ORIGINAL RESEARCH

Ring Finger Protein 213 Variant and Plaque Characteristics, Vascular Remodeling, and Hemodynamics in Patients With Intracranial Atherosclerotic Stroke: A High-Resolution Magnetic Resonance Imaging and Hemodynamic Study

Eun-Hyeok Choi, MD;* Hanul Lee, MD;* Jong-Won Chung, MD; Woo-Keun Seo, MD, PhD; Gyeong-Moon Kim, MD, PhD; Chang-Seok Ki, MD, PhD; Yoon-Chul Kim, PhD; Oh Young Bang, MD, PhD

***Background*—**Intracranial atherosclerotic stroke is prevalent in Asians. We hypothesized that patients with the ring finger protein 213 (*RNF213*) variant, a susceptibility locus for moyamoya disease in Asians, have different neuroimaging characteristics in terms of the vessel wall and hemodynamics.

***Methods and Results*—**We analyzed consecutive patients with ischemic events in middle cerebral artery distribution and relevant plaques of the distal internal carotid artery or proximal middle cerebral artery on high-resolution magnetic resonance imaging. Patients with carotid/cardiac sources of embolism or moyamoya disease were excluded. High-resolution magnetic resonance imaging features (eg, outer vessel diameters and plaque characteristics) and fractional flow (as measured by adjusted signal intensity ratio on time-of-flight magnetic resonance angiography) were compared between *RNF213* p.Arg4810Lys variant carriers and noncarriers. Among 144 patients included, 44 (29.9%) had the *RNF213* variant. Clinical characteristics, including age, sex, body mass index, and vascular risk factors, were not significantly different between *RNF213* variant carriers and noncarriers. However, the outer vessel diameter was smaller in *RNF213* variant carriers than in noncarriers ($P<0.0001$ for middle cerebral artery of relevant stenosis [2.05-mm analysis of RNF213 gene for moyamoya disease in the Chinese HAN population 2.75 mm]; $P<0.0001$ for contralateral side [2.42 versus 3.00 mm] and $P<0.001$ for basilar artery [3.19 versus 3.53 mm]). Other high-resolution magnetic resonance imaging features, including plaque morphology and eccentricity, were not significantly different. Fractional flow was diminished in patients with smaller-diameter intracranial arteries with a similar degree of stenosis.

***Conclusions*—**The *RNF213* variant may be associated with vasculogenesis, but not with atherogenesis. Patients with this variant had small intracranial arteries predisposing hemodynamic compromise in the presence of intracranial atherosclerosis. In addition to antiatherosclerotic strategies, further studies are warranted to develop novel therapeutic strategies against *RNF213* vasculopathy in Asians. **(*J Am Heart Assoc*. 2019;8:e011996. DOI: 10.1161/JAHA.119.011996.)**

Key Words: atherosclerosis • genetic association • intracranial stenosis • ring finger protein • stroke

Intracranial atherosclerotic stroke (ICAS) is more prevalent in East Asians than in Westerners. However, the reason for the racial-ethnic differences is unknown. Possible explanations include inherited susceptibility to intracranial vessel atherosclerosis,[1] acquired differences in risk-factor prevalence,[2,3] and differential responses to the same risk factors.[4–6] No genetic factors specific to ICAS have been reported. Recent genome-wide association studies have shown stroke-subtype–sensitive genetic factors.[7] However, they merged intra- and extracranial atherosclerosis for these studies.

Moyamoya disease is an idiopathic intracranial arterial disease characterized by progressive stenosis of the distal

From the Department of Neurology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea (E.-H.C., H.L., J.-W.C., W.-K.S., G.-M.K., O.Y.B.); Translational and Stem Cell Research Laboratory on Stroke (J.-W.C., O.Y.B.), and Clinical Research Institute (Y.-C.K.), Samsung Medical Center, Seoul, Korea; Department of Laboratory Medicine and Genetics, Green Cross Genome, Youngin, Korea (C.-S.K.).

*Dr Choi and Dr Lee contributed equally to this work.

Correspondence to: Oh Young Bang, MD, PhD, Department of Neurology, Samsung Medical Center, Sungkyunkwan University, 50 Irwon-dong, Gangnam-gu, Seoul 135-710, South Korea. E-mail: ohyoung.bang@samsung.com

Received January 10, 2019; accepted August 5, 2019.

Clinical Perspective

What Is New?

- In this prospective study, plaque characteristics did not differ depending on the presence of the ring finger protein 213 (RNF213) variant, but patients with this variant had smaller intracranial arteries. Smaller intracranial arteries may impact brain blood flow, and this effect may be magnified in the presence of intracranial atherosclerosis.

What Are the Clinical Implications?

- Our findings suggest that intracranial atherosclerosis and moyamoya disease share a common genetic variant, and among these variants, *RNF213* variant may have a role in arterial caliber and brain flow hemodynamics in Asians.

internal carotid artery (ICA) and a hazy network of basal collaterals called moyamoya vessels. The main pathological changes of the stenotic segment in moyamoya disease are the fibrocelluar thickening of the intima, irregular undulation of the internal elastic laminae, medial thinness, and a decrease in the outer vessel diameter, whereas focal thickness of the intima attributed to atheroma and subintimal hemorrhage is the main feature of ICAS. The ring finger protein 213 (*RNF213*), p.Arg4810Lys, is exclusively found in East Asian countries where the prevalence of this variant has been reported to be up to 2.5%.[8,9] Although a genome-wide linkage analysis and exome analysis recently identified the *RNF213* gene on 17q25.3 as the strongest susceptibility gene for moyamoya disease in East Asian populations,[10,11] this genetic variant associated with moyamoya disease was also observed in patients with nonmoyamoya intracranial stenosis.[12,13,14]

We hypothesized that this genetic variant of the *RNF213* gene influences the intracranial vessels and is related to development of ICAS in Asians. Thus, we compared the plaque characteristics and vascular remodeling pattern on high-resolution magnetic resonance imaging (HR-MRI) and hemodynamic changes related to intracranial plaques depending on the presence or absence of this variant.

Patients and Methods

Data Availability

Anonymized data will be shared by the corresponding author upon reasonable request from any qualified investigator.

Study Population

From January 2012 to September 2017, patients with ischemic cerebrovascular events in the middle cerebral artery (MCA) distribution who were admitted to a university medical center were prospectively recruited. Potential participants were defined as patients experiencing focal or lateralizing symptoms within the MCA distribution within 7 days of admission and showing ≥30% stenosis or occlusion at terminal portions of the ICA and/or proximal MCA on conventional or magnetic resonance angiography. All patients underwent HR-MRI, and only those who had relevant plaques on HR-MRI were included in this study. Based on the SSS-TOAST (Stop Stroke Study Trial of Org 10 172 in Acute Stroke Treatment), patients with potential sources of cardioaortic embolism, extracranial atherosclerosis with significant (≥50%) stenosis of the relevant extracranial arteries, other stroke mechanisms (coagulopathy, vasculitis, arterial dissection, etc), or incomplete evaluations were excluded. Patients who showed typical luminal or HR-MRI features of intracranial arterial dissection (ie, the presence of intimal flap or double lumen, intravascular hematoma, or aneurysmal formation) were excluded.[15,16] Additionally, conventional angiography was performed in cases where moyamoya disease was suspected. Patients who showed typical features of moyamoya disease in terms of the luminal features of conventional/magnetic resonance angiography (ie, presence of basal collaterals) or typical vessel wall changes on HR-MRI (ie, circular enhancement without plaque) were excluded.[17,18] Details of patient selection are provided in Figure 1. The local institutional review board approved this study (approval number, 2016-08-064). All patients or patient guardians provided written informed consent for participation in this study.

Workups and Genetic Study

Clinical information, including age, sex, height, body mass index, and vascular risk factors, was collected. All patients underwent standardized diagnostic tests that included routine blood tests (total cholesterol, triglyceride, high- and low-density lipoprotein-cholesterol, C-reactive protein, etc) and cardiac workups (electrocardiography, ≥24-hour cardiac telemetry, or echocardiography). Hemostatic markers of prothrombotic tendency, including antiphospholipid antibodies, were measured in patients who were aged <50 years.

Genomic DNA was extracted from peripheral blood leukocytes using a Wizard Genomic DNA Purification kit and following the manufacturer's instructions (Promega, Madison, WI). The c.14429G>A (p.Arg4810Lys) variant of the *RNF213* gene (GenBank accession number, NM_001256071.1) was amplified using primer sets designed by the authors (available upon request). A polymerase chain reaction was performed with a thermal cycler (model 9700; Applied Biosystems, Foster City, CA), and direct sequencing was performed with a BigDye Terminator Cycle Sequencing Ready Reaction kit

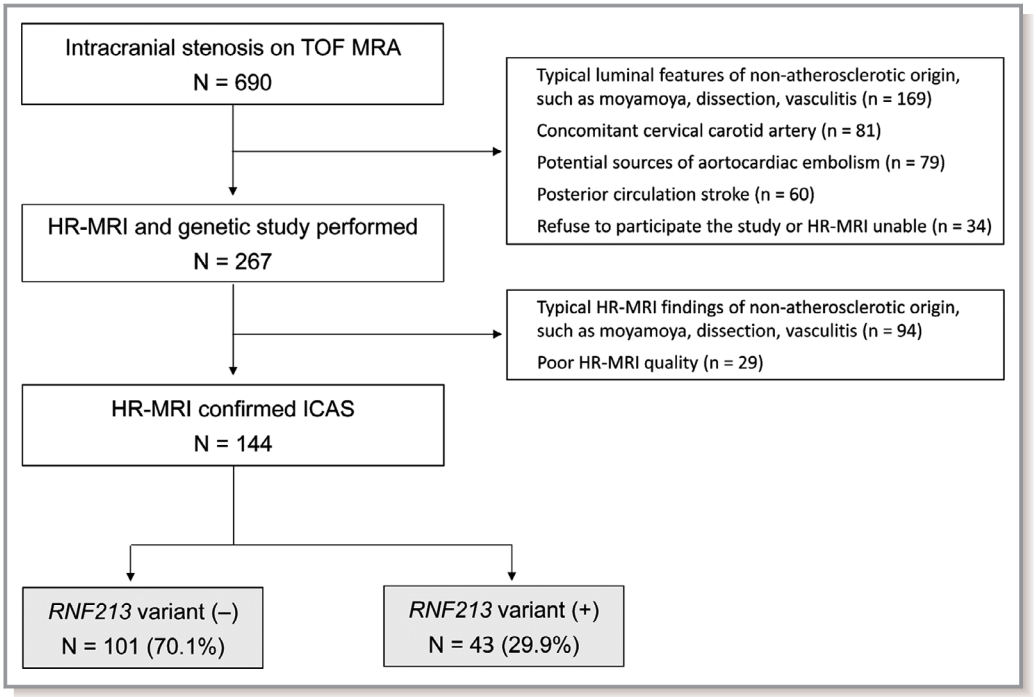

Figure 1. Patient selection. HR-MRI indicates high-resolution magnetic resonance image; ICAS, intracranial atherosclerotic stroke; MRA, magnetic resonance angiography; *RNF*, ring finger protein; TOF, time of flight.

(Applied Biosystems) on an ABI Prism 3730*xl* genetic analyzer (Applied Biosystems).

HR-MRI and Hemodynamic Study

HR-MR images were analyzed to evaluate the vessel walls. Details of the HR-MRI parameters are described elsewhere.[19,20] HR-MRI was performed using a 3T system (Achieva; Phillips Medical Systems, Best, The Netherlands) with a standard 8-channel head coil. The neuroradiologist (J.C.) selected the vessel and site of evaluation based on clinical presentation and 3D time-of-flight magnetic resonance angiography findings and chose the combination of acquisition orientations (axial only or axial and sagittal). Black-blood HR-MRI, using the spatial presaturation technique, was performed as follows: (1) axial and sagittal proton density (repetition time/echo time=2150/12.5 ms, echo train length=10, slice thickness=2 mm, flip angle=90 degrees, matrix=280×280, field of view=14 cm, and number of excitations=2); (2) axial and sagittal T_2-weighted images (repetition time/echo time=2150/100 ms, echo train length=10, slice thickness=2 mm, flip angle=90 degrees, matrix=280×280, field of view=14 cm, and number of excitations=2); (3) sagittal T_1 fluid-attenuated inversion recovery, precontrast and postcontrast (repetition time/echo time=2100/10 ms, echo train length=6, slice thickness=2 mm, flip angle=90 degrees, matrix=280×280, field of view=14 cm, and number of excitations=2); and (4) axial postcontrast 3D T_1-weighted volumetric isotropic turbo spin echo acquisition (repetition time/echo time=350/20 ms, turbo spin echo factor=25, 0.5 mm isotropic voxel, flip angle=90 degrees, matrix=360×360, field-of-view=18 cm, and number of excitations=2).

Outer vessel diameter was measured manually on T_2 proton-density–weighted images. Outer vessel diameter of the most stenosed site (or maximal plaque site in case of total occlusion) was measured. In addition, normal vessels located contralateral to the mid portion of the MCA (or proximal to the stenotic portion in case of a stenotic contralateral side) and basilar artery (BA) were also assessed at the mid portion of the BA as reference values. Saggital images were used to measure MCA diameter and axial images for BA diameter. In this study, the wall area, the difference between areas of the vessel and lumen, was not evaluated because it could not be measured in cases with a small outer vessel diameter. Precontrast and postcontrast T_1 fluid-attenuated inversion recovery images were compared to determine the presence of enhancement and enhancement pattern (concentric versus eccentric), using a Medical Image Processing Analysis and Visualization program. Presence of enhancement was defined as a >20% increase in normalized signal intensity of the plaque after contrast agent injection. Normalized signal intensity was calculated as the signal intensity of the BA. Enhancement was considered concentric if it was uniform or circumferential. Enhancement was regarded as eccentric if it was not 360 degrees circumferential. When multiple plaques were detected in a symptomatic intracranial artery, the plaque

causing the highest degree of stenosis was considered symptomatic and selected for analysis. Two neurologists (H.L. and E.-H.C.) interpreted the HR-MRI images. A third reader (J.H.) was invited to resolve any disagreements between the 2 interpretations. All quantitative data were remeasured 2 weeks later by a neurologist (E.-H.C.) to estimate intraobserver variability. Intraclass correlation coefficients for the measured HR-MRI parameters were examined for both inter- and intraobserver agreement, and the final database was locked after achieving intraclass correlation coefficients >0.80.

The adjusted signal intensity ratio on time-of-flight magnetic resonance angiography was measured as a surrogate marker of fractional flow, as previously reported by the WASID/SONIA (Warfarin Aspirin Symptomatic Intracranial Disease/Stroke Outcomes and Neuroimaging of Intracranial Atherosclerosis) collaboration investigators.[21,22] In brief, the region of interest distal and proximal to the stenosis was visualized on a maximum intensity project view. The mean signal intensity (SI) for the region of interest was measured. The fractional flow ratio (FFR), the adjusted signal intensity ratio, was calculated as (distal SI−background SI)/(proximal SI−background SI). Data analysis was performed semiautomatically with software developed in-house using MATLAB code (The MathWorks, Inc, Natick, MA; Y.C.K.).

Statistical Analysis

Differences in discrete variables among the groups were examined by χ^2, Fisher's exact, and Mann-Whitney U tests. Differences in continuous variables were examined using 1-way ANOVA, Kruskal-Wallis tests, and *t* tests, as appropriate. Correlations of FFR with degree of stenosis and outer vessel diameter were analyzed using Spearman's correlation analysis. In addition, independent factors for outer vessel diameter (quartiles for the stenotic segment of the MCA and distal ICA) and FFR related to the *RNF213* variant were evaluated using multivariate linear regression analysis. Commercially available software (SPSS, Version 18.0; SPSS, Inc, Chicago, IL) was used for the statistical analyses. $P<0.05$ was considered statistically significant. For continuous variables, we used Youden's index to consider optimal cut-off values that maximize likelihood. This was done using R software (R Core Team, 2017).

Results

Among the 267 participants with intracranial stenosis on time-of-flight MRI who underwent both HR-MRI and genetic study, 94 patients who showed typical HR-MRI findings of nonatherosclerotic origin, such as moyamoya (n=36), dissection (n=43), reversible vasoconstriction syndrome (n=4), vasculitis (n=4), or others (n=7), and 29 patients with poor image quality were excluded from this study. Finally, 144 patients with intracranial plaques on HR-MRI and absence of basal collaterals (moyamoya vessels) were included; 58 (40.3%) were female and the average age was 55.5±13.5 years (range, 25–89). Relevant stenosis of the MCA was observed in 62 (40.5%) patients, and the remaining patients had distal ICA or combined stenosis.

Based on the results of the genetic study, 101 (70.1%) patients with ICAS confirmed by HR-MRI were classified as *RNF213* variant noncarriers and 44 (29.9%) as *RNF213* variant carriers (heterozygote in all cases). Details of the baseline characteristics of patients and conventional neuroimaging findings of *RNF213* variant noncarriers and carriers are summarized in Table 1. There was no significant difference in the characteristics of patients, including age, sex, height, and body mass index, and vascular risk factors, infarct pattern, and site/degree of steno-occlusive segments, between carriers and noncarriers.

Table 2 shows the HR-MRI features. The outer vessel diameter of the stenotic segment was significantly smaller in carriers than in noncarriers ($P<0.001$ in both MCA and distal ICA). Interestingly, outer vessel diameters of nondiseased segments (plaque negative on HR-MRI), that is, contralateral side and BA, were smaller in carriers than in noncarriers ($P<0.01$ in all cases). The cut-off value of the outer vessel diameter of the stenotic segment between the groups was 2.55 mm for the MCA and 2.80 mm for the distal ICA. Cut-off values were 2.87 mm for asymptomatic MCA, 2.72 mm for asymptomatic distal ICA, and 3.68 mm for asymptomatic BA. The multivariate test showed that the association between outer vessel diameter and the genetic variant remained significant after adjusting for age, sex, height, and body mass index (Table 3). For example, patients with outer diameter <2.55 mm were almost 9 times more likely to have *RNF213* variant (odds ratio, 8.991; 95% CI, 3.486–23.189). On the contrary, the characteristics of plaques, in terms of the presence of plaque enhancement and eccentricity, were not significantly different between the groups. A typical example of *RNF213* variant carriers is shown in Figure 2.

Fractional flow was measured at the stenotic segments of relevant arteries (n=79 for MCA and n=50 for distal ICA). FFR was significantly correlated not only with degree of stenosis (Spearman's correlation, $r=0.563$; $P<0.001$), but also with outer vessel diameter (Spearman's correlation, $r=0.370$; $P<0.001$). As shown in Figure 3, FFR markedly decreased with the decrease in outer vessel diameter among patients with similar degrees of stenosis, especially in those with a severe degree of stenosis (Figure 3B).

A multiple regression analysis was performed to further evaluate the independent factors associated with a smaller diameter of stenotic segments and reduced fractional flow

Table 1. Patient Characteristics of the *RNF213* Variant Carriers and Noncarriers

	RNF213 Variant Noncarrier (n=101)	*RNF213* Variant Carrier (n=43)	*P* Value
Sex, female	43 (42.57%)	15 (34.88%)	0.3892
Age, y	54.06±12.1	51.6±10.62	0.2504
Height, cm	162.89±8.96	165.63±9.87	0.1054
Body mass index	24.84±3.14	25.98±3.61	0.1926
Vascular risk factors			
Hypertension	53 (52.48%)	17 (39.53%)	0.1551
Diabetes mellitus	28 (27.72%)	11 (25.58%)	0.7913
History of dyslipidemia	62 (61.39%)	26 (60.47%)	0.9174
Family history of moyamoya	6 (5.94%)	5 (11.63%)	0.3045
NIHSS	0.75±2.188	1.51±4.108	0.6630
Infarct patterns on DWI			
Branch occlusive disease	21 (20.79%)	3 (6.81%)	0.0701
Nonbranch occlusive disease	48 (47.52%)	22 (50.0%)	
Transient ischemic attacks	32 (31.68%)	19 (43.18%)	
TOF-MRA findings			
Site of index stenosis*			
Distal ICA	45 (38.79%)	16 (32.65%)	0.3404
MCA	70 (60.34%)	32 (65.31%)	
Degree of stenosis of index site			
Occlusion	33 (32.67%)	18 (41.86%)	0.4157
>70%	40 (39.6%)	15 (34.88%)	
>50%	9 (8.91%)	1 (2.33%)	
<50%	19 (18.81%)	9 (20.93%)	

ACA indicates anterior cerebral artery; DWI, diffusion-weighted image; HR-MRI, high-resolution magnetic resonance image; ICA, internal carotid artery; ICAS, intracranial atherosclerotic stroke; MCA, middle cerebral artery; NIHSS, National Institutes of Health Stroke Scale; *RNF*, ring finger protein; TOF, time of flight.
*Some patients had tandem enhancing plaques in both the distal ICA and MCA.

across the stenotic segment. The *RNF213* variant was independently associated with a smaller diameter of the stenotic segment (β=−0.788; $P<0.001$), after adjustment for clinical characteristics (age, sex, and vascular risk factors), degree of stenosis, and plaque characteristics (presence of enhancement and eccentricity). In addition, outer vessel diameter of stenotic segments was independently associated with the FFR (β=0.145; $P<0.001$). Other factors, including clinical characteristics (age, sex, and vascular risk factors), *RNF213* variant, degree of stenosis, and plaque characteristics (presence of enhancement and eccentricity), did not significantly contribute to the FFR ($P>0.05$ for all).

Discussion

The major findings of this study are as follows. First, the *RNF213* variant was observed in around 30% of HR-MRI–confirmed cases of ICAS. This frequency was much higher than that observed in stroke patients without intracranial stenosis (3.6% of 137 patients) and healthy control patients (1.05% of 1100 subjects).[23,24] Second, outer vessel diameters of the intracranial arteries were smaller in *RNF213* carriers than in noncarrier in both symptomatic and asymptomatic sites, including the posterior circulation. Such small intracranial arteries could be vulnerable to hemodynamic compromise, resulting in stroke in the presence of intracranial atherosclerosis. Third, no significant difference in plaque characteristics was observed regardless of the presence or absence of this variant.

This study shows that the *RNF213* variant is common in patients with HR-MRI–confirmed intracranial atherosclerotic stroke and that the outer vessel diameter of intracranial arteries was smaller in carriers than in noncarriers. Very recently, Hongo et al measured the outer dimeter of the MCA in a small cohort (the *RNF213* variant 19 carriers and 28 noncarriers) and showed that the outer diameter of the MCA was smaller in the

Table 2. HR-MRI Features of *RNF213* Carriers and Noncarriers

	RNF213 Variant Non-Carrier	*RNF213* Variant Carrier	*P* Value
Outer vessel diameter, mm			
MCA (disease site)	2.78±0.78	2.05±0.63	<0.001
MCA (intact site)	3.00±0.58	2.42±0.51	<0.001
Distal ICA (disease site)	3.25±0.57	2.56±0.53	<0.001
Distal ICA (intact site)	3.22±0.52	2.76±0.46	<0.001
Basilar artery	3.53±0.59	3.19±0.42	0.0010
Plaque enhancement pattern			
No enhancement	19 (18.81%)	9 (20.93%)	0.7699
Enhancement	82 (81.19%)	34 (79.07%)	0.2232
Eccentric	48 (58.54%)	10 (29.41%)	
Concentric	34 (41.46%)	24 (70.59%)	

ICA indicates internal carotid artery; HR-MRI, high-resolution magnetic resonance image; MCA, middle cerebral artery; *RNF*, ring finger protein.

RNF213 variant carriers.[25] Our present study also showed that negative remodeling involves all the intracranial arteries measured. Not only the stenotic MCA segments, but also contralateral MCA, distal ICA, and BA showed negative remodeling in patients with this variant. Such findings raise the possibility that the *RNF213* variant may play a role in posterior circulation stroke. The results of the present study are in line with recent studies of systemic vascular disease.[26,27] *RNF213* p.Arg4810Lys variant causes a classical moyamoya disease when present in a heterozygous state, but the exact same variant results in moyamoya disease and systemic vascular diseases when present in a homozygous state in a gene-dosage–dependent manner.[26] In addition, *RNF213* homozygous patients show a very unique pattern of diffuse narrowing of the aorta and iliofemoral arteries, together with stenosis of the renal, celiac, or peripheral pulmonary arteries.[27] These findings, together with the present data (all were heterozygous patients), suggest that *RNF213* p.Arg4810Lys leads to vasculopathy characterized by negative remodeling involving not only major intracranial vessels, but also systemic vessels and intracranial vessels in general.

Such small diameter and thinness of intracranial vessels could be caused by several pathogenic processes related to the *RNF213* variant. First, impaired circulating vascular progenitor cells (endothelial and smooth muscle progenitor cells) have been reported in patients with moyamoya disease.[28,29] Defects in circulating cells that originate from the bone marrow and help maintain the vasculature may cause impaired angiogenesis and consequent intimal thickening and medial thinness. Second, *RNF213* encodes a relatively large protein with dual AAA^+ ATPase and E3 ligase activities.[8] In vitro and in vivo experiments suggest that *RNF213* is related to angiogenesis and vascular inflammation, although the complete physiologic functions of *RNF213* remain unknown.[8] Hitomi et al established a model of induced pluripotent stem cells derived from vascular endothelial cells, and showed that the angiogenic activity from patients with moyamoya disease and *RNF213* carriers was lower than that of control subjects. The overexpression of the *RNF213* variant downregulated Securin and inhibited angiogenic activity.[30] Third, caveolin-1, a scaffolding protein of the caveolae plasma membrane, is involved in the pathogenesis of cancers and vascular diseases.[31] In our recent data, caveolin-1 level was markedly decreased in patients with moyamoya disease, especially in patients with the *RNF213* variant, and to a lesser degree in those with ICAS.[32] Caveolin-1 overexpression enhanced caveolae generation and accelerated capillary tube formation by nearly 3-fold, whereas caveolin-1 downregulation reduced in vitro and in vivo capillary formation and increased cell death of smooth muscle cells, and was associated with pathological angiogenesis.[31,33,34,35]

The WASID (Warfarin-Aspirin Symptomatic Intracranial Disease) trial investigators showed that degree of stenosis (presence of severe stenosis of ≥70%) was associated with subsequent ischemic stroke in the territory of the stenotic artery.[36] They also showed that besides degree of stenosis,

Table 3. Multivariate Testing for the Association Between the Outer Vessel Diameters and the *RNF213* Variant

		No. of Patients Who Did Meet the Cut-Off Value		Univariate		Multivariate*	
Segment	Cut-Off Values	*RNF213* Variant Noncarrier	*RNF213* Variant Carrier	OR [95% CI]	*P* Value	OR [95% CI]	*P* Value
MCA (disease site)	<2.55 mm	39 (38.61%)	37 (86.05%)	9.132 [3.593, 23.208]	<0.001	8.991 [3.486, 23.189]	<0.001
MCA (intact site)	<2.87 mm	36 (35.64%)	38 (88.37%)	12.565 [4.663, 33.857]	<0.001	12.763 [4.643, 35.088]	<0.001
Distal ICA (disease site)	<2.55 mm	23 (22.77%)	35 (81.40%)	13.952 [5.744, 33.89]	<0.001	12.679 [5.136, 31.301]	<0.001
Distal ICA (intact site)	<2.72 mm	15 (14.85%)	26 (60.47%)	8.451 [3.732, 19.137]	<0.001	8.061 [3.505, 18.54]	<0.001
Basilar artery	<3.68 mm	62 (63.92%)	39 (92.86%)	6.409 [1.968, 20.875]	0.002	7.094 [2.092, 24.053]	0.001

ICA indicates internal carotid artery; MCA, middle cerebral artery; *RNF213*, ring finger protein 213.
*Adjusting for age, sex, height, and body mass index.

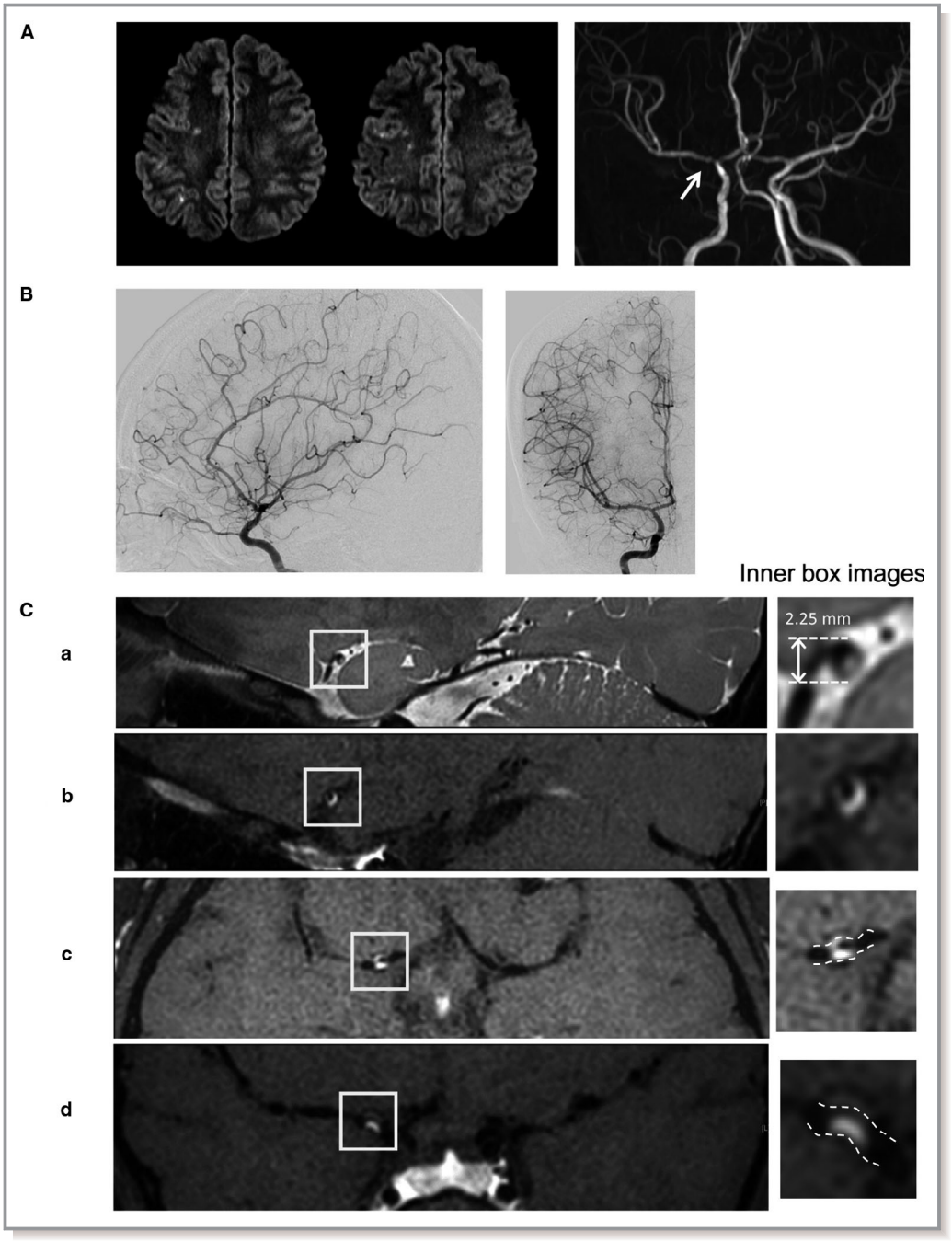

Figure 2. A typical example of intracranial atherosclerosis and the *RNF213* variant. A 35-year-old woman presented with recurrent transient left arm weakness. **A**, Diffusion-weighted images show multiple small cortical infarcts in the right middle cerebral artery (MCA) territory. Magnetic resonance angiography showed focal stenosis of the right proximal MCA (arrow). **B**, Conventional angiographic findings of mild right proximal MCA and distal internal carotid artery (ICA) stenosis and no basal collaterals (moyamoya vessels). **C**, High-resolution magnetic resonance imaging showed an eccentric plaque and thin outer vessel diameter (2.25 mm) on T_2 proton-density–weighted images (a), and an eccentric enhancing plaque in the thin MCA on sagittal (b), axial (c), and coronal views (d). Distal ICAs were normal, except small outer vessel diameters.

hemodynamic factors, such as those measured by the FFR, may be associated with subsequent stroke; diminished FFR was associated with recurrent stroke in stenosis <70%.[21] In the present study, hemodynamic impairment, as measured by diminished FFR, was severe in patients with smaller diameter intracranial arteries, even though they had similar degrees of stenosis. Such a finding suggests that a smaller diameter could be a predisposing factor in terms of hemodynamics in

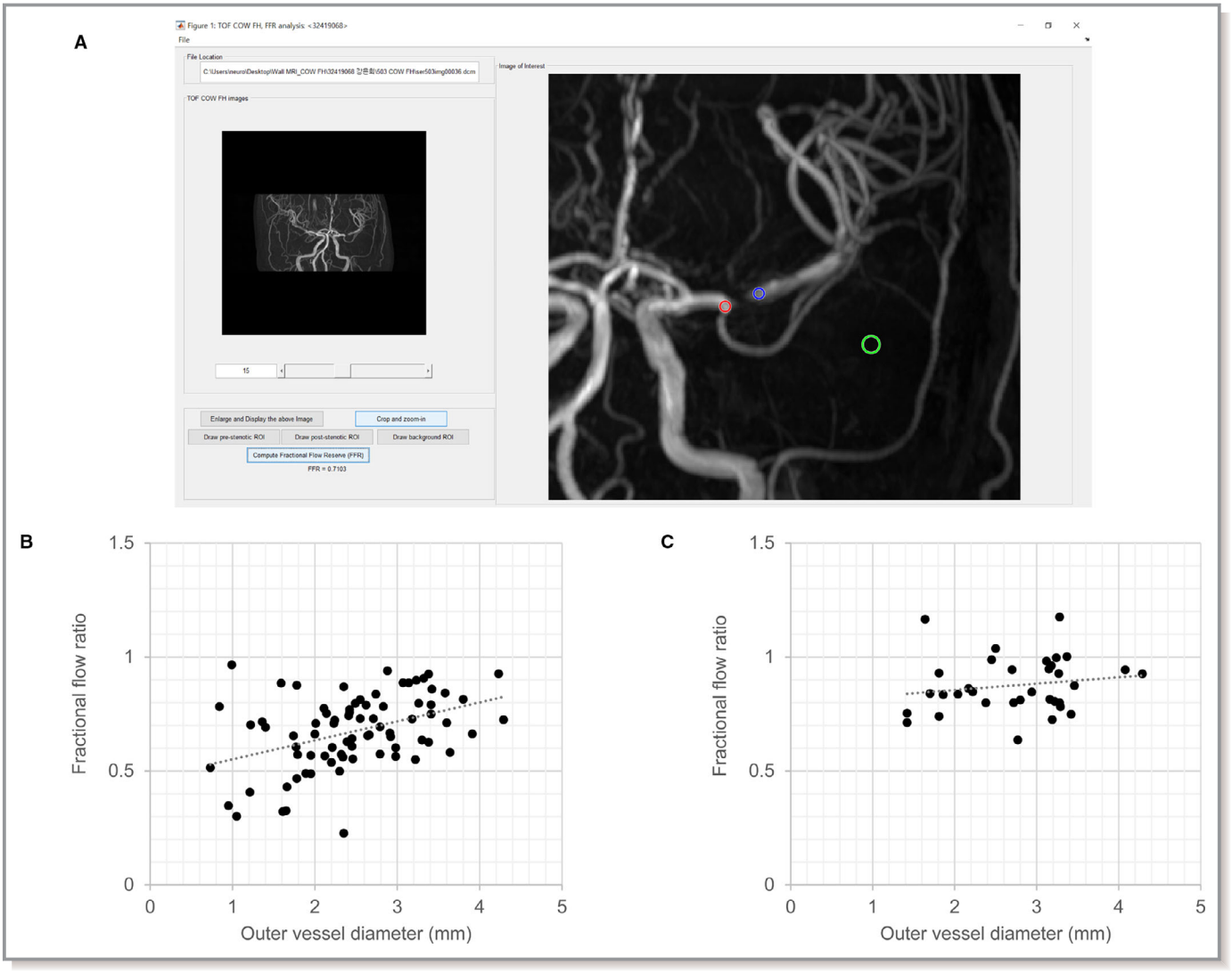

Figure 3. Fractional flow ratio. **A**, In-house user interface for semiautomatic measurement of the fractional flow ratio. Correlation between the outer vessel diameter and fractional flow ratio in patients with (**B**) a severe degree of stenosis (>70%) and (**C**) a milder degree of stenosis (30–69%). Red circle is an area of interest which measures prestenotic time-of-flight signal intensity; blue circle is an area of interest which measures poststenotic time-of-flight signal intensity; and green circle is an area of interest which measures the background signal intensity. COW indicates Circle Of Willis; FFR, fractional flow ratio; FH, Foot to head; ROI region of interest; TOF, time of flight.

the presence of intracranial plaque. Besides, the smaller diameter of intracranial arteries may affect the results of treatment of ICAS. The outer diameter of the MCA of patients with this variant was relatively small in our present (2.05±0.63 mm) and previous (2.09±0.32 mm) cohorts.[25] The results of the SAMMPRIS (Stenting and Aggressive Medical Management for Preventing Recurrent Stroke in Intracranial Arterial Stenosis) trial showed that treating very small vessels (<2.5–2.75 mm in diameter) was associated with higher complication rates, because small vessels are more likely to have restenosis or acute thrombosis and they may also be more prone to injury with stenting.[37]

Several possible mechanisms of enhanced atherogenesis related to the *RNF213* variant exist. First, small diameters associated with the *RNF213* variant may be associated with intracranial atherosclerosis. Obi et al studied diagnostic coronary arteriography in 884 patients and showed that a coronary artery diameter was a significant independent predictor of atherosclerotic lesions, suggesting that small coronary artery size is a risk factor for atherosclerosis.[38] They suggested that the effect of coronary size on hemodynamics was the underlying reason.[38] Volume flow rate, flow velocity, and shear stress, variables that suppress atherogenesis, all increase with vessel radius. Second, absence of compensatory/positive remodeling and presence of negative remodeling may result in a more-severe stenosis and hemodynamic disturbance in the presence of the same plaque burden and accelerated atherogenesis. Third, the

RNF213 variant could lead to vascular fragility, which may render vessels more vulnerable to hemodynamic stress and secondary insults.[39,40] Last, the *RNF213* gene is reportedly associated with vascular risk factors, such as blood pressure.[41] However, our study showed that atherogenic features, such as the plaque characteristics on HR-MRI and vascular risk factors, were not different between carriers and noncarriers.

This study had several limitations. First, the study was cross-sectional and had a limited sample size. A long-term follow-up HR-MRI study of a large cohort is needed. However, this is one of the largest series of ICAS cases examined using HR-MRI. Second, most patients with *RNF213* variant showed a diameter smaller than the cut-off value. However, CIs were relatively wide, which may be attributed to the relatively high counts for the number of patients without *RNF213* variant who had a smaller diameter lower than the cut-off value. As a result, the study suffered from lack of desired precision, combined with the small sample study, resulting in wider CIs. Third, the results of this study cannot be generalized outside of East Asians because the *RNF213* p.Arg4810Lys variant has not been reported in Westerners or South Asians. Several non-p.Arg4810Lys *RNF213* variants (rs148731719 and rs397514563) were recently found in whites and East and South Asian cases with moyamoya disease,[9,11,42,43] which need further studies in non-Asian populations. Last, genetic variants other than *RNF213* were not tested in the present study. Very recently, a multiancestry genome-wide association study identified several loci associated with stroke subtypes.[44] In this study, the *RNF213* p.Arg4810Lys was not the identified locus for large artery atherosclerotic stroke. However, in this study, a relatively small number of East Asians were included, and they merged extra- and intracranial atherosclerotic stroke as large artery atherosclerotic stroke.

In conclusion, our data showed that plaque characteristics did not differ depending on presence of the *RNF213* variant, but patients with this variant had smaller intracranial arteries predisposing hemodynamic compromise in the presence of intracranial atherosclerosis. These findings suggest that the *RNF213* p.Arg4810Lys variant is associated with vasculogenesis, but not with atherogenesis. In addition, ICAS and moyamoya disease share a common genetic variant. The results of the present study raise the possibility that this variant could contribute to the high prevalence of ICAS in Asians. The size of the population carrying the *RNF213* variant was estimated to be 16.16 million people in East Asian countries.[45] Considering the high prevalence of the *RNF213* variant in Asians, especially in patients with ICAS, further studies are warranted to develop novel therapeutic strategies against *RNF213* vasculopathy as well as antiatherosclerotic strategies in Asian patients.

Acknowledgments

We thank Keumhee Cho and Minji Kim of the Statistics and Data Center, Samsung Medical Clinic, who assisted in the Statistical analysis.

Author Contributions

Eun-Hyeok Choi: study concept and design, analysis and interpretation of data, drafting/revising the manuscript for content, and statistical analysis. Hanul Lee: study concept and design, analysis and interpretation of data, and drafting/revising the manuscript for content. Chung, Seo, Kim, Ki, and Kim: study concept and design, acquisition of data, and analysis and interpretation of data. Bang: study concept and design, acquisition of data, analysis and interpretation of data, and drafting/revising the manuscript for content.

Sources of Funding

This study was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (No. 2018R1A2B2003489).

Disclosures

None.

References

1. Mak W, Cheng TS, Chan KH, Cheung RT, Ho SL. A possible explanation for the racial difference in distribution of large-arterial cerebrovascular disease: ancestral European settlers evolved genetic resistance to atherosclerosis, but confined to the intracranial arteries. *Med Hypotheses*. 2005;65:637–648.
2. Sacco RL, Kargman DE, Gu Q, Zamanillo MC. Race-ethnicity and determinants of intracranial atherosclerotic cerebral infarction. The Northern Manhattan Stroke Study. *Stroke*. 1995;26:14–20.
3. Forouhi NG, Sattar N. CVD risk factors and ethnicity—a homogeneous relationship? *Atheroscler Suppl*. 2006;7:11–19.
4. Boden-Albala B, Sacco RL, Lee HS, Grahame-Clarke C, Rundek T, Elkind MV, Wright C, Giardina EG, DiTullio MR, Homma S, Paik MC. Metabolic syndrome and ischemic stroke risk: Northern Manhattan study. *Stroke*. 2008;39:30–35.
5. Bang OY, Saver JL, Liebeskind DS, Pineda S, Yun SW, Ovbiagele B. Impact of metabolic syndrome on distribution of cervicocephalic atherosclerosis: data from a diverse race-ethnic group. *J Neurol Sci*. 2009;284:40–45.
6. Bang OY. Intracranial atherosclerosis: current understanding and perspectives. *J Stroke*. 2014;16:27–35.
7. Traylor M, Farrall M, Holliday EG, Sudlow C, Hopewell JC, Cheng YC, Fornage M, Ikram MA, Malik R, Bevan S, Thorsteinsdottir U, Nalls MA, Longstreth W, Wiggins KL, Yadav S, Parati EA, Destefano AL, Worrall BB, Kittner SJ, Khan MS, Reiner AP, Helgadottir A, Achterberg S, Fernandez-Cadenas I, Abboud S, Schmidt R, Walters M, Chen WM, Ringelstein EB, O'Donnell M, Ho WK, Pera J, Lemmens R, Norrving B, Higgins P, Benn M, Sale M, Kuhlenbaumer G, Doney AS, Vicente AM, Delavaran H, Algra A, Davies G, Oliveira SA, Palmer CN, Deary I, Schmidt H, Pandolfo M, Montaner J, Carty C, de Bakker PI, Kostulas K, Ferro JM, van Zuydam NR, Valdimarsson E, Nordestgaard BG, Lindgren A, Thijs V, Slowik A, Saleheen D, Pare G, Berger K, Thorleifsson G, Hofman A, Mosley TH, Mitchell BD, Furie K, Clarke R, Levi C, Seshadri S, Gschwendtner A, Boncoraglio GB, Sharma P, Bis JC, Gretarsdottir S, Psaty BM, Rothwell PM, Rosand J, Meschia JF, Stefansson K, Dichgans M, Markus HS. Genetic risk factors for ischaemic stroke and its subtypes (the metastroke collaboration): a meta-analysis of genome-wide association studies. *Lancet Neurol*. 2012;11:951–962.

8. Koizumi A, Kobayashi H, Hitomi T, Harada KH, Habu T, Youssefian S. A new horizon of moyamoya disease and associated health risks explored through RNF213. *Environ Health Prev Med*. 2016;21:55–70.
9. Ma J, Liu Y, Ma L, Huang S, Li H, You C. Rnf213 polymorphism and moyamoya disease: a systematic review and meta-analysis. *Neurol India*. 2013;61:35–39.
10. Kamada F, Aoki Y, Narisawa A, Abe Y, Komatsuzaki S, Kikuchi A, Kanno J, Niihori T, Ono M, Ishii N, Owada Y, Fujimura M, Mashimo Y, Suzuki Y, Hata A, Tsuchiya S, Tominaga T, Matsubara Y, Kure S. A genome-wide association study identifies RNF213 as the first moyamoya disease gene. *J Hum Genet*. 2011;56:34–40.
11. Liu W, Morito D, Takashima S, Mineharu Y, Kobayashi H, Hitomi T, Hashikata H, Matsuura N, Yamazaki S, Toyoda A, Kikuta K, Takagi Y, Harada KH, Fujiyama A, Herzig R, Krischek B, Zou L, Kim JE, Kitakaze M, Miyamoto S, Nagata K, Hashimoto N, Koizumi A. Identification of RNF213 as a susceptibility gene for moyamoya disease and its possible role in vascular development. *PLoS One*. 2011;6:e22542.
12. 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;43:3371–3374.
13. Miyawaki S, Imai H, Shimizu M, Yagi S, Ono H, Mukasa A, Nakatomi H, Shimizu T, Saito N. Genetic variant RNF213 c.14576 g>A in various phenotypes of intracranial major artery stenosis/occlusion. *Stroke*. 2013;44:2894–2897.
14. Bang OY, Ryoo S, Kim SJ, Yoon CH, Cha J, Yeon JY, Kim KH, Kim GM, Chung CS, Lee KH, Shin HJ, Ki CS, Jeon P, Kim JS, Hong SC. Adult moyamoya disease: a burden of intracranial stenosis in East Asians? *PLoS One*. 2015;10:e0130663.
15. Shin J, Chung JW, Park MS, Lee H, Cha J, Seo WK, Kim GM, Bang OY. Outcomes after ischemic stroke caused by intracranial atherosclerosis vs dissection. *Neurology*. 2018;91:e1751–e1759.
16. Park MS, Cha J, Chung JW, Seo WK, Kim GM, Bang OY. Arterial dissection as a cause of intracranial stenosis in East Asians. *J Am Coll Cardiol*. 2017;70:2205–2206.
17. Fukui M. Guidelines for the diagnosis and treatment of spontaneous occlusion of the circle of Willis ('moyamoya' disease). Research Committee on Spontaneous Occlusion of the Circle of Willis (moyamoya disease) of the Ministry of Health and Welfare, Japan. *Clin Neurol Neurosurg*. 1997;99(suppl 2):S238–S240.
18. Ryoo S, Cha J, Kim SJ, Choi JW, Ki CS, Kim KH, Jeon P, Kim JS, Hong SC, Bang OY. High-resolution magnetic resonance wall imaging findings of moyamoya disease. *Stroke*. 2014;45:2457–2460.
19. Ryoo S, Lee MJ, Cha J, Jeon P, Bang OY. Differential vascular pathophysiologic types of intracranial atherosclerotic stroke: a high-resolution wall magnetic resonance imaging study. *Stroke*. 2015;46:2815–2821.
20. Chung JW, Hwang J, Lee MJ, Cha J, Bang OY. Previous statin use and high-resolution magnetic resonance imaging characteristics of intracranial atherosclerotic plaque: the intensive statin treatment in acute ischemic stroke patients with intracranial atherosclerosis study. *Stroke*. 2016;47:1789–1796.
21. Liebeskind DS, Kosinski AS, Lynn MJ, Scalzo F, Fong AK, Fariborz P, Chimowitz MI, Feldmann E. Noninvasive fractional flow on MRA predicts stroke risk of intracranial stenosis. *J Neuroimaging*. 2015;25:87–91.
22. Leng X, Wong LK, Soo Y, Leung T, Zou X, Wang Y, Feldmann E, Liu L, Liebeskind D. Signal intensity ratio as a novel measure of hemodynamic significance for intracranial atherosclerosis. *Int J Stroke*. 2013;8:E46.
23. Bang OY, Chung JW, Cha J, Lee MJ, Yeon JY, Ki CS, Jeon P, Kim JS, Hong SC. A polymorphism in RNF213 is a susceptibility gene for intracranial atherosclerosis. *PLoS One*. 2016;11:e0156607.
24. Jang MA, Chung JW, Yeon JY, Kim JS, Hong SC, Bang OY, Ki CS. Frequency and significance of rare RNF213 variants in patients with adult moyamoya disease. *PLoS One*. 2017;12:e0179689.
25. Hongo H, Miyawaki S, Imai H, Shinya Y, Ono H, Mori H, Nakatomi H, Kunimatsu A, Saito N. Smaller outer diameter of atherosclerotic middle cerebral artery associated with RNF213 c.14576g>A Variant (rs112735431). *Surg Neurol Int*. 2017;8:104.
26. Fukushima H, Takenouchi T, Kosaki K. Homozygosity for moyamoya disease risk allele leads to moyamoya disease with extracranial systemic and pulmonary vasculopathy. *Am J Med Genet A*. 2016;170:2453–2456.
27. Chang SA, Song JS, Park TK, Yang JH, Kwon WC, Kim SR, Kim SM, Cha J, Jang SY, Cho YS, Kim TJ, Bang OY, Song JY, Ki CS, Kim DK. Nonsyndromic peripheral pulmonary artery stenosis is associated with homozygosity of RNF213 p.Arg4810lys regardless of co-occurrence of moyamoya disease. *Chest*. 2018;153:404–413.
28. Jung KH, Chu K, Lee ST, Park HK, Kim DH, Kim JH, Bahn JJ, Song EC, Kim M, Lee SK, Roh JK. Circulating endothelial progenitor cells as a pathogenetic marker of moyamoya disease. *J Cereb Blood Flow Metab*. 2008;28:1795–1803.
29. Kang HS, Moon YJ, Kim YY, Park WY, Park AK, Wang KC, Kim JE, Phi JH, Lee JY, Kim SK. Smooth-muscle progenitor cells isolated from patients with moyamoya disease: novel experimental cell model. *J Neurosurg*. 2014;120:415–425.
30. Hitomi T, Habu T, Kobayashi H, Okuda H, Harada KH, Osafune K, Taura D, Sone M, Asaka I, Ameku T, Watanabe A, Kasahara T, Sudo T, Shiota F, Hashikata H, Takagi Y, Morito D, Miyamoto S, Nakao K, Koizumi A. Downregulation of securin by the variant RNF213 R4810k (rs112735431, g>A) reduces angiogenic activity of induced pluripotent stem cell-derived vascular endothelial cells from moyamoya patients. *Biochem Biophys Res Commun*. 2013;438:13–19.
31. Frank PG, Woodman SE, Park DS, Lisanti MP. Caveolin, caveolae, and endothelial cell function. *Arterioscler Thromb Vasc Biol*. 2003;23:1161–1168.
32. Bang OY, Chung JW, Kim SJ, Oh MJ, Kim SY, Cho YH, Cha J, Yeon JY, Kim KH, Kim GM, Chung CS, Lee KH, Ki CS, Jeon P, Kim JS, Hong SC, Moon GJ. Caveolin-1, ring finger protein 213, and endothelial function in moyamoya disease. *Int J Stroke*. 2016;11:999–1008.
33. Liu J, Wang XB, Park DS, Lisanti MP. Caveolin-1 expression enhances endothelial capillary tubule formation. *J Biol Chem*. 2002;277:10661–10668.
34. Chang SH, Feng D, Nagy JA, Sciuto TE, Dvorak AM, Dvorak HF. Vascular permeability and pathological angiogenesis in caveolin-1-null mice. *Am J Pathol*. 2009;175:1768–1776.
35. Chung JW, Kim DH, Oh MJ, Cho YH, Kim EH, Moon GJ, Ki CS, Cha J, Kim K, Jeon P, Yeon JY, Kim GM, Kim JS, Hong SC, Bang OY. Caveolin-1 and arterial remodeling in adult moyamoya disease. *Stroke*. 2018;49:2597–2604.
36. Kasner SE, Chimowitz MI, Lynn MJ, Howlett-Smith H, Stern BJ, Hertzberg VS, Frankel MR, Levine SR, Chaturvedi S, Benesch CG, Sila CA, Jovin TG, Romano JG, Cloft HJ; Warfarin Aspirin Symptomatic Intracranial Disease Trial Investigators. Predictors of ischemic stroke in the territory of a symptomatic intracranial arterial stenosis. *Circulation*. 2006;113:555–563.
37. Abou-Chebl A, Steinmetz H. Critique of "stenting versus aggressive medical therapy for intracranial arterial stenosis" by chimowitz et al. in the New England Journal of Medicine. *Stroke*. 2012;43:616–620.
38. Nwasokwa ON, Weiss M, Gladstone C, Bodenheimer MM. Effect of coronary artery size on the prevalence of atherosclerosis. *Am J Cardiol*. 1996;78:741–746.
39. Fujimura M, Sonobe S, Nishijima Y, Niizuma K, Sakata H, Kure S, Tominaga T. Genetics and biomarkers of moyamoya disease: significance of RNF213 as a susceptibility gene. *J Stroke*. 2014;16:65–72.
40. Bang OY, Fujimura M, Kim SK. The pathophysiology of moyamoya disease: an update. *J Stroke*. 2016;18:12–20.
41. Koizumi A, Kobayashi H, Liu W, Fujii Y, Senevirathna ST, Nanayakkara S, Okuda H, Hitomi T, Harada KH, Takenaka K, Watanabe T, Shimbo S. P.R4810k, a polymorphism of RNF213, the susceptibility gene for moyamoya disease, is associated with blood pressure. *Environ Health Prev Med*. 2013;18:121–129.
42. Wu Z, Jiang H, Zhang L, Xu X, Zhang X, Kang Z, Song D, Zhang J, Guan M, Gu Y. Molecular analysis of RNF213 gene for moyamoya disease in the Chinese Han population. *PLoS One*. 2012;7:e48179.
43. Cecchi AC, Guo D, Ren Z, Flynn K, Santos-Cortez RL, Leal SM, Wang GT, Regalado ES, Steinberg GK, Shendure J, Bamshad MJ, Grotta JC, Nickerson DA, Pannu H, Milewicz DM. RNF213 rare variants in an ethnically diverse population with moyamoya disease. *Stroke*. 2014;45:3200–3207.
44. Malik R, Chauhan G, Traylor M, Sargurupremraj M, Okada Y, Mishra A, Rutten-Jacobs L, Giese AK, van der Laan W, Gretarsdottir S. et al. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. *Nat Genet*. 2018;50:524–537.
45. Liu W, Hitomi T, Kobayashi H, Harada KH, Koizumi A. Distribution of moyamoya disease susceptibility polymorphism p.R4810k in RNF213 in East and Southeast Asian populations. *Neurol Med Chir (Tokyo)*. 2012;52:299–303.