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Short Communication

Association of inflammatory response gene polymorphism with atherothrombotic stroke in Northern Han Chinese

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Atherosclerosis is an important pathophysiological basis of atherothrombotic stroke (ATS), and inflammation plays a significant role in atherosclerosis formation. In this study, single-nucleotide polymorphisms (SNPs) in three key inflammation-related genes, 5-lipoxygenase activating protein (*ALOX5AP*), phosphodiesterase 4D (*PDE4D*), and interleukin-1α (*IL-1α*), were investigated to determine their association with ATS in Northern Han Chinese. Six-hundred and eighty-two ATS patients and 598 unrelated controls were recruited. Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism and matrix-assisted laser desorption ionization time-of-flight mass spectrometry primer extension. The genotype and allele frequencies of each SNP were statistically analyzed. Risk of ATS was found for the *ALOX5AP* SG13S114A/T AA genotype ($P = 0.040$) and A allele ($P = 0.033$), *PDE4D* SNP83C/T TT genotype ($P = 0.010$) and T allele ($P = 0.008$) and SNP219A/G GG genotype ($P = 0.025$) and G allele ($P = 0.022$), and the *IL-1α*-889C/T T allele ($P = 0.035$). The differences still remained significant after adjustment. The *ALOX5AP* HapA haplotype was not correlated with ATS ($P = 0.834$), but GCGA represented an at-risk haplotype ($P = 0.008$). Furthermore, the *PDE4D* AA haplotype at SNP219-220 might be an at-risk haplotype ($P = 0.013$), while GA might be a protective haplotype ($P = 0.005$). The *ALOX5AP* (SG13S114A/T), *PDE4D* (SNP83C/T, 219A/G), and *IL-1α* (-889C/T) SNPs were associated with an increased risk of ATS in Northern Han Chinese.

Keywords inflammation; polymorphism; atherothrombotic stroke; ALOX5AP; PDE4D

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Introduction

Stroke is the third leading cause of mortality and a major cause of functional disability among the older people worldwide [1]. According to the World Health Organization estimates, nearly 5.5 million people died of stroke in 2002, and >50% of these deaths occurred in the Asian countries like China, India, Pakistan, Bangladesh, Korea, and Japan (WHO report: http://www.who.int/cardiovascular diseases/). The prevalence of stroke in China has risen significantly over the past few decades and is currently estimated at 170.3/100,000. Geographical epidemiologic studies have revealed that the prevalence rate of stroke in northern China was higher than other regions [2]. In general, ischemic stroke (IS) accounts for >70% of these stroke cases [3].

IS is a complex multifactorial disorder that is thought to result from interactions between an individual's genetic background and lifetime exposure to various environmental factors. Studies involving family members, siblings, and twins have suggested that IS has a strong genetic component [4,5]. IS is caused by a number of different pathologies, which may result from different genetic predispositions. Therefore, accurate stroke subtyping is likely to be important to identify genetic associations. Atherothrombotic stroke (ATS) is an important subtype of IS, and its pathophysiological mechanism has been defined as a combination of vascular endothelial damage, platelet aggregation, and thrombosis [6]. Inflammation plays a key role in establishing the initial atherosclerotic lesion, and increases the risk of ATS to a greater degree compared with other stroke subtypes [7]. A genetic variation in the components of the inflammatory response has also been implicated as a risk factor, particularly *via* interaction with proinflammatory conventional risk factors [8,9].

In this study, we selected the genes encoding 5-lipoxygenase activating protein (ALOX5AP), phosphodiesterase 4D (PDE4D), and interleukin-1α (IL-1α), all of which are involved in the inflammatory mechanisms, and are reported to be involved in the pathogenesis of stroke and cardiovascular diseases. ALOX5AP is characterized as an essential regulator of the biosynthesis of leukotriene A4 (LTA4) hydrolase, an important proinflammatory enzyme

that has been implicated in the pathogenesis and progression of atherosclerosis [6]. The PDE4D hydrolase degrades second messenger cyclic adenosine monophosphate (cAMP), which is the key signal transduction molecule of many cell types, including smooth muscle, vascular endothelial, and inflammatory cells [10]. Low cAMP level is known to enhance cell migration and proliferation, and this mechanism is believed to underlie atherosclerosis. IL-1α is another important inflammatory response factor that has been shown to promote the synthesis of fibrinogen, C-reactive protein, and other proinflammatory components, all of which participate in the pathogenesis of atherosclerosis [11]. Indeed, high levels of *IL-1α* mRNA transcripts have been detected in atherosclerotic plaques from human patients [12]. The single-nucleotide polymorphisms (SNPs) present in the three inflammatory genes have been found to be associated with an increased risk of ATS in different populations [13–17]. But, conversely, other genetic studies have produced conflicting results [18–21]. Thus, the ethnic-specific relationship of these SNPs with ATS remains unclear. Therefore, this case-control study was designed to investigate the ATS-associated inflammatory genes' SNPs in a high-risk, ethnically homogeneous Chinese population to determine their association with ATS susceptibility.

Materials and Methods

Subjects

ATS patients ($n = 682$) and unrelated controls ($n = 598$) were recruited between March 2008 and May 2011 from the First Affiliated Hospital of China Medical University, Shengjing Hospital and the Jinzhou Central Hospital in tandem. All patients resided in the northern China region (Liaoning Province) and identified themselves as Han ethnicity. ATS diagnosis was made by the onset of sudden loss of global or focal cerebral function and corresponding infarction detected by brain imaging with computed tomography (CT) or magnetic resonance imaging (MRI). Classification by the Trial of Org 10 172 in Acute Stroke Treatment (TOAST) [22] indicated that all ATS patients were of the aortic atherosclerotic subtype.

The control subjects were recruited simultaneously from the same geographical area and had normal brain CT/MRI imaging studies following the same diagnostic criteria as cases. The controls had no clinical evidence of neurological diseases and were matched by age, sex, and ethnic origin. The controls included inpatients with minor illnesses and people undergoing annual medical examination, free of neurovascular and cardiovascular history or family history of stroke, ascertained by direct interview before recruitment.

All study participants underwent extensive clinical examination and were shown to be free of renal or liver insufficiency, hematopathy, cancer, autoimmune disease, and occlusive arterial disease or phlebothrombosis of the limbs. A team of professional physicians reviewed each participant's medical records to ensure that the eligibility criteria were met. Subjects who had a history of or current hypertension, diabetes, smoking habits, and/or hypercholesterolemia were noted. Hypertension was defined as having a previous diagnosis of hypertension or if systolic or diastolic blood pressure was $\geq$140 or $\geq$90 mmHg, respectively, or both on at least two different occasions. Diabetes was defined if fasting plasma glucose level was $>$126 mg/dl or if they had any history of being diagnosed with the disease. Hypercholesterolemia was defined as having $>$200 mg/dl total cholesterol in blood. Smoking was defined if smoked $\geq$10 cigarettes per day for $>$6 months. Written informed consent was obtained from all study participants or their family, and the experimental protocols were approved by the Ethics Committees of each hospital.

DNA isolation and polymorphism genotyping

Venous blood (5 ml) was collected from all patients and controls and subjected to Ficoll gradient centrifugation to isolate the peripheral blood mononuclear cells (PBMCs). Genomic DNA was extracted from each participant's PBMCs using the Wizard Genomic DNA Purification Kit (Promega, Madison, USA) following the manufacturer's instructions. DNA samples were stored at −20°C until used.

The SNPs selected for study were: *ALOX5AP*: SG13S25 (rs17222814), SG13S114 (rs10507391), SG13S89 (rs4769874), SG13S32 (rs9551963); *PDE4D*: SNP83 (rs966221), SNP87 (rs2910829), SNP219 (rs6450512), SNP220 (rs425384); and *IL-1α* gene promoter area-889 (rs1800587). The DNA sequences for each of the SNPs, the surrounding genomic DNA, and the genomic region were obtained from the dbSNP database (www.ncbi.nlm.nih.gov/SNP/), the Human Genome Browser (http://genome.ucsc.edu/), and electronically-linked databases [National Center for Biotechnology Information (NCBI), Celera's Human SNP Reference Database, SNP Consortium, and Human Genome Variation Database]. The sequences were then used to design SNP-specific *Taq*Man genotyping assays (synthesized by Applied Biosystems, Foster City, USA). The SNPs were genotyped by polymerase chain reaction-restriction fragment length polymorphism and matrix-assisted laser desorption ionization time-of-flight mass spectrometry primer extension (performed by Sequenom, San Diego, USA). The parameters of the SNP-specific genotyping assays are presented in **Tables 1** and **2**, respectively.

Statistical analysis

Statistical analyses were performed with SPSS software for Windows (ver13.0; SPSS Inc., Chicago, USA). Data are presented as mean ± SD or percent frequency. For each

Table 1 Primer sequences, PCR conditions, and genotyping profiles

SNP	Primer sequences, 5′–3′	Restriction enzyme	Annealing temperature (°C)/extension (s)/cycles	Profiles of DNA fragments
ALOX5AP SG13S32A/C	F: CAAGGGGCTTTATGGTTA	*Taq*I	53.3/74/36	AA: 370; CC: 177, 193
	R: AAGCCTGAAGGAAGAAGC			AC: 370, 177, 193
ALOX5AP SG13S89A/G	F: CCCACTTTCC TCGCTGTGCT	*BSh*1236I	53.3/74/36	AA: 610; GG: 230, 380
	R: CCGAAAGGGGACCAAAAGTA			AG: 610, 230, 380
PDE4D SNP83C/T	F: TTGTTTCTAGTTAGCCTTG	*Tai*I	50/60/40	T/T: 359; C/C: 264, 95
	R: ATTTGGCCTTGCAATATAC			C/T: 359, 264, 95
PDE4D SNP87T/C	F: AAGATGAGGAAGAATAATGG	*SSP*1	52/60/40	T/T: 486; C/C: 264, 222
	R: ATGAAGACACCTGAAAGATC			C/T: 486, 264, 222
*IL-1*α-889C/T	F: GGGGGCTTCACTATGTTGCCCACACTGGACTAA	*Nco*I	57/60/40	T/T: 309; C/C: 266, 43
	R: GAAGGCATGGATTTTTACATATGACCTTCCATG			C/T: 309, 266, 43

F, forward; R, reverse.

Table 2 Primer sequences and peak value of MALDI-TOF-PEX

SNP	Primer sequences, 5′–3′ (extension primer sequences, 5′–3′)	Primer peak value (Da)	SNP signal peak value (Da)
ALOX5AP SG13S25A/G	F: ACGTTGGATGTGTGTCCATACATAGCCCTC	5072.3	A: 5343.5
	R: ACGTTGGATGATCAGCTAGTCTCTTTCCCC (TTTCCCCAGCCACTGTT)		G: 5395.5
ALOX5AP SG13S114A/T	F: ACGTTGGATGCTCTTAAGGTAGGTCT	5704.7	A: 5975.9
	R: ACGTTGGATGTCCAGATGTATGTCCAAGCC (GCCTCTCTTTGCAATTCTA)		T: 6031.8
PDE4D 219A/G	F: ACGTTGGATGGCACTCAGGTTAAATCTACCA	5728.7	G: 5975.9
	R: ACGTTGGATGCTAATGACTTTTGTTCAACTG (TGTTCAACTGTATCACTCT)		A: 6055.8
PDE4D 220A/C	F: ACGTTGGATGTGGTGAGCACAATCCTTGAG	5014.3	C: 5301.5
	R: ACGTTGGATGTTCCCTTGGGTGGAAAACTC (CTCCTCTCTCTGTTCCT)		A: 5341.4

F, forward; R, reverse.

gene variant, the odds ratio (OR) was calculated along with the 95% confidence interval (CI) to measure the extent of genetic association with ATS. The allele and genotype frequencies between the patients and controls were compared by a chi-squared (χ^2) test or Fisher's exact test using codominant models. The Hardy–Weinberg equilibrium (HWE) test was assessed by HWE software. Risk factors were screened by Student's *t*-test or χ^2 test. Then, the relation between the genotypes and ATS was evaluated by logistic regression analysis, after being adjusted for potentially confounding covariates. Haplotype analysis of *ALOX5AP* and *PDE4D* SNPs was performed using the SHEsis program

(http://analysis.Bio-x.cn/my analysis.php). $P < 0.05$ was considered statistically significant.

Results

Characteristics of the population

The characteristics of the ATS patients and healthy controls are shown in **Table 3**. No significant difference existed between the two groups for age, sex, or body mass index (BMI). The prevalence of hypertension, diabetes, hypercholesterolemia, and smoking was significantly higher for ATS patients than for the controls. These data may suggest that hypertension, diabetes, hypercholesterolemia, and smoking may contribute to the development of ATS in Northern Han Chinese.

ATS-associated genotypes and alleles

All genotype distributions were in HWE in both groups (patient group: $\chi^2 = 0.180$, $P = 0.671$; control group: $\chi^2 = 0.332$, $P = 0.564$). The distributions of the *ALOX5AP*, *PDE4D*, and *IL-1α* genotypic polymorphisms and the allelic frequencies in patients and controls are presented in **Table 4**. There was no significant difference in the distribution of *ALOX5AP* SNPs SG13S25A/G, SG13S32A/C, SG13S89A/G, and *PDE4D* SNPs 87T/C and 220A/C between ATS patients and control subjects. In contrast, the frequencies of ATS patients with the AA genotype and A allele of *ALOX5AP* SG13S114A/T, the TT genotype and T allele of *PDE4D* SNP83C/T, and the GG genotype and G allele of *PDE4D* SNP219A/G were significantly higher than those in controls. Although there was no significant difference in the genotype of *IL-1α*-889C/T between ATS patients and control subjects, the T allele was significantly higher in ATS patients. After adjustment for conventional risk factors (age, sex, BMI, hypertension, diabetes, hypercholesterolemia, and smoking habit), logistic regression analysis revealed that the differences remained significant (**Table 4**).

Table 3 Characteristics of subjects

	Cases ($n = 682$)	Controls ($n = 598$)	P value
Age (years)	62.09 ± 9.43	61.84 ± 10.12	0.873
Male, *n* (%)	403 (59.1)	336 (56.2)	0.308
BMI (kg/m^2)	24.46 ± 3.07	24.37 ± 3.27	0.727
Hypertension, *n* (%)	299 (43.9)	169 (28.3)	0.000
Diabetes, *n* (%)	160 (23.4)	95 (15.9)	0.001
Hypercholesterolemia, *n* (%)	149 (21.8)	89 (14.9)	0.002
Smoking, *n* (%)	203 (29.8)	136 (22.8)	0.005

Note: Continuous and categorical variables were tested by Student's *t*-test and χ^2 analysis, respectively.

BMI, body mass index.

Haplotype

The haplotype of the *ALOX5AP* gene was composed of four sites: SG13S25, SG13S114, SG13S89, and SG13S32. We found that haplotype HapA (SG13S25G-SG13S114T-SG13S89G-SG13S32A) had no relevance in the Northern Chinese Han population. In contrast, the frequency of the GCGA haplotype was significantly higher in our ATS patients than in controls (OR = 1.683, 95% CI = 1.132–2.479, $P = 0.008$). For the *PDE4D* gene, two haplotypes were composed of SNP83, SNP87 and SNP219, SNP220, respectively. A two-point haplotype analysis of SNP83C/T and SNP87T/C demonstrated that there was no significant association with ATS risk in our study population. The second two-point haplotype analysis of SNP219A/G and SNP220A/C demonstrated that the frequency of AA was higher in ATS patients than in controls (OR = 1.296, 95% CI = 1.056–1.591, $P = 0.013$), and the frequency of GA was lower in the ATS patients than in controls (OR = 0.650, 95% CI = 0.482–0.878, $P = 0.005$; **Table 5**).

Discussion

ALOX5AP is an important regulator in the biosynthesis of proinflammatory LTs. A genetic variation in the *ALOX5AP* genes has been shown to contribute to the risk of stroke and MI in the Icelandic and Scottish populations [20,23]. Similar results were reported by Lõhmussaar *et al*. [15] in a Central European population and Kaushal *et al*. [16] in an American Caucasian population. In the Chinese population, a common genetic variant (SG13S114A/T) in the *ALOX5AP* gene is reportedly associated with an increased risk of ATS in males [24]. Wang *et al*. [25] found no significant association of variants of *ALOX5AP* with ATS in Eastern Han Chinese populations. But they discovered that levels of LTB4, a key product of the 5-lipoxygenase (5-LO)/5-LO-activating protein (FLAP) pathway, were increased significantly in ATS patients, and they also found that carriers of the T allele were associated with the higher plasma LTB4 levels. In our hospital-based case-control study, we found that the *ALOX5AP* SG13S114A/T might be an independent risk factor for ATS in Northern Han Chinese. It's pathogenesis may be that ALOX5AP is a regulator of the LT biosynthetic pathway [26], which plays an important role in the pathogenesis of atherosclerosis and inflammatory diseases. Genetic effects in the LT biosynthetic pathway could be an important contributor to the development of atherosclerosis and to an increasing risk of ATS through the formation of the proinflammatory LTB4 and/or through an increase in vascular permeability caused by cysteinyl leukotrienes (LTC4 and its metabolites LTD4,

Table 4 Genotype and allele distributions among the cases and controls

	Case, *n* (%)	Control, *n* (%)	Unadjusted		Adjusted	
			OR (95% CI)	*P* value	OR (95% CI)	*P* value
ALOX5AP SG13S25A/G						
Genotype						
GG	521 (76.5)	471 (78.8)	Ref		Ref	
AG	138 (20.2)	117 (19.5)	1.052 (0.845–1.311)	0.649	1.048 (0.812–1.591)	0.791
AA	23 (3.3)	10 (1.7)	2.034 (0.978–4.229)	0.052	2.027 (0.814–4.305)	0.051
Allele						
G	1180 (86.5)	1059 (88.5)	Ref		Ref	
A	184 (13.5)	137 (11.5)	1.178 (0.957–1.449)	0.121	1.169 (0.847–1.413)	0.125
ALOX5AP SG13S114 A/T						
Genotype						
TT	324 (47.5)	297 (49.6)	Ref		Ref	
AT	260 (38.2)	249 (41.7)	0.976 (0.858–1.110)	0.714	0.956 (0.845–1.217)	0.718
AA	98 (14.3)	52 (8.7)	1.559 (1.149–2.114)	0.040	1.508 (1.113–2.346)	0.043
Allele						
T	908 (66.6)	843 (70.5)	Ref		Ref	
A	456 (33.4)	353 (29.5)	1.133 (1.009–1.271)	0.033	1.127 (1.012–1.278)	0.035
ALOX5AP SG13S89A/G						
Genotype						
GG	626 (91.8)	539 (90.2)	Ref		Ref	
AG	50 (7.3)	55 (9.1)	0.799 (0.554–1.153)	0.229	0.713 (0.464–1.174)	0.238
AA	6 (0.9)	4 (0.7)	1.289 (0.366–4.543)	0.692	1.211 (0.352–4.463)	0.785
Allele						
G	1302 (95.5)	1133 (94.7)	Ref		Ref	
A	62 (4.5)	63 (5.3)	0.863 (0.613–1.215)	0.398	0.848 (0.609–1.223)	0.402
ALOX5AP SG13S32A/C						
Genotype						
AA	309 (45.3)	282 (47.2)	Ref		Ref	
AC	190 (27.8)	153 (25.6)	0.955 (0.866–1.053)	0.358	0.942 (0.851–1.049)	0.342
CC	183 (26.9)	163 (27.2)	0.991 (0.899–1.093)	0.858	0.915 (0.827–1.094)	0.867
Allele						
A	808 (59.2)	717 (59.9)	Ref		Ref	
C	556 (40.8)	479 (40.1)	0.988 (0.927–1.053)	0.714	0.976 (0.912–1.043)	0.782
PDE4D SNP83C/T						
Genotype						
CC	210 (30.8)	138 (23.1)	Ref		Ref	
CT	320 (46.9)	310 (51.8)	1.286 (1.081–1.531)	0.004	1.274 (1.085–1.614)	0.005
TT	152 (22.3))	150 (25.1)	1.211 (1.043–1.405)	0.010	1.202 (1.041–1.609)	0.018
Allele						
C	740 (54.3)	586 (49.0)	Ref		Ref	
T	624 (45.7)	610 (51.0)	1.107 (1.027–1.194)	0.008	1.104 (1.021–1.254)	0.015
PDE4D SNP87T/C						
Genotype						
CC	302 (44.3)	262 (43.8)	Ref		Ref	
CT	220 (32.2)	222 (37.2)	0.919 (0.799–1.056)	0.235	0.816 (1.004–1.492)	0.278
TT	160 (23.5)	114 (19.0)	1.142 (0.937–1.392)	0.186	1.134 (0.921–1.534)	0.172
Allele						

Continued

Table 4. *Continued*

	Case, *n* (%)	Control, *n* (%)	Unadjusted		Adjusted	
			OR (95% CI)	*P* value	OR (95% CI)	*P* value
C	824 (60.4)	746 (62.4)	Ref		Ref	
T	540 (39.6)	450 (37.6)	1.052 (0.954–1.161)	0.309	1.042 (0.948–1.247)	0.412
PDE4D SNP219A/G						
Genotype						
AA	198 (29.1)	139 (23.2)	Ref		Ref	
AG	325 (47.6)	300 (50.2)	1.196 (1.003–1.426)	0.045	1.145 (1.006–1.517)	0.038
GG	159 (23.3)	159 (26.6)	1.189 (1.020–1.386)	0.025	1.188 (1.022–1.158)	0.026
Allele						
A	721 (52.9)	578 (48.3)	Ref		1.087 (1.011–1.307)	0.020
G	643 (47.1)	618 (51.7)	1.094 (1.013–1.181)	0.022	Ref	
PDE4D SNP220A/C						
Genotype						
AA	271 (39.8)	226 (37.8)	Ref		Ref	
AC	265 (38.9)	243 (40.7)	1.049 (0.925–1.190) 0.453		1.047 (0.917–1.208)	0.468
CC	146 (21.3)	129 (21.5)	1.021 (0.919–1.134)	0.701	1.023 (0.824–1.132)	0.612
Allele						
A	807 (59.2)	695 (58.1)	Ref		Ref	
C	557 (40.8)	501 (41.9)	1.018 (0.954–1.087)	0.589	1.012 (0.941–1.098)	0.624
*IL-1*α-889C/T						
Genotype						
CC	534 (78.3)	490 (81.9)	Ref		Ref	
CT	104 (15.2)	81 (13.6)	1.149 (0.879–1.502)	0.308	1.113 (0.754–1.782)	0.451
TT	44 (6.5)	27 (4.5)	1.458 (0.916–2.319)	0.109	1.441 (0.903–2.447)	0.113
Allele						
C	1172 (85.9)	1061 (88.7)	Ref		Ref	
T	192 (14.1)	135 (11.3)	1.247 (1.015–1.532)	0.035	1.235 (1.024–1.792)	0.038

Note: Unadjusted (without covariates) and adjusted (for age, sex, BMI, hypertension, diabetes, hypercholesterolemia, and smoking habit) logistic regression analysis was performed using codominant models between controls and ATS patients.

OR, odds ratio; CI, confidence interval; Ref, reference group.

LTE4) [27]. Unfortunately, the levels of LTB4 in blood neutrophils were not measured in the current study, and we cannot speculate further on the potential functional effects of the *ALOX5AP* polymorphism. In haplotype analysis, we found no association of the *ALOX5AP* HapA haplotype, which was linked to ATS in a northern European population, with ATS in our population, but GCGA haplotype might represent a special at-risk haplotype. Several factors may have influenced these different findings: (i) Haplotype structure and haplotypes could be different among different populations, rendering the uncritical use of haplotype tagging SNPs impossible. (ii) Different mutation rates of multiple alleles in different populations may result in different output which therefore lacks statistical power.

In the present study, *PDE4D* SNP83and SNP219 were found to be significantly associated with ATS. A Pakistani study also found that SNP83 had a strong association with ATS [28]. Staton *et al*. [29] have found a significant association of SNP83 with ATS in an Australian population. A recent study from North India also found SNP83 to be significantly associated with ATS in the North Indian population [30]. Our finding adds to the growing number of evidences that this gene variant exerted a significant, independent effect on individual predisposition to ATS occurrence. About SNP219, to the best of our knowledge this is the first study to investigate the association of *PDE4D* SNP219A/G variants with ATS in Chinese population. We concluded that SNP219A/G may be a specific variation site in our Northern Han population. Our analysis of the *PDE4D* gene haplotypes revealed no differences in frequency distribution for the two-point SNP83–87 haplotype, in contrast to the deCODE findings. It is possible that different research designs and/or our limited sample size underlie the inconsistency. However, in the two-point

Table 5 Haplotype analysis results

Haplotype	Cases (%)	Control, (%)	*P* value	OR	95% CI
ALOX5AP					
HapA	38.1	36.5	0.834	0.975	0.788–1.213
AAGT	5.8	5.3	0.513	1.159	0.739–1.812
GAGA	15.3	19.4	0.061	0.761	0.581–0.983
GCGA	9.8	6.2	0.008	1.683	1.132–2.479
GCGT	19.9	21.4	0.512	1.017	0.852–1.514
PDE4D SNP83–87					
CC	39.7	33.9	0.182	1.132	0.878–1.431
CT	13.8	11.8	0.074	1.339	0.991–1.973
TC	23.1	26.7	0.183	0.809	0.612–1.113
TT	23.4	27.6	0.114	0.921	0.663–1.134
PDE4D SNP219–220					
AA	47.8	41.4	0.013	1.296	1.056–1.591
AC	5.6	7	0.262	0.785	0.515–1.198
GA	11.2	16.3	0.005	0.65	0.482–0.878
GC	35.4	35.3	0.979	1.003	0.811–1.241

Note: Only haplotypes with 3% or above are presented. The global haplotype test for association with ATS was not significant ($\chi^2 = 7.63$ with 3 degrees of freedom, $P = 0.061$).

OR, odds ratio; CI, confidence interval; Ref, reference group.

SNP219–220 haplotype, we found that the frequency of the AA haplotype was higher in the ATS patients than in the control group, suggesting that it may represent an at-risk haplotype. In contrast, the frequency of the GA haplotype was lower in the case group, suggesting that it may represent a protective haplotype. The results for the SNP219–220 haplotype are consistent with a previous study of Caucasian Americans [31] and the deCODE project.

Um *et al*. [13] and Banerjee *et al*. [14] discovered that the *IL-1α*-889 T alleles were associated with ATS susceptibility in Asian Indian and South Korea populations, which is consistent with our current results. Thus, we suspect that the *IL-1α*-889 T allele may be associated with ATS in Asian ethnicities. Since the -889 sites are located in the *IL-1α* gene promoter area, mutations may affect the affinity or availability of binding sites for trans-acting factors. It is also possible that mutations in this region alter the DNA subprime structure, thereby affecting the initiation of *IL-1α* gene transcription and mRNA stability. Either of these changes may result in increases or decreases of gene and protein expression. We speculate that even if genetic mutations of the *IL-1α*-889 sites do not directly influence ATS formation, they may aggravate the inflammatory response, which in turn could promote the atherosclerotic process and lead to ATS.

Careful evaluations were required for assessing some confounding factors such as hypertension, diabetes mellitus, hypercholesterolemia, and heavy smoking. In this study, the rate of cases suffered from hypertension is 43.9%, significantly higher than those in controls, this is consistent with traditional standpoint that hypertension is the risk factor of ATS. At the same time, the proportion of hypercholesterolemia, diabetes, and smokers in cases is also significantly higher than those in controls. Meanwhile, our logistic regression analysis also indicated that, in the absence of risk factors (hypertension, hypercholesterolemia, diabetes, and smoking), the significance and relative degree of risk were decreased. Therefore, life habits and co-morbidity risk factors have important impact on genetic predisposition to ATS.

The study also presents several limitations. First, the sample size in the present study (682 cases and 598 controls) might not be large enough to detect a small effect of potential low-penetrance SNPs. Additionally, there was possible selection bias since the controls were partly recruited from hospital. Second, each single susceptible polymorphism might only contribute to a modest effect; thus analysis of a single SNP could be confused by unstudied SNPs that influence the phenotype. The combined effects of multiple variants of a gene or multiple genes would provide more information about ATS risk and provide a more comprehensive evaluation of genetic contribution to the risk of ATS. Therefore, more studies are needed to demonstrate the gene–gene interaction affecting the susceptibility to ATS.

In summary, in the present study, the results suggest that some common genetic variants in inflammatory response genes are associated with an increased risk for ATS in Chinese Northern Han populations, which strengthens the evidence that inflammatory response genes play a key role in ATS pathogenesis. A future meta-analysis of ethnically diverse study populations may help elucidate the precise role of genetic variation in ATS. If the ethnicity-specific genetic mechanisms are ultimately confirmed, novel effective pharmacological interventions may be developed to lower the risk of ATS in targeted populations.

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