Association between common genetic variants of α2A-, α2B- and α2C-adrenoceptors and the risk of silent brain infarction

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Received June 27, 2013; Accepted March 4, 2014

DOI: 10.3892/mmr.2014.2072

Abstract. Silent brain infarction (SBI) is an asymptomatic cerebrovascular disorder. The aim of the present study was to investigate the association between adrenoceptor-α2 (*ADRA2*) gene polymorphisms and SBI. A total of 361 patients with SBI and 467 healthy control subjects were examined. The polymerase chain reaction was performed to genotype the *ADRA2A* 1780G>A, *ADRA2B* 301-303 insertion/deletion (I/D) and *ADRA2C* 322-325I/D polymorphisms. The frequency of the *ADRA2C* 322-325I/D polymorphism was significantly different between patients with SBI and control subjects. When interaction analyses were performed for vascular risk factors, the *ADRA2C* 322-325ID genotype increased the risk for SBI in the presence of hypertension and elevated plasma homocysteine levels. The *ADRA2C* 322-325ID genotype and plasma homocysteine levels showed a significant synergistic effect for SBI. In addition, the *ADRA2A* 1780AA genotype was associated with elevated plasma homocysteine levels. Although further analysis of the association between *ADRA2* polymorphisms and clinical risk factors of SBI is required, the present study of a limited set of SBI risk factors with *ADRA2* polymorphisms provides the first evidence of the involvement of *ADRA2* gene family members in the development of SBI. Further studies using larger and more heterogeneous populations are required to validate the association of *ADRA2* polymorphisms with SBI.

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Key words: α2-adrenoceptor, silent brain infarction, genetic variants, polymorphism, vascular risk factors

Introduction

Silent brain infarction (SBI) is a cerebrovascular disorder. The clinical and pathological aspects of SBI are distinct from those of ischemic stroke; while vascular brain lesions are evident using magnetic resonance imaging (MRI), SBI is clinically asymptomatic (1,2). SBI and white matter lesions are frequently observed using brain MRI in healthy elderly individuals and are associated with an increased risk of stroke and dementia (1-6). SBI is a risk factor for stroke (7). SBI has been associated with several cardiovascular risk factors. Previous studies strongly suggest that SBI can be significantly influenced by multiple risk factors, including hypertension, homocysteine levels, cigarette smoking and metabolic syndrome (7-11). However, relatively little is known about the genes involved in SBI pathogenesis. The present study was a candidate gene association study focusing on the association between the adrenoceptor-α2 (*ADRA2*) genes and SBI. The rationale was two-fold: i) *ADRA2* genes are responsible for blood flow vasoconstriction, which is linked to thrombosis; ii) *ADRA2* defects are associated with an increased likelihood of ischemic stroke (12,13).

There are three subtypes of α2-adrenoceptors (α2-ARs), α2A, α2B and α2C, which are encoded by the *ADRA2A*, *ADRA2B* and *ADRA2C* genes, respectively (10,11). The α2-AR, a membrane receptor for norepinephrine and epinephrine, mediates physiological responses to endogenous catecholamine. The α2-AR is involved in blood pressure reduction, inhibition of presynaptic neurotransmitter release, lipolysis and insulin secretion, as well as augmentation of platelet aggregation (13). The three α2-AR subtypes exhibit similar affinities for endogenous catecholamines, but differ in their pharmacological properties, tissue distribution and sensitivity to receptor desensitization and phosphorylation (13).

Although the clinical significance of the α2-AR subtypes and their genetic variants for human disease has yet to be fully elucidated, several case-control and population-based studies have reported positive associations among genetic

polymorphisms of *ADRA2A*, *ADRA2B* and *ADRA2C* and several vascular diseases as follows: i) *ADRA2A* 1780G>A polymorphism (rs553668) and hypertension (14) and diabetes mellitus (15), ii) *ADRA2B* 301-303 insertion/deletion (I/D) polymorphism (rs28365031) and hypertension and coronary arterial disease (14,16); and iii) *ADRA2C* 322-325I/D polymorphism (rs61767072) and congestive heart failure (17).

The objective of the present study was to investigate associations of *ADRA2A* 1780G>A, *ADRA2B* 301-303I/D and *ADRA2C* 322-325I/D polymorphisms with SBI and its risk factors.

Materials and methods

Subjects. The study population comprised 361 patients with SBI (151 males, 210 females) and 448 control subjects (186 males, 262 females). The patients were enrolled from July 1, 2000 to February 28, 2008 in the Neurology Department at CHA Bundang Medical Center (Seongnam, South Korea) by consecutive referral. Control subjects were enrolled from individuals who came to CHA Bundang Medical Center for health examinations.

Patients with a known history of stroke or cardiovascular disease were excluded. MRI was performed with a 1.5-T superconducting magnet (Siemens Magnetom Symphony, Erlangen, Germany). Transverse T1-weighted, T2-weighted, and fluid-attenuated inversion recovery (FLAIR) images were obtained with a slice thickness of 7 mm. SBI was diagnosed based on the following criteria (17,18): i) Spotty areas ≥3 mm in diameter in the area supplied by deep perforating arteries, showing high intensity in the T2 and FLAIR images and low intensity in the T1 image; ii) absence of neurological symptoms corresponding to the MRI lesions; iii) no history of clinical stroke, including transient ischemic attack; iv) no prior ischemic heart disease; v) ≥40 years of age and vi) Korean descendance (residency in Seoul or Kyeonggi, South Korea). Among the initial 1,000 participants evaluated, 191 met the exclusion criteria, leaving 448 controls and 361 cases. The diagnosis of SBI was made when two independent researchers agreed on the diagnosis. Small, punctate hyperintense lesions (1-2 mm in diameter) were more likely to represent a dilated perivascular space and were not considered in the present study.

Control subjects were selected from gender- and age- (within 4.5 years) matched individuals presenting at the hospital for a health examination that included biochemical assessment, an electrocardiogram and brain MRI during the same period, and who had no history of cerebrovascular disease (including SBI) or of myocardial infarction. A total of 448 age- and gender-matched control subjects (mean age ± standard deviation, 64.07±11.30 years; males, 41.5%) were included in the present study. The enrollment was conducted by matching age and gender in the control group to the mean age and frequency of gender in the stroke group. Baseline demographic data and a history of conventional vascular risk factors were obtained from each control subject. With regard to the patient group, patients with a known history of stroke or cardiovascular disease were excluded.

Hypertension was diagnosed as a high baseline blood pressure (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg) on more than one occasion or current treatment with antihypertensive medication. Diabetes mellitus was defined as high fasting plasma glucose levels (≥126 mg/dl) or current treatment with an oral hypoglycemic agent or insulin. Hyperlipidemia was defined as high fasting serum total cholesterol levels (≥240 mg/dl) or a history of antihyperlipidemic treatment.

Informed consent was obtained from all study participants once a full explanation of the study had been provided. The institutional review board of CHA Bundang Medical Center approved the present genetic study in June 2000.

Assessment of homocysteine, vitamin B12 and folate concentrations. Blood was collected in a tube containing anticoagulant after 12 h of fasting. The tube was centrifuged for 15 min at 1,000 x g and the plasma was separated from the whole blood. The concentration of homocysteine in the plasma was measured using a fluorescent polarizing immunoassay with IMx (Abbott Laboratories, Abbott Park, IL, USA). Folate and vitamin B12 were assessed in plasma using the ACS:180 radioassay (Bayer Industries, Tarrytown, NY, USA).

Genotyping of ADRA2A 1780G>A, ADRA2B 301-303I/D and ADRA2C 322-325I/D. Genomic DNA was extracted from anticoagulant-treated peripheral blood using the G-DEX blood extraction kit (Intron Biotechnology, Seongnam, South Korea). *ADRA2A* 1780G>A polymorphisms were determined by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis with the following primers, generating a 211-bp product: Forward, 5'-ACT GGA CTA CAA GGG CAT GG-3' and reverse, 5'-ACA TCA AAA CCA AGG CCA AG-3'. The PCR product was incubated with 5 units *Dra*I (New England Biolabs, Beverly, MA, USA) at 37˚C for 16 h. The *ADRA2A 1780A* allele was cut into two fragments of 113 and 98 bp, whereas the *ADRA2A 1780G* allele was uncut.

ADRA2B and *ADRA2C* polymorphisms were screened by PCR using the following primers: *ADRA2B* forward, 5'-AGG GTG TTT GTG GGG CAT CT-3' and reverse, 5'-CAA GCT GAG GCC GGA GAC ACT-3'; *ADRA2C* forward, 5'-AGC CGG ACG AGA GCA GCG CA-3' and reverse, 5'-AGG CCT CGC GGC AGA TGC CGT ACA-3'. The sizes of *ADRA2B* and *ADRA2C* with the insertion polymorphisms were 112 and 384 bp, whereas those of *ADRA2B* and *ADRA2C* with deletion polymorphisms were 103 and 372 bp, respectively. The products were electrophoretically resolved on a 4.5% agarose gel stained with ethidium bromide and visualized under ultraviolet illumination. For three studied polymorphisms, 30% of the PCR assays were randomly selected for a second PCR assay followed by DNA sequencing to validate the RFLP findings. Sequencing was performed using an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The concordance of the quality control samples was 100%.

Statistical analysis. To estimate the relative risk for SBI for the various genotypes, an adjusted odds ratio (AOR) and 95% confidence interval (CI) were calculated. Case and control groups were compared using the Mann Whitney-test for continuous variables and the χ^2 test for categorical variables.

Table I. Baseline characteristics of controls (n=488) and patients with SBI (n=361).

Characteristic	Controls	Patients with SBI	P-value
Male, n (%)	186 (41.5)	151 (41.8)	0.929
Age in years (mean ± SD)	64.07±11.30	64.29±11.65	0.784
tHCy in μmol/l, mean ± SD (total n)	10.11±4.21 (444)	11.48±6.46 (359)	**<0.001**
Folate in nmol/l, mean ± SD (total n)	9.69±8.99 (329)	9.07±5.94 (343)	0.289
Vitamin B12 in pg/ml, mean ± SD (total n)	751.86±733.84 (321)	763.90±1419.53 (342)	0.890
Total cholesterol in mg/dl, mean ± SD (total n)	192.63±44.73 (433)	204.76±41.91 (340)	**<0.001**
Triglyceride in mg/dl, mean ± SD (total n)	142.37±88.10 (433)	159.20±121.87 (338)	**0.033**
Hypertension, n (%)	217 (48.4)	184 (51.0)	0.480
Diabetes mellitus, n (%)	71 (15.8)	53 (14.7)	0.695
Hyperlipidemia, n (%)	99 (22.1)	92 (25.5)	0.279

P-values were calculated using the two-sided t-test for continuous variables and the χ^2 test for categorical variables. Bold print indicates statistical significance. SBI, silent brain infarction; tHCy, total homocysteine; SD, standard deviation.

For the multivariate analysis, logistic regression analysis was used to adjust for possible confounders, including age, gender, hypertension, diabetes mellitus and hyperlipidemia. The analyses were performed using GraphPad Prism 4.0 (GraphPad Software, Inc., San Diego, CA, USA), StatsDirect Statistical Software 2.4.4 (StatsDirect Ltd., Altrincham, UK) and MedCalc 12.0 (Frank Schoonjans, Ostend, Belgium). Multiple hypothesis testing was performed using the Benjamini-Hochberg method to control for false discovery rate (FDR) in the logistic regression analysis (19). The calculation of the FDR is a technique to address the problems associated with multiple comparisons and provides a measure of the expected proportion of false-positives among the data.

Results

The demographic characteristics of the 361 patients with SBI and 448 controls are shown in Table I. The SBI and control populations consisted of 41.8 and 41.5% males, respectively. The mean ages of the SBI and control populations were 64.29±11.65 and 64.07±11.30 years, respectively. Few significant differences were observed between the two groups. However, total plasma homocysteine levels were significantly higher in patients with SBI than those in the controls. Table II presents a comparison of genotype frequencies of *ADRA2A* 1780G>A, *ADRA2B* 301-303I/D and *ADRA2C* 322-325I/D polymorphisms between the case and control groups. The frequency of the *ADRA2C* 322-325I/D polymorphism was significantly associated with an increased risk of SBI (AOR, 2.026; 95% CI, 1.335-3.075; P=0.001).

To clarify the clinical significance of the *ADRA2* polymorphisms, interaction analyses were performed for vascular risk factors hypertension, diabetes mellitus, hyperlipidemia and total plasma homocysteine levels according to the *ADRA2* genotypes (Table III). The *ADRA2C* 322-325I/D genotype was associated with an increased risk for SBI in the presence of hypertension (AOR, 2.952; 95% CI, 1.561-5.584) and also in the presence of elevated total plasma homocysteine levels (AOR, 9.223; 95% CI, 2.037-41.759). Fig. 1 shows

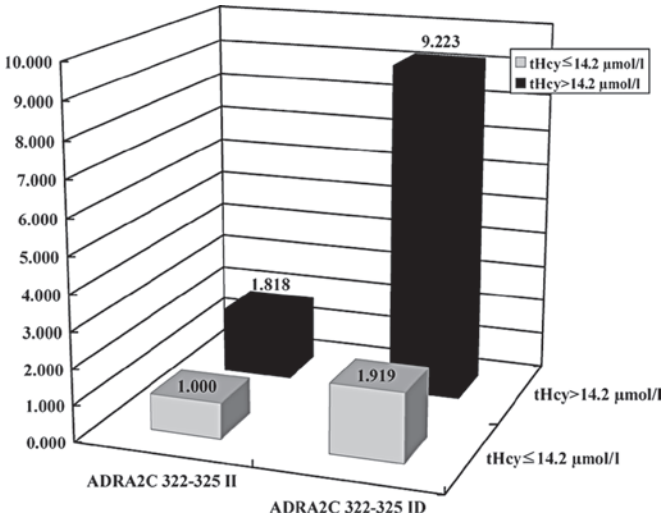

Figure 1. Combined effects of the *ADRA2C* 322-325ID genotype with elevated plasma tHcy. tHcy, total homocysteine; *ADRA2*, adrenoceptor-α2.

the combined effects of *ADRA2C* 322-325I/D and plasma homocysteine levels.

ADRA2 polymorphisms were associated with plasma homocysteine and vitamin B12. The *ADRA2A* 1780AA genotype was associated with elevated plasma homocysteine levels (Table IV), while the *ADRA2B* 301-303DD genotype was associated with lower vitamin B12 levels (Table V).

Discussion

In the present study, the risk for SBI in the Korean population was evaluated by investigating the associations between SBI and the three polymorphisms of the *ADRA2* gene family alone and in combination with individual vascular risk factors. The results indicate that the *ADRA2C* 322-325ID and *ADRA2A* 1780AA genotypes were associated with a risk of SBI. The *ADRA2C* 322-325ID polymorphism was associated with increased plasma homocysteine levels. Furthermore,

Table II. Genotype frequencies of *ADRA2A*, *ADRA2B* and *ADRA2C* polymorphisms.

Characteristics	Controls, n (%)	Patients with SBI, n (%)	AOR (95% CI)	β-Coefficient	Standard error	P-value	FDR-P	Statistical power (%)
ADRA2A 1780 G>A								
GG	149 (33.3)	123 (34.1)	1.000					
GA	230 (51.3)	180 (49.9)	0.932 (0.683-1.272)	-0.070	0.159	0.659	0.659	6.4
AA	69 (15.4)	58 (16.0)	1.015 (0.660-1.560)	0.015	0.219	0.946	0.946	5.1
Dominant, GG vs. GA+AA			0.953 (0.710-1.280)	-0.048	0.151	0.750	0.750	5.7
Recessive, GG+GA vs. AA			1.054 (0.720-1.544)	0.053	0.195	0.787	0.787	5.7
ADRA2B 301-303 I/D								
II	176 (39.3)	149 (41.3)	1.000					
ID	224 (50.0)	157 (43.5)	0.814 (0.602-1.099)	-0.206	0.154	0.179	0.269	23.7
DD	48 (10.7)	55 (15.2)	1.373 (0.878-2.145)	0.317	0.228	0.165	0.329	27.3
Dominant, II vs. ID+DD			0.913 (0.687-1.213)	-0.091	0.145	0.530	0.750	8.9
Recessive, II+ID vs. DD			1.506 (0.994-2.283)	0.410	0.212	0.054	0.107	48.1
ADRA2C 322-325 I/D								
II	405 (90.4)	298 (82.5)	1.000					
ID	43 (9.6)	63 (17.5)	**2.026 (1.335-3.075)**	0.706	0.213	**0.001**	0.003	**90.7**

Controls, n=448; patients with SBI, n=361. Adjusted for age, gender, hypertension, diabetes mellitus and hyperlipidemia. Bold print indicates statistical significance. *ADRA2*, adrenoceptor-α2; AOR, adjusted odds ratio; CI, confidence interval; FDR, false discovery rate; SBI, silent brain infarction.

Table III. Combinatorial effects of *ADRA2* genotypes with individual vascular risk factors for silent brain infarction occurrence.

	ADRA2A 1780 G>A		*ADRA2B* 301-303 I>D		*ADRA2C* 322-325 I>D	
Genotypes	GG+GA	AA	II+ID	DD	II	ID
Hypertension						
(-)	1.000 (reference)	0.756 (0.444-1.288)	1.000 (reference)	0.860 (0.547-1.352)	1.000 (reference)	1.068 (0.782-1.458)
(+)	1.042 (0.760-1.428)	1.596 (0.891-2.860)	0.752 (0.500-1.130)	0.956 (0.627-1.457)	1.656 (0.921-2.978)	**2.952 (1.561-5.584)**
Diabetes mellitus						
(-)	1.000 (reference)	0.922 (0.611-1.392)	1.000 (reference)	0.804 (0.516-1.253)	1.000 (reference)	0.833 (0.536-1.296)
(+)	0.760 (0.491-1.177)	2.014 (0.711-5.705)	1.474 (0.932-2.332)	1.367 (0.544-3.435)	2.102 (1.325-3.335)	1.420 (0.560-3.601)
Hyperlipidemia						
(-)	1.000 (reference)	0.865 (0.557-1.344)	1.000 (reference)	1.335 (0.932-1.910)	1.000 (reference)	1.449 (1.016-2.068)
(+)	1.125 (0.783-1.616)	2.124 (0.977-4.619)	1.735 (1.074-2.802)	1.334 (0.594-2.999)	2.600 (1.616-4.181)	1.130 (0.464-2.757)
tHCy[a]						
≤14.2 μmol/l	1.000 (reference)	1.068 (0.707-1.615)	1.000 (reference)	1.815 (1.162-2.833)	1.000 (reference)	1.919 (1.228-3.000)
>14.2 μmol/l	2.002 (1.248-3.213)	1.982 (0.735-5.344)	2.332 (1.467-3.708)	1.212 (0.364-4.040)	1.818 (1.145-2.887)	**9.223 (2.037-41.759)**

[a]14.2 μmol/l is based on the top 15% of the plasma tHCy levels in the patients and control group. Values are presented as the adjusted odds ratio (95% of confidence intervals). Bold print indicates statistical significance. *ADRA2*, adrenoceptor-α2; tHCy, total homocysteine.

Table IV. Genetic associations of *ADRA2A*, *ADRA2B* and *ADRA2C* polymorphisms with plasma Hcy levels.

	ADRA2A 1780G>A			*ADRA2B* 301-303 I/D			*ADRA2C* 322-325 I/D		
Hcy decile (μmol/l)	AA/GG+GA	AOR_{trend} (95% CI)	P-$value_{trend}$	DD/II+ID	AOR_{trend} (95% CI)	P-$value_{trend}$	ID/II	AOR_{trend} (95% CI)	P-$value_{trend}$
Hcy≤6.69	7/74	**1.160 (1.025-1.313)**[a]	**0.019**[a]	11/70	1.099 (0.960-1.258)[a]	0.173[a]	11/70	1.016 (0.889-1.160)[a]	0.816[a]
6.69<Hcy≤7.56	12/68			5/75			12/68		
7.56<Hcy≤8.34	14/66			11/69			9/71		
8.34<Hcy≤8.95	13/67			7/73			6/74		
8.95<Hcy≤9.75	10/70			9/71			9/71		
9.75<Hcy≤10.42	13/68			13/68			13/68		
10.42<Hcy≤11.40	18/63			14/67			15/66		
11.40<Hcy≤12.60	9/72			14/67			9/72		
12.60<Hcy≤15.14	14/64			9/69			13/65		
Hcy>15.14	14/64			9/71			8/72		

Hcy levels in recruited patients ranged between 27.15 and 5.31 μmol/l. [a]Adjusted for age, gender, hypertension, diabetes mellitus and hyperlipidemia. Bold print indicates statistical significance. Hcy, homocysteine; AOR, adjusted odds ratio; CI, confidence interval; *ADRA2*, adrenoceptor-α2.

Table V. Genetic associations of *ADRA2A*, *ADRA2B* and *ADRA2C* polymorphisms with VB12 levels.

	ADRA2A 1780G>A			*ADRA2B* 301-303 I/D			*ADRA2C* 322-325 I/D		
VB12 decile (pg/ml)	AA/GG+GA	AOR_{trend} (95% CI)	P-$value_{trend}$	DD/II+ID	AOR_{trend} (95% CI)	P-$value_{trend}$	ID/II	AOR_{trend} (95% CI)	P-$value_{trend}$
VB12≤351	9/58	1.091 (0.957-1.245)[a]	0.193[a]	6/61	**0.862 (0.750-0.991)**[a]	**0.037**[a]	9/58	1.081 (0.937-1.246)[a]	0.285[a]
351<VB12≤435	5/62			11/56			7/60		
435<VB12≤490	10/55			9/56			7/58		
490<VB12≤547	13/54			17/50			10/57		
547<VB12≤600	16/50			8/58			10/56		
600<VB12≤669	14/52			11/55			8/58		
669<VB12≤745	14/54			11/57			12/56		
45<VB12≤880	11/55			8/58			9/57		
880<VB12≤1123	6/59			9/56			11/54		
VB12>1123	11/55			3/63			8/58		

Homocysteine levels in recruited patients ranged between 27.15 and 5.31 μmol/l. [a]Adjusted for age, gender, hypertension, diabetes mellitus and hyperlipidemia. Bold print indicates statistical significance. VB12, vitamin B12; AOR, adjusted odds ratio; CI, confidence interval; *ADRA2*, adrenoceptor-α2.

the *ADRA2A* 1780AA genotype was associated with higher plasma homocysteine levels.

Hyperhomocysteinemia is usually caused by a mutation in the methylenetetrahydrofolate reductase gene and is linked with vascular diseases associated with thrombosis, induced hypertension and angiopathy. In addition, for the past decade, mildly elevated plasma homocysteine levels have been recognized as a risk factor for a number of occlusive vascular diseases. Hyperhomocysteinemia is associated with an increased risk for SBI (8,9).

The *ADRA2* gene family is well known to have several vascular functions, including maintenance of basal cerebral blood flow, cerebral vasodilation, vascular integrity and the normal functions of vascular smooth muscle cells (20-23). High levels of α2-AR activity are found in blood vessels, where these receptors act as temperature receptors to mediate cold-induced vasoconstriction. α2-AR stimulation causes calcium mobilization in the vascular smooth muscle cells, which is involved in the contraction and relaxation of blood vessels, affecting blood pressure (24). However, the ADRA2 proteins are differentially distributed in various tissue types and have different physiological functions and pharmacological activities. mRNAs encoding three subtypes of α2-ARs have been observed in the central nervous system; however, while the distribution of α2B-ARs is limited to the thalamus, α2A- and α2C-ARs are widely distributed throughout the brain (13). These data suggest that α2A- and α2C-ARs are more likely than α2B-ARs to be associated with physiological conditions of the whole brain.

In previous studies, the A allele of *ADRA2A* 1780G>A was associated with increased α2A-AR expression, hypertension, diabetes mellitus and decreased insulin secretion (14,15). The *ADRA2B* 301-303I/D polymorphism has been associated with impaired agonist-dependent phosphorylation, loss of desensitization and with sustained signaling of α2B-AR, despite continued activation by α2-agonists and subsequent prolonged vasoconstriction (25). Several studies have reported that the *ADRA2B* 301-303I/D polymorphism is associated with clinical vascular diseases, including cardiovascular diseases (26,27). Although the clinical impact of the *ADRA2C* 322-325I/D polymorphism on human disease is inconclusive, the D allele of *ADRA2C* 322-325 has been associated with cardiac disorder (17) and may be linked to blood flow or vascular function as a result of its association with decreased function *in vitro* (28).

In the present study, the importance of the *ADRA2C* 322-325I/D polymorphism was demonstrated by several associations. The interaction analysis of hypertension and the *ADRA2C* 322-325I/D genotype showed a significant association. It is suggested that there may be a positive interaction of the *ADRA2C* 322-325I/D polymorphism with hypertension or cerebral blood flow to increase the risk of SBI. Furthermore, the *ADRA2C* 322-325I/D polymorphism was associated with elevated plasma levels of homocysteine. It is proposed that the combination of *ADRA2C* 322-325I/D and higher plasma levels of homocysteine has a strong synergistic effect that increases the risk for SBI. *ADRA2C* 322-325I/D and higher plasma levels of homocysteine are risk factors that induce SBI. The interaction of the two factors increases the risk of developing SBI. The *ADRA2A* 1780G>A polymorphism was also associated with elevated plasma levels of homocysteine, with the *ADRA2A* 1780AA genotype tending to be associated with higher levels.

Several limitations of the present study warrant consideration. As the participation rate for the present study was low, the recruitment phase was extended over a long time period. Furthermore, the control subjects in the present study were not completely healthy, since some of them had sought medical attention. According to previous experience, recruitment of healthy participants with imaging and laboratory tests would markedly reduce the enrollment rate. However, enrollment of participants without imaging and laboratory tests may produce another bias in vascular risk factor assessment. Finally, the present study did not collect sufficient data on smoking, a risk factor associated with SBI. Therefore, no correlation test was performed for smoking. As smoking is a risk factor for vascular diseases, including stroke and white matter lesion, it is expected to be associated with SBI. However, the *ADRA2* gene family is known to affect vascular disorders regardless of smoking (24). It is suggested that a single RFLP approach of *ADRA2C* 322–325 I/D polymorphism may have considerable clinical utility.

In conclusion, it was observed that the *ADRA2C* 322-325I/D genotype increased the risk of SBI. Furthermore, the combination of the *ADRA2C* 322-325I/D genotype and high plasma levels of homocysteine had a strong synergistic effect that increased the risk for SBI. It is suggested that the *ADRA2C* 322-325I/D genotype is a novel genetic risk marker for SBI among individuals with hyperhomocysteinemia. Further studies using larger and more heterogeneous cohorts are required to validate the association of *ADRA2* polymorphisms with SBI.

Acknowledgements

The present study was supported by grants from the Basic Science Research Program (NRF-2012R1A1A2007033 and NRF-2013R1A1A2008177) and the Priority Research Centers Program (2009-0093821) through the National Research Foundation of Korea, funded by the Ministry of Education, Science and Technology, Republic of Korea.

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