(1) Full title

Characterization of moyamoya and middle cerebral artery diseases by carotid canal diameter and *RNF213* p.R4810K genotype

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MMD Characterized by Carotid Canal and *RNF213*

(7) Keywords

moyamoya disease; middle cerebral artery disease; bony carotid canal; *RNF213*; genotype

(8) Abbreviations

MMD moyamoyadisease

MCAD middlecerebralarterydisease

MCA middlecerebralartery

ICA internalcarotidartery

CCD carotidcanaldiameter

CT computedtomography

B-MMD bilateralmoyamoyadisease

U-MMD unilateralmoyamoyadisease

DSA digitalsubtractionangiography

ACA anteriorcerebralartery

ROC receiveroperatingcharacteristics

HR-MRI high resolution magnetic resonance imaging

Abstract

Objectives: It is sometimes difficult to differentiate middle cerebral artery disease from moyamoya disease because the two can present similarly yet have different treatment strategies. We investigated whether the presence of a narrow carotid canal and the *RNF213* mutation can help differentiate between the two phenotypes.

Population and Methods: We analyzed 78 patients with moyamoya disease, 27 patients with middle cerebral artery disease, and 79 controls from 2 facilities. The carotid canal diameter was measured using computed tomography. The p.R4810K mutation was genotyped by TaqMan assay. A receiver operating characteristics analysis was performed to assess the significance of the carotid canal diameter for the accurate diagnosis of moyamoya disease.

Results: The carotid canal diameter was significantly narrower in patients with moyamoya disease than in controls. The optimal cutoff values were 5.0 mm for adult males and 4.5 mm for adult females and children (sensitivity: 0.82; specificity: 0.92). Among the patients with middle cerebral artery disease, 18.5% and 25.0% of the affected hemispheres had the p.R4810K mutation and narrow canal (i.e., below the cutoff), respectively, whereas only 3.1% of those had both. Contrastingly, 68.8% of the affected hemispheres in patients with moyamoya disease had both these characteristics. Among the patients with moyamoya disease, those with the p.R4810K mutation tended to have narrower carotid canals.

Conclusions: Although the presence of a narrow carotid canal or the p.R4810K mutation alone could not be used to distinguish those with moyamoya disease from those with middle cerebral artery disease, the combination of these factors could better characterize the two phenotypes.

Introduction:

Moyamoya disease (MMD) and middle cerebral artery disease (MCAD) are characterized by stenoocclusive lesions of the large intracranial arteries. They are more prevalent in East Asian countries and usually present with middle cerebral artery (MCA) stenosis (1,2). MMD can be distinguished from MCAD based on the presence of stenosis in the distal ends of the internal carotid artery (ICA) and the development of fine collaterals at the base of the brain called moyamoya vessels. However, MCAD is also accompanied by moyamoya-like collaterals in some cases (3–5), and some patients with MCAD can also develop MMD (6). Likewise, some patients with MMD have typical features of MMD in one hemisphere and only stenosis in the MCA in the other hemisphere. Thus, it is sometimes difficult to differentiate between the two diseases based on radiological examinations alone. Nevertheless, it is crucial to distinguish MMD from MCAD because revascularization surgery is effective for MMD, but not for MCAD (7,8). Cerebral angiography is the gold standard for differentiating between the two, but this procedure is invasive. Thus, it would be beneficial to identify biomarkers to characterize and differentiate between these two phenotypes with less invasive modalities.

The *RNF213* p.R4810K mutation (rs112735431) has been identified as a major susceptibility gene mutation for MMD (9–11) and was found in 90%, 79%, and 23% of Japanese, Korean, and Chinese patients with MMD, respectively (11). However, the p.R4810K mutation was also shown to be associated with MCAD. Miyawaki et al. reported that the mutation was found in 23.8% of non-MMD cases involving intracranial major artery stenosis/occlusion, including MCAD (12). Similarly, Kim et al. reported that 26.7% of patients with atherosclerotic MCAD had *RNF213* mutations, including the p.R4810K mutation (13). Thus, the mutation itself cannot be a biomarker for differentiating between the two phenotypes.

On the other hand, it has been reported that the outer diameter of the MCA was significantly smaller in patients with MMD than in those with atherosclerotic MCA stenosis or occlusion (14), suggesting that the remodeling of the major intracranial arteries could be a biomarker to differentiate between these two phenotypes. However, studies investigating these aspects typically had a limited study population, and few studies have directly compared the two diseases. Therefore, it remains unelucidated whether MMD and MCAD can be distinguished based on the outer vessel diameter with a specific cutoff value.

In the present study, we investigated whether MMD and MCAD can be characterized more efficiently based on the narrow outer diameter alone or in combination with the p.R4810K mutation. We used the bony carotid canal diameter (CCD) to evaluate negative remodeling owing to several advantages of computed tomography (CT). It has good accessibility, a large amount of sequential data is available not only for cases but also for control individuals (15), and protocol for CCD measurement is simple and reproducible (15,16), which enabled inter-institutional comparison.

Study Population and Methods:

Study population

This study was approved by the Ethics Committee of the Institutional Review Board of Kyoto University (approval numbers: G1109 and G0342). We retrospectively examined patients with bilateral MMD (B-MMD), those with unilateral MMD (U-MMD), and those with MCAD who received a genotyping of the *RNF213* p.R4810K mutation and underwent CT examination between April 2009 and March 2014 at Kobe City Medical Center General Hospital and between July 2011 and January 2019 at Kyoto University Hospital. We analyzed 60 patients with B-

MMD, 18 patients with U-MMD, and 27 patients with MCAD. We also included a control group of 79 subjects whose brain CT was taken for the examination of other diseases, who had no intracranial artery stenosis, and who gave permission for p.R4810K genotyping. Among these, 25 patients with B-MMD, 6 patients with U-MMD, 18 patients with MCAD, and 39 controls were from Kobe City Medical Center General Hospital, whereas the others were from Kyoto University Hospital. We examined the following factors: age, sex, p.R4810K mutation status, the CCD, hypertension, diabetes, smoking, drinking, and family history. Individuals aged <17 years were considered children. Written informed consent was obtained from all patients and from legal guardians if the patient was aged <20 years.

Carotid canal measurement and p.R4810K genotyping

The CCD was evaluated by measuring the major axis of the horizontal part of the carotid canal using 0.3–0.6 mm thin-slice CT (Supplemental Fig. 1), as described previously (16). The carotid canal can be sectioned perpendicularly to its long axis, enabling the measurement of its maximum diameter and perpendicular diameter, but to increase the versatility of the method, our analysis was conducted by measuring the CCD along the horizontal axis of the carotid canal. The inter-rater reliability between the two examiners for this measurement method was analyzed using the intraclass correlation coefficient.

Genotyping of the p.R4810K was performed using a TaqMan® probe (Custom TaqMan® SNP Genotyping Assays; Applied Biosystems, Foster city, CA, USA) and StepOnePlus Real-Time PCR System (Applied Biosystems) according to the manufacturer's protocols after extracting DNA from whole blood using the QIAamp DNA Blood Mini Kit® (Qiagen, Valencia, CA, USA). The amino acid position of the p.R4810K was evaluated

according to NP_001243000.2, the major isoform of RNF213 lacking exon 4. This is also known as c.14429G>A (NM_001256071.2) or rs112735431 (ENST00000582970.5). This was identical to the p.R4859K or c.14576G>A used in some previous reports according to ENST00000508628.6 (17).

Phenotype definition

Both B-MMD and U-MMD were diagnosed according to the diagnostic criteria and evaluated using digital subtraction angiography (DSA). MMD was defined as either 1) the presence of stenosis or occlusion of the terminal portion of the intracranial ICA and the proximal portions of the anterior cerebral artery (ACA) and/or MCA; or 2) the development of abnormal vascular networks (moyamoya vessels) near the occlusive or stenotic lesions in the arterial phase (18). MCAD was defined as a non-MMD disease and was diagnosed based on the presence of >50% stenosis or obstruction of M1 evaluated via magnetic resonance angiography or DSA. Those who had either moyamoya-like collaterals or stenosis in the terminal portion of the ICA were not included in our patient cohort. No patient had any signs of cardiac embolism, dissection, vasculitis, or any of the other syndromes. Most patients with MCAD did not have wall irregularities due to atherosclerosis, suggesting that the etiology is not necessarily clear and some cases with MCAD may share pathophysiological background with those with MMD.

Statistical analysis

Statistical analyses were performed using the GraphPad Prism software (version 8.2.1) and JMP Pro statistical software (version 14.0.0; SAS Institute Inc., Cary, NC). Clinical characteristics were compared between cases and controls using Student’s t-test for continuous

variables and Fisher's exact test for categorical variables. A p-value of <0.05 was considered statistically significant. A receiver operating characteristics (ROC) analysis was performed to assess the significance of the CCD for the accurate diagnosis of MMD.

Results:

Reliability of the CCD measurement

The inter-rater reliability between the two examiners in the measurement of the bony internal carotid canal was assessed, and the intraclass correlation coefficients for patients with B-MMD, patients with U-MMD, and controls were found to be 0.845, 0.909, and 0.880, respectively. All of them were >0.8, indicating high reliability.

Factors affecting the CCD

To characterize the CCD, we analyzed the factors affecting it. Among controls, the CCD was significantly narrower in females than in males (mean: 5.11 mm vs. 5.75 mm, respectively, $p < 0.001$; Fig. 1A) and in children than in adults (mean: 4.80 mm vs. 5.52 mm, respectively, $p < 0.001$; Fig. 1B). The CCD also increased gradually from childhood to young adulthood. Among adults, the CCD tended to narrow with increasing age, although there was no significant difference between younger adults and older adults ($r = -0.152$, $p = 0.070$; Fig. 1C). This was also the case among patients with MMD; the CCD was significantly narrower in females than in males (mean: 4.10 mm vs. 4.45 mm, respectively, $p = 0.004$; Fig. 2A) and in children than in adults (mean: 3.93 mm vs. 4.28 mm, respectively, $p = 0.034$; Fig. 2B). The CCD in the patients with the p.R4810K mutation also tended to be narrower than in those without the mutation, albeit not statistically significant (mean: 4.19 mm vs. 4.53 mm, respectively, $p = 0.070$; Fig. 2C).

Among patients with MCAD, the CCD was not statistically different between those with and without the p.R4810K mutation (mean: 5.19 mm vs. 5.17 mm, respectively, $p = 0.939$; Fig. 2D).

Characteristics of the study population

The characteristics of the study population are shown in Table 1. Compared with the controls, the proportion of patients with the p.R4810K mutation was significantly higher among patients with B-MMD, U-MMD, and MCAD ($p < 0.001$). Both right and left carotid canals were significantly narrower in patients with B-MMD than in controls ($p < 0.001$). There was no significant difference between the right CCD and left CCD both in patients with B-MMD and in controls. When comparing the CCD between the affected and unaffected sides, the CCD on the affected side was significantly narrower in patients with U-MMD ($p < 0.001$), but not in patients with MCAD ($p = 0.292$). Hypertension was also more prevalent in patients with MCAD ($p = 0.004$), but not in patients with B-MMD or U-MMD, compared with controls. A family history of MMD was observed not only in patients with B-MMD and U-MMD but also in those with MCAD (4 of 27, $p = 0.004$ vs. controls).

Cutoff values for diagnosing MMD

Patients with B-MMD had a significantly narrower CCD than controls (mean 4.23 mm vs. 5.46 mm, respectively, $p < 0.001$). We also performed age- and sex-matched analyses because of the age and sex differences in the carotid canal and observed significant differences in the CCD between patients and controls (Supplemental Table 1). The ROC curves showed areas under the curves of 0.94 for adult males, 0.91 for adult females, and 1.00 for children. Subsequently, we aimed to determine the optimal cutoff value to distinguish between patients

with MMD and controls. First, the optimal cutoff values were calculated using the data from Kobe City Medical Center General Hospital. The values were 4.94 mm for adult males, 4.59 mm for adult females, 4.36 mm for male children, and 4.43 mm for female children (Supplemental Fig. 2). The cutoff values of 5.0 mm for adult males and 4.5 mm for adult females and children had a sensitivity of 0.94 and specificity of 0.90. The same cutoff values were applied to the replication cohort from Kyoto University Hospital, and the sensitivity and specificity were found to be 0.73 and 0.94, respectively, showing a slight reduction in sensitivity. Nevertheless, when the data from both institutions were combined, the sensitivity was 0.82 and the specificity was 0.92, confirming the robustness of this cutoff value. In fact, the cutoff values calculated directly from the combined cohort were 5.00 mm for adult males, 4.52 mm for adult females, 4.53 mm for male children, and 4.43 mm for female children (Fig. 3). Similar cutoff values were obtained by age-matched and sex-matched analyses (Supplemental Fig. 3).

Difference between B-MMD, U-MMD, and MCAD

When the left and right CCDs were represented graphically, the distribution varied depending on the groups. The carotid canal tended to be narrower on both sides in patients with B-MMD. On the other hand, the affected side was narrower in patients with U-MMD. The CCD was largely within the normal range on both sides in patients with MCAD (Fig. 4A). Compared with controls, the affected hemispheres in patients with MMD and MCAD had a significantly narrower carotid canal ($p < 0.001$ and $p = 0.035$, respectively), with the CCD in the affected hemispheres in the former being significantly narrower than in the latter ($p < 0.001$; Fig. 4B). The carotid canal in the unaffected side was significantly narrower in patients with U-MMD than in controls ($p = 0.002$), but this was not the case in patients with MCAD ($p = 0.245$; Fig 4C).

Moreover, the CCD tended to be narrower in the unaffected hemispheres of patients with MMD than in those with MCAD, but this finding was not significant ($p = 0.057$). With the cutoff values of 5.0 mm for adult males and 4.5 mm for adult females and children, carotid canal stenosis was found in the affected side in 78.9% of the patients with B-MMD and U-MMD, but only in 25.0% of the patients with MCAD. The p.R4810K mutation was found in 88.3% of the patients with B-MMD and 18.5% of the patients with MCAD, demonstrating that the CCD or the presence of the p.R4810K mutation alone cannot be used to effectively distinguish MMD from MCAD. However, when considered together, the presence of both the p.R4810K mutation and carotid canal stenosis was observed in 68.8% of the affected hemispheres in patients with MMD, but only in 3.1% of those in patients with MCAD (Table 2), indicating that patients who have both the p.R4810K mutation and a narrow carotid canal are more likely to have MMD rather than MCAD.

Discussion:

In the present study, we demonstrated the reliability of measuring the bony carotid canal, as evidenced by its high inter-rater reliability and inter-institutional reproducibility. The CCD measured via CT was a useful biomarker that distinguishes MMD and controls, with a high sensitivity of 0.82 and specificity of 0.92. Intriguingly, patients with both the p.R4810K mutation and a narrow carotid canal were predominantly patients with MMD, and only a few of them were patients with MCAD. Although the p.R4810K mutation or narrow carotid canal alone could not be used to distinguish MMD from MCAD, the combination of these two biomarkers was effective in doing so.

Narrowing of the outer vessel diameter is becoming increasingly recognized as a feature of MMD. This is most accurately measured using high resolution magnetic resonance imaging (HR-MRI), which can directly assess the negative remodeling of the arteries. Several reports on the evaluation of the outer diameter of the ICA in patients with MMD or other intracranial arterial stenoses using HR-MRI have been published in recent years (Supplemental Table 2) (1,13,14,19–23). However, a specific cutoff value to distinguish patients with MMD from normal subjects and patients with other cerebrovascular diseases is yet to be established. The consistency of data across institutes and studies also remains questionable. Due to the lack of a direct comparison between the discriminating capacity of the outer diameter of the ICA measured by HR-MRI and the CCD measured by CT, we cannot conclude which technique is superior. Our data suggests that the CCD measured via CT was useful and reliable in differentiating between patients with MMD and controls with high sensitivity and specificity. Furthermore, the mean CCD among controls was similar to the findings of many previous studies (Supplemental Table 3) (17,24,25), further supporting the reliability of the CCD.

Kim T et al. reported that deep learning using plain skull radiography, with particular attention to the lower face, can distinguish patients with MMD from controls with high accuracy (26). In MMD, the CCD measured by CT shows a high correlation with the outer diameter of the ICA evaluated by HR-MRI in our previous study (15) and therefore bone remodeling including the bony carotid canal may occur due to the narrowing of the outer diameter of the ICA.

In the assessment of the negative remodeling of the large intracranial arteries, it should be noted that the cutoff value of the CCD to distinguish patients with MMD from controls is age- and sex-dependent, as partly mentioned by Motoshima et al. (25). This would be the case with the measurement of the outer diameter of the ICA and MCA using HR-MRI. Using the age- and

sex-specific CCD cutoff values (4.5 mm for adult females and children and 5.0 mm for males), we investigated whether the presence of both a narrow carotid canal and the *RNF213* p.R4810K mutation increases the diagnostic accuracy for B-MMD, U-MMD, and MCAD. The p.R4810K mutation was significantly associated with B-MMD, U-MMD, and MCAD, which was consistent with the previous reports. The frequency of the mutation was different among the phenotypes: 88.3% in B-MMD, 66.7% in U-MMD, and 18.5% in MCAD. A narrow carotid canal was observed in 78.9% of the affected hemispheres in patients with B-MMD and U-MMD, but only in 25.0% of the affected hemispheres in patients with MCAD. These findings suggest that the presence of the p.R4810K mutation or a narrow carotid canal alone cannot be used to distinguish between MMD and MCAD. However, when taken together, 68.8% of the affected hemispheres in patients with B-MMD or U-MMD had both risk factors, whereas only 3.1% of those in patients with MCAD had both risk factors. Interestingly, only 4.3% of the affected hemispheres in patients with MMD, but 50.0% of those in patients with MCAD, had neither of the two risk factors. Although it is still difficult to distinguish between the two phenotypes among those who only have one risk factor, the presence of both the p.R4810K mutation and a narrow carotid canal can help in differentiating between the diseases.

There are several reports showing that *RNF213* affects the outer diameter of blood vessels. The p.R4810K mutation was associated with a reduced diameter in patients with MCAD (19,27), whereas there was no association between the p.R4810K mutation and the outer diameter of the large intracranial arteries in patients with MMD (21). In contrast, our data showed that the p.R4810K mutation did not affect the CCD in patients with MCAD, whereas the mutation tended to show negative correlation with the CCD in patients with MMD, albeit not statistically significant. The discrepancy in these findings may be because the change in the outer

diameter of the ICA caused by MCAD was too small to alter the CCD. Further studies are warranted to investigate the association of both the CCD and the outer diameter of the large intracranial arteries with the p.R4810K mutation among patients with MMD and MCAD.

Our speculations on the mechanism of these phenomena are as follows. The CCD and the outer diameter of the ICA are correlated. In MMD, the blood flow in the ICA decreases due to the peripheral narrowing of the ICA (including both the ACA and MCA), and thus the carotid canal can be plastically narrowed. However, because the blood flow to the ACA remains intact in MCAD, the outer diameter of the ICA and the CCD are affected to a small degree (Fig. 5). In addition, considering that the CCD was also significantly narrowed on the unaffected side in patients with U-MMD compared with controls, it is possible that a reduction in blood flow to large intracranial arteries may have already occurred prior to the development of moyamoya vessels, which may have manifested as a narrow carotid canal. In this case, the narrower diameter of the large intracranial arteries among those with the p.R4810K mutation may be a result of reduced flow demand. Interestingly, the transient occlusion of the MCA in mice increased the expression of *RNF213* in neurons, which might affect the blood flow demand of neurons (28).

The strength of the present study is that we confirmed high inter-rater reliability for CCD measurement as well as the reproducible accuracy of the cutoff value using an external validation cohort. We also performed sex- and age-specific analyses to eliminate the effect of confounding variables. However, our study also has several limitations. First, compared with HR-MRI, it may be difficult to distinguish patients with MMD from those with MCAD or controls using CT in the early stage of the disease. This is because subtle changes in the outer diameter of the ICA may not significantly alter the diameter of the bony carotid canal. Indeed, our previous study

showed a negative correlation between the disease stage of MMD and the CCD, and narrowing of the carotid canal was small in earlier stages, i.e., Suzuki's stages 0 (unaffected side of U-MMD) and 1 (15). In addition, HR-MRI would be more accurate in the assessment of outer vessel diameter, and future validation with HR-MRI is warranted. Second, the present study did not include either patients with MCAD with moyamoya-like collaterals or those with twig-like MCA. The latter phenotype shows an angioarchitecture in which a plexiform arterial network exists with a steno-occlusive MCA (29,30). Pathogenesis of twig-like MCA is supposed to be different from MMD in which normal MCA becomes progressively stenotic at the ICA terminus and/or proximal MCA, but differentiating these conditions is not necessarily easy. Patients with MMD and MCAD as well as patients with twig-like MCA have been found to be positive for the p.R4810K mutation (31), and this mutation alone is still indistinguishable. Although both MCAD with moyamoya-like collaterals and twig-like MCA are rare, the assessment of the discriminating capacity of the p.R4810K mutation combined with negative vascular remodeling could offer better disease categorization. Many of our cases with MCAD did not show atherosclerotic changes. Although they did not have either moyamoya-like collaterals or twig-like MCA, they may have similar characteristics with these conditions. Because MCAD consists of various phenotypes, further research on this disease state is warranted. Last, there is a possibility of selection bias because of a retrospective study design. To minimize the bias, we performed an age- and sex-matched analysis and the validation study using an independent cohort.

Conclusions:

The presence of a narrow CCD is a reliable indicator for the negative remodeling of the large intracranial arteries in patients with MMD. After accounting for age and sex in the cutoff values, the CCD was very effective for identifying patients with MMD. Although the CCD or the p.R4810K mutation alone cannot be used to distinguish MMD from MCAD, the combination of these factors can be used to effectively distinguish the two disease phenotypes.

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Declarations of interest:

none.

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Figure legends

Fig. 1. Effect of age and sex on the carotid canal diameter among controls

(A) Among controls, the carotid canal diameter (CCD) was significantly narrower in females than in males (mean: 5.11 mm vs. 5.75 mm, respectively, $p < 0.001$).

(B) Among controls, the CCD was significantly narrower in children than in adults (mean: 4.80 mm. vs. 5.52 mm, respectively, $p < 0.001$).

(C) Distribution of CCD according to age. The CCD tended to increase gradually from childhood to young adulthood and narrowed with increasing age in adults, but these findings were not statistically significant.

Fig. 2. Confirmation of the effect of age and sex on the carotid canal diameter in patients with bilateral moyamoya disease

(A) Among patients with bilateral moyamoya disease (B-MMD), the carotid canal diameter (CCD) was significantly narrower in females than in males (mean: 4.10 mm vs. 4.45 mm, respectively, $p = 0.004$)

(B) Among patients with B-MMD, the CCD was significantly narrower in children than in adults (mean: 3.93 mm vs. 4.28 mm, respectively, $p = 0.034$).

(C) Among patients with B-MMD, the CCD tended to be narrower in patients with the p.R4810K mutation than in those without the mutation, but this was not statistically significant (mean: 4.19 mm vs. 4.53 mm, respectively, $p = 0.070$).

(D) Among patients with middle cerebral artery disease (MCAD), the CCD was not significantly different between those with and without the p.R4810K mutation (affected hemispheres:

mean: 5.19 mm vs. 5.17 mm, respectively, $p = 0.939$; unaffected hemispheres: mean: 5.13 mm vs. 5.30 mm, respectively, $p = 0.623$).

Fig. 3. Optimal cutoff values for the carotid canal diameter to distinguish between the patients with bilateral moyamoya disease and controls.

The carotid canal diameter (CCD) was significantly narrower among patients with bilateral moyamoya disease (B-MMD) than in controls. Receiver operating characteristics analysis revealed that the area under the curve was 0.912 for adult females, 0.939 for adult males, 0.929 for female children, and 1.000 for male children. The cutoff values calculated directly from the combined cohort were 4.52 mm for adult females (A), 5.00 mm for adult males (B), 4.43 mm for female children (C), and 4.53 mm for male children (D). These are very close to the optimal cutoff values calculated using the data from Kobe City Medical Center General Hospital.

Fig. 4. Distribution of the carotid canal diameters for each disease phenotype

(A) Patients with bilateral moyamoya disease (B-MMD), unilateral moyamoya disease (U-MMD), and middle cerebral artery disease (MCAD) and controls showed distinct distributions of the right and left carotid canal diameters (CCDs). The carotid canal tended to be narrower on both sides in patients with B-MMD, but only on the affected side in patients with U-MMD. In patients with MCAD, the carotid canal was not narrow in either side, but still relatively smaller than that in controls.

(B) Compared with controls, significantly narrower carotid canals were observed in the affected hemispheres of patients with MMD and those with MCAD ($p < 0.001$ and $p = 0.035$,

respectively). The CCD was significantly narrower in the affected hemispheres of patients with MMD than in those with MCAD ($p < 0.001$).

(C) The carotid canal on the unaffected side was significantly narrower in patients with U-MMD than in controls ($p = 0.002$), but the CCD on the unaffected side was not significantly different between patients with U-MMD and those with MCAD ($p = 0.245$). The CCD tended to be narrower in the unaffected hemispheres of patients with MMD than in those with MCAD, albeit not statistically significant ($p = 0.057$).

Fig. 5. Hypothetical mechanism of carotid canal narrowing in patients with moyamoya disease

The carotid canal diameter (CCD) was smaller both in patients with moyamoya disease (MMD) and in patients with middle cerebral artery disease (MCAD) than in controls. However, the CCD was much smaller in patients with MMD than in patients with MCAD. This could be because the blood flow of the internal carotid artery (ICA) decreases due to the narrowing of the peripheral part of the ICA, including both anterior cerebral artery (ACA) and middle cerebral artery. The reduced blood flow in the ICA causes the narrowing of the outer diameter of the ICA, leading to the narrowing of the bony carotid canal (A). However, because the blood flow to the ACA remains intact in MCAD, the outer diameter of the ICA and the CCD are only minimally affected (B).

Figure 1

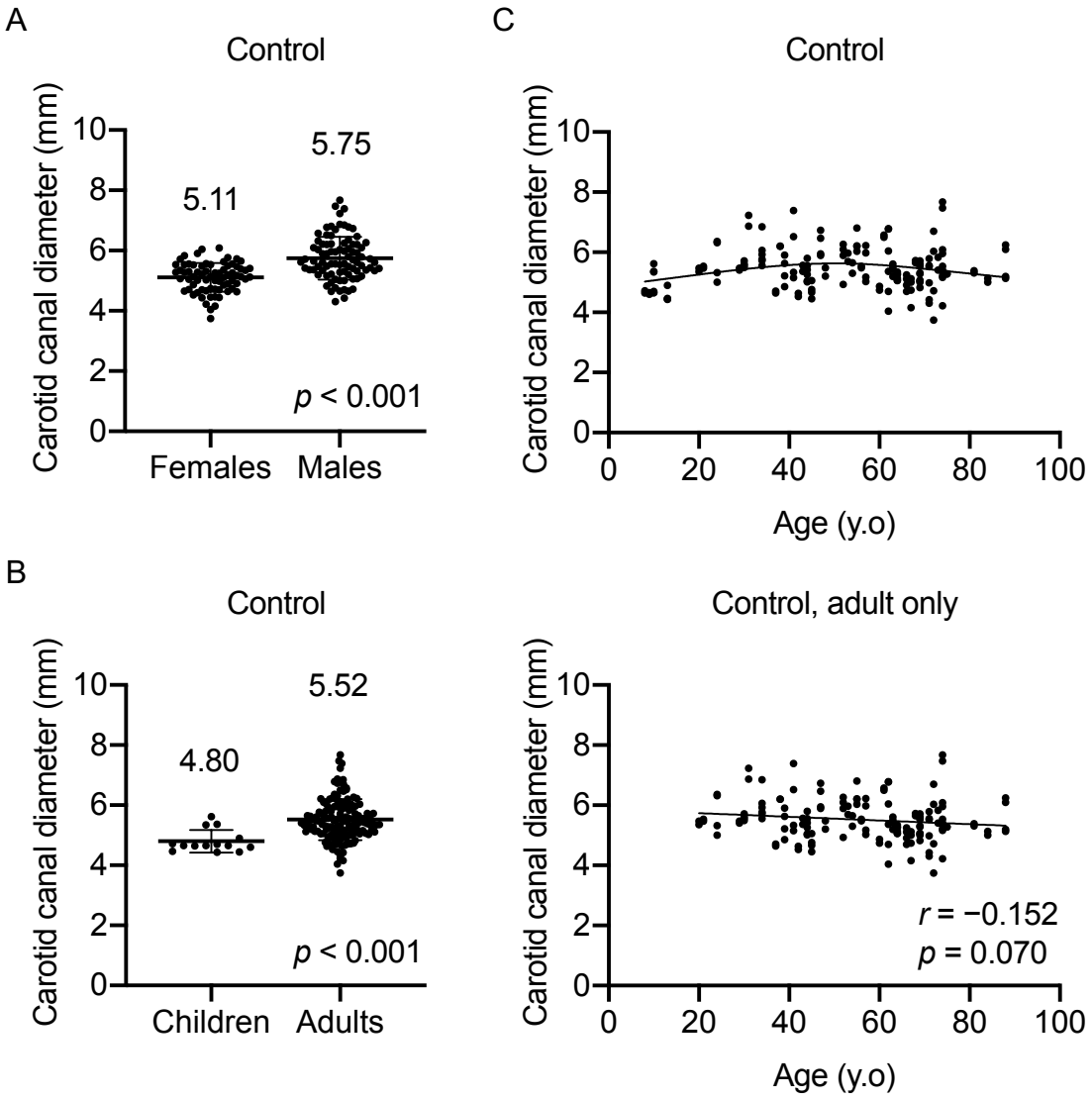

A
Control
Carotid canal diameter (mm)
10
8
6
4
2
0
5.11
5.75
p < 0.001
Females
Males
B
Control
Carotid canal diameter (mm)
4.80
5.52
p < 0.001
Children
Adults
C
Control
Carotid canal diameter (mm)
0
20
40
60
80
100
Age (y.o)
Control, adult only
Carotid canal diameter (mm)
r = −0.152
p = 0.070
Age (y.o)

Figure 2

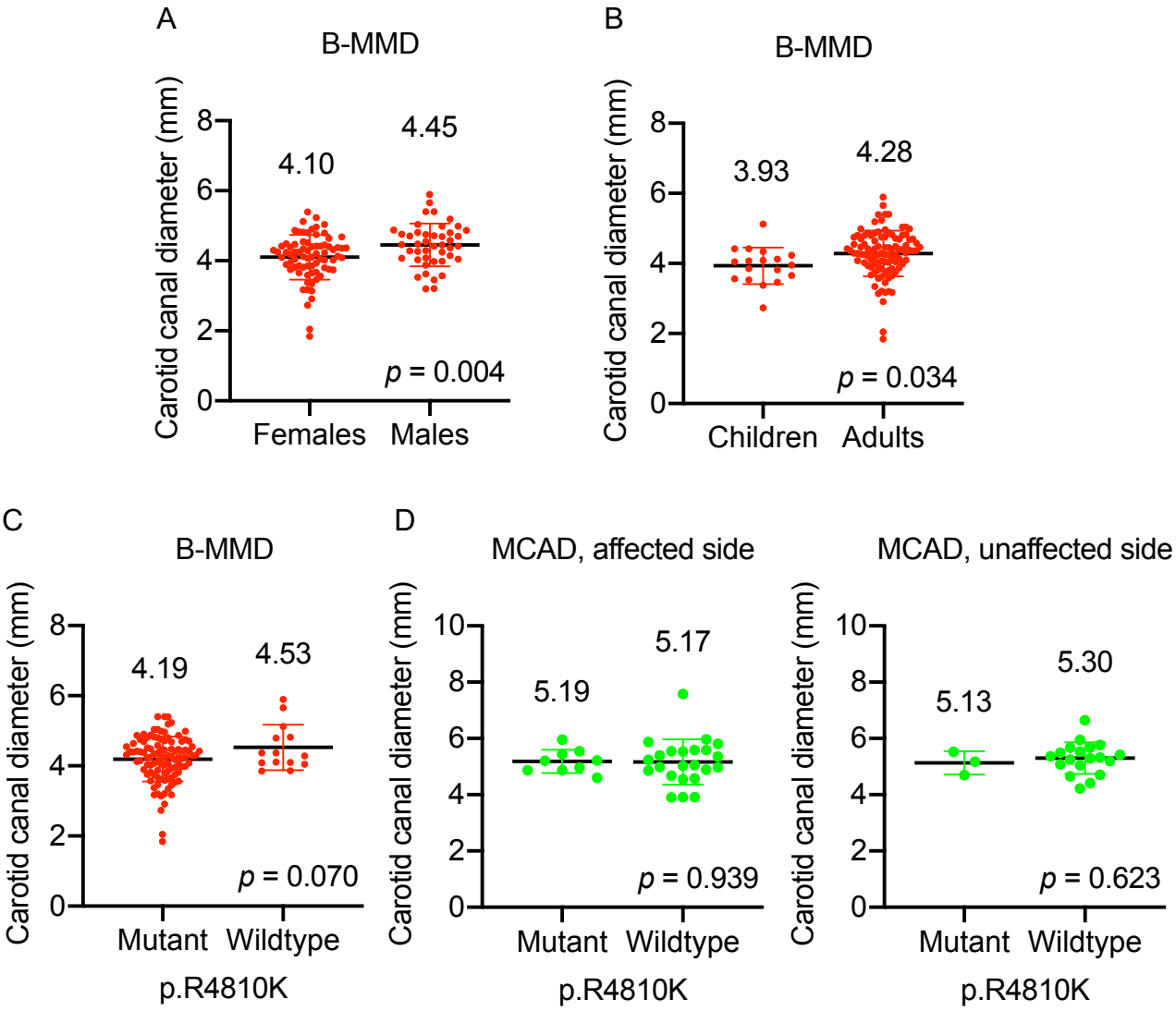

A
B-MMD
Carotid canal diameter (mm)
8
6
4
2
0
4.10
4.45
p = 0.004
Females
Males
B
B-MMD
Carotid canal diameter (mm)
3.93
4.28
p = 0.034
Children
Adults
C
B-MMD
Carotid canal diameter (mm)
4.19
4.53
p = 0.070
Mutant
Wildtype
p.R4810K
D
MCAD, affected side
Carotid canal diameter (mm)
10
8
6
4
2
0
5.19
5.17
p = 0.939
Mutant
Wildtype
p.R4810K
MCAD, unaffected side
Carotid canal diameter (mm)
5.13
5.30
p = 0.623
Mutant
Wildtype
p.R4810K

Figure 3

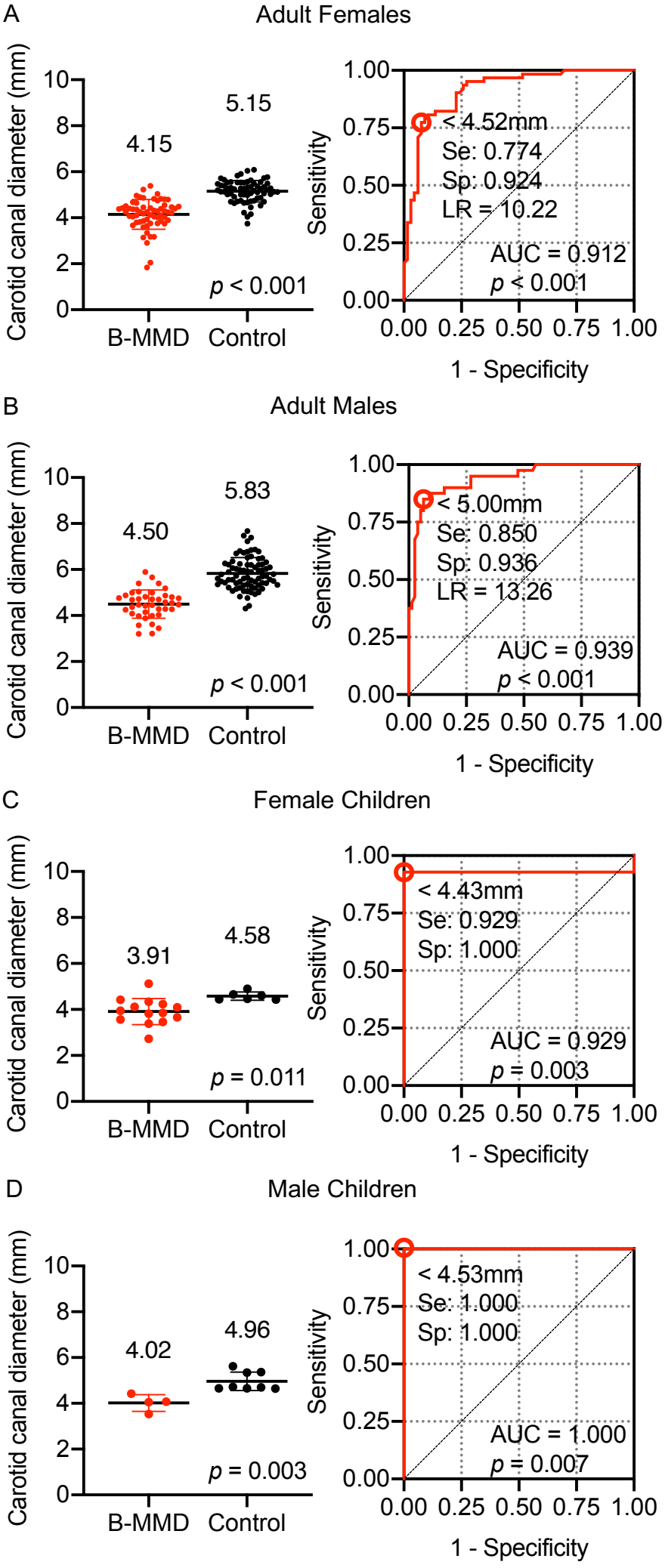

A
Adult Females
Carotid canal diameter (mm)
10
8
6
4
2
0
4.15
5.15
p < 0.001
B-MMD
Control
Sensitivity
1.00
0.75
0.50
0.25
0.00
< 4.52mm
Se: 0.774
Sp: 0.924
LR = 10.22
AUC = 0.912
p < 0.001
0.00 0.25 0.50 0.75 1.00
1 - Specificity
B
Adult Males
Carotid canal diameter (mm)
4.50
5.83
p < 0.001
B-MMD
Control
Sensitivity
< 5.00mm
Se: 0.850
Sp: 0.936
LR = 13.26
AUC = 0.939
p < 0.001
1 - Specificity
C
Female Children
Carotid canal diameter (mm)
3.91
4.58
p = 0.011
B-MMD
Control
Sensitivity
< 4.43mm
Se: 0.929
Sp: 1.000
AUC = 0.929
p = 0.003
1 - Specificity
D
Male Children
Carotid canal diameter (mm)
4.02
4.96
p = 0.003
B-MMD
Control
Sensitivity
< 4.53mm
Se: 1.000
Sp: 1.000
AUC = 1.000
p = 0.007
1 - Specificity

Figure 4

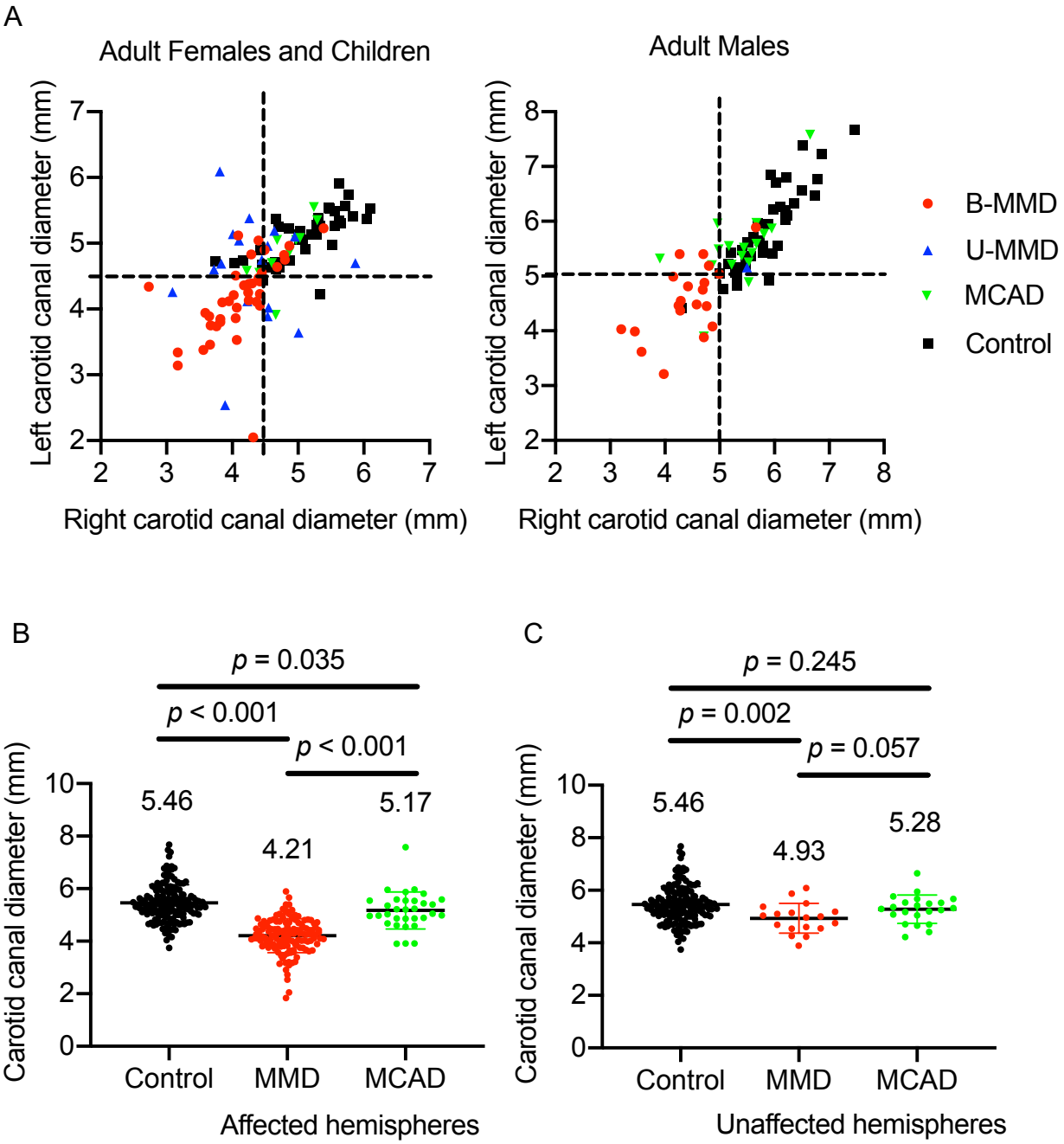

A
Adult Females and Children
Adult Males
Left carotid canal diameter (mm)
Right carotid canal diameter (mm)
B-MMD
U-MMD
MCAD
Control
B
p = 0.035
p < 0.001
p < 0.001
5.46
4.21
5.17
Carotid canal diameter (mm)
Control
MMD
MCAD
Affected hemispheres
C
p = 0.245
p = 0.002
p = 0.057
5.46
4.93
5.28
Unaffected hemispheres

Figure 5

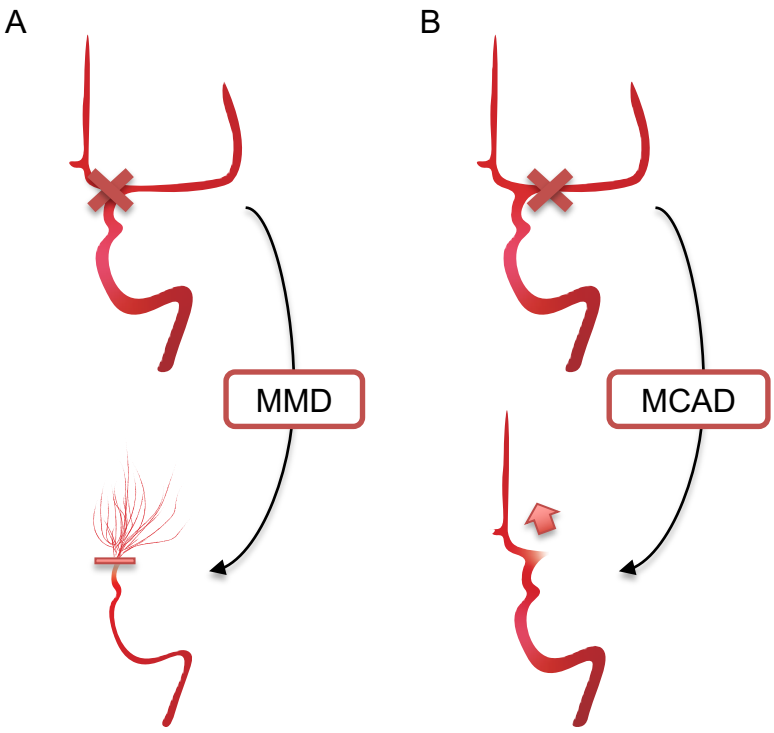


Table 1. Characteristics of the study population

	Control	B-MMD	*p*-value	U-MMD	*p*-value	MCAD	*p*-value
N	79	60		18		27	
Age, median (range)	55 (8–88)	44 (8–86)	0.013	43 (5–77)	0.307	56 (18–80)	0.318
Female, n (%)	36 (45.6)	38 (63.3)	0.041	17 (94.4)	<0.001	10 (37.0)	0.505
p.R4810K mutant, n (%)	0 (0)	53 (88.3)	<0.001	12 (66.7)	<0.001	5 (18.5)	<0.001
Right carotid canal diameter (mm), mean (SD)	5.46 (0.69)	4.18 (0.62)	<0.001	N/A		N/A	
Left carotid canal diameter (mm), mean (SD)	5.46 (0.71)	4.28 (0.68)	<0.001	N/A		N/A	
R-CCD vs L-CCD, *p*-value	0.985	0.371					
Carotid canal diameter in the affected hemisphere (mm), mean (SD)	N/A	N/A		4.08 (0.64)		5.16 (0.83) *	
Carotid canal diameter in the unaffected	N/A	N/A		4.93 (0.57)		5.28 (0.54) *	

hemisphere (mm), mean (SD)						
CCD on affected side vs CCD on unaffected side, *p*-value			<0.001		0.292 *	
Hypertension, n (%)	14/78 (17.9)	19/60 (31.7)	0.072	7/18 (38.9)	0.064	13/27 (48.1)	0.004
Diabetes mellitus, n (%)	5/79 (6.33)	7/59 (11.9)	0.363	2/18 (11.1)	0.611	5/27 (18.5)	0.119
Smoking habit, n (%)	27/76 (35.5)	14/59 (23.7)	0.187	1/17 (5.89)	0.018	12/26 (46.2)	0.358
Drinking habit, n (%)	30/76 (39.5)	15/59 (25.4)	0.100	5/17 (29.4)	0.582	12/26 (46.2)	0.646
Family history of MMD, n (%)	0/75 (0)	23/60 (38.3)	<0.001	3/18 (16.7)	0.006	4/27 (14.8)	0.004

MMD; moyamoya disease, B-MMD; bilateral moyamoya disease, U-MMD; unilateral moyamoya disease, MCAD; middle cerebral artery disease, CCD; carotid canal diameter, N/A; not available

* Measured in 22 patients with unilateral MCAD (5 patients with bilateral MCAD were excluded).

Table 2. Association of narrow carotid canal and *RNF213* mutation with moyamoya disease and middle cerebral artery disease

		MMD (affected hemispheres)	MMD (unaffected hemispheres)	MCAD (affected hemispheres)	MCAD (unaffected hemispheres)	Control
p.R4810K mutation	Canal stenosis (+)	95 (68.8%)	1 (5.6%)	1 (3.1%)	0 (0%)	0 (0%)
	No canal stenosis	23 (16.7%)	11 (61.1%)	8 (25.0%)	3 (13.6%)	0 (0%)
Wild type	Canal stenosis (+)	14 (10.1%)	2 (11.1%)	7 (21.9%)	3 (13.6%)	12 (7.6%)
	No canal stenosis	6 (4.3%)	4 (22.2%)	16 (50.0%)	16 (72.7%)	146 (92.4%)

MMD; moyamoya disease, MCAD; middle cerebral artery disease

Supplemental Table 1. Characteristics of the study population with age-matched and sex-matched analysis

	Control	B-MMD	*p*−value
N	40	40	
Age, median (range)	44.5 (9–88)	44.5 (9–86)	0.998
Female, n (%)	22 (55.0)	22 (55.0)	1.000
p.R4810K mutant, n (%)	0 (0)	34 (85.0)	<0.001
Right carotid canal diameter (mm), mean (SD)	5.47 (0.74)	4.29 (0.50)	<0.001
Left carotid canal diameter (mm), mean (SD)	5.45 (0.72)	4.26 (0.71)	<0.001
All carotid canal diameter (mm), mean (SD)	5.46 (0.73)	4.27 (0.61)	<0.001
Hypertension, n (%)	5/40 (12.5)	12/40 (30.0)	0.099
Diabetes mellitus, n (%)	1/39 (2.6)	4/39 (10.3)	0.358
Smoking habit, n (%)	15/40 (37.5)	8/39 (20.5)	0.137
Drinking habit, n (%)	16/40 (40.0)	11/39 (28.2)	0.344
Family history of MMD, n (%)	0/39 (0)	14/40 (35.0)	<0.001

MMD; moyamoya disease, B-MMD; bilateral moyamoya disease

Supplemental Table 2. The outer diameter of cranial arteries of patients with moyamoya disease or intracranial artery stenosis/occlusion in the literature (1/2)

	Reference	Imaging sequence	Disease	Group	N	Measured site	Mean (SD)	Statistical analysis	*RNF213* variant rate
1	Choi E et al, 2019	T2 proton-density–weighted images	ICAS	RNF213 variant carrier	43	MCA (disease site)	2.05 (0.63)	$p < 0.001$	1 (except for MMD)
						MCA (intact site)	2.42 (0.51)		
						Distal ICA (disease site)	2.56 (0.53)		
						Distal ICA (intact site)	2.76 (0.46)		
						BA	3.19 (0.42)	$p = 0.0010$	
				RNF213 variant non carrier	101	MCA (disease site)	2.78 (0.78)	$p < 0.001$	0
						MCA (intact site)	3.00 (0.58)		
						Distal ICA (disease site)	3.25 (0.57)		
						Distal ICA (intact site)	3.22 (0.52)		
						BA	3.53 (0.59)	$p = 0.0010$	
2	Cheng W et al, 2019	3D SPACE	MMD and ICASO	RNF213 variant	9	disease site (ACA or MCA)	N/A	N/A	1
3	Chung JW et al, 2018	T2 proton-density–weighted images	MMD (affected ICA)	<34	29	ICA (immediately after the branching Ophthalmic artery)	2.46 (0.65)	$p = 0.658$	N/A
				35-47	35		2.47 (1.14)		
				48-53	33		2.64 (0.96)		
				≥54	29		2.32 (1.09)		
				Asymptomatic	44		2.70 (1.31)	$p = 0.035$	
				Transient ischemic attack	28		2.66 (0.60)		
				Ischemic stroke	48		2.24 (0.72)		
				Intracerebral hemorrhage	6		1.81 (1.00)		
				RNF213 variant absent	41		2.58 (0.95)	$p = 0.428$	0
				RNF213 variant present	85		2.43 (1.00)		1
				Cav-1 1Q	31		1.92 (0.94)	$p < 0.001$	N/A
				Cav-1 2Q	31		2.37 (0.91)		
				Cav-1 3Q	32		2.87 (1.05)		
				Cav-1 4Q	32		2.73 (0.76)		
4	Xue S et al, 2018	3D SPACE	RNF213 R4810K variant related ICASO	variant	8	disease site (ICA or MCA)	N/A	N/A	1
5	Yu LB et al, 2016	T2 proton-density–weighted images	MMD, ICAD	MMD	12 (22 sides)	ICA (lesion site)	2.23 (0.54)	$p < 0.01$	N/A
						ICA (reference site)	3.81 (0.42)	$p = 0.058$	
						MCA (lesion site)	1.81 (0.42)	$p < 0.01$	
						MCA (reference site)	2.72 (0.13)	$p = 0.058$	
						BA	4.12 (0.62)	$p = 0.855$	
				ICAD	6 (10 sides)	ICA (lesion site)	4.80 (0.53)	$p < 0.01$	
						ICA (reference site)	5.07 (1.56)	$p = 0.058$	
						MCA (lesion site)	4.05 (0.99)	$p < 0.01$	
						MCA (reference site)	3.44 (0.27)	$p = 0.058$	
						BA	4.06 (0.69)	$p = 0.855$	
6	Kim YJ et al, 2016	proton-density–weighted images	Symptomatic MCAD	Atherosclerosis Group (AG)	45	MCA (lesion site)	2.75 (0.58)	$p < 0.0001$	4/15
				Nonatherosclerosis Group (NG)	36		1.83 (0.64)		10/16
				NG-Suspected MMD	28		1.67 (0.57)	N/A	9/13
				NG-Others	8		2.41 (0.52)		1/3

Supplemental Table 2. The outer diameter of cranial arteries of patients with moyamoya disease or intracranial artery stenosis/occlusion in the literature (2/2)

7	Kuroda S et al, 2015	3D-CISS	MMD, M1 stenosis, Control	Control	17	C1	3.8 (0.5)	n.s.	N/A
						M1	3.0 (0.3)		
						A1	2.0 (0.3)		
						BA	3.4 (0.5)		
				M1 stenosis ipsilateral	6	C1	3.8 (0.8)		
						M1	2.7 (0.4)		
						A1	1.8 (0.2)		
						BA	3.6 (0.4)		
				M1 stenosis contralateral	6	C1	3.9 (0.4)		
						M1	2.7 (0.3)		
						A1	2.1 (0.6)		
						BA	3.7 (0.4)		
				MMD Stage 0	15	C1	3.6 (0.6)		
						M1	2.7 (0.5)		
						A1	1.6 (0.5)		
						BA	2.9 (0.6)		
				MMD Stage 1-6	91	C1	2.3 (0.7)	$p < 0.01$	
						M1	1.3 (0.5)		
						A1	1.0 (0.4)		
						BA	3.1 (0.6)	n.s.	
				MMD Stage 1	1	C1	2.7	n.s.	
						M1	2.1		
						A1	1.1		
						BA	3.2		
				MMD Stage 2	3	C1	2.8 (0.3)	$p < 0.01$	
						M1	2.3 (0.4)	n.s.	
						A1	1.6 (0.2)		
						BA	3.3 (0.2)		
				MMD Stage 3	34	C1	2.4 (0.6)	n.s.	
						M1	1.4 (0.4)	$p < 0.01$	
						A1	1.1 (0.4)		
						BA	2.9 (0.6)	n.s.	
				MMD Stage 4	18	C1	2.7 (0.7)	n.s.	
						M1	1.3 (0.4)		
						A1	1.0 (0.6)		
						BA	3.2 (0.6)		
				MMD Stage 5	26	C1	1.9 (0.5)	$p < 0.01$	
						M1	1.1 (0.4)	n.s.	
						A1	0.9 (0.3)		
						BA	3.3 (0.5)		
				MMD Stage 6	9	C1	1.6 (0.4)	n.s.	
						M1	1.2 (0.4)		
						A1	0.8 (0.3)	$p < 0.01$	
						BA	3.3 (0.6)	n.s.	
8	Kim YJ et al, 2013	proton-density–weighted images	Symptomatic MCA stenosis	MMD	12	MCA (lesion site)	1.61 (0.43)	$p < 0.0001$	N/A
				ICAD	20		3.03 (0.53)		

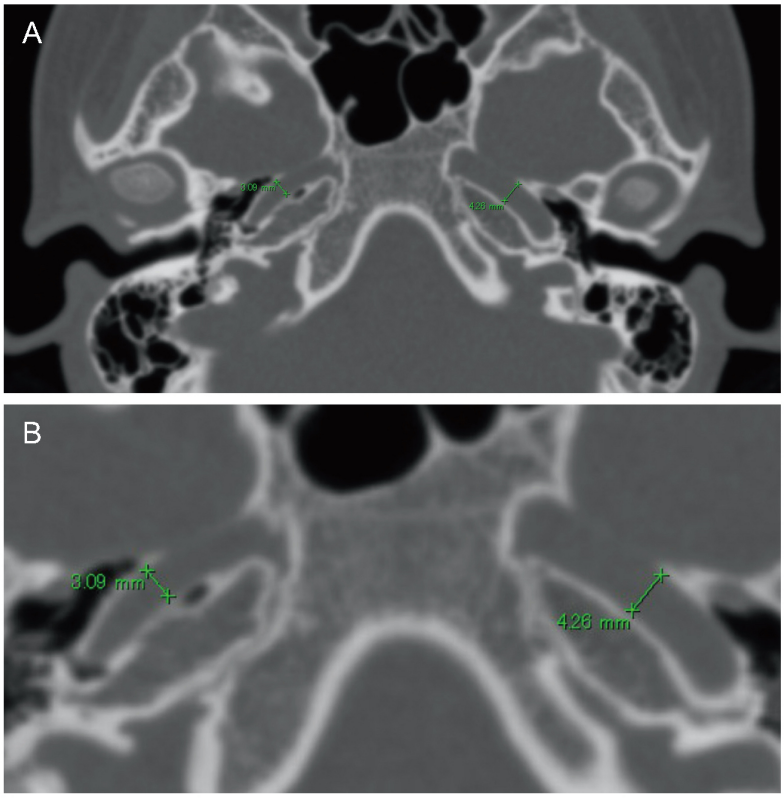
MMD; moyamoya disease, ICAS; intracranial atherosclerotic stroke, ICASO; intracranial major artery stenosis/occlusion, ICAD; intracranial atherosclerotic disease, MCAD; middle cerebral artery disease, ICA; internal carotid artery, MCA; middle cerebral artery, ACA; anterior cerebral artery, BA; basilar artery, N/A; not available, n.s.; not significant

Supplemental Table 3. The carotid canal diameter of patients with moyamoya disease in the literature

	Reference	Disease	Group	N	Measured position	Mean (SD)	Statistical analysis, compared with Control	*RNF213* variant rate
1	Faheem N et al, 2019	MMD, Control	MMD	45	R-CCD	4.802 (0.861)	Significant difference (95%CI was used)	N/A
				45	L-CCD	4.693 (0.899)		
			Control	45	R-CCD	5.410 (0.765)	N/A	
				45	L-CCD	5.457 (0.678)		
2	Motoshima S et al, 2012	MMD, Control	MMD total	35	CCD	4.7 (0.6)	$p < 0.01$	N/A
			MMD ≤15	16		4.4 (0.5)		
			MMD >15	19		5.0 (0.6)		
			Control total	35		5.6 (0.6)	N/A	
			Control ≤15	16		5.5 (0.7)		
			Control >15	19		5.7 (0.5)		
3	Watanabe A et al, 2010	MMD, Control	MMD	11	CCD	3.31 (0.44)	$p < 0.01$	N/A
			Control	60		5.27 (0.62)	N/A	
4	Oichi Y et al, 2022 (present study)	MMD, MCAD, Control	B-MMD	60	R-CCD	4.18 (0.62)	$p < 0.001$	0.883
					L-CCD	4.28 (0.68)		
			U-MMD	18	CCD on affected side	4.46 (0.68)	$p < 0.001$	0.667
					CCD on unaffected side	4.63 (0.71)	$p = 0.002$	
			MCAD	27	CCD on affected side	5.15 (0.57)	$p = 0.007$	0.185
					CCD on unaffected side	5.28 (0.69)	$p = 0.245$	
			Control	79	R-CCD	5.46 (0.69)	N/A	0
					L-CCD	5.46 (0.71)		

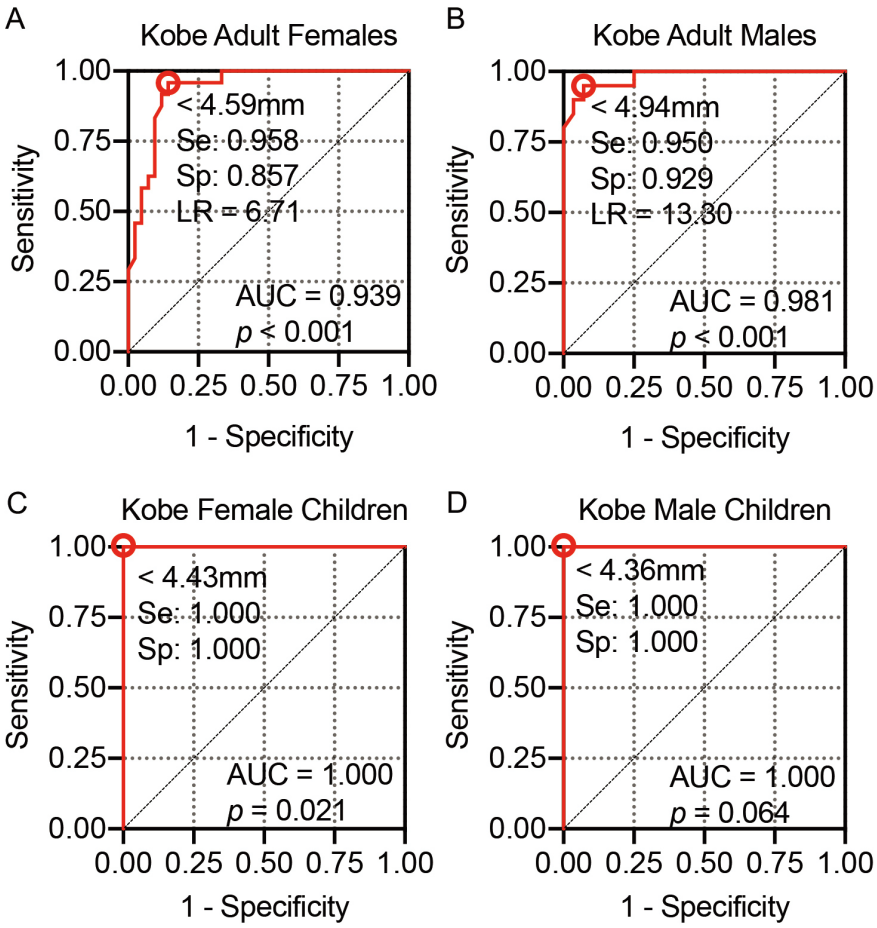
MMD; moyamoya disease, B-MMD; bilateral moyamoya disease, U-MMD; unilateral moyamoya disease, MCAD; middle cerebral artery disease, CCD; carotid canal diameter, N/A; not available

Supplemental Fig. 1. The representative images showing the measurement of the carotid canal diameter



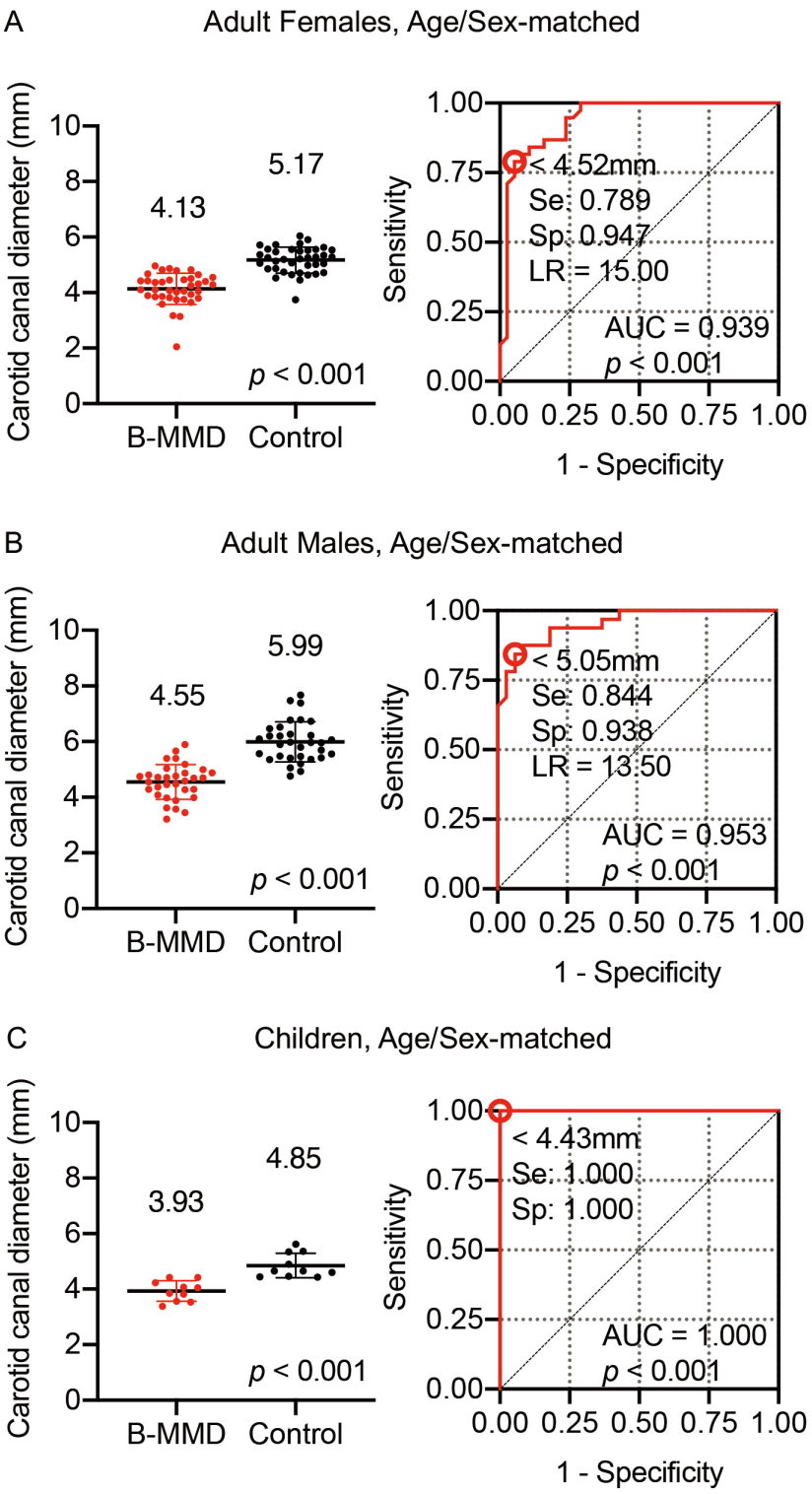
The carotid canal diameter was evaluated by measuring the major axis of the horizontal part of the carotid canal using thin slice CT. (A) A representative CT image of a patient with unilateral moyamoya disease. The carotid canal is narrower on the affected (right) side than on the unaffected (left) side (3.09 mm vs 4.26 mm). (B) The enlarged image.

Supplemental Fig. 2. Optimal cutoff values for the carotid canal diameter to distinguish between the patients with bilateral moyamoya disease and controls calculated using the data from Kobe City Medical Center General Hospital



The carotid canal diameter was compared between patients with bilateral moyamoya disease and controls using the data from Kobe City Medical Center General Hospital. (A) adult females, (B) adult males, (C) female children, and (D) male children. The optimal cutoff values were around 4.5 mm for adult females, and around 5.0 mm for adult males.

Supplemental Fig. 3. Optimal cutoff values for the carotid canal diameter to distinguish between patients with moyamoya disease and age-matched and sex-matched controls



The carotid canal diameter was significantly narrower in patients with bilateral moyamoya disease than in controls, and the receiver operating characteristics analysis revealed that the area under the curve was 0.939 for adult females (A), 0.953 for adult males (B), and 1.000 for children (C). The optimal cutoff values were 4.52 mm for adult females, 5.05 mm for adult males, and 4.42 mm for children. These results were similar to Fig. 3.