

Identification of a Genetic Variant Common to Moyamoya Disease and Intracranial Major Artery Stenosis/Occlusion

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Background and Purpose—The c.14576G>A variant in ring finger protein 213 (*RNF213*) was recently identified as a susceptibility gene variant for moyamoya disease (MMD). The occurrence of c.14576G>A variant was evaluated in patients with intracranial major artery stenosis/occlusion (ICASO) without signs of MMD (non-MMD ICASO), as well as in patients with MMD and other cerebrovascular diseases as controls.

Methods—This single-hospital-based case-control study was completed in 7 months (from October 2011–April 2012) at Department of Neurosurgery, The University of Tokyo Hospital. The occurrence of c.14576G>A variant was analyzed in 41 patients with non-MMD ICASO, in 48 with MMD, in 21 with cervical disease, in 61 with cerebral aneurysm, and in 25 normal subjects.

Results—Nine of 41 patients (21.9%) with non-MMD ICASO and 41 of 48 (85.4%) with MMD had the c.14576G>A variant. One of 61 patients (1.6%) with cerebral aneurysm and no patients with cervical disease or normal subjects had the variant. Comparison of each phenotype group with the normal subjects showed that presence of c.14576G>A variant had significant associations with MMD (odds ratio [OR], 292.8; 95% confidence interval [CI], 15.4–5153.0; $P<0.0001$) and with non-MMD ICASO (OR, 14.9; 95% CI, 0.82–268.4; $P=0.01$), but no association with either cerebral aneurysm (OR, 1.2; 95% CI, 0.04–32.0; $P=1.00$) or cervical disease (OR, 1.1; 95% CI, 0.02–62.3; $P=1.00$).

Conclusions—The present study indicates that a particular subset of Japanese patients with non-MMD ICASO has a genetic variant associated with MMD. Therefore, we propose the existence of a new entity of ICASO caused by the c.14576G>A variant in *RNF213*. (*Stroke*. 2012;43:3371–3374.)

Key Words: genetics ■ intracranial stenosis ■ moyamoya ■ stroke

Recently, a susceptibility gene for moyamoya disease (MMD) was identified in the East Asian population. Three individual studies of MMD patients revealed high frequencies of the same single base substitution (nonsynonymous mutation), c.14576G>A (p.R4859K) variant in ring finger protein 213 (*RNF213*; a gene located in chromosome 17q; based on the National Center for Biotechnology Information Reference sequence NP_065965.4).^{1–3} *RNF213* encodes a protein with 5256 amino acids harboring a RING (really interesting new gene) finger motif and an AAA (ATPases associated with a variety of cellular activities) domain, indicating the presence of both E3 ubiquitin ligase activity and energy-dependent unfoldase.^{1,2} E3 ubiquitin ligase, which has several subtypes, is an enzyme that ubiquitinates specific target proteins, resulting in degeneration by proteasomes. Such degeneration of specific proteins controlled by E3 ubiquitin ligase is known to be involved in various physiological activities such as the cell cycle, signal transduction, DNA repair, and transcriptional regulation.⁴

Unfoldase is an enzyme that catalyzes protein unfolding.⁵ Knockdown of *RNF213* in zebrafish leads to abnormal sprouting and irregular diameter of intracranial vessels, suggesting some contribution to vascular formation.² However, the biochemical function and pathological role of *RNF213* have not been completely clarified.

Previous clinical studies revealed that a wide spectrum of phenotypes could occur within a family unit despite the members having the identical c.14576G>A variant, with some individuals showing the typical phenotype of MMD such as bilateral stenosis/occlusion of the terminal portion of the internal carotid arteries, some showing only unilateral or middle cerebral artery stenosis/occlusion, and others with no abnormalities.²

Intracranial major artery stenosis/occlusion (ICASO) is an important cause of stroke and is considered to have several etiologies, mainly involving atherosclerosis or cardiac embolism, but occasionally involves MMD, vasculitis, or dissection, and rarely involves autoimmune diseases and other

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conditions.⁶ We assumed that the c.14576G>A variant could be the cause of a wide spectrum of phenotypes of ICASO including MMD, and so some cases of ICASO originally considered to originate from atherosclerosis might be associated with the c.14576G>A variant in *RNF213*. Therefore, a new entity of *RNF213* variant-related ICASO might be identified that should be categorized as MMD based on the genetic background.

The present study investigated the occurrence of c.14576G>A variant in patients with ICASO not diagnosed as MMD compared with the frequency in patients with MMD and other cerebrovascular diseases associated with cervical carotid stenosis/occlusion and cerebral aneurysm as control groups.

Materials and Methods

Study Population

This study was a single-hospital-based case-control study conducted at Department of Neurosurgery, The University of Tokyo Hospital that was completed in 7 months (from October 2011–April 2012). A total of 196 Japanese patients who agreed to participate in this study were enrolled among both new and revisiting outpatients in this period as follows: 41 patients with ICASO in the absence of MMD (non-MMD); 48 patients with MMD (both bilateral and unilateral types) diagnosed according to published guidelines;⁷ 21 patients with cervical carotid artery stenosis/occlusion (cervical disease); and 61 patients with cerebral aneurysm. Twenty-five patients without cerebrovascular lesions also were enrolled as normal controls (Supplementary Table I).

The diagnosis of cerebrovascular lesion was made based on the findings of magnetic resonance angiography (MRA). MRA images were interpreted by ≥ 2 physicians, including at least 1 radiologist and 1 neurosurgeon. MMD was diagnosed by the Research Committee on Moyamoya Disease of the Ministry of Health, Welfare, and Labor, Japan criteria.⁷ Briefly, the Research Committee on Moyamoya Disease of the Ministry of Health, Welfare, and Labor, Japan criteria require all of the following findings: (1) steno-occlusive lesions around the terminal portions of the internal carotid arteries (including the proximal portions of the anterior cerebral arteries and middle cerebral arteries); (2) moyamoya vessels at the base of the brain appearing as abnormal vascular networks on conventional angiography or MRA; (3) findings 1 and 2 are present bilaterally (unilateral finding is defined as probable MMD); and (4) known diseases with similar angiographic findings are excluded (ie, arteriosclerosis, autoimmune disease, meningitis, brain neoplasm, Down syndrome, neurofibromatosis type 1, head trauma, irradiation to the head, protein C deficiency, protein S deficiency, and other diseases). In this study, we included patients with probable MMD (or unilateral MMD) in the MMD group.

Patients with intracranial major artery stenosis or occlusion that did not satisfy the MMD criteria were categorized as having non-MMD ICASO. In this study, the non-MMD ICASO group included 41 patients. Most of these patients showed only partial stenosis or occlusion of the intracranial major artery without abnormal vascular networks in the basal ganglia on MRA. Most lesions were located in the unilateral middle cerebral artery or internal cerebral artery. Most patients had atherosclerotic features on MRA, with history of hypertension, hyperlipidemia, and/or diabetes in relatively elderly patients. Some patients with non-MMD ICASO were relatively young and had fewer risk factors for atherosclerosis, and they had no abnormal vascular networks in the basal ganglia. In this study, no patients had signs of cardiac embolism, dissection, vasculitis, or any other basic diseases that cause ICASO.

Identification of *RNF213* Mutations

Peripheral blood samples were obtained from all enrolled patients. Genomic DNA was obtained from the peripheral blood leukocytes

at SRL Inc using DNA Extraction Kit (Talent). Mutation analysis of exon 61, which includes the c.14576G>A variant of *RNF213* (GenBank accession number, NM_020914.4), was performed by direct sequencing at FASMAC Co, Ltd, using an ABI Genetic Analyzer 3130XL or ABI DNA Analyzer 3730xL (Applied Biosystems) and analyzed with Sequence Scanner version 1.0 (Applied Biosystems). The same primer sequences and polymerase chain reaction conditions were used as described previously.²

Statistical Analysis

Fisher exact test was used to compare the rate of the patients with c.14576G>A variant in each phenotype group with the rate in the control group. Because our data included few individual values, appropriate corrections were used to calculate the odds ratio and 95% confidence interval. All analyses were performed on JMP Pro version 9.0.2 (SAS Institute). $P < 0.05$ was considered statistically significant.

Ethical Considerations

This study was approved by the Human Genome, Gene Analysis Research Ethics Committee of the Faculty of Medicine, The University of Tokyo (approval number, 3516; approval date, September 12, 2011). Written informed consents were obtained from all participants in this study.

Results

The non-MMD ICASO group included 9 patients with the c.14576G>A variant (8 heterozygotes and 1 homozygote) among 41 patients (21.9%). The MMD group included 41 patients with the c.14576G>A variant (40 heterozygotes and 1 homozygote) among the 48 patients (85.4%). In contrast, only 1 patient of 61 patients (1.6%) with cerebral aneurysm and no patients with cervical disease or normal subjects had the c.14576G>A variant (Supplementary I). Comparison of each phenotype group with the normal subjects showed that presence of c.14576G>A variant had significant associations with MMD (odds ratio, 292.8; 95% confidence interval, 15.4–5153.0; $P < 0.0001$) and with non-MMD ICASO (odds ratio, 14.9; 95% confidence interval, 0.82–268.4; $P = 0.01$), but had no association with either cerebral aneurysm (odds ratio, 1.2; 95% confidence interval, 0.04–32.0; $P = 1.00$) or cervical disease (odds ratio, 1.1; 95% confidence interval, 0.02–62.3; $P = 1.00$; Table).

The Figure shows the MRA images of the 4 patients with non-MMD ICASO identified with the c.14576G>A variant (the MRA images of the other 5 patients with the c.14576G>A variant in the non-MMD ICASO group are shown in Supplementary Figure I). The reasons for the diagnosis of non-MMD ICASO in each case are described in the Figure legend.

Case 1 had no risk factors for atherosclerosis or baseline disease, so the diagnosis was ICASO of unknown etiology. Cases 2 to 4 had atherosclerosis diagnosed based on the presence of risk factors. In particular, case 4 had undergone coronary artery bypass for angina pectoris.

Discussion

The major finding of this study is the identification of a certain number of patients (9/41; 21.9%) with c.14576G>A variant among patients with ICASO that did not meet the diagnostic criteria for MMD. The c.14576G>A variant was common in patients with MMD (41/48; 85.1%) but was almost completely absent in patients with other cerebrovascular

Table. Comparison of the Rate of the Occurrence of c.14576G>A Variant in Each Phenotype Group With the Control Group

	MMD	ICASO Non-MMD	Cerebral Aneurysm	Cervical Disease	Control
Total n of patients	48	41	61	21	25
N of patients with c.14576G>A variant (G/A+A/A)	41	9	1	0	0
Rate (%)	85.4	21.9	1.6	0	0
P Value	<0.0001	0.010	1.00	1.00	
OR	282.2	14.9	1.2	1.1	
95% CI	15.4–5153.8	0.82–268.4	0.04–32.0	0.02–62.3	

CI indicates confidence interval; ICASO, intracranial main artery stenosis/occlusion; MMD, moyamoya disease; OR, odds ratio.

Cervical disease here indicates cervical carotid artery stenosis/occlusion.

diseases and normal control subjects. These findings strongly indicate that some cases of ICASO ascribed to unknown etiology or atherosclerosis might be part of the MMD spectrum caused by the c.14576G>A variant in *RNF213*.

The present study included 9 patients with non-MMD ICASO associated with c.14576G>A variant. Case 1 in the Figure was a relatively young patient without atherosclerotic risk factors, indicating unknown etiology except for genetic background. The other 3 patients (cases 2–4) in the Figure were elderly patients with some atherosclerotic risk factors such as hypertension, diabetes mellitus, hyperlipidemia, and others. The angiographic findings of ICASO could not be distinguished from the conventional atherosclerotic ICASO characteristics in these elderly patients. Therefore, atherosclerosis would be diagnosed according to the angiographic findings in such elderly patients without knowledge of the presence or absence of the c.14576G>A variant. Consequently, genetic analysis is the only way to establish the differential diagnosis between such ICASO caused by atherosclerosis and by MMD.

Epidemiological studies have shown that Asians have development of atherosclerotic ICASO with significantly higher rates than Caucasians,^{8,9} suggesting that a genetic factor peculiar to Asians might be responsible. The c.14576G>A in *RNF213* is present in ≈2% of east Asian populations, a relatively higher rate compared with Caucasians,^{1–3} so our present findings may have identified the reason for the epidemiological difference. The limitation of this study is that the actual ratio of the population with the c.14576G>A variant in *RNF213* associated with ICASO remains uncertain because the study included too few subjects. However, the findings strongly indicate that c.14576G>A variant in *RNF213* is important in the pathogenesis of ICASO. A larger clinical study is needed to establish the importance of genetic analysis of the c.14576G>A variant in *RNF213* in the differential diagnosis of ICASO.

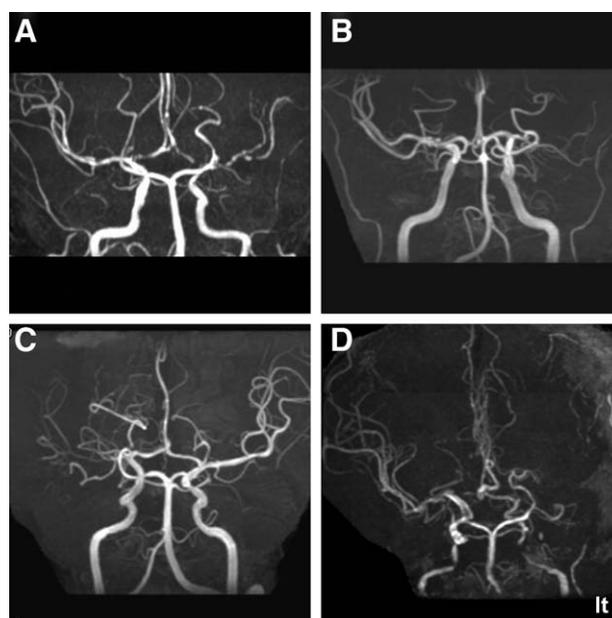


Figure. Magnetic resonance angiography (MRA) images of the 4 patients with the c.14576G>A variant in the non-moyamoya disease (MMD) intracranial major artery stenosis/occlusion (ICASO) group. **A**, Case 1 was a 43-year-old man with diffuse stenosis of the M1 segment of the left middle cerebral artery (left M1). Non-MMD ICASO was diagnosed because the lesion was only unilateral diffuse stenosis of the M1 segment and no abnormal vascular network was present in the basal ganglia. The patient was relatively young and had no risk factor for atherosclerosis, so the diagnosis was spontaneous or unknown etiology intracranial major artery stenosis. **B**, Case 2 was a 61-year-old woman with occlusion of the left M1. Non-MMD ICASO was diagnosed because the lesion was only unilateral occlusion of the M1 and no abnormal vascular network was present in the basal ganglia. The patient was also relatively elderly and had risk factor such as hypertension, so the diagnosis was atherosclerosis. **C**, Case 3 was a 65-year-old man with occlusion of the right M1. Non-MMD ICASO was diagnosed because the lesion was only unilateral occlusion of the M1 and no abnormal vascular network was present in the basal ganglia. The patient was also relatively elderly and had risk factors such as hypertension, so the diagnosis was atherosclerosis. **D**, Case 4 was a 74-year-old man with severe diffuse stenosis of the left internal carotid artery (ICA), occlusion of the left M1, and diffuse stenosis of the right ICA. Non-MMD ICASO was diagnosed because no abnormal vascular network was present in the basal ganglia. The patient was also relatively elderly and had undergone coronary artery bypass for angina pectoris. He also had risk factors such as hypertension, hyperlipidemia, history of smoking, and arteriosclerosis obliterans of the lower extremities, so the diagnosis was atherosclerosis.

Conclusion

The present study indicates that a particular subset of Japanese patients with ICASO without signs of MMD has a genetic variant associated with MMD. Therefore, we propose the existence of a new entity of ICASO caused by the c.14576G>A variant in *RNF213*, *RNF213* c.14576G>A variant-related ICASO, which can be differentiated from ICASO caused by atherosclerosis only by using genetic analysis. Accurate clinical diagnosis will require screening for the c.14576G>A variant in patients with ICASO.

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Disclosure

None.

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Supplemental Table

Table S1. Characteristics of Study Population and Distribution of the c.14576G>A Variant in the Study Population

Characteristics	All Patients	MMD	ICASO Non MMD	Cerebral Aneurysm	Cervical Disease	Control
No. of Patients	196	48	41	61	21	25
Age, y, mean±SD	59.8±15.9	48.4±18.7	62.3±11.3	65.6±11.1	70.4±7.8	49.8±16.1
(Range, y)	(6 – 93)	(6 – 85)	(24 – 76)	(35 – 93)	(49 – 80)	(8 – 77)
Female, n (%)	108 (55.1)	34 (70.8)	17 (41.4)	42 (68.8)	2 (9.5)	13 (52.0)
c.14576G>A genotype						
Wild type: G/G (%)	145 (74.0)	7 (14.6)	32 (78.1)	60 (98.4)	21 (100)	25 (100)
Heterozygous: G/A (%)	49 (25.0)	40 (83.3)	8 (19.5)	1 (1.6)	0	0
Homozygous A/A (%)	2 (1.0)	1 (2.1)	1 (2.4)	0	0	0

MMD indicates moyamoya disease; ICASO, intracranial main artery stenosis/occlusion; Cervical Disease, cervical carotid artery stenosis/occlusion.

Figure S1.

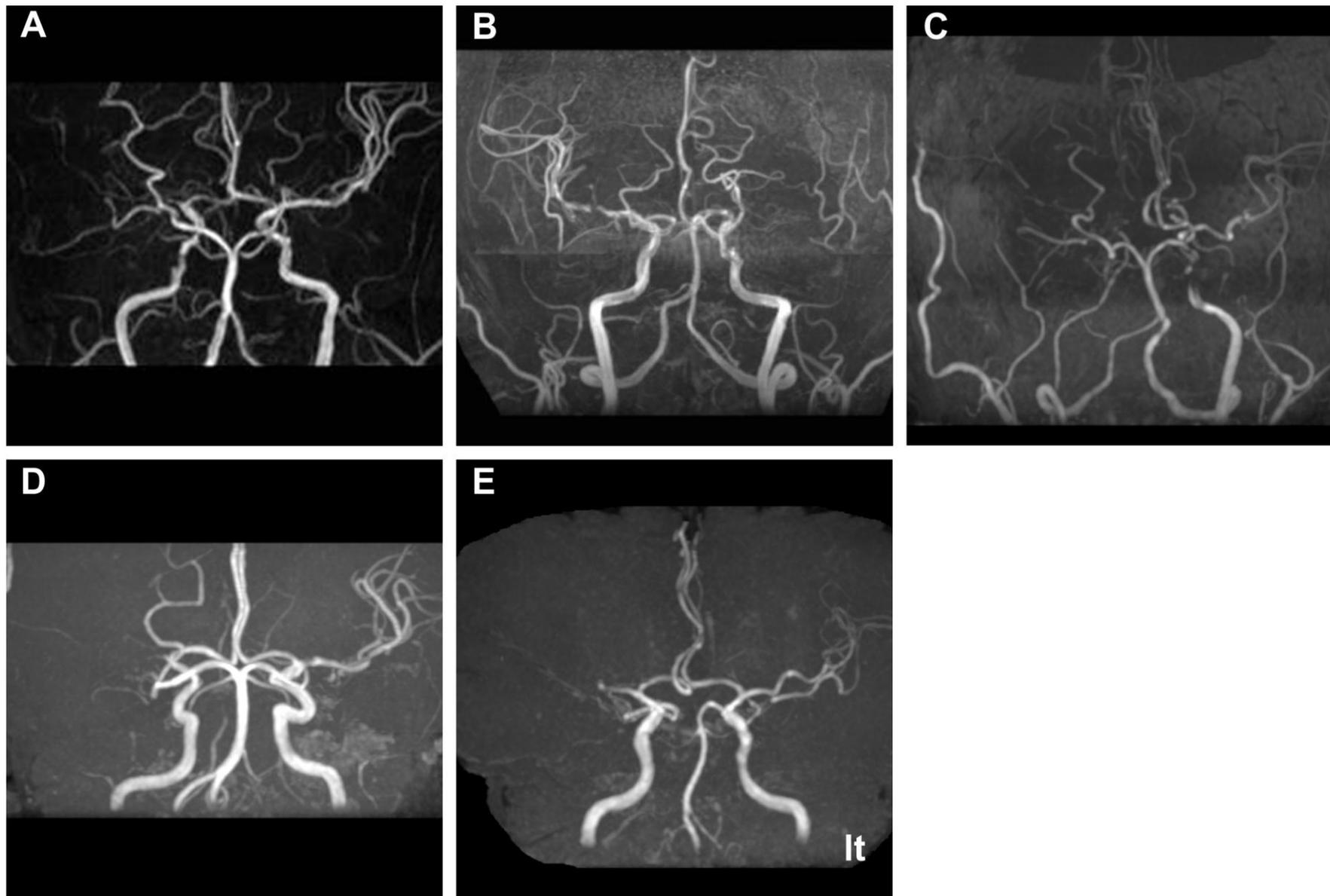


Figure Legends

FigureS1. Magnetic resonance angiography images of the other 5 patients with the c.14576G>A variant in the nonMMD ICASO group.

A: Case 5, a 61-year-old female with occlusion of the M1 segment of the right middle cerebral artery (right M1) and diffuse stenosis of the proximal right internal carotid artery (ICA). NonMMD ICASO was diagnosed as no abnormal vascular network was present in the basal ganglia. The patient was relatively elderly and had risk factors such as hypertension, so the diagnosis was atherosclerosis.

B: Case 6, a 66-year-old female with diffuse stenosis of the proximal left ICA, occlusion of the left M1, and segmental stenosis of the right M1. NonMMD ICASO was diagnosed as no abnormal vascular network was present in the basal ganglia. The patient was relatively elderly and had risk factors such as diabetes and hyperlipidemia, so the diagnosis was atherosclerosis.

C: Case 7, a 68-year-old female with diffuse stenosis of the right ICA, right M1, and proximal left ICA. NonMMD ICASO was diagnosed as no abnormal vascular network was present in the basal ganglia. The patient was relatively elderly and had risk factors such as hypertension, so the diagnosis was atherosclerosis.

D: Case 8, a 34-year-old male with occlusion of the right M1 and segmental stenosis/occlusion of the left M1. NonMMD ICASO was diagnosed as no abnormal vascular network was present in the basal ganglia. The patient was relatively young but had risk factors such as hypertension, obesity, and history of smoking, so the diagnosis was atherosclerosis.

E: Case 9, a 75-year-old male with occlusion of the right M1. NonMMD ICASO was diagnosed as no abnormal vascular network was present in the basal ganglia. The patient was elderly and had risk factors such as hypertension, diabetes, hyperlipidemia, and history of smoking, so the diagnosis was atherosclerosis.