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Original Article

The first intron polymorphism (Rs9579646) of *ALOX5AP* gene is associated with ischemic stroke subtype in two independent Chinese populations

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Abstract: The first intron of human genes is likely to be involved in transcriptional regulation, which is potentially related to the susceptibility of ischemic stroke (IS). The purpose of the present study was to explore the association between the first intron polymorphism (rs9579646) of *ALOX5AP* gene and the risk of IS in Chinese Han population. We investigated the rs9579646 polymorphism by TaqMan genotyping in two independent Chinese Han populations: the first cohort comprised of 517 IS patients and 530 healthy inhabitants, while the second cohort included 589 IS patients and 616 healthy controls. After adjusting for conventional risk factors, the G allele frequencies in the small-artery occlusion (SAO) subgroups were significantly higher than those in control groups of the two Chinese cohorts (P=0.033 and P=0.030, respectively). The GG genotype frequencies were significantly higher in the SAO subtypes compared with control groups in two Chinese cohorts (P=0.044 and P=0.037, respectively). Additionally, the effect of rs9579646 on the risk of SAO subtype was best described with recessive and additive genetic models ($P < 0.05$). In conclusion, our results suggested that the first intron polymorphism rs9579646 may be a potential genetic risk factor for SAO subtype in Chinese Han population.

Keywords: *ALOX5AP*, ischemic stroke, intron, polymorphism

Introduction

The pathogenesis of ischemic stroke (IS) relies on a complex interaction of genetic and environmental factors [1]. Several studies have confirmed that increased 5-lipoxygenase-activating protein (ALOX5AP) activity could lead to the accumulation of leukotrienes (LTs) in fatty deposits on the arterial wall [2]. The subsequent breakdown of these deposits by the immune system may then lead to the development of atherosclerosis and an increased risk of IS [3].

Introns, which are removed during RNA splicing, have been considered as "junk DNA" for a long time. However, some preliminary studies have revealed that introns have important biological functions. Particularly, the first intron of human genes is likely to be involved in transcriptional regulation [4, 5]. Intron polymorphisms, especially in the first intron, might affect gene transcriptional regulation and the susceptibility to IS.

However, the role of the first intron polymorphisms in the susceptibility to IS has not been extensively explored. Therefore, the aim of the present study was to investigate the first intron polymorphism (rs9579646) of *ALOX5AP* gene and its association with IS in two independent Chinese populations.

Materials and methods

Study population

A two-stage study design was used to investigate the association between the rs9579646 polymorphism of *ALOX5AP* and IS risk. In the initial study, 517 patients with IS (271 men and 246 women, mean age of 56.4±9.7 years) were

recruited from two medical centers in Zhengzhou and Xinxiang. The diagnosis of IS was based on a loss of global or focal cerebral function persisting for > 24 h with corresponding infarction on brain imaging with probable vascular causes [6]. All IS patients underwent computed tomography (CT), magnetic resonance imaging (MRI) and thorough neurological examinations. Based on the imaging examination, the IS patients were classified into three subtypes according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST), including large-artery atherosclerosis (LAA), small-artery occlusion lacunar (SAO), and stroke of other undetermined etiology (SUE). Patients with atrial fibrillation, cerebral hemorrhage, peripheral vascular diseases, or kidney diseases were excluded from the present study. The control group comprised of 530 unrelated Henan Han individuals (283 men and 247 women, mean age 55.4±10.8 years), selected from the same demographic area and matched with the cases by age, sex, and residency. The control group comprised subjects without a history of neurovascular and cardiovascular diseases, or a family history of stroke, ascertained by direct interview before recruitment.

For the purpose of verification, another independent case-control cohort containing 589 IS patients and 616 controls were recruited from two medical centers in Nanyang and Xinyang, respectively. IS and control recruitment criteria were identical to those of the first population. There were no overlapping participants between the two populations.

A questionnaire was designed to collect detailed information concerning the risk factors of stroke, including gender, age, hypertension, diabetes mellitus, hyperlipidemia, coronary heart disease, atrial fibrillation, smoking, and alcohol abuse for both cases and controls. This study was approved by the Ethics Committee on Human Research of Zhengzhou University and each subject enrolled in this study provided informed consent.

Genotyping of the rs9579646 polymorphism

Genomic DNA was extracted from peripheral blood using the AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biotechnology, Union City, CA, USA) according to the manufacturer's instructions. TaqMan-MGB probes were used to analyze the rs9579646 polymorphism. The genotype was determined according to the relative fluorescence intensity of the probe detected with the real-time PCR system. The primers were forward (F), 5'-GCACTGGAGATAGTTATGAAAGTGGTC-3' and reverse (R), 5'-GATCTGGAAAAGGAGAATTGTGTAGAG-3' (Sangon, Shanghai, China). PCR was performed in 10 μL reactions containing 5 μL of 2 × TaqMan Universal PCR Master Mix (Applied Biosystems), 0.2 μL of forward primer, 0.2 μL of reverse primer, 0.6 μL of FAM-labeled probe, 0.6 μL of HEX-labeled probe, 0.4 μL of ROXII, 1.0 μL of DNA template (1~20 ng/μL), and 2 μL of ddH_2O. The PCR reaction conditions included pre-degeneration for 2 min at 95°C, followed by denaturation at 95°C for 15 s, and 40 cycles of annealing and extension for 30 s at 60°C, with a final extension at 60°C for 1 min. After PCR amplification, the sample genotypes were determined after measuring the allele-specific fluorescence with the ABI Prism 7700 Sequence Detection System using SDS 1.7 software for allele discrimination (Applied Biosystems). A total of 10% of all genotypes were sequenced to examine consistency and no discrepancies were detected.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was tested using a chi-squared test. Demographic and clinical characteristics were compared between the IS patients and controls using Student's *t*-test and chi-squared test for continuous and categorical variables, respectively. Allele and genotype distributions were compared using the chi-squared test. HWE testing was conducted using SHEsis software (http://analysis.bio-x.cn) [7]. The strength of the association between *ALOX5AP* polymorphisms and IS was estimated by odds ratio (*OR*) with 95% confidence intervals (*CI*). The multivariate logistic regression model was used to exclude the effects of potential confounding factors including sex, age, smoking, hypertension and diabetes. All statistical analyses were performed using the SPSS 21.0 package (SPSS, Chicago, IL, USA). *P* values less than 0.05 (two-tailed) were considered statistically significant.

Results

Clinical characteristics of the subjects

We included two populations in our study. And the IS cases and controls were all recruited from Henan Province in China. The clinical and

Table 1. Characteristics of the study populations

	Population 1			Population 2		
	Cases (n=517)	Controls (n=530)	*P* value	Cases (n=589)	Controls (n=616)	*P* value
Gender (males/females)	271/246	283/247	0.751	343/246	334/282	0.160
Age (mean ± SD, years)	56.4±9.7	55.4±10.8	0.101	56.3±9.6	55.4±10.8	0.120
Total cholesterol (mmol/L)	5.09±0.89	4.80±0.80	0.000*	5.03±0.81	4.81±0.94	0.000*
Total triglyceride (mmol/L)	2.00±0.90	1.68±0.76	0.000*	1.90±0.62	1.66±0.87	0.000*
Hypertension, (n, %)	303 (58.6)	70 (13.2)	0.000*	357 (60.6)	68 (11.0)	0.000*
Diabetes, (n, %)	64 (12.4)	18 (3.4)	0.000*	88 (14.9)	38 (6.2)	0.000*
Smokers, (n, %)	86 (16.6)	42 (7.9)	0.000*	106 (18.0)	51 (8.3)	0.000*
Alcohol, (n, %)	75 (14.5)	39 (7.4)	0.000*	73 (12.4)	38 (6.2)	0.000*

*$P < 0.05$ denotes statistical significance.

Table 2. Genotype and allelic distribution of rs9579646 in IS and control subjects

Population 1	Rs9579646	IS subjects (n=517, n (%))	Control subjects (n=530, n (%))	*P* value	Adjusted *OR* (95% *CI*)
	AA	108 (20.9)	115 (21.7)		
	AG	251 (48.5)	271 (51.1)	0.931	0.986 (0.721-1.350)
	GG	158 (30.6)	144 (27.2)	0.379	1.168 (0.826-1.652)
	A allele	467 (45.2)	501 (47.3)		
	G allele	567 (54.8)	559 (52.7)	0.335	1.088 (0.916-1.292)
Population 2	Rs9579946	IS subjects (n=589, n (%))	Control subjects (n=616, n (%))	*P* value	Adjusted *OR* (95% *CI*)
	AA	123 (20.9)	135 (21.9)		
	AG	291 (49.4)	314 (51.0)	0.909	1.017 (0.760-1.362)
	GG	175 (29.7)	167 (27.1)	0.397	1.150 (0.832-1.589)
	A allele	537 (45.6)	584 (47.4)		
	G allele	641 (54.4)	648 (52.6)	0.371	1.076 (0.917-1.263)

P value and *OR* (95% *CI*) were adjusted for confounding factors.

pathological characteristics of Population 1 and Population 2 were shown in **Table 1**. Cases and controls were well matched in age and gender ($P > 0.05$). Compared with the control groups, IS groups had a higher percentage of hypertension, diabetes mellitus, smokers, individuals who consumed alcohol, total triglyceride (TG) and total cholesterol (TC) ($P < 0.05$).

Association analysis of the rs9579646 polymorphism

The rs9579646 genotype frequency distributions were both consistent with HWE (P=0.554 and P=0.581, respectively) in the two populations. In the first population, the G allele frequencies and the GG genotype frequencies showed no significant differences between the IS and control groups ($P > 0.05$). See **Table 2**.

We compared the frequency of the rs9579646 genotype within genders between cases and controls. The stratified analysis results showed that there were still no significant differences in the rs9579646 allele and genotype frequencies between different sexes ($P > 0.05$). See **Table 3**.

Subgroup analysis and inherited model test

However, subgroup analysis indicated that the G allele frequency and GG genotype frequencies of rs9579646 were significantly different between SAO case and control (P=0.033 and P=0.044, respectively). This association was not observed in both LAA (OR=1.018, 95% CI=0.838-1.237, P=0.857) and SUE subtypes (OR=0.896, 95% CI=0.559-1.436, P=0.649). See **Table 4**. To assess the effect of rs9579646

Table 3. Stratified analysis of the relationship between rs9579646 Genotypes and susceptibility of IS

Population 1	Case (n, %)	Control (n, %)	*P* value	*OR* (95% *CI*)	Population 2	Case (n, %)	Control (n, %)	*P* value	*OR* (95% *CI*)
Male	n=271	n=283			Male	n=343	n=334		
AA	56 (20.7)	65 (23.0)			AA	71 (20.7)	80 (24.0)		
AG	131 (48.3)	148 (52.3)	0.901	1.027 (0.670-1.576)	AG	154 (44.9)	160 (47.9)	0.682	1.085 (0.735-1.600)
GG	84 (31.0)	70 (24.7)	0.174	1.393 (0.864-2.246)	GG	118 (34.4)	94 (28.1)	0.104	1.414 (0.930-2.151)
A allele	243 (44.8)	278 (49.1)			A	296 (43.1)	320 (47.9)		
G allele	299 (55.2)	288 (50.9)	0.153	1.188 (0.938-1.504)	G	390 (56.9)	348 (52.1)	0.079	1.212 (0.978-1.501)
Female	n=246	n=247			Female	n=246	n=282		
AA	52 (21.1)	50 (20.2)			AA	52 (21.1)	55 (19.5)		
AG	120 (48.8)	123 (49.8)	0.787	0.938 (0.591-1.490)	AG	137 (55.7)	154 (54.6)	0.788	0.941 (0.604-1.466)
GG	74 (30.1)	74 (30.0)	0.879	0.962 (0.581-1.592)	GG	57 (23.2)	73 (25.9)	0.465	0.826 (0.494-1.380)
A allele	224 (45.5)	223 (45.1)			A	241 (49.0)	264 (46.8)		
G allele	268 (54.5)	271 (54.9)	0.903	0.985 (0.766-1.265)	G	251 (51.0)	300 (53.2)	0.480	0.917 (0.719-1.168)

P value and *OR* (95% *CI*) were adjusted for confounding factors.

Table 4. Genotype and allelic distribution of rs9579646 in IS subtypes and controls

Population 1	Control (n=530)	Patients with ischemic stroke		
		LAA (n=330)	SAO (n=150)	SUE (n=37)
AA	115 (21.7)	74 (22.4)	25 (16.7)	9 (24.3)
AG	271 (51.1)	161 (48.8)	71 (47.3)	19 (51.4)
GG	144 (27.2)	95 (28.8)	54 (36.0)	9 (24.3)
OR (95% *CI*), AA vs AG		0.923 (0.650-1.312)	1.205 (0.727-1.998)	0.896 (0.394-2.039)
P value		0.656	0.469	0.793
OR (95% *CI*), AA vs GG		1.025 (0.694-1.515)	1.725 (1.012-2.942)	0.799 (0.307-2.077)
P value		0.900	0.044*	0.644
G allele (%)	52.7	53.2	59.7	50.0
P value		0.857	0.033*	0.649
OR (95% *CI*)		1.018 (0.838-1.237)	1.326 (1.022-1.720)	0.896 (0.559-1.436)
Population 2	Control (n=616)	LAA (n=383)	SAO (n=167)	SUE (n=39)
AA	135 (21.9)	86 (22.5)	28 (16.8)	9 (23.1)
AG	314 (51.0)	190 (49.6)	80 (47.9)	21 (53.8)
GG	167 (27.1)	107 (27.9)	59 (35.3)	9 (23.1)
OR (95% *CI*), AA vs AG		0.950 (0.686-1.315)	1.228 (0.764-1.976)	1.003 (0.448-2.247)
P value		0.756	0.396	0.994
OR (95% *CI*), AA vs GG		1.006 (0.699-1.446)	1.703 (1.029-2.819)	0.808 (0.312-2.093)
P value		0.975	0.037*	0.661
G allele (%)	52.6	52.7	59.3	50.0
P value		0.950	0.030*	0.656
OR (95% *CI*)		1.006 (0.840-1.205)	1.312 (1.027-1.677)	0.901 (0.570-1.424)

P value and *OR* (95% *CI*) were adjusted for confounding factors. *Express the adjusted *P* value for significance $P < 0.05$.

on patients with SAO subtype, we compared additive, dominant, and recessive models. The effect of rs9579646 was best described with recessive and additive models (**Table 5**).

Replication study

The effects observed in the first population of rs9579646 variant were then analyzed in the second population. Similar to the first population, we observed that both GG genotype and G allele frequencies in the SAO subtype of the second population were significantly higher than those in control group after adjusting for conventional risk factors ($P < 0.05$, **Table 4**). Rs9579646 was also associated with the SAO subtype in recessive and additive genetic models (**Table 5**). The rs9579646 polymorphism showed no significant difference between different genders ($P > 0.05$). See **Table 3**.

Table 5. Detailed association of rs9579646 between IS and control groups under different genetic models

	Model	Genotype	Case (n, %)	Control (n, %)	χ^2	*P* value	*OR* (95% *CI*)
Population 1	Dominant	AA	25 (16.7)	115 (21.7)			
		AG+GG	125 (83.3)	415 (78.3)	1.810	0.178	1.386 (0.860-2.231)
	Recessive	AA+AG	96 (64.0)	386 (72.8)			
		GG	54 (36.0)	144 (27.2)	4.417	0.036*	1.508 (1.027-2.215)
	Additive	AA	25 (16.7)	115 (21.7)			
		AG	71 (47.3)	271 (51.1)	0.525	0.469	1.205 (0.727-1.998)
		GG	54 (36.0)	144 (27.2)	4.060	0.044*	1.725 (1.012-2.942)
Population 2	Dominant	AA	28 (16.8)	135 (21.9)			
		AG+GG	139 (83.2)	481 (78.1)	2.113	0.146	1.393 (0.890-2.182)
	Recessive	AA+AG	108 (64.7)	449 (72.9)			
		GG	59 (35.3)	167 (27.1)	4.322	0.038*	1.469 (1.021-2.113)
	Additive	AA	28 (16.8)	135 (21.9)			
		AG	80 (47.9)	314 (51.0)	0.721	0.396	1.228 (0.764-1.976)
		GG	59 (35.3)	167 (27.1)	4.348	0.037*	1.703 (1.029-2.819)

P value and *OR* (95% *CI*) were adjusted for confounding factors. *express the adjusted *P* value for significance $P < 0.05$.

Discussion

A previous work revealed that the first intron is generally longer than the non-first introns and is enriched in C+G and CG dinucleotides [8]. The high contents of C+G, CG dinucleotides and unmethylated CpG islands could provide potential binding sites for some important transcription factors, suggesting that the first intron could harbor more functional elements and is more likely to be involved in transcriptional regulation [9] Therefore, the aim of the present study was to investigate the association between the first intron polymorphism (rs9579646) of *ALOX5AP* gene and the risk of IS.

An advantage of the present study was the two-stage design, which greatly reduced the possibility of identifying false-positive findings in genetic association studies [10]. For the rs9579646 polymorphism, the G allele and AG/GG genotype frequencies showed no significant differences between IS cases and controls in two independent Chinese cohorts. After stratifying these data by IS subtypes, We observed that the GG genotype and G allele frequencies of the rs9579646 polymorphism were markedly increased in the SAO subtype compared with control groups in two Chinese cohorts ($P < 0.05$). The multivariate logistic regression analysis showed that the GG genotype was associated with a 1.725-fold increased risk for the SAO subtype after adjusting the conventional risk factors (95% *CI*, 1.012-2.942; *P*=0.044).

Next, genotype association tests with dominant, recessive and additive models were performed and rs9579646 was associated with SAO in recessive and additive genetic models in both Chinese cohorts ($P < 0.05$). This association was consistent with previous studies of Zhang *et al*, who showed that the rs9579646 GG genotype was associated with an increased risk of IS in the northern Chinese Han population (*OR*=1.73; 95% *CI*, 1.01-2.97; *P*=0.047) [11]. Based on these preliminary results, we proposed a potential molecular mechanism to explain the role of rs9579646 in individual susceptibility to IS. It is likely that the G nucleotide provides a potential binding site that might upregulate *ALOX5AP* transcription through certain mechanism. The increased *ALOX5AP* transcription results in the activation of the 5-LO pathway and the accumulation of leukotrienes, which has been implicated in the development of atherosclerosis and an increased risk of IS.

However, the association was inconsistent with the results reported by Sun *et al*, who showed that the rs9579646 AG genotype was associated with a marginally decreased risk of stroke [12]. While Wang *et al* observed that the rs9579646 AG genotype significantly decreased the risk of stroke in male subjects [13]. In addition, our results were also different from

those of Kaushal *et al*, who reported that the rs9579646 polymorphism was associated with a decreased risk of IS, particularly with the large vessel subtype in US Caucasians [14]. The results of a meta-analysis showed that the rs9579646 polymorphism had marginal association with IS (*OR*=1.23; 95% *CI*, 1.03-1.46) [15]. The controversial results of rs9579646 among these studies may be attributed to the different ethnic backgrounds, sample sizes, statistical analysis and so on. Therefore, further studies with different populations are required to confirm these findings.

The limitation of the present study was that we did not perform the functional characterization of the rs9579646 polymorphism using an *in vitro* luciferase assay. Moreover, we did not examine the mRNA levels of *ALOX5AP* gene in different rs9579646 genotypes in both IS and control groups.

Conclusions

In conclusion, we suggested that the first intron polymorphism (rs9579646) of *ALOX5AP* gene was associated with SAO subtype in two independent Chinese populations. And rs9579646 was associated with the SAO subtype in recessive and additive genetic models in both Chinese cohorts. Further studies are needed to identify other potentially causative polymorphisms and gene-gene interactions involved in the leukotriene pathway to fully understand the genetics of stroke susceptibility. Moreover, studies with functional evaluation are warranted to confirm our findings.

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Disclosure of conflict of interest

None.

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References

[1] O'Donnell MJ, Xavier D, Liu L, Zhang H, Chin SL, Raomelacini P, Rangarajan S, Islam S, Pais P and Mcqueen MJ. Risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the INTERSTROKE study): a case-control study. Lancet 2010; 376: 112-123.

[2] Evans JF, Ferguson AD, Mosley RT and Hutchinson JH. What's all the FLAP about?: 5-lipoxygenase-activating protein inhibitors for inflammatory diseases. Trends Pharmacol Sci 2008; 29: 72-78.

[3] Quarta G, Stanzione R, Evangelista A, Zanda B, Di Angelantonio E, Marchitti S, Di Castro S, Di Vavo M, Volpe M and Rubattu S. Phosphodiesterase 4D and 5-lipoxygenase activating protein genes and risk of ischemic stroke in Sardinians. Eur J Hum Genet 2009; 17: 1448-1453.

[4] Li H, Chen D and Zhang J. Analysis of intron sequence features associated with transcriptional regulation in human genes. PLoS One 2012; 7: e46784.

[5] Ying S, Kojima T, Kawada A, Nachat R, Serre G, Simon M and Takahara H. An intronic enhancer driven by NF-kappaB contributes to transcriptional regulation of peptidylarginine deiminase type I gene in human keratinocytes. J Invest Dermatol 2010; 130: 2543-2552.

[6] Saleheen D, Bukhari S, Haider SR, Nazir A, Khanum S, Shafqat S, Anis MK and Frossard P. Association of phosphodiesterase 4D gene with ischemic stroke in a Pakistani population. Stroke 2005; 36: 2275-2277.

[7] Yong Y and Lin HE. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 2005; 15: 97-98.

[8] Kalari KR, Casavant M, Bair TB, Keen HL, Comeron JM, Casavant TL and Scheetz TE. First exons and introns--a survey of GC content and gene structure in the human genome. Silico Biol 2006; 6: 237.

[9] Majewski J and Ott J. Distribution and characterization of regulatory elements in the human genome. Genome Res 2002; 12: 1827-1836.

[10] Raitoharju E, Seppala I, Levula M, Kuukasjarvi P, Laurikka J, Nikus K, Huovila AP, Oksala N, Klopp N, Illig T, Laaksonen R, Karhunen PJ, Viik

J, Lehtinen R, Pelto-Huikko M, Tarkka M, Kahonen M and Lehtimaki T. Common variation in the ADAM8 gene affects serum sADAM8 concentrations and the risk of myocardial infarction in two independent cohorts. Atherosclerosis 2011; 218: 127-133.

[11] Zhang SY, Xu ML, Zhang CE, Qu ZY, Zhang BB, Zheng ZY and Zhang LM. Association of ALOX5AP gene single nucleotide polymorphisms and cerebral infarction in the Han population of northern China. BMC Med Genet 2012; 13: 61.

[12] Sun H, Wu H, Zhang J, Wang J, Lu Y, Ding H, Xiao H and Zhang J. A tagging SNP in ALOX5AP and risk of stroke: a haplotype-based analysis among eastern Chinese Han population. Mol Biol Rep 2011; 38: 4731-4738.

[13] Wang Y, Wang G, Sun H, Chen C, Xiao H and Zhang J. Association of ALOX5AP with ischemic stroke in eastern Chinese. World J Emerg Med 2012; 3: 108-113.

[14] Kaushal R, Pal P, Alwell K, Haverbusch M, Flaherty M, Moomaw C, Sekar P, Kissela B, Kleindorfer D, Chakraborty R, Broderick J, Deka R and Woo D. Association of ALOX5AP with ischemic stroke: a population-based case-control study. Hum Genet 2007; 121: 601-607.

[15] Zintzaras E, Rodopoulou P and Sakellaridis N. Variants of the arachidonate 5-lipoxygenase-activating protein (ALOX5AP) gene and risk of stroke: a HuGE gene-disease association review and meta-analysis. Am J Epidemiol 2009; 169: 523-532.