Tables 2 The genotype and allele distributions of the SNPs rs2389995 and rs2240419 of HDAC9 for the cases and controls

SNP	Genotype	N (Case/Control)	Model	OR (95 % CI)	*P*-value
rs2389995 (A/G)	AA	194/182	Codominant	1	/
	AG	58/103	/	0.53 (0.36–0.77)	$P < 0.001$
	GG	10/15	/	0.63 (0.27–1.43)	$P < 0.001$
	AA	194/182	Dominant	1	/
	AG + GG	68/118	/	0.54 (0.38–0.78)	$P < 0.001$
	AA + AG	252/285	Recessive	1	/
	GG	10/15	/	0.75 (0.33–1.71)	$P < 0.001$
rs2240419 (C/T)	CC	104/168	Codominant	1	/
	CT	115/106	/	1.75 (1.22–2.51)	$P < 0.001$
	TT	43/26	/	2.67 (1.55–4.61)	$P < 0.001$
	CC	104/168	Dominant	1	/
	CT + TT	158/132	/	1.93 (1.38–2.71)	$P < 0.001$
	CC + CT	219/274	Recessive	1	/
	TT	43/26	/	2.07 (1.23–3.47)	$P < 0.001$

Fig. 1 The linkage disequilibrium analysis between rs2389995 and rs2240419 of HDAC9

population based on the data from HapMap. A recent study performed in China also demonstrated the similar resuts, moreover, the other two polymorphisms rs2240419 and rs2389995which are 23,271 and 25,442 bp upstream of the above mentioned SNP, were significantly associated with the risk of atherosclerosis and ischemic stroke [7]. The present study also demonstrated the SNP rs11984041 of HDAC9 was nonpolymorphic at all in Chinese Han population. And the minor G allele of rs2389995 was found significantly associated with decreased risk of ischemic stroke, but the minor T allele of rs2240419 significantly increased the risk of ischemic stroke.

The mechanism by which the gene HDAC9 increases the risk of large vessel stroke is still unknown, it may be associated with the accelerate of atherosclerosis [14]. There are 18 mammalian HDACs, which fall into 4 classes on the basis of their structural and biochemical characteristics [15]. HDAC9 is expressed in heart, pancreatic islets, neuron, spinal cord, teeth, smooth and skeletal muscles, T lymphocytes, endothelium, and adipose tissues [15]. The HDAC9 protein is able to inhibit myogenesis, which is involved in the development of heart [16]. But the deleterious effects on systemic arteries has not been demonstrated yet. On the other hand, it has an effect on neuronal survival by altering brain ischaemic responses. The protein has been shown to protect neurons from apoptosis, both by inhibiting JUN phosphorylation and by repressing JUN transcription. Therefore, HDAC inhibitors have been used as a treatment for stroke [17, 18]. Another study also found the macrophage may be involved in atherosclerosis and stroke [19]. HDAC9 is most abundantly expressed during macrophage differentiation among class IIa HDACs. Systemic and macrophage HDAC9 deficiency reduces atherosclerosis development in different sites. The molecular mechanisms behind the decreased atherosclerosis are likely multifactorial, including increased macrophage cholesterol efflux and phenotypic switching of macrophages from a proinflammatory M1 to a less inflammatory M2 state via PPAR-γ [19]. Also a recent study suggestted that HDAC9 represents the disease-relevant gene at the stroke and coronary artery disease risk locus on 7p21.1, and that risk alleles in this region mediate their effects through increased HDAC9 expression. Targeted inhibition of HDAC9 might be a viable strategy to prevent atherosclerosis.

However, there were some limitation in this study. Firstly is the relative small population number. And a case control study is not of high level of evidence for supporting the conclusion. Second, no functional analysis was performed to understand the mechanism of the association between the SNPs with ischemic stroke. Moreover, besides the SNPs explored in this study, there may have more other polymorphisms in developing atherosclerosis, a genome-wide association study with a large sample size is a better choice for it.

In conclusion, the present study demonstrated the SNP rs11984041 of HDAC9 was nonpolymorphic in Chinese Han population. The minor G allele of rs2389995 significantly decreased and the minor T allele of rs2240419 significantly increased the risk of ischemic stroke.

Declaration of Interest The authors report no conflicts of interest.

Author Contribution Guo qingxu: carried out the molecular genetic studies, participated in the sequence alignment, and drafted the manuscript.

Zhang Yan: carried out the molecular genetic studies, participated in the sequence alignment.

Xu Jiannan: carried out the molecular genetic studies, participated in the sequence alignment.

Liu Yunlong: conceived of the study, participated in its design and coordination, and helped to draft the manuscript.

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