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Research Article

HDAC9 Polymorphism is Associated with Carotid Plaque in a Korean Population

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Abstract

Background: An association between histone deactylase (HDAC) polymorphism and large vessel ischemic stroke was identified in two genome-wide association studies. The aim of this study was to investigate the association between *HDAC9*rs 2107595 polymorphism and carotid atherosclerosis in Korean adults.

Methods and Findings: The study population consisted of 1954 subjects aged 50 years or more from the Dong-gu Study. Carotid ultrasonography was performed to measure common carotid (CCA) intima-media thickness (IMT) and the presence of carotid plaques. Genotyping was performed by high-resolution melting analysis. After adjustment for age, diabetes, and total cholesterol, carriers of AA and AG had a higher risk ofcarotid plaquethan the GG genotype (odds ratio $[OR]_{AG}$= 1.38, 95%confidence interval [CI]=1.12-1.70; OR_{AA} = 1.46:1.05-2.02). After further adjustment forcovariates, ORs of AA and AG for carotid plaque were slightly increased (OR_{AG}= 1.44: 1.17-1.78; OR_{AA} = 1.57: 1.12-2.20). However, *HDAC9* genotype was not associated with CCA-IMT.

Conclusions: We found that the *HDAC9*rs 2107595 polymorphism is associated with carotid plaque. These results suggest that the *HDAC9* polymorphism may be an independent risk factor for cardiovascular disease.

Keywords: *HDAC9*; Polymorphism; Carotid artery plaque; Intima-media thickness

Introduction

Stroke is one of the most common causes of morbidity and mortality in the world [1, 2]. In Korea, it is the third common cause of death, after cancer and heart disease [2]. Strokes are classified as is chemic or hemorrhagic, with more than 80% of them being ischemic [3]. Animal models and twin studies have suggested a significant genetic component in ischemic stroke [4]. Many candidate gene studies and several genome-wide association studies (GWAS) have been performed to identify common genetic variants [5-9]. However, these results are inconsistent. Polymorphisms of the β-fibrinogen (FGβ) and phosphodiesterase 4 (PDE4D) genes were associated with the risk of ischemic stroke [7, 8, 10], which is a finding thatwas not confirmed in other studies [9, 11]. Carotid intima-media thickness (IMT) is used to detect atherosclerotic disease and isarisk factor for cardiovascular disease (CVD) and stroke [12-16]. Genetic studies have shown that several genes were associated with carotid IMT [17-19].

Two genome-wide association study (GWAS) identified an association between two single nucleotide polymorphisms (SNPs) (rs11984041 and rs2107595) in histone deactylase 9 (*HDAC9)* genes and large vessel ischemic stroke [5, 6]. The underlying mechanism of this association remains unclear. HDAC genes encode proteins that deacetylate nucleosomal histones and alter the chromatin structure to affect the gene transcription factor [20]. *HDAC9*, one of the class II HDACs, is ubiquitously expressed in the brain, skeletal muscles and cardiac tissue. Additionally, it is expressed in healthy human cerebral and systemic arteries and is upregulated in human atherosclerotic plaques in various arteries [21]. To explore the mechanisms underlying the 7p21.1 association with large artery stroke, a meta-analysis demonstrated thatthese two SNPswere also associated with common carotid artery intima-media thickness (CCA-IMT) and with the presence of carotid plaques, as well [21].

The aim of this study was to evaluate the association between the HDAC9 polymorphism and carotid atherosclerosis in Korean adults. This finding has not been replicated in Asian populations.

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Methods

Subjects

The Dong-gu Study is an ongoing prospective study designed to investigate the prevalence, incidence and risk factors for chronic diseases in urban populations. The details ofthe study subjects and measurements have been published previously [22]. In the Dong-gu Study, 9260 subjects aged 50 years and older were enrolled between 2007 and 2010 in the Dong-gu district of Gwangju Metropolitan City in South Korea. In total, 2000 subjects were randomly selected from the cohort of the Dong-gu Study cohort, with stratification by sex. Of the 2000 randomly selected subjects, 46 subjects were excluded because of missing data for carotid ultrasonography, blood lipid tests and life-style. Total 1954participants (978 men and 976 women) were included in the final analyses.

Ethics statement

This study was approved by the Institutional Review Board of Chonnam National University Hospital, and written informed consent was obtained from each subject.

HDAC9 genotyping

Genomic DNA was extracted from peripheral blood with a QIA amp DNA Mini Kit (Qiagen Inc., Chatsworth, CA, USA) according to the manufacturer's protocol. Because rs11984041 is not polymorphic in the Asian population in the Hap Map project, only rs2107595 genotyping was performed by high-resolution melting analysis as described previously [23]. The primer pair was F: 5'-TTTTGTGTGCTTGTACATTCTTTTT-3' and R: 5'-ACTCATTGAGAAGGATGAGGAG-3'. The cycling conditions were 5min at 95 °C, followed by 40 cycles of 94 °C for 30 s, 59 °C for 30 s, and 72 °C for 30 s. In the melting analysis, the temperature was increased from 70 to 81 °C at a rate of 0.1 °C per second.

Carotid ultrasound

Details of the ultrasound methods were published previously [24]. Carotid ultrasonography was performed using ahigh-resolution mode B ultrasound system (SONOACE 9900, Medison, Korea) with an electrical linear array transducer (7.5 MHz). The CCA-IMTwas determined as the average of the maximum IMT values for the left and right CCAs. The presence of carotid plaques was defined as focal structures that encroached into the lumen by at least 100% of the surrounding IMT value.

Other clinical variables

Smoking status was classified as non-smoker or former smoker versus current smoker. The body mass index (BMI) was calculated by dividing the weight (in kilograms) by the height squared (in meters squared). Diabetes was defined as having a fasting glucose level >126 mg/dl (7 mmol/l) or use of hypoglycemic medication. Hypertension was defined by systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥90 mmHg, or using of antihypertensive medication. Blood samples were drawn from an antecubital vein in the morning after a 12-h overnight fast. Serum total cholesterol, high-density lipoprotein (HDL) -cholesterol, triglyceride and fasting blood glucose levels were measured by enzymatic methods using an automatic analyzer (model 7600 chemical analyzer; Hitachi Ltd, Tokyo, Japan).

Table 1: Characteristics of the study subjects according to *HDAC9* genotype.

	GG	AG	AA	p-value
N	918	835	201	
Age (years)	64.8 ± 8.3	65.7 ± 7.9	64.9 ± 8.0	0.060
Men (%)	442 (48.1)	432 (51.7)	104 (51.7)	0.285
Body mass index (kg/m²)	24.3 ± 2.8	24.3 ± 2.9	24.4 ± 3.0	0.906
Current smoking (%)	115 (12.5)	108 (12.9)	31 (15.4)	0.541
Hypertension (%)	427 (46.5)	385 (46.1)	85 (42.3)	0.546
Diabetes mellitus (%)	204 (22.2)	150(18.0)	37 (18.4)	0.070
Total cholesterol (mg/dl)	201.1 ± 40.9	197.4 ± 38.3	194.3 ± 34.8	0.036
HDL cholesterol (mg/dl)	51.9 ± 12.2	51.4 ± 12.2	50.5 ± 12.2	0.307
Triglyceride (mg/dl)	140.1 ± 120.0	142.6 ± 96.7	137.8 ± 84.5	0.808
CCA-IMT (mm)	0.721 ± 0.155	0.727 ± 0.150	0.734 ± 0.164	0.488
Carotid plaque (%)	309 (33.7)	353 (42.3)	85 (42.3)	<0.001

Values are mean ± SD or number (percentage).
HDL, high-density lipoprotein; CCA-IMT, common carotid artery intima-media thickness.

Statistical analysis

The data are presented as the means ± standard deviation (SD), or as percentages for the categorical variables. Analysis of variance and Pearson's chi-square test were used to compare the baseline characteristics across the *HDAC9* genotypes. Multiple linear regression and logistic regression modelswere used to evaluate the associations of *HDAC9* genotype with CCA-IMT and carotid plaque formation. The analyses were adjusted for age, diabetes, and total cholesterol with a P-value of less than 0.2 in association with *HADC9* genotype. In addition, we conducted a stratified analysis by age to assess effect modification by age (50-64 years and ≥65 years). Statistical analyses were performed using SPSS version 21.0 (SPSS, Inc., an IBM Company, Chicago, Illinois, USA). The significance level was set at a P value of <0.05.

Results

The *HDAC9* genotype frequencies were 47.0% for *GG*, 42.7% for AG and 10.3% for AA. The overall mean age was 65.2±8.1years. The *HDAC9* genotype frequencies were consistent with Hardy-Weinberg equilibrium (p= 0.55). There were no significant differences in the proportion of males, smoking, hypertension, and diabetes, serum HDL-cholesterollevel, and triglyceride levelsexcept for total cholesterol level across the *HDAC9* genotypes (Table 1).

HDAC9 polymorphism was not associated with CCA-IMT (Table 2). However, there was a significant association between *HDAC9* polymorphism and carotid plaque formation. The carriers of AA and AG had higher risk for carotid plaque formation than the GG genotype (odds ratio $[OR]_{AG}$= 1.44, 95% confidence interval [CI] = 1.19-1.75; OR_{AA} = 1.44, 95% CI = 1.06-1.97, respectively). After adjustment forage, diabetes, and total cholesterol, ORs for carotid plaque were slightly increased of AA and AG (OR_{AG} = 1.43, 95% CI = 1.17-1.75; OR_{AA} = 1.52, 95% CI = 1.10-2.10, respectively) (Table 3). In a stratified analysis by age, although there was no significant interaction by age (p=0.332), this association was more evident in the younger group (50-64 years) than in the elderly (≥65 years) (Table 4).

Table 2: Association of *HDAC9* genotype with intima-media thickness.

Genotype	Model I	Model II
GG	0.725 ± 0.005	0.723 ± 0.005
AG	0.723 ± 0.005	0.725 ± 0.005
AA	0.736 ± 0.010	0.737 ± 0.010
P for trend	0.522	0.301

Data are presented as mean ± standard error.
Model I, adjusted age and sex.
Model II, further adjusted for BMI, smoking, diabetes, hypertension, total cholesterol, HDL cholesterol, and log-transformed triglycerides.

Table 3: Odds ratio and 95% confidence interval of HDAC9 genotype for carotid plaque.

Genotype	Model I	Model II
GG	1 (reference)	1 (reference)
AG	1.38 (1.13-1.70)	1.44 (1.17-1.77)
AA	1.45 (1.05-2.02)	1.56 (1.12-2.18)
P for trend	0.002	<0.001

Data are presented as mean ± standard error.
Model I, adjusted age and sex.
Model II, further adjusted for BMI, smoking, diabetes, hypertension, total cholesterol, HDL cholesterol, and log-transformed triglycerides.

Table 4: Odds ratios and 95% confidence intervals of *HDAC9* genotype for carotid plaque according to age group.

Genotype	<65 years	≥ 65 years	*P*for interaction
GG	1 (reference)	1 (reference)	0.332
AG	1.59 (1.17-2.17)	1.31 (1.00-1.72)	
AA	1.96 (1.22-3.14)	1.22 (0.79-1.90)	
*P*for trend	0.001	0.102	

*Adjusted for age, diabetes, and total cholesterol.

Discussion

In this study, we demonstrated that the *HDAC9*rs 2107595 is associated with an increased risk for carotid plaques. Although this finding is not novel, to our knowledge, this study is the first to investigate the association of *HDAC9* polymorphism and carotid atherosclerosis in an Asian population.

AGWAS revealed that rs11984041 minor A allele in *HDAC9* had an increased risk of large vessel ischemic stroke [6]. In a Chinese study [25], the rs2389995A allele and rs2240419 T allelein *HDAC9* were associated with an increased risk of large-vessel stroke. The underlying mechanism by which *HDAC9* variants increase the risk of large vessel stroke is not clear yet.A meta-analysis of 31,210 participants of European ancestry revealed that both SNPs (rs11984041 and rs2107595) were associated with CCA-IMT and with the presence of carotid plaques [21]. They also found that *HDAC9* expression was up regulated in carotid plaque formations compared with left internal thoracic controls and that *HDAC9* mRNA expression was greater in carotid plaque than in femoral plaques. Hence, they suggested that alterations in *HDAC9* expression promoting atherosclerosis could mediate the association between *HDAC9* polymorphism and the risk of large vessel ischemic stroke. In accordance with thisstudy, we report that *HDAC9* polymorphism was associated with carotid plaques, even though it was not associated with carotid IMT.

Sodium valproate, an antiepileptic drug, is one of HDAC inhibitors that have been shown to attenuate atherosclerosis in animal models [26]. HDAC inhibitors have been suggested as a treatment for stroke [27, 28]. The functional mechanism show *HDAC9* gene variants influence atherosclerosis is unknown. Recent evidence suggests that the roles of *HDAC9* in adipogenic differentiation and macrophage development could explain this mechanism. *HDAC9* is an endogenous negative regulator of adipogenic differentiation [29]. *HDAC9* gene deletion improves adipogenic differentiation and systemic metabolic state during a high-fat feeding [30]. Moreover, *HDAC9* gene deletion upregulated the expression of beige adipocyte marker genes in association with increased energy expenditure and adaptive thermogenesis. Cao *et al.* have showed that systemic and macrophage *HDAC9* deficiency resulted decreased atherosclerosis, increased macrophage cholesterol efflux by *ABCA1* and *ABCG1*, and induced phenotypic switching of macrophages from a proinflammatory M1 state to a less inflammatory M2 state via peroxisome proliferator-activated receptors (PPAR)-γ [31]. Therefore, they concluded that macrophage *HDAC9* upregulation is atherogenic via suppression of cholesterol efflux and generation of alternatively activated macrophages in atherosclerosis.

Our study has potential limitations. We mightnot have sufficient power to detect small to moderate effects because of our relatively small sample [21]. The power of our study was 32.3% to detect a difference of 0.077mm in CCA-IMT, which was shown in a meta-analysis. In this study, carriers of AA and AG tended to have higher, although not significant, IMT values than those ofthe GG genotype which was in accordance with the results of a meta-analysis [21].

We found that HDAC9 polymorphism was associated with carotid atherosclerosis in Korean adults. These results suggest that the *HDAC9* polymorphism may be an independent risk factor for cardiovascular disease.

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