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ORIGINAL RESEARCH

To Investigate the Influence of Smoking Cessation Intention and Common Downstream Variants of *HDAC9* Gene on Large Artery Atherosclerotic Cerebral Infarction

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Objective: To investigate the association of smoking cessation intention and single nucleotide polymorphism of *HDAC9* gene with LAA-S in Han people in Hainan province.

Methods: A case-control study was conducted. Six single nucleotide polymorphisms (SNPS) of HDAC9 gene were genotyped by SNPscan genotyping technique in 248 patients with LAA-S and 237 controls in Hainan Han population. SNP loci (rs10227612, rs12669496, rs1548577, rs2074633, rs2526626, and rs2717344) were genotyped, and the genotype and allele frequencies were compared between the case and control group. At the same time, the distribution of smoking between the case and control group was compared, and the 3-year and 7-year follow-up smoking cessation between the case and control group was compared, so as to find out the effects of smoking cessation intention and HDAC9 SNP on LAA-S.

Results: (1) The GT genotype at rs10227612, GG genotype at rs2717344, and GA genotype at rs1548577 in the case group were significantly higher than those in the control group, and the differences were statistically significant. (2) There were significant differences in the distribution of smoking between the case and control group ($P < 0.05$), and there were significant differences in the smoking cessation after 3 years and 7 years of follow-up between the case and control group ($P < 0.05$). The intention to quit smoking was positively correlated with the incidence of LAA-S.

Conclusion: (1) The rs10227612, rs1548577, rs2074633, rs2717344 of HDAC9 gene may be significantly related to atherosclerotic cerebral infarction of great arteries in Hainan Han population, while rs12669496 and rs2526626 may not be related. (2) According to the statistics of smoking in the case and control group, smoking was related to large artery atherosclerotic cerebral infarction, and the intention to quit smoking was a very important factor affecting the success of smoking cessation.

Keywords: *HDAC9*, SNP, LAA-S, Hainan nationality, willingness to quit smoking

Introduction

Cerebral infarction (CI) is a common disease in neurology, with the characteristics of high incidence, high disability rate, high mortality rate, and high recurrence rate, which causes a great burden to patients and their families. In the TOAST (trial of org 10,172 in acute stroke treatment) classification, large artery atherosclerosis stroke (LAA-S) is mainly caused by atherosclerosis of carotid artery, vertebrobasilar artery, anterior cerebral artery, middle cerebral artery, and posterior cerebral artery. The most common cause of cerebral infarction is atherosclerosis. In addition to common risk factors, such as hypertension, diabetes, hyperlipidemia, smoking, and drinking, it may be related to environmental factors, lifestyle, and genetic factors, among which genetic factors play an important role in the occurrence of cerebral infarction. Studies based on twins, families, and molecular epidemiology have found that the average heritability of cerebral infarction is about 37.9%, of which the heritability of large artery atherosclerosis is about 40%, the heritability of cardio embolism is about 32.6%, and the heritability of small artery occlusion is about 16.1%, which suggests that there is genetic

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heterogeneity in cerebral infarction. Moreover, genetic factors have a greater impact on cerebral infarction with large artery atherosclerosis.[1,2]

Recent genome-wide association studies (GWAS) have found that different genes are associated with different subtypes of cerebral infarction.[3–8] For example, PITX2 and ZFHX3 genes are associated with cardiogenic embolism type.[3,4,6,7] While histone deacetylase 9 (HDAC9) is mainly associated with LAA-S.[6–10]

Some susceptibility loci of HDAC9 gene have been found to be associated with LAA-S, but the results are different. The results of A European study[6] showed that the A allele of HDAC9 gene rs11984041 was the susceptible allele of LAA-S, while a study in Shanghai, China,[11] showed that there was no significant correlation between HDAC9 gene rs11984041 and LAA-S, and a report in Beijing[12] showed that there is no significant correlation between HDAC9 gene single nucleotide polymorphism rs11984041 and large artery atherosclerotic cerebral infarction. Therefore, it is speculated that the association between HDAC9 gene single nucleotide polymorphism and large artery atherosclerotic cerebral infarction may have regional differences. Hainan Island is a relatively isolated region with rich geomorphological features and diverse climate types and is also known as the "longevity Island". There are obvious regional differences between Hainan Island and other areas in China. Therefore, the Han population of Hainan Island was selected as the research object to analyze the association between single nucleotide polymorphisms (SNPs) of HDAC9 gene and LAA-S through a case-control study in Hainan province. In the latest genome-wide association analysis, the gene detection results of 2167 European LAA-S patients and 49,159 controls showed that the downstream SNP loci of HDAC9 gene were significantly associated with large artery atherosclerotic cerebral infarction in the European population.[9] In this study, six downstream SNPs of HDAC9 gene were selected to explore the association between HDAC9 gene SNPs and LAA-S.

Smoking is one of the controllable risk factors for LAA-S, and the test report from the Chinese Center for Disease Control and Prevention shows that the success rate of smoking cessation in Chinese smokers is only 7.8%.[13] Therefore, this study explored the effect of smoking cessation intention on the success of smoking cessation, and then concluded the correlation between smoking cessation intention and large artery atherosclerotic cerebral infarction.

Subjects and Methods

Subjects of Study

Patients diagnosed with LAA-S (TOAST classification) in the Department of Neurology of Haikou People's Hospital, the First Affiliated Hospital of Hainan Medical College, the Second Affiliated Hospital of Hainan Medical College, Hainan Provincial People's Hospital, the Third People's Hospital of Haikou City, and People's Liberation Army 187 Hospital from August 2015 to December 2016 were selected as the case group.

The control group was selected from the physical examination center of the First Affiliated Hospital of Hainan Medical College, the Second Affiliated Hospital of Hainan Medical College and Hainan Cadre Sanatorium during the same period.

All participants were, originally, Han residents of Hainan, and all participants were informed and given written consent before participating in the study. The study was approved and supported by the Ethics Committee of Hainan Medical College as well as local data protection authorities.

(1) Case group

Inclusion criteria: ① Hainan Han residents; ② Aged 40–89 and able to provide a blood sample; ③ Patients met the TOAST criteria and were diagnosed with LAA-S.

Exclusion criteria: ① combined atrial fibrillation; ② Complicated with hematological diseases, vasculitis, hypercoagulable state, or autoimmune diseases; ③ Patients with LAA-S who refused to participate in this study.

(2) Control group

Inclusion criteria: ① Hainan Han residents; ② Aged 50–89 years and able to provide a blood sample; ③ No history of cerebral infarction or other major diseases.

Exclusion criteria: Persons who met the inclusion criteria and refused to participate in this study.

Study Methods

Data Collection

Data collected included gender, age, native place, ethnic group, past medical history, blood pressure, blood glucose, total cholesterol, triglyceride, high-density lipoprotein, low-density lipoprotein, head CT or MRI, carotid ultrasound, CTA, TCD, MRA or DSA, smoking, smoking cessation (telephone follow-up), and other data.

Venous Blood Extraction and DNA Extraction

The subjects who met the inclusion criteria were screened, and their informed consent was obtained. Two cubital venous blood tubes were drawn using a purple blood routine tube equipped with EDTA anticoagulant, each tube was 2mL, which was divided into 4 aliquots and placed in a frozen storage tube and stored at −20°C refrigerator. One sample (about 1mL) of the collected peripheral anticoagulant whole blood was taken out and thawed at room temperature. DNA was extracted from whole blood in strict accordance with the instructions of DNA extraction kit (Yaneng Biotechnology Co., LTD.).

Determination of DNA Concentration and Purity

Prepare a lot of functional nucleic acid tester, operate on the computer to determine the concentration and purity, and read the ratio of OD260/OD280, that is, the purity of the extracted DNA, if the ratio is 1.7–2.0, it means that the measured DNA purity is high, containing low impurities. The ratio of OD260/OD280 obtained in this study was in the above range, and the purity was relatively high, which met the requirements of this study. The extracted DNA samples were stored in the refrigerator at −20°C for a long time.

Selection of SNP Sites of HDAC9 Gene

GWAS results in the Caucasian population showed SNPs rs11984041 (chromosome 7:18992312)[8] and rs2107595 (chromosome 7:18992312). 19009765) (human genome reference assembly GRCh38/hg38)[11] were associated with large-artery atherosclerotic cerebral infarction, so we initially selected the region between these two loci and the region near them (3kbp upstream and downstream of the two loci). The region chromosome 7:18989312–19012765 (23.45kbp) (human genome reference assembly GRCh38/hg38) was selected as the research object. In the 1000 Genomes Project database of Han Chinese in Beijing (CHB) and Southern Han Chinese (CHS), Setting $r^2 \geq 0.90$ and minor allele frequency (MAF) ≥0.05, six tag SNPs (rs10227612, rs12669496, rs1548577, rs2074633, rs2526626, and rs2717344) were selected in this region. Because the MAF of rs11984041 in CHB and CHS was 0, that is, rs11984041 was monomorphous in Chinese Han population, so rs11984041 was not included in the six SNPs.

Genotyping

Genotyping of all SNPS was performed with a custom-designed 48-heavy SNPscan™ kit (catalog number: G0104; Tianhao Biological Technology Co., LTD., Shanghai, China), this kit was developed according to the patented SNP genotyping technology of Shanghai Tianhao Biological Technology Co., LTD. The basic principle of SNPscan genotyping technology is to use the high specificity of ligase ligation reaction to realize the identification of SNP alleles, and then by introducing non-specific sequences of different lengths at the end of the ligation probe and obtaining ligation products of different lengths corresponding to the loci through ligase addition reaction. The ligation products were amplified by PCR using fluorescence-labeled universal primers, and the amplification products were separated by fluorescence capillary electrophoresis. Finally, the genotypes of each SNP locus were obtained by analyzing the electrophoresis patterns.

Statistical Methods

SPSS19.0 statistical software was used to analyze the data. The measurement data were expressed as mean ± standard deviation (X ± S), *t* test was used to compare the mean of the two samples, and chi-square test was used to compare the count data. When the genotype and allele frequency were less than 5, Fisher's exact test was used. The relative risk of alleles was expressed as Odds ratio (OR) and 95% confidence interval (CI). $P < 0.05$ was considered statistically significant. Logistic regression model was used to analyze the relationship between allele frequency distribution and LAA-S, and $P < 0.005$ was considered statistically significant.

Results

Basic Information of Genotyping SNPs Loci

The basic information of the six SNP sites in the *HDAC9* gene is shown in Table 1. The minor allele frequencies (MAF) of the six SNPs were between 0.2215 and 0.6109, with MAF ≥0.05.

Hardy-Weinberg Equilibrium Test of Genotype Distribution Frequency of HDAC9 Gene Locus

The gene frequencies of six SNPs (rs10227612, rs12669496, rs1548577, rs2074633, rs2526626, and rs2717344) in 248 LAA-S patients and 237 controls were tested for Hardy-Weinberg equilibrium. Table 2 shows that the distribution frequencies of the 6 SNPs in HDAC9 gene in the case group and the control group were in accordance with the Hardy-Weinberg law of genetic equilibrium, and the two groups were both genetic equilibrium populations (P > 0.005). The gene frequencies of the selected study population were representative of the population.

Table 1 Basic Information of SNPs for Genotyping

Sequence No.	SNP	A Location on a Chromosome	A location on a gene	Major > Minor Allelic Gene	Minor Alleles	MAF Case Health
1	rs10227612	Chr7: 18,994,956	Intron region	G>T	G	0.4173 0.3544
2	rs12669496	Chr7: 19,008,849	3' end	T>C	T	0.2359 0.2278
3	rs1548577	Chr7: 19,009,539	3' end	G>A	A	0.6109 0.5443
4	rs2074633	Chr7: 18,996,297	3' untranslated region	C>T	C	0.3750 0.3059
5	rs2526626	Chr7: 19,001,714	3' untranslated zone	A>G	A	0.2339 0.2215
6	rs2717344	Chr7: 18,991,230	Intron region	A>G	A	0.2964 0.3629

Abbreviation: MAF stands for minor allele frequency; [a]The reference genome sequence GRCh38/hg38 was used to determine the chromosomal position.

Table 2 Hardy-Weinberg Equilibrium Test for Genotype Distribution Frequencies of SNPs in the Case Group and the Control Group

Serial No.	Locus	Groups	χ^2	*P*
1	rs10227612	Case group	0.0973	0.7551
		Control group	4.2104	0.0402
2	rs12669496	Case group	0.0793	0.7782
		Control group	0.3922	0.5311
3	rs1548577	Case group	0.8991	0.3430
		Control group	1.5702	0.2102
4	rs2074633	Case group	0.7168	0.3972
		Control group	4.3554	0.0369
5	rs2526626	Case group	0.3074	0.5793
		Control group	0.2664	0.6058
6	rs2717344	Case group	0.0569	0.8115
		Control group	3.4249	0.0642

Comparison of Genotype Frequency and Allele Frequency Between Case Group and Control Group

The success rate of genotyping for each SNP was 100%. The genotype consistency of repeated samples was more than 99.8%, and the genotype distribution of all SNPs did not deviate from Hardy-Weinberg equilibrium in all study individuals ($P > 0.005$). The genotype frequency and allele frequency distribution of the six SNPs are shown in Table 3. The genotype frequencies and allele frequencies of six SNPs (rs10227612, rs12669496, rs1548577, rs2074633, rs2526626, and rs2717344) in the case group and the control group were statistically analyzed. Four SNPs (rs10227612, rs1548577, rs2074633, and rs2717344) were found to be significantly associated with LAA-S.

For rs10227612, the genotype frequencies of GG, TT, and GT were 16.94%, 33.47%, and 49.60% in the case group and 15.61%, 44.73%, and 39.66% in the control group, respectively. The allele frequencies of G and T were 41.73% and 58.27% in the case group and 35.44% and 64.56% in the control group, respectively. The frequencies of GT genotype and G allele in the case group were significantly higher than those in the control group, and the differences were statistically significant ($P < 0.05$), as shown in Table 3.

For rs12669496, the frequencies of CC, TT, and CT genotypes were 58.06%, 5.24%, and 36.69% in the case group, and 60.34%, 5.91%, and 33.76% in the control group, respectively. The C and T allele frequencies were 76.41% and 23.59% in the case group and 77.22% and 22.78% in the control group, respectively. The frequencies of CT genotype and T allele in the case group were slightly higher than those in the control group, but there was no significant difference after statistical analysis ($P > 0.05$), as shown in Table 3.

For rs1548577, the frequencies of GG, AA, and GA genotypes in the case group were 13.71%, 35.89%, and 50.40%, and those in the control group were 22.78%, 31.65%, and 45.57%, respectively. The allele frequencies of G and A were 38.91% and 61.09% in the case group and 45.57% and 54.43% in the control group, respectively. The frequencies of GA

Table 3 Comparison of Genotype Frequency and Allele Frequency Between Case Group and Control Group

Sequence No.	Locus	Genotype Alleles	Case Group (n=248) Genotype/Allele Frequency	Control Group (n=237) Genotype/Allele Frequencies	χ^2	P
1	rs10227612	TT	83 (33.47%)	106 (44.73%)	6.745	0.034
		GT	123 (49.60%)	94 (39.66%)		
		GG	42 (16.94%)	37 (15.61%)		
		T	289 (58.27%)	306 (64.56%)	4.045	0.044
		G	207 (41.73%)	168 (35.44%)		
2	rs12669496	CC	144 (58.06%)	143 (60.34%)	0.499	0.779
		CT	91 (36.69%)	80 (33.76%)		
		TT	13 (5.24%).	14 (5.91%).		
		C	379 (76.41%)	366 (77.22%)	0.088	0.767
		T	117 (23.59%)	108 (22.78%)		
3	rs1548577	AA	89 (35.89%)	75 (31.65%)	6.735	0.034
		GA	125 (50.40%)	108 (45.57%)		
		GG	34 (13.71%).	54 (22.78%).		
		A	303 (61.09%)	258 (54.43%)	6.063	0.014
		G	193 (38.91%)	226 (45.57%)		

(*Continued*)

Table 3 (Continued).

Sequence No.	Locus	Genotype Alleles	Case Group (n=248) Genotype/Allele Frequency	Control Group (n=237) Genotype/Allele Frequencies	χ^2	*P*
4	rs2074633	TT	100 (40.32%)	121 (51.05%)	5.643	0.060
		CT	110 (44.35%)	87 (36.71%)		
		CC	38 (15.32%).	29 (12.24%).		
		T	310 (62.50%)	329 (69.41%)	5.147	0.023
		C	186 (37.50%)	145 (30.59%)		
5	rs2526626	GG	144 (58.06%)	145 (61.18%)	0.783	0.676
		GA	92 (37.10%)	79 (33.33%)		
		AA	12 (4.84%).	13 (5.49%).		
		G	380 (76.61%)	369 (77.85%)	0.210	0.647
		A	116 (23.39%)	105 (22.15%)		
6	rs2717344	GG	122 (49.19%)	103 (43.46%)	6.660	0.036
		GA	105 (42.34%)	96 (40.51%)		
		AA	21 (8.47%).	38 (16.03%).		
		G	349 (70.36%)	302 (63.71%)	4.856	0.028
		A	147 (29.64%)	172 (36.29%)		

genotype and A allele in the case group were significantly higher than those in the control group, and the differences were statistically significant ($P < 0.05$), as shown in Table 3.

For rs2074633, the frequencies of CC, TT, and CT genotypes were 15.32%, 40.32% and 44.35% in the case group and 12.24%, 51.05%, and 36.71% in the control group, respectively. The C and T allele frequencies were 37.50% and 62.50% in the case group and 30.59% and 69.41% in the control group, respectively. The frequencies of CT genotype and C allele in the case group were slightly higher than those in the control group, but there was no significant difference in CT genotype ($P > 0.05$), while the difference in C allele was statistically significant ($P < 0.05$), as shown in Table 3.

For rs2526626, the frequencies of GG, AA, and GA genotypes were 58.06%, 4.84%, and 37.10% in the case group, and 61.18%, 5.49%, and 33.33% in the control group, respectively. The allele frequencies of G and A were 76.61% and 23.39% in the case group and 77.85% and 22.15% in the control group, respectively. The frequencies of GA genotype and A allele in the case group were slightly higher than those in the control group, but there was no significant difference after statistical analysis ($P > 0.05$), as shown in Table 3.

For rs2717344, the frequencies of GG, AA, and GA genotypes were 49.19%, 8.47%, and 42.34% in the case group, and 43.46%, 16.03%, and 40.51% in the control group, respectively. The allele frequencies of G and A were 70.36% and 29.64% in the case group and 63.71% and 36.29% in the control group, respectively. The frequencies of GG genotype and G allele in the case group were significantly higher than those in the control group, and the differences were statistically significant ($P < 0.05$), as shown in Table 3.

Correlation Analysis Between SNPs and LAA-S

To further evaluate the pathogenic degree of four statistically significant SNPs in *HDAC9* gene in LAA-S, Logistic regression analysis was used, and the results are shown in Table 4. The table shows that T allele of rs10227612 is a protective factor for LAA-S (OR = 0.767[0.591–0.993]), and the difference is statistically significant (P = 0.044). The

Table 4 Logistic Regression Analysis of HDAC9 Gene SNP Alleles and LAA-S

Serial No.	Locus	Risk Allele	OR (95% CI)	P
1	rs10227612	T	0.767 (0.591–0.993)	0.044
2	rs12670036	A	0.771 (0.596–0.998)	0.048
3	rs1548577	A	1.375 (1.067–1.773)	0.014
4	rs2074633	T	0.735 (0.563–0.959)	0.023

A allele of rs12670036 was a protective factor for LAA-S (OR = 0.771[0.596–0.998]), and the difference was statistically significant (P = 0.048). An allele of rs1548577 was a risk factor for LAA-S (OR = 1.375[1.067–1.773]), and the difference was statistically significant (P = 0.014). T allele of rs2074633 was a protective factor for LAA-S (OR = 0.735 [0.563–0.959]), and the difference was statistically significant (P = 0.023).

Statistics of the Distribution of Smokers in the Case Group and the Control Group

According to Table 5, the P value of K–S test and S-W test is 0, then P < 0.05, the difference is statistically significant, and the distribution is skewed, which indicates that there is a significant difference in the number of smokers in the two groups. The K–S test W = 0.365, and the S-W test W = 0.633, which indicated that the number of smokers in the case group was more than that in the healthy group.

Statistics of Smoking Cessation in Case Group and Control Group at 3- and 7-Years Follow-Up

Statistics of Successful Smoking Cessation in Case Group and Control Group at 3 and 7 Years Follow-Up

According to Table 6, the P value of K–S test and S-W test is 0, then P < 0.05, the difference is statistically significant, and the distribution is skewed, which indicates that there is a significant difference in the number of smokers in the four groups. In the third year after successful smoking cessation, $W_{健康}$=0.389 was higher than W = 0.388 in K–S test, and W = 0.706 was higher than W = 0.680 in S-W test. In the seventh year of successful smoking cessation, $W_{健康}$=0.387 was higher than W = 0.323 in K–S test, W = 0.798 was higher than W = 0.743 in S-W test. This indicated that the success rate of smoking cessation in the case group was higher than that in the healthy group.

3.6.2 Statistics of the correlation between smoking cessation success and quitting intention of smokers in case group and control group.

Table 5 Normality Tests of Smoking in Healthy and Case Groups

	Kolmogorov–Smirnova		Shapiro–Wilk	
	Statistics	Sig.	Statistics	Sig.
The healthy and case groups smoked	0.365	0.000	0.633	0.000

Table 6 Normality Tests for Smoking Cessation in Case and Healthy Groups

	Kolmogorov–Smirnova		Shapiro–Wilk	
	Statistics	Sig.	Statistics	Sig.
Successful smoking cessation in the case group (3 years of follow-up)	0.389	0.000	0.706	0.000
Successful smoking cessation in the case group (at 7th year of follow-up)	0.387	0.000	0.798	0.000
Successful smoking cessation in the healthy group (3 years of follow-up)	0.388	0.000	0.680	0.000
Successful smoking cessation in the healthy group (at 7 years of follow-up)	0.323	0.000	0.743	0.000

Table 7 Spearman Correlation Coefficient of Smoking Cessation Intention in Case Group

Coefficient of Correlation	Successful Smoking Cessation (at 3 Years of Follow-up)	Successful Smoking Cessation (7 Years of Follow-up)	Intention to Quit Smoking
Successful smoking cessation (3-year follow-up)	1.000	0.518**	0.498**
Successful smoking cessation (at 7 years of follow-up)	0.518**	1.000	0.978**
Willingness to quit smoking	0.498**	0.978**	1.000

Notes: **The correlation is significant at a confidence (two-test) of 0.01.

Table 8 Spearman Correlation Coefficient of Intention to Quit Smoking in Healthy Group

Correlation Coefficient	Successful Smoking Cessation (at 3 Years of Follow-up)	Successful Smoking Cessation (7 Years of Follow-up)	Intention to Quit Smoking
Successful smoking cessation (3-year follow-up)	1.000	0.834**	0.438**
Successful smoking cessation (at 7 years of follow-up)	0.834**	1.000	0.657**
Willingness to quit smoking	0.438**	0.657**	1.000

Notes: **The correlation is significant at a confidence (two-test) of 0.01.

Table 7 shows that the correlation coefficient between the willingness to quit smoking and the successful follow-up in the third year was 0.498, and the correlation coefficient between the willingness to quit smoking and the successful follow-up in the seventh year was 0.978.

Table 8 shows that the correlation coefficient between the willingness to quit smoking and the successful follow-up of the third year was 0.438, and the correlation coefficient between the willingness to quit smoking and the successful follow-up of the seventh year was 0.657. According to Tables 7 and 8, when the confidence level (double test) is 0.01, the correlation between the willingness to quit smoking and the success of quitting smoking is significant, that is, the success rate of quitting smoking of the willingness to quit smoking is higher than that of the non-willingness to quit smoking.

Discussion

Single nucleotide polymorphism (SNP) refers to DNA sequence polymorphism caused by single nucleotide variation at the genome level. This variation involves only a single base variation and is generally caused by a single base transition or transversion. SNP is the most common type of human heritable variation. It is widely distributed in the human genome, and there is one SNP every 300 to 1000 bp in human DNA.

Histone deacetylases (HDacs) are a large family of enzymes that, together with histone acetyltransferases (HAT), regulate the acetylation of histone and non-histone proteins, keeping the process of histone acetylation and deacetylation in a dynamic balance and regulating the gene expression of cells. So far, a total of 18 HDAC isoforms have been found in the human body.[14] HDAC9 gene, a subtype of HDAC gene family, is located at 7p21 and consists of 26 exons. The encoded HDAC9 consists of 1069 amino acids. The main effect of HDAC9 is by deacetylating the expression of key node genes in histone 3 (H3) and histone 4 (H4). In addition, there are a variety of HDAC9 isoforms, which are expressed in brain, skeletal muscle, heart muscle, placenta, kidney, and other tissues.[15,16] In terms of angiogenesis, HDAC9 can promote the proliferation and angiogenesis of vascular endothelial cells.[17] Lan Q et al[18] reported that four single nucleotide polymorphisms (rs1700874, rs10248565, rs11761619, and rs9316119) of HDAC9 gene were significantly associated with lung cancer. Some studies[19,20] have also proved that HDAC9 gene is related to kidney disease and ovarian disease. Genome-wide association analysis and case-control studies have shown that some single nucleotide polymorphisms (SNPs) of HDAC9 are associated with LAA-S,[6–9] and the expression of HDAC9 in carotid, aortic, and femoral atherosclerotic plaques is significantly higher than that in normal controls.[8] These results suggest that HDAC9 may increase the risk of cerebral infarction by promoting atherosclerosis, but the molecular mechanism of its association and the molecular mechanism in the development of large artery atherosclerosis needs to be further elucidated.

In this study, the adjacent probes paired on the same template were specifically linked under the action of ligase-chain reaction to produce ligation products. The ligation products were amplified by multiplex fluorescent PCR, and finally the genotypes of each SNP site were obtained by analysis of electrophoresis patterns. Finally, the genotypes of each SNP were obtained by

electrophoresis analysis. The correlation between single nucleotide polymorphisms of HDAC9 gene and large artery atherosclerotic cerebral infarction was studied. Ligase chain reaction (LCR) is a promising in vitro nucleic acid amplification technology after PCR. Four oligonucleotide primers can be used to amplify nucleic acids in vitro through denaturation, renature and ligation under the action of thermostable ligases, which can accurately detect single or a few base mutations in the target gene. It has been widely used in the fields of life science, genetic engineering, disease diagnosis, forensic medicine, and other fields. Multiplex PCR, also known as multiplex primer PCR or compound PCR, is a PCR reaction in which more than two pairs of primers are added to the same PCR reaction system to amplify multiple nucleic acid fragments at the same time. Its reaction principle, reaction reagents, and operation process are the same as those of general PCR. Because multiplex PCR amplifies multiple-target genes at the same time, it has the advantages of saving time, reducing cost and improving efficiency.

Genome-wide association analysis showed that the downstream SNPs of HDAC9 gene were significantly associated with large artery atherosclerotic cerebral infarction.[6] By browsing the human genome data of NCBI database, six SNPS (rs10227612, rs12669496, rs1548577, rs2074633, rs2526626, rs) between 18,989,312 and 19,012,765 located in the downstream of HDAC9 gene on Chr7 were selected 2,717,344) to investigate the association between single nucleotide polymorphisms (SNPs) of HDAC9 and LAA-S. A total of 248 patients with large artery atherosclerotic cerebral infarction and 237 controls from Hainan were enrolled in this study. The association between *HDAC9* gene SNPs and LAA-S was investigated. One of these SNPS (rs10227612) has also been reported at home and abroad, and the research results are different. A study in Shanghai reported that the rs10227612 locus of *HDAC9* gene was not significantly associated with LAA-S, while the results of this study showed that the rs10227612 locus of *HDAC9* gene was significantly associated with LAA-S. The T allele was a protective factor for LAA-S (OR = 0.767[0.591–0.993]). The results of the two studies were different. It is speculated that the single nucleotide polymorphism of *HDAC9* gene may be different due to the differences between ethnic groups.[11] On the other hand, it may be due to the inconsistency of the inclusion and exclusion criteria of the selected subjects. It is also possible that different studies included different sample sizes, resulting in inconsistent statistical validity.

In conclusion, by comparing the results of Hainan Island residents with those of other studies at home and abroad, the association between *HDAC9* gene single nucleotide polymorphism rs10227612 and LAA-S has obvious regional differences, resulting in genetic heterogeneity. However, the sample size of this study is small, and further research with a larger sample size is needed to confirm the single nucleotide polymorphism of HDAC9 gene and genetic heterogeneity of atherosclerotic cerebral infarction in large arteries of Han people in Hainan. In addition, the follow-up interval of the study object is long, so the follow-up frequency should be increased.

At the same time, this study compared the distribution of smoking between the case group and the healthy group, and the number of smokers in the case group was much more than that in the healthy group, indicating the correlation between smoking and LAA-S. The 3-year and 7-year telephone follow-up showed that the success rate of smoking cessation in the case group was higher than that in the control group, and those who had the intention to quit smoking had a higher success rate of smoking cessation. They were more worried about the impact of smoking on health, disease recovery, and quality of life, which would prompt smokers to consider smoking cessation. Therefore, the intention to quit smoking is the most important influencing factor for smokers to quit smoking.

Smoking is largely a psychological need, a habit and a psychological dependence. Developing healthy lifestyle habits, such as regular exercise, eating a balanced diet, and maintaining a good quality of sleep can help ease the discomfort during smoking cessation. At the same time, the whole society should pay attention to and vigorously publicize the harm of smoking, and medical institutions should take the initiative to open and publicize smoking cessation clinics, encourage and help quitters to establish firm confidence and perseverance, provide regular follow-up services, and continue to strengthen the effect of smoking cessation, so that more and more smokers are free from the harm of tobacco. In this study, there were not many smokers collected, and the sample size of smokers was relatively small. Further investigation will expand the sample size to more fully understand the influencing factors of smoking and quitting intention and provide theoretical basis for comprehensive tobacco control.

Ethics Statement

All participants are Han Chinese indigenous residents of Hainan, and all participants were notified and given written consent before participating in the study. This study was approved and supported by the Ethics Committee of Hainan

Medical College and local data protection institutions. Project application code: H0906. And our study complies with the Declaration of Helsinki.

Disclosure

The authors report no conflicts of interest in this work.

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