

Original Article

The impact of the AHSB genetic polymorphism on the risk of ischemic stroke: a case-control study

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Received February 6, 2018; Accepted April 10, 2018; Epub October 1, 2018; Published October 15, 2018

Abstract: Ischemic stroke (IS) is a complex disease caused by an obstruction within a brain-supplying blood vessel that involves both genetic and environmental factors. In this study, we evaluated the association of genetic polymorphisms in the AHSB gene with ischemic stroke risk in the Chinese population. A case-control study was conducted that included 477 nephropathy patients and 490 healthy controls. Chi-squared tests and a genetic model were used to evaluate associations. In the genetic model analysis, we identified that the SNP of rs2070634 in the AHSB gene was associated with a 1.37-fold increase the risk of stroke in the co-dominant model (adjusted, the "G/T" genotype), and a 1.40-fold increase the risk of stroke in the Over-dominant model (adjusted, the "G/T" genotype), respectively. The rs2518136 in the AHSB gene was associated with a 1.37-fold increase the risk of stroke in the co-dominant model (adjusted, the "T/C" genotype) and a 1.41-fold decrease the risk of stroke in the over-dominant model (adjusted, the "T/C" genotype), respectively. We found four SNPs (rs2248690, rs2070634, rs4917 and rs2518136) show a strong linkage, but the AHSB haplotype was not found to be associated with a risk of ischemic stroke. The present study suggests that the AHSB polymorphism may contribute to an increased risk of ischemic stroke.

Keywords: Ischemic stroke, AHSB, genetics polymorphism, Chinese population

Introduction

Stroke, as a serious cerebrovascular disease, is a leading cause of death and long-term adult disability throughout the world [1]. Because stroke involves high morbidity and high mortality [2], the medical community has linked it with coronary heart disease and cancer as one of the three major diseases that threaten human life and health [3-5]. Ischemic stroke (IS), the most common type, results from a local cerebral ischemia that occurs with an obstruction within a brain-supplying blood vessel [6, 7]. The specific reasons for the patho-physiological cause of Ischemic stroke are still not clear. Previous studies have demonstrated that Ischemic stroke is a complex and heterogeneous disease influenced by both genetic and environmental factors (such as age, smoking, high blood pressure, abdominal obesity, cardiac arrhythmias, diabetes mellitus, and lack of physical activities) [8-10].

Until now, large-scale genome-wide association studies (GWAS) have identified several genetic polymorphisms that are associated with the risk of ischemic stroke. For example, XingYang [11] et al. found that *CYP2C8*, *EPHX2*, and *CYP4A11* gene variants could increase the risk for ischemic stroke. Nan Zhao [12] et al. studied inflammatory gene polymorphisms with ischemic stroke. Qiaoya [13] et al. found that *IMPA2* can increase the risk of ischemic stroke. Qing [14] et al. found that the *CYP2J2* gene can increase the risk of ischemic stroke. Qu Z [15] et al. studied the association of a tagging *ALOX5AP* polymorphism with the risk of ischemic stroke.

AHSB is a hepatic secretory protein that inhibits arterial calcium deposition in vitro [16]. AHSB is an important circulating inhibitor of ectopic mineral calcification, abundant in blood plasma, and which may be related to the pathogenesis of cardiovascular and cerebrovascular

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Table 1. General characteristics the of this study population

Variable	Cases (n = 477)	%	Controls (n = 490)	%	p-value
Sex					> 0.05
Male	310	65.00%	320	65.30%	
Female	167	35.00%	170	34.70%	
Age, yr (mean ± SD)	62.40±9.22		53.95±11.20		< 0.001

Genotyping

The candidate SNPs in the *AHSG* gene were selected from previously published polymorphisms associated with ischemic stroke. The minor allele frequencies (MAF) of all the selected SNPs were > 5% in the

diseases [17]. Previous studies have reported the *AHSG* gene is associated with various diseases, including coronary atherosclerosis [18, 19], coronary heart disease [20, 21], and atherosclerosis disease [22, 23]. However, few studies have examined the association between the *AHSG* gene and ischemic stroke. In the current study, we evaluated a significant association between 5 SNPs in the *AHSG* gene and the ischemic stroke risk in a Han Chinese population.

Materials and methods

Ethics statement

The study protocol was approved by the institutional ethics committee. All blood samples were collected following informed written consent of the participating individuals. The experimental protocol was implemented in accordance with the approved guidelines.

Subjects

We recruited a total of 477 patients, who were diagnosed with ischemic stroke (310 males and 167 females; age at diagnosis: 62.40±9.22 years) according to the fourth edition of Cerebrovascular Disease Diagnostic Standards [24]. These patients from Northwestern China were enrolled in the study at the Second Affiliated Hospital of Xi'an Jiaotong University from March 2011 to April 2016. These patients have no genetic relationship. The control group was composed of 490 healthy subjects (320 males and 170 females; age: 53.95±11.20 years) who were recruited from routine healthy examinations in the same hospitals. The controls were not related to the case group and had no history of cerebral infarction or transient ischemic attack. The first and second degree relatives of the controls had no cerebrovascular diseases or hypertension.

HapMap Chinese population. Some SNPs should be excluded ($P < 0.01$ for deviations from HWE). All the selected SNPs in the study were successfully genotyped with an average call rate of 99.68%. Blood samples were collected in tubes containing Ethylenediaminetetraacetic acid (EDTA) and stored at -80C after centrifuging at 1,500 rpm for 10 min. DNA was extracted from whole blood using the GoldMag-Mini Whole Blood Genomic DNA Purification Kit (GoldMag Co. Ltd. Xi'an City, China). We used NanoDrop 2000 (Thermo Scientific, Waltham, Massachusetts, USA) to measure the DNA concentration. The design of SNP genotyping and data processing were performed by Sequenom MassARRAY platform Software (Sequenom Co. Ltd., San Diego, California, USA) [25]. Genotype calling was carried out with version 3.0 of MassARRAY RT software and analyzed by version 3.4 of MassARRAY Typer software [26]. Sequenom Typer 4.0 software was used for data management and analysis.

Statistical analysis

Microsoft Excel (Microsoft, Redmond, WA) and SPSS Statistics (version 17.0, SPSS, Chicago, IL) were used for statistical analyses. $P \leq 0.05$ was considered to be statistically significant. SNP genotype frequencies in the case and control groups were calculated by Chi-square test, and the Hardy-Weinberg equilibrium (HWE) was used to check the genotype frequency of the control group. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were tested using unconditional logistic regression analysis with adjustment for age and gender [27]. Three models (dominant, recessive, log-additive) were applied by PLINK software (<http://pngu.mgh.harvard.edu/purcell/plink/>) to assess the association of SNPs with the risk of ischemic stroke [28]. Furthermore, Haploview (version 4.2, Broad Institute, Cambridge, MA) and SHEsis software were used for checking the linkage disequilibrium structure [29].

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Table 2. Allele frequencies in cases and controls and odds ratio estimates for ischemic stroke risk

SNP	Gene(s)	Locus	Alleles A/B	MAF		HWE <i>p</i>	OR	95% CI	<i>p</i> -value
				Case	Control				
rs2248690	AHSG	3q27.3	T/A	0.166	0.161	0.133	1.04	0.82-1.32	0.763
rs2070633	AHSG	3q27.3	T/C	0.374	0.352	0.002*	1.10	0.92-1.33	0.304
rs2070634	AHSG	3q27.3	T/G	0.374	0.352	0.023*	1.10	0.92-1.33	0.304
rs4917	AHSG	3q27.3	T/C	0.267	0.259	0.638	1.04	0.85-1.27	0.704
rs2518136	AHSG	3q27.3	T/C	0.374	0.354	0.023	1.09	0.91-1.32	0.351

SNP: single-nucleotide polymorphism; MAF: minor allele frequency; HWE: Hardy-Weinberg equilibrium; OR: odds ratio; CI: confidence interval. *Sites with HWE $p \leq 0.05$ were excluded.

Table 3. Relationships between AHSG polymorphism and ischemic stroke risk

SNP	Model	Genotype	Control	Case	Before adjusted		After adjusted		AIC	BIC	
					OR (95% CI)	<i>P</i> ^a -value	OR (95% CI)	<i>P</i> ^b -value			
rs2070634	Codominant	G/G	217 (44.4%)	181 (38%)	1	0.033*	1	0.052*	1191.5	1215.8	
		G/T	200 (40.9%)	235 (49.3%)	1.41 (1.07-1.85)		1.37 (1.02-1.84)				
		T/T	72 (14.7%)	61 (12.8%)	1.02 (0.68-1.51)		0.92 (0.60-1.40)				
	Dominant	G/G	217 (44.4%)	181 (38%)	1	0.042*	1	0.12	1193	1212.5	
		G/T-T/T	272 (55.6%)	296 (62%)	1.30 (1.01-1.69)		1.24 (0.94-1.64)				
	Recessive	G/G-G/T	417 (85.3%)	416 (87.2%)	1	0.38	1	0.22	1193.8	1213.3	
		T/T	72 (14.7%)	61 (12.8%)	0.85 (0.59-1.23)		0.78 (0.53-1.16)				
	Overdominant	G/G-T/T	289 (59.1%)	242 (50.7%)	1	0.0089*	1	0.016*	1189.6	1209.1	
		G/T	200 (40.9%)	235 (49.3%)	1.40 (1.09-1.81)		1.40 (1.06-1.85)				
	Log-additive	--	--	--	1.10 (0.92-1.32)	0.31	1.05 (0.86-1.28)	0.63	1195.1	1214.6	
rs2518136	Codominant	C/C	216 (44.2%)	180 (37.7%)	1	0.023*	1	0.042*	1191	1215.4	
		T/C	200 (40.9%)	237 (49.7%)	1.42 (1.08-1.87)		1.37 (1.02-1.84)				
		T/T	73 (14.9%)	60 (12.6%)	0.99 (0.66-1.46)		0.88 (0.58-1.35)				
	Dominant	C/C	216 (44.2%)	180 (37.7%)	1	0.042*	1	0.14	1193.2	1212.7	
		T/C-T/T	273 (55.8%)	297 (62.3%)	1.31 (1.01-1.69)		1.23 (0.93-1.62)				
	Recessive	C/C-T/C	416 (85.1%)	417 (87.4%)	1	0.29	1	0.15	1193.3	1212.8	
		T/T	73 (14.9%)	60 (12.6%)	0.82 (0.57-1.18)		0.75 (0.50-1.11)				
	Overdominant	C/C-T/T	289 (59.1%)	240 (50.3%)	1	0.0061*	1	0.014*	1189.4	1208.8	
		T/C	200 (40.9%)	237 (49.7%)	1.43 (1.11-1.84)		1.41 (1.07-1.86)				
		Log-additive	--	--	--	1.09 (0.91-1.31)	0.36	1.03 (0.85-1.26)	0.75	1195.3	1214.8

Abbreviations: SNP: Single nucleotide polymorphism; OR: odds ratio; 95% CI: 95% confidence interval. ^a*P* values were calculated from unconditional logistic regression analysis. ^b*P* values were calculated by unconditional logistic regression analysis with adjustments for age and gender. * $P \leq 0.05$ indicates statistical significance.

Results

Characteristics of the participants

This study involved 967 subjects, including 477 patients (310 males and 167 females; age at diagnosis: 62.40 ± 9.22 years) and 490 healthy controls (320 males and 170 females; age: 53.95 ± 11.20 years). The stroke cases and controls were matched by sex, but there was a significant difference in age between ischemic stroke cases and controls ($P < 0.001$) (Table 1).

The associations between AHSG and ischemic stroke

Table 2 shows the basic information of candidate SNPs in this study, including chromosomal

position, gene, allele, Hardy-Weinberg equilibrium (HWE) test results, and minor allele frequency (MAF), 95% CI and the *p*-value of allele. One SNP (rs2070633) was excluded for significant deviation from the Hardy-Weinberg equilibrium ($P < 0.01$). We used the chi-squared test to assess the risk of gene polymorphism in the allele model, but there is no statistically significant association between the allele and ischemic stroke risk, and the Bonferroni correction also confirms this result (Table 2).

Associations between genotype frequencies and ischemic stroke risk

As shown in Table 3, we found that the rs2070634 in the AHSG gene was associated with a 1.37-fold increase the risk of ischemic

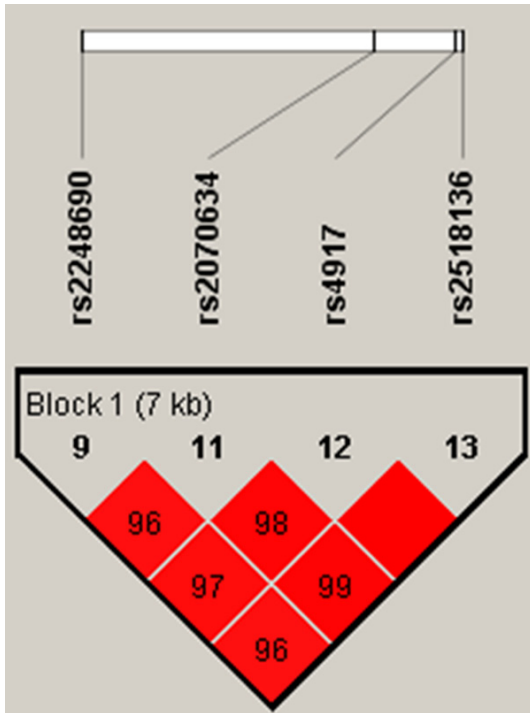


Figure 1. Linkage disequilibrium (LD) plots containing four SNPs from the AHSG gene.

stroke in the co-dominant model (adjusted OR = 1.37, 95% CI = 1.02-1.84, $P = 0.049$ for the “G/T” genotype) and a 1.40-fold increase the risk of ischemic stroke in the over-dominant model (adjusted OR = 1.40, 95% CI = 1.06-1.85, $P = 0.016$ for the “G/T” genotype), respectively. The rs2518136 in the AHSG gene was associated with a 1.37-fold increase the risk of ischemic stroke in the co-dominant model (OR = 1.37, 95% CI = 1.02-1.84, $P = 0.042$ for the “T/C” genotype) and a 1.41-fold increase the risk of ischemic stroke in the over-dominant model (OR = 1.41, 95% CI = 1.07-1.86, $P = 0.014$ for the “T/C” genotype), respectively.

Associations between haplotype analyses and ischemic stroke risk

LD and haplotype analyses of the SNPs in the case and control samples were further studied. The haplotype analysis detected the block in the AHSG genes (**Figure 1**). Although the four candidate SNPs (rs2248690, rs2070634, rs4917 and rs2518136) in the AHSG gene have shown strong linkage (**Figure 1**), the result for the AHSG haplotype was not found to be associated with a risk of ischemic stroke, because the p value had no statistical difference (**Table**

4). In addition, we did not find any association between the TF haplotype and the risk of stroke.

Discussion

Genetic studies have provided insight into numerous cardiovascular and cerebrovascular diseases, including ischemic stroke. In our study, we selected 5 SNPs in AHSG in the Chinese population to investigate their associations with the risk of ischemic stroke. Our results suggest that two SNPs (rs2070634, rs2518136) were associated with an increased risk of ischemic stroke. These results suggest that genetic polymorphisms in the AHSG gene may play an important role in increasing the risk of ischemic stroke in the Chinese population.

The AHSG (alpha₂-Heremans-Schmid glycoprotein), also called Fetuin A, is a 63-kDa glycoprotein synthesized by the liver and secreted into the serum [17], which is located at chromosome 3q27, and its locus is associated with cardiovascular disease [21, 30-32]. The AHSG gene is mainly secreted by hepatocytes and is abundant in blood plasma [33]. Previous studies about genome-wide association studies (GWAS) have confirmed that the AHSG polymorphism was associated with the risk of cardiovascular and cerebrovascular diseases [34]. For example, Eva Fisher [35] et al. researched the association of five tagging SNPs (rs2248690, rs2070633, rs2070635, rs4917, and rs6787344) in the AHSG gene and the risk of cardiovascular disease, the study results suggesting an involvement of fetuin-A in the pathogenesis of cardiovascular disease. Lehtinen [36] et al. studied the AHSG gene polymorphisms and the subclinical atherosclerosis risk, and the results indicated that the AHSG gene is related to death by cardiovascular disease. Several studies also have reported that the AHSG gene plays a key role in ischemic stroke (IS) and rs4917 is considered AHSG’s strongest marker in plasma levels. For example, Ma [37] et al. studied the association of the AHSG polymorphism and the risk of ischemic stroke, and these result suggest that the rs4918 SNPs of the AHSG gene can increase the risk of ischemic stroke in the Northern Han Chinese population. In this study, we found that two tSNPs (rs2070634, rs2518136) were associated with an increased risk of ischemic stroke in the genetic model analysis, and the four candidate

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Table 4. Haplotype analysis results in this study

	Haplotypes				Before adjusted			After adjusted	
	rs2248690	rs2070634	rs4917	rs2518136	Freq	OR (95% CI)	P-value	OR (95% CI)	P ^a -value
1	A	G	C	C	0.63	1	—	1	—
2	T	T	T	T	0.1605	1.07 (0.83-1.36)	0.61	0.99 (0.76-1.29)	0.96
3	A	T	T	T	0.1005	1.05 (0.77-1.43)	0.75	1.06 (0.76-1.48)	0.74
4	A	T	C	T	0.0998	1.16 (0.86-1.57)	0.34	1.10 (0.79-1.53)	0.57
Rare	*	*	*	*	0.0091	0.96 (0.32-2.89)	0.94	1.07 (0.32-3.62)	0.91

Abbreviations: CI = confidence interval; OR = odds ratio; SNP = single nucleotide polymorphism. P^a: Adjusted by gender and age.

SNPs (rs2248690, rs2070634, rs4917 and rs2518136) in the AHSG gene showed a strong linkage, so this provides a new theoretical basis for the ischemic stroke study. Therefore, these results indicate that *the* AHSG gene plays a pivotal role in ischemic stroke. More samples and functional tests are required to confirm our results, and we will be working on them.

Some potential limitations of our study should be considered when analyzing the results. Firstly, the sample size of our study was small. Secondly, selection bias was inevitable. Thirdly, associations between AHSG polymorphisms and clinicopathological disease type were not evaluated in this study. Additional studies are needed to illuminate the genetic mechanisms underlying ischemic stroke by fine-mapping the susceptibility regions of the variants.

To sum up, in this study, we confirmed that the AHSG gene was associated with the risk of ischemic stroke in the Chinese population once again, a finding which may provide new data and theoretical bases to facilitate earlier diagnosis and prevention, and illuminate the new candidate genes and new ideas for the study of subsequent occurrence mechanisms of ischemic stroke. Therefore, more studies should investigate these SNPs using more clinical data with bigger samples. And in the future, we will continue to collect samples, perfect the clinical information, and do a comprehensive study of the impact of polymorphisms on ischemic stroke.

Acknowledgements

This work was supported by Major project development plan funding project of Xizang Minzu University (No.18MDZ04). We thank all the patients and individuals for their participation and all the physicians and nurses of the

Second Affiliated Hospital of Xi'an Jiaotong University for their offer of ischemic stroke blood samples.

Disclosure of conflict of interest

None.

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