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Original Article

Association analysis of *ALOX5* gene polymorphisms with stroke risk: a case-control study in a Chinese Han population

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Abstract: Background: Stroke is a major cause of death and disability in the world. Genetic factors have been implicated in stroke risk but few have been reported. This study aimed to assess the association of *ALOX5* gene polymorphisms and stroke risk in a Chinese Han population. Methods: We conducted a case-control study in ischemic stroke and its subtypes in 488 cases and 504 controls, all of Chinese Han ancestry. We selected 9 single nucleotide polymorphisms (SNPs) of the *ALOX5* gene to test the association. All these SNPs were genotyped using Sequenom Mass-ARRAY technology. For each SNP, genotypic frequencies in controls were tested for departure from Hardy-Weinberg Equilibrium (HWE) using an exact test. A *P*-value of 0.05 was considered the threshold for statistical significance. We compared the allele frequencies of cases and controls using the chi-squared (χ^2) test. Associations between the gene and the risk of stroke were tested using various genetic models (allele, dominant, and recessive) and analysis by SNP stats. Odds ratios and 95% confidence intervals (CIs) were calculated by unconditional logistic regression with adjustments for age and gender. Results: We identified that the SNP rs3740107 were associated with a decreased risk stroke in the recessive model (OR=0.51; 95% CI=0.28-0.91; *P*=0.02). Additionally, very strong linkage was found between rs6593482, rs3824612, rs2029253, rs7918542, rs7919239, rs1369214, and rs10900213; and between rs3740107 and rs3780914 by Haploview analysis. Conclusion: The present study suggested that gene polymorphisms in the *ALOX5* gene may exert influences stroke susceptibility in a Chinese Han population.

Keywords: *ALOX5*, stroke, gene polymorphisms, case-control study, Chinese Han population

Introduction

Cerebrovascular disease (stroke) is the third leading cause of death worldwide, and the leading cause of adult chronic disability in most regions [1, 2]. Stroke represents an increasing health problem throughout the world as the proportion of elderly increases, and is an important cause of dementia and age-related cognitive decline. Countries of low and middle income have the largest burden of stroke, accounting for more than 85% of stroke mortality worldwide, but few reliable data are available to identify risk factors for stroke in most of these regions, and particularly for hemorrhagic stroke [3-6]. Stroke is a syndrome rather than a single disease, and subtypes of stroke are caused by a number of different specific disease processes. About 80% of stroke is ischemic; the three most common ischemic stroke subtypes are large vessel, cardioembolic and small vessel (lacunar) stroke [7].

Epidemiological studies have addressed that conventional cardiovascular risk factors such as age, sex, obesity, smoking, hypertension, diabetes and abnormal lipid metabolism accounting for only 50-60% of disease susceptibility [8, 9]. However, these risk factors cannot fully account for the overall risk of stroke. A large amount of genetic epidemiological studies have consistently suggested genetic factors

Table 1. General characteristics of case and control subjects

Variables	Case (n=488)		Control (n=504)		*P*-value
	No.	%	No.	%	
Median age	63.96		50.36		$P<0.001^b$
Sex					$P<0.001^a$
Male	325	66.6	308	61.1	
Female	163	33.4	196	38.9	
Total	488		504		

[a]*P* values were calculated by Student t tests; [b]*P* values were calculated from two-sided chi-square tests.

contribute to the susceptibility to stroke [10] and have already identified many predisposing genes that are associated with stroke risk, including *HDAC9* [7], *LTC4S* and *ALOX5* [11].

The *ALOX5* gene maps on chromosome 10, which induce a major enzyme called 5-lipoxygenase, a kind of lipoxygenase that metabolize arachidonic acid into inflammatory molecules called prostaglandins and leukotrienes. Previous studies have demonstrated that *ALOX5* gene polymorphisms as factors in vascular pathology and Alzheimer's disease [12]. Wang's study also proved that *ALOX5* gene polymorphisms associated with ischemic stroke risk in a cohort of Chinese in east China [11]. Moreover, in Africans this polymorphism was associated with an increased susceptibility to pulmonary tuber culosis [13]. However, whether these polymorphisms associated with stroke in the northwest Chinese Han population is unknown.

However, knowledge about the clinical importance of *ALOX5* genetics in stroke is still limited. In order to investigate the contribution of genetic variations to the risk of stroke, a case-control study including 488 cases and 503 controls was carried out to clarify the involvement of *ALOX5* single nucleotide polymorphisms (SNPs) as risk factors for the pathogenesis of stroke in the northwest Chinese Han population.

Materials and methods

Study subjects

We performed a case-control study to determine the association between gene polymorphism of *ALOX5* and stroke risk. All the study populations were Han Chinese selected from Xi'an and its surrounding regions. Finally, a total of 488 stroke cases, and 504 controls were recruited from the Haikou City People's Hospital between January 2011 and February 2014. All patients were newly diagnosed with stroke and were histologically confirmed. Control subjects were randomly selected from the medical examination center at the same hospital during the similar period, and these controls were cancer-free individuals and genetically unrelated to the patients. All protocols and methods were approved by the ethics committees of the local participating hospitals. Written informed consent was obtained from all participants. Experiments were conducted according to the principles expressed in the Declaration of Helsinki.

Polymorphisms selection and genotyping assays

Candidate t SNPs in the ALOX5 gene was selected from previously published polymorphisms associated with stroke. Validated t SNPs was selected with a MAF >5% in the Hap Map Asian population. A total of 9 t SNPs in the ALOX5 gene were selected for further genotyping. Genomic DNA was extracted from peripheral blood leukocytes using the GoldMag® nanoparticles method (GoldMag Ltd. Xi'an, China) according to the manufacturer's instructions, and DNA concentration was measured using the NanoDrop 2000 (ThermoScientific, Waltham, Massachusetts, USA). We used Sequenom MassARRAY Assay Design 3.0 Software to design Multiplexed SNP Mass EXTEND assays [14]. SNP genotyping was performed using the Sequenom Mass ARRAYRS1000 with a standard protocol recommended by the manufacturer [14]. Data management and analyses were performed using the Sequenom Typer 4.0 software as previously described [14, 15].

Statistical analysis

Statistical analyses were performed using the SPSS 19.0 statistical package (SPSS, Chicago, IL, USA) and Microsoft Excel (Redmond, WA, USA). All *P* values presented in this study are two-sided, and we used $P \leq 0.05$ as the threshold of statistical significance. An exact test was used to assess the departure of each SNP frequency from Hardy-Weinberg equilibrium (HWE) in controls. We compared allele frequencies between cases and controls using a χ^2 test

Table 2. PCR primers used for this study

SNP_ID	1st-PCR primer sequences	2nd-PCR primer sequences	UEP sequences
rs6593482	ACGTTGGATGTATATCCTGGATAGAAGTC	ACGTTGGATGAAAGTGAAAACACAACCCGC	AAATGTCTGTAAGTCATGTATCC
rs3824612	ACGTTGGATGTGCTCCCAGTTCCCTAAATG	ACGTTGGATGTGGAAGACAGAGTGTAGAGG	GAGCAAAAAAGAAGGGAGA
rs2029253	ACGTTGGATGGCATGGTATGAAATAAACCC	ACGTTGGATGCTGGTGCCTTGGTGACTTTC	cccGCCTTGGTGACTTTCCAGCAG
rs7918542	ACGTTGGATGTAATCTCATCCCCAGAGGAC	ACGTTGGATGTAGGTGAGTAGTTGCCTAGC	gCTAGGAGTTGGGGAGGGG
rs7919239	ACGTTGGATGTGGTAGTAGCAGCAGTTCAC	ACGTTGGATGGCAGCAAGTATACAAAAGGTG	ggAGGTGTTCAACTTCATTAGGA
rs1369214	ACGTTGGATGTGACAAATGTATGGATGGGC	ACGTTGGATGCCTTTCACAAAATTGTATCG	TCACAAAATTGTATCGTAATTTGC
rs10900213	ACGTTGGATGGAATAGGGCAGCCCCTAAAC	ACGTTGGATGTCTGCCCATAAATCCTATCC	aatagGAGACAGCATCAAAGTCACTC
rs3740107	ACGTTGGATGACGAAAGATGAGAGACCGAC	ACGTTGGATGCACATCTATTTCACCTAACC	CCTAACCTCTTTCTTCTTAGT
rs3780914	ACGTTGGATGTGGGACAGGAAATACCACCG	ACGTTGGATGTGGGAAGTTCCAGAAGTATC	TGGAATCAGAAGAGCCT

Table 3. Basic information about nine SNPs examined in the ALOX5gene

SNP ID	Chromosome	Position	Band	Allele	Gene (s)	Role	HWE *P*
				A/B			
rs6593482	10	45188455	10q11.21	T/G	ALOX5	Promoter	0.84
rs3824612	10	45192870	10q11.21	T/C	ALOX5	Intron	0.89
rs2029253	10	45211490	10q11.21	A/G	ALOX5	Intron	0.54
rs7918542	10	45216257	10q11.21	G/A	ALOX5	Intron	0.61
rs7919239	10	45218362	10q11.21	A/C	ALOX5	Intron	0.63
rs1369214	10	45220735	10q11.21	G/A	ALOX5	Intron	0.82
rs10900213	10	45224720	10q11.21	G/T	ALOX5	Intron	0.86
rs3740107	10	45243776	10q11.21	A/G	ALOX5	Intron	0.96
rs3780914	10	45255750	10q11.21	C/T	ALOX5	Intron	0.67

Table 4. Genotypic model analysis of relationship between ALOX5 t SNPs and stroke Risk

SNP ID	Minor Allele	MAF		Allele model		Dominant model		Recessive model	
		Case	Control	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
rs6593482	T	0.177	0.180	0.98 (0.78-1.24)	0.550	1.03 (0.79-1.35)	0.826	0.68 (0.32-1.42)	0.299
rs3824612	T	0.474	0.487	0.95 (0.80-1.13)	0.327	0.89 (0.67-1.17)	0.388	1.00 (0.74-1.33)	0.978
rs2029253	A	0.400	0.386	1.06 (0.89-1.27)	0.375	1.09 (0.85-1.42)	0.489	1.06 (0.76-1.48)	0.745
rs7918542	G	0.187	0.183	1.03 (0.82-1.29)	0.543	1.11 (0.85-1.45)	0.428	0.61 (0.29-1.26)	0.175
rs7919239	A	0.185	0.183	1.02 (0.81-1.28)	0.544	1.10 (0.84-1.43)	0.486	0.61 (0.29-1.26)	0.179
rs1369214	G	0.151	0.139	1.10 (0.86-1.41)	0.590	1.10 (0.83-1.46)	0.495	1.29 (0.50-3.29)	0.595
rs10900213	G	0.376	0.365	1.05 (0.87-1.26)	0.389	1.07 (0.83-1.39)	0.577	1.04 (0.73-1.48)	0.837
rs3740107	A	0.234	0.260	0.87 (0.71-1.07)	0.482	0.93 (0.72-1.19)	0.552	0.51 (0.28-0.91)	0.02*
rs3780914	C	0.386	0.390	0.98 (0.82-1.18)	0.378	0.95 (0.73-1.23)	0.698	1.02 (0.72-1.46)	0.906

*The *P* values ≤0.05 and have statistical significance.

[16]. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were tested using unconditional logistic regression analysis with adjustment for age and gender [17]. We did not divide subjects into subgroups because of the limited sample size. The possibility of sex differences as a source of population sub-structure was evaluated by a genotype test for each SNP in male and female controls, and the number of significant results at the 5% level was compared with the number expected by the χ^2 test. We did not detect population stratification because all participants' ethnicity was Han Chinese.

The three genetic models (dominant, recessive and additive) were applied by PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/) to assess the association of single t SNPs with the risk of stroke. ORs and 95% CIs were calcu-

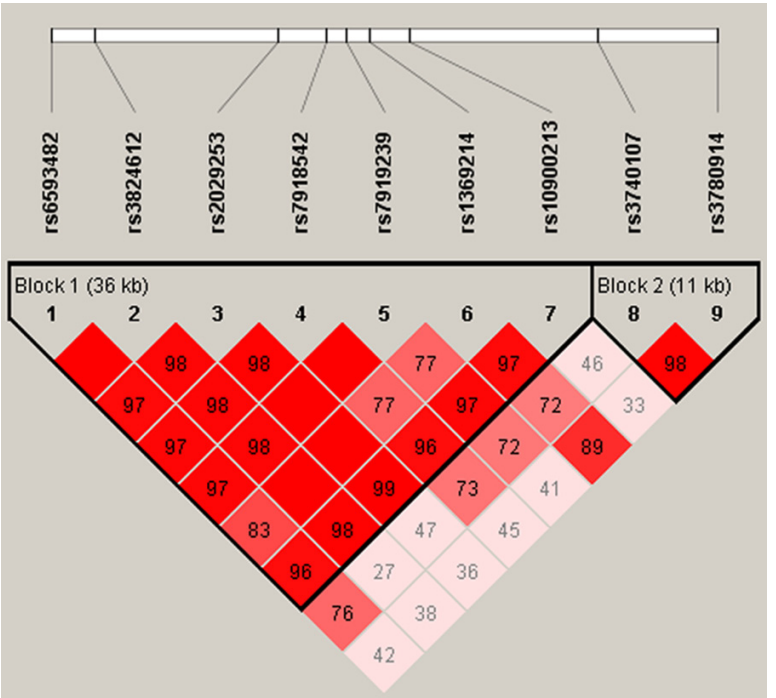

Figure 1. Haplotype block map for all the t SNPs of the *ALOX5* gene. Block 1 includes rs6593482, rs3824612, rs2029253, rs7918542, rs7919239, rs1369214, and rs10900213; Block 2 includes rs3740107 and rs3780914. The LD between two SNPs is standardized D9 (red schemes).

lated by unconditional logistic regression analyses adjusted for age and sex [17, 18].

Results

In total, 992 subjects were recruited in the current study, including 488 cases (325 male, 163 female; median age at diagnosis 63.96 years) and 504 controls (308 male, 196 female; median age 50.36 years). General characteristics of the cases and controls were listed in **Table 1**. Statistically significant differences were observed between stroke cases and controls in terms of age and gender (P<0.001).

A total of nine SNPs were selected for further genotyping. Each SNP/sample call rate was 98.5% in both cases and controls. The primer sequences are presented in **Table 2**. **Table 3** summarizes the basic information about nine SNPs examined in the study population. All of the tested t SNPs are in Hardy-Weinberg equilibrium (HWE) (P>0.05) in the control population of this study (**Table 3**).

We further analyzed the associations between SNPs and stroke risk under three genetic models (allele, dominant and recessive). **Table 4** shows that the SNP rs3740107 were associated with a decreased risk stroke: in the recessive model, (OR=0.51; 95% CI= 0.28-0.91; P=0.02).

ALOX5 polymorphisms were further characterized using linkage disequilibrium (LD) and haplotype analyses. LD was determined pair wise among all 9 SNPs and the haplotype structure of *ALOX5* gene was analyzed (D' and r^2). Haplotype block divided by D' confidence interval method, D'value of 95% CI 0.70~0.98 in adjacent SNPs were classified as the same haplotype block. Haplotype analysis identified two LD blocks within *ALOX5*, and very strong linkage was found between rs6593482, rs3824612, rs2029253, rs7918542, rs7919239, rs1369214, and rs10900213; and between rs3740107 and rs3780914 (**Figure 1**).

Discussion

Cerebrovascular disease are the leading causes of death and disability in the developed world, with an increasing prevalence due to the aging of the population and obesity [19]. Stroke is a major cause of death and disability in most populations of eastern Asia, and the incidence, particularly of hemorrhagic stroke, is generally higher than in western populations [20]. In our study, we evaluated nine SNPs of the *ALOX5* gene to investigate whether they are associated with stroke in the northwest Chinese Han population. We found rs3740107 was associated with a decreased risk of stroke in the study population.

The SNP rs3740107 at chromosome 16q12 located in the *ALOX5* gene. Previous studies have demonstrated *ALOX5* gene polymorphisms as risk factors in ischemic stroke [11], vascular pathology and Alzheimer's disease [12], and human pulmonary tuberculosis [13]. Another study revealed the pharmacogenetic

association between *ALOX5* promoter genotype and the response to anti-asthma treatment [21]. Additionally, the *ALOX5* gene is proved to be a novel therapeutic target in cancer stem cells of chronic myeloid leukemia [22].

Despite reported relevant associations, the molecular consequences of *ALOX5* variants have only incompletely been characterized so far. In the present study, we identified rs3740107 as a protective locus for stroke in the northwest Chinese Han population. The SNP rs3740107 in *ALOX5* was first described to be associated with a decreased risk of stroke in this population. Although the polymorphisms of *ALOX5* has been identified as risk of coronary artery disease [23]. However, the SNP has not been studied before, so this hypothesis needs to be investigated in future studies. Further investigations are warranted to verify and discover more loci associated with risk for stroke in other ethnic populations.

There are several inherent limitations in our study should be considered. First, the sample size of our study was relatively small. The statistical power may be limited because of the sample size. Second, all subjects of this study were limited to the Han Chinese population; therefore, it is unknown whether the results are applicable to other ethnicities. Third, this was a hospital-based study; therefore, selection bias may be unavoidable. So larger well-designed studies combined with stroke classification are needed to confirm the associations and clarify the potentially biological mechanisms of these polymorphisms in stroke.

In conclusion, the current findings suggested that a common susceptibility locus rs3740107 was associated with a decreased risk of stroke risk in a Chinese Han population and might be used as a molecular marker for evaluating stroke risk. Further investigations are warranted to verify and discover more susceptible loci to other cardiovascular diseases and to elucidate the underlying molecular mechanism.

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Disclosure of conflict of interest

None.

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References

[1] Bonita R. Epidemiology of stroke. Lancet 1992; 339: 342-344.
[2] Committee PA. Reducing brain damage: faster access to better stroke care. Fifty-Second Report of Session 2005; 6.
[3] Feigin VL. Stroke in developing countries: can the epidemic be stopped and outcomes improved? Lancet Neurol 2007; 6: 94-97.
[4] Strong K, Mathers C and Bonita R. Preventing stroke: saving lives around the world. Lancet Neurol 2007; 6: 182-187.
[5] O'Donnell M and Yusuf S. Tackling the global burden of stroke: the need for large-scale international studies. Lancet Neurol 2009; 8: 306-307.
[6] Ariesen M, Claus S, Rinkel G and Algra A. Risk factors for intracerebral hemorrhage in the general population a systematic review. Stroke 2003; 34: 2060-2065.
[7] International Stroke Genetics Consortium (ISGC); Wellcome Trust Case Control Consortium 2 (WTCCC2), Bellenguez C, Bevan S, Gschwendtner A, Spencer CC, Burgess AI, Pirinen M, Jackson CA, Traylor M, Strange A, Su Z, Band G, Syme PD, Malik R, Pera J, Norrving B, Lemmens R, Freeman C, Schanz R, James T, Poole D, Murphy L, Segal H, Cortellini L, Cheng YC, Woo D, Nalls MA, Müller-Myhsok B, Meisinger C, Seedorf U, Ross-Adams H, Boonen S, Wloch-Kopec D, Valant V, Slark J, Furie K, Delavaran H, Langford C, Deloukas P, Edkins S, Hunt S, Gray E, Dronov S, Peltonen L, Gretarsdottir S, Thorleifsson G, Thorsteinsdottir U, Stefansson K, Boncoraglio GB, Parati EA, Attia J, Holliday E, Levi C, Franzosi MG, Goel A, Helgadottir A, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin A, Duncanson A, Jankowski J, Mathew CG, Palmer CN, Plomin R,

Rautanen A, Sawcer SJ, Trembath RC, Viswanathan AC, Wood NW, Worrall BB, Kittner SJ, Mitchell BD, Kissela B, Meschia JF, Thijs V, Lindgren A, Macleod MJ, Slowik A, Walters M, Rosand J, Sharma P, Farrall M, Sudlow CL, Rothwell PM, Dichgans M, Donnelly P, Markus HS. Genome-wide association study identifies a variant in HDAC9 associated with large vessel ischemic stroke. Nat Genet 2012; 44: 328-333.

[8] O'Donnell MJ, Xavier D, Liu L, Zhang H, Chin SL, Rao-Melacini 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.

[9] Sacco R, Ellenberg J, Mohr J, Tatemichi T, Hier D, Price T and Wolf P. Infarcts of undetermined cause: the NINCDS Stroke Data Bank. Ann Neurol 1989; 25: 382-390.

[10] Dichgans M. Genetics of ischaemic stroke. Lancet Neurol2007; 6: 149-161.

[11] Wang GN, Zhang JS, Cao WJ, Sun H, Zhang J, Wang Y and Xiao H. Association of ALOX5, LTA4H and LTC4S gene polymorphisms with ischemic stroke risk in a cohort of Chinese in east China. World J Emerg Med 2013; 4: 32.

[12] Manev H and Manev R. 5-Lipoxygenase (ALOX5) and FLAP (ALOX5AP) gene polymorphisms as factors in vascular pathology and Alzheimer's disease. Med Hypotheses 2006; 66: 501-503.

[13] Herb F, Thye T, Niemann S, Browne EN, Chinbuah MA, Gyapong J, Osei I, Owusu-Dabo E, Werz O, Rüsch-Gerdes S, Horstmann RD, Meyer CG. ALOX5 variants associated with susceptibility to human pulmonary tuberculosis. Hum Mol Genet 2008; 17: 1052-1060.

[14] Gabriel S and Ziaugra L. SNP genotyping using Sequenom MassARRAY 7K platform. Curr Protoc Hum Genet 2004; Chapter 2: Unit 2.12.

[15] Thomas RK, Baker AC, DeBiasi RM, Winckler W, LaFramboise T, Lin WM, Wang M, Feng W, Zander T and MacConaill LE. High-throughput oncogene mutation profiling in human cancer. Nat Genet 2007; 39: 347-351.

[16] Adamec C. [Example of the use of the non-parametric test. Test X^2 for comparison of 2 independent examples]. Cesk Zdrav 1964; 12: 613-619.

[17] Bland JM and Altman DG. The odds ratio. BMJ 2000; 320: 1468.

[18] Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, De Bakker PI and Daly MJ. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81: 559-575.

[19] Bonow RO, Smaha LA, Smith SC, Mensah GA and Lenfant C. World Heart Day 2002 the international burden of cardiovascular disease: responding to the emerging global epidemic. Circulation 2002; 106: 1602-1605.

[20] Blood pressure, cholesterol, and stroke in eastern Asia. Eastern Stroke and Coronary Heart Disease Collaborative Research Group. Lancet 1998; 352: 1801-1807.

[21] Drazen JM, Yandava CN, Dubé L, Szczerback N, Hippensteel R, Pillari A, Israel E, Schork N, Silverman ES and Katz DA. Pharmacogenetic association between ALOX5 promoter genotype and the response to anti-asthma treatment. Nat Genet 1999; 22: 168-170.

[22] Chen Y, Li D and Li S. The Alox5 gene is a novel therapeutic target in cancer stem cells of chronic myeloid leukemia. Cell Cycle 2009; 8: 3488-3492.

[23] Assimes TL, Knowles JW, Priest JR, Basu A, Volcik KA, Southwick A, Tabor HK, Hartiala J, Allayee H and Grove ML. Common polymorphisms of ALOX5 and ALOX5AP and risk of coronary artery disease. Hum Genet 2008; 123: 399-408.