RGS5 rs4657251 polymorphism is associated with small vessel occlusion stroke in Taiwan Han Chinese

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Abstract

Background: The regulator of G-protein signaling protein 5 (RGS5) has been demonstrated to play a role in regulating blood pressure and cardiovascular function. Studies have shown that RGS5 polymorphisms exhibit susceptibility to hypertension. However, no study has yet been performed among stroke patients.

Methods: To evaluate whether RGS5 rs4657251 is a susceptibility gene for stroke, we performed a case-control association study involving 714 large-artery atherosclerosis (LAA) patients, 383 small vessel occlusion (SVO) patients, 401 hypertensive intracranial hemorrhages (HICH), and 626 controls. The RGS5 rs4657251 polymorphism was analyzed through polymerase chain reaction.

Results: The TC genotype was significantly higher in the SVO group compared with that in the control group (odds ratio [OR] = 1.34, 95% confidence interval [CI] = 1.02-1.76, $p = 0.035$). In addition, the dominant phenotype (TC + CC vs TT) was also significantly different between the SVO and the control groups (OR = 1.31, 95% CI = 1.01-1.70, $p = 0.046$). However, no association was found between RGS5 rs4657251 and LAA an HICH. After adjustment with gender, diabetes, smoking, cholesterol and low-density lipoprotein levels, RGS5 rs4657251 polymorphism remained an independent risk factor for SVO (OR = 1.49; 95% CI = 1.12-1.98) but not for LAA or HICH.

Conclusion: Our findings, obtained among Taiwan Han Chinese subjects, provide the first evidence that RGS5 rs4657251 polymorphism is an independent risk factor for SVO.

Keywords: Genetic; Polymorphism; RGS5; Stroke

1. INTRODUCTION

A family of proteins, which was classified as regulators of G protein signaling (RGS) proteins, was recently identified. RGS proteins can interact with the G subunit through the RGS domain, facilitates the GTPase activities of the G subunit, and diminishes G protein signals.[1,2] The RGS5, one of the members of this protein family, is predominantly expressed in the heart, lungs, small intestine, vascular smooth muscle cells, and pericytes. Recent study had demonstrated that RGS5 was involved in the regulation of Gαi and Gαq activities.[3] It also inhibited the signals of angiotensin II type I receptor and endothelin-1 receptor.[3] RGS5 overexpression attenuates the angiotensin-induced activation of mitogen-activated protein kinase in smooth muscle cells of the human aorta.[4] Through cDNA microarray, Adams et al[5] demonstrated that RGS5 was predominantly expressed in the aortic media than in the venous media.

Recently, it has been shown that RGS5 was associated with blood pressure regulation and cardiovascular functions.[1] Kirsch et al[6] demonstrated that RGS5 was downregulated in stroke-prone spontaneously hypertensive rats compared with stroke-resistant spontaneously hypertensive rats. Chang et al[7] also showed that the Na^+/K^+-transporting ATPase beta-1 polypeptide, selectin E, and RGS5 located in chromosome 1 were involved in hypertension formation. Significant downregulations of RGS2 and RGS5 were observed in the arteries of spontaneously hypertensive rats and adrenocorticotropic hormone-induced hypertensive rats.[8] Blood pressure was significantly decreased without the concomitant cardiac dysfunction in RGS5 knockout (RGS5 –/–) mice.[9] The aortas of RGS5 –/– mice were dilated compared with those of the normal control mice.[9] Prolonged angiotensin II perfusion resulted in increased blood pressure accompanied by the loss of RGS5 expression.[10] Treatment with Rho-kinase and MEK inhibitors reversed RGS5 deficiency-induced hypertension.[10] Xiao et al[11] found that the haplotype-based association of RGS5 polymorphisms (rs12041294C/T, rs10917690A/G, rs10917695T/C, rs10917696T/C, and rs2662774G/A) was correlated with essential hypertension. Faruque et al[12] also showed that among African Americans, six and five RGS5 polymorphisms were associated with systolic blood pressure (SBP) and

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diastolic blood pressure (DBP), respectively. Overall, RGS5 is possibly involved in hypertension regulation.

Stroke is one of the leading causes of disability and mortality worldwide.[13] Several vascular risk factors, such as hypertension, diabetes, and smoking, are correlated with stroke.[13] Previous reports have demonstrated the association of RGS5 polymorphisms with hypertension, one of the major risk factors for stroke. However, knowledge on the association between RGS5 polymorphisms and stroke remains scarce. In the present study, we aim to investigate the association of RGS5 rs4657251 polymorphism with strokes and its subtypes among Han Chinese population in Taiwan.

2. METHODS

2.1. Study population

Patients with first-ever stroke hospitalized in the Chang Gung Memorial Hospital were enrolled in this study. In addition, individuals without prior history of coronary artery disease or stroke were recruited as normal controls. The study protocol complied with the Helsinki Declaration and was approved by the internal review board of the Chang Gung Memorial Hospital (approved number: 103--3254C). An informed consent was provided to and signed by all eligible patients and control participants.

Based on the results of investigative examinations, including head computerized tomography or magnetic resonance imaging scan, those patients with ischemic stroke were subclassified on the basis of the definitions presented by the Trial of Org 10172 in Acute Stroke Treatment[14]: large-artery atherosclerosis (LAA, hypodense lesions with a diameter of >15 mm and >50% stenosis in the appropriate extra- or intra-cranial arteries) and small vessel disease (small vessel occlusion [SVO], small, subcortical, hypodense lesions with a diameter of <15 mm and corresponding to clinical lacunar syndrome). Cardioembolic as well as other or undetermined strokes were excluded from this study because these cases could result from different etiologies. In addition, hypertensive intracranial hemorrhages (HICH) were identified by a hematoma located in the basal ganglion (putamen and thalamus), the cerebellum, and the pons, by whether the hematoma appeared to be mainly caused by hypertension.

A detailed medical history was obtained, and all participants were subjected to a thorough neurological examination. Demographic data, laboratory findings, and established vascular risk factors were all recorded. Arterial hypertension was documented when patients yielded SBP of ≥140 mmHg or DBP of ≥90 mmHg in at least two occasions in office; or when a patient was receiving antihypertensive medications at the time of the study. Diabetes mellitus was diagnosed when the fasting plasma glucose was >126 mg/dL, HbA1C ≥6.5%, or when a patient was receiving antidiabetic drug treatment at the time of the study. Hypercholesterolemia was defined as a fasting total cholesterol >200 mg/dL. High levels of low-density lipoprotein (LDL) cholesterol and triglyceride were diagnosed when their values were >100 mg/dL and 400 mg/dL, respectively. Smoking, active or had quit within the last 3 months, was considered as a current habit.

2.2. Genotyping

RGS5 rs4657251 polymorphism was determined using a Bruker SNP GENOTYPING system (Bruker Corporation, Billerica, Massachusetts, USA). First, genomic DNA was prepared from the peripheral blood samples of the patients using Gentra PUREGENER DNA purification kits (Qiagen, Frankfurt, Germany). Subsequently, 50 ng of genomic DNA was amplified via polymerase chain reaction, followed by allele-specific primer extension reaction. The primer extension products were purified using a GENEPURE OLIG purification system (Bruker Corporation, Billerica, Massachusetts, USA) and subjected to matrix-assisted laser desorption ionization time-of-flight mass spectrometry. All the aforementioned procedures were performed in the National Center for Genome Medicine (Academia Sinica, Taipei, Taiwan; http://ncgm.sinica.edu.tw/).

2.3. Statistical analysis

The genotype and allele frequencies of the control and stroke subjects were analyzed via the χ^2 test and the Hardy–Weinberg equilibrium. Logistic regression analysis was performed to estimate the odds ratio (OR) and 95% confidence interval (CI), and these parameters were then adjusted using several risk factors as confounders. All data were analyzed with the Statistical Product and Service Solutions version 14.0 (SPSS, IBM, NY, USA) software for Windows. A significant difference was set to a p value of <0.05.

3. RESULTS

A total of 2124 participants, including 626 normal controls, 714 LAA patients, 383 SVO, and 401 HICH patients, were enrolled in this study. The clinical characteristics are listed in Table 1. Significantly older patients were found in the LAA group (66.6 ± 11.4) than in the control group (62.2 ± 11.9). On the contrary, younger age of onset was observed in the SVO group (59.3 ± 12.2) and HICH group (56.4 ± 12.7). The number of males, as well as the numbers of individuals with a history of hypertension and smoking, was significantly higher in the three stroke subtypes than in the control group ($p < 0.001$). Total cholesterol levels in the LAA and SVO groups were significantly lower than those in the control group. LDL level was significantly lower in HICH groups.

To address the genetic susceptibility of RGS5 polymorphisms to stroke, RGS5 rs4657251 was genotyped in this study. As listed in Table 2, the frequency of TT/TC/CC was

Table 1

Clinical parameters in control and different ischemic stroke subtypes

	Control (n = 626)	LAA (n = 714)	SVO (n = 383)	HICH (n = 401)
Age	62.2 ± 11.9	66.6 ± 11.4***	59.3 ± 12.2***	56.6 ± 12.6***
Gender				
Female	281 (44.9%)	141 (19.7%)***	128 (33.4%)***	108 (26.9%)***
Male	345 (55.1%)	573 (80.3%)	255 (66.6%)	293 (73.1%)
Hypertension				
No	300 (47.9%)	140 (19.6%)***	83 (21.7%)***	0***
Yes	326 (52.1%)	574 (80.4%)	300 (78.3%)	401 (100%)
Diabetes				
No	507 (81.0%)	443 (62.0%)***	268 (70.0%)***	322 (80.3%)
Yes	119 (19.0%)	271 (38.0%)	115 (30.0%)	79 (19.7%)
Cholesterol				
Low	334 (53.4%)	461 (64.6%)***	237 (61.4%)**	270 (67.3%)***
High	292 (46.6%)	253 (35.4%)	146 (38.6%)	131 (32.7%)
Smoking				
No	472 (75.4%)	306 (42.9%)***	206 (53.8%)***	239 (59.6%)***
Yes	154 (24.6%)	408 (57.1%)	177 (46.2%)	162 (40.4%)
LDL				
Low	192 (30.7%)	240 (33.6%)	114 (29.8%)	169 (49.1%)***
High	434 (69.3%)	474 (66.4%)	269 (70.2%)	232 (50.9%)

HICH = hypertensive intracranial hemorrhage; LAA = large artery atherosclerosis; LDL = low-density lipoprotein; SVO = small vessel disease.
**$p < 0.01$; ***$p < 0.001$.

Table 2

The genotype distribution of rs4657251 in control and different stroke patients

	Con (n = 626)	LAA (n = 714)	OR (95% CI)	SVO (n = 383)	OR (95% CI)	HICH (n = 401)	OR (95% CI)
TT	407 (65.0%)	451 (63.2%)	1	225 (58.7 %)	1	252 (62.8%)	1
TC	192 (30.7%)	233 (32.6%)	0.92 (0.72-1.15)	142 (37.1%)	**1.34 (1.02-1.76)**	132 (32.9%)	1.11 (0.85-1.46)
CC	27 (4.3%)	30 (4.2%)	0.99 (0.53-1.71)	16 (4.2%)	1.07 (0.57-2.03)	17 (4.2%)	1.03 (0.55-1.93)
TT	407 (65.0%)	451 (63.2%)	1	225 (58.7%)	1	252 (62.8%)	1
TC + CC	219 (35.0%)	263 (36.8%)	1.08 (0.87-1.36)	158 (41.3%)	**1.31 (1.01-1.70)**	149 (37.2%)	1.009 (0.847-1.426)

Significance was denoted by bold.

CI = confidence interval; HICH = hypertensive intracranial hemorrhage; LAA = large artery atherosclerosis; OR = odds ratio; SVO = small vessel disease.

65.0%/30.7%/4.3% in the control group, 63.5%/44.1%/4.4% in the LAA group, 58.7%/37.1%/4.2% in the SVO group, and 62.8%/32.9%/4.3% in HICH, respectively. Using the TT genotype as reference (dominant model), the control and LAA groups showed no significant difference. By contrast, the TC genotype was significantly higher than the TT genotype in the SVO group than in the control group (37.1% vs 30.7%, OR = 1.34, 95% CI = 1.02-1.76, $p = 0.035$). In addition, compared with TT genotype, TC + CC genotype exhibited significant association with SVO (OR = 1.31, 95% CI = 1.01-1.70, $p = 0.046$). Using TT + TC as reference (recessive model), no significant association of the CC genotype was found for the stroke groups (data not shown). Our findings suggested that RGS5 rs4657251 C allele might be a risk factor for SVO.

Hypertension is one of the major risk factors of stroke. RGS5 polymorphisms also have been shown to be associated with hypertension. In Table 3, the frequency of hypertension was significantly lower in control group with TC + CC genotype compared to TT genotype (42.9% vs 57.0%; OR = 0.57, 95% CI = 0.41-0.79, $p = 0.001$). Intriguingly, no any association was found in LAA or SVO.

Finally, multiple logistic regression analysis was performed to evaluate the correlation of RGS5 rs4657251 polymorphism with stroke subtypes. As listed in Table 4, using the TT genotype as reference, the presence of TC + CC significantly increased the risk of SVO (OR = 1.49; 95% CI = 1.12-1.98) after adjustment of the clinical variables such as gender, hypertension, diabetes, smoking, cholesterol, and LDL levels. The polymorphisms of rs4657251 did not influence LAA and HICH susceptibility in this study.

Table 3

The association of RGS5 rs4657251 with hypertension in control and stroke patients

	Hypertension			
	No	Yes	*p*	OR (95% CI)
Control			**0.001**	
TT	175 (43.0%)	232 (57.1%)		1
TC + CC	125 (57.1%)	94 (42.9%)		**0.57 (0.41-0.79)**
LAA			0.993	
TT	88 (62.9%)	363 (63.2%)		1
TC + CC	52 (37.1%)	211 (36.8%)		0.98 (0.67-1.45)
SVO			0.952	
TT	49 (59.0%)	176 (58.7%)		1
TC + CC	34 (41.0%)	124 (41.3%)		1.02 (0.62-1.66)
HICH			NA	
TT	0	252 (62.8%)		NA
TC + CC	0	149 (37.2%)		NA

Significance was denoted by bold.

CI = confidence interval; HICH = hypertensive intracranial hemorrhage; LAA = large artery atherosclerosis; OR = odds ratio; SVO = small vessel disease.

4. DISCUSSION

Stroke is one of the major causes of mortality and disability worldwide. RGS5, which regulates the activities of Gαi and Gαq, has been shown to be involved in cardiovascular disease and hypertension.[1,15] In this study, we highlighted the association of RGS5 rs4657251 polymorphism with the risk for ischemic stroke among Taiwan Han Chinese patients.

In the current study, we observed that RGS5 rs4657251 TT genotype frequency was significantly lower in the SVO group than in the control group, whereas no association was found between the LAA group and the control group. One possible explanation for this discrepant finding is that LAA and SVO are caused by different pathological factors. Reports have shown that LAA and SVO have both common and distinct genetic backgrounds.[16] Fan et al[17]demonstrated that rs17222919, which is a promoter polymorphism 5-lipoxygenase-activating protein, was associated with LAA but not with SVO after adjusting cardiovascular risk factors. The -1195G>A polymorphism and the A-1195-G-765 haplotype of cyclooxygenase 2 were associated with ischemic stroke and SVO but not with LAA.[18] Subtilisin/kexin type 9, which is the E670G polymorphism of proprotein convertase, was correlated with LAA.[19] Another possible explanation is the function of RGS5 in arteriogenesis. Arteriogenesis can overcome artery occlusion through growth of collateral arterioles, subsequently preventing several artery diseases.[20] RGS5 expression was significantly increased in collateral arteriolar smooth muscle cells during arteriogenesis.[20] RGS5 overexpression triggered the growth of collateral arterioles via activation of RhoA signaling pathway, whereas RGS5 downregulation blocked this phenomenon.[20] Moreover, a previous report demonstrated that RGS5 downregulation accelerated the formation of atherosclerosis in ApoE-deficient mice.[21] Very recently, Ozen et al[22] had reported that loss of RGS5 expression was associated with elevated pericyte numbers and their endothelial coverage, which was accompanied with increased length and density of capillary. This phenomenon caused less blood-brain barrier

Table 4

Multiple regression analysis of clinical covariates and RGS5 rs4657251 in LAA, SVO, and HICH

	LAA OR (95% CI)	SVO OR (95% CI)	HICH OR (95% CI)
Gender (male)	**1.97 (1.47-2.65)**	1.02 (0.74-1.42)	**1.82 (1.35-2.46)**
Hypertension	**3.60 (2.73-4.71)**	**3.20 (2.36-4.32)**	NA
Diabetes	**2.17 (1.65-2.87)**	**1.53 (1.11-2.10)**	1.08 (0.78-1.49)
Smoking	**3.18 (2.40-4.22)**	**2.65 (1.90-3.69)**	**1.60 (1.18-2.15)**
rs4657251 (TT vs TC + CC)	1.26 (0.98-1.63)	**1.49 (1.12-1.97)**	1.11 (0.86-1.46)

Significance was denoted by bold.

CI = confidence interval; HICH = hypertensive intracranial hemorrhage; LAA = large artery atherosclerosis; LDL = low-density lipoprotein; NA = nonanalysis; OR = odds ratio; SVO = small vessel disease.

(BBB) damages after cerebral vascular occlusion.[22] In addition, decreased vascular leakage and maintained tight junctions and exerted partial neuronal protection were found in RGS5 –/– pericyte in the infarct area.[22] The increased risk for development of SVO in patients with C allele may result from enhanced RGS5 activity leading to delay of the repair of pericyte and caused SVO. However, further study is needed to elucidate the effects of rs4657251 on RGS5 cellular function.

Hypertension is one of the major risk factors for the development of stroke. RGS5 polymorphisms have been shown to play a critical role in controlling blood pressure and hypertension. Faruque et al[12] demonstrated that RGS5 rs2815272 was correlated with hypertension and blood pressure levels (both SBP and DBP). Four haplotypes constructed using five RGS5 polymorphisms, namely, rs12041294, rs10917690, rs10917695, rs10917696, and rs2662774, were correlated with essential hypertension.[11] RGS5 rs16849802 polymorphism was associated with essential hypertension in the Mongolian population.[22] The GAA haplotypes rs2255642, rs2456899, and 16849802 of RGS5 were significantly increased in the essential hypertension group in the Mongolian population.[22] Unlike in previous reports, RGS5 rs4657251 C allele was correlated with SBP but not with hypertension among African Americans.[12] In the current study, we discovered that compared with TT genotype subjects, the control group with CT + CC genotype had nearly half the risk for hypertension (OR = 0.57; 95% CI = 0.41-0.79, p = 0.001). The dissimilar finding possibly resulted from the different and distinct ethnic genetic backgrounds. In addition, Faruque et al demonstrated that the C allele of RGS5 rs4657251 was correlated with SBP.[12] Decreased of around 2.3 mmHg of SBP was found in people with C allele of rs4657251.[12] Previous reports have indicated that loss of RGS5 may trigger hypertension.[8,10] In this study, we observed that C allele frequency was lower in health hypertensive group. It provided one reasonable explanation that C allele may slightly enhance the activity of RGS5. Intriguingly, no association of RGS rs4657251 with hypertension was found in LAA and SVO patients. These findings suggested that RGS5 rs4657251 may play a protection role in development of hypertension in health control but not in stroke patients.

The current study has some limitations. First, this was a single-center, retrospective study with a small-scale sample size. However, we recruited as many patients as possible. In addition, our study population contained pure Taiwanese Han descendants to prevent contamination. Second, although we included only first-episode acute stroke subjects, some patients might have previously undetected silent brain infarcts. We excluded these patients through neuroimaging results as much as possible. Third, whether or not RGS5 rs4657251 affected RG5 activities remained to be elucidated.

In conclusion, our findings provide the first evidence that RGS5 rs4656251 polymorphism is significantly associated with SVO subtype but not with LAA subtype. This polymorphism may be an independent risk factor for the development of SVO. Future large-scale research is necessary to validate our findings.

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