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Associations between *GUCY1A3* genetic polymorphisms and large artery atherosclerotic stroke risk in Chinese Han population: a case-control study

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Abstract

Background: Previous genome-wide association studies have found two single nucleotide polymorphisms (SNP) rs7692387 and rs1842896 located on or near the *GUCY1A3* gene were associated with coronary artery disease (CAD). *GUCY1A3* was considered to be involved in the process of atherosclerosis, but there was little information about the association between genotypic polymorphisms of the *GUCY1A3* and large artery atherosclerotic (LAA) stroke. This study aimed to investigate the associations between the *GUCY1A3* rs7692387, rs1842896 polymorphisms and LAA stroke susceptibility.

Methods: A total of 298 LAA stroke patients and 300 control subjects from a southern Chinese Han population were included. SNaPshot technique was used for genotype analysis. Associations between genotypes and LAA stroke susceptibility were analyzed with logistic regression model.

Results: Our study found that under the recessive model (TT vs. GT + GG), the *GUCY1A3* rs1842896 polymorphism was significantly correlated with LAA stroke (OR = 1.48, 95%CI: 1.07–2.04, $P = 0.018$). After adjustment for its effects on age, gender, cigarette smoking, total cholesterol, low-density lipoprotein cholesterol, HbA1c, hypertension, diabetes mellitus, and CAD, the rs1842896 TT genotype retained association with increased susceptibility to LAA stroke (recessive model: adjusted OR = 1.96, 95%CI: 1.22–3.17, $P = 0.006$). However, association between rs7692387 polymorphism with LAA stroke was not observed.

Conclusion: Our results indicate that the *GUCY1A3* rs1842896 polymorphism is an LAA stroke risk factor in Southern Han Chinese.

Keywords: *GUCY1A3*, Polymorphism, Large artery atherosclerotic stroke, Atherosclerosis

Background

Ischemic stroke (IS) is a disease with high morbidity and mortality, which affects people across the world and causes heavy economic burden for families and the whole society [1]. According to the TOAST (Trial of Org 10,172 in Acute Stroke Treatment) classification [2], IS can be divided into five etiologic subtypes, including

large artery atherosclerotic (LAA) stroke. LAA stroke carries the highest risk of early neurological deterioration and early recurrent stroke in comparison to other IS subtypes [3, 4]. LAA stroke is attributed to atherosclerosis, where the development of thrombosis may cause occlusion of cerebral artery, and lead to irreversible brain damage. Atherosclerosis is a typical complex disease process caused by multiple environmental and genetic factors and their interactions [5]. However, the causative genes are largely unknown. To date, genome-wide association studies (GWAS) have identified several genomic loci are associated with atherosclerosis-related diseases, such as LAA stroke and coronary artery disease

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(CAD). Some of these variants on chromosome 4q32.1 tags the *GUCY1A3* gene (according to a new nomenclature also known as *GUCY1A1*) [6, 7].

The *GUCY1A3* gene encodes the $\alpha 1$ subunit of the soluble guanylyl cyclase (sGC). The sGC complex, a heterodimeric enzyme comprising a $\beta 1$ subunit and either an $\alpha 1$ or $\alpha 2$ subunit, is the only known receptor for nitric oxide (NO) and catalyzes the formation of the second messenger cyclic guanosine 3',5'-monophosphate (cGMP). cGMP regulates a vast array of physiological processes, including neurotransmission [8], vascular smooth muscle cells (VSMCs) relaxation [9], and inhibition of platelet aggregation [10]. Previous GWAS have found rs7692387 [11] and rs1842896 [6] single nucleotide polymorphisms (SNPs) located on or near the *GUCY1A3* gene were associated with coronary artery disease (CAD). Although the nidi of CAD and LAA stroke are different, they share the same pathophysiological basis: atherosclerosis.

GUCY1A3 was considered to be involved in the process of atherosclerosis, but there was little information about the association between genotypic polymorphisms of the *GUCY1A3* and large artery atherosclerotic (LAA) stroke. To our knowledge, the genetic evidence on the associations between the *GUCY1A3* rs7692387, rs1842896 polymorphisms and susceptibility of LAA stroke has not been reported previously. Therefore, the present study was conducted to evaluate the associations between the two SNPs and LAA stroke susceptibility in a Southern Chinese Han population.

Methods

Study population

A total of consecutive unrelated 298 LAA stroke patients were enrolled from September 2016 to December 2017 in this study. IS was defined according to the World Health Organization definition as "rapidly developing clinical signs of focal (or global) disturbance of cerebral function lasting more than 24 hours with no apparent cause other than of vascular origin", with evidence of cerebral infarction in

clinically relevant areas of the brain confirmed by computed tomography (CT) and/or magnetic resonance imaging (MRI). LAA stroke was diagnosed by experienced neurologists according to the TOAST classification [2]. Patients were enrolled if they: 1) aged 18 years or older; 2) were Southern Han Chinese; 3) had available blood samples. The following exclusion criteria were applied: the presence of other types of cerebrovascular disease (e.g. intracranial hemorrhage, subarachnoid hemorrhage, transient ischemic attack, cerebral aneurysm or cerebrovascular malformation), known embolic source (aortic arch, cardiac or carotid), severe systemic diseases such as neoplasms, autoimmune or inflammatory diseases, and serious chronic diseases (e.g. hepatic or renal failure). Three hundred symptom-free individuals were recruited as the control group. The inclusion criteria of control subjects were as follows: 1) aged 18 years or older; 2) were Southern Han Chinese; 3) had no history of cerebrovascular diseases; 4) had no history of atherosclerotic diseases; 5) had regular physical examinations. Exclusion criteria were the same as the criteria used for the patient group. The study was approved by the ethics committee of the First Affiliated Hospital of Guangxi Medical University and was carried out in accordance with the Declaration of Helsinki. All participants provided informed consent. All methods were performed in accordance with the relevant guidelines and regulations.

Genotyping

Genomic DNA was isolated from a 300 μ L blood sample using the TIANamp Blood DNA Kit (Tiangen Biotec [Beijing] Co., China) according to the manufacturer's instructions. Extracted genomic DNA samples were stored at -80°C until genotyping was performed. Two SNPs in *GUCY1A3* gene, rs1842896 and rs7692387, were genotyped using the method of SNaPshot kit commercialized by Applied Biosystems, as described previously [12]. The sequences of primers used for polymerase chain reaction (PCR) and minisequencing extension were listed in Table 1.

Table 1 Primer sequences for *GUCY1A3* SNaPshot genotyping

Primer description	Sequence
rs1842896 PCR primer	Forward: GAATTATACAGTTACTTGCCTCAGGTTGAC Reverse: GAAAAGGCAGATTAAGATGACGGATTT
rs7692387 PCR primer	Forward: CACTTCACTGTGGAAACCGAGTTG Reverse: CAAAGCAAGTAAGGCAGAGAACAAGA
rs1842896 SR1 primer	CATGATAACTAGTGCTTTTAAGGGAATG
rs1842896 SR2 primer	TTTTTTTTTTTTCATGATAACTAGTGCTTTTAAGGGAATG
rs1842896 SR3 primer	TTTTTTTTTTTTTTTTTTTTTTCATGATAACTAGTGCTTTTAA-GGGAATG
rs7692387 SF1 primer	GGCCAAGGGCAGAGACATTT
rs7692387 SF2 primer	TTTTTTTTTTTTTGCCAAGGGCAGAGACATTT
rs7692387 SF3 primer	TTTTTTTTTTTTTTTTTTTTTTCGCCAAGGGCAGAGACATTT

Fluorescent-labeled PCR fragments were resolved by capillary electrophoresis on an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The data produced were analyzed with GeneMapper 4.1 software (Applied Biosystems).

Statistical analysis

SPSS 22.0 software (IBM Inc., Chicago, IL, USA) was used for the statistical analysis. Alleles were tested for deviation from Hardy-Weinberg equilibrium (HWE) using a chi-square test. Continuous variables were expressed as mean \pm SD. Normality of the sample distribution of each continuous variable was tested with the Kolmogorov-Smirnov test. Differences of continuous variables were evaluated by the student's t-test or Mann-Whitney U test. Categorical variables were expressed as proportions and compared using a chi-square test. The genetic association was estimated using a univariate logistic regression analysis. A multivariate logistic regression analysis was performed to adjust for potential confounding factors, such as age, gender, cigarette smoking, total cholesterol, low-density lipoprotein cholesterol, Hemoglobin A1c (HbA1c), hypertension history, diabetes mellitus history, and coronary artery disease history. All tests were two-tailed and a *P*-value of 0.05 was considered statistically significant.

Results

Clinical characteristics of the study population

The clinical characteristics of LAA stroke patients and control subjects are summarized in Table 2. A total of 298

Table 2 Clinical characteristics of cases and controls

	Cases (<i>n</i> = 298)	Controls (<i>n</i> = 300)
Age, years	67.58 \pm 12.62*	63.29 \pm 11.80
Male, <i>n</i> (%)	206 (69.13%)	194 (64.67%)
BMI, kg/m ²	23.72 \pm 3.53	23.32 \pm 3.22
SBP, mmHg	148.39 \pm 21.90*	128.87 \pm 19.99
DBP, mmHg	85.58 \pm 14.68*	77.78 \pm 13.14
Total Cholesterol, mmol/L	4.93 \pm 1.28	4.88 \pm 1.28
Triglyceride, mmol/L	1.51 \pm 0.91	1.49 \pm 0.93
Low-Density Lipoprotein Cholesterol, mmol/L	3.29 \pm 1.09	3.21 \pm 1.06
Hemoglobin A1c, %	6.69 \pm 1.53*	6.24 \pm 1.24
Cigarette smoking, <i>n</i> (%)	110 (36.91%)*	71 (23.67%)
Coronary artery disease history, <i>n</i> (%)	24 (8.05%)*	11 (3.67%)
Hypertension history, <i>n</i> (%)	198 (66.44%)*	98 (32.67%)
Diabetes mellitus history, <i>n</i> (%)	65 (21.81%)*	30 (10.00%)

Age, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol, triglyceride, low-density lipoprotein cholesterol, glucose and Hemoglobin A1c (HbA1c) values are shown as the mean (standard deviation), and other values as the number of individuals (*n*) with percentage (*n*/total *N*) in parentheses.

**P* < 0.05 vs. control

patients with LAA stroke, 206 (69.13%) males and 92 (30.87%) females, were enrolled in the case group. A total of 300 volunteers, 194 (64.67%) males and 106 (35.33%) females, were enrolled in the control group. The mean age was 67.58 \pm 12.62 years in the case group and 63.29 \pm 11.80 years in the control group. As shown in Table 2, the prevalence of conventional risk factors such as hypertension, diabetes mellitus, and cigarette smoking, was found to be higher in patients compared to the controls. Patients with LAA stroke had a higher prevalence of coronary artery disease history (8.05 vs. 3.67%) than controls. The level of HbA1c was significantly higher in LAA stroke patients when compared to controls.

Genotype distributions of the *GUCY1A3* SNPs

The genotype distribution of the *GUCY1A3* rs1842896 and rs7692387 in LAA stroke patients and controls are given in Table 3. The distribution of rs1842896 genotypes in LAA stroke patients significantly differed from the control group (*P* = 0.028), while the distribution of rs7692387 genotypes was similar between case and control individuals (*P* = 0.915). The TT, GT and GG genotype frequencies of rs1842896 were 54.36, 36.58 and 9.06% in patients, 44.67, 47.33, and 8.00% in controls, respectively. Obtained genotype frequencies for each SNP did not significantly deviate from Hardy-Weinberg equilibrium expectations (*P* > 0.05).

Associations between the *GUCY1A3* SNPs and LAA stroke risk

We assessed the associations of the *GUCY1A3* rs1842896 and rs7692387 polymorphisms with LAA stroke in different genetic models, including dominant, recessive, and additive models. Genotypic association between rs1842896 and LAA stroke was significant in the recessive model (recessive model: OR = 1.48, 95% CI: 1.07–2.04, *P* = 0.018, Table 4), there was no statistical significance in the other models (Table 4). After adjusted for age, gender, cigarette smoking, total cholesterol, low-density lipoprotein cholesterol,

Table 3 Distributions of the *GUCY1A3* rs1842896 and rs7692387 genotypes

Genotypes	Cases (<i>n</i> = 298)	Controls (<i>n</i> = 300)	χ^2 -value	<i>P</i> -value
rs1842896				
TT (%)	162 (54.36%)	134 (44.67%)	7.157	0.028*
GT (%)	109 (36.58%)	142 (47.33%)		
GG (%)	27 (9.06%)	24 (8.00%)		
rs7692387				
GG (%)	184 (61.74%)	190 (63.33%)	0.178	0.915
GA (%)	102 (34.23%)	99 (33.00%)		
AA (%)	12 (4.03%)	11 (3.67%)		

P-values are based on chi-square test for genotype comparison

The entries in boldface are statistically significant

**P* < 0.05

Table 4 Associations between the *GUCY1A3* rs1842896 and rs7692387 with LAA stroke

Model	Crude OR (95% CI)	P-value	Adjusted OR (95% CI)	Adjusted P-value
rs1842896				
Dominant (TT + GT vs. GG)	0.87 (0.49–1.55)	0.643	0.79 (0.34–1.83)	0.586
Recessive (TT vs. GT + GG)	1.48 (1.07–2.04)	0.018*	1.96 (1.22–3.17)	0.006*
Additive (TT vs. GG)	1.08 (0.59–1.95)	0.813	1.22 (0.51–2.93)	0.656
rs7692387				
Dominant (GG + GA vs. AA)	0.91 (0.39–2.09)	0.819	1.48 (0.51–4.33)	0.472
Recessive (GG vs. GA + AA)	0.93 (0.67–1.30)	0.688	0.71 (0.43–1.17)	0.177
Additive (GG vs. AA)	0.89 (0.38–2.06)	0.782	1.28 (0.43–3.75)	0.657

Adjusted for age, gender, cigarette smoking, total cholesterol, low-density lipoprotein cholesterol, HbA1c, hypertension history, diabetes mellitus history, and coronary artery disease history

The entries in boldface are statistically significant

* $P < 0.05$

HbA1c, hypertension history, diabetes mellitus history, and coronary artery disease history, TT genotype of rs1842896 was still associated with an increased risk of LAA stroke in the recessive model (adjusted OR = 1.96, 95% CI: 1.22–3.17, $P = 0.006$, Table 4). However, the rs7692387 polymorphism failed to show significant association in all three genetic models.

Discussion

We designed this study to investigate the associations between the *GUCY1A3* polymorphisms and LAA stroke in Chinese Han population. Our results showed that, under the recessive model, the *GUCY1A3* rs1842896 TT genotype was significantly correlated with LAA stroke. After adjustment for its effects on age, gender, cigarette smoking, total cholesterol, low-density lipoprotein cholesterol, HbA1c, hypertension, diabetes mellitus, and CAD, the rs1842896 TT genotype retained association with increased susceptibility to LAA stroke (recessive model: adjusted OR = 1.96, 95%CI: 1.22–3.17, $P = 0.006$), indicating the SNP might be associated with LAA stroke independently of its effects on hypertension, diabetes mellitus and CAD. This association suggested that the risk for LAA stroke in the rs1842896 TT genotype carriers were increased 1.96-fold when compared with GG/GT genotype carriers. However, association between the rs7692387 polymorphism with LAA stroke was not observed.

The rs1842896 is located 76.4 kb upstream of the *GUCY1A3* locus. The *GUCY1A3* gene encodes the $\alpha 1$ subunit of sGC, the major cellular receptor for NO that is implicated in pathogenesis of atherosclerosis. sGC acts as a heterodimer composed of an α subunit and a β subunit. Although two isoforms of each subunit ($\alpha 1$, $\alpha 2$, $\beta 1$ and $\beta 2$) exist, only the $\alpha 1\beta 1$ heterodimer and the $\alpha 2\beta 1$ heterodimer are functional enzymes [13]. The $\alpha 1\beta 1$ heterodimer is the most abundant isoform distributed ubiquitously and displays higher activity than $\alpha 2\beta 1$, so it is regarded as canonical sGC [14]. As a key enzyme of

the NO signaling pathway, sGC can change the conversion of GTP into cGMP, leading to increased production of cGMP. Increasing intracellular levels of cGMP and the subsequent intracellular processes lead to relaxation of VSMCs and inhibition of platelet aggregation [14, 15], preventing the formation of atherosclerosis. Previous studies have explored the therapeutic potential of sGC stimulators [16].

The impact of genetic variation in the *GUCY1A3* gene on human atherosclerosis has been highlighted by previous studies. In 2012, a meta-analysis of two GWAS in Chinese Han population with 1515 CAD cases and 5019 controls identified rs1842896 at 4q32.1 near *GUCY1A3* showed significant association with CAD [6]. Later, the CARDIoGRAMplusC4D Consortium [11] also detected a strong association between a variant at the *GUCY1A3* locus and CAD. Recently, an extended meta-analysis [7] which considerably expanded the coverage of human genetic variation especially for low-frequency variants to determine whether these variants mediate risk for IS found that *GUCY1A3* showed suggestive association with LAA stroke. Besides, it has been shown that rare coding variants in the *GUCY1A3* gene were linked in families to myocardial infarction [17] and stroke [18] risk.

The risk variants in the *GUCY1A3* gene leading to reduced sGC availability and activity may be one reason of increased risk in atherosclerotic events. Kessler et al. found *GUCY1A3* gene variant was associated with both reduced platelet $\alpha 1$ sGC protein levels and increased atherosclerotic disease burden [19]. It has also been shown that absence or reduction of $\alpha 1$ subunit protein was detectable in variants in the *GUCY1A3* gene identified in sporadic young myocardial infarction cases [20]. There are several limitations of our study that have to be taken into account. First, our study population was limited to southern Chinese individuals. As genetic influence is strongly relevant to ethnic background, our findings need to be validated in other ethnic populations. Second, our study

sample size was relatively small. Third, biological functions were not conducted which were deserved further research. Fourth, it has been demonstrated that nutraceuticals and functional food ingredients can reduce the IS risk [21, 22]. However, we were not able to collect the important risk factor for subjects who participated in the study. Further studies solving these limitations are needed.

Conclusion

To our knowledge, this is the first study to demonstrate an association between the *GUCY1A3* rs1842896 and LAA stroke. We found that the *GUCY1A3* rs1842896 TT genotype was associated with an increased susceptibility to LAA stroke in a Chinese Han population. This finding indicates a potential link between *GUCY1A3* and risk of LAA stroke. Further research will be required to explore the role of *GUCY1A3* in the pathogenesis of LAA stroke.

Abbreviations

BMI: Body mass index; CAD: Coronary artery disease; cGMP: cyclic guanosine 3',5'-monophosphate; DBP: Diastolic blood pressure; GWAS: Genome-wide association studies; HbA1c: Hemoglobin A1c; HWE: Hardy-Weinberg equilibrium; IS: Ischemic stroke; LAA: Large artery atherosclerotic; MRI: Magnetic resonance imaging; NO: Nitric oxide; PCR: Polymerase chain reaction; SBP: Systolic blood pressure; sGC: Soluble guanylyl cyclase; SNP: Single nucleotide polymorphisms; TOAST: Trial of Org 10,172 in Acute Stroke Treatment; VSMCs: Vascular smooth muscle cells

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Authors' contributions

LM designed and revised the manuscript. LJJ and LLY performed experiments and drafted the manuscript. JDD helped to perform experiments and performed statistical analysis. JYY, ZGQ and MDC helped to perform experiments. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Ethics approval and consent to participate

The study was approved by the ethics committee of the First Affiliated Hospital of Guangxi Medical University and was carried out in accordance with the Declaration of Helsinki. All participants provided informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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