Association of apolipoprotein E gene polymorphism with small-vessel lesions and stroke type in moyamoya disease: a preliminary study

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OBJECTIVE The present study was conducted to investigate whether microbleeds or microinfarcts are associated with apolipoprotein E (*APOE*) gene polymorphisms in patients with moyamoya disease (MMD), and if so, whether *APOE* gene polymorphisms are also associated with stroke type in patients with MMD.

METHODS This cross-sectional, multicenter study included 86 consecutive patients with MMD who underwent T2*-weighted gradient echo or susceptibility-weighted MR imaging and 83 healthy control volunteers. Baseline clinical and radiological characteristics were recorded at diagnosis, and inter- and intragroup differences in the *APOE* genotypes were assessed. Multivariate binary logistic regression models were used to determine the association factors for small-vessel lesions (SVLs) and hemorrhagic presentation in patients with MMD.

RESULTS There was no difference in *APOE* gene polymorphism and the incidence of SVLs between patients with MMD and healthy controls ($p > 0.05$). In the MMD group, 7 (8.1%) patients had microbleeds and 32 (37.2%) patients had microinfarcts. Microbleeds were more frequently identified in patients with hemorrhagic-type than in nonhemorrhagic-type MMD ($p = 0.003$). *APOE* genotypes differed according to the presence of microbleeds ($p = 0.024$). *APOE* $\epsilon 2$ or $\epsilon 4$ carriers also experienced microbleeds more frequently than *APOE* $\epsilon 3/\epsilon 3$ carriers ($p = 0.013$). In the multivariate regression analysis in patients with MMD, microbleeds were significantly related to *APOE* $\epsilon 2$ or $\epsilon 4$ carrier status (OR 7.86; 95% CI 1.20–51.62; $p = 0.032$) and cerebral aneurysm (OR 17.31; 95% CI 2.09–143.57; $p = 0.008$). Microinfarcts were independently associated with hypertension (OR 3.01; 95% CI 1.05–7.86; $p = 0.007$). Hemorrhagic presentation was markedly associated with microbleeds (OR 10.63; 95% CI 1.11–102.0; $p = 0.041$).

CONCLUSIONS These preliminary results did not show a difference in *APOE* gene polymorphisms between patients with MMD and healthy persons. However, they imply that *APOE* gene polymorphisms may play certain roles in the presence of microbleeds but not microinfarcts in patients with MMD. A further confirmatory study is necessary to elucidate the effect of *APOE* gene polymorphisms and SVLs on the future incidence of stroke in patients with MMD.

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KEY WORDS apolipoprotein E; genotypes; moyamoya disease; microbleeds; microinfarcts; vascular disorders

MOYAMOYA disease (MMD) is a progressive cerebrovascular occlusive disorder consisting of bilateral stenosis or occlusion of the supraclinoid internal carotid artery (ICA) and its major branches, with net-like vessels in the area surrounding the circle of Willis.²⁷ Many studies have demonstrated a higher prevalence of asymptomatic cerebral microbleeds in patients with

MMD compared with the normal population.¹¹ In addition, a recent meta-analysis showed that a high incidence of asymptomatic cerebral microbleeds appeared to be correlated with the hemorrhagic onset type in patients with MMD.²⁰ Kikuta et al.¹² identified the presence of multiple microbleeds as a predictor of subsequent hemorrhages in patients with MMD.

ABBREVIATIONS APOE = apolipoprotein E; GRE = gradient-recalled echo; ICA = internal carotid artery; MMD = moyamoya disease; SVL = small-vessel lesion; T2WI = T2*-weighted imaging.

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To date, apolipoprotein E (*APOE*) gene polymorphisms have been reported to be associated with lobar microbleeds, vascular A β deposition, loss of smooth muscle, and vessel wall thickening.^{19,25,28} In contrast to cerebral amyloid angiopathy, cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy, and Alzheimer's disease, the associations between *APOE* gene polymorphisms and cerebral microbleeds or microinfarcts have never been studied in patients with MMD. Therefore, we hypothesized that associations might exist between *APOE* gene polymorphisms and cerebral microangiopathy, such as microbleeds or microinfarcts, in patients with MMD. For this purpose, we investigated the associations between clinical and radiological features and *APOE* gene polymorphisms in patients with MMD.

Methods

Population and Participant Selection

This study protocol was approved by the institutional review board of the Catholic Medical Center at the Catholic University of Korea. Between 2012 and 2014, 3 neurovascular experts who have diagnosed and treated patients with MMD for more than 10 years participated in the screening of subjects in 3 affiliated hospitals. MMD was confirmed according to the guidelines for diagnosis and treatment of MMD (i.e., spontaneous occlusion of the circle of Willis).²² Patients with moyamoya syndrome or probable MMD, an ethnicity other than Korean, incomplete radiological data, or brain MR images obtained after revascularization surgery were excluded from the analysis. The baseline characteristics of the patients were recorded, such as age at initial symptom development, sex, the presence of hypertension or an antihypertensive medication history, the presence of diabetes mellitus or an antidiabetic medication history, the presence of hyperlipidemia or a lipid-lowering drug medication history, current smoking status, and the use of antithrombotic medication. Among 92 eligible subjects identified between 2012 and 2014, 6 patients with MMD were excluded due to incomplete MR scanning. Therefore, 86 (93.5%) patients with MMD were included in the final analysis. Among them were 27 patients with hemorrhagic MMD and 59 with nonhemorrhagic MMD (56 patients with ischemic MMD and 3 with asymptomatic MMD). Eighty-three healthy volunteers without stroke episodes and MMD, whose radiological images did not show any stenosis or occlusion of intracranial major arteries, were selected for the control group.

Radiological Image Acquisition and Analysis

We analyzed brain MR images and digital subtraction angiography obtained when patients initially visited the participating hospital. An acute ischemic stroke was confirmed using MR diffusion-weighted imaging, and a hemorrhagic stroke was identified using CT or T2*-weighted imaging (T2WI) or gradient-recalled echo (GRE) MRI of abnormal lesions, with a low signal indicating a hemosiderin rim that was larger than 10 mm. Stroke frequency was determined as the number of separate radiological abnormal lesions on CT or MRI scans. Microbleeds were definitively identified as small, round, well-defined, hypointense

foci on GRE images or susceptibility-weighted images with diameters of 2–10 mm in the brain parenchyma. Linear or elongated lesions in the subarachnoid space of the cortical, juxta-cortical, or air-bone interfaces of the frontal or temporal lobes and hemosiderin rims close to a large intracerebral hemorrhage or an infarct were excluded.⁵ In addition, symmetrical hypointense signal changes in the basal ganglia on GRE or T2WI sequences were examined for calcification and then excluded.⁵ Microinfarcts were defined as minute foci with gliosis, pallor, or cystic lesions with diameters of 2–5 mm and a high signal intensity on T2WI or FLAIR images; however, lesions with high signal intensity measuring more than 5 mm on T2WI or FLAIR were regarded as old or recent subacute infarcts. Representative small-vessel lesions (SVLs) are displayed in Fig. 1. Angiographic details of all hemispheres included the angiographic stage, as suggested by Suzuki and Takaku.²⁷ Two independent neurosurgeons reviewed all of the radiological data. Disagreements between the reviewers regarding the diagnosis or measurements were thoroughly discussed and resolved by consensus.

Genetic Analysis

All DNA samples were acquired from the oral mucosa, and *APOE* genotyping was conducted using a polymerase chain reaction sequencing method. All genetic analysis was performed by an independent facility (BIOFACT Co., Ltd.), which was completely unaware of each subject's clinical and radiological information. The distributions of *APOE* genotypes and allele frequencies in the MMD and control groups were all in Hardy-Weinberg equilibrium ($p = 0.87$ and $p = 0.17$, respectively).

Statistics

For the descriptive analysis, all baseline values are displayed as percentages, and the mean values $\pm$ SDs and the ranges are shown. For categorical variables, the chi-square test or Fisher's exact test was performed between 2 independent groups. A binary logistic regression method was used to calculate the association predictors for SVLs and hemorrhagic presentation. If the p value of a potential fac-

FIG. 1. Representative radiological features of microbleeds or microinfarcts in MMD are shown. A 51-year-old patient with hemorrhagic MMD had left periventricular hemorrhage and intraventricular hemorrhage concurrent with a microbleed at the periventricular white matter near the left occipital horn on MR susceptibility-weighted image (left), and a 54-year-old patient with ischemic MMD had multiple microinfarcts and old infarcts at the subcortical area on MR T2-weighted image (right).

tor was less than 0.05 in the univariate analysis, it was entered into the multivariate analysis model but was backward eliminated to $p < 0.1$. The Hosmer-Lemeshow test was used as a goodness-of-fit test. Probability values less than 0.05 were considered statistically significant. Two-tailed methods were used in all statistical analyses. All statistical calculations were performed using SAS version 9.13 (SAS Institute, Inc.).

Results

Baseline Characteristics and APOE Gene Polymorphisms of Patients with MMD and Healthy Controls

Table 1 shows the comparison of demographic data and APOE gene polymorphisms between 86 patients with MMD and 83 healthy controls. The mean age was higher in the control group than in the MMD group ($p < 0.001$). Regarding APOE genotype and presence of $\epsilon 2$, $\epsilon 3$, or $\epsilon 4$, there was no difference between the MMD group and the control group ($p > 0.05$). Microbleeds were observed in 7 (8.1%) patients with MMD and 2 (2.4%) healthy controls without significant difference ($p = 0.17$), and the incidence of microinfarcts was similar in both groups ($p = 0.9$). The detailed demographic data for the 86 patients with MMD according to the presence of SVLs are summarized in Table 2. The mean age was greater in patients with MMD with SVLs than in patients with MMD without SVLs ($p = 0.01$). Hypertension was more frequently identified in a group with SVLs than in a group without SVLs ($p = 0.042$). The other baseline clinical and angiographic details were not significantly different between patients with MMD with and without SVLs.

Distribution of APOE Gene Polymorphism According to SVLs and Stroke Type in Patients with MMD

The distribution of APOE genotypes in the patients with MMD was as follows: $\epsilon 2/\epsilon 2$ in 1 (1.2%) patient, $\epsilon 2/\epsilon 3$ in 9 (10.5%) patients, $\epsilon 3/\epsilon 3$ in 63 (73.3%) patients, $\epsilon 3/\epsilon 4$ in 12 (14.0%) patients, and $\epsilon 4/\epsilon 4$ in 1 (1.2%) patient. The difference in the APOE genotype distribution with respect to the presence of SVLs in patients with MMD showed borderline significance ($p = 0.071$) (Fig. 2A). In particular, there was a significant difference in the APOE genotype distribution according to the presence of microbleeds but not microinfarcts ($p = 0.024$ and $p = 0.31$, respectively) (Fig. 2B and C). The APOE genotype of patients with hemorrhagic MMD did not differ from that of patients with nonhemorrhagic MMD ($p = 0.3$) (Fig. 2D). APOE $\epsilon 2$ allele carriers tended to be more abundant among patients with MMD with SVLs than patients with MMD without SVLs ($p = 0.086$) (Fig. 3A), and APOE $\epsilon 2$ allele frequency differed between the 2 groups with and without SVLs ($p = 0.031$) (Fig. 3B). Although APOE $\epsilon 4$ allele carrier status was marginally associated with the presence of microbleeds in patients with MMD ($p = 0.067$) (Fig. 3D), APOE $\epsilon 2$ or $\epsilon 4$ allele carriers exhibited microbleeds more often than APOE $\epsilon 2$ or $\epsilon 4$ allele noncarriers (Fig. 3C, $p = 0.013$).

Details of SVLs and Association with Stroke Type and Cerebral Aneurysms in Patients with MMD

There were 36 patients with SVLs. Seven (8.1%) pa-

TABLE 1. Comparison of APOE gene polymorphisms between 86 patients with MMD and 83 healthy volunteers*

Variable	MMD	Control	p Value†
Age in yrs, mean $\pm$ SD‡	46.9 $\pm$ 14.2	54.0 $\pm$ 10.2	<0.001
Sex, F	63 (73.3)	60 (72.3)	0.8
APOE genotype			0.5
$\epsilon 2/\epsilon 2$	1 (1.2)	0	
$\epsilon 2/\epsilon 3$	9 (10.5)	14 (16.9)	
$\epsilon 2/\epsilon 4$	0	0	
$\epsilon 3/\epsilon 3$	63 (73.3)	53 (63.9)	
$\epsilon 3/\epsilon 4$	12 (14.0)	15 (18.1)	
$\epsilon 4/\epsilon 4$	1 (1.2)	1 (1.2)	
APOE $\epsilon 2$ carriers	10 (11.6)	14 (16.9)	0.3
APOE $\epsilon 2$ allele frequency			0.3
0	76 (88.4)	69 (83.1)	
1	9 (10.5)	14 (16.9)	
2	1 (1.2)	0	
APOE $\epsilon 3$ carriers	84 (97.7)	80 (96.4)	0.6
APOE $\epsilon 3$ allele frequency			0.5
0	2 (2.3)	3 (3.6)	
1	21 (24.4)	27 (32.5)	
2	63 (73.3)	53 (63.9)	
APOE $\epsilon 4$ carriers	13 (15.1)	18 (21.7)	0.3
APOE $\epsilon 4$ allele frequency			0.4
0	73 (84.9)	65 (78.3)	
1	13 (15.1)	17 (20.5)	
2	0	1 (1.2)	
APOE $\epsilon 2$ or $\epsilon 4$ carriers	23 (26.7)	30 (36.1)	0.19
Microbleeds	7 (8.1)	2 (2.4)	0.17
Microinfarcts	32 (37.2)	30 (36.1)	0.9

* All values are reported as the number of patients (%) unless otherwise stated.

† The Fisher exact test or Student's t-test was performed.

‡ Age at gene sampling.

tients had 11 microbleeds (multiple microbleeds in 2 patients and solitary microbleeds in 5 patients), which were located in the deep brain area (Table 3), and 32 (37.2%) patients presented with microinfarcts. Only 3 (3.5%) patients displayed concurrent microbleeds and microinfarcts. Six patients with MMD had cerebral aneurysms. Microbleeds were detected more frequently in patients with hemorrhagic MMD than in patients with nonhemorrhagic MMD ($p = 0.003$) (Fig. 4A). The presence of microinfarcts was not different between patients with hemorrhagic and nonhemorrhagic MMD ($p = 0.98$) (Fig. 4B). The rate (50%) of microbleeds in 6 patients with MMD with cerebral aneurysms was higher than that (5%) of microbleeds in 80 patients with MMD without cerebral aneurysms ($p = 0.006$) (Fig. 4C).

Association Factors for Microbleeds, Microinfarcts, and Hemorrhagic Presentation in Patients with MMD

In the multivariate analysis, APOE $\epsilon 2$ or $\epsilon 4$ carrier sta-

TABLE 2. Comparison of baseline characteristics of patients with MMD, with or without SVLs*

Variable	MMD w/ SVLs, n = 36	MMD w/o SVLs, n = 50	p Value†
Age in yrs, mean $\pm$ SD (range)‡	46.2 $\pm$ 13.7 (9–72)	37.4 $\pm$ 16.5 (5–64)	0.01
Sex, F	28 (77.8)	35 (70)	0.42
Hypertension	17 (47.2)	13 (26)	0.042
Diabetes	2 (5.6)	5 (10)	0.69
Hyperlipidemia	5 (13.9)	2 (4)	0.12
Current smoker	4 (11.1)	5 (10)	1.0
Antiplatelet medication	1 (2.8)	5 (10)	0.39
Family history of MMD	0	4 (8)	0.14
Stroke history			
No. of ischemic strokes			0.064
1	15 (41.7)	11 (22)	
≥ 2	5 (13.9)	16 (32)	
No. of hemorrhagic strokes			0.4
1	12 (33.3)	14 (28)	
≥ 2	1 (2.8)	0	
Angiographic stage§			0.67
1	1 (2.8)	4 (8)	
2	4 (11.1)	8 (16)	
3	13 (36.1)	12 (24)	
4	7 (19.4)	14 (28)	
5	7 (19.4)	7 (14)	
6	4 (11.1)	5 (10)	
Cerebral aneurysms	4 (11.1)	2 (4)	0.23

* All values are reported as the number of patients (%) unless otherwise stated.

† The Fisher exact test or Student's t-test was performed.

‡ Age at presentation of initial symptoms.

§ According to Suzuki and Takaku.²⁷

tus (OR, 7.86; 95% CI 1.20–51.62; $p = 0.032$) and cerebral aneurysm (OR 17.31; 95% CI 2.09–143.57; $p = 0.008$) were strongly associated with cerebral microbleeds in patients with MMD (Table 4). By contrast, hypertension (OR 3.01; 95% CI 1.05–7.86; $p = 0.007$) was an independently associated factor for microinfarcts in patients with MMD, but age at symptom onset (OR 1.03; 95% CI 0.99–1.06; $p = 0.075$) demonstrated only a borderline association with microinfarcts in patients with MMD (Table 4). On the other hand, microbleeds were markedly associated with hemorrhagic presentation among patients with MMD (OR 10.63; 95% CI 1.11–102.0; $p = 0.041$).

Discussion

To the best of our knowledge, this study is the first to examine the relationships between *APOE* gene polymorphisms and SVLs in patients with MMD. *APOE* gene polymorphisms have been widely investigated in the fields of neurovascular disease and stroke.^{9,13,21,24,29} According to a recent systematic review, *APOE* $\epsilon 4$ allele carrier status was associated with white matter hyperintensity burden and lobar microbleeds in cerebrovascular disease.²⁴ However, *APOE* $\epsilon 2$ allele carrier status was associated with white matter hyperintensity and the risk of

brain infarcts.²⁴ In a hospital-based, multicenter study of cerebral microbleeds and *APOE* gene polymorphisms in Korean patients who had suffered strokes, the presence of the *APOE* $\epsilon 2$ or $\epsilon 4$ allele was associated with lobar microbleeds but not with nonlobar microbleeds.¹³ The *APOE* $\epsilon 2$ allele is known to be associated with fibrinoid necrosis, and the *APOE* $\epsilon 4$ allele has been associated with the loss of smooth muscle and vessel wall thickness, as well as with vascular A β deposition.^{2,16,28} Although there was no difference in *APOE* genotype between patients with MMD and the control group (Table 1), our results revealed a difference in *APOE* genotype according to the presence of microbleeds (Fig. 2B). *APOE* $\epsilon 2$ or $\epsilon 4$ allele carrier status was strongly associated with microbleeds, but not with microinfarcts in patients with MMD (Table 4). Therefore, we speculate that *APOE* gene polymorphisms could be associated with SVLs, particularly with microbleeds in patients with MMD.

To date, there have been several reports concerning cerebral microbleeds in patients with MMD, with the prevalence ranging from 14.8% to 51.9%.^{7,10–12,20} Kikuta et al. demonstrated a higher prevalence (44%) of cerebral microbleeds in patients with MMD than in healthy individuals (5.8%).¹¹ Our detection rate (8.1%) of microbleeds is the first reported in Korean patients with MMD and was rela-

FIG. 2. Bar graphs showing APOE genotype distribution according to SVLs and stroke type in patients with MMD is shown. The APOE genotype distribution is marginally different between 2 groups with and without SVLs (A). There is a significant difference in APOE genotype distribution between 2 groups with and without microbleeds (B), but not between 2 groups with and without microinfarcts (C). APOE genotype also does not differ according to stroke type in patients with MMD (D).

FIG. 3. Bar graphs showing allele status according to SVLs in patients with MMD is shown. APOE $\epsilon 2$ allele frequency shows a significant association with SVLs in patients with MMD (B). The APOE $\epsilon 2$ and $\epsilon 4$ alleles also display a significant association with microbleeds (C). APOE $\epsilon 2$ allele carriers include the genotypes $\epsilon 2/\epsilon 2$ and $\epsilon 2/\epsilon 3$ (A); APOE $\epsilon 4$ allele carriers include the genotypes $\epsilon 3/\epsilon 4$ and $\epsilon 4/\epsilon 4$ (D). The allele frequency indicates the number of alleles (B).

TABLE 3. Summary of microbleeds and aneurysms in patients with MMD*

Case No.	Age (yrs), Sex	Microbleeds	Hemorrhage†	Aneurysm
1	51, F	Lt PVWM	Lt PVWM, IVH	No
2	55, F	Lt PVWM	Rt BG	UIA at rt PCA
3	42, F	Rt PVWM, lt BG	Lt thalamoputamen	RA at lt AChA
4	54, F	Lt BG, lt PVWM	Lt PVWM	RA at lt AChA
5	37, F	Rt PVWM	Rt PVWM and IVH	No
6	33, F	Lt thalamus, rt PVWM, rt Cbll	Lt BG	No
7	45, M	Lt PVWM	Rt AWSi	No
8	48, F	No	Rt IVH	RA at rt AChA
9	61, F	No	Lt temporal lobe	UIA at lt LPChA
10	15, F	No	Lt ACAi, rt PCAi	UIA at ICA

ACAi = anterior cerebral artery infarct; AChA = anterior choroidal artery; AWSi = anterior watershed infarct involvement between the ACA and middle cerebral artery territory; BG = basal ganglia; Cbll = cerebellum; IVH = intraventricular hemorrhage; LPChA = lateral posterior choroidal artery; PCA = posterior cerebral artery; PCAi = PCA infarct; PVWM = periventricular white matter; RIA = ruptured intracranial aneurysm; UIA = unruptured intracranial aneurysm.

* There were 11 microbleeds in 7 patients; there were 6 aneurysms in 6 patients.

† Case 7 and Case 10 presented with cerebral infarction.

tively lower than those in the aforementioned studies. This might have been due to detection bias arising from the use of heterogeneous MR apparatuses and scanning protocols or ethnicity differences. The present study showed that microbleeds were a strong association factor for hemorrhagic presentation in patients with MMD (Table 4). This finding was consistent with the other previous study.²⁰ Although our study did not investigate subsequent hemorrhages in patients with MMD with microbleeds, several studies have reported the presence of microbleeds as a predictor of subsequent hemorrhagic stroke in patients with MMD.^{12,14,23,26}

Microbleeds in patients with MMD have mainly been reported in deep brain areas, such as the periventricular white matter, basal ganglia, or thalami, and they mainly occur in arteries displaying the subependymal–leptomeningeal artery anastomosis pattern.¹⁰ In the present study, all microbleeds were identified in deep brain areas (Table 3). In addition, a previous histopathological report described ruptured moyamoya vessels showing fibrin deposits in the wall, fragmented elastic laminae, attenuated media, microaneurysm formation, focal fibrin deposits, and marked attenuation of vessel wall thickness with diminution of the elastic lamina in patients with MMD.³¹ Many researchers have reported that angiographic features related to hemorrhagic stroke in patients with MMD consist of anterior cerebral artery occlusion, cerebral aneurysms, and dilation of the anterior choroidal artery or posterior communicating artery.^{8,17} In particular, microbleeds in deep and periventricular white matter have been reported as a probable predictor of subsequent intraventricular hemorrhage in pa-

FIG. 4. Bar graphs showing SVLs according to stroke type and cerebral aneurysms in patients with MMD. Microbleeds are more common in patients with hemorrhagic MMD than in patients with nonhemorrhagic MMD (A), but microinfarcts are not associated with stroke type (B). More cerebral aneurysms were detected in patients with MMD with microbleeds than in patients with MMD without microbleeds (C).

TABLE 4. Multivariate analysis for microbleeds, microinfarcts, and hemorrhagic presentation in patients with MMD

Variable	Microbleeds		Microinfarcts		Hemorrhagic Presentation	
	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value
Age at symptom onset			1.03 (0.99–1.06)	0.075		
Hypertension			3.01 (1.05–7.86)	0.007		
<i>APOE</i> ϵ 2 or ϵ 4 carriers*	7.86 (1.20–51.62)	0.032				
Cerebral aneurysm	17.31 (2.09–143.57)	0.008			7.21 (0.69–76.03)	0.1
Microbleeds					10.63 (1.11–102.0)	0.041

* Reference: ϵ 3/ ϵ 3.

tients with MMD.²⁶ As mentioned above, cerebral microbleeds in patients with MMD have mainly been identified in the deep brain structure (and not in the cerebral cortex). These findings could indicate that hemodynamic stress is the main mechanism causing the rupture of moyamoya vessels.

Nevertheless, it is notable that in the brain, APOE is secreted primarily from astrocytes under conditions such as injury to the CNS; APOE not only plays a main role in lipid metabolism, but it also acts as a ligand for neuronal receptors.^{1,15,18} MMD is a chronic, cerebral steno-occlusive disease with an ever-present risk of hypoxia or hemodynamic stress. Therefore, in the presence of the *APOE* ϵ 2 or ϵ 4 allele, chronic hypoxic or hemodynamic stress might trigger the occurrence of SVLs, such as microbleeds, in patients with MMD. Our study demonstrated that *APOE* ϵ 2 or ϵ 4 allele carriers and cerebral aneurysm were strongly associated with microbleeds (Table 4). Here, we hypothesize a shared genetic pathomechanism for SVLs in patients with MMD that is consistent with other small-vessel diseases, such as arteriosclerosis, cerebral amyloid angiopathy, Alzheimer's disease, and brain aging.

Although the *APOE* gene polymorphisms were not related to microinfarcts, there have been no reports regarding microinfarcts in patients with MMD in the literature. Microinfarcts possibly result from heterogeneous disease entities, including white matter hyperintensities of presumed vascular origin, lacunae of presumed vascular origin, and the perivascular space, as suggested by the STandards for Reporting Vascular changes on nEuroimaging (STRIVE) group.³⁰ We classified high-density lesions in cortical or subcortical areas and the brainstem as microinfarcts based on the sizes of the lesions, as suggested by a previous report.⁶ Recently, an observational study regarding acute microinfarcts as the cause of leukoaraiosis has been published.³ Despite the heterogeneity of this entity, our results indicated that hypertension might be involved in the pathomechanism of microinfarcts in patients with MMD.

To our knowledge, the present study is the first report regarding the associations between *APOE* gene polymorphisms and SVLs in patients with MMD. Moreover, subjects were recruited from multiple centers. However, this study has several limitations. First, the control group might not be representative due to a hospital-based sampling without age matching. Second, we did not study the impact of SVLs on subsequent strokes in patients with MMD. In addition, we could not use the same MR ap-

paratuses or scanning protocols in all of the participating hospitals. Last, we did not measure the number and location of microinfarcts or use a standardized measuring tool for microinfarcts (such as the Fazekas scale).⁴

Conclusions

There was no difference in *APOE* gene polymorphisms between patients with MMD and healthy controls. However, this study showed that microbleeds were significantly associated with hemorrhagic presentation in patients with MMD, and cerebral aneurysm and *APOE* ϵ 2 or ϵ 4 allele carrier status showed a strong association with microbleeds. Therefore, these findings imply that there may be certain roles of *APOE* gene polymorphism in the development of microbleeds in patients with MMD. In addition, hypertension in patients with MMD may be associated with the development of microinfarcts. A prospective observational study is necessary to confirm the impact of SVLs and *APOE* gene polymorphism for future stroke events in patients with MMD.

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Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: all authors. Acquisition of data: all authors. Analysis and interpretation of data: Huh, Jang. Drafting the article: Jang. Reviewed submitted version of manuscript: Huh, Jang. Approved the final version of the manuscript on behalf of all authors: Huh. Statistical analysis: Jang. Study supervision: Huh, Lee.

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