의학박사 학위논문

두개강내 동맥경화증, 두개강외 동맥 경화증 및 소혈관 폐색 간의 유전자 변이에 관한 비교 연구

Comparison of genetic variants among intracranial atherosclerosis, extracranial atherosclerosis, and small vessel occlusion

울 산 대 학 교 대 학 원

의 학 과

김 연 정

두개강내 동맥경화증, 두개강외 동맥 경화증 및 소혈관 폐색 간의 유전자 변이에 관한 비교 연구

지 도 교 수　　김 종 성

이 논문을 의학박사 학위 논문으로 제출함

2018년 12월

울 산 대 학 교　대 학 원

의　학　과

김 연 정

김연정의 의학박사학위 논문을 인준함

심사위원 김 종 성 (인)

심사위원 권 순 억 (인)

심사위원 강 동 화 (인)

심사위원 전 사 일 (인)

심사위원 김 범 준 (인)

울 산 대 학 교 대 학 원

2018년 12월

Abstract

Although the distribution of cervicocephalic arterial atherosclerosis varies with race, the reason behind this difference remains unclear. As conventional vascular risk factors alone cannot explain this variability in the location of cerebral atherosclerosis, genetic differences involving vascular tortuosity and ICAS have been suggested to play a role. We aimed to investigate the association between polymorphisms of genes related to vascular tortuosity, *RNF213*, and *MMP2*, and the location of cervicocephalic arterial stenosis.

A prospective case-control study was conducted on patients with cerebral infarction or transient ischemic attack and age- and sex-matched stroke-free controls. The stroke patients were categorized into those with intracranial artery atherosclerosis (ICAS), extracranial artery atherosclerosis (ECAS), and small vessel occlusion (SVO) according to the presence and location of vascular stenosis. The location, number, and degree of steno-occlusive lesions were assessed by magnetic resonance angiography. Six single nucleotide polymorphisms (SNPs) including rs2118181 (*FBN1*), rs2179357 (*SLC2A10*), rs1036095 (*TGFBR2*), rs243865 (*MMP2*), rs1800470 (*TGFB1*), and rs112735431 (*RNF213*) were analyzed with the TaqMan Genotyping Assay, and the distribution of genotype was compared.

None of the six SNPs was associated with stroke in our initial analysis comparing the 449 stroke patients (71 with ECAS, 169 with ICAS, and 209 with SVO) with the 447 controls. In the analysis looking at stroke subgroups, the adjusted odds ratios (aORs) for age and sex indicated a significant association between rs112735431 and ICAS in the allele comparison (aOR=2.79, 95% CI: 1.17-6.66, p=0.0206) and in the additive and dominant models (aOR=2.86, 95% CI: 1.19-6.90, p=0.0192). rs2179357 was significantly associated with ICAS in the recessive model (aOR=1.65, 95% CI: 1.03-2.64, p =0.0383), and rs1800470 was significantly associated with ECAS in the recessive model (aOR=0.47, 95% CI: 0.22-0.99, p=0.0467) when compared to the controls. The analysis of the location of vascular stenosis showed that rs112735431 was associated with anterior circulation (aOR=4.94, 95% CI: 1.33-18.41, p=0.0172) and both anterior and posterior circulation (aOR=7.01, 95% CI: 1.29-38.16, p=0243), especially in the middle cerebral artery, but not with posterior circulation alone. Finally, the analysis of the burden of vascular stenosis revealed that the number (aOR=3.09, 95% CI: 1.23-7.76, p=0.0161) and degree (aOR=3.77, 95% CI: 1.48-9.58, p=0.0053) of the stenotic vessel were increased in patients with rs112735431 polymorphism.

rs112735431 was associated with ICAS in the Korean population. The variant allele of rs112735431 was shown to be associated with anterior circulation vascular stenosis and an increased burden of vascular stenosis in ICAS. rs2179357 and rs1800470 was

associated with ICAS and ECAS, respectively. Further large-scale studies are needed to confirm the effect of rs2179357 and rs1800470.

Key words: RNF213, intracranial arterial atherosclerosis, ischemic stroke, polymorphism

Contents

Tables and figures

Abbreviations

ACA: anterior cerebral artery

aOR: adjusted odds ratio

CI: confidence interval

ECAS: extracranial artery atherosclerosis

GWAS: genome-wide association study

ICA: internal carotid artery

ICAS: intracranial artery atherosclerosis

LAA: large artery atherosclerosis

MCA: middle cerebral artery

MMD: moyamoya disease

MMP: matrix metalloproteinase

MRA: magnetic resonance angiography

MRI: magnetic resonance imaging

SNP: single nucleotide polymorphism

SVO: small vessel occlusion

TOAST: Trial of. Org 10172 in Acute Stroke Treatment

Introduction

The distribution of cervicocephalic arterial atherosclerosis varies with race. Intracranial artery atherosclerosis (ICAS) is more common in Asians, whereas extracranial artery atherosclerosis (ECAS) is more prevalent among Caucasians[1-8], although the reason for this difference remains unclear. Previous studies have suggested that conventional vascular risk factors known to be associated with ICAS or ECAS may contribute to this racial variability. For example, hyperlipidemia[3] has been shown to be more related with ECAS, while hypertension, diabetes mellitus, and metabolic syndrome[9] seem to be more associated with ICAS. Prior study results are inconsistent[10, 11], however, and do not provide sufficient evidence to explain the differences in the distribution of cervicocephalic arterial atherosclerosis by conventional risk factors alone.

One possible factor that may account for this variability is inherited genetic variation. A considerable amount of research has been done recently on the association between ischemic stroke subtype and genetic variation. However, the stroke subtypes in these studies are mainly divided into large artery atherosclerosis (LAA), small vessel occlusion (SVO), and cardioembolic stroke[12, 13], and the genetic variation specifically associated with ICAS or ECAS have not been well studied. A few studies do suggest an association between ICAS and the T(-344)C polymorphism of the aldosterone system[14] and TT genotype of phosphodiesterase[15], as well as an association between ECAS and the C-reactive protein

1059G>C polymorphism[16] and APOE e4 allele[17]. Such studies, however, are still sporadic, and the polymorphisms specifically related to ICAS have not been sufficiently elucidated to date.

Vascular tortuosity is known to cause vascular stenosis, and genetic variation associated with vascular tortuosity may play a role in determining the location of cervicocephalic arterial atherosclerosis. It is well known that atherosclerosis in the carotid artery is associated with geometric properties such as radius and bifurcation angle[18]. Intravascular laminar flow is disrupted at bifurcation sites or curved portions, resulting in hemodynamic shear stress and endothelial dysfunction[19]. The relationship between ICAS and vascular tortuosity has not been well studied compared to that between ECAS and tortuosity. Recent studies on the geometric properties of ICAS have shown that having a high degree of tortuosity and a small diameter are related to middle cerebral artery atherosclerosis, probably by altering the hemodynamics[20]. Such results suggest that arterial tortuosity may be a factor determining atherosclerotic location. Although the exact cause and timing of arterial tortuosity is unclear, several mechanisms including the effect of increased TGF activity on the degree of arterial tortuosity have been postulated[21]. Genetically abnormal vessel walls may also have lower manifest axial tension, which has been shown in manipulated rabbit carotid arteries to result in increased tortuosity[22]. Arterial tortuosity has been previously described in several genetically mediated conditions associated with aortopathy, such as Marfan syndrome

(*FBN1*), Loeys-Dietz syndrome (*TGFBR1, TGFBR2*), arterial tortuosity syndrome (*SLC2A10*), and Osteoarthritis-aneurysm syndrome (*SMAD3*). However, the studies examining the association between polymorphisms of these genes and arterial tortuosity were performed mainly on patients with aortic dissection or aneurysm[23-26].

The ring finger protein 213 (*RNF213*) gene in the 17q25-ter region is well known as the susceptibility gene for Moyamoya disease (MMD)[27], which is an uncommon cerebrovascular disease characterized by the progressive stenosis of the large intracranial arteries and a hazy network of basal collaterals. Although the *RNF213* variants are frequently found in patients with MMD, it is also seen in non-moyamoya intracranial arterial stenosis, such as non-MMD ICAS[28, 29] and dissection[30], and predominantly involve the intracranial rather than the extracranial artery. The exact function of *RNF213* in blood vessels is unknown. Microscopic findings of the larger intracranial arteries in MMD included segmental narrowing, thickening of the intimal and medial layers, proliferation or degradation of smooth muscle cells, and tortuosity or fragmentation of the internal elastic lamina. However, according to several preclinical and clinical study data, vascular stenosis as well as these pathologic findings were not fully induced even in those with *RNF213* variation. Additionally, the results showed various penetrance rates of *RNF213* depending on the environment and the race of the subjects. Although exactly how *RNF213* is associated

with the arterial stenosis has not yet been clarified, it may be one of the genes potentially responsible for the racial variability observed in cervicocephalic arterial stenosis.

The family of matrix metalloproteinase (MMPs) are zinc-dependent endopeptidases that mediate extracellular matrix turnover and capillary permeability[31]. MMPs are important in vascular remodeling and atherosclerosis[32-35]. Among MMP subgroups, MMP-2 and MMP-9 have been widely studied[36]. In animal models, MMP-2 and MMP-9 have been shown to increase after stroke and be related to blood-brain barrier disruption, hemorrhagic transformation, and brain edema[37-39]. Genetic studies have shown the polymorphisms of these MMPs to be associated with ischemic stroke[40, 41]. However, there have not been many studies looking at MMPs and stroke subtype. A previous genetic study evaluating the association between *MMP2* polymorphism and stroke subtype found *MMP2* polymorphism to be associated with SVO and not with LAA[42]; this study, however, did not analyze ICAS and ECAS separately. Recent studies have suggested an association between low MMP-2 plasma levels and the intracranial location of cerebral atherosclerosis[43]. Considering these reports, the genetic polymorphism of *MMP2* may also play a role in causing varying cervicocephalic arterial stenosis in different racial groups.

We aimed to investigate the association between polymorphisms of genes related with vascular tortuosity (i.e., *SLC2A10*, *TGFB1*, *FBN1*, and *TGFBR2*), *RNF213,* and *MMP2,* and the location of cervicocephalic arterial stenosis.

Method

Study population

From December 2013 to December 2017, a prospective case-control study was conducted on patients with cerebral infarction or transient ischemic attack and age- and sex-matched stroke-free controls.

Among the patients with stroke or transient ischemic attack admitted to Asan Medical Center and Kyung Hee University Hospital during the study period, those who underwent imaging studies including brain magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) of the intracranial and extracranial cerebral arteries were chosen. Out of these, patients classified as having LAA and SVO by the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification[44] were enrolled. Because the purpose of the present study was to clarify the pathophysiology of ECAS, ICAS, and SVO, we excluded patients who had both ECAS and ICAS, and those with stroke subtypes other than LAA or SVO. Stroke-free controls with matching age and sex were enrolled. Controls were recruited from stroke day events, community welfare centers, and the outpatient clinic of Kyung Hee University Hospital. Those who were enrolled from the outpatient clinic had a headache or dizziness of non-vascular origin. Subjects who had previously been diagnosed with stroke or cerebral vascular stenosis were excluded.

This study was approved by the Institutional Review Board of Asan Medical Center, Kyung

Hee University Hospital, and Seoul National University. Informed consent for this study was obtained from the patients or their family members.

Radiological assessments

MR examinations were performed with a 1.5-tesla (GE Medical Systems, Milwaukee, WI) or 3.0-tesla (Philips Medical Systems, The Netherlands) MRI unit employing standard neurovascular coils. Common MRI parameters were 5-mm slice thickness, 2-mm interslice gap, and 20 axial slices. 1.5-teslathree-dimensional time-of-flight MRA of circle of Willis parameters were 20°flip angle, 512 ×512 matrix number, 250-mm field-of-view, 25-msec repetition time(TR), and 2-msec echo time(TE). 3.0-tesla three-dimensional time-of-flight MRA of circle of Willis parameters were 20°flip angle, 768 ×768 matrix number, 250-mm field-of-view, 25-msec repetition time (TR), and 4.1-msec echo time (TE). 1.5-tesla three-dimensional contrast-enhanced MRA from the aortic arch to the level of the central skull parameters were 30°flip angle, 512 ×512 matrix number, 320-mm field-of-view, 5.7-msec TR, and 1.4-msec TE. 3.0-tesla three-dimensional contrast-enhanced MRA from the aortic arch to the level of the central skull parameters were 30°flip angle, 1024 ×1024 matrix number, 320-mm field-of-view, 4.9-msec TR, and 1.85-msec TE. For the contrast-enhanced MRA, 20 ml of intravenous gadopentetate dimeglumine were injected at a speed of 3-4 ml/sec.

Patients with LAA were further classified into ECAS and ICAS depending on the location

of vascular stenosis. Patients with stenosis in the proximal to middle internal carotid artery (ICA, C1) or vertebral artery (V1~3) were classified as ECAS, and patients with stenosis of the distal ICA (above the C2), anterior cerebral artery (ACA), middle cerebral artery (MCA), posterior cerebral artery, basilar artery, or distal vertebral artery (V4) were classified as ICAS. Cases where the vascular stenosis was less than 50% were also included if the stenosis was obvious. The location, number, and degree of steno-occlusive lesions were assessed by MRA[45]. The number and degree of steno-occlusive lesions on MRA were used to assess the burden of vascular stenosis. The degree of vascular stenosis was visually graded along the following scale: 0, indicating no stenosis; 1, indicating mild stenosis less than 50%; 2, indicating moderate stenosis of 50 to 99%; and 3, indicating occlusion. The number of stenosis was assessed by counting the number of involved vessels. All images were analyzed independently by two experienced reviewers who were blinded to the diagnosis. Any differences in the assessments between the two reviewers were resolved by reaching a consensus through discussion.

Gene polymorphism analysis

Single nucleotide polymorphisms (SNP) are genetic variants consisting of a single DNA base-pair change usually resulting in two possible allelic identities at that position. Among the genes related to arterial tortuosity, we searched for SNPs of genes that were studied in

relation to arterial aneurysm and dissection, coronary artery disease, and peripheral artery disease. Out of these, the following nine SNPs were selected: rs2179357 (*SLC2A10*)[46], rs1626340 (*TGFBR1*)[47], rs1036095, rs4522809 (*TGFBR2*)[47-50], rs1800470 (*TGFB1*)[21, 48], rs2118181, rs1036477 (*FBN1*)[51, 52], rs17293632 and rs56062135 (*SMAD3*)[53-55]. Six SNPs were chosen in addition to these: rs112735431 (*RNF213*)[9, 28-30], rs243865 (*MMP2*)[43], and rs3918242 (*MMP9*), which have been studied in association with ICAS, and rs12122341 (*TSPAN2*), rs11984041 (*HDAC9*), and rs10744777 (*ALDH2*), which have been showed to be associated with LAA (rs12122341, rs11984041) or SVO (rs10744777) in a genome-wide association study (GWAS)[12, 13]. In the first stage, the aforementioned fifteen SNPs were analyzed in 193 participants (131 cases and 62 controls). During the second stage, we analyzed a total of 896 participants for six SNPs, rs2118181, rs2179357, rs1036095, rs243865, rs1800470, and rs112735431, which were shown to be associated with stroke and its subtypes during the first stage. Although a meta-analysis of GWASs has previously demonstrated an association between rs12122341 and rs11984041 and LAA, these genetic variations were not observed in this study.

Venous blood was collected into tubes containing ethylenediamine tetraacetic acid (EDTA), and genomic DNA was isolated with the G-DEX IIc Genomic DNA Extraction kit. Approximately 10 ng of genomic DNA was used for the TaqMan SNP Genotyping Assay. In total, fifteen SNPs were analyzed with the TaqMan Genotyping Assay on a 7900HT real-time

polymerase chain reaction system (Applied Biosystems, Foster City, CA, USA) with software version 2.4. Amplification mixtures (5 μl) for each target contained template DNA (10 ng), 2X TaqMan Genotyping Master Mix, and 40X TaqMan SNP Genotyping Assay reagent in a 384-well plate. The cycling conditions were 10 min at 95°C, followed by 40 cycles of 15 s at 95°C, and 60 s at 60°C. Genotyping for four SNPs (rs112735431, rs12122341, rs11984041, and rs3918242) were completed using the Sanger sequencing method (Applied Biosystems, Foster City, CA, USA) due to the failure of TaqMan probes to work. For sequencing analysis, genomic sequences were obtained from the GenBank (http://www.ncbi/nlm.nih.gov/) database, and polymerase chain reaction primers were designed using the Primer3 software (http://frodo.wi.mit.edu/primer3/) to amplify target regions. Each fragment amplified by polymerase chain reaction was sequenced with an ABI Prism 3730 sequencer (Applied Biosystems, Foster City, CA, USA). DNA polymorphisms were identified using the PolyPhred program (http://droog.gs.washington.edu/polyphred/).

Statistical analysis

Group comparisons were performed by Chi-square test, Student's t-test, Fisher's exact test, ANOVA, Wilcoxon rank sum test, and CMH shift test according to the characteristics of the data. The results are shown as number (%), mean ± standard deviation, and median

(interquartile range). Ordinal logistic regression was performed to assess the number and degree of vascular stenosis.

To test genetic associations between groups, four different genetic models have been applied: allelic (recessive allele vs. dominant allele), additive (the additive effect that occurs from homozygous dominant to heterozygote to homozygous recessive), recessive (homozygous recessive vs. homozygous dominant + heterozygote) and dominant (homozygous dominant vs. heterozygote + homozygous recessive) models. We used a χ^2-test to compare allele frequencies. Multinomial logistic regression with adjustment for age and sex was used to compare the genetic association between patients and controls under additive, dominant, and recessive genetic models. The strength of association was evaluated and interpreted as odds ratio (OR) with its corresponding 95% confidence interval (CI). A p value <0.05 was considered to indicate a significant difference. SAS version 9.4 (SAS Institute Inc, Cary, NC, USA) was used for all statistical analyses.

Results

During the study period, a total of 896 participants consisting of 449 cases and 447 controls were enrolled. The age, sex, and body mass index were not significantly different between the case and control groups. Systolic (p<0.0001) and diastolic (p<0.001) blood pressure, and the prevalence of hypertension (p<0.0001) and diabetes (p<0.0001) were significantly higher in the case patients.

In the analysis of LAA and SVO, the mean age was higher in patients with LAA (64.0 ± 12.6 years) than those with SVO (61.6 ± 10.1 years, p=0.024). There was no significant difference in the sex of the patients between the two groups. BMI (p=0.018) and diastolic BP (p=0.032) were significantly higher in the SVO group. Patients with LAA were then further divided into ECAS and ICAS groups. The mean age was highest in the ECAS group (66.4 ± 10.1 years) followed by the ICAS group (63.0 ± 13.5 years), with the SVO group coming in last (61.6±10.1 years, p=0.011). The proportion of males was highest in those with ECAS (84.5 %) and lowest in those with ICAS (57.4%, p<0.001) (Table 1).

Table 1. Baseline characteristics of subjects

	Case (n=449)	Control (n=447)	P-value	LAA (n=240)	SVO (n=209)	P-value	ECAS (n=71)	ICAS (n=169)	SVO (n=209)	P-value
Age (years)	62.9±11.6	63.6±11.0	0.385	64.0±12.6	61.6±10.1	0.024	66.4±10.1	63.0±13.5	61.6±10.1	0.011
Sex (male)	287 (63.9)	285 (63.8)	0.960	157 (65.4)	130 (62.2)	0.479	60 (84.5)	97 (57.4)	130 (62.2)	<0.001
BMI (kg/m^2)	24.1±2.9	24.2±3.2	0.644	23.8±2.8	24.5±3.0	0.018	23.6±2.4	23.9±3.0	24.5±3.0	0.051
Systolic BP (mmHg)	150.0±25.4	136.0±21.4	<0.000	149.4±26.1	150.7±24.6	0.595	148.5±27.3	149.8±25.7	150.7±24.6	0.816
Diastolic BP (mmHg)	83.7±14.2	79.9±13.3	<0.001	82.3±14.1	85.2±14.1	0.032	81.0±14.9	82.9±13.8	85.2±14.1	0.067
Hypertension	301 (67.0)	166 (37.4)	<0.000	166 (69.2)	135 (64.6)	0.304	52 (73.2)	114 (67.5)	135 (64.6)	0.404
Diabetes	129 (28.7)	56 (12.6)	<0.000	68 (28.3)	61 (29.2)	0.842	16 (22.5)	52 (30.8)	61 (29.2)	0.429

Values are shown as number (%) and mean ± standard deviation.

LAA, large artery atherosclerosis; SVO, small vessel occlusion; ECAS, extracranial artery atherosclerosis; ICAS, intracranial artery atherosclerosis; BMI, body mass index; BP, blood pressure

The genetic distributions of the six SNPs were not significantly different between the case and control subjects. In the stroke subgroup analysis, however, the distribution of rs112735431, the polymorphism of *RNF213*, was significantly different among subgroups. Between patients with LAA and patients with SVO, heterozygotes were found in 13 of the 240 LAA patients (5.4%) compared to 3 out of the 209 SVO patients (1.5%, p=0.026). ICAS was significantly prevalent among the 13 heterozygote LAA patients (n=11, p=0.033). There were no subjects who had rs112735431 polymorphism of homozygous recessive pattern (Table 2).

The initial genetic association analysis showed that none of the six SNPs were associated with stroke when compared with the control. In the analysis comparing stroke subgroups with the control, rs112735431 was significantly associated with LAA in the allelic {adjusted odds ratio (aOR)=2.46, 95% confidence interval (CI): 1.07-5.66, p=0.034} and additive and dominant model (aOR=2.51, 95% CI: 1.08-5.82, p=0.033). Further analysis showed rs112735431 to be significantly associated with ICAS in the allelic comparison (aOR=2.79, 95% CI: 1.17-6.66, p=0.021) and additive and dominant model (aOR=2.86, 95% CI: 1.19-6.90, p=0.019), but not with ECAS (Table 3).

rs2179357, the polymorphism of *SLC2A10*, showed a significant association with ICAS in the recessive model (aOR=1.65, 95% CI: 1.03-2.64, p=0.038). rs1800470, the

polymorphism of *TGFB1*, was significantly associated with ECAS in the recessive model (aOR=0.47, 95% CI: 0.22-0.99, p=0.047) (Table 3).

Table 2. Genotype distribution

Gene symbol	SNPs	Case (n=449)	Control (n=447)	P-value	LAA (n=240)	SVO (n=209)	P-value	ECAS (n=71)	ICAS (n=169)	SVO (n=209)	P-value
SLC2A10	rs2179357			0.322			0.240				0.163
	CC	164 (37.1)	161 (36.7)		96 (40.3)	68 (33.3)		32 (45.1)	64 (38.3)	68 (33.3)	
	CT	205 (46.4)	220 (50.1)		102 (42.9)	103 (50.5)		32 (45.1)	70 (41.9)	103 (50.5)	
	TT	73 (16.5)	58 (13.2)		40 (16.8)	33 (16.2)		7 (9.9)	33 (19.8)	33 (16.2)	
TGFBR2	rs1036095			0.403			0.604				0.890
	CC	18 (4.1)	25 (5.7)		11 (4.6)	7 (3.4)		3 (4.3)	8 (4.8)	7 (3.4)	
	CG	132 (29.9)	139 (31.7)		74 (31.2)	58 (28.3)		21 (30.0)	53 (31.7)	58 (28.3)	
	GG	292 (66.1)	275 (62.6)		152 (64.1)	140 (68.3)		46 (65.7)	106 (63.5)	140 (68.3)	
TGF-b1	rs1800470			0.302			0.458				0.210
	AA	124 (28.1)	103 (23.8)		66 (27.7)	58 (28.6)		17 (24.3)	49 (29.2)	58 (28.6)	
	AG	223 (50.6)	226 (52.2)		126 (52.9)	97 (47.8)		44 (62.9)	82 (48.8)	97 (47.8)	
	GG	94 (21.3)	104 (24.0)		46 (19.3)	48 (23.6)		9 (12.9)	37 (22.0)	48 (23.6)	
FBN1	rs2118181			0.507			0.379				0.570
	CC	31 (7.0)	39 (8.9)		15 (6.3)	16 (7.8)		6 (8.6)	9 (5.4)	16 (7.8)	
	CT	189 (42.7)	191 (43.5)		96 (40.3)	93 (45.4)		26 (37.1)	70 (41.7)	93 (45.4)	
	TT	223 (50.3)	209 (47.6)		127 (53.4)	96 (46.8)		38 (54.3)	89 (53.0)	96 (46.8)	

Gene symbol	SNPs	Case (n=449)	Control (n=447)	P-value	LAA (n=240)	SVO (n=209)	P-value	ECAS (n=71)	ICAS (n=169)	SVO (n=209)	P-value
MMP-2	rs243865			0.345			0.450				0.705
	CC	369 (82.7)	379 (85.9)		202 (84.5)	167 (80.7)		60 (85.7)	142 (84.0)	167 (80.7)	
	CT	71 (15.9)	59 (13.4)		35 (14.6)	36 (17.4)		9 (12.9)	26 (15.4)	36 (17.4)	
	TT	6 (1.3)	3 (0.7)		2 (0.8)	4 (1.9)		1 (1.4)	1 (0.6)	4 (1.9)	
RNF213	rs112735431			0.249			0.026				0.033
	AG	16 (3.6)	10 (2.3)		13 (5.4)	3 (1.5)		2 (2.9)	11 (6.5)	3 (1.5)	
	GG	426 (96.4)	425 (97.7)		226 (94.6)	200 (98.5)		68 (97.1)	158 (93.5)	200 (98.5)	

Genotype distributions are shown as number (%)

LAA, large artery atherosclerosis; SVO, small vessel occlusion; ECAS, extracranial artery atherosclerosis; ICAS, intracranial artery atherosclerosis

Table 3. Association between SNPs and stroke subtypes

	Stroke vs. Control		LAA vs. Control		ECAS vs. Control		ICAS vs. Control		SVO vs. Control	
SNPs/Models	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value
rs2179357										
Allelic	1.06 (0.88-1.28)	0.551	1.00 (0.79-1.26)	0.991	0.78 (0.53-1.14)	0.203	1.11 (0.86-1.43)	0.436	1.14 (0.90-1.45)	0.291
Additive	1.06 (0.87-1.29)	0.548	1.00 (0.79-1.26)	0.991	0.78 (0.54-1.15)	0.210	1.11 (0.86-1.44)	0.431	1.14 (0.90-1.46)	0.285
Dominant	0.98 (0.74-1.28)	0.858	0.86 (0.62-1.19)	0.360	0.74 (0.44-1.24)	0.254	0.92 (0.64-1.33)	0.671	1.14 (0.80-1.62)	0.461
Recessive	1.31 (0.90-1.90)	0.162	1.32 (0.85-2.05)	0.215	0.68 (0.30-1.57)	0.365	1.65 (1.03-2.64)	0.038	1.29 (0.81-2.06)	0.279
rs1036095										
Allelic	0.85 (0.68-1.08)	0.184	0.93 (0.70-1.22)	0.587	0.86 (0.54-1.35)	0.504	0.95 (0.69-1.29)	0.736	0.77 (0.57-1.04)	0.093
Additive	0.86 (0.68-1.08)	0.195	0.93 (0.71-1.22)	0.597	0.86 (0.56-1.34)	0.516	0.95 (0.70-1.29)	0.742	0.78 (0.58-1.05)	0.100
Dominant	0.86 (0.65-1.13)	0.278	0.94 (0.68-1.31)	0.715	0.88 (0.51-1.50)	0.633	0.96 (0.66-1.39)	0.833	0.77 (0.54-1.10)	0.146
Recessive	0.71 (0.38-1.32)	0.276	0.80 (0.38-1.65)	0.542	0.65 (0.19-2.27)	0.502	0.84 (0.37-1.91)	0.681	0.59 (0.25-1.40)	0.233
rs1800470										
Allelic	0.86 (0.71-1.04)	0.121	0.84 (0.67-1.05)	0.128	0.79 (0.55-1.13)	0.197	0.87 (0.67-1.12)	0.269	0.89 (0.70-1.13)	0.3389
Additive	0.86 (0.71-1.04)	0.116	0.83 (0.66-1.05)	0.118	0.78 (0.53-1.13)	0.187	0.86 (0.67-1.12)	0.261	0.89 (0.70-1.13)	0.330
Dominant	0.79 (0.58-1.07)	0.132	0.81 (0.56-1.16)	0.247	0.95 (0.52-1.73)	0.871	0.77 (0.51-1.15)	0.199	0.77 (0.53-1.13)	0.186
Recessive	0.84 (0.61-1.16)	0.299	0.76 (0.51-1.12)	0.168	0.47 (0.22-0.99)	0.047	0.89 (0.58-1.37)	0.598	0.95 (0.64-1.41)	0.808

	Stroke vs. Control		LAA vs. Control		ECAS vs. Control		ICAS vs. Control		SVO vs. Control	
SNPs/Models	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value
rs2118181										
Allelic	0.90 (0.73-1.10)	0.294	0.82 (0.64-1.05)	0.113	0.87 (0.58-1.31)	0.506	0.80 (0.60-1.06)	0.118	0.99 (0.77-1.28)	0.966
Additive	0.89 (0.72-1.10)	0.285	0.81 (0.63-1.05)	0.109	0.87 (0.58-1.30)	0.501	0.79 (0.59-1.05)	0.110	0.99 (0.77-1.29)	0.968
Dominant	0.90 (0.69-1.17)	0.417	0.80 (0.58-1.10)	0.165	0.83 (0.49-1.38)	0.466	0.79 (0.55-1.13)	0.200	1.03 (0.73-1.43)	0.884
Recessive	0.78 (0.48-1.27)	0.316	0.69 (0.37-1.27)	0.233	0.89 (0.36-2.21)	0.805	0.59 (0.28-1.26)	0.174	0.90 (0.49-1.65)	0.723
rs243865										
Allelic	1.29 (0.92-1.81)	0.141	1.13 (0.74-1.70)	0.576	1.09 (0.55-2.13)	0.810	1.13 (0.71-1.79)	0.609	1.49 (1.00-2.23)	0.052
Additive	1.28 (0.92-1.78)	0.150	1.12 (0.75-1.69)	0.580	1.08 (0.56-2.10)	0.811	1.12 (0.71-1.77)	0.617	1.47 (0.99-2.18)	0.057
Dominant	1.28 (0.89-1.84)	0.191	1.13 (0.73-1.76)	0.585	1.03 (0.50-2.15)	0.927	1.15 (0.70-1.89)	0.572	1.46 (0.94-2.26)	0.093
Recessive	2.01 (0.50-8.09)	0.326	1.21 (0.20-7.30)	0.837	2.12 (0.21-21.28)	0.522	0.89 (0.09-8.58)	0.916	2.98 (0.66-13.51)	0.156
rs112735431										
Allelic	1.57 (0.71-3.48)	0.269	2.46 (1.07-5.66)	0.034	1.37 (0.29-6.45)	0.693	2.79 (1.17-6.66)	0.021	0.61 (0.17-2.22)	0.448
Additive	1.58 (0.71-3.53)	0.265	2.51 (1.08-5.82)	0.033	1.38 (0.29-6.57)	0.690	2.86 (1.19-6.90)	0.019	0.60 (0.16-2.22)	0.445
Dominant	1.58 (0.71-3.53)	0.265	2.51 (1.08-5.82)	0.033	1.38 (0.29-6.57)	0.690	2.86 (1.19-6.90)	0.019	0.60 (0.16-2.22)	0.445
Recessive	Non-estimable		Non-estimable		Non-estimable		Non-estimable		Non-estimable	

SNP, single nucleotide polymorphism; LAA, large artery atherosclerosis; SVO, small vessel occlusion; ECAS, extracranial artery atherosclerosis; ICAS, intracranial artery atherosclerosis; aOR, adjusted odds ratio for age and sex; CI, confidence interval

We conducted additional analysis comparing heterozygotes (AG) and homozygous dominant (GG) of rs112735431, which had shown a strong association with ICAS. The baseline characteristics of the total 896 case and control subjects did not show any significant difference. Within the 449 stroke cases, there was no significant difference in initial National Institutes of Health Stroke Scale and modified Rankin Scale between the heterozygote and homozygous dominant groups. Radiological analysis of vascular stenosis location, however, revealed that patients with heterozygotes predominantly showed stenosis in the anterior or both the anterior and posterior circulations (p=0.021). On the other hand, patients with homozygous dominant displayed an even distribution of vascular stenosis. Subsequent multinomial logistic regression analysis showed that rs112735431 was associated with anterior circulation (aOR=4.76, 95% CI: 1.30-17.51, p=0.019 in allelic comparison; aOR=4.94, 95% CI: 1.33-18.41, p=0.017 in additive and dominant models) and both anterior and posterior circulation (aOR=6.68, 95% CI: 1.26-35.34, p=0.026 in allelic comparison; aOR=7.01, 95% CI: 1.29-38.16, p=0.024 in additive and dominant models). In the analysis of involved vessels, only the MCA was associated with heterozygotes (p=0.003). The analysis of the burden of vascular stenosis showed that the number (aOR=2.99, 95% CI: 1.27-7.39, p=0.018 in allelic comparison; aOR=3.09, 95% CI: 1.23-7.76, p=0.016 in additive and dominant models) and degree (aOR=3.62, 95% CI: 1.45-9.07, p=0.006 in allelic comparison; aOR=3.77,

95% CI: 1.48-9.58, p=0.005 in additive and dominant models) of the stenotic vessel were increased in patients with rs112735431 polymorphism (Table 4).

Table 4. Characteristic comparison between heterozygotes and homozygous dominant of rs112735431

(a) Baseline characteristics

	Heterozygotes (n=26)	Homozygous dominant (n=851)	p-value
Age (years)	59.9±12.2	63.3±11.3	0.130
Sex (male)	15 (57.7)	546 (64.2)	0.499
BMI (Kg/m2)	24.1±3.0	24.1±3.0	0.994
Systolic BP (mmHg)	152.0±28.9	144.4±24.8	0.160
Diastolic BP (mmHg)	83.7±14.7	82.2±13.9	0.611
Hypertension	15 (57.7)	442 (52.1)	0.575
Diabetes	6 (23.1)	171 (20.2)	0.716

Values are shown as number (%) and mean ± standard deviation.

BMI, body mass index; BP, blood pressure

(b) Clinical characteristics and imaging analysis in stroke patients

	Heterozygotes (n=16)	Homozygous dominant (n=426)	p-value
Age (years)	59.4±10.7	63.1±11.6	0.213
Sex (male)	8 (50)	278 (65.3)	0.210
BMI (Kg/m2)	23.4±2.4	24.0±3.1	0.547
Systolic BP (mmHg)	155.8±29.2	149.9±25.4	0.369
Diastolic BP (mmHg)	85.2±15.6	53.7±14.2	0.682
Hypertension	12 (75.0)	282 (66.2)	0.454
Diabetes	5 (31.3)	119 (27.9)	0.780
History of stroke	2 (12.5)	64 (15.1)	1.000

	Heterozygotes	Homozygous dominant	p-value
Smoking			0.717
None	3 (37.5)	136 (46.3)	
Ex-smoker	1 (12.5)	52 (17.7)	
Current smoker	4 (50.0)	106 (36.1)	
WBC	8486.2±2298.3	7983.5±2552.7	0.438
Hb	14.1±1.9	14.2±1.7	0.822
Platelet	258.1±47.3	239.5±66.5	0.268
PT	1.1±0.3	1.0±0.1	0.301
CRP	0.4±0.6	0.3±1.2	0.962
AST	22.3±5.3	25.2±10.5	0.275
ALT	20.6±8.5	23.1±15.0	0.515
BUN	17.4±16.6	14.7±6.5	0.135
Cr	1.3±2.2	0.9±0.9	0.096
Total cholesterol	187.8±38.1	178.0±41.1	0.347
Triglyceride	160.1±118.6	136.1±90.3	0.303
HDL-Cholesterol	41.7±12.2	44.8±11.9	0.305
LDL-Cholesterol	125.3±34.5	115.4±35.3	0.270
HbA1C	6.3±1.3	6.4±1.4	0.806
Fasting glucose	114.1±31.1	115.8±39.4	0.861
Admission NIHSS	3 (2 - 5)	3 (2 - 6)	0.616
Admission mRS			0.116
0	5 (31.3)	141 (33.1)	
1	8 (50.0)	66 (15.5)	
2	2 (12.5)	85 (20.0)	
3	0 (0.0)	82 (19.2)	
4	1 (6.3)	48 (11.3)	
5	0 (0.0)	4 (0.9)	
Location of vascular stenosis			0.021

No	3 (18.8)	200 (46.9)	
Anterior	10 (62.5)	140 (32.9)	
Posterior	0 (0.0)	43 (10.1)	
Both	3 (18.8)	43 (10.1)	
Number of stenotic vessels	1 (1 - 2)	1 (0 - 1)	0.018
Degree of stenosis			0.035
No	3 (18.8)	200 (46.9)	
Mild	0 (0.0)	28 (6.6)	
Moderate	7 (43.8)	126 (29.6)	
Occlusion	6 (37.5)	72 (16.9)	
Involved vessel			
CCA	1 (6.3)	7 (1.6)	0.257
Proximal ICA	2 (12.5)	53 (12.4)	1.000
Distal ICA	3 (18.8)	28 (6.6)	0.094
MCA	10 (62.5)	111 (26.1)	0.003
ACA	0 (0.0)	19 (4.5)	1.000
PCA	3 (18.8)	31 (7.3)	0.117
Proximal VA	0 (0.0)	27 (6.3)	0.613
Distal VA	0 (0.0)	23 (5.4)	1.000
BA	0 (0.0)	32 (7.5)	0.619

Values are shown as number (%), mean ± standard deviation and median (interquartile range). Comparisons were performed by Chi-square test, Fisher's exact test, Wilcoxon rank sum test and CMH shift test.

BMI, body mass index; BP, blood pressure; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; ECAS, extracranial artery atherosclerosis; ICAS, intracranial artery atherosclerosis; SVO, small vessel occlusion; CCA, common carotid artery; ICA, internal carotid artery; MCA, middle cerebral artery; ACA, anterior cerebral artery; PCA, posterior cerebral artery; VA, vertebral artery; BA, basilar artery

(c) Location of vascular stenosis

	anterior vs. no stenosis		posterior vs. no stenosis		both vs. no stenosis	
Model	aOR (95% CI)	P-value	aOR (95% CI)	P-value	aOR (95% CI)	P-value
Allelic	4.76 (1.30-17.51)	0.019	NE	NE	6.68 (1.26-35.34)	0.026
Additive	4.94 (1.33-18.41)	0.017	NE	NE	7.01 (1.29-38.16)	0.024
Dominant	4.94 (1.33-18.41)	0.017	NE	NE	7.01 (1.29-38.16)	0.024
Recessive	NE	NE	NE	NE	NE	NE

P-value by multinomial logistic regression adjusted for age and sex.

aOR, adjusted odds ratio for age and sex; CI, confidence interval; NE, non-estimated

(d) Burden of vascular stenosis

	Number of stenosis		Degree of stenosis	
Model	aOR (95% CI)	P-value	aOR (95% CI)	P-value
Allelic	2.99 (1.21-7.39)	0.018	3.62 (1.45-9.07)	0.006
Additive	3.09 (1.23-7.76)	0.016	3.77 (1.48-9.56)	0.005
Dominant	3.09 (1.23-7.76)	0.016	3.77 (1.48-9.56)	0.005
Recessive	NE	NE	NE	NE

P-value by ordinal logistic regression adjusted for age and sex. The common odds ratios is indicated by shifting to a higher value.

aOR, adjusted odds ratio for age and sex; CI, confidence interval; NE, non-estimate

Discussion

In this study, we have demonstrated that rs112735431, the polymorphism of RNF213, was significantly associated with ICAS in all genetic models, and with anterior or both anterior and posterior circulation in terms of the location of vascular stenosis. Among the cervicocephalic arteries, only MCA showed a statistically significant association with rs112735431 polymorphism. Finally, we found that rs112735431 was associated with an increased burden of vascular stenosis as measured by the number and degree of stenosis.

The ring finger protein 213 (RNF213) gene is a well-known susceptibility gene for MMD. The genetic variation of *RNF213* is reportedly present in 73% of definite MMD patients and in 50% of unilateral MMD patients[56]; however, it is not specific to MMD. This variant is also associated with non-MMD vascular diseases, particularly with intracranial arterial diseases such as ICAS and dissection[28-30]. Several reports from Japan and Korea have shown that approximately 22%–24% of patients with non-MMD ICAS possess the *RNF213* variant[29, 56, 57]. The exact function of *RNF213* and its effect on the intracranial artery is yet unknown. One possible hypothesis is that the *RNF213* genetic variant leads to vascular fragility, thereby making vessels more vulnerable to hemodynamic stress and ultimately progress to ICAS[27]. Since the aim of our study was to elucidate the difference between ICAS and ECAS, we excluded diagnosed MMD patients. In other words, subjects with rs112735431 polymorphism in this study belong to the non-MMD ICAS group and do not

meet the diagnostic criteria for MMD. It is difficult to confirm at this point whether these non-MMD ICAS patients in fact have an undiagnosed or early stage adult-onset MMD or atherosclerosis or both[58]. However, given its prevalence among Asians, the genetic variation of *RNF213* may be a factor accounting for the high prevalence of ICAS in East Asia as well as the difference in the location of cervicocephalic arterial stenosis between Asians and Caucasians.

All cases with rs112735431 polymorphism included in this study had heterozygous variation; homozygous recessive variation was not found. This may be due to the exclusion of confirmed MMD patients from this study. Previous studies on MMD patients have reported that the homozygous p.R4810K variant of *RNF213* predicted an early onset and severe form of MMD[59, 60]. In the present study, we found that patients with the *RNF213* variant showed an increased burden of vascular stenosis both in terms of the number and degree of stenosis in all analyzed models. This result suggests that the variant allele of rs112735431 has the effect of increasing the burden of atherosclerosis in non-MMD ICAS.

Interestingly, patients with the *RNF213* variant showed an association with anterior and both anterior and posterior circulation with regard to the location of vascular stenosis in the present study. There were no cases where only the posterior circulation exhibited vascular stenosis without involving the anterior circulation. It is possible that non-MMD ICAS patients with rs112735431 polymorphism involve the anterior circulation first as do the

typical cases of MMD. Among the various blood vessels of anterior circulation, only the MCA showed a statistically significant association with the heterozygous variant of *RNF213* in this study (Figure 1). This is different from typical MMD where the involvement of the distal ICA is an important finding that is also included in the diagnostic criteria. Since the diagnostic criteria and known clinical features of typical MMD are based on studies of childhood-onset MMD, the clinical manifestations of adult-onset MMD are not well known, nor are they easy to study. Some studies have recently shown that adult-onset MMD has less prominent basal collaterals and may present as unilateral MMD or display stenotic lesions in the MCA or ACA with a relatively intact distal ICA[57, 61, 62]. These results suggest that the heterozygous variant of rs112735431 in this study may be an early manifestation of adult-onset MMD, although it would be difficult to diagnose these cases as adult-onset MMD without long term follow-up.

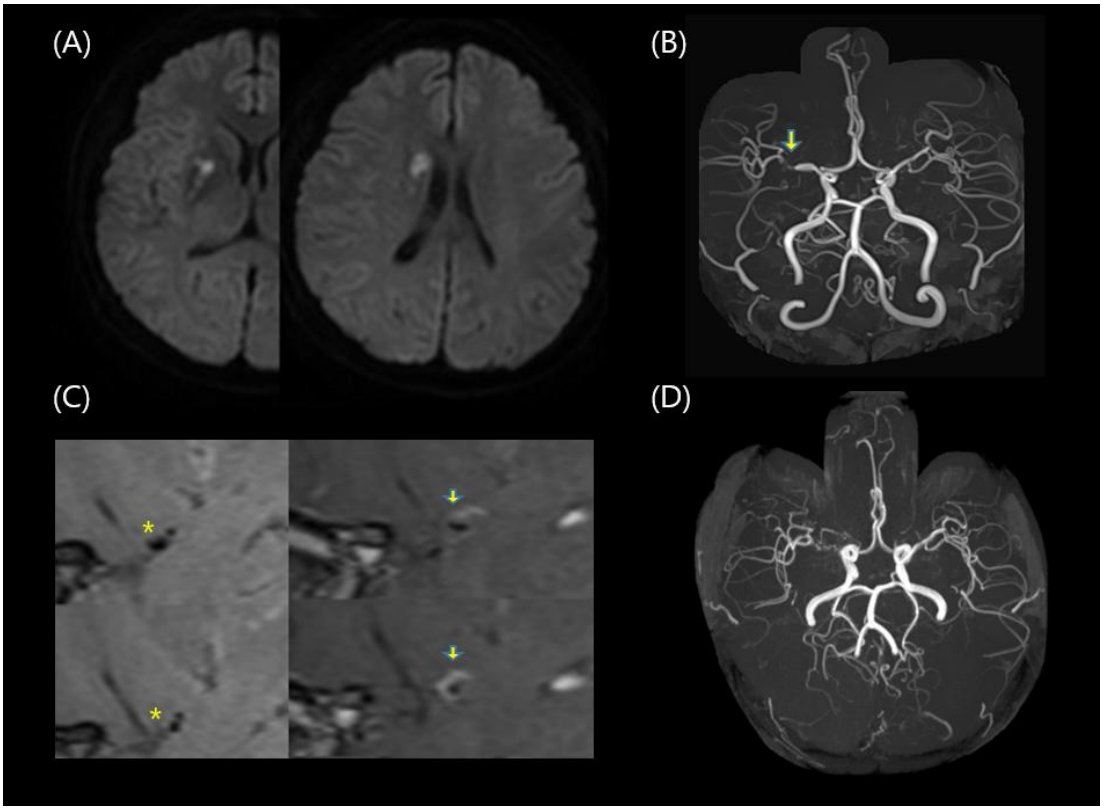

Figure 1. Magnetic resonance imaging and magnetic resonance angiography findings of patient with rs112735431 polymorphism

A 54-year-old female presented with transient dysarthria and weakness of right arm and leg. Imaging studies showed findings of an acute stroke in the right basal ganglia on the diffusion-weighted image (A) and severe focal stenosis in the right middle cerebral artery (B). In the 3.0-tesla high resolution-magnetic resonance imaging, there was segmental stenosis in the right distal middle cerebral artery with eccentric wall thickening and enhancement in the pre- and post-T1 enhancement images (C). One year later, an increase of the extent of segmental irregular stenosis involving the whole right M1 segment was observed in the follow-up magnetic resonance angiography (D).

Both the recessive models of rs2179357, the polymorphism of *SLC2A10*, and rs1800470, the polymorphism of *TGFB1,* showed a significant association with ICAS and ECAS, respectively. The *SLC2A10* gene provides the instructions for making a protein called GLUT10, a glucose transporter that contributes to maintaining glucose homeostasis. Genetic mutation of *SLC2A10* causes arterial tortuosity syndrome. The level of GLUT10 appears to be involved in the regulation of the TGF- β signaling pathway, which affects cell growth, proliferation, and differentiation. The TGF-β signaling pathway is also involved in blood vessel development. *SLC2A10* gene mutations induce an upregulation of TGF-β signaling by reducing or eliminating GLUT10 function, and this excessive growth signaling results in vascular tortuosity. *SLC2A10* and *TGFB1* polymorphisms have not received much attention in the field of ischemic stroke. In view of previous study results showing MCA tortuosity to increase the risk of ICAS[20], the results of the present study suggest that the recessive models of *SLCA10* and *TGFB1* polymorphisms may also be involved in vascular tortuosity and thus affect the risk of ICAS and ECAS, respectively. The evidence so far is not clear, however, and further large-scale studies analyzing both vascular tortuosity and genetic variants are needed to confirm the effect of these polymorphisms.

The results of this study have implications for future study designs. Studies that do not differentiate between various races or different stroke subtypes in their analysis may not produce meaningful results. During the first stage of the two genetic analysis we conducted

in this study, our analysis of fifteen SNPs did not detect the rs12122341 or rs11984041 polymorphisms, both of which a meta-analysis of GWASs has shown earlier to be associated with LAA. As the GWASs included other racial populations, the majority being non-Asian, this discrepancy suggests that differences in genotype among races need to be considered. The analysis of rs112735431 also initially showed no significant difference when the stroke patients were compared with the control subjects. The effect of rs112735431 on cervicocephalic arteries was confirmed only after comparing stroke subtypes. As genetic data around the world continues to accumulate, more studies reflecting the possibility of race-specific genetic variations and the different pathomechanism among stroke subtypes will need to be designed. Future studies conducting genetic analysis by specific ancestry groups and stroke subtype will take on more importance.

This study has several limitations. First, the sample size is relatively small. Because we analyzed data according to stroke subtype, we had a limited number of cases for each stroke subtype. The number of heterozygote cases of rs112735431 was also small. Second, we were unable to directly identify the different distribution of cervicocephalic arterial stenosis by race since all participating subjects of this study were Korean. Third, there is a lack of histopathological evidence with which to compare the rs112735431 variation. Long term follow-up will be needed to confirm the diagnosis of the non-MMD patients with rs112735431 polymorphism. Lastly, we did not have brain

images of those in the control group. Unlike the stroke patients, the control group was recruited from the general community by enrolling those fulfilling the criteria of not having a history of stroke or cerebrovascular disease. We were thus unable to identify their cerebrovascular status by brain imaging. Considering these limitations, additional studies of larger samples that include subjects of different races should be conducted to confirm our findings. Nevertheless, the results of our study will help elucidate the underlying genetic factors involved in the varying location of cervicocephalic arterial atherosclerosis observed among different racial populations.

Conclusion

rs112735431 was associated with ICAS in the Korean population. The variant allele of rs112735431 showed an association with anterior circulation vascular stenosis, especially in the MCA, and an increased burden of vascular stenosis in ICAS.

rs2179357 and rs1800470 showed an association with ICAS and ECAS, respectively. Further large-scale studies are needed to confirm the effect of rs2179357 and rs1800470.

References

1. Kim JS, Kim YJ, Ahn SH, Kim BJ. Location of cerebral atherosclerosis: Why is there a difference between East and West? Int J Stroke 2018;13(1):35-46.

2. Yang WJ, Wong KS, Chen XY. Intracranial Atherosclerosis: From Microscopy to High-Resolution Magnetic Resonance Imaging. J Stroke 2017;19(3):249-60.

3. Kim JS, Nah HW, Park SM, Kim SK, Cho KH, Lee J, et al. Risk factors and stroke mechanisms in atherosclerotic stroke: intracranial compared with extracranial and anterior compared with posterior circulation disease. Stroke 2012;43(12):3313-8.

4. Sacco RL, Kargman DE, Gu Q, Zamanillo MC. Race-ethnicity and determinants of intracranial atherosclerotic cerebral infarction. The Northern Manhattan Stroke Study. Stroke 1995;26(1):14-20.

5. Inzitari D, Hachinski VC, Taylor DW, Barnett HJ. Racial differences in the anterior circulation in cerebrovascular disease. How much can be explained by risk factors? Arch Neurol 1990;47(10):1080-4.

6. Caplan LR, Gorelick PB, Hier DB. Race, sex and occlusive cerebrovascular disease: a review. Stroke 1986;17(4):648-55.

7. Nishimaru K, McHenry LC, Jr., Toole JF. Cerebral angiographic and clinical differences in carotid system transient ischemic attacks between American Caucasian and Japanese patients. Stroke 1984;15(1):56-9.

8. Kuller L, Reisler DM. An explanation for variations in distribution of stroke and arteriosclerotic heart disease among populations and racial groups. Am J Epidemiol 1971;93(1):1-9.

9. Bang OY. Intracranial atherosclerosis: current understanding and perspectives. J Stroke 2014;16(1):27-35.

10. Kim YD, Choi HY, Jung YH, Nam CM, Yang JH, Cho HJ, et al. Classic risk factors for atherosclerosis are not major determinants for location of extracranial or intracranial cerebral atherosclerosis. Neuroepidemiology 2009;32(3):201-7.

11. Wityk RJ, Lehman D, Klag M, Coresh J, Ahn H, Litt B. Race and sex differences in the distribution of cerebral atherosclerosis. Stroke 1996;27(11):1974-80.

12. Loci associated with ischaemic stroke and its subtypes (SiGN): a genome-wide association study. Lancet Neurol 2016;15(2):174-84.

13. Traylor M, Farrall M, Holliday EG, Sudlow C, Hopewell JC, Cheng YC, et al. Genetic risk factors for ischaemic stroke and its subtypes (the METASTROKE collaboration): a meta-analysis of genome-wide association studies. Lancet Neurol 2012;11(11):951-62.

14. Munshi A, Sharma V, Kaul S, Rajeshwar K, Babu MS, Shafi G, et al. Association of the -344C/T aldosterone synthase (CYP11B2) gene variant with hypertension and stroke. J Neurol Sci 2010;296(1-2):34-8.

15. Kalita J, Somarajan BI, Kumar B, Kumar S, Mittal B, Misra UK. Phosphodiesterase 4 D gene polymorphism in relation to intracranial and extracranial atherosclerosis in ischemic stroke. Dis Markers 2011;31(4):191-7.

16. Liu ZZ, Lv H, Gao F, Liu G, Zheng HG, Zhou YL, et al. Polymorphism in the human C-reactive protein (CRP) gene, serum concentrations of CRP, and the difference between intracranial and extracranial atherosclerosis. Clin Chim Acta 2008;389(1-2):40-4.

17. Chutinet A, Suwanwela NC, Snabboon T, Chaisinanunkul N, Furie KL, Phanthumchinda K. Association between genetic polymorphisms and sites of cervicocerebral artery atherosclerosis. J Stroke Cerebrovasc Dis 2012;21(5):379-85.

18. Phan TG, Beare RJ, Jolley D, Das G, Ren M, Wong K, et al. Carotid artery anatomy and geometry as risk factors for carotid atherosclerotic disease. Stroke 2012;43(6):1596-601.

19. Chatzizisis YS, Coskun AU, Jonas M, Edelman ER, Feldman CL, Stone PH. Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: molecular, cellular, and vascular behavior. J Am Coll Cardiol 2007;49(25):2379-93.

20. Kim BJ, Kim SM, Kang DW, Kwon SU, Suh DC, Kim JS. Vascular tortuosity may be related to intracranial artery atherosclerosis. Int J Stroke 2015;10(7):1081-6.

21. Franken R, El Morabit A, de Waard V, Timmermans J, Scholte AJ, van den Berg MP, et al. Increased aortic tortuosity indicates a more severe aortic phenotype in adults with Marfan syndrome. Int J Cardiol 2015;194:7-12.

22. Jackson ZS, Dajnowiec D, Gotlieb AI, Langille BL. Partial off-loading of longitudinal tension induces arterial tortuosity. Arterioscler Thromb Vasc Biol 2005;25(5):957-62.

23. Kim HW, Stansfield BK. Genetic and Epigenetic Regulation of Aortic Aneurysms. Biomed Res Int 2017;2017:7268521.

24. Morris SA. Arterial tortuosity in genetic arteriopathies. Curr Opin Cardiol 2015;30(6):587-93.

25. De Backer J, Campens L, De Paepe A. Genes in thoracic aortic aneurysms/dissections - do they matter? Ann Cardiothorac Surg 2013;2(1):73-82.

26. Thompson AR, Drenos F, Hafez H, Humphries SE. Candidate gene association studies in abdominal aortic aneurysm disease: a review and meta-analysis. Eur J Vasc Endovasc Surg 2008;35(1):19-30.

27. Fujimura M, Sonobe S, Nishijima Y, Niizuma K, Sakata H, Kure S, et al. Genetics and Biomarkers of Moyamoya Disease: Significance of RNF213 as a Susceptibility Gene. J Stroke 2014;16(2):65-72.

28. Bang OY, Fujimura M, Kim SK. The Pathophysiology of Moyamoya Disease: An Update. J Stroke 2016;18(1):12-20.

29. Miyawaki S, Imai H, Shimizu M, Yagi S, Ono H, Mukasa A, et al. Genetic variant RNF213 c.14576G>A in various phenotypes of intracranial major artery stenosis/occlusion. Stroke 2013;44(10):2894-7.

30. Kim JS, Lee HB, Kwon HS. RNF213 Polymorphism in Intracranial Artery Dissection. J Stroke 2018;20(3):404-6.

31. Nagase H, Woessner JF, Jr. Matrix metalloproteinases. J Biol Chem 1999;274(31):21491-4.

32. Henney AM, Wakeley PR, Davies MJ, Foster K, Hembry R, Murphy G, et al. Localization of stromelysin gene expression in atherosclerotic plaques by in situ hybridization. Proc Natl Acad Sci U S A 1991;88(18):8154-8.

33. Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest 1994;94(6):2493-503.

34. Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circ Res 2002;90(3):251-62.

35. Newby AC. Dual role of matrix metalloproteinases (matrixins) in intimal thickening and atherosclerotic plaque rupture. Physiol Rev 2005;85(1):1-31.

36. Lucivero V, Prontera M, Mezzapesa DM, Petruzzellis M, Sancilio M, Tinelli A, et al. Different roles of matrix metalloproteinases-2 and -9 after human ischaemic stroke.

Neurol Sci 2007;28(4):165-70.

37. Heo JH, Lucero J, Abumiya T, Koziol JA, Copeland BR, del Zoppo GJ. Matrix metalloproteinases increase very early during experimental focal cerebral ischemia. J Cereb Blood Flow Metab 1999;19(6):624-33.

38. Kelly MA, Shuaib A, Todd KG. Matrix metalloproteinase activation and blood-brain barrier breakdown following thrombolysis. Exp Neurol 2006;200(1):38-49.

39. Shigemori Y, Katayama Y, Mori T, Maeda T, Kawamata T. Matrix metalloproteinase-9 is associated with blood-brain barrier opening and brain edema formation after cortical contusion in rats. Acta Neurochir Suppl 2006;96:130-3.

40. Nie SW, Wang XF, Tang ZC. Correlations between MMP-2/MMP-9 promoter polymorphisms and ischemic stroke. Int J Clin Exp Med 2014;7(2):400-4.

41. Niu F, Wei B, Yan M, Li J, Ouyang Y, Jin T. Matrix metalloproteinase-2 gene polymorphisms are associated with ischemic stroke in a Hainan population. Medicine (Baltimore) 2018;97(39):e12302.

42. Fatar M, Stroick M, Steffens M, Senn E, Reuter B, Bukow S, et al. Single-nucleotide polymorphisms of MMP-2 gene in stroke subtypes. Cerebrovasc Dis 2008;26(2):113-9.

43. Jeon SB, Chun S, Choi-Kwon S, Chi HS, Nah HW, Kwon SU, et al. Biomarkers and location of atherosclerosis: matrix metalloproteinase-2 may be related to

intracranial atherosclerosis. Atherosclerosis 2012;223(2):442-7.

44. Adams HP, Jr., Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke 1993;24(1):35-41.

45. Arenillas JF, Molina CA, Chacon P, Rovira A, Montaner J, Coscojuela P, et al. High lipoprotein (a), diabetes, and the extent of symptomatic intracranial atherosclerosis. Neurology 2004;63(1):27-32.

46. Jiang YD, Chang YC, Chiu YF, Chang TJ, Li HY, Lin WH, et al. SLC2A10 genetic polymorphism predicts development of peripheral arterial disease in patients with type 2 diabetes. SLC2A10 and PAD in type 2 diabetes. BMC Med Genet 2010;11:126.

47. Ruigrok YM, Baas AF, Medic J, Wijmenga C, Rinkel GJ. The transforming growth factor-beta receptor genes and the risk of intracranial aneurysms. Int J Stroke 2012;7(8):645-8.

48. Yang M, Zhu M, Tang L, Zhu H, Lu Y, Xu B, et al. Polymorphisms of TGFbeta-1 and TGFBR2 in relation to coronary artery disease in a Chinese population. Clin Biochem 2016;49(12):873-8.

49. Ruiz-Franco A, Barboza MA, Jara-Prado A, Canizales-Quinteros S, Leon-Mimila P, Arguelles-Morales N, et al. TGFBR2 mutation and MTHFR-C677T polymorphism in

a Mexican mestizo population with cervico-cerebral artery dissection. J Neurol 2016;263(6):1066-73.

50. Biros E, Norman PE, Jones GT, van Rij AM, Yu G, Moxon JV, et al. Meta-analysis of the association between single nucleotide polymorphisms in TGF-beta receptor genes and abdominal aortic aneurysm. Atherosclerosis 2011;219(1):218-23.

51. Lesauskaite V, Sepetiene R, Jariene G, Patamsyte V, Zukovas G, Grabauskyte I, et al. FBN1 polymorphisms in patients with the dilatative pathology of the ascending thoracic aorta. Eur J Cardiothorac Surg 2015;47(4):e124-30.

52. Iakoubova OA, Tong CH, Rowland CM, Luke MM, Garcia VE, Catanese JJ, et al. Genetic variants in FBN-1 and risk for thoracic aortic aneurysm and dissection. PLoS One 2014;9(4):e91437.

53. Turner AW, Martinuk A, Silva A, Lau P, Nikpay M, Eriksson P, et al. Functional Analysis of a Novel Genome-Wide Association Study Signal in SMAD3 That Confers Protection From Coronary Artery Disease. Arterioscler Thromb Vasc Biol 2016;36(5):972-83.

54. Garcia-Bermudez M, Lopez-Mejias R, Genre F, Castaneda S, Gonzalez-Juanatey C, Llorca J, et al. SMAD3 rs17228212 gene polymorphism is associated with reduced risk to cerebrovascular accidents and subclinical atherosclerosis in anti-CCP negative Spanish rheumatoid arthritis patients. PLoS One 2013;8(10):e77695.

55. Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mayer B, et al. Genomewide association analysis of coronary artery disease. N Engl J Med 2007;357(5):443-53.

56. Miyawaki S, Imai H, Takayanagi S, Mukasa A, Nakatomi H, Saito N. Identification of a genetic variant common to moyamoya disease and intracranial major artery stenosis/occlusion. Stroke 2012;43(12):3371-4.

57. Bang OY, Ryoo S, Kim SJ, Yoon CH, Cha J, Yeon JY, et al. Adult Moyamoya Disease: A Burden of Intracranial Stenosis in East Asians? PLoS One 2015;10(6):e0130663.

58. Jiang T, Perry A, Dacey RG, Jr., Zipfel GJ, Derdeyn CP. Intracranial atherosclerotic disease associated with moyamoya collateral formation: histopathological findings. J Neurosurg 2013;118(5):1030-4.

59. Kim EH, Yum MS, Ra YS, Park JB, Ahn JS, Kim GH, et al. Importance of RNF213 polymorphism on clinical features and long-term outcome in moyamoya disease. J Neurosurg 2016;124(5):1221-7.

60. Miyatake S, Miyake N, Touho H, Nishimura-Tadaki A, Kondo Y, Okada I, et al. Homozygous c.14576G>A variant of RNF213 predicts early-onset and severe form of moyamoya disease. Neurology 2012;78(11):803-10.

61. Scott RM, Smith ER. Moyamoya disease and moyamoya syndrome. N Engl J Med 2009;360(12):1226-37.

62. Kim YJ, Lee JK, Ahn SH, Kim BJ, Kang DW, Kim JS, et al. Nonatheroscleotic Isolated Middle Cerebral Artery Disease May Be Early Manifestation of Moyamoya Disease. Stroke 2016;47(9):2229-35.

Korean abstract

경두개 동맥 죽상 경화증의 분포는 인종에 따라 다르지만, 이러한 차이의 원인은 분명하지 않다. 동맥경화를 일으키는 것으로 알려진 기존의 혈관 위험 인자만으로는 인종간 뇌동맥 혈관 협착의 위치 차이를 설명하기 쉽지 않기 때문에, 혈관의 만곡도(tortuosity)와 두개강내 동맥경화증과 관련된 유전적 차이가 중요한 역할을 할 수 있다. 이 연구에서는 혈관의 만곡도, RNF213, 그리고 MMP2 유전자의 단일 유전자 변이와 경두개 동맥 협착 위치와의 연관성을 연구하였다.

뇌경색 또는 일과성 뇌허혈 발작 환자들과 뇌경색 기왕력이 없는 나이, 성별을 맞춘 대조군을 대상으로 전향적 연구를 시행하였다. 환자군은 혈관 협착의 유무와 그 위치에 따라 두개강내 동맥경화증과 두개강외 동맥경화증 및 소혈관 폐쇄로 분류하였다. 혈관 협착의 위치, 수 그리고 정도는 자기 공명 혈관 영상을 통해 평가하였다. TaqMan Genotyping Assay로 rs2118181 (*FBN1*), rs2179357 (*SLC2A10*), rs1036095 (*TGFBR2*), rs243865 (*MMP2*), rs1800470 (*TGFB1*), 그리고 rs112735431 (*RNF213*)을 포함한 6개의 단일 염기 다형성 유전자형을 비교하였다.

전체 449명의 뇌졸중 환자들(두개강외 동맥경화증 71명, 두개강내 동맥경화증 169명, 소혈관 폐색 209명)과 447명의 대조군의 비교 분석에서, 6개의 단일 염기 다형성 중 어떤 것도 대조군에 비해 뇌졸중 환자군과 유의한 연관성을 보이지 않았다. 뇌졸중 아형에 따른 분석에서 rs112735431는 분석한 모든 모델에서

두개강내 동맥경화증과 유의한 연광성을 보였다(aOR=2.79, 95% Confidence interval (CI): 1.17-6.66, p=0.0206 in the allele comparison; aOR=2.86, 95% CI: 1.19-6.90, p=0.0192 in the additive and dominant models). rs2179357은 recessive model에서 대조군에 비해 두개강내 동맥경화증과 유의한 연관성을 보였고(aOR=1.65, 95% CI: 1.03-2.64, p =0.0383), rs1800470은 recessive model에서 대조군에 비해 두개강외 동맥경화증과 유의한 관련성을 보였다(aOR=0.47, 95% CI: 0.22-0.99, p=0.0467). 혈관 협착의 위치 분석에서 rs112735431은 전방 뇌혈관(aOR=4.94, 95% CI: 1.33-18.41, p=0.0172) 또는 전방과 후방 뇌혈관 모두를 침범하는 경우(aOR=7.01, 95% CI: 1.29-38.16, p=0243)와 연관성을 보였으며, 후방 뇌혈관과는 관련성이 없었다. 전방 뇌혈관 중에서는 중대뇌동맥과 특히 관련이 있었다. 마지막으로 혈관 협착에 미치는 영향에 대한 분석에서, rs112735431 유전자 다형성을 갖는 환자는 혈관 협착의 수(aOR=3.09, 95% CI: 1.23-7.76, p=0.0161)와 정도(aOR=3.77, 95% CI: 1.48-9.58, p=0.0053)가 모두 증가하는 것을 확인하였다.

본 연구에서 rs112735431은 한국인의 두개강내 동맥경화증과 연관성이 있었다. rs112735431 유전자 다형성은 두개강내 동맥 경화증 중에서도 전방 뇌혈관 또는 전방과 후방 뇌혈관 모두를 침범하는 경우와 관련이 있었고, 혈관 협착의 수와 정도를 높여 혈관 협착 부담을 증가시켰다. rs2179357과 rs1800470은 각각 두개강내 동맥경화증, 그리고 두개강외 동맥경화증과 연관성을 보였으나, 이

유전자 다형성의 영향을 확인하기 위해서는 더 큰 규모의 후속 연구가 필요하다.

핵심어: RNF213, 두개강내 동맥경화증, 뇌경색, 유전자 다형성.