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Moyamoya disease, 2nd Edition

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Editorial: Moyamoya disease

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KEYWORDS

moyamoya disease (MMD), cerebral vascular disease, stroke, EC-IC bypass, hemodynamics

Editorial on the Research Topic
Moyamoya disease

Moyamoya disease is a rare cause of stroke characterized by progressive stenosis of the terminal carotid arteries and compensatory collaterals. In the last decade, new techniques in clinical contrast-enhanced imaging have allowed for a more accurate and useful diagnosis using CT, MRI, and DSA images. However, Moyamoya disease remains to this day an unknown etiology, mainly because diagnosis is based on the exclusion of all other possible underlying medical conditions.

This Research Topic covers some recent investigations in research on Moyamoya disease, covers original research, and reviews at the physiological, etiology, and surgical treatment levels. It contains 10 articles, including 3 reviews.

Mineharu and Miyamoto present a review focused on RNF213/Mysterin and GUCY1A3 and their strong linker, calcineurin/NFAT signaling, and caveolin to understand the pathophysiology of moyamoya disease. Although intimal thickening with fibrosis and damaged vascular smooth muscle cells are the distinguishing features of moyamoya disease, the origin of the fibrous tissue and the mechanism of smooth muscle cell damage remains not fully elucidated. The review also points out that endothelial cells and smooth muscle cells have long been a focus of interest, but other vascular components such as immune cells and the extracellular matrix also need to be investigated in future studies.

Moyamoya disease is a complex and incompletely-understood cerebrovascular pathological entity that requires thorough clinical and imaging evaluation. Larson et al. described their institution's implementation of, rationale for, and experience with a comprehensive multidisciplinary collaboration and evaluation strategy for adult patients with moyamoya.

A major difficulty in treating moyamoya is the lack of effective methods to detect novel or progressive disease prior to the onset of disabling stroke. More importantly, a tool to better stratify operative candidates and quantify response to therapy could substantively complement existing methods. Sesen et al. present proof-of-principle data supporting the use of urinary biomarkers as diagnostic adjuncts in pediatric moyamoya patients. The authors summarized that urinary proteins are useful predictors of the presence of moyamoya and may provide a basis for a novel, non-invasive method to identify new disease and monitor known patients following treatment.

Fox et al. present a review of the pathological features of the stenosis associated with MMD. Neointimal hyperplasia, disruption of the internal elastic lamina, and medial attenuation, which ultimately lead to progressive decreases in both luminal and external arterial diameter. The authors summarize the molecular pathways which have been implicated in the pathophysiology of stenosis in MMD with functions in cellular proliferation and migration, extracellular matrix remodeling, apoptosis, and vascular inflammation. The author also raised several questions for further investigation.

In the study by Hurth et al., the clinical value of early postoperative computed tomographic angiography (CTA) after direct extracranial-intracranial (EC-IC) bypass surgery in moyamoya patients was investigated. The authors summarized that early postoperative CTA has a high predictive value to confirm the patency of a bypass. On the other hand, a high false positive rate of (according to CTA) occluded bypasses after direct EC-IC bypass surgery can be seen.

Lucia et al. presented research aimed at characterizing the cases of bypass failure and repeat revascularization at a single center. A rescue surgery should be considered in those with neurological symptoms and decreased CVRC. Intermediate flow bypass using a radial artery graft is a reliable technique for patients requiring repeat revascularization.

In the study by Chen et al., A bibliometric analysis to examine the development of and research trends in MMA research was carried out. The research trends of global scientific research on MMA over the past decade were systematically analyzed. The study can provide guidance for scholars who want to understand current trends in research in this area and new research frontiers.

Inflammation has been shown to play a pivotal role in the pathogenesis of moyamoya disease. Ma et al. investigated the relationship between Platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR) in Chinese patients with newly diagnosed MMD. In patients with MMD, there seemed to be a positive association between PLR and NLR. This may help to further explain the role of inflammation in the occurrence and development of MMD.

In the study by Zheng et al., the hemodynamic changes using ultrasound according to digital subtraction angiography (DSA) findings and the association between ultrasound parameters and clinical symptoms of moyamoya disease (MMD) were investigated. The authors evaluated Hemodynamic parameters of the extracranial internal carotid artery (EICA) and posterior cerebral artery (PCA) by ultrasound, and they found that Ultrasound parameters were related to DSA findings, ultrasound may be useful in predicting the clinical symptoms of patients with MMD.

Ye et al. presented a study aimed to investigate the effectiveness and safety of antiplatelet therapy compared with conservative treatment and surgical revascularization in ischemic MMD patients. Antiplatelet agents were effective and safe in preventing further cerebral ischemic attacks in adult patients with ischemic MMD. They may be a replacement therapy for patients with surgical contraindications and patients prior to revascularization.

Author contributions

The author confirms being the sole contributor of this work and has approved it for publication.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Efficacy and Safety of Antiplatelet Agents for Adult Patients With Ischemic Moyamoya Disease

Fei Ye[1,2†], Jiaoxing Li[1†], Tianzhu Wang[3,4†], Kai Lan[5,6†], Haiyan Li[7], Haoyuan Yin[8], Tongli Guo[9], Xiong Zhang[10], Tingting Yang[11], Jie Liang[1,2], Xiaoxin Wu[1], Qi Li[3,4] and Wenli Sheng[1,2*]*

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Background: The use of antiplatelet agents in ischemic moyamoya disease (MMD) is controversial. This study aimed to investigate the effectiveness and safety of antiplatelet therapy compared with conservative treatment and surgical revascularization in ischemic MMD patients.

Methods: Ischemic MMD patients were retrospectively enrolled from eight clinical sites from January 2013 to December 2018. Follow-up was performed through clinical visits and/or telephone interviews from first discharge to December 2019. The primary outcome was the episodes of further ischemic attacks, and the secondary outcome was the individual functional status. Risk factors for future stroke were identified by the LASSO-Cox regression model. Propensity score matching was applied to assemble a cohort of patients with similar baseline characteristics using the *TriMatch* package.

Results: Among 217 eligible patients, 159 patients were included in the analyses after a 1:1:1 propensity score matching. At a mean follow-up of 33 months, 12 patients (7.5%) developed further incident cerebral ischemic events (surgical:antiplatelet:conservative = 1:3:8; $p = 0.030$), 26 patients (16.4%) developed a poor functional status (surgical:antiplatelet:conservative = 7:12:7; $p = 0.317$), and 3 patients (1.8%) died of cerebral hemorrhage (surgical:antiplatelet:conservative = 1:2:0; $p = 0.361$). The survival curve showed that the risk of further cerebral ischemic attacks was lowest with surgical revascularization, while antiplatelet therapy was statistically significant for preventing recurrent risks compared with conservative treatment ($\chi^2 = 8.987$; $p = 0.011$). No significant difference was found in the functional status and bleeding events. The LASSO-Cox regression model revealed that a family history of MMD (HR = 6.93; 95% CI: 1.28–37.52; $p = 0.025$), a past history of stroke or transient ischemic attack

(HR = 4.35; 95% CI: 1.09–17.33; $p = 0.037$), and treatment (HR = 0.05; 95% CI: 0.01–0.32; $p = 0.001$) were significantly related to the risk of recurrent strokes.

Conclusions: Antiplatelet agents were effective and safe in preventing further cerebral ischemic attacks in adult patients with ischemic MMD. They may be a replacement therapy for patients with surgical contraindications and for patients prior to revascularization.

Keywords: Moyamoya disease, antiplatelet agents, efficacy, safety, recurrent stroke prevention

INTRODUCTION

Moyamoya disease (MMD) is an unusual cerebrovascular disease characterized by progressive steno-occlusive changes in the distal internal carotid artery (ICA) and its major branches with the development of an abnormal vascular network at the base of the brain visible on angiography (1). The Asian population has a bimodal age of presentation and is classified into two major categories: ischemic type and hemorrhagic type (2). Although current evidence has demonstrated the epidemiological and clinical characteristics of MMD, the cause of this disease is not well-known (3–8). Currently, revascularization surgery is recommended as a standard treatment for MMD patients to prevent future strokes (9). However, there are still many MMD patients who are not willing to undergo revascularization or have surgical contraindications. Consequently, it is necessary to identify a replacement therapy.

Antiplatelet agents are considered to be a vital tool in acute ischemic stroke and transient ischemic attack (TIA) management for reducing and preventing recurrent stroke risks (10, 11). To date, limited reports have focused on antiplatelet therapy for MMD, and no randomized controlled trial has suggested a lack of strongly supporting evidence indicating the benefits of this therapy. In addition, there is still controversy regarding the use of antiplatelet agents among ischemic MMD patients. Many non-Asian experts advise their use for improving microcirculation and preventing recurrent strokes, while most Asian physicians hold the opposite view, namely, that they are useless in improving blood supply and carry the potential risk of hemorrhage (12, 13). Thus, we conducted this study to evaluate the effectiveness and safety outcomes of treatment with antiplatelet agents compared with conservative treatment and surgical revascularization in ischemic MMD patients.

MATERIALS AND METHODS

Study Design

This study was a multicenter retrospective cohort study involving adult patients with ischemic MMD who took an oral antiplatelet agent, underwent surgical revascularization, and received conservative management between January 2013 and December 2018 in eight Chinese teaching hospitals. The study was approved by the independent ethics committee of each participating teaching hospital [(2020)137]. Patient informed consent was waived due to the use of deidentified data by the clinical research review board. This study was carried out on the basis of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement (14).

Patient Selection

Patients with a clinical diagnosis of MMD were identified from the national health care system using the diagnosis-specific codes by the department of medical records in each participating hospital, including International Classification of Disease Ninth revision (ICD-9) code 437.5 and ICD-10 code I67.5. Cerebral angiography was carried out for all the patients. The diagnostic criteria for definitive or probable MMD were based on the 2012 Tokyo guideline (9, 15): (1) stenosis and/or occlusion of the terminal portion of the ICA and the proximal portion of the anterior cerebral artery (ACA) and/or middle cerebral artery (MCA), (2) abnormal vascular networks near the lesion, and (3) unilateral or bilateral involvement. Patients were eligible for inclusion in this study if they met the following criteria: (1) aged 18 or older, (2) clinical presentations of TIA or cerebral infarction, (3) no history of prior antiplatelet agents or neurosurgery, and (4) complete clinical data available. Patients who were diagnosed with moyamoya syndrome, were lost to follow-up, refused to participate, died before discharge, or had malignant tumors were excluded.

Covariates

Electronic medical records were carefully reviewed, including hospital charts, clinic notes, operative notes, radiographic data, and therapeutic medications. All the clinical data were retrospectively collected by the data coordinators at each participating hospital in December 2019. The baseline characteristics mainly included age at symptom onset, age at diagnosis, sex, vascular risk factors (hypertension, diabetes mellitus, hyperlipidemia, active smoking, and alcohol consumption), a past history of stroke or TIA, a family history of MMD, a modified Rankin Scale (mRS) score (16), angiography findings (bilateral steno-occlusive change, Suzuki classification, and intracranial aneurysm), and clinical manifestation (TIA, lacunar infarction, and cerebral infarction). In this study, if a patient presented with an initial symptom of TIA, we did not add it to the past history of TIA. Although the RNF213 gene polymorphism and cerebral perfusion changes can influence the prognosis, these factors were excluded because they are not routine examinations in clinical practice (17, 18).

Treatment

There are currently no clear guidelines for surgical revascularization in the treatment of MMD, but it holds a comparatively higher level of evidence than non-surgical treatment (9). Thus, randomized assignment is not applicable and unethical in clinical practice. In this study, surgical revascularization was recommended to patients with a non-emergency status, markedly ischemic symptoms, and no surgical contraindications, while non-surgical treatment was recommended to patients with mild ischemic symptoms and/or surgical contraindications. The treatment choice was independently determined by patients themselves based on their obtained medical information about the benefits and risks of different treatments. Treatments were classified into antiplatelet therapy, conservative treatment, and surgical revascularization. The treatment for patients who received antiplatelet therapy included oral aspirin 100 mg daily, oral clopidogrel 75 mg daily, and combined aspirin and clopidogrel for the first 3 weeks, followed by aspirin daily. The treatment for patients who underwent surgical revascularization procedures mainly includes direct bypass, indirect bypass, and combined bypass. Patients who had undergone surgical treatment but not surgical revascularization were excluded in this study. The conservative treatment group also included untreated participants and participants who received other medical management.

Clinical Follow-Up

The follow-up was performed through clinical visits and/or telephone interviews from first discharge to December 2019. If patients could not be contacted in December 2019, the follow-up was determined from their last clinical visit. In this study, the primary outcome was episodes of further ischemic attacks. It was defined as an acute focal infarction of the brain or retina, including sudden onset of a new focal neurological deficit lasting 24 h or more with clinical and/or imaging evidence of infarction, sudden onset of a new focal neurological deficit lasting <24 h, or rapid worsening of an existing focal neurological deficit lasting more than 24 h but accompanied by new ischemic changes on MRI or CT of the brain (19). The secondary outcome was the individual functional status assessed by the mRS score. mRS scores ≤ 2 were regarded as a good outcome, and scores >2 were regarded as a poor outcome. The safety outcome was evaluated according to episodes of serious bleeding events based on the definition of the Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries (GUSTO) (20). The follow-up period was defined as the time from initial discharge to the main outcome or December 2019. All reported clinical outcomes were confirmed by data coordinators who were unaware of the study-group assignments.

Sample Size

The G*Power software (*version* 3.1.9.7) was applied to determine the sample size for the current study (21). Calculations were performed for three groups according to the initial treatment choice. The statistical program was set to the *F* family of tests, to a one-way analysis of variance (ANOVA), and to the "A Prior" power analysis necessary to identify sample size (22). The effect size, type I error (α), and statistical power (1 – β) were set at 0.25, 0.05, and 0.80, respectively. Based on the setup above, the total sample size was calculated to be at least 159 patients with an actual power of 0.805 (**Figure 1**).

Statistical Analysis

Propensity-score matching (PSM) was widely used to adjust the between-group variations and remove the biases for non-randomized trials with two treatment alternatives (23). The propensity score (PS) is a conditional probability of receiving a particular treatment, which is calculated by a non-parsimonious multivariable logistic regression model with the treatment choice as the dependent variable and all baseline characteristics as covariates (23). In this study, a 1:1:1 matching was performed with an optimal matching algorithm and a caliper width equal to 0.25 of the standard deviation of the logit of the PS using *TriMatch* package (23, 24). In detail, the PS is estimated by logistic regression separately for all possible pairs of three groups. Each member of the antiplatelet group is matched to all surgical groups

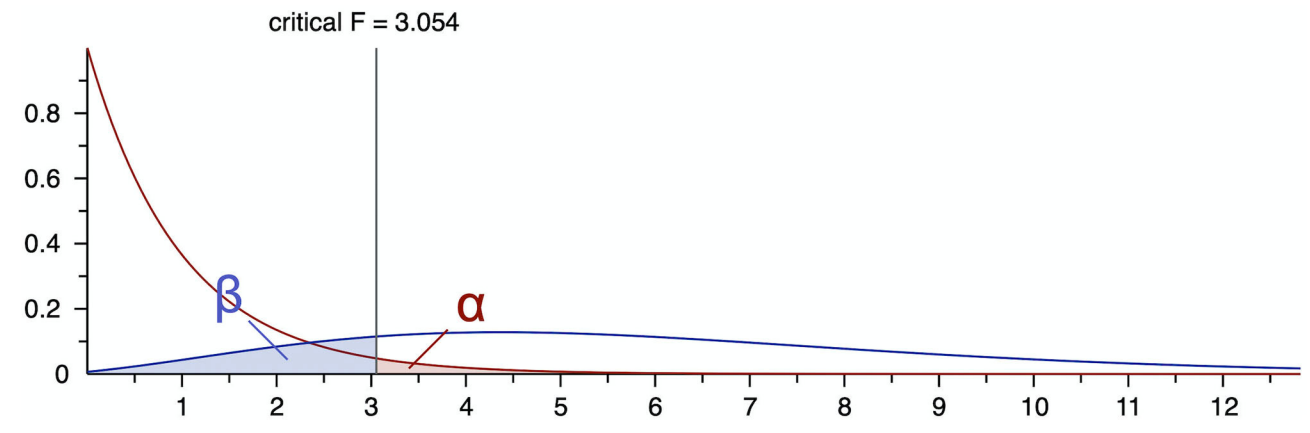

FIGURE 1 | The sample size for this study based on the G*Power software.

within a pre-specified caliper, and each member of the antiplatelet group is also matched to all conservative groups within the caliper. Those patients who do not have any match are removed from the sample. The PS distance is also calculated for all possible pairs between the remaining conservative group and surgical group members. The pairs with a PS distance greater than the caliper value are further eliminated. Only the unique triplets with the smallest total PS distance are finally retained in the match sample. Additionally, the standardized differences were estimated for all the baseline covariates before and after matching to assess the pre-match imbalance and post-match balance, which indicated a relatively good balance with an acceptable range of <25.0% (23).

In the matched cohort, continuous variables were reported as the median ± standard deviation. Proportions were calculated for categorical variables. ANOVA was used to analyze continuous variables. Pearson's chi-square test was used to analyze categorical variables. Univariate Cox regression analyses were performed to estimate the comparative risks of primary and secondary outcomes. Pre-specified subgroup analysis was

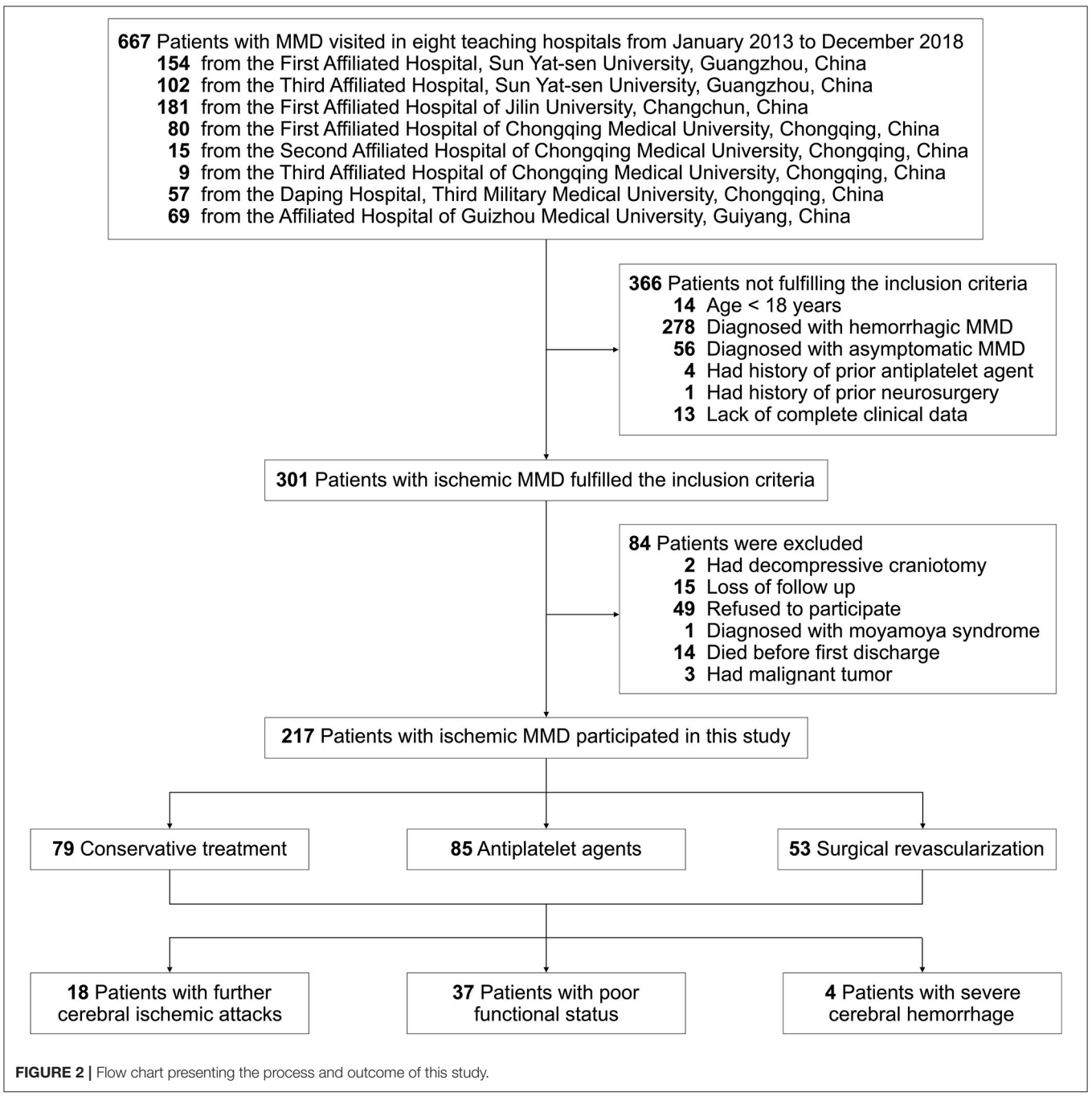

FIGURE 2 | Flow chart presenting the process and outcome of this study.

performed on the basis of type of antiplatelet therapy (aspirin, clopidogrel, and combined antiplatelet agents). Tests for interaction were performed to assess for heterogeneity of treatment effect among subgroups. Additionally, the least absolute shrinkage and selection operator (LASSO) method was used to identify major covariates associated with future strokes from all the covariates. The major covariates were filtered via non-zero coefficients. Risk factors were then selected by multivariate Cox regression analysis using a forward stepwise method. These selected risk factors were reported as hazard ratios (HRs) with 95% confidence intervals (CIs) and *p*-values.

All statistical analyses were performed with the R Project for Statistical Computing (*version 4.0.2*) and Statistical Program for Social Science (SPSS) Statistics (*version 26*). The significance level was set at 0.05 in all tests.

RESULTS

Baseline Characteristics

This study was carried out according to the flow chart shown in **Figure 2**. A total of 667 MMD patients were identified at the eight Chinese teaching hospitals from January 2013 to December 2018. After applying the inclusion and exclusion criteria, 217 patients with ischemic MMD were enrolled in this study. All the baseline characteristics of these patients are summarized in **Table 1**. The average ages at symptom onset and diagnosis were 46 ± 12 and 47 ± 11 years, respectively. The female-to-male ratio was 0.9 (100:117). Vascular risk factors were noted in 143 patients (65.9%), including 45.6% with hypertension, 19.8% with diabetes mellitus, 7.8% with hyperlipidemia, 33.6% with active smoking, and 27.2% with alcohol consumption. The family history of MMD was approximately 5.5%. A past history of stroke or TIA was identified in 27 (12.4%) patients. The most common initial symptoms were cerebral infarction (61.3%) and TIA (32.7%). On angiography, most patients had bilateral steno-occlusive changes with Suzuki stage III–VI, but nearly 10% of them had intracranial aneurysms. Among them, 85 (39.2%) patients received antiplatelet therapy: 47 were treated with oral aspirin 100 mg daily, 18 with oral clopidogrel 75 mg daily, and 20 with combined aspirin and clopidogrel for the first 3 weeks, followed by aspirin daily. Fifty-three (24.4%) patients had undergone surgical revascularization procedures. The remaining 79 patients (36.4%) were conservatively treated.

TABLE 1 | Baseline characteristics of the 217 patients with ischemic moyamoya disease.

Characteristic	All ($n = 217$)
Age at symptom onset (years)	46 ± 12
Age at diagnosis (years)	47 ± 11
Female-to-male ratio	0.9 (100:117)
Vascular risk factors	
Hypertension	99 (45.6%)
Diabetes mellitus	43 (19.8%)
Hyperlipidemia	17 (7.8%)
Active smoking	73 (33.6%)
Alcohol consumption	59 (27.2%)
Past history of stroke or TIA	27 (12.4%)
Family history of MMD	12 (5.5%)
mRS score at baseline	
0–2	179 (82.5%)
>2	38 (17.5%)
Angiography findings	
Bilateral	195 (89.9%)
Suzuki stage ≥III	191 (88.0%)
Intracranial aneurysm	20 (9.2%)
Clinical manifestation	
TIA	71 (32.7%)
Lacunar infarction	13 (6.0%)
Cerebral infarction	133 (61.3%)
Treatment	
Conservative treatment	79 (36.4%)
Antiplatelet therapy	85 (39.2%)
Surgical revascularization	53 (24.4%)

Long-Term Outcomes

Of the enrolled patients, seven patients (3.2%) could not be contacted in December 2019, so the follow-up was determined from their last clinical visits. During a follow-up of 34 ± 18 months, 18 patients (8.3%) had acute ischemic strokes, 37 patients (17.1%) developed a poor functional status, and 4 patients (1.8%) died of cerebral hemorrhage (**Figure 2**). The median interval from first discharge to subsequent ischemic stroke was 36 ± 25 months. Eleven of the 79 (13.9%) conservatively treated patients, 6 of the 85 (7.1%) antiplatelet-treated patients, and 1 of the 53 (1.9%) surgically treated patients had future strokes (**Table 2**).

Development and Validation of 1:1:1 PSM

There were significant differences between the three groups in several of the baseline characteristics before PSM (**Table 3**). With the use of 1:1:1 PSM via the TriMatch package, 53 patients with antiplatelet agents were matched with 53 conservative patients and 53 patients underwent surgical revascularization (**Table 3**). After matching, the between-group variations were successfully removed using the optimal matching algorithm and a caliper width equal to 0.25 (**Figure 3**). The standardized differences were <25.0% for almost all potential risk factors, indicating only small differences between these groups (**Supplementary Tables 1, 2**).

Primary Outcome

Among the matched groups, 12 patients (7.5%) developed incident ischemic stroke after an average follow-up of 33 ± 17 months. All of them had an initial symptom of cerebral infarction with a median interval period of 29 ± 25 months. The shortest interval from first discharge to the episode of cerebral infarction was 5 months, and the longest was 72 months. Of these patients, one who took antiplatelet agents died due to severe cerebral hemorrhage, and eight patients had a poor functional status. Additionally, further cerebral ischemic attack occurred in three patients (5.7%) in the antiplatelet group,

TABLE 2 | Summary of 18 patients with recurrent ischemic stroke.

No	Age at symptom onset (years)	Vascular risk factor	mRS score at baseline	Initial symptom	Outcome			
					Recurrent ischemic event	Internal (months)	Functional status	Bleeding event
Untreated group ($n = 11$)								
1	36–40	None	>2	Cerebral infarction	Cerebral infarction	5	Good	No
2	20–25	Hypertension	>2	Cerebral infarction	Cerebral infarction	26	Good	No
3	26–30	Hypertension	>2	Cerebral infarction	Cerebral infarction	64	Good	No
4	20–25	None	>2	Cerebral infarction	Cerebral infarction	50	Good	No
5	36–40	None	>2	Cerebral infarction	Cerebral infarction	31	Good	No
6	46–50	Hypertension	0–2	Cerebral infarction	Cerebral infarction	5	Good	No
7	30–35	Smoking, drinking	>2	Cerebral infarction	Cerebral infarction	44	Poor	No
8	46–50	None	>2	Cerebral infarction	Cerebral infarction	6	Poor	No
9	56–60	Hypertension, diabetes	0–2	Cerebral infarction	Cerebral infarction	20	Poor	No
10	50–55	None	>2	Cerebral infarction	Cerebral infarction	6	Poor	No
11	56–60	Hypertension, diabetes	0–2	Cerebral infarction	Cerebral infarction	33	Good	No
Antiplatelet group ($n = 6$)								
1	40–45	Hypertension, hyperlipidemia	>2	Cerebral infarction	Cerebral infarction	65	Poor	Cerebral hemorrhage
2	50–55	Smoking	0–2	Cerebral infarction	Cerebral infarction	13	Poor	No
3	36–40	None	>2	Cerebral infarction	Cerebral infarction	58	Poor	Cerebral hemorrhage
4	56–60	Hypertension, diabetes, smoking	0–2	Cerebral infarction	Cerebral infarction	63	Good	No
5	30–35	Hyperlipidemia	>2	Cerebral infarction	Cerebral infarction	16	Poor	No
6	56–60	Hypertension, diabetes, drinking	>2	Cerebral infarction	Cerebral infarction	64	Poor	No
Surgical group ($n = 1$)								
1	20–25	None	>2	Cerebral infarction	Cerebral infarction	72	Poor	No

compared with one patient (1.9%) in the surgical group and eight patients (15.1%) in the conservative group ($p = 0.030$). The univariate Cox regression analysis of recurrent ischemic events is shown in **Figure 4**, demonstrating that the probability of further cerebral ischemic attacks was lowest with surgical revascularization, while antiplatelet therapy was also statistically significant for preventing and reducing recurrent risks compared with the effects of conservative treatment. The omnibus tests of model coefficients revealed that there was a significant difference between the three risk curves ($\chi^2 = 8.987$; $p = 0.011$). No significant difference in recurrence risk was found between subgroups of different antiplatelet agents ($p > 0.05$; **Table 4**).

Secondary Outcome

In the matched groups, 26 patients (16.4%) had a poor functional status at follow-up. Of these patients, 12 (46.2%) had moderate-to-severe neurological dysfunction at baseline, and these patients

TABLE 3 | Comparison of clinical features and outcomes between different groups before and after matching.

Characteristic	Before matching				After matching			
	Surgical (n = 53)	Antiplatelet (n = 85)	Conservative (n = 79)	p-value	Surgical (n = 53)	Antiplatelet (n = 53)	Conservative (n = 53)	p-value
Age at symptom onset (years)	44 ± 12	48 ± 10	45 ± 13	0.064	44 ± 12	47 ± 11	47 ± 13	0.271
Age at diagnosis (years)	45 ± 12	49 ± 10	46 ± 12	0.108	45 ± 12	48 ± 11	48 ± 13	0.255
Female-to-male ratio	1.1	0.5	1.2	0.018	1.1	0.7	0.8	0.493
Vascular risk factors								
Hypertension	19 (35.8%)	45 (52.9%)	35 (44.3%)	0.140	19 (35.8%)	23 (43.4%)	23 (43.4%)	0.659
Diabetes mellitus	5 (9.4%)	23 (27.1%)	15 (19.0%)	0.040	5 (9.4%)	8 (15.1%)	8 (15.1%)	0.610
Hyperlipidemia	0 (0.0%)	11 (12.9%)	6 (7.6%)	0.023	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.000
Active smoking	13 (24.5%)	32 (37.6%)	28 (35.4%)	0.260	13 (24.5%)	16 (30.2%)	19 (35.8%)	0.447
Alcohol consumption	12 (22.6%)	26 (30.6%)	21 (26.6%)	0.587	12 (22.6%)	13 (24.5%)	15 (28.3%)	0.792
Past history of stroke or TIA	8 (15.1%)	13 (15.3%)	6 (7.6%)	0.262	8 (15.1%)	8 (15.1%)	5 (9.4%)	0.610
Family history of MMD	4 (7.5%)	4 (4.7%)	4 (5.1%)	0.757	4 (7.5%)	2 (3.8%)	2 (3.8%)	0.591
mRS score at baseline								
0–2	47 (88.7%)	63 (74.1%)	69 (87.3%)	0.033	47 (88.7%)	43 (81.1%)	45 (84.9%)	0.555
>2	6 (11.3%)	22 (25.9%)	10 (12.7%)		6 (11.3%)	10 (18.9%)	8 (15.1%)	
Angiography findings								
Bilateral	51 (96.2%)	69 (81.2%)	75 (94.9%)	0.003	51 (96.2%)	48 (90.6%)	51 (96.2%)	0.346
Suzuki stage ≥ III	46 (86.8%)	73 (85.9%)	72 (91.1%)	0.556	46 (86.8%)	46 (86.8%)	49 (92.5%)	0.569
Intracranial aneurysm	6 (11.3%)	5 (5.9%)	9 (11.4%)	0.395	6 (11.3%)	4 (7.5%)	6 (11.3%)	0.757
Clinical manifestation								
TIA	21 (39.6%)	23 (27.1%)	27 (34.2%)	0.273	21 (39.6%)	15 (28.3%)	15 (28.3%)	0.580
Lacunar infarction	3 (5.7%)	3 (3.5%)	7 (8.9%)		3 (5.7%)	2 (3.8%)	4 (7.5%)	
Cerebral infarction	29 (54.7%)	59 (69.4%)	45 (57.0%)		29 (54.7%)	36 (67.9%)	34 (64.2%)	
Follow-up (months)	32.2 ± 16.7	36.6 ± 20.5	33.2 ± 15.5	0.291	32.2 ± 16.7	35.6 ± 20.0	32.2 ± 14.1	0.488
Outcomes								
Future ischemic stroke	1 (1.9%)	6 (7.1%)	11 (13.9%)	0.042	1 (1.9%)	3 (5.7%)	8 (15.1%)	0.030
Poor functional status	7 (13.2%)	21 (24.7%)	11 (13.9%)	0.248	7 (13.2%)	12 (22.6%)	7 (13.2%)	0.317
Serious bleeding event	1 (1.9%)	3 (3.5%)	0 (0.0%)	0.244	1 (1.9%)	2 (3.8%)	0 (0.0%)	0.361

accounted for a greater proportion than the 24 patients (15.1%) among all subjects. In the present study, a poor functional status occurred in 12 patients (22.6%) in the antiplatelet group, compared with 7 patients (13.2%) in the surgical group and 7 patients (13.2%) in the conservative group (**Table 3**). Although antiplatelet therapy was found to be associated with a comparatively higher risk of a poor outcome, the univariate Cox regression analysis showed no significant difference between groups ($\chi^2 = 0.400$; $p = 0.819$).

Safety Outcome

In the matched groups, three deaths (1.8%) occurred because of severe cerebral hemorrhages, including two deaths among patients who were treated with antiplatelet agents and one patient who was treated with surgical revascularization (**Table 3**). No hemorrhagic events were observed in the conservative groups. No significant difference was observed among groups and subgroups, but antiplatelet therapy showed a relatively higher risk of bleeding (3.8%) in two patients treated with aspirin (**Table 4**).

Risk Factors for Further Cerebral Ischemic Attacks

All the baseline characteristics and treatment choices were entered as covariates. Five potential major factors with non-zero coefficients were selected from the 16 clinical features in terms of the 159 patients via the LASSO regression model (1:3 ratio, **Figure 5**), including a family history of MMD, a past history of stroke or TIA, an mRS score at baseline, clinical manifestation, and treatment choice. A family history of MMD (HR = 6.93; 95% CI: 1.28–37.52; $p = 0.025$), a past history of stroke or TIA (HR = 4.35; 95% CI: 1.09–17.33; $p = 0.037$), and treatment (HR = 0.05; 95% CI: 0.01–0.32; $p = 0.001$) were identified as risk factors in multivariate Cox regression analysis using the forward stepwise method. No significant correlations were observed between further cerebral ischemic attacks and other factors ($p > 0.05$).

DISCUSSION

In the present study, we evaluated whether antiplatelet agents showed a potential effect in adult patients with ischemic MMD.

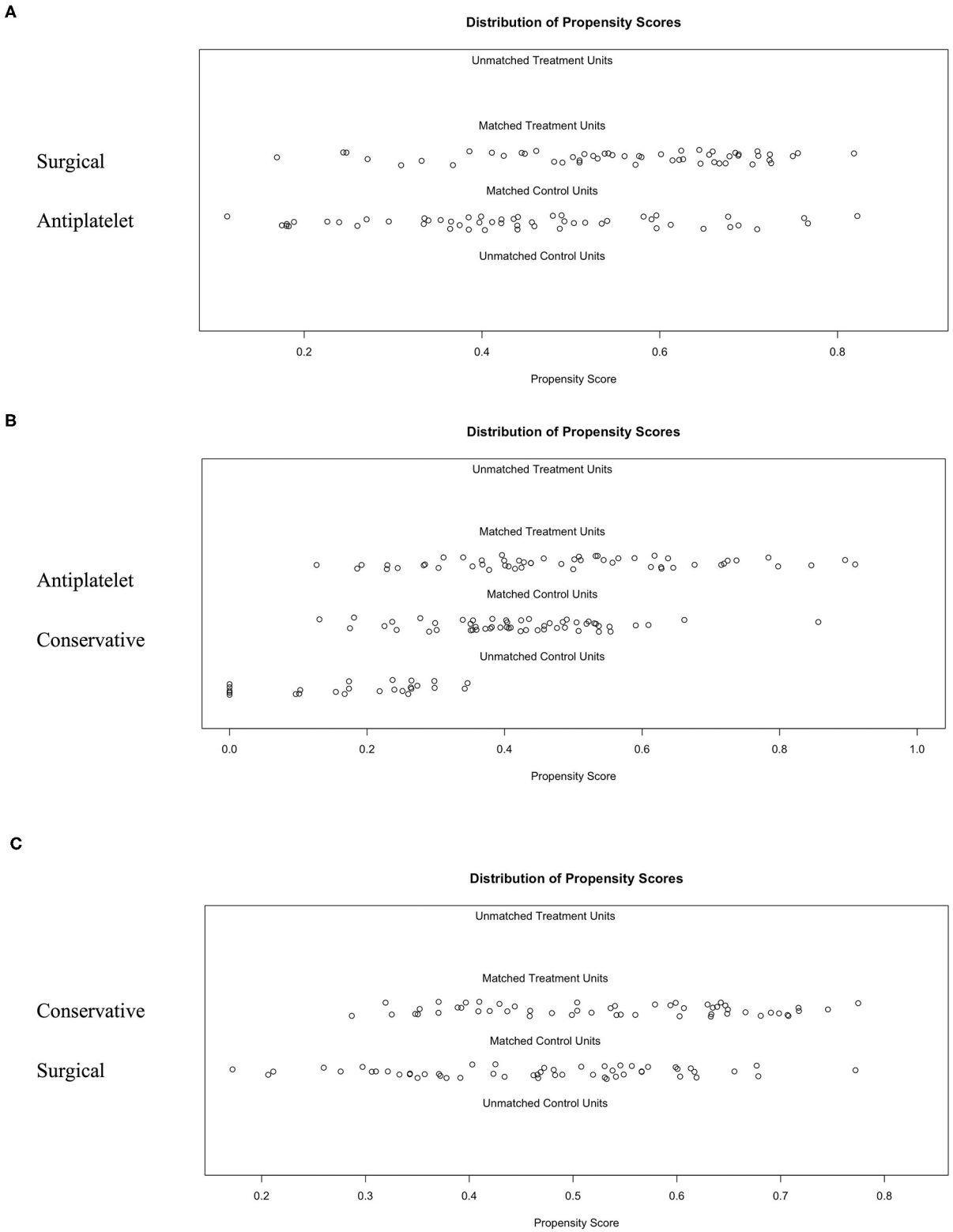

FIGURE 3 | The 1:1:1 PSM results for different groups using the optimal matching algorithm and a caliper width equal to 0.25. **(A)** Surgical group matched to the antiplatelet group. **(B)** Antiplatelet group matched to the conservative group. **(C)** Identification of the accuracy between the matched conservative group and surgical group.

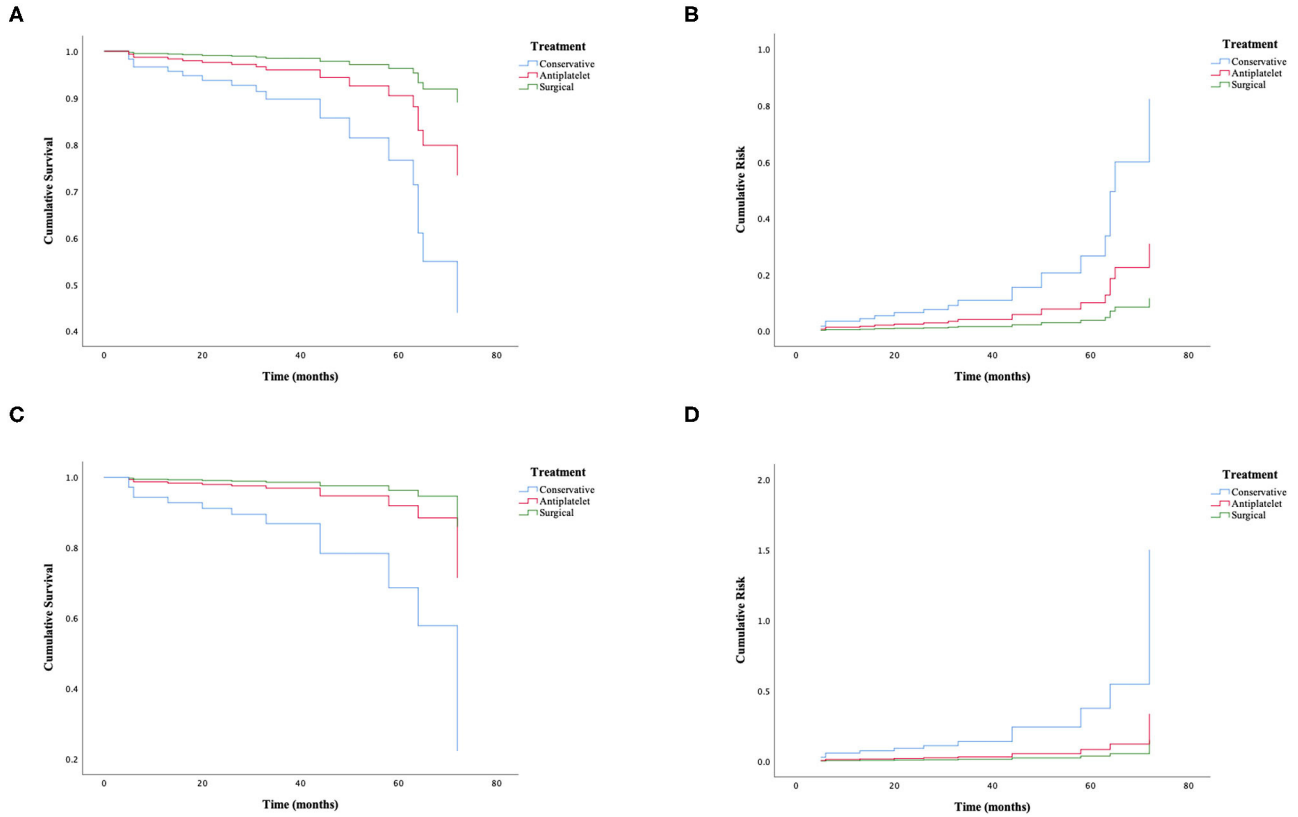

FIGURE 4 | Cox regression analysis of further cerebral ischemic attacks in adult patients with ischemic moyamoya disease treated by different methods. **(A,B)** The cumulative survival before ($\chi^2 = 7.188$; $p = 0.027$) and after matching ($\chi^2 = 8.987$; $p = 0.011$). **(C,D)** The cumulative risk before ($\chi^2 = 7.188$; $p = 0.027$) and after matching ($\chi^2 = 8.987$; $p = 0.011$).

To clarify the efficacy and safety of antiplatelet agents in ischemic MMD, we performed this retrospective cohort study and assessed the outcomes of 217 patients during a follow-up of 34 ± 18 months; the participants included 79 conservatively treated patients, 85 patients treated with antiplatelet agents, and 53 patients treated with surgical revascularization. Our results showed that antiplatelet agents were effective in preventing and reducing further cerebral ischemic attacks in these patients.

The baseline characteristics were similar to those of samples in previous studies, and the ratio of women to men (0.9) and family history of MMD (5.5%) in this study appeared to be similar to the respective parameters in the study by Duan et al. (6), which indicated an important influence of heredity. Most of the patients were diagnosed with bilateral steno-occlusive changes (89.9%), and a few were diagnosed with intracranial aneurysms (9.2%) on angiography, a finding that was consistent with a previous study by Kraemer et al. (25) and remained unclear. In addition, as Hervé et al. (26) found, the most common symptom was cerebral infarction (61.3%), followed by TIA (32.7%). However, the average ages at symptom onset (46 ± 12 years) and diagnosis (47 ± 11 years) in our study were comparably older than those in previous studies (3–8) because our strict criteria excluded a large portion of pediatric patients and adult patients with hemorrhagic MMD, which may explain the different results. Furthermore, our study revealed that a family history of MMD, a past history of stroke or TIA, and treatment choice could be independent risk factors for additional cerebral ischemic events based on a LASSO-Cox regression model.

Similarly, the univariate Cox regression analysis showed that revascularization surgery performed best for the treatment of ischemic MMD, with the lowest rate of further cerebral ischemic events (1.9%) and longest interval period between strokes (72 months) in this study, which was generally employed as the first choice to treat MMD patients to prevent future strokes with strong evidence (9, 27, 28). Interestingly, our study also showed that compared with conservative treatment, antiplatelet therapy was beneficial for reducing further cerebral ischemic events (5.7 vs. 15.1%) and extending the interval period (45 months vs. 18 months) among these patients. It was obvious that conservative treatment was inferior to surgical revascularization with regard to the prognosis of MMD patients. In the past, observational studies categorized antiplatelet therapy as conservative treatment for statistical analysis. Thus, the effectiveness of antiplatelet agents could be weakened due to the mixture of no treatment and treatment with other medications. In addition, current studies suggested that aspirin was effective in the postoperative

TABLE 4 | Comparison of the clinical features and outcomes between subgroups with different antiplatelet agents.

Characteristic	Aspirin ($n = 33$)	Clopidogrel ($n = 10$)	Aspirin combined with clopidogrel ($n = 10$)	*p*-value
Age at symptom onset (years)	47 ± 12	46 ± 10	52 ± 9	0.393
Age at diagnosis (years)	48 ± 12	46 ± 10	52 ± 10	0.463
Female-to-male ratio	0.8	0.7	0.4	0.682
Vascular risk factors				
Hypertension	14 (42.4%)	3 (30.0%)	6 (60.0%)	0.393
Diabetes mellitus	4 (12.1%)	1 (10.0%)	3 (30.0%)	0.339
Hyperlipidemia	0 (0.0%)	0 (0.0%)	0 (0.0%)	1.000
Active smoking	9 (27.3%)	3 (30.0%)	4 (40.0%)	0.745
Alcohol consumption	6 (18.2%)	2 (20.0%)	5 (50.0%)	0.115
Past history of stroke or TIA	7 (21.2%)	0 (0.0%)	1 (10.0%)	0.229
Family history of MMD	2 (6.1%)	0 (0.0%)	0 (0.0%)	0.533
mRS score at baseline				
0–2	26 (78.8%)	8 (80.0%)	9 (90.0%)	0.726
>2	7 (21.2%)	2 (20.0%)	1 (10.0%)	
Angiography findings				
Bilateral	31 (93.9%)	8 (80.0%)	9 (90.0%)	0.417
Suzuki stage ≥ III	29 (87.9%)	8 (80.0%)	9 (90.0%)	0.769
Intracranial aneurysm	1 (3.0%)	1 (10.0%)	2 (20.0%)	0.195
Clinical manifestation				
TIA	8 (24.2%)	3 (30.0%)	4 (40.0%)	0.734
Lacunar infarction	2 (6.1%)	0 (0.0%)	0 (0.0%)	
Cerebral infarction	23 (69.7%)	7 (70.0%)	6 (60.0%)	
Follow-up (months)	36.9 ± 22.6	31.8 ± 17.1	35.4 ± 13.4	0.787
Outcomes				
Future ischemic stroke	2 (6.1%)	0 (0.0%)	1 (10.0%)	0.618
Poor functional status	8 (24.2%)	3 (30.0%)	1 (10.0%)	0.530
Serious bleeding event	2 (6.1%)	0 (0.0%)	0 (0.0%)	0.533

management of ischemic MMD (29, 30). However, the use of antiplatelet agents in the treatment of MMD is controversial, and few studies have reported the efficacy of antiplatelet agents among non-surgical patients or before surgery. The majority of non-Asian experts advised using them for improving microcirculation and preventing recurrent strokes, while most Asian physicians held the opposite view that they were useless in improving blood supply and carried the potential risk of hemorrhages (12, 13). A recent study in Japan demonstrated that antiplatelet agents were effective in improving cerebral perfusion in adult patients with symptomatically ischemic MMD (31, 32). Likewise, our study focused on the efficacy and safety of antiplatelet agents used for adult MMD patients with the initial symptom of ischemia and compared their efficacy and safety with those of conservative treatment and surgical revascularization. Regarding the primary outcome, the recurrence rate of cerebral ischemic attack in the antiplatelet group was 5.7%, 1.9% in the surgical group, and 15.1% in the conservative group ($p = 0.030$), indicating that antiplatelet treatment could be effective for preventing ischemic stroke or TIA. However, regarding the secondary outcome, the patients in the antiplatelet group account for a higher proportion of poor neurological function than the other two groups, although statistical significance was absent among them. We noticed that the number of patients with mRS > 2 at baseline in the antiplatelet group was also higher than that in the other two groups. Therefore, we could not conclude that antiplatelet agents are responsible for a poorer functional status. We re-analyzed these 12 patients with recurrent strokes. Among these patients, four patients presented with a baseline mRS of 0–2 (surgical:antiplatelet:conservative = 0:1:3), suggesting a trend to benefit these patients with mild symptoms at baseline from both surgical revascularization and antiplatelet therapy could be compared with conservative treatment. However, because of our small sample size, we still needed a large sample size randomized control study to prove this finding. Thus, these differences may influence the secondary outcome. Because of the limitation of the retrospective study, some covariates could not be adjusted by the propensity score matching method. Moreover,

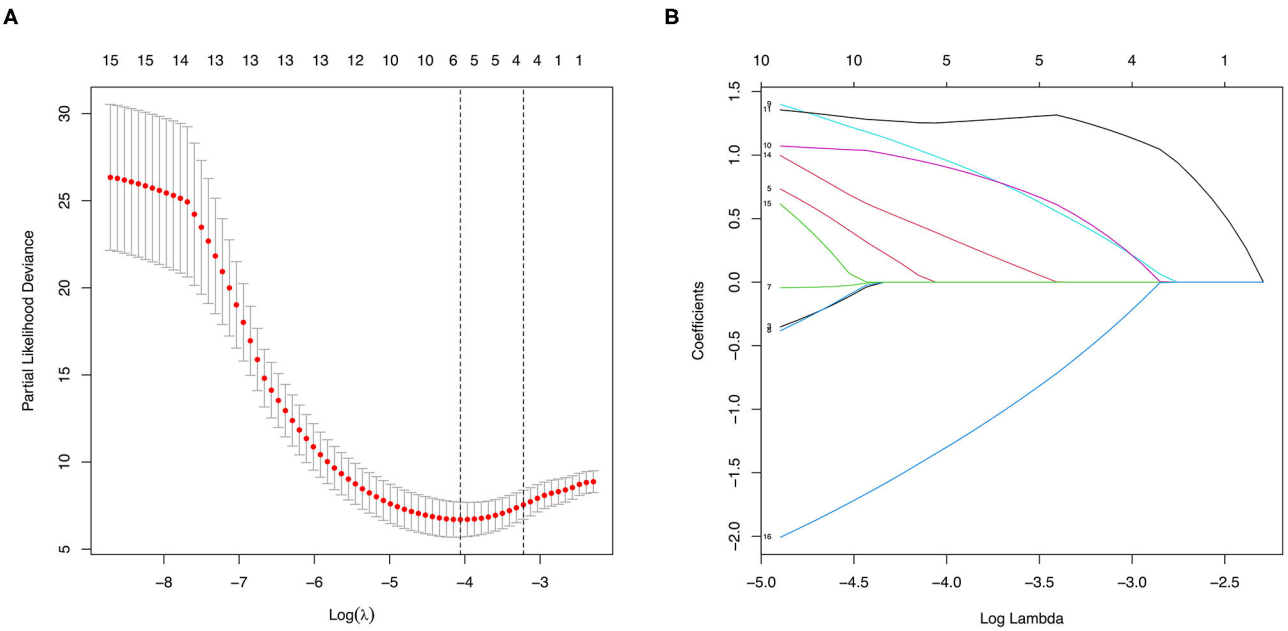

FIGURE 5 | Features selection using the least absolute shrinkage and selection operator (LASSO) method. **(A)** Tuning parameter (λ) selection in the LASSO model used five-fold cross-validation via minimum criteria. The partial likelihood deviance (binomial deviance) curve was plotted vs. log(λ). Dotted vertical lines were drawn at the optimal values by using the minimum criteria and the 1 standard error of the minimum criteria (the 1-SE criteria). **(B)** LASSO coefficient profiles of the 16 covariates. A coefficient profile plot was produced against the log (λ) sequence. Vertical line was drawn at the value selected using five-fold cross-validation, where optimal λ resulted in 5 non-zero coefficients.

the insufficient follow-up period weakened the statistical power in the secondary outcome comparison. Although the mechanism of MMD is still unclear, pathological studies have demonstrated that endovascular thrombosis and hyperplasia of smooth muscle cells lead to the progressive stenosis or occlusion of the distal ICA, which could explain the cause of ischemic symptoms (1). In addition, antiplatelet agents are widely used for the primary or secondary preventive management of acute ischemic stroke and TIA patients (10, 11). Aspirin inhibits the metabolism of arachidonic acid to prostacyclin 2 (PGI2) and serum thromboxane A2 (TXA2) by blocking cyclooxygenase-1 (COX-1) to reduce thrombosis. Clopidogrel is an adenosine diphosphate (ADP) receptor inhibitor that selectively interacts with P2Y12 receptor to depress platelet aggregation. Indeed, antiplatelet agents have been effectively applied to prevent further cerebral ischemic events because of their ability to improve the development of luminal thrombosis.

There were also several limitations of this study. First, this study is a non-randomized observational study with a small sample size. Second, although PSM is used to correct baseline differences, selection biases may not be removed because our patients were enrolled from eight clinical sites. Professional performances at different clinical centers could also affect the validity of the conclusions. Third, our subjects were all Asians; thus, whether our results are applicable to Western populations is unknown. Finally, genetic factors were not assessed in our study. Thus, a large randomized controlled trial is necessary.

In brief, we found that surgical revascularization was the first treatment choice, but antiplatelet therapy was a relatively significant management strategy for adult patients with ischemic MMD for reducing and preventing future strokes. Therefore, antiplatelet therapy may be a replacement therapy for patients with surgical contraindications and for patients before revascularization. We believe that this information about actual therapeutic practices in China will be helpful for the management of ischemic MMD.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials,** further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the independent ethics committe of the First Affiliated Hospital of Sun Yat-sen University and each participating medical center's ethics committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

FY designed the study, drafted the manuscript, and contributed to the discussion. JLi, TW, and KL designed the study, collected the data, and analyzed the data. HL, TG, XZ, and TY collected the data and contributed to the discussion. JLia and XW collected the data. QL and WS designed the study, reviewed the manuscript, and contributed to the discussion. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.608000/full#supplementary-material

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Mapping Trends in Moyamoya Angiopathy Research: A 10-Year Bibliometric and Visualization-Based Analyses of the Web of Science Core Collection (WoSCC)

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Chen D, Zhang G, Wang J, Chen S, Wang J, Nie H and Tang Z (2021) Mapping Trends in Moyamoya Angiopathy Research: A 10-Year Bibliometric and Visualization-Based Analyses of the Web of Science Core Collection (WoSCC). Front. Neurol. 12:637310. doi: 10.3389/fneur.2021.637310

Background: Moyamoya angiopathy (MMA), which includes moyamoya disease (MMD) and moyamoya syndrome (MMS), is an uncommon cerebrovascular condition characterized by recurrent stroke. We carried out a bibliometric analysis to examine the development of and research trends in MMA research.

Methods: Studies published between 2010 and 2019 on MMA were retrieved from the Web of Science Core Collection (WoSCC) on August 14, 2020, and bibliometric and visualization-based analyses were performed by using three different scientometric tools: HistCite, VOSviewer, and CiteSpace.

Results: A total of 1,896 publications published in 384 journals by 6,744 authors, 1,641 institutions and 56 countries/regions were included in the analyses. Annual publication outputs increased from 2010 to 2019. The USA, Japan and China were three key contributors to this study field. Capital Medical University, Seoul National University, and Stanford University were three major institutions with larger numbers of publications. Zhang D, World Neurosurgery, Kuroda S, and STROKE were the most prolific author, prolific journal, top co-cited author and top co-cited journal, respectively. The top five keywords during this period were moyamoya disease, revascularization, stroke, children and surgery, while revascularization surgery and RNF213 were the most common frontier topics.

Conclusions: In this study, the research trends of global scientific research on MMA over the past decade were systematically analyzed. The study can provide guidance for scholars who want to understand current trends in research in this area and new research frontiers.

Keywords: bibliometrics, CiteSpace, emerging topics, visualization, HistCite, VOSviewer, moyamoya, Web of Science

INTRODUCTION

Moyamoya angiopathy (MMA) is an infrequent, chronic, and disabling cerebrovascular condition. Clinical features include ongoing stenosis and occlusion of the distal part of internal carotid arteries and the proliferation of moyamoya-associated collaterals (1, 2). MMA can be divided into moyamoya disease (MMD) and moyamoya syndrome (MMS). The specific pathological mechanism of MMA remains unclear (3). Revascularization surgery is an effective MMA treatment (2).

In the field of bibliometrics, quantitative and visualization-based analyses were performed on scientific publication data and citation data (4, 5). Through the quantitative analysis of publications available in a library, the research trends in a specific field can be investigated. The Web of Science Core Collection (WoSCC) is the most commonly used database in bibliometric studies (6). Scientometric applications used in bibliometric studies include HistCite (7), VOSviewer (8), and CiteSpace (9). For the past few years, bibliometric analysis has been applied in various biomedical fields (10–12).

As far as we know, no published bibliometric study has focused on MMA research. Here, we visually analyzed the development of and trends in MMA research from 2010 to 2019 using HistCite, VOSviewer, and CiteSpace.

MATERIALS AND METHODS

Data Source and Information Retrieval Strategy

A literature search was carried out by using the WoSCC database to collect publications on MMA. All data obtained were appropriate for the bibliometric analysis. All searches were performed under identical conditions to minimize the bias on August 14, 2020. The search strategy was unanimously agreed on by all members of our research team. The advanced search was conducted using the following formula: TS = ("moyamoya"). The detailed search processes and analysis procedures were shown in **Figure 1** (13).

Inclusion Criteria

(a) Peer-Reviewed Articles on MMA
(b) Documents types: articles, reviews, letters, and proceeding papers
(c) Year of publication: 2010–2019
(d) Language type: all languages
(e) Database: Web of Science Core Collection (WoSCC).

Exclusion Criteria

(a) Articles that required a manual search
(b) Unpublished papers.

Statistical and Analytical Methods

The full records and cited references of identified publications were exported as plain text and tab-delimited (Win, UTF-8) files for bibliometric analysis and visualization. HistCite (Clarivate Analytics, Philadelphia, PA, USA), CiteSpace 5.3.R4 (Drexel University, Philadelphia, USA) and VOSviewer 1.6.14 (Leiden University, Leiden, Netherlands) were used for data analysis. HistCite is a program used to quickly summarize and analyse publications. In this study, HistCite was applied to confirm annual output, total number of citations, language type and document type. VOSviewer was employed to construct visual maps and summarize prolific countries/regions, institutions, journals, and authors, as well as the top co-cited journals, authors, keywords and references. For VOSviewer, the full counting method was used, and the minimum threshold of the data selected to construct the visual maps depended on specific items such as the institution or journal. CiteSpace, which runs in a Java environment, was used to construct the category map and detect the burst terms of keywords and co-cited references. The parameters used with CiteSpace were set as follows: time slice (2010–2019 by year), text processing (term source: all selection), term type (burst terms), node type (set based on the item), links (strength: cosine; scope: within slices), selection criteria (top 50 objects), and pruning (pathfinder and pruning sliced networks). The linear forecasting model can be described as $f(x) = ax + b$, where x is the publication year and $f(x)$ is the number of publications. The linear fit of the annual number of publications by year was graphed using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA), and the output was predicted for 2020. Pearson's correlation analysis of the year and annual output was conducted using IBM SPSS Statistics 25.0 software (SPSS Inc., Chicago, USA). Journal impact factors (IFs) were identified according to the 2019 Journal Citation Reports (JCR) released by Clarivate Analytics on June 29, 2020.

A review of ethics was unnecessary because all original data used in this study were from a public database.

RESULTS

General Publication Data and the Upward Trend of Annual Output

A total of 1,896 studies published from 2010 to 2019 were used for our bibliometric data analysis. The studies were published in 384 journals by 6,744 authors, 1,641 institutions and 56 countries/regions. There were 20,050 citations in total. The article was the primary document type (n = 1,552, 81.86%), followed by the review (n = 208, 10.97%), letter (n = 132, 6.96%), and proceedings paper (n = 4, 0.21%). Languages included English (n = 1,873, 98.79%), French (n = 10, 0.53%), German (n = 7, 0.37%), and Spanish (n = 6, 0.32%). Three Eastern Asian languages Japanese, Korean and Chinese, were not included in the database.

It was suggested that the annual output varied by years, and there was a slight increase in output over the last 10 years (**Figure 2A**). The most prolific year was 2019, in which 273 papers were published, and the minimum output occurred in 2011 (n = 121, 6.38%). In terms of total citations, there was a peak in 2014 (n = 2,899) (**Figure 2A**), and 2013 (n = 163, GR = 14.79%), 2014 (n = 194, GR = 19.02%), 2016 (n = 218, GR = 11.22%), and 2019 (n = 273, GR = 15.68%) were considered "remarkable" years (14) [note that a year is defined as remarkable

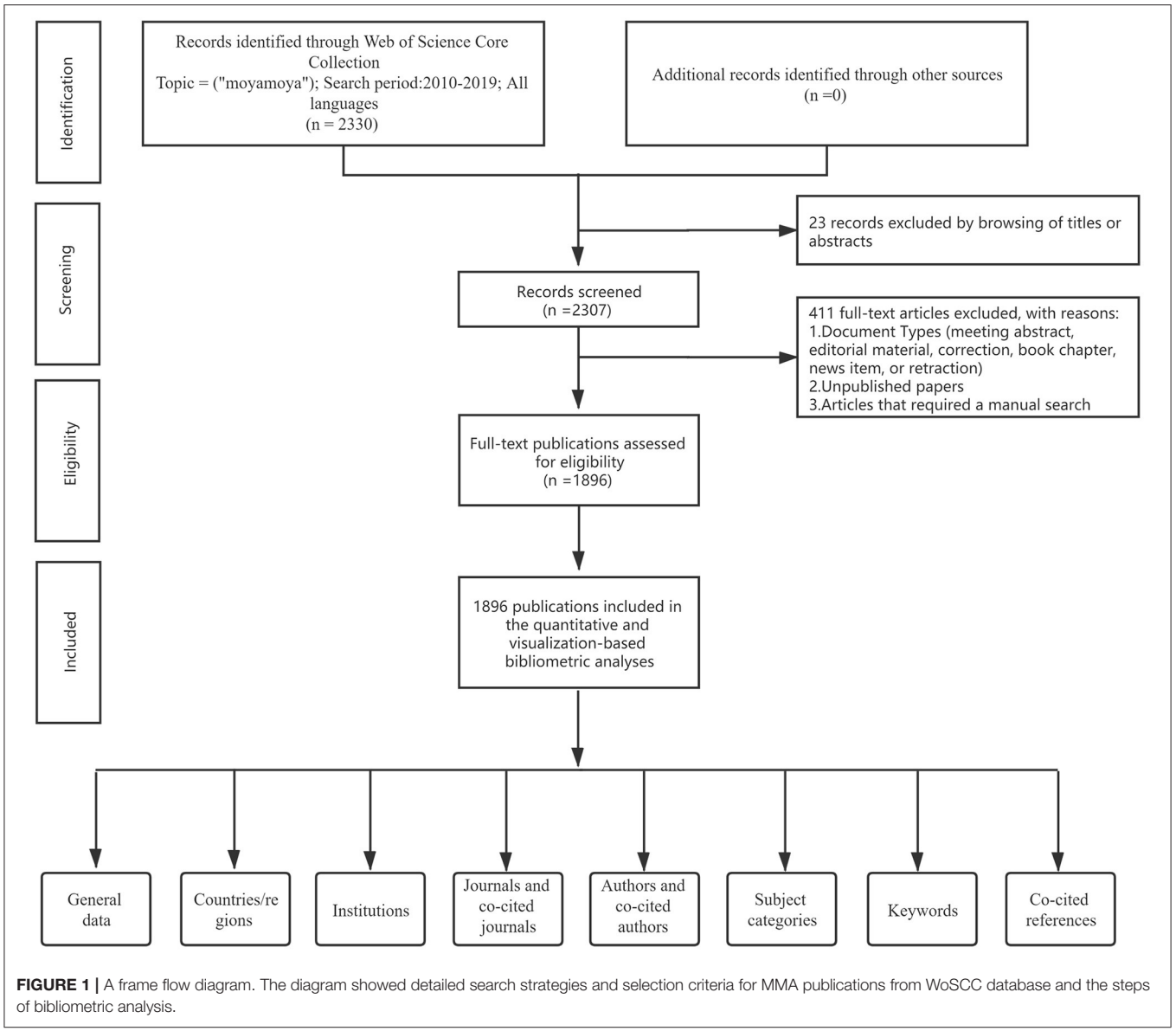

FIGURE 1 | A frame flow diagram. The diagram showed detailed search strategies and selection criteria for MMA publications from WoSCC database and the steps of bibliometric analysis.

if more than 150 papers were published and if there was a year-over-year growth rate (GR) >10% (**Figure 2B**)].

Pearson's correlation analysis showed that the annual output was positively correlated with the year ($r = 0.983$, $P < 0.0001$), and the linear fit (**Figure 2C**) between the year and the number of published MMA studies was significantly correlated ($R^2 = 0.9654$, $P < 0.0001$). According to the mathematical model, publication output will reach 279 in 2020.

Countries/Regions

A total of 56 countries/regions contributed to the published MMA research ($n = 1{,}896$). The USA ($n = 473$, 24.95%) was the most prolific country/region. The top 10 productive countries/regions were shown in **Table 1**. A co-authorship network was constructed for the countries/regions using VOSviewer. The map of the co-authorship network (**Figure 3**) includes 23 countries, and the smallest node has 6 publications. The USA, Japan, China, and South Korea were represented by the four largest nodes, which is consistent with the result in **Table 1**. The USA had the strongest collaboration network, with the maximum total link strength (TLS = 127). The strongest collaboration networks were between the USA and China (TLS = 21) and between the USA and Canada (TLS = 21).

Institutions

The top 10 institutions in terms of productivity were presented in **Table 2**. Among these institutions, Capital Medical University (China, 95 publications) was the most prolific institution, followed by Seoul National University (South

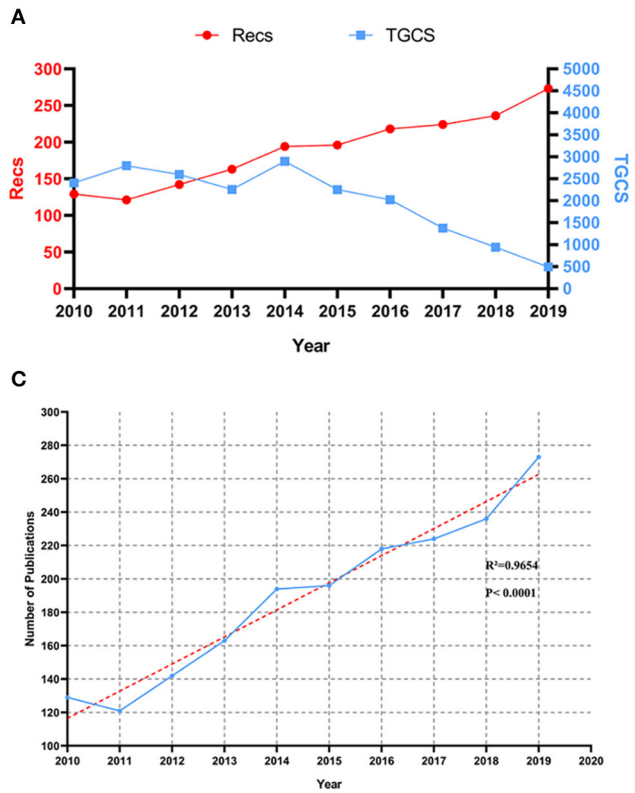

FIGURE 2 | (A,B) The annual output, citations and growth rate on a year-on-year basis with regard to MMA during 2010–2019. **(C)** The linear model of the upward trend in the field of MMA ($R^2 = 0.9654$, $P < 0.0001$). Recs: Number of Records, namely the number of publications for a given year; TGCS: Total Global Citation Score, namely the total count of citations.

TABLE 1 | The top 10 countries according to total publications during 2010–2019.

Rank	Country	Number of publications	Proportion (%)	Total citations	Citations/ paper
1	The USA	473	24.95%	5,892	12.5 (7)
2	Japan	458	24.16%	5,906	12.9 (5)
3	China	347	18.30%	2,993	8.6 (8)
4	South Korea	231	12.18%	3,205	13.9 (3)
5	Germany	102	5.38%	1,304	12.8 (6)
6	Canada	72	3.80%	1,308	18.2 (1)
7	India	62	3.27%	191	3.1 (10)
8	France	59	3.11%	766	13 (4)
9	England	51	2.69%	745	14.6 (2)
10	Italy	47	2.48%	341	7.3 (9)

Data were retrieved from 1,896 publications with VOSviewer on August 14, 2020.

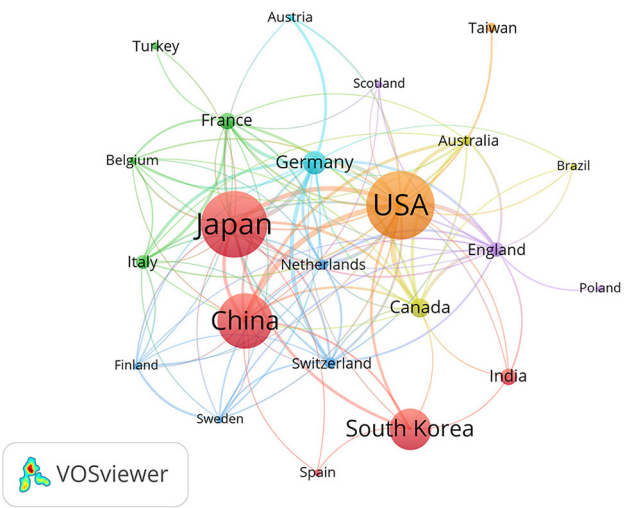

FIGURE 3 | The co-authorship network visualization map of countries/regions related to MMA. Lines between clusters represented occurrence and the size of node was positively associated with the number of publications.

Korea, 84 publications) and Stanford University (the USA, 71 publications). In terms of citations, Tohoku University (Japan, 1,633 times), Seoul National University (South Korea, 1,453 times), Kyoto University (Japan, 1,408 times) and Stanford University (the USA, 1,300 times) exceeded 1,000 citations. Cooperative relationship among 63 institutions is shown in **Figure 4**. The co-authorship institutional analysis network had a minimum threshold of 10 publications. As shown in the network map, a variety of institutions closely cooperation with each other.

Journals and Co-cited Journals

Peer-reviewed journals publishing literature on MMA were identified with VOSviewer (n = 384). The top 10 journals and co-cited journals were shown in **Table 3**. The top 10 journals in terms of productivity collectively produced 714 publications, accounting for 37.7% of all papers, and STROKE had the highest impact factor (IF = 7.19). World Neurosurgery was the most prolific journal, with 168 publications. The top 3 co-cited journals were as follows: STROKE (5,721 co-citations), *Journal of Neurosurgery* (3,289 co-citations) and *Neurosurgery* (2,410 co-citations).

TABLE 2 | The top 10 most productive institutions between 2010 and 2019.

	2010–2019			
Rank	**The name of institution**	**Publications**	**Citations**	**Location**
1	Capital Med Univ	95	661	China
2	Seoul Natl Univ	84	1,453	Korea
3	Stanford Univ	71	1,300	The USA
4	Tohoku Univ	63	1,633	Japan
5	Kyoto Univ	57	1,408	Japan
6	Hokkaido Univ	55	821	Japan
7	Beijing Inst Brain Disorders	45	221	China
8	Sungkyunkwan Univ	41	703	Korea
9	Natl Cerebral & Cardiovasc Ctr	39	994	Japan
10	China Natl Clin Res Ctr Neurol Dis	37	187	China

Data were retrieved from 1,896 publications with VOSviewer on August 14, 2020.

Authors and Co-cited Authors

A total of 6,744 authors contributed to MMA-related research, with an average of 3.6 authors per study. The top 12 contributing authors involved in the research were listed in **Table 4**. Zhang D was the most prolific author, with 61 publications, followed by Tominaga T (n = 59) and Fujimura M (n = 52). Co-cited authors were authors who have been co-cited in publications, and co-citation is a key measurement of the contribution degree of an author. The top 12 co-cited authors were shown in **Table 4**.

Visual maps of authors and co-cited authors can effectively display powerful research teams and potential partners (15). A co-authorship analysis of authors was carried out by VOSviewer. The minimum number of publications of each author was set to 10, and 106 authors were screened. Among them, some authors were not connected to others. To improve the visualization, the largest subnetwork (67 authors) was identified, as illustrated

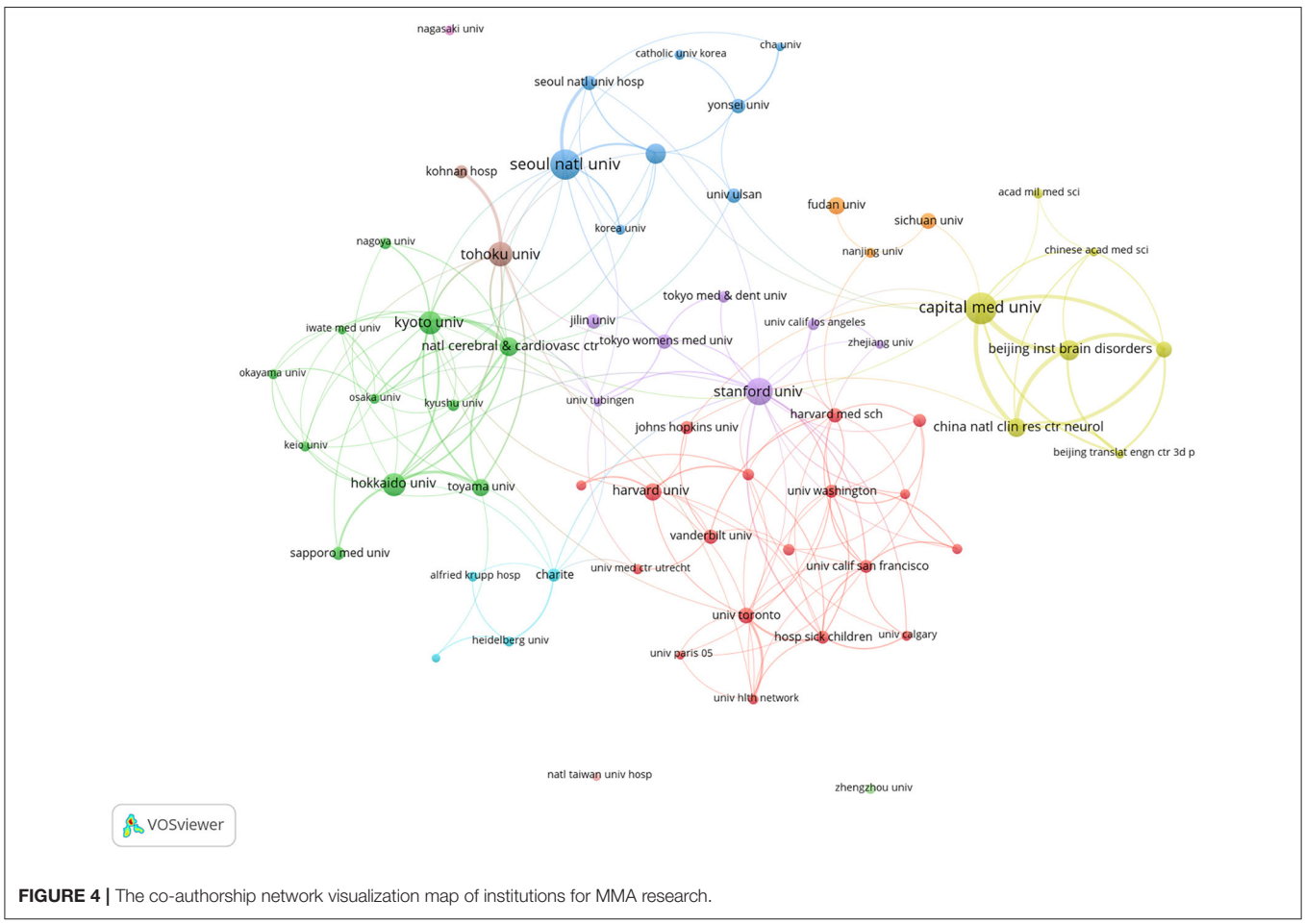

FIGURE 4 | The co-authorship network visualization map of institutions for MMA research.

TABLE 3 | The top 10 journals and co-cited journals on MMA research between 2010 and 2019.

Rank	Journal	Publication number	Citation	IF[#]	Co-cited journal	Co-citation	IF
1	*World Neurosurgery*	168	796	1.829	*Stroke*	5,721	7.19
2	*Journal of Stroke and Cerebrovascular Diseases*	84	555	1.787	*Journal of Neurosurgery*	3,289	3.968
3	*Journal of Neurosurgery*	80	1,108	3.968	*Neurosurgery*	2,410	4.853
4	*Stroke*	74	2,080	7.19	*World Neurosurgery**	1,585	1.829
5	*Journal of Neurosurgery-Pediatrics*	58	575	2.117	*American Journal of Neuroradiology*	1,504	3.381
6	*Childs Nervous System*	56	323	1.298	*Clinical Neurology and Neurosurgery*	1,415	1.53
7	*Neurologia medico-chirurgica*	54	704	1.836	*Neurology*	1,293	8.77
8	*Acta Neurochirurgica*	48	522	1.817	*New England Journal of Medicine*	1,024	74.699
9	*American Journal of Neuroradiology*	47	708	3.381	*Acta Neurochirurgica*	986	1.817
10	*Neurosurgery*	45	844	4.853	*Cerebrovascular Diseases*	948	2.698

*Data were retrieved from 1,896 publications with VOSviewer on August 14, 2020. (*Surgical Neurology was renamed World Neurosurgery in 2010).*
[#]Abbreviation for Impact Factor.

TABLE 4 | The top 12 prolific authors and co-cited authors on MMA research from 2010 to 2019.

	Author				Co-cited authors		
Rank	Name	Publications	Citations	Country	Name	Co-citations	Country
1	Zhang D	61	474	China	Kuroda S	878	Japan
2	Fujimura M	52	1,140	Japan	Suzuki J	854	Japan
3	Tominaga T	50	1,268	Japan	Fujimura M	747	Japan
4	Wang R	49	459	China	Scott RM	704	The USA
5	Houkin K	45	753	Japan	Fukui M	449	Japan
6	Zhao JZ	44	397	China	Houkin K	416	Japan
7	Kuroda S	42	702	Japan	Miyamoto S	335	Japan
8	Miyamoto S	41	1,099	Japan	Liu WY	264	China
9	Zhang Y	40	254	China	Kim SK	233	South Korea
10	Steinberg GK	36	686	The USA	Matsushima T	211	Japan
11	Kim SK	36	575	South Korea	Karasawa J	206	Japan
12	Zhang Q	36	224	China	Matsushima Y	206	Japan

Data were retrieved from 1,896 publications with VOSviewer on August 14, 2020.

in **Figure 5A**. **Figure 5B** displays the network map of co-cited authors with more than 230 co-citations. In the map, Kuroda S was the most notable author in terms of co-citations.

Subject Categories

The map of publication categories (**Figure 6**) was generated using CiteSpace software. Centrality indicates the importance of an item in CiteSpace. All 1,896 publications were associated with 40 disciplines. The top 10 subject categories ranked by publication or centrality were shown in **Table 5**, and NEUROSCIENCES AND NEUROLOGY and BIOCHEMISTRY AND MOLECULAR BIOLOGY tied for first.

Keywords

High-frequency keywords could reflect research hot spots. A thesaurus was used to clean the data, and 4,619 keywords were extracted from the 1,896 publications. Ultimately, 62 keywords with more than 30 occurrences were identified. The top 5 keywords ranked by number of occurrences were as following: moyamoya disease (n = 1,081), revascularization (n = 421), stroke (n = 372), children (n = 331), and surgery (n = 258). The co-occurrence network of keywords is displayed in **Figure 7A**. As shown in **Figure 7A**, the keywords were grouped into four clusters. Notably, the primary keywords for cluster 1 (red) referred to epidemiology and genetics and included epidemiologic features, Japan, prevalence, gene, genetics, and RNF213. Cluster 2 (green) referred to surgical treatments and included revascularization, surgery, pial synangiosis, encephaloduroarteriosynangiosis, synangiosis, and indirect revascularization. Cluster 3 (blue) referred to imaging diagnosis and included blood flow, MRI, hemodynamics, perfusion, PET, cerebrovascular reactivity, and SPECT, and cluster 4 (yellow) referred to clinical presentation and prognosis and comprised hemorrhage, aneurysm, subarachnoid hemorrhage, and natural history.

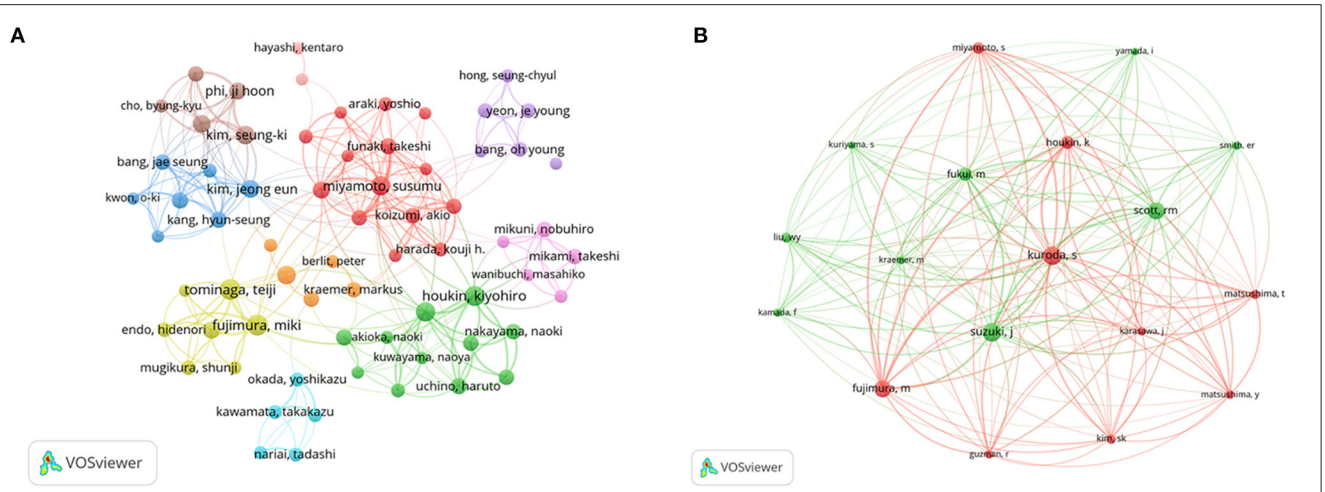

FIGURE 5 | (A) Co-authorship network visualization map of authors on MMA research. **(B)** Co-citation network visualization map of authors on MMA research.

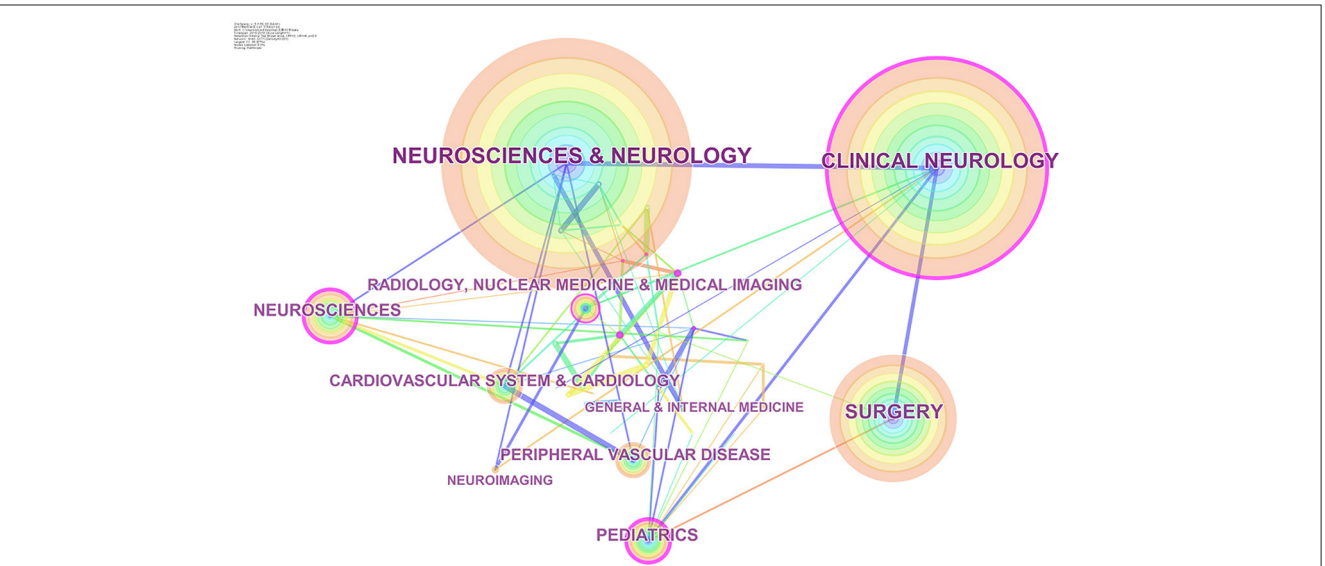

FIGURE 6 | The visualization map of subject categories on MMA. The tree ring-shaped nodes represented different subject categories. The lines between two nodes meant co-occurrence. The area of the nodes referred to the number of publications. Nodes with high centrality (purple ring) were deemed as the hot field.

Burst terms were identified with CiteSpace to indicate new research trends and frontier topics (16). The node type was set as "Keyword," and other parameters were set in accordance with the description in the Materials and Methods section. The minimum duration was set to the default value of 2. As **Figure 7B** shows, 46 keywords with strong citation bursts were found. Among the top keywords, revascularization surgery had the highest burst strength (8.4439). Other keywords with high burst strengths from 2017 to 2019 include revascularization surgery, cerebral vascular reactivity, outcome, synangiosis, association, and stenosis.

Co-cited References

Co-cited references were references cited collectively in the reference lists of other literature (17). Among the 1896 publications, 24,041 co-cited references were identified. The top ten co-cited references were presented in **Table 6**. The article published in *Arch Neurol-Chicago* (18) written by Suzuki J and colleagues was the most co-cited reference ($n = 615$), followed by the review written by Scott RM in The New England Journal of Medicine ($n = 452$) (2) and the review written by Kuroda S in *Lancet Neurology* ($n = 354$) (1). References with more than 100 co-citations were used to form a co-citation network map. In **Figure 8A**, the study published in *Arch Neurol-Chicago* (18) has the highest weight and an active relationship with other studies.

Citation bursts can be analyzed by identifying references that researchers focused on during certain periods of time (26). References with the strongest citation bursts were identified using CiteSpaceV, and the minimum burst duration was confined to

TABLE 5 | Top 10 subject categories in terms of publication number and centrality related to MMA research.

Rank	Publications	Category	Centrality	Category
1	1,364	NEUROSCIENCES AND NEUROLOGY	0.37	BIOCHEMISTRY AND MOLECULAR BIOLOGY
2	1,188	CLINICAL NEUROLOGY	0.35	PEDIATRICS
3	710	SURGERY	0.25	CLINICAL NEUROLOGY
4	302	NEUROSCIENCES	0.23	BIOPHYSICS
5	256	PEDIATRICS	0.21	NEUROSCIENCES
6	237	CARDIOVASCULAR SYSTEM AND CARDIOLOGY	0.19	RADIOLOGY, NUCLEAR MEDICINE & MEDICAL IMAGING
7	224	PERIPHERAL VASCULAR DISEASE	0.18	HEMATOLOGY
8	184	RADIOLOGY, NUCLEAR MEDICINE AND MEDICAL IMAGING	0.18	CELL BIOLOGY
9	83	NEUROIMAGING	0.13	CARDIAC AND CARDIOVASCULAR SYSTEMS
10	74	GENERAL AND INTERNAL MEDICINE	0.09	CARDIOVASCULAR SYSTEM AND CARDIOLOGY GENETICS AND HEREDITY ONCOLOGY

Data were retrieved from 1,896 publications with CiteSpaceV on August 14, 2020.

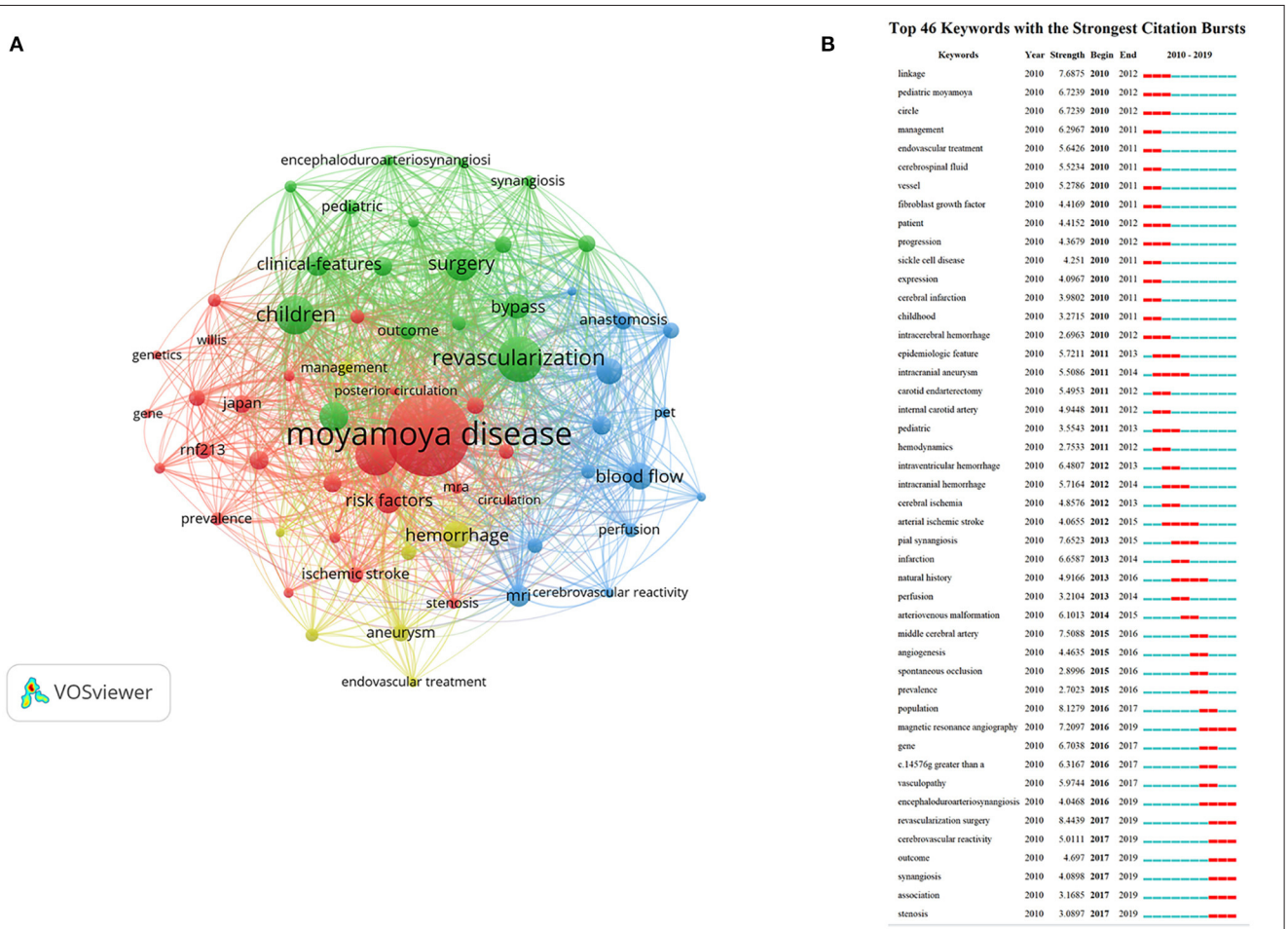

FIGURE 7 | (A) The co-occurrence network visualization map of keywords in the MMA research field from 2010 to 2019. Keywords in the same color represent were sorted into the same cluster. **(B)** The top 46 keywords with the strongest citation bursts on MMA research between 2010 and 2019. The red segment of the blue line denoted the burst duration of a keyword.

4 years. The node type was set as "Cited Reference," and the other parameters were set in accordance with the description in the Materials and Methods section. As shown in **Figure 8B**, burst strength values of the top 25 references with the strongest citation bursts ranged from 2.9728 to 18.0315. "Hallemeier CL, 2006, *Stroke*, V37, P1490 (27)" had the highest burst strength (18.0315), and three co-cited references had recent bursts: "Dusick JR, 2011, *Neurosurgery*, V68, P34 (28)," "Bao XY, 2012, *Cerebrovasc Dis*, V34, P305 (29)," and "Sonobe S, 2014, *Brain Res*, V1552, P64 (30)." Significantly, the third article by Sonobe S showed the highest burst strength (8.5243) among studies with citation bursts ending in 2019. Dusick JR demonstrated that indirect revascularization surgeries by encephaloduroarteriosynangiosis (EDAS) and multiple burr-hole operation provided effective prevention for recurrent ischemia and hemorrhage in 95% children and adults (28). Bao XY revealed EDAS in Chinese adult patients with MMD was relatively safe and effective at preventing future ischemia and improving quality of life (29). SONOBE S and colleagues found that the functional deficiency of RNF213 did not sufficiently cause the emergence of the MMD-like phenotype (30).

Revascularization surgery researches and RNF213-related researches were selected as recent frontier topics. "Revascularization surgery" had the highest burst strength among the six recent keywords. It was also described in the first article with citation bursts ending in 2019 by Dusick JR (28). "RNF213" was discussed in the third article by Sonobe S (30), which also represented the hot spots of MMA.

TABLE 6 | The top 10 co-cited reference with regard to MMA during 2010–2019.

Rank	Co-cited reference	Count
1	Suzuki J, 1969, *Arch Neurol-Chicago*, V20, P288 (18)	615
2	Scott RM, 2009, *New Engl J Med*, V360, P1226 (2)	452
3	Kuroda S, 2008, *Lancet Neurol*, V7, P1056 (1)	354
4	Fukui M, 1997, *Clin Neurol Neurosur*, V99, PS238 (19)	269
5	Scott RM, 2004, *J Neurosurg*, V100, P142 (20)	204
6	Kamada F, 2011, *J Hum Genet*, V56, P34 (21)	193
7	Suzuki J, 1983, *Stroke*, V14, P104 (22)	190
8	Liu WY, 2011, *PLoS ONE*, V6 (23)	188
9	Guzman R, 2009, *J Neurosurg*, V111, P927 (24)	187
10	Kuriyama S, 2008, *Stroke*, V39, P42 (25)	166

Data were retrieved from 1,896 publications with VOSviewer on August 14, 2020.

DISCUSSION

Although MMA is a rare cerebrovascular disease, it is one of the main causes of pediatric and young adult stroke (31, 32). The etiology and pathogenesis mechanisms of MMA remained unclear, and there is currently a lack of effective medical treatments. Surgical intervention is currently the main therapeutic strategy. However, surgical treatment mainly aims to improve cerebral hypoperfusion rather than combat the pathogenic mechanisms (33). Timely diagnosis and early intervention have benefitted MMA patients (34), but it is still necessary to establish a sufficient understanding of this disease. Therefore, we made a novel attempt to examine MMA publications from 2010 to 2019 and to provide a full view of the research trends by using a bibliometric analysis.

The data of annual output and growth rate could reflect the development of a given research area in terms of scientific data.

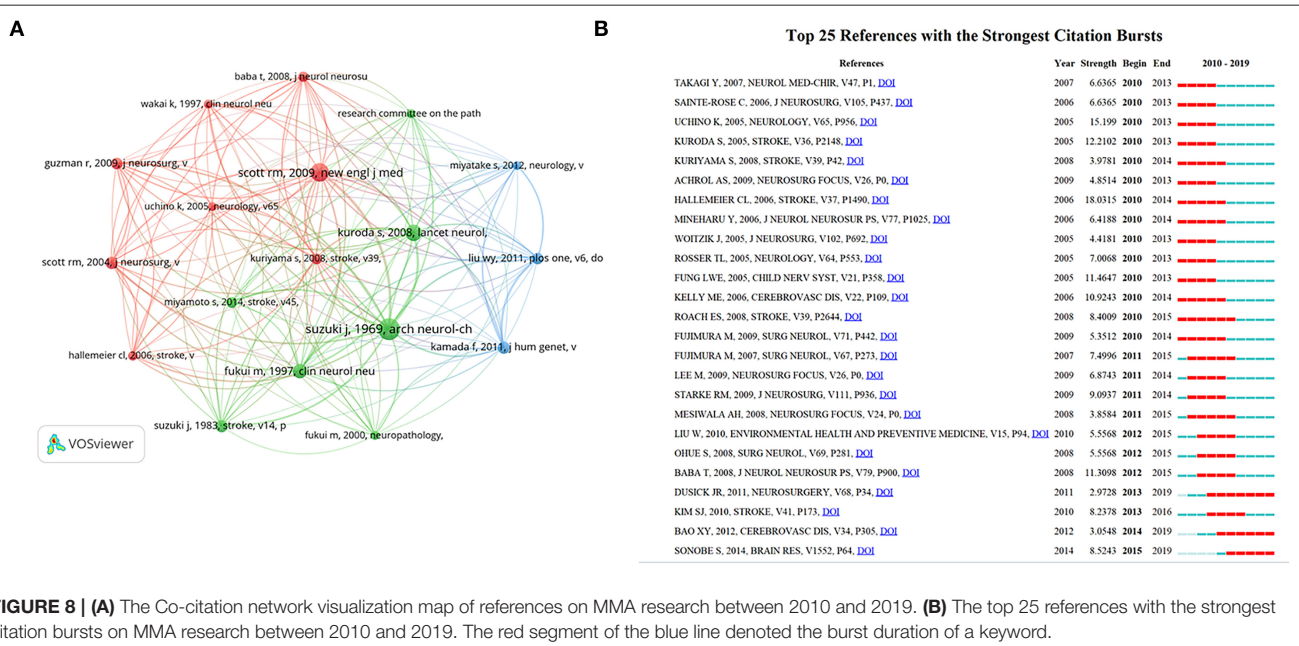

FIGURE 8 | (A) The Co-citation network visualization map of references on MMA research between 2010 and 2019. **(B)** The top 25 references with the strongest citation bursts on MMA research between 2010 and 2019. The red segment of the blue line denoted the burst duration of a keyword.

In 2011, RNF213 was reported as the first susceptibility gene by Kamada F et al. and Liu WY et al. respectively (21, 23). This discovery advanced the study of genetic factors in MMD pathogenesis. More RNF213-related articles were published after 2011. These two articles were also shown in **Table 6**. This may be the reason why the growth rate rapidly increased in 2011–2012. And in 2012, Japan released new guidelines for diagnosis and treatment of MMD (35). This guideline was recognized worldwide and drove research discovery in related area.

Japan, China, and South Korea belong to the same cluster, as shown in **Figure 3**, and they were ranked the highest in **Table 1**. Furthermore, nine of the top 10 productive institutions were in East Asian countries, which shows that East Asian institutions occupied key positions in MMA research, which is consistent with the epidemiological understanding that MMA has a higher incidence rate in East Asia (36).

As shown in **Table 3**, World Neurosurgery had the largest number of publications, and STROKE had the most co-cited article. An understanding of prolific journals could help researchers choose journals for draft submissions, and publications from top co-cited journals could be used as authoritative references. Moreover, there were six journals that were in both the top 10 prolific journals and the top 10 co-cited journals: *World Neurosurgery, Journal of Neurosurgery, Stroke, Acata Neurochirurgica, American Journal of Neuroradiology*, and *Neurosurgery*. All six journals were strongly recommended by researchers in the field.

In terms of subjects in **Figure 6** and **Table 5**, NEUROSCIENCES AND NEUROLOGY and BIOCHEMISTRY AND MOLECULAR BIOLOGY occupied pivotal positions in this field, indicating that both clinical practice and mechanism-related studies were of vital importance for MMA research.

Generally, a co-cited reference that ranked higher represented an "intellectual base" in this area (9, 37). These publications could provide a foundation for scholars who want to acquire quick insight in a particular field.

As shown in Result section, revascularization surgery and RNF213 were chosen as recent frontier topics in our study.

(1) Revascularization surgery played a significant role in the treatment of MMA patients. Surgical revascularization surgeries could be divided into direct revascularization, indirect revascularization, and the combination of both (38). As for adult patients with MMD, direct revascularization and combined revascularization were recommended (39, 40). Indirect revascularization was feasible for certain patient subgroups such as pediatric patients as well (41). The Japanese MMD trial (JAM) by Miyamoto S, et al. provided the highest-level evidence of the preventive effect of direct anastomosis for hemorrhagic MMD (42). Remarkably, the paper appeared in **Figure 8A**.

There were still some lingering questions remained to be elucidated. Although revascularization surgery might well be the most effective method for ischemic MMD in clinical practice, randomized clinical trials were still lacking (43, 44). The small sample size and lacking of neurological function assessment restrained the clinical applications of the JAM trial. Besides, ischemic events, especially transient ischemic attacks, were the main clinical manifestation for children and hemorrhagic stroke was more generally seen in adults (45–47). The occurrence rate of hemorrhagic stroke in adults varied among different region (47). Furthermore, cognition preservation was also of great importance. Revascularization surgery has been proved to be beneficial to patients with concomitant cognitive impairment (48), while a recent study revealed that cerebral hyperperfusion in the acute phase after revascularization surgery could result in cognitive impairment (49). Comprehensive evaluation and precise diagnosis were desperately needed to provide reliable basis for surgery opportunity choice and clinical treatments. Hence, large scale multicenter and multination RCTs were needed to illustrate the role of revascularization surgery among various populations of different epidemiological backgrounds. However, it was noteworthy that RCTs for ischemic MMD were ethically difficult to be carried out. Ongoing trials, such as the Adult Hemorrhagic Moyamoya Surgery Study (AHMMS), might provide insights to some of these questions (50). The aim of the AHMSS study was to replicate the therapeutic efficacy for preventing rebleeding in Chinese adult patients with hemorrhagic MMD and to reveal whether extracranial–intracranial (EC–IC) bypass surgery could improve neurological function, which was not evaluated in the JAM trial.

(2) MMA has been reported to be related to genetic factors, and numerous studies have revealed that the RNF213 gene on chromosome 17q25.3 played a role in the pathogenesis of MMA (51). Screening for RNF213 gene susceptibility to MMA patients and their families might provide evidence for the early diagnosis of MMA disease (52). A variant in RNF213 that altered arginine at position 4810 (p.R4810K) was associated with MMD in Asian populations. The homozygote of the p.R4810K variant on RNF213 exhibited an early onset age and severe form of moyamoya disease (53). Alterations in RNF213 predisposed patients of diverse ethnicities to MMD, but the p.R4810K variant predisposed individuals of Asian populations only (54). R4810K was an AAA(+) ATPase and decreased ATPase activity, suggesting its antiangiogenic activity through stabilizing oligomers. Therefore, a specific inhibitor of ATP binding to the first AAA(+) could be a promising therapeutic candidate for MMD (55).

This article also had some limitations:

1) Publication bias: To match the data type requirements of scientometric tools such as CiteSpace and VOSviewer, we extracted data from WoSCC, one of the most extensive and comprehensive global databases and the most commonly used source of publications in scientometry. Articles not indexed in WoSCC could not be involved in our study. As a result, the bias is hard to be avoided objectively.
2) Language bias: Identified literature were primarily published in English (ratio = 0.9879) which might lead to a language bias. English was not the main language used in East Asia, where MMA had a high incidence rate. MMA research written in Chinese, Japanese, and Korean had high reference value.

3) The larger number of publications was not always more important or informative. Researchers were recommended to pay attention to both the quantity of publications and their citation when they followed certain authors or institutions.

Bibliometric analysis could be used as a synopsis to help researchers to gain initial and general insights into a specific field. With the expansion of researchers' exploration of MMA, the hot spots of research on MMA have gradually changed. The brief contrasts between bibliometric data in 2000–2009 and 2010–2019 have been presented in **Supplementary Material**. Despite certain limitations, our study provided a comprehensive assessment and a preliminary understanding of the trends in MMA research. We sincerely hope that bibliometric and visualization-based analyses of global literature can provide an in-depth view of the disease mechanisms, epidemiology and treatments.

Prospects

To our knowledge, this article presents the first bibliometric study that systematically analyses the global trends in MMA research over the past 10 years. This analysis could guide scholars in the selection of new research directions and help them to understand research hot spots and frontiers. Hopefully, high-quality clinical evidence will be obtained in the future. Further cooperation between authors, institutions and countries is expected to accelerate the treatment of MMA.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

DC and GZ: study conception, design and data analysis. DC: paper writing. JiaW, SC, JinW, HN, and ZT: language polishing, paper review and editing. All authors read and approved the final version of the paper.

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SUPPLEMENTARY MATERIAL

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ORIGINAL RESEARCH
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Early Post-operative CT-Angiography Imaging After EC-IC Bypass Surgery in Moyamoya Patients

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Objective: To evaluate the clinical value of early post-operative computed tomographic angiography (CTA) after direct extracranial-intracranial (EC-IC) bypass surgery in moyamoya patients.

Methods: A retrospective analysis of all adult moyamoya patients treated at our center from 2013 to 2019 with a direct EC-IC bypass was performed. Early post-operative CTA (within 24 h after surgery) was compared with conventional digital subtraction angiography (DSA) 6–12 months after surgery. If available, magnetic resonance time-of-flight angiography (MR-TOF) was evaluated 3 months and 6–12 months post-operatively as well. Imaging results were analyzed and compared with CTA, MR-TOF and DSA, whereat DSA was used as the final and definite modality to decide on bypass patency.

Results: A total of 103 direct EC-IC bypasses in 63 moyamoya patients were analyzed. All inclusion criteria were met in 32 patients (53 direct bypasses). In 84.9% the bypass appeared definitively, in 5.7% uncertainly and in 9.4% not patent according to early post-operative CTA. MR-TOF suggested definitive bypass patency in 86.8% 3 months after surgery and in 93.5% 6–12 months after surgery. DSA 6–12 months post-operatively showed a patency in 98.1% of all bypasses. The positive predictive value (to correctly detect an occluded bypass) on post-operative CTA was 12.5%, the negative predictive value (to correctly detect a patent bypass) was 100% with a sensitivity of 100% and a specificity of 86.5%.

Conclusion: Early post-operative CTA has a high predictive value to confirm the patency of a bypass. On the other hand, a high false positive rate of (according to CTA) occluded bypasses after direct EC-IC bypass surgery can be seen. This must be considered critically when initiating possible therapeutic measures.

Keywords: moyamoya, revascularization, computed tomographic angiography, CTA, magnetic resonance time-of-flight angiography, MR-ToF, EC-IC bypass, neurosurgery

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INTRODUCTION

Surgical revascularization with direct extracranial-intracranial (EC-IC) bypass is the most common therapy in adult moyamoya patients (1, 2). Long-term bypass patency has been shown to be between 88 and 97% in these patients (3–6). The risk of bypass occlusion appears highest during the first week after surgery and declines over time as the bypass matures (3).

Digital subtraction angiography (DSA) is the "gold standard" for the imaging of cerebral vessels (3, 4, 7, 8). However, non-invasive techniques, such as computed tomography angiography (CTA) or time-of-flight magnetic resonance angiography (MR-TOF) can also be used to examine the cerebral vessels with high precision (9–13). CTA has been reported to have a specificity of 88% to 100% and even higher sensitivity for the detection and follow-up of intracranial aneurysms compared to DSA (7, 10, 13). MR-TOF has been shown to have a specificity of up to 94% and sensitivity of 88% in the same context (9, 11, 12). Little is known about the clinical value of early post-operative CTA after EC-IC bypass surgery in moyamoya patients. A small cohort with 11 patients revealed a slightly lower specificity of post-operative CTA compared to DSA but an identical sensitivity regarding early-post-operative bypass patency (8). A comparison of early post-operative CTA and MR-TOF revealed a similar visualization of EC-IC bypasses in moyamoya patients (14).

Aim of this study was to analyze the clinical value of early post-operative CTA compared to long-term results of MR-TOF and DSA regarding the bypass patency in adult moyamoya patients after EC-IC bypass surgery.

METHODS

A single-center retrospective analysis was performed including all consecutive adult moyamoya patients treated at our center between 2013 and 2019. Patients who had received a direct STA-MCA bypass, an early post-operative CTA and a DSA after 6 to 12 months were included. The primary aim of the study was to calculate sensitivity, specificity, positive and negative predictive value of early post-operative CTA. In addition, the association of bypass patency with patients' characteristics was investigated.

The presence of moyamoya angiopathy was proven in all cases angiographically. All patients received pre- and post-operative diagnostic imaging according to a routine protocol (which has been adapted over time) including pre-operative H2 15O PET CT with acetazolamide challenge, early post-operative CTA within 24 h, MR-TOF ∼3 months and DSA as well as MR-TOF approximately 6 to 12 months after surgery. Indication for revascularization was based on the results of H2 15O PET CT with acetazolamide challenge in all cases. Inclusion criteria for this study were the availability of early post-operative CTA and DSA after 6 to 12 months post-operatively. Patients with direct bypasses other than STA-MCA were excluded.

Bypass patency was evaluated independently for each imaging modality and each bypass blinded for the longitudinal imaging findings of each patient. Results were categorized as "patent," "uncertain" or "occluded." Bypasses were rated as uncertain if only the proximal donor branch was visible but could not be traced to the anastomosis. Bypasses were considered occluded if neither the proximal nor the distal part of the donor vessel could be visualized. Occluded bypasses and those with uncertain patency were reevaluated by a second reviewer according to the same criteria. Divergent results were finally decided in consensus. For statistical processing, uncertain bypasses were classified as non-patent. Additionally, MR-TOF images 3 months and 6 to 12 months after surgery were analyzed if available, following the same criteria as for CTA and DSA. Results of 6 to 12 months post-operative DSA were used as the final criterion for bypass patency. General clinical data was collected from the patients' medical files.

Statistics

Data acquisition and statistical analyses were performed with IBM® SPSS Statistics 21 (IBM Corporation, Armonk, NY, USA) and Microsoft® Excel 16.16.11 (Microsoft Corporation, Redmond, WA, USA). Due to the exploratory character of the study no a-priori case number calculation was performed. Metric variables were tested for normal distribution using the Shapiro-Wilk test. Normally distributed data was compared by means of *t*-tests for dependent or independent variables, as applicable. Nominally scaled data was analyzed with the Chi-Square or Fisher's-Exact test. P-values < 0.05 were considered as significant. The confidence interval was assumed to be 95%. Sensitivity (truly occluded/occluded in imaging modality), specificity (truly patent/patent in imaging modality), negative (truly occluded/truly and falsely occluded in imaging modality) and positive (truly patent/truly and falsely patent in imaging modality) predictive values were calculated for CTA and MR-TOF.

CTA Imaging

CT images were acquired on a 128-slice CT (Somatom Definition AS+, Siemens Healthcare AG, Forchheim, Germany). CT angiography was performed using bolus tracking after intravenous injection of 50 ml iodinated contrast agent (Imeron®, 400 mg iodine/ml, Bracco Imaging, Konstanz, Germany) followed by a 60-ml saline chaser at a flow rate of 4 ml/s. For the CTA the following scanning parameters were used: automatic tube current modulation (CARE Dose 4D, Siemens Healthcare AG, Forchheim, Germany) with a reference mAs setting of 120, Care kV with a reference setting of 120 kV, 0.75 mm collimation, 0.3 s rotation time, 0.55 spiral pitch factor. Axial MPRs were reconstructed from the raw data with a FOV of 240 mm, matrix size 512 × 512 and a slice thickness of 1.5 mm using the reconstruction kernel I26f in conjunction with advanced modeled iterative reconstruction (ADMIRE) software (Siemens Healthcare AG) strength 2.

MR-TOF Imaging

All follow-up MR scans at our hospital were done on a clinical 3T Scanner (Magnetom Skyra, Siemens Healthcare, Erlangen, Germany) using a 3D TOF-MRA sequence with the following parameters: TE 3.73 ms, TR 22 ms, flip angle 18°, FoV 177 × 210 mm, slice thickness 0.60 mm, pixel spacing 0.23 mm. The acquisition time was 3:36 min.

TABLE 1 | Characteristics of all included patients.

Patient characteristics		
Gender	Female	Male
% (*N*) of patients	75.8% (25)	24.2% (8)
Unilateral/bilateral angiopathy	Unilateral	Bilateral
% (*N*) of patients	34.4% (11)	65.6% (21)

TABLE 2 | Bypass characteristics. Cross table showing percentage and number of bypasses in which an intraoperative temporary thrombosis (which was resolved before suturing the anastomosis) of the donor vessel was observed and bypass blood flow at the end of surgery.

Bypass characteristics		**Intraoperative blood flow**			
% of bypasses (*N*)		**Promptly**	**Delayed**	**Occluded**	**Total**
Intraoperative thrombosis	Yes	3.8% (2)	3.8% (2)	0% (0)	**7.5%** (4)
	No	84.9% (45)	7.5% (4)	0% (0)	**92.5%** (49)
	Total	**88.7%** (47)	**11.3%** (6)	**0%** (0)	**100%** (53)

Intraoperative blood flow is described as written by the surgeon (NK and CR) in the surgical report based on visual inspection only without invasive measurement as promptly, delayed or occluded.

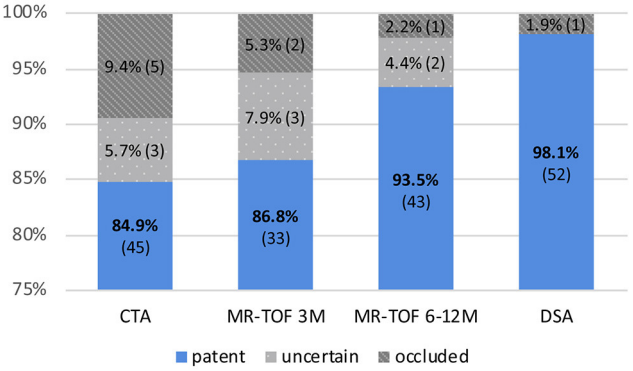

FIGURE 1 | Percentage and number of bypasses shown as "patent," "uncertain," and "occluded" in CTA post-operatively, MR-TOF after 3 months (3M), MR-TOF after 6 to 12 months (6–12 M) and DSA after 6 to 12 months.

Ethics

Ethical approval for this study was obtained from the Ethics Committee at the Medical Faculty of the University of Tuebingen (909/2020BO2).

RESULTS

A total of 103 direct EC-IC bypasses in 63 moyamoya patients were analyzed. Of those, 32 patients with 53 bypasses met the inclusion criteria and were reviewed in detail. The main reason for exclusion from the analysis was a non-available CTA or DSA in the defined time period. Four bypasses other than STA-MCA were excluded. The mean patient age at the time of surgery was 41.1 years (sd = 15.5) with an age range of 17 to 67 years. 78.1% ($n = 25$) were female and 21.9% ($n = 7$) were male (**Table 1**). Twenty-eight bypasses were on the right, 25 on the left side. Twenty-one patients (65.6%) received bilateral bypasses.

Intraoperatively, the bypasses were described as patent (proven by duplex sonography and ICG) in all cases at the end of surgery. In four cases the blood flow of the donor vessel was limited after preparation caused by vasospasm or thrombosis. Blood flow was restored successfully in all four cases by local medical therapy (heparin, papaverine) before placing the anastomosis. The intraoperative blood flow was described as promptly in 88.7% ($n = 47$) and delayed in 11.3% ($n = 6$) of the cases (**Table 2**).

Bypass Patency According to CTA

In 84.9% ($n = 45$) of all cases the bypass was clearly patent in the early post-operative CTA. In 5.7% ($n = 3$) patency was uncertain and in 9.4% ($n = 5$) CTA was suspicious for an occluded bypass (**Figure 1**). Examples for patent, uncertainly patent and suspiciously occluded bypasses are shown in **Figure 2**.

Bypass Patency According to MR-TOF

MR-TOF after 3 months was available for 38, after 6 to 12 months for 46 bypasses. Three-month post-operative MR-TOF showed the bypass to be clearly patent in 86.8% ($n = 33$), uncertainly patent in 7.9% ($n = 3$) and suspicious for occlusion in 5.3% ($n = 2$).

MR-TOF 6 to 12 months after surgery showed a clearly patent bypass in 93.5% ($n = 43$), an uncertain patency in 4.3% ($n = 2$) and occlusion in 2.2% ($n = 1$) of all cases.

Bypass Patency According to DSA

The result of DSA 6 to 12 months after surgery was the final criterion for bypass patency and showed a patency rate of 98.1% ($n = 52$) of all direct revascularizations. One STA-MCA bypass was occluded at that time (1.9%), which was concordant with the previous imaging of this patient (see **Figure 3**). In this single case, intraoperatively impaired blood flow of the donor vessel was seen caused by local thrombosis which, however, was restored to normal blood flow after medical therapy with local intraluminal heparin.

There was no significant difference in bypass patency regarding gender, age or side of the hemisphere for the post-operative CTA, MR-TOF after 3 months or MR-TOF after 6 to 12 months [CTA: gender—Fisher's exact, $p = 0.51$; age − 95%-CI(−11.28, 12.78), $t(51) = 0.13$, $p = 0.90$; side—Fisher's exact, $p = 86$; MR-TOF after 3 months: gender—Fisher's exact, $p = 1$; age − 95%-CI(−21.96, 8.73), $t(36) = -0.87$, $p = 0.39$; side—Fisher's exact, $p = 0.78$; MR-TOF after 6 to 12 months: gender—Fisher's exact, $p = 1$; age–95%-CI(−26.06, 12.51), $t(44) = -0.71$, $p = 0.48$; side—Fisher's exact, $p = 0.73$].

The overall sensitivity to detect an angiographically proven, truly occluded bypass was 100% for CTA and MR-TOF examinations. The specificity of detecting an angiographically

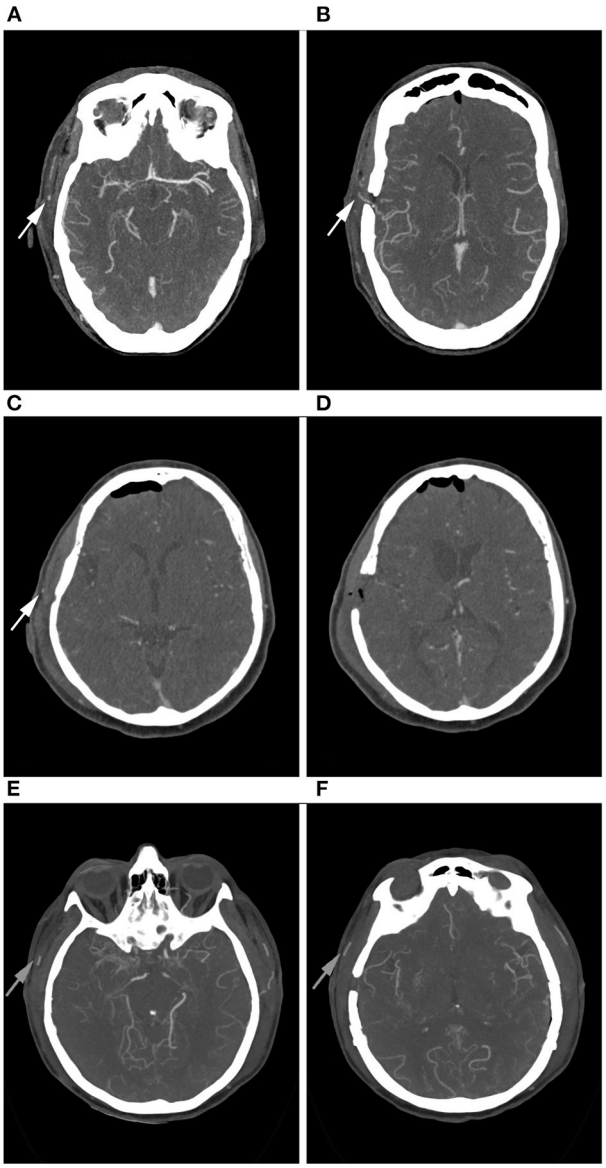

FIGURE 2 | Example of right-sided STA-MCA bypasses in CTA. **(A,B)**: Bypass is patent. The proximal **(A)** and the distal **(B)** parietal branch of the STA are visible (arrows). **(C,D)**: Bypass of uncertain patency. Only the proximal branch of the STA is visible (**C**, arrow), the distal branch is not clearly contrasted **(D)**. **(E,F)**: Bypass appears occluded. Neither the proximal **(E)** nor the distal **(F)** parietal branch of the STA are contrasted. Good contrast enhancement of the frontal branch of the STA (arrows).

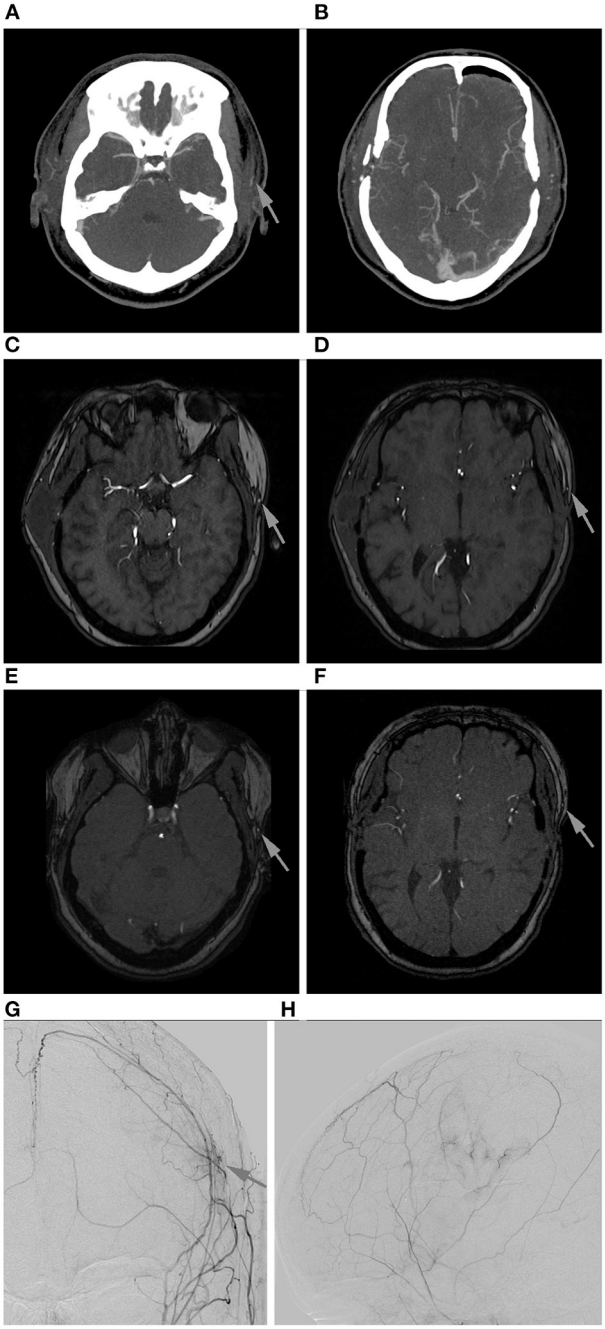

FIGURE 3 | Follow-up of a left-sided STA-MCA bypass shown to be occluded in DSA. **(A,B)**: Early post-operative CTA. Only the frontal branch of the STA is contrasted (arrow). **(C,D)**: MR-TOF after 3 months. Only the frontal branch of the STA is contrasted (arrows). **(E,F)**: MR-TOF after 6 months. Only the frontal branch of the STA is contrasted (arrows). **(G,H)**: Angiogram of the external carotid artery anterior-posterior **(G)** and lateral **(H)** with only little cortical perfusion at the site of revascularization (arrow; vessel sprouting caused by indirect revascularization with additional EGPS (MCA territory) and in the ACA territory (spontaneous collateralization caused by the middle meningeal artery).

proven patent bypass was 86.5% for post-operative CTA, 89.2% for MR-TOF after 3 months and 95.6% for MR-TOF after 6 to 12 months.

The positive predictive value (as the probability for a bypass to be truly occluded when shown uncertainly or non-patent in the post-operative CTA) was 12.5%. For MR-TOF the positive predictive value was 20% after 3 months and 33% after 6 to 12 months. The negative predictive value (the probability for a bypass to be truly patent in DSA when shown patent in CTA or MR-TOF) was 100%.

DISCUSSION

In this study we were able to show that the post-operative CTA after well-indicated EC-IC bypass surgery is highly reliable to confirm the patency of a bypass compared to the 6 to 12-month post-operative DSA. On the other hand, a relatively high false positive rate of bypasses appearing as occluded in early post-operative CTA was seen.

Overall definite bypass patency after 6 to 12 months was excellent with 98.1% and, therefore, slightly higher than reported in the literature (3, 4). One bypass which was confirmed to be occluded in the follow-up DSA was already displayed as non-patent in the post-operative CTA. This assumed very early occlusion of the bypass supports data from earlier studies that showed, that risk of bypass occlusion is highest during the first post-operative week (3). As we always place an additional indirect bypass by encephalo-galea-periost-synangiosis (EGPS) on the brain when doing a direct revascularization, the patient's body can build collaterals through the indirect bypass, as also seen in this case (**Figures 3G,H**). This, however, takes many months until the revascularization has reached relevant blood flow.

Our data show a sensitivity of 100% for the post-operative CTA as well as for MR-TOF on follow-up examinations with a slightly lower specificity. This is consistent with data from smaller cohorts investigating EC-IC bypasses by means of CTA (8, 15). However, sensitivity rates might be falsely high due to the low number of occluded bypasses in our cohort. We found a continuous increase of specificity from 86.5% in the direct post-operative examination to 95.6% after 6 to 12 months. Although different imaging modalities are compared in this case (CTA and MR-TOF) earlier examinations showed similar results on bypass visualization regarding its patency for both examination methods (14). Also, the MR-TOF specificity rate of 95.6% after 6 to 12 months almost equals findings from Park et al. who reported a specificity for CTA compared to DSA of 95.5% regarding bypass patency. In that study CTA was performed 5 to 9 months after surgery (15), which is comparable to our data.

A possible explanation for an initially higher rate of an incorrectly implied bypass occlusion is the low-flow nature of single-branch STA-MCA bypasses (16, 17). Since the time-point of the CT scan after application of the contrast agent is determined to optimally display the intracranial arteries (based on the common carotid artery), time of contrast wash-in might be delayed for the STA with a slow flow in the early post-operative phase. This, however, improves over time as the blood flow through the bypass usually increases over time depending on the intracranial blood demand (3). Therefore, a higher residual anterograde blood flow at the site of the anastomosis competing with the applied retrograde STA-MCA perfusion (2) might lead to a slower blood flow during the early post-operative period leading to false positive results on CTA imaging.

In some cases, initially uncertainly patent bypasses were displayed patent in the DSA after 6 to 12 months but didn't mature as much over time as expected. This might be a consequence of a lower intracranial blood demand than pre-operatively estimated despite extended pre-operative examinations (18).

The question arises, which clinical consequences should be derived from these results. In our institution, usually no emergency revision surgeries are performed after detecting a possibly occluded bypass on CTA. Adequate volume management should be maintained in the early post-operative phase in all patients. Extended continuous blood-pressure monitoring and intravascular volume supplementation might be considered. Also, indirect revascularization might develop even if direct revascularization fails as we usually place an EGPS in addition to direct EC-IC bypass surgery on the brain to possibly enhance indirect vessel sprouting, if needed.

In conclusion, our findings do not reveal any substantial benefits of early post-operative CTA due to a high false-positive rate for bypass occlusion. Considering the additional costs and exposure to radiation routine early post-operative CTA might not be recommended in the early post-operative course.

LIMITATIONS

This is a retrospective single-center study in moyamoya patients. Imaging was only rated by one person as patent bypasses are clearly identifiable. For uncertain or possibly occluded bypasses a second rater was asked for final consensus. We routinely do not use ultrasound examinations in the direct post-operative phase, which however might be a good tool to monitor bypass-patency but has several limitations in the early post-operative phase caused by local swelling and the wound healing. Only one bypass was seen to be truly occluded after 6 to 12 months. Therefore, sensitivity rates in this study might appear higher due to the low number of occluded bypasses. A relatively high number of overall drop-outs is based on the fact that the routine imaging protocol developed over time and, therefore, not all imaging was available for each patient at all time-points.

CONCLUSIONS

Our analysis shows a high reliability of the post-operative CTA to confirm the patency of a bypass compared to the 1-year post-operative DSA. On the other hand, a relatively high false positive rate of bypasses appearing as occluded after direct EC-IC bypass surgery is shown in early post-operative CTA. This should be critically considered when initiating early surgical revision or extended blood pressure and intravascular volume-management based on CTA. Routine early post-operative CTA might not be recommended for the evaluation of bypass patency in the early post-operative course.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Medical Faculty

of the University of Tuebingen (909/2020BO2). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Data analysis was performed by HH and CR. The first draft of the manuscript was written by HH and all authors commented on previous versions of the manuscript. All authors contributed to the study conception, design, material preparation, and data collection. All authors read and approved the final manuscript.

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frontiers in Neurology

ORIGINAL RESEARCH
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Non-invasive Urinary Biomarkers in Moyamoya Disease

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Introduction: A major difficulty in treating moyamoya disease is the lack of effective methods to detect novel or progressive disease prior to the onset of disabling stroke. More importantly, a tool to better stratify operative candidates and quantify response to therapy could substantively complement existing methods. Here, we present proof-of-principle data supporting the use of urinary biomarkers as diagnostic adjuncts in pediatric moyamoya patients.

Methods: Urine and cerebrospinal fluid specimens were collected from pediatric patients with moyamoya disease and a cohort of age and sex-matched control patients. Clinical and radiographic data were paired with measurements of a previously validated panel of angiogenic proteins quantified by ELISA. Results were compared to age and sex-matched controls and subjected to statistical analyses.

Results: Evaluation of a specific panel of urinary and cerebrospinal fluid biomarkers by ELISA demonstrated significant elevations of angiogenic proteins in samples from moyamoya patients compared to matched controls. ROC curves for individual urinary biomarkers, including MMP-2, MMP-9, MMP-9/NGAL, and VEGF, showed excellent discrimination. The optimal urinary biomarker was MMP-2, providing a sensitivity of 88%, specificity of 100%, and overall accuracy of 91%. Biomarker levels changed in response to therapy and correlated with radiographic evidence of revascularization.

Conclusions: We report, for the first time, identification of a panel of urinary biomarkers that predicts the presence of moyamoya disease. These biomarkers correlate with presence of disease and can be tracked from the central nervous system to urine. These data support the hypothesis that urinary proteins are useful predictors of the presence of moyamoya disease and may provide a basis for a novel, non-invasive method to identify new disease and monitor known patients following treatment.

Keywords: pediatric, biomarker, urine, cerebrospinal fluid, non-invasive, stroke, angiogenensis, moyamoya

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INTRODUCTION

Moyamoya disease is an increasingly recognized cause of pediatric stroke, found in ∼6% of cases in the United States (1, 2). Surgical revascularization is an effective treatment, but clinicians face challenges in successfully identifying disease prior to disabling stroke, predicting optimal timing for operative intervention and tailoring specific surgical approaches to a given patient. Follow-up is important to ensure successful engraftment and to monitor for progressive disease in other vascular territories. These challenges are further exacerbated in children, who may not be able to articulate symptoms clearly or tolerate detailed imaging more easily and safely performed in adults.

Our laboratory has had a longstanding interest in the development of non-invasive biomarkers designed to aid in the diagnosis, prognosis and therapy of tumors and cerebrovascular disease, including biomarker "fingerprints" that can distinguish between central nervous system tumors, moyamoya disease and arteriovenous malformations (3–10). Here we present initial proof-of-principle data demonstrating that a novel, non-invasive panel of urinary biomarkers can identify the presence of moyamoya disease and that the biomarkers track from the central nervous system in cerebrospinal fluid (CSF) to urine. Importantly, we also show that levels of these biomarkers vary in response to therapeutic intervention and correlate with radiographic changes post-surgery. To our knowledge, this is the first report of this application of urinary biomarkers in this population and we hope that these data provide a foundation for expanded study of this approach.

MATERIALS AND METHODS

Patient Population

Specimens and records were collected as part of an institutional review board-approved protocol at Boston Children's Hospital (BCH) and patients underwent sample collection and surgery between 2009 and 2016. All moyamoya disease patients were 18 years of age or younger at time of specimen collection and all moyamoya disease diagnoses were confirmed with MRI and catheter angiography with independent verification by board-certified neuroradiologists as part of routine clinical practice and documented in the medical records. To standardize patient populations as much as possible, all patients were pediatric, met the diagnosis of moyamoya disease (not syndromic) with bilateral disease, Suzuki grade II-V and at least 6 weeks out from any documented acute stroke or hemorrhage at time of collection to minimize the risk of confounding biomarker profiles from acute stroke (11, 12). This timeline was based on previous data that indicates that any alterations in the levels of the biomarkers examined in this study—including MMPs and VEGF—that could potentially be affected by acute stroke are typically normalized within this time window (11, 13–15). No patients had known histories of other vascular malformations or recent surgery (within 3 months of specimen collection).

Control patients were healthy, age- and sex-matched and had undergone previous unremarkable imaging of the head and brain as part of routine clinical care (typically negative studies following evaluations to rule out congenital pathologies such as Chiari I malformation or tethered spinal cord).

Urine and CSF Collection and Analysis

Urine from moyamoya disease patients was collected in the morning before surgery (or at the 6–12 month postoperative visit) and CSF was collected at time of surgery from the craniotomy site. Urine and CSF of control patients was collected from children undergoing operation for simple fatty filum/tethered cord or collected as part of routine clinical care. Once collected, urine and CSF were transported on ice to our laboratory and stored frozen (−20°C) until assay. Aliquots of each sample were centrifuged at 4,000 rpm for 5 min at 4°C and the supernatants were collected, as previously described by us (4).

ELISA (Quantikine kits; R&D Systems, Inc.) were used to quantify levels of MMP-2, MMP-9, MMP-9/NGAL, and VEGF. Specimens, standards and reagents were prepared according to manufacturer's instructions. Protein concentration was determined via the Bradford method using bovine serum albumin as the standard. Levels were determined as nanogram per milliliter (ng/mL) for MMP-2, MMP-9, and MMP-9/neutrophil gelatinase—associated lipocalin (NGAL) or picogram per liter (pg/L) for VEGF.

This work is presented in accordance with reporting recommendations for biomarker prognostic studies (16).

Statistical Analysis

Statistical analysis was performed by our biostatistician (DZ). Patients with moyamoya disease and age-matched controls were compared with respect to urinary MMPs (ng/mL) and VEGF (pg/L) by the univariate Mann–Whitney *U*-test and presented using medians and interquartile ranges (17). Percentages of individuals positive for MMP-9, MMP-9/NGAL, MMP-2, and VEGF were compared between the two groups using Fisher's exact test for binomial proportions. Receiver operating characteristic (ROC) curve analysis was performed to calculate area under the curve (AUC) for the independent predictors and to identify threshold values (i.e., cut-off points) that provide the optimal tradeoff between sensitivity of specificity (18). AUC values and 95% confidence intervals (CI) were used to summarize diagnostic performance of MMPs and VEGF.

The AUC value (maximum 1, also known as the c-index) was used as a measure of predictive accuracy of each biomarker. Multivariable logistic regression modeling was applied to determine significant independent predictive biomarkers of moyamoya disease (19). Statistical analysis was conducted using SPSS software version 24.0 (IBM Corporation, Armon, NY). Power analysis indicated that the sample sizes of moyamoya disease patients and age-matched controls provided 80% power ($\alpha = 0.05, \beta = 0.20$) to detect differences of 50% in the proportion of individuals with positive expression (20). Two-tailed $P < 0.05$ were considered statistically significant. AUC values over 0.700 were considered good and values over 0.800 were regarded to indicate excellent predictive accuracy.

TABLE 1 | Comparison of urinary MMPs and VEGF for moyamoya disease patients and controls.

	Moyamoya group ($n = 32$)			Control group ($n = 14$)			
Biomarker	**Median**	**IQR**	**Range**	**Median**	**IQR**	**Range**	***P*-value**
MMP-2	11.7	3–19.6	0–91	0	0–0	0–0	<0.001*
MMP-9	0.2	0–9.7	0–273	0	0–0	0–6.2	0.005*
MMP-9/NGAL	1.0	0.4–5.3	0.2–149	0	0–0.1	0–12.8	<0.001*
VEGF	420	163–1,112	0–4,000	0	0–113	0–391	<0.001*

Units are ng/mL for MMPs and pg/L for VEGF. IQR, interquartile range.
MMP-9/NGAL and VEGF are based on 20 moyamoya patients.
**Statistically significant.*

RESULTS

Demographics

A total of 32 patients with moyamoya disease (17 females, 15 males) and 14 healthy controls (7 females, 7 males) from 1 to 18 years of age were included in this study. Median age (interquartile range) of patients and controls were 8 (7–14) years and 7 (4–13) years, respectively ($P = 0.20$). Gender distribution did not differ between the two groups analyzed for urinary biomarkers ($P = 0.85$) (**Table 1**). The CSF subset of this cohort was smaller ($n = 19$ patients, $n = 5$ controls) and were also age and sex-matched ($P = 0.49$ and 0.90, respectively). Of the 32 moyamoya disease patients, 22 had experienced previous radiographic stroke (69%) and 26 (81%) had experienced transient ischemic attacks (TIAs), but no patients had new strokes within 6 weeks of sample collection (as documented by diffusion weighted imaging on MRI).

Urinary Biomarkers Are Elevated in Moyamoya Disease Patients

Median levels of urinary MMPs and VEGF were significantly higher among moyamoya disease patients (all $P < 0.001$, except MMP-9, $P = 0.005$, Mann–Whitney U-tests). As shown in **Table 1**, median levels of MMP-2 were 11.7 ng/mL (IQR: 3.0–19.6) for patients and 0 ng/mL (IQR: 0–0) for controls. Median VEGF levels were 420 pg/L (IQR: 163–1,112) and 0 pg/L (IQR: 0–113) for patients and controls, respectively. As illustrated in **Figure 1**, a significantly higher percentage of moyamoya disease patients (17 of 32 = 53%) were positive for MMP-9 compared to controls (1/14 = 7%) ($P = 0.003$). Similarly, whereas only 2 of 14 controls were positive for MMP-9/NGAL, all 20 of the moyamoya disease patients tested were positive ($P < 0.001$). A total of 88% of moyamoya disease patients were positive for MMP-2 (28 of 32) compared to 0 of 14 controls ($P < 0.001$). For VEGF, 95% of patients (19 of 20) were positive compared to 43% (6 of 14) controls ($P < 0.01$).

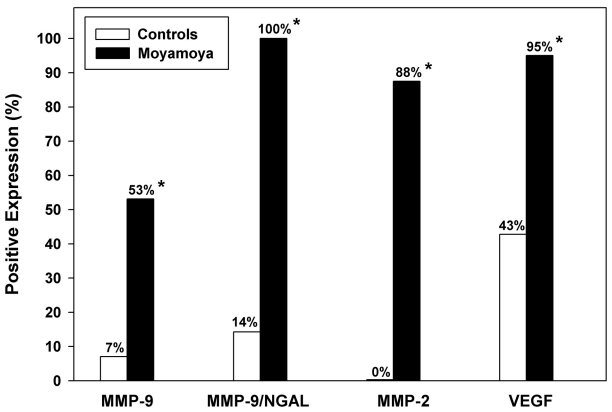

FIGURE 1 | Comparison of the percentage of moyamoya disease patients and controls with positive expression for urinary MMPs and VEGF. Significantly higher positive expression rates were observed for each biomarker as denoted by asterisks. The most useful biomarker according to both multivariable logistic regression and ROC analysis was MMP-2 demonstrating excellent predictive accuracy.

Receiver-operating characteristic (ROC) curve analysis indicated that all urinary MMPs and VEGF were significant in differentiating moyamoya disease patients from healthy controls as indicated by the area under the curve (AUC) values (**Table 2**). AUC for MMP-2, indicating diagnostic performance, was excellent (AUC = 0.938, 95% CI: 0.870–1.000, $P < 0.001$). ROC analysis revealed the optimal cut-off value for MMP-2 as 1.5 ng/mL, which is associated with a sensitivity of 0.88 or 88% (28 of 32 moyamoya disease patients correctly classified), a specificity of 1.00 or 100% (all 14 controls correctly classified), and an accuracy of 91% (42/46). Any non-zero cut-off value or criterion for MMP-2 would have produced false negatives since four moyamoya disease patients did not have measurable MMP-2 in their urine. **Figure 2** depicts the ROC curve and shows the relationship between the true-positive rate and the false-positive rate with the optimal urinary MMP-2 cut point of >1.5 ng/mL.

Multiple stepwise logistic regression revealed that, independent of age and gender, urinary MMP-2 was the only independent biomarker that significantly differentiates moyamoya disease patients from controls (c-index = 0.938, $P < 0.001$). Although MMP-9 and MMP-9/NGAL also demonstrate significant diagnostic performance, as indicated by the statistics in **Table 2**, the results of the ROC analysis and logistic regression modeling clearly showed that MMP-2 prevailed as the most accurate urinary biomarker in differentiating moyamoya disease from normal healthy controls.

TABLE 2 | ROC Analysis of urinary biomarkers in predicting moyamoya disease.

Biomarker	AUC	95% Confidence Interval	*P*-value
MMP-2	0.938	0.870–1.000	<0.001*
MMP-9	0.731	0.587–0.877	0.013*
MMP-9/NGAL	0.933	0.818–1.000	<0.001*
VEGF	0.875	0.756–0.994	<0.001*

ROC, receiver operating characteristic; AUC, area under the curve.
**Statistically significant.*

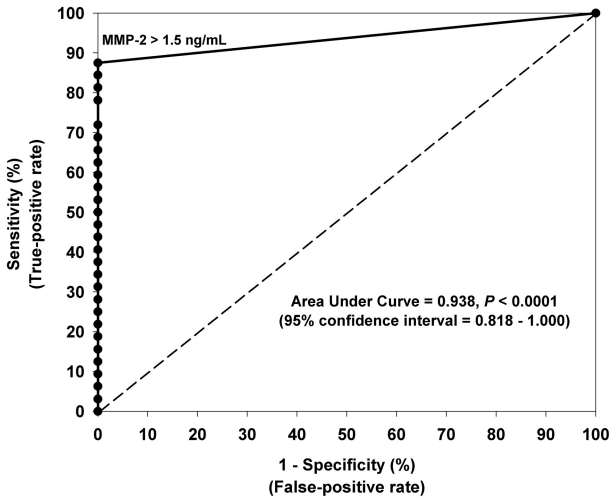

FIGURE 2 | Receiver-operating characteristic (ROC) curve analysis indicating the optimal urinary MMP-2 cut-off value (>1.5 ng/mL) for differentiating moyamoya disease patients and controls. The 45° line represents the line of nondiscrimination which would be equivalent to a coin toss. The area under the ROC curve for MMP-2 indicates excellent diagnostic predictive accuracy (AUC = 0.938, 95% CI: 0.870–1.000, $P < 0.0001$). Sensitivity was 88% and specificity was 100% using the optimal cut-off value of 1.5 ng/mL.

CSF Biomarkers Are Elevated in Moyamoya Disease Patients

CSF analysis was carried out in a similar fashion to that done on the urine and was collected from the same individuals. CSF analysis was constrained by both the number of patients available for the study and the volume of useable CSF per patient (due to fewer controls and the limitations in getting adequate volumes of non-bloody CSF for collection per individual, both controls and moyamoya disease). These limitations meant that we had a smaller "*n*" of patients, and the volume of CSF per patient meant that we had to limit our CSF biomarker analysis. Consequently, we focused on the MMP species identified as most promising from the urinary studies and were forced to exclude VEGF from CSF analysis.

We applied ROC analysis to evaluate the diagnostic performance of MMPs in CSF (**Table 3**). Of the three MMPs studied, only MMP-9 was predictive (AUC = 1.000, $P = 0.002$), although MMP-2 and MMP-9/NGAL also had strong trends toward significance. The optimal cut-off value for MMP-9 in CSF was 2.6 ng/mL which was associated with a sensitivity and specificity of 100%. All 5 controls had MMP-9 levels <2.6 ng/mL (three had no detectable levels of MMP-9, one was 0.2 ng/mL, and one subject had a value of 2.2 ng/mL). Of the 19 moyamoya disease patients with MMP-9 measurements in CSF, levels in all patients ranged from 2.9 to 27.0 ng/mL. Multiple stepwise logistic regression confirmed that, controlling for age and gender, MMP-9 was independently predictive of moyamoya disease ($P = 0.003$).

TABLE 3 | ROC analysis of markers in cerebral spinal fluid for differentiating patients with moyamoya disease from controls.

CSF marker	AUC	95% CI	*P*-value
MMP-2	0.274	0.000–0.600	0.126
MMP-9	1.000	1.000–1.000	0.002*
MMP-9/NGAL	0.776	0.569–0.984	0.066

ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval.
**Significant multivariable predictor.*
Optimal cut-off value for MMP-9 is 2.6 ng/mL or greater. This cut-off value provides a sensitivity of 100%, specificity of 100%, and accuracy of 100%.

Urinary Biomarkers Change and Correlate With Postoperative Radiographic Response

Urine was collected at 6–12 months postoperatively in patients within this group. A total of 7/32 patients had urine available for analysis. Biomarker levels were compared to preoperative samples (MMP-9/NGAL ELISA kits were no longer unavailable at the time of this delayed analysis, so MMP-2, MMP-9, and VEGF were performed). Pre- and post-operative angiograms were assessed for surgical collaterals (as measured by Matsushima grade) and pre- and post-operative axial FLAIR MRI images were compared for changes in ivy sign (a marker of slow cortical blood flow). All patients had Matsushima A or B collaterals and had reductions in global ivy sign as independently read by neuroradiologists as part of routine clinical care. MMP-2 levels decreased in 6/7 (86%) patients, MMP-9 and VEGF decreased in 5/7 (71%) patients. Representative data from a patient is presented in **Figure 3**.

DISCUSSION

Rationale for Urinary Biomarkers: *Why urine?*

There is ample precedence for the successful use of urinary biomarkers to identify physiologic states such as pregnancy and to monitor disease, such as diabetes. Use of urinary biomarkers offers advantages particularly relevant to moyamoya disease. Current methods of diagnosis and follow-up rely on infrequent clinical examinations and expensive radiographic studies (such as MRI and angiograms) that also often require sedation or anesthesia in children (2, 21). In contrast, urine collection can be done frequently, without sedation and is relatively inexpensive. Collection of urine is easy and non-invasive, avoiding the difficulties and risk inherent to biomarkers studied in spinal fluid

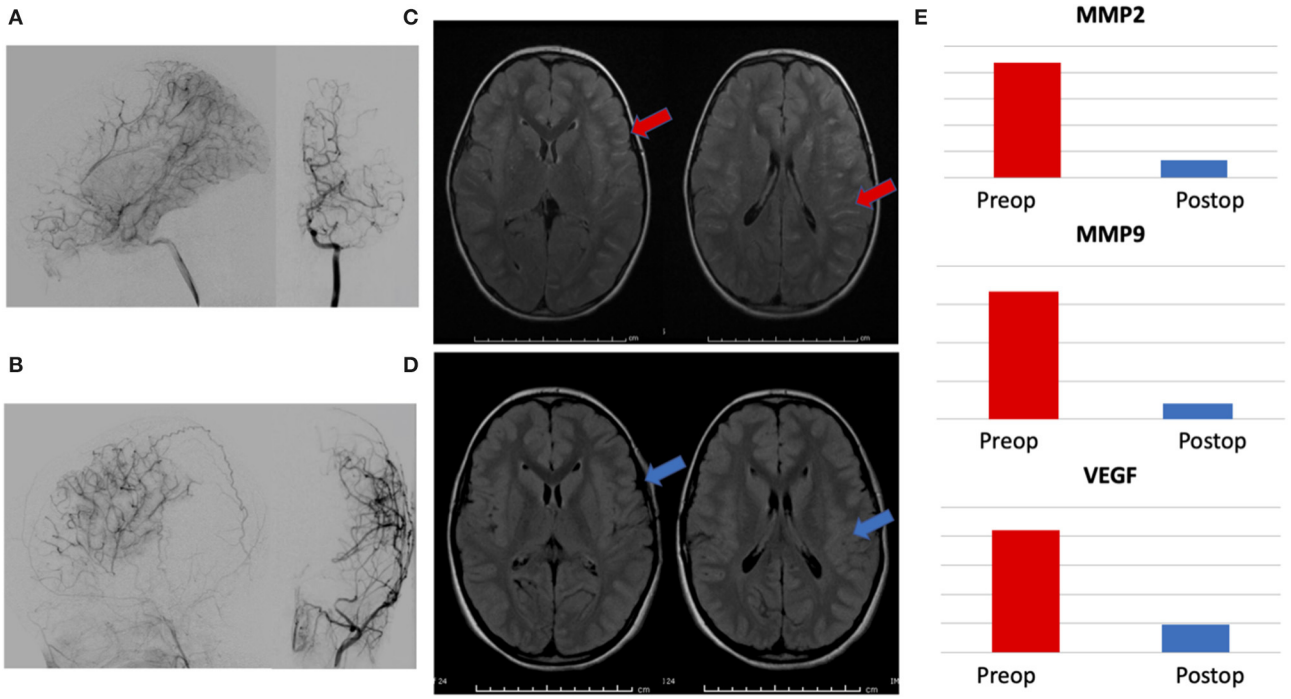

FIGURE 3 | Representative patient demonstrating correlation between radiographic changes and biomarker levels. **(A)** Preoperative angiogram with lateral and AP views of internal carotid artery injection showing Suzuki II-III moyamoya, **(B)** 1 year postoperative angiogram with lateral and AP views of external carotid injection showing Matsushima A surgical collaterals, **(C)** preoperative axial FLAIR MRI images demonstrating hyperintense sulcal signal—ivy sign, (red arrows) and **(D)** postoperative images showing marked resolution of ivy sign (blue arrows). **(E)** Reduction in postoperative levels of urinary biomarkers MMP-2, MMP-9, and VEGF, correlating with radiographic evidence of successful revascularization.

or blood, which require lumbar punctures and venipuncture. Urine collection can be done locally and mailed, saving families travel to hospitals (10). Most importantly, biomarkers provide a method of diagnosis that relies on biological activity; a novel, quantifiable—and complementary—approach to the current method of evaluation with imaging studies.

Selection of Moyamoya Disease Biomarker Panel: *Why choose these proteins?*

Background

Our laboratory has extensive experience with the identification and validation of urinary biomarkers in cerebrovascular disease, cancer and the central nervous system (CNS), including the first report of successfully applying this novel methodology specifically to brain tumors in a multicenter trial (3–10). Current data from our lab and others supports the hypothesis of angiogenesis and extracellular matrix (ECM) remodeling as essential processes in many CNS disorders, including moyamoya disease, involving vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs), a multigene family of degradative enzymes and neutrophil gelatinase associated lipocalin (NGAL), an enzyme that binds with MMP-9 to protect it from degradation—and elevated levels of MMPs and VEGF have been reported in the serum of moyamoya disease patients (2, 4, 5, 22–30). Our group was the first to demonstrate that human urinary MMP levels are sensitive biomarkers of various systemic disorders, including cancer and vascular anomalies (4, 5, 21, 31–40). Recent studies from our laboratory, now confirmed by others, support the utility of urinary levels of growth factors and MMPs as markers of disease, including our work first describing the use of this technique specifically in the CNS (4, 5, 11, 21, 26, 37, 40–48).

Rationale

In the study presented here, we focused on MMPs, NGAL, and VEGF as proteins known to be involved in the pathogenesis of moyamoya disease and easily detectable in urine with commercially available methods (in contrast to more complex techniques, such as mass spectrometry). It is important to note that these are useful as general markers of ischemia/ECM remodeling—and therefore potentially directly applicable to monitoring clinical factors relevant to moyamoya disease status and progression—but are not unique to this condition. In addition, some markers—like the ones in this study—may be elevated immediately after stroke, but typically normalize after a short window of a few weeks, making the timing of biomarker sampling important and which we have addressed in the selection of our patient cohort to minimize confounding (11, 13–15). Diagnostic specificity can be improved by looking at a broader range of molecules and multiplexing several proteins in combination to create a biomarker "fingerprint" that is unique to a given disease, and we have done this for other CNS vascular

and tumor cohorts, including moyamoya disease (3–5, 10, 49). In particular, we anticipate that molecules that regulate mechanisms of vascular morphogenesis and arteriogenesis (including, but not limited to, axon guidance factors, for example), may be useful as future areas of investigation to enhance the functionality of biomarker profiling in moyamoya disease.

Correlation of Urine to CNS Disease

Another key point of this study is the ability to link the levels of these putative biomarkers with the disease. A unique problem with moyamoya disease is the inability to harvest source tissue for analysis, distinct from other pathologies such as tumor and AVM, that afford primary lesional tissue for confirmation with biomarker staining. We addressed this challenge with three strategies. First, we subjected the urinary data to rigorous statistical analysis, performed by a biostatistician, in order to ensure the robustness of our results. Second, we obtained matched CSF samples from the same patients, in order to establish that the biomarker profile in the CNS matched the same pattern seen in the urine, a technique validated in the literature and—to our knowledge—unique in its application to moyamoya disease as described here (3–5). Third, we provide longitudinal data that demonstrates that the urinary biomarker profile changes in direct response to changes in disease status after treatment, as corroborated by radiographic studies and also with precedent in the literature (3–6, 10, 49).

Potential Applications to Moyamoya Disease: *How might this approach be used?*

Currently there are no methods available that can accurately predict how a given child's disease will progress or how that child may respond to surgical intervention (2). While the precedent of prognostic biomarkers has proven immensely valuable in other fields of medicine to predict outcomes or response to therapy, nothing like this exists for moyamoya disease (50–52). While in need of validation, the diagnostic potential of this panel could be expanded to incorporate a prognostic function by correlating high pre-operative levels of biomarker with good potential for revascularization and concomitant reduction in post-operative stroke risk. Development of a prognostic biomarker panel as outlined in this proposal would bring something entirely new—and needed—to the armamentarium of clinicians treating patients with moyamoya disease.

This study fulfills the necessary initial criteria in clinical biomarker development; statistical validation of a panel of markers that can be accurately measured with widely available techniques and associated with a biologically relevant pathway that connects the biomarkers to the disease process (9, 16). In addition, this work is unique in linking these markers from CSF to urine and also presenting their utility in a "real-world" clinical scenario of longitudinal follow-up. Ultimately, we would anticipate a panel of biomarkers for moyamoya disease (not a single protein), with distinct combinations employed based on the clinical need. For example, one fingerprint might be applied for screening, while a different group of biomarkers might help to stratify ischemia and follow response to surgery.

There is precedent to use biomarkers as tools to monitor response to therapy. Specifically, in moyamoya disease and other vascular diseases, evidence exists that levels of MMPs and VEGF are elevated in the setting of chronic ischemia and—once successful revascularization occurs and the ischemia is reduced—the ischemic stimulus driving the upregulation of MMP and VEGF is decreased, with a concomitant reduction in the levels of these markers (27, 30, 49, 53–55). As documented in previous work from our lab and others, the CNS levels of these molecules are directly related to urinary levels, with previous reports linking source tissue, CSF, serum and urine (3–6, 21, 37, 56). The working model is that the end-organ (brain) experiences chronic ischemia from the moyamoya arteriopathy and is elaborating these angiogenic factors at baseline in order to develop compensatory collateral development. Once surgical revascularization occurs, transient elevations in these factors enhance surgical collateral growth until the ischemia is corrected (a process well-documented by postoperative imaging studies, showing improved perfusion and surgical collaterals on angiogram), at which point the ischemic stimulus no longer exists, and the production of pro-angiogenic molecules decreases. While we do not have sampling from the immediate postoperative period (days to weeks), we would hypothesize that we would see marked elevations in pro-angiogenic biomarkers (such as VEGF and MMPs) within this window until enough revascularization occurred to normalize perfusion (usually several weeks to months after indirect revascularization). In support of this hypothesis, our data shows that these biomarkers are elevated pre-surgery and decrease post-operatively in concordance with radiographic evidence of effective surgical revascularization on angiogram and improved hemodynamic perfusion on MRI with reduced ivy sign (**Figure 3**).

To be clear, the rarity of moyamoya disease suggests that widespread screening of the general population to identify *de novo* cases is not a realistic goal. Utility would be greater in screening a defined, at-risk population, such as Down syndrome (which has a 26-fold higher incidence of moyamoya syndrome), sickle cell disease or patients with known family histories of moyamoya (1, 2, 9, 57–59). This targeted approach could reduce risk of scanning, cost of screening and aid in better detection of disease. Another important role for biomarkers in moyamoya disease may be aiding in stratification of risk and operative strategy in already identified moyamoya patients, with biomarker-based risk reassessment on an ongoing basis. Longitudinal data from this study suggest that these biomarkers change in response to ischemia, and further work may allow clinicians to better select operative candidates by adding biomarker profiling to complement image analysis. This could be especially useful in the growing number of asymptomatic or early-stage moyamoya disease cases that are presently without a clear clinical equipoise.

Finally, the role of urinary biomarkers may extend beyond diagnostic or prognostic adjuncts and actually inform the development of novel, biologically-based therapies. This approach of combining a specific therapy with immediate feedback on efficacy—theranostics—has rapidly expanded in

medicine. Our lab has started to merge the fields of diagnostic biomarkers with targeted therapeutics in brain tumors (60, 61). It is tempting to consider that a similar approach with moyamoya disease, using biomarker-informed delivery of pro-angiogenic therapeutics, then following response to therapy, as shown in this paper.

Limitations and Future Directions

Although much of the data revealed by this work is promising, there are clearly a number of limitations inherent to this study. First, a challenge is the rarity of the disease, making it difficult to collect CSF and urine from the same patient in great numbers. For example, while only one of the CSF markers in this study reached significance, the others clearly trended as expected and we anticipate that validation would have been achieved with larger datasets. It will be important in future work to substantially increase the CSF sampling if it is to be as robust as urinary data. Also relevant to the rarity of patients is expanding the number of post-operative urine samples to validate this approach, as minimizing variation related to timing and recovery from surgery will be important. To address this issue, it would be ideal to validate these findings in much larger populations, ideally as multi-center effort. Second, there is potential heterogeneity in moyamoya, with both disease and syndromic populations (2). While we have tried to minimize this variability by standardizing age, disease status and linked specimens, it would be helpful to expand this study to examine defined cohorts of moyamoya (RNF213, Down syndrome, post-radiation, etc.) and determine if there are unique biomarker signatures across populations. Third, expanding the number of molecules to evaluate as putative biomarkers for specific clinical applications could be done with larger numbers—and this has been successfully achieved in multiple diseases, by our group and others. For example, a panel of biomarkers designed specifically to quantify ischemia could be developed in conjunction with radiographic data.

CONCLUSIONS

We report a novel panel of urinary and CSF biomarkers that can identify the presence of moyamoya disease with a high degree of sensitivity and accuracy. Urinary MMP-2 emerged as the optimal marker, with a sensitivity of 87.5%, specificity of 100%, and accuracy of 91.3%. We demonstrate that these biomarkers track from CSF to urine and correlate with response to therapeutic interventions, including evidence of radiographic revascularization. These proteins can be assessed non-invasively, offering unique advantages in safety, ease of monitoring and reduced cost, along with a new quantifiable, biological approach that complements existing clinical and radiographic practice. Together, these proof-of-principle data support the ongoing investigation of urinary biomarkers as tools that may have utility in the diagnosis, prognosis, and treatment of moyamoya disease.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because they are linked to patient clinical images and histories and the assays are commercially available, as noted in the Methods section. Requests to access the datasets should be directed to edward.smith@childrens.harvard.edu.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Boston Children's Hospital Institutional Review Board. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

ES and JS contributed to conception and design of the study. JD and AM-G analyzed the data. DZ performed the statistical analysis. ES wrote the first draft of the manuscript. ES, DZ, and DO wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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REVIEW
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Implementation and Rationale for a Unified Clinical and Imaging Protocol for Evaluation and Treatment of Moyamoya Angiopathy: A Single Institutional Experience

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Moyamoya disease (MMD) is a complex and incompletely-understood cerebrovascular pathological entity that requires thorough clinical and imaging evaluation. Moyamoya is rare, thereby making the establishment of an effective, thorough and interdisciplinary patient evaluation protocol challenging, even within specialized referral centers. Nevertheless, implementation of such a protocol is crucial in order to provide the best possible evaluation and treatment for MMD patients. Here, we describe our institution's implementation of, rationale for, and experience with a comprehensive multidisciplinary collaboration and evaluation strategy for adult patients with moyamoya. This evaluation course consists of, first of all, a thorough clinical and laboratory evaluation with a vascular neurologist. This is followed by a comprehensive imaging assessment which evaluates angiographic and parenchymal features, in addition to cerebrovascular functionality. Finally, appropriate referrals are made to consulting services as indicated, which includes vascular neurosurgery. These steps are described in detail herein.

Keywords: moyamoya, revascularization, vessel wall imaging, cerebrovascular reactivity, BOLD, protocol

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INTRODUCTION

Moyamoya disease (MMD) is an idiopathic steno-occlusive vasculopathy affecting the intracranial arteries with formation of fragile collateral neo-vessels (1). MMD is distinct from moyamoya syndrome (MMS), in which patients have a similar radiographic appearance of blood vessel narrowing, but caused by different mechanisms than the genetic mutations that leads to MMD (2). Despite important discoveries relating to the genetic underpinnings of MMD in recent years, a complete picture of the underlying pathophysiology remains to be uncovered (3–6).

Contemporary evaluation of moyamoya vasculopathy involves assessing for the presence of underlying conditions associated with MMS rather than MMD. Next is assessing the risk of ischemic or hemorrhagic intracranial events to determine whether preventative therapeutic measures are indicated. Many different imaging modalities are available for assessment of the severity and extent of MMD, including conventional angiography, various MRI and MRA techniques, CT and CTA techniques, and nuclear medicine studies. These include both anatomic and functional methods, assessing the caliber of the vessels, degree of collateral circulation, brain

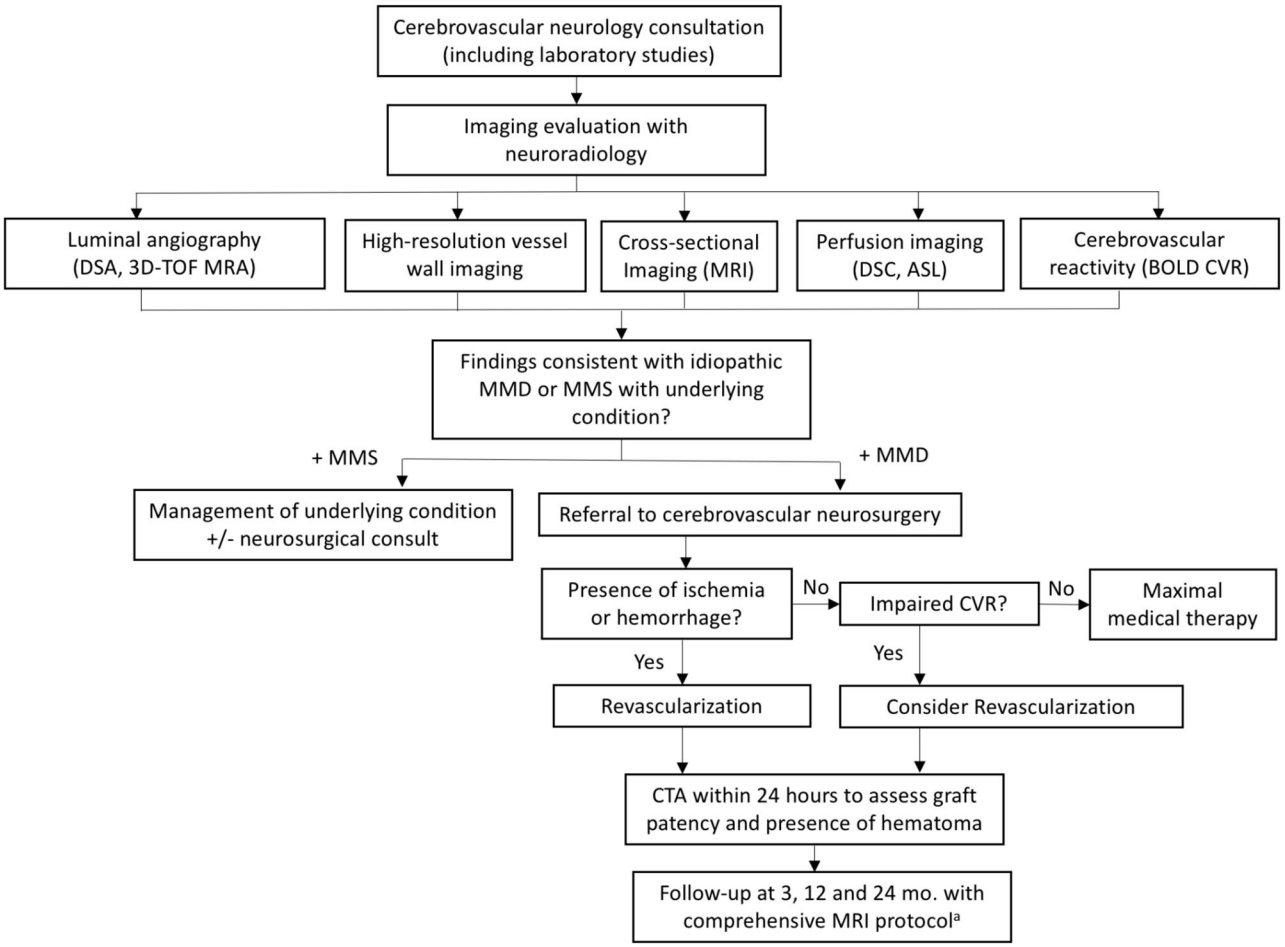

FIGURE 1 | Flowchart summarizing our multidisciplinary evaluation and management process. [a]Comprehensive protocol includes: 3D TOF MRA, vessel wall imaging, standard brain parenchymal imaging, DSC/ASL perfusion imaging, and BOLD CVR. ASL, arterial spin labeling; BOLD CVR, blood oxygen level dependent cerebrovascular reactivity; DSC, dynamic susceptibility contrast; MMD, moyamoya disease; MMS, moyamoya syndrome; TOF, time of flight.

perfusion, and determining cerebral vascular reserve. The precise imaging techniques employed vary considerably amongst, and sometimes within, institutions. Information gathered from such studies typically guides the decision to address any existing underlying syndromes, pursue medical therapy, or to proceed with surgical revascularization (5).

As the pathomechanisms of MMD and MMS continue to be elucidated, so too does our realization of the complex, and sometimes systemic, nature of these disease processes, including possible involvement of the extracranial internal and external carotid arteries, aorta, pulmonary artery, coronary artery, celiac trunk, and renal artery. As an example, some cases of MMS may require evaluation by a specialist other than a cerebrovascular neurologist or neurosurgeon in order to manage any concomitant extracranial disease. Given our incomplete understanding of the disease as well as the relative rarity, establishment of an evidence-based evaluation and management paradigm remains challenging. It is possible, however, that many existing evaluation paradigms for moyamoya patients are inadequate to address the complexity of the disease. Patients who are ultimately diagnosed with MMD likely require lifelong cerebrovascular care with potential for invasive surgical interventions. It is therefore prudent for specialized centers involved in the care of MMD patients to thoroughly and consistently evaluate each case in order to optimize management strategy.

With a lack of data regarding evaluation and management protocols for moyamoya, descriptions of such protocols from

Abbreviations: ASL, arterial spin labeling; BOLD, blood oxygen level dependent; CBF, cerebral blood flow; CBV, cerebral blood volume; CTP, CT perfusion; CVR, cerebrovascular reactivity; DSC, dynamic susceptibility contrast; DWI, diffusion-weighted imaging; FLAIR, fluid-attenuated inversion recovery; HR-VWI, high resolution vessel wall imaging; MIP, maximal intensity projection; MMD, Moyamoya disease; MMS, Moyamoya syndrome; MPRAGE, magnetization-prepared rapid acquisition with gradient echo; MTT, mean transit time; PET, positron emission tomography; PLD, post label delay; SPECT, single photon emission computed tomography; STA-MCA, superficial temporal artery to middle cerebral artery; SWI, susceptibility-weighted imaging; TOF, time of flight; TTP, time to peak.

TABLE 1 | Blood/serum and CSF tests obtained at initial evaluation to screen for associated conditions.

	Sample	
	Blood/serum	**Cerebrospinal fluid (CSF)**
Test	CBC with differential	Cell count and differential
	Basic metabolic panel	Total protein
	Peripheral smear (morphology evaluation)	Glucose (CSF and serum)
	Thrombophilia profile*	Gram stain
	Inflammatory markers (ESR, CRP)	HSV (PCR)
	Connective tissue disease cascade	Parvovirus B19 (PCR)
	ANCA vasculitis panel	VZV (PCR)
	Thyroid function cascade	Oligoclonal banding panel
	Hemoglobin A1c	CSF IgG index panel
	Homocysteine	IgG/Albumin ratio
	Lipid panel	

*Includes lupus anticoagulant and antiphospholipid antibodies. HSV, herpes simplex virus; PCR, polymerase chain reaction; VZV, varicella zoster virus.

centers with specialized moyamoya care may aid other centers in establishing their own paradigms. Herein, we describe our institution's development of, rationale for and experience with a comprehensive interdisciplinary evaluation strategy for adult MMD patients.

MULTIDISCIPLINARY MOYAMOYA MANAGEMENT AND STUDY GROUP

The first and perhaps most important step taken at our institution was the establishment of a dedicated multidisciplinary moyamoya team including representatives from vascular neurology, vascular neurosurgery, genetics, and neuroradiology. Together, we devised a comprehensive evaluation paradigm to work up or exclude potential causes of moyamoya syndrome, to direct treatment, and to follow patients after conservative or surgical management. This included introduction of a dedicated moyamoya clinic whereby all patients presenting to our institution identified with possible or known moyamoya are evaluated directly by, or occasionally in conjunction with, a dedicated vascular neurologist. This process ensures that patients are evaluated consistently by an experienced clinician and have their work-up streamlined. In conjunction, we standardized and optimized our imaging evaluation. These processes are detailed in the following sections. An outline of our multidisciplinary evaluation and management process is provided in **Figure 1**.

INITIAL EVALUATION AND REFERRAL TO CONSULTING SERVICES

A key step in evaluating a patient with moyamoya angiopathy is determining whether their overall clinical picture fits more-so with idiopathic MMD, or with MMS. This is determined by an initial evaluation with a vascular neurologist with experience in caring for patients with moyamoya. In cases of MMS, patients will have the presence of an underlying condition that may require evaluation by a specialist in that particular area. MMS is known to be associated with several genetic conditions such as Down syndrome (7), neurofibromatosis type-1 (8) and sickle cell disease, (9) autoimmune conditions including systemic lupus erythematosus (10), Hashimoto's thyroiditis (11) and Grave's disease (12), various prothrombotic conditions (10) as well as connective tissue syndromes such as Marfan syndrome (13). Furthermore, infectious etiologies such as vasculitis, encephalitis, or meningitis may either precede the development of a Moyamoya-like angiographic pattern or be present concurrently as evidenced by lumbar puncture or other laboratory studies (14–17). As such, at the time of initial assessment, all patients undergo standardized serum and CSF testing to screen for these associated conditions (**Table 1**). As a general rule, all patients will undergo testing with this full panel given that our population consists of a large proportion of North American/Non-Asian patients, and therefore evaluating for mimics or underlying conditions associated with MMS is important. In a small percentage of cases however, individuals may not undergo the complete laboratory workup if a high degree of suspicion is present for idiopathic MMD.

The concomitance of such conditions with an angiographic and clinical picture of moyamoya requires a balanced care approach, in which the patient is treated by the referring neurologist while at the same time undergoing evaluation by a specialist for the condition that underlies the MMS. For example, patients with underlying diagnoses of neurofibromatosis type-1 and systemic lupus erythematosus would benefit from evaluation by physicians specializing in genetic medicine and rheumatology, respectively. This type of collaboration is crucial, as treating an underlying disease process may improve vascular patency in MMS, therefore obviating the need for revascularization (18). This is particularly true in cases of acquired syndromes such as lupus or antiphospholipid syndrome, whereas chronic, genetic conditions with MMS may eventually require revascularization (7). Our institutional practice therefore relies on early referral to and evaluation by specialists in the specific underlying condition associated with MMS.

IMAGING EVALUATION

Imaging studies are crucial for diagnosis, disease staging, and treatment planning. This information, in turn, guides the clinician's decision to pursue medical therapy or to proceed with a revascularization procedure. A neuroradiologist with experience interpreting imaging studies for the specific diagnosis of moyamoya is an essential part of the treatment team. For the purposes of our protocol, imaging assessment of MMD can be classified into five categories: (1) luminal angiography, (2) vessel wall imaging, (3) standard brain parenchymal imaging, (4) perfusion imaging, and (5) assessment of cerebrovascular reactivity. There are several effective techniques to evaluate each of these imaging categories. For our protocol, we consolidated all five imaging categories into a single comprehensive MRI examination. The advantages of this approach includes the

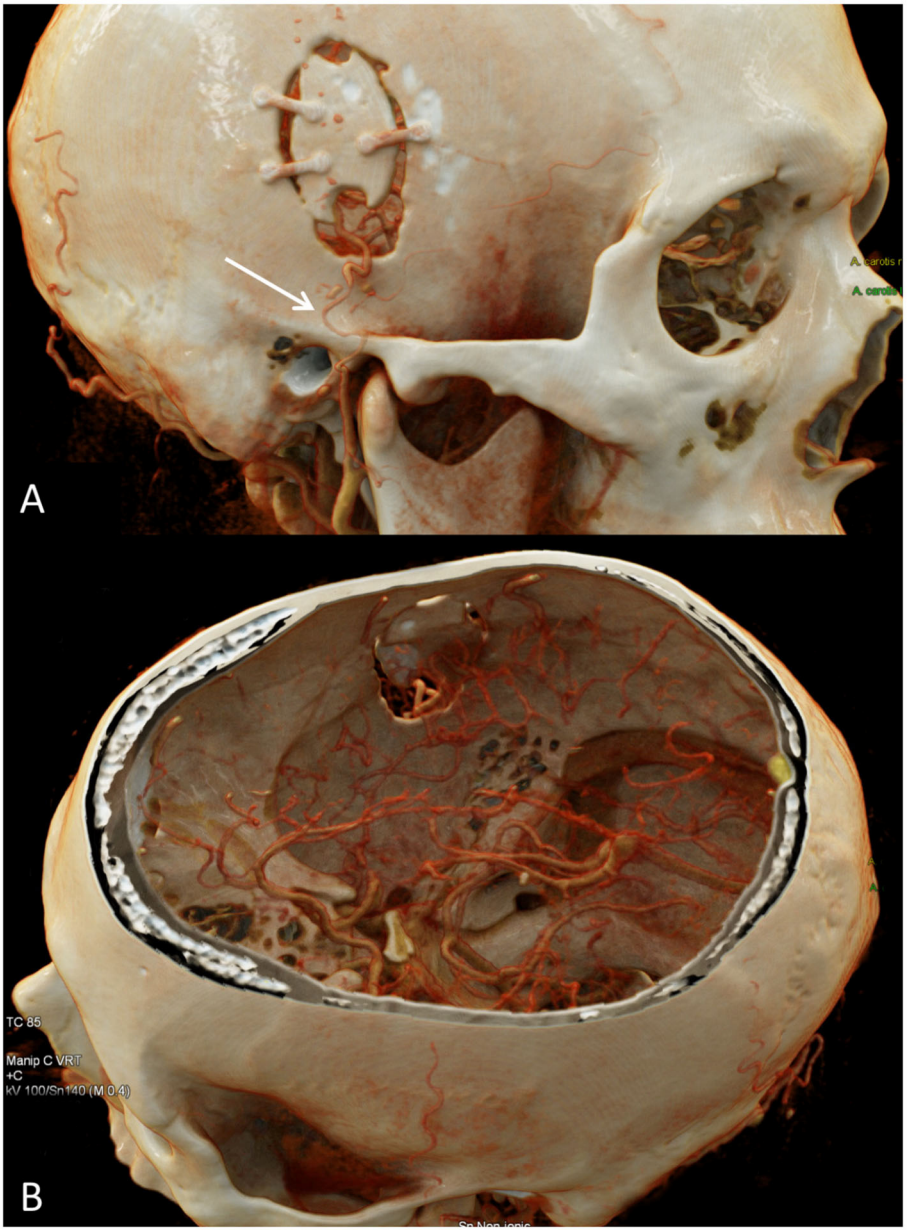

FIGURE 2 | CTA of the head and cinematic rendering provides photorealistic depiction of the STA-MCA bypass postsurgical changes. **(A)** Oblique view of the head shows a right frontal craniotomy with craniotomy flap in satisfactory position. An osseous cut-out within the inferior craniotomy flap permits unobstructed intracranial passage of the STA. There is segmental narrowing of the superficial temporal artery in the vicinity of the zygomatic arch, likely on the basis of vasospasm (arrow). **(B)** Intracranial view with top of calvarium segmented clearly depicts the STA-MCA anastomosis. STA-MCA, superficial temporal artery to middle cerebral artery.

relative availability of MRI, the ability to obtain all the required imaging information in a single visit, lack of radiation, and a superior examination of the brain parenchyma. Additionally, among imaging modalities, MRI provides the unique ability to demonstrate important moyamoya features such as the ivy sign and vascular mural abnormalities. This approach also ensures consistent evaluation of all five categories of imaging information by a small group of neuroradiologists familiar with the pulse sequences and the disease. A minor disadvantage of this approach is that the overall scan time is ∼90 min, which can be difficult for claustrophobic patients, although we believe that the advantage of consolidating the imaging evaluation into a single appointment offsets this factor. We perform this protocol at our baseline MRI examination and at routine follow-up examinations.

Importantly, patients who are referred to our service for evaluation, unless contraindicated, undergo digital subtraction angiography (DSA) in order to interrogate the extent of disease

including the degree of arterial stenosis as well as the pattern of basal and leptomeningeal collateralization. Evaluation of these patterns, in addition to determining the diameter and contribution to the intracranial blood supply by various external carotid artery branches enables for planning of potential surgical intervention in cases where it may be indicated. Indeed, DSA is largely considered as the gold standard for evaluation of MMD given its high spatial and temporal resolution (19). Additionally, CT examinations, including CTA and computed tomography perfusion (CTP) may be used in select circumstances such as detailed evaluation of a direct bypass (**Figure 2**) or emergency evaluations (**Figure 3**).

ANGIOGRAPHIC ASSESSMENT OF THE LUMEN

While patients typically undergo a baseline catheter angiogram, non-invasive approaches are incorporated into our routine follow-up imaging protocol. For MRI examinations 3-D time of flight (3D-TOF) MRA is used. This technique is widely available and allows assessment of the basal arteries, moyamoya collaterals, and direct bypass grafts. CTA offers similar capability but exposes the patient to radiation–an undesirable attribute in a patient population that commonly requires numerous imaging evaluations over a lifetime. Thus, we typically reserve CTA assessment for urgent indications. Physician groups could also consider additional incorporation of a dynamic gadolinium bolus MRA examination such as TRICKS (Time Resolved Imaging of Contrast Kinetics) or TWIST (Time-resolved angiography with Interleaved Stochastic Trajectories) depending on time constraints and local expertise.

HIGH RESOLUTION VESSEL WALL IMAGING

High-resolution vessel wall imaging (HR-VWI) consists of a variety of MRI techniques that provide sub-millimeter resolution and suppression of surrounding signal from flowing blood which enables assessment of the arterial wall (20). The most consistent feature of MMD on HR-VWI is negative remodeling of the involved segments (decreased outer wall diameter), although vessel wall enhancement and thickening are also reported (19, 21, 22). The primary utility of HR-VWI lies in diagnosing and distinguishing idiopathic MMD from other disease processes which may appear similar on conventional luminal imaging modalities, such as intracranial atherosclerosis (23). This distinction is beneficial from a management perspective, as patients with idiopathic MMD are more likely to benefit from surgical revascularization, whereas patients with distinct intracranial pathologies (such as atherosclerosis and vasculitis) are typically managed with medical therapy (24). However, neuroradiologist experience is important since the imaging findings such as vessel wall enhancement are variable and could be misinterpreted. Additionally, emerging evidence indicates that vessel wall enhancement may predict disease activity including progression of arterial stenosis and risk for territorial infarct, although the potential role of HR-VWI in this regard requires more data (20, 25).

Given the potential utility for HR-VWI, we include a robust multicontrast vessel wall protocol similar to that described by the University of Washington group (**Figure 4**) (23, 26). This allows for evaluation of the wall diameter, thickness, contrast enhancement, and T2-weighted features which are thought to help differentiate atherosclerosis, vasculitis, and MMD. Additionally, in our experience, subtle findings such as small areas of atherosclerotic plaque may be more conspicuous on vessel wall imaging than other imaging modalities including 3D-TOF angiography. Incorporation of vessel wall imaging into a standard protocol requires radiologist expertise and is dependent on the availability of requisite MRI hardware and software, although commercially available pulse sequence techniques and experience have become more commonplace in recent years.

When using information from vessel wall imaging examinations to make clinical decisions, it is useful to keep some of the current limitations in mind. First, there is a paucity of pathologic correlation with VWI findings, in particular for MMD. More prospective longitudinal data is needed, including description of findings at initial presentation, over time, and in post-treatment states. Findings may vary amongst the different available VWI techniques, which differ in spatial resolution, methods of blood signal suppression, and other technical aspects. MMD may be a heterogeneous entity with similar radiographic and clinical findings which may depend on the population studied; most publications for MMD are currently from Eastern regions with fewer reports from Western regions including the United States.

CROSS-SECTIONAL IMAGING

Standard cross-sectional MRI images allow assessment of numerous important findings including the "ivy sign," white matter T2 hyperintensity burden, ischemic or hemorrhage infarct, volume loss amongst other findings (19). The precise pulse sequences used will rely on availability and local practice patterns. Our protocol includes standard pulse sequences with an emphasis on high resolution volumetric sequences. For example, volumetric T1 magnetization- prepared rapid acquisition with gradient echo (MPRAGE) allows assessment of T1 characteristics and cerebral volumetric calculations. Volumetric (rather than 2D) T2 fluid-attenuated inversion recovery (FLAIR) images facilitate fusion with blood-oxygen level dependent cerebrovascular reactivity (BOLD CVR) maps and potential for white matter T2 hyperintensity quantification. An echo-planar gradient echo sequence is used instead of a susceptibility-weighted imaging sequence due to time considerations, although some features of MMD may be more conspicuous on the latter (19). Of course, standard diffusion-weighted images (DWI) are obtained in addition to the aforementioned. The primary alternate modality to assess the parenchyma is CT, although MRI is advantageous as it provides more detailed assessment and avoids radiation dose.

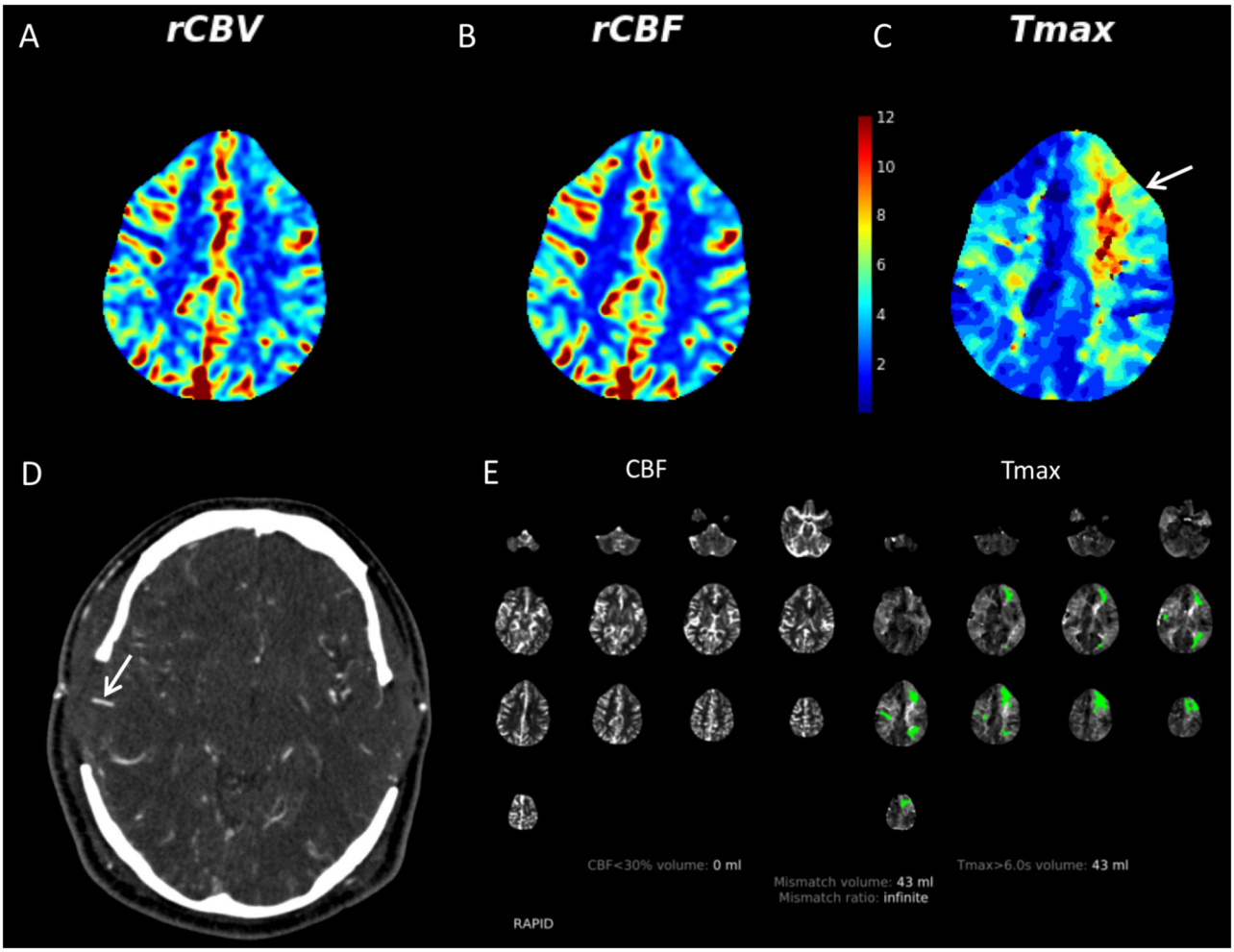

FIGURE 3 | 36-year-old male with multiple vascular risk factors presented to our institution with bilateral internal carotid artery terminus narrowing. He had previously undergone a left-sided STA-MCA bypass. He developed aphasia on postoperative day 4 following a right direct STA-MCA bypass (done in conjunction with an indirect right bypass). Given the acuity of the clinical features, the patient was evaluated with CT/CTA/CTP. There was no evidence of acute infarct or hemorrhage on non-contrast CT (not shown). CTP demonstrates mildly increased CBV **(A)** and CBF **(B)** in the right MCA territory compared to the left. There is a region of prolonged Tmax in the left frontal lobe (yellow and red colors, white arrow) **(C)**. Tmax, which represents the timepoint at which the residue of the perfusion deconvolution function reaches its maximum value, has been used in recent years to identify areas of brain parenchyma at risk for infarct (so-called penumbra) in the setting of acute stroke. The CTA demonstrates that both direct bypasses are patent (right shown with white arrow) **(D)**. RAPID software (RapidAI, Menlo Park, CA, USA) indicates that there is no region of CBF < 30%, suggesting lack of acute infarct **(E)**. Substantial areas of prolonged Tmax (green regions) suggesting ischemic tissue was present. Lack of acute infarct was confirmed on subsequent MRI. This case demonstrates that it is important to have readily available methods to evaluate acute parenchymal, angiographic, and perfusion changes in patients with MMD as part of a MMD program. CBF, cerebral blood flow; CBV, cerebral blood volume; CTP, CT perfusion; MMD, moyamoya disease; STA-MCA, superficial temporal artery to middle cerebral artery.

PERFUSION IMAGING

Well-established techniques useful for characterization of perfusion in MMD include dynamic susceptibility contrast (DSC) perfusion and arterial spin labeling (ASL). Our standardized protocol utilizes DSC perfusion. DSC perfusion is commonly performed in evaluation of glial brain tumors and is a method familiar to MR technologists. The DSC perfusion pulse sequence is performed concomitantly with an intravenously administered gadolinium bolus. In the context of our MMD imaging protocol, patients only require a single bolus of gadolinium for both HR-VWI as well as DSC perfusion which are performed in a single study.

DSC perfusion allows assessment of numerous perfusion parameters: Cerebral blood flow (CBF) enables us to understand if blood delivery to the brain is adequate, mean transit time (MTT), time to peak (TTP), and Tmax aid in understanding the performance of moyamoya induced collateral flow and/or surgically created collateral pathways and their role in maintaining CBF, relative cerebral blood volume (rCBV) helps in understanding the volume of small collateral vessels in any portion of the brain. Together, these parameters create

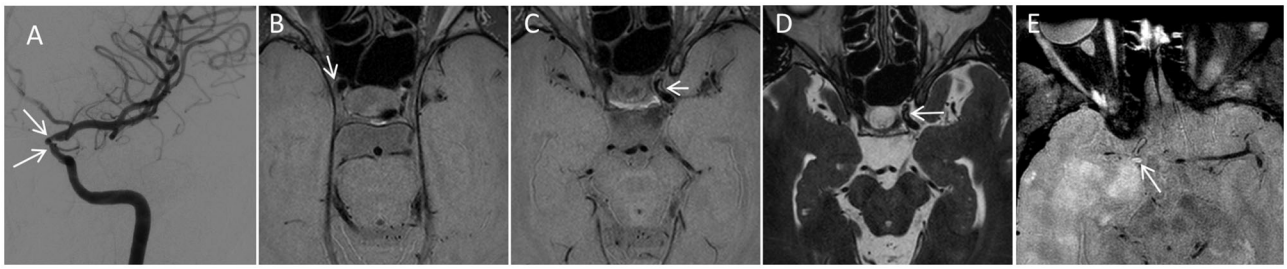

FIGURE 4 | Utility of multicontrast VWI to help establish the etiology of stenosis. A 36-year-old female presented to our institution with areas of bilateral intracranial internal carotid artery (ICA) narrowing, left greater than right. A catheter angiogram from the left ICA injection **(A)** Demonstrates stenosis. The angiographic pattern is atypical for idiopathic moyamoya disease, including stenoses of the clinoid and ophthalmic segments of the left ICA (white arrows) with sparing of the ICA terminus and proximal M1 segments. VWI as part of the comprehensive protocol demonstrated multifocal areas of subtle eccentric vessel wall thickening in both ICAs, Including subtle plaques such one along the lateral wall (white arrow) on axial proton density VWI **(B)**. Eccentric multifocal plaques along the left ICA were more prominent (arrowhead in **C,D**) with rims of non-enhancing intermediate signal on proton density VWI **(C)** and high signal on T2 weighted VWI **(D)**. Such rim of high T2 signal is described as a feature of atherosclerosis. Overall, the anatomic distribution of stenoses, lack of negative mural remodeling, and signal characteristics support atherosclerosis as the etiology. 55-year-old male with a 5-year history of primary CNS vasculitis with prior diffuse segmental involvement of the intracranial arteries **(E)**. He experienced a relapse while on maintenance mycophenolate mofetil, with new stenosis of the right supraclinoid ICA and high-grade circumferential vessel wall enhancement (arrow). While the location and circumferential involvement of the vessel wall are similar to that seen in MMD, the marked degree of unilateral contrast enhancement in conjunction with the clinical scenario are most compatible with vasculitis. ICA, internal carotid artery; VWI, vessel wall imaging.

a picture of cerebral hemodynamics when a patient is at their baseline cerebrovascular function.

We will occasionally use ASL perfusion as either a supplement to DSC perfusion or when gadolinium cannot be administered. ASL does not require gadolinium as it relies on extracranial magnetic tagging of inflowing blood as a means of measuring cerebral perfusion. ASL allows assessment of CBF but requires selection of an appropriate post-labeling delay (PLD), which represents the difference in time between "tagging" of inflowing blood and image acquisition. Too short a PLD leads to acquisition before magnetically labeled blood has made its way to the brain, and with an excessively long PLD, there is insufficient labeled blood available to contribute perfusion information. Newer multi-delay ASL techniques are now becoming commercially available and have potential to improve evaluation of processes such as MMD. Decreased perfusion from stenosis itself can lead to exaggerated areas of apparent decreased flow, particularly with short PLD intervals (**Figure 5**).

There are several other methods to assess perfusion as well which could be considered depending on the clinical scenario, availability, and local expertise. CT perfusion allows assessment of the same parameters, but with the drawback of potentially harmful ionizing radiation exposure. There are also several nuclear medicine techniques including ^{99m}Technecium (Tc)-labeled radiopharmaceutical single photon emission computed tomography (SPECT) and ^{15}O-water positron emission tomography (PET). ^{15}O-water PET has the advantage in that it offers potential for absolute quantification of perfusion rather than just evaluation of relative perfusion in comparison to other brain regions. However, ^{15}O-water has a half-life of ∼2 min, requiring not just an on-site cyclotron for ^{15}O-water production, but also an efficient system for transporting the tracer directly from the cyclotron into the PET exam room (27). Other disadvantages of these modalities include separate appointment times, radiation exposure, and the fact that they are difficult to incorporate into a single unified imaging protocol. If nuclear medicine approaches are employed, it may be useful to have a dedicated representative on a moyamoya treatment team with a solid understanding of the clinical considerations and pathology.

ANALYSIS OF CEREBROVASCULAR REACTIVITY

Our protocol includes assessment of CVR since this is one factor we consider for recommending surgical revascularization and for following patients after treatment (**Figure 6**). Analysis of CVR in combination with perfusion imaging is important: Perfusion imaging provides information about a patient's baseline perfusion status but does not provide information regarding cerebral hemodynamics in response to a stressor. Likewise, assessing CVR provides information regarding a patient's cerebrovascular response to a hemodynamic stressor but provides little insight into resting state perfusion. These two modalities performed in combination with one another provide a more complete picture of cerebral hemodynamics at baseline and in context of a stressor.

Again, there are several methods to assess CVR. The most significant change that has taken place over the years at our institution is the utilization of MRI-based BOLD imaging as opposed to SPECT for evaluation of CVR. BOLD CVR imaging is an excellent way to introduce a hypercapnic stimulus and determine if it elicits a vasodilatory response from baseline. It is effectively a way to determine if the underlying vascular tension has been disrupted by pathology. Brain territories that are at risk for infarction due to poor blood flow will have elevated regional carbon dioxide in order to produce local vasodilation and recruit more oxygenated blood. With progressive disease, at some point this compensatory mechanism will no longer be able to keep up and brain function becomes impaired with risk of infarction.

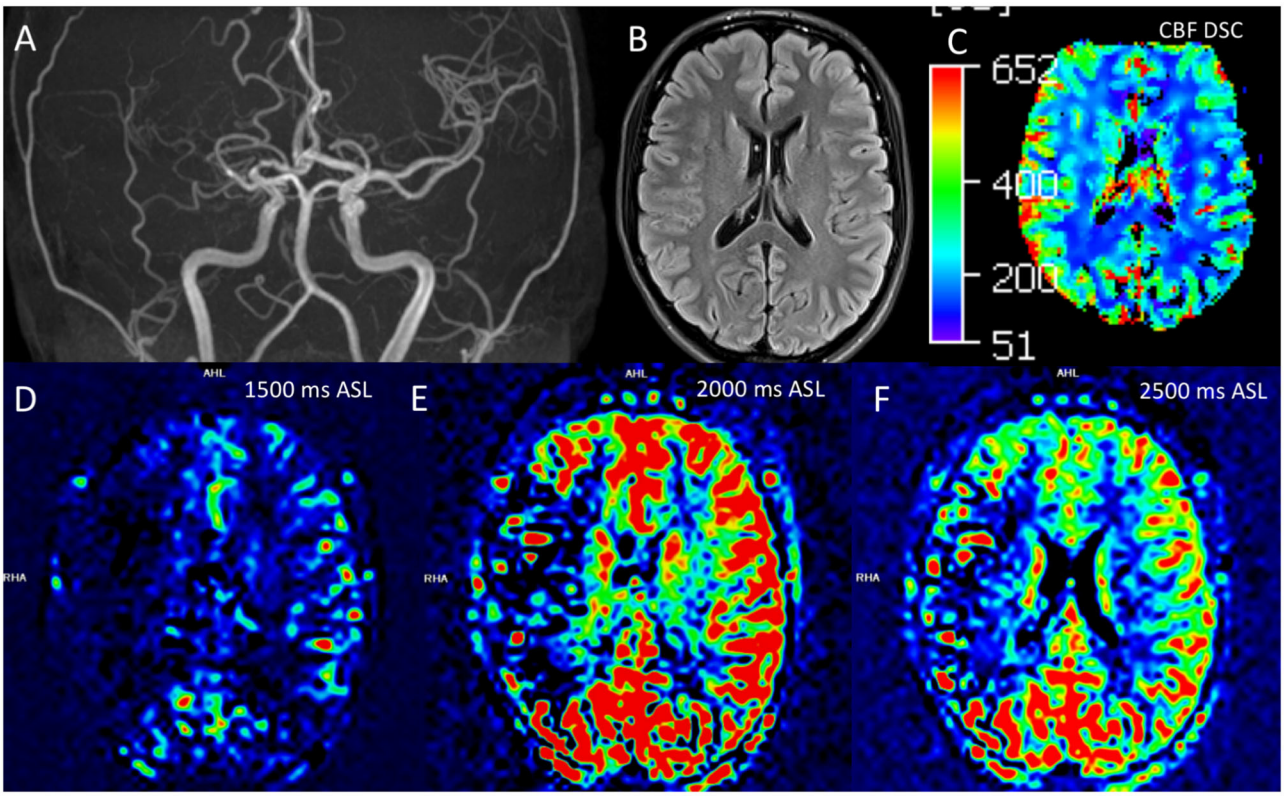

FIGURE 5 | Comparison of ASL and DSC assessments of perfusion in moyamoya disease. Correct interpretation of ASL is heavily dependent on knowledge of the technical parameters and underlying basis for signal generation. A 21 year-old-female had findings of right sided MMD, including stenosis of the right M1, M2, and A1 segments on this coronal 3D-TOF MIP **(A)**. Routine cross-sectional images such as this representative 2D T2 FLAIR image did not demonstrate chronic changes, including lack of infarct, white matter T2 hyperintensity, or Ivy sign **(B)**. DSC perfusion shows preserved or slightly increased CBF in the right cerebral hemisphere **(C)**. ASL perfusion was also obtained with multiple tag delay time points including 1,500 ms **(D)**, 2,000 ms **(E)**, and 2,500 ms **(F)**. The ASL images suggest decreased flow in the right MCA territory; this appearance is more marked at the 2,000 ms timepoint. However, this low ASL signal represented delayed transit through collateral pathways rather than low CBF, demonstrating the ASL must be interpreted in context of the technique and pathophysiology. ASL, arterial spin labeling; CBF, cerebral blood flow; DSC, dynamic susceptibility contrast; FLAIR, fluid-attenuated inversion recovery; MIP, maximal intensity projection; TOF, time of flight.

BOLD CVR effectively provides a functional assessment of the clinical significance of observed perfusion abnormalities.

The mechanism of BOLD contrast allows imaging of local variability in brain perfusion based on susceptibility differences between paramagnetic deoxygenated hemoglobin and diamagnetic oxygenated hemoglobin. With task-based fMRI examinations (e.g., motor or language tasks), there is overcompensation of arterial inflow increasing weakly diamagnetic oxygenated hemoglobin to metabolically active brain a few seconds after task initiation. This locally increases BOLD T2 signal and provides the contrast for such fMRI examinations. For cerebrovascular reactivity assessment, a 20 s breath hold task can be employed although the onset of BOLD signal increase is significantly delayed in comparison with traditional fMRI tasks. Normal cortical arterioles will dilate in response to increased serum CO_2 concentration as a result of breath holding, increasing blood flow in brain regions where there is preserved cerebrovascular reserve and thereby causing an increase in BOLD signal. However, arterioles that are already maximally dilated at baseline cannot respond to the CO_2 challenge and lack the ability to increase local cortical perfusion. Consequently, in regions of brain where blood vessels are maximally dilated, a CO_2 stimulus will result in reduced or absent BOLD signal. In the worst-case scenario, this may lead to a steal phenomenon in which the CO_2 stimulus will result in redirection of blood from areas with absent vasoreactivity to areas with preserved vascular reserve. This results in paradoxically negative BOLD signal in the zone(s) of poor vasoreactivity.

The advantages of the breath-hold BOLD cerebral reactivity imaging technique include technical simplicity as well as the ability to easily incorporate this exam as a subcomponent of a larger comprehensive moyamoya MRI exam. However, this technique does require pre-exam coaching, patient compliance, facile MR technologists, and radiologist post-processing time and effort. Also, magnetic susceptibility effects from the skull base impair the ability of this BOLD fMRI technique to evaluate the basal frontal and temporal lobes. Advanced, related BOLD contrast techniques that are theoretically superior to breath-holding have also been used at some facilities. These include gas delivery via mask to enhance the CO_2 stimulus and computer

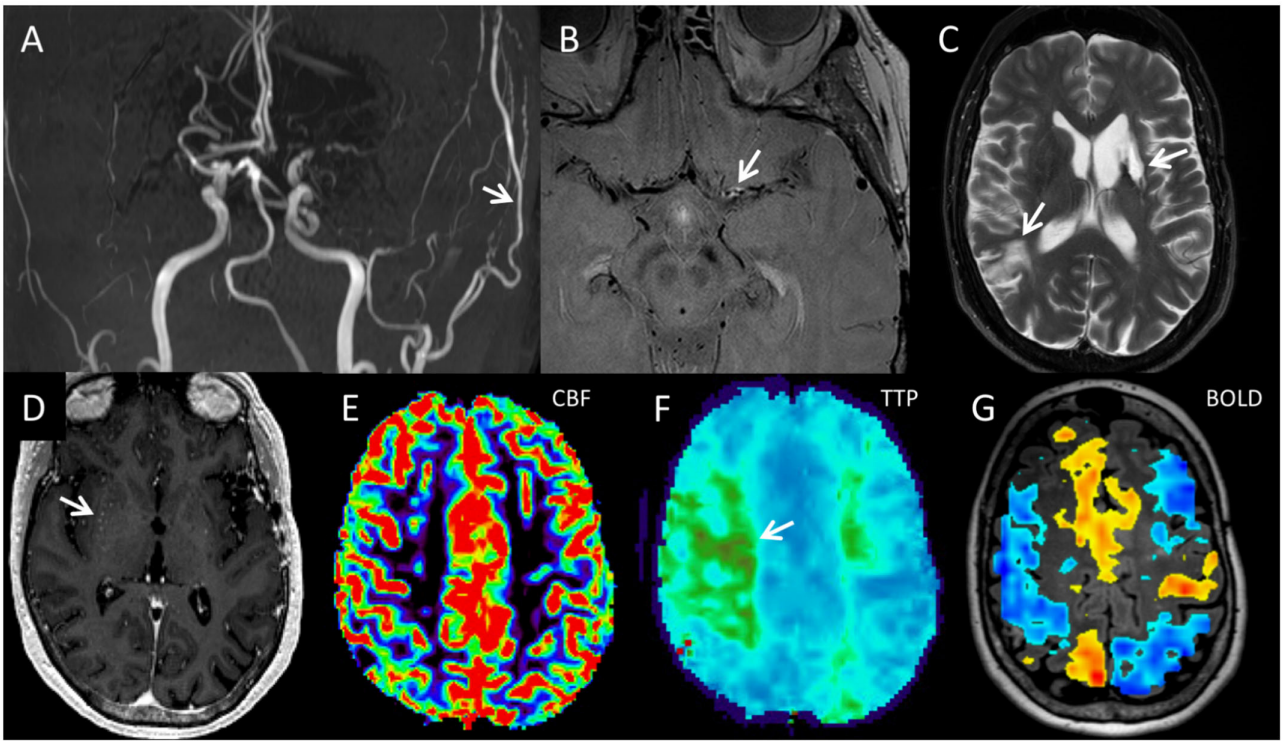

FIGURE 6 | The five components of the comprehensive moyamoya disease imaging protocol include **(A)** luminal angiography, **(B)** vessel wall imaging, **(C,D)** standard cross-sectional imaging, **(E,F)** perfusion imaging, and **(G)** BOLD CVR map, and are illustrated in this 65-year-old female diagnosed with MMD after a comprehensive work-up. Coronal 3D MIP from a 3D-TOF image **(A)** demonstrates narrowing of the bilateral supraclinoid ICAs, M1 segments, and left A1 segment with decreased to absent flow. There is a patent left direct STA-MCA bypass with relatively prominent flow in the left STA (white arrow). Axial proton density vessel wall imaging demonstrates circumferential enhancement of the proximal left M1 segment (white arrow) with negative remodeling **(B)**. Distal to the area of circumferential enhancement, the left M1 segment is narrowed with negative remodeling but no vessel wall enhancement. **(C)** Axial T2-weighted fast spin echo image demonstrates chronic infarcts in the left basal ganglia and right parietal lobe (arrows). **(D)** Axial post-gadoliniumT1 weighted MPRAGE (magnetization prepared gradient echo) image demonstrates dots of enhancement in the right basal ganglia consistent with moyamoya collateral arteries (white arrow). **(E)** Axial DSC CBF images demonstrates preserved blood flow throughout. **(F)** Axial TTP image demonstrates regional delayed transit time within the posterior right frontal lobe and left centrum semiovale region (region of green color, white arrow), consistent with delayed, but preserved flow supplied from collateral moyamoya vessels. A 20 s breath-hold BOLD cerebral vascular reactivity map superimposed on a 3D axial T2 FLAIR image shows large regions of decreased reactivity in both MCA territories including areas that had normal CBF as well as increased reactivity in other regions including the ACA territories. That is, decreased reactivity could either show up on the map as non-activation, which represents decreased reactivity without steal, or blue, which represents decreased reactivity with steal. Areas of steal paradoxically receive less blood during the CO_2 challenge. Such decreased cerebrovascular reactivity imply lack of hemodynamic reserve in which brain tissue is perfused normally at rest but receive inadequate blood flow when the patient is experiencing a hemodynamic stressor. ACA, anterior cerebral arteries; BOLD CVR, blood oxygen level dependent cerebrovascular reactivity; CBF, cerebral blood flow; CBV, cerebral blood volume; CTP, CT perfusion; DSC, dynamic susceptibility contrast; ICA, internal carotid artery; MMD, moyamoya disease; MPRAGE, magnetization prepared gradient echo; STA-MCA, superficial temporal artery to middle cerebral artery; TTP, time to peak.

temporally controlled inhaled gas delivery. However, these methods significantly increase the complexity and time of the MRI examinations and are not as widely available. Another method that is more commonly employed is ^{99m}technecium (Tc)-labeled radiopharmaceutical perfusion SPECT with and without administration of acetazolamide (Diamox) to dilate the intracranial arterioles. Disadvantages of this approach include radiation requirement, a lengthier examination, and the need for two separate patient appointments to assess cerebrovascular reactivity.

Transcranial Doppler (TCD) is used to assess vasoreactivity in some Institutions. It is a valid technique but highly operator dependent. The introduction of BOLD MRI in our protocol, has allowed us to assess vasoreactivity during MRI eliminating the need of additional techniques. An additional advantage of the protocol we propose is that the physician interpreting the study has imaging and physiological data available in a single study.

SURGICAL EVALUATION AND PROCEDURES

Although patients treated at high-volume centers are, in general, more likely to have better outcomes as compared to those treated at low-volume centers, this effect is magnified in cases where revascularization procedures are performed (28). All patients who present to our moyamoya clinic with a potential diagnosis of moyamoya receive a referral for neurosurgical evaluation, following which the decision to proceed with a revascularization procedure is made in conjunction with the team members. In recent years, a

randomized trial has demonstrated the role for revascularization surgery in patients with hemorrhagic moyamoya (29). While a randomized trial has yet to be performed in regards to the benefit of revascularization for ischemic-type moyamoya, existing literature suggests that revascularization may reduce future ischemic events in such patients (30–33). Based on these data, in addition to recommendations made in published guidelines, patients with hemorrhagic or ischemic events or those with impaired cerebral hemodynamics are considered for revascularization as opposed to conservative management alone (2).

After a patient has been selected for revascularization surgery at our institution, it is necessary to determine which specific revascularization technique is most suited to the particular patient. Revascularization procedures for moyamoya can be categorized into two main subtypes: direct and indirect. Direct cerebral revascularization consists of immediate flow augmentation to the affected hemisphere by supplying an additional source of blood flow via a direct surgical anastomosis between an extracranial donor vessel and intracranial recipient (34).

Indirect revascularization procedures utilize the vascular parasitization capabilities of the hypoxic brain by placing a vascularized graft directly on the pial surface (34). Alternatively, direct and indirect anastomosis may be performed in combination with one another and may have a theoretical advantage to performing either direct or indirect alone: Performing a direct bypass provides immediate hemodynamic improvement, and the indirect bypass improves the midterm result and as a possible fallback strategy in case the direct bypass fails (34). When compared to indirect revascularization alone, combined approaches seem to be superior in terms of improving functional cerebrovascular reserve, angiographic collateralization, as well as reducing the rate of future ischemic events (30, 35).

Although which revascularization technique to utilize should be determined within the context of each unique patient, current literature demonstrates the superiority of direct bypass to indirect bypass in terms of improving angiographic collateralization, functional cerebrovascular hemodynamics, as well as decreasing the rate of post-operative cerebral events (30, 32, 35–38). This pattern seems to be true for pediatric MMD patients as well (31, 39), although, as compared to adults, pediatric patients are more likely to benefit from indirect revascularization procedures alone (36). Given these data, our institutional protocol calls for direct revascularization, either alone or with combined indirect revascularization, in cases of an acceptable donor artery as a first-line option in patients who are surgical candidates. In cases were an undersized or otherwise unacceptable donor artery is not present, indirect revascularization is an acceptable alternative and is considered superior to medical therapy alone.

FOLLOW-UP CONSIDERATIONS

Clinical Management

In general, patients are scheduled for follow-up visits 3, 12, and 24 months following a revascularization procedure. The initial follow-up visit consists of a thorough evaluation with the treating neurologist and neurosurgeon. The use of anti-platelets following revascularization suffers from a paucity of data (34). Nevertheless, some data have indicated a potential association of anti-platelets with improved clinical status at mid and long-term follow-up. In these patients, an increased risk of hemorrhagic events was not observed (40). Other data have failed to suggest an association between anti-platelet use and graft patency or ischemic events (40, 41). Despite a lack of robust evidence, our institutional protocol typically utilizes low-dose aspirin in patients who have undergone cerebral revascularization, unless otherwise contraindicated.

Imaging Assessment

Patients who have undergone revascularization procedures undergo CT and CTA within 24 h following the procedure in order to assess for an extra-axial hematoma (as patients are most often on aspirin) and to assess graft patency. Following discharge after a revascularization procedure, the primary concern is the assessment of bypass graft patency along with development of adequate collateralization. Furthermore, assessing for the presence of interval ischemic events is also important, along with interrogating for expected improvements in cerebrovascular reserve capacity. Our comprehensive MRI protocol allows assessment of these considerations. In addition, duplex ultrasound is first used to examine bypass graft patency and can be used in patients who have undergone either direct or indirect revascularization procedures (42, 43).

Catheter-based DSA is not routinely utilized in follow-up assessments unless the patient is being considered for graft revision, or to plan for contralateral revascularization in cases of bilateral moyamoya.

CONCLUSIONS

Moyamoya patients are likely to have the best outcomes when evaluated and treated at high-volume centers with a specialized care team. Inter-disciplinary collaboration between treating physicians involving the medical, radiological and surgical management of moyamoya patients is crucial in order to offer the best possible care. Here, we describe the development and rational for our institutional adult moyamoya clinical and imaging protocol with the hope that this information may aid other, specialized centers in implementing their own collaborative care protocols. We hope this serves as an example of the value of a multi-disciplinary team and standardized approach. In the future, improved guidelines and increased standardization of approaches amongst institutions would be useful.

AUTHOR CONTRIBUTIONS

GL, VL, and JK: study conception. AL: draft writing. AL, VL, LS, GL, NC, KW, and JK: draft editing. All authors contributed to the article and approved the submitted version.

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ORIGINAL RESEARCH
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Clinical Significance of Ultrasound-Based Hemodynamic Assessment of Extracranial Internal Carotid Artery and Posterior Cerebral Artery in Symptomatic and Angiographic Evolution of Moyamoya Disease: A Preliminary Study

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Objective: To investigate the hemodynamic changes using ultrasound according to digital subtraction angiography (DSA) findings and explore the association between ultrasound parameters and clinical symptoms of moyamoya disease (MMD).

Methods: Hemodynamic parameters of extracranial internal carotid artery (EICA) and posterior cerebral artery (PCA) were evaluated by ultrasound. According to DSA findings, EICA parameters among Suzuki stages (stage I-II, III-IV, and V-VI), and PCA parameters among leptomeningeal system scores (score 0–2, 3–4, and 5–6) were compared, respectively. ROC analysis was performed based on the ultrasound parameters to distinguish stroke from non-stroke patients.

Results: Forty patients with MMD were included in our study (16 men; median age, 37 years). The diameter (D), peak systolic velocity (PSV), end diastolic velocity (EDV) and flow volume (FV) of EICA decreased as the Suzuki stage advanced (D: $P < 0.001$; PSV: $P < 0.001$; EDV: $P < 0.001$; FV: $P < 0.001$). The PSV and EDV of PCA increased as the leptomeningeal system scores advanced (PSV: $P < 0.001$; EDV: $P < 0.001$). ROC analysis showed that the area under the curves (AUCs) based on the D and FV of EICA, the PSV and EDV of PCA and their combination were 0.688, 0.670, 0.727, 0.684, and 0.772, respectively, to distinguish stroke from non-stroke patients.

Conclusions: Ultrasound parameters were related to Suzuki stages and leptomeningeal system scores. Ultrasound may be useful in predicting the occurrence of stroke in patients with MMD. Future prospective studies with large sample sizes and long-term follow-up are needed to confirm our preliminary findings.

Keywords: moyamoya disease, ultrasound, stroke, extracranial internal carotid artery, posterior cerebral artery

INTRODUCTION

Moyamoya disease (MMD) is a rare disease of unknown etiology, it is characterized by progressive stenosis of the bilateral terminal portions of internal carotid arteries, and their main branches with compensatory abnormal vascular networks at the base of the brain (1). The incidence of moyamoya disease is high in East Asia countries such as Japan and Korea. In Japan, the annual incidence was 0.35 per 100,000. Cerebral ischemia and intracranial hemorrhage due to the rupture of fragile collateral vessels that compensate for ischemia are the main hazards of MMD (2–5). MMD is an important cause of stroke in children and adults. Affected first-degree relatives and those who cannot receive revascularization surgery for a period of time, undergoing imaging at regular intervals can reduce the risk of permanent disability caused by stroke and improve long-term prognosis (6, 7).

Digital subtraction angiography (DSA) plays an important role in guiding the clinical treatment and assessing the prognosis of MMD. Suzuki et al. proposed a conventional criterion for the diagnosis and grading of MMD based on vascular morphology by using DSA (1, 8), however, due to the abundant collateral networks in MMD patients, MMD patients with the same Suzuki stage may have different cerebrovascular reserves and clinical symptoms. Recently, Liu et al. combined the leptomeningeal system from the posterior cerebral artery (PCA) territory to the anterior cerebral artery (ACA) and middle cerebral artery (MCA) territory, which is the most important compensatory system in patients with MMD, and Suzuki stage, proposed a new grading system to better evaluate the clinical characteristics, guide treatment, and predict prognosis (9, 10). However, due to the invasive procedure, high radiation exposure and time consumption of angiography, long-term monitoring has many limitations. Ultrasonography as a non-invasive, economical, and repeatable technique has shown certain value in the diagnosis and prognostic assessment of MMD (11–13). Hong et al. (14) demonstrated that flow volume (FV) of the internal carotid artery (ICA) detected by ultrasound was inversely correlated with Suzuki's grade. Yasuda et al. (15) showed the ratio of the extracranial ICA (EICA) and common carotid artery diameters tended to be lower in symptomatic arteries than in asymptomatic arteries, the ratio decreased as cerebral vasoreactivity decreased. Wang et al. (16) indicated that as the velocity of the PCA decrease, the ischemic lesions spread to a wider range and perfusion levels decreased from good to poor. Based on the above studies, we aim to explore the association between ultrasound parameters, DSA findings and clinical symptoms of patients with MMD. Carotid ultrasound and transcranial color-coded duplex sonography (TCCS) were used to detect hemodynamic changes in the EICA and PCA to realize real-time monitoring of patients with MMD, providing more information for evaluating clinical symptoms in patients with MMD.

MATERIALS AND METHODS

Study Population

We prospectively enrolled consecutive patients with MMD at our institution between July 2019 and February 2020. The inclusion criteria for our study were: (1) patients who were diagnosed with MMD according to the MMD guidelines (17); (2) patients who received ultrasound, DSA, computed tomography (CT) and magnetic resonance imaging (MRI) examinations with intervals of these examinations was <1 month. The exclusion criteria were: (1) patients who were diagnosed with moyamoya syndrome with identified causes (17, 18); (2) patients refused ultrasound examination or with poor temporal acoustic bone window; (3) patients had a history of hypertension, hyperlipidemia, diabetes or smoking; (4) patients with proximal internal carotid artery or vertebrobasilar disease; (5) patients who had diseases that affected cardiac output; (6) MMD patients who had prior revascularization surgery; and (7) patients with unilateral MMD. Finally, a total of 40 patients were included in our study (**Figure 1**). Informed consent was obtained from all eligible patients (or their parent or legal guardian in the case of children under 16 years), and the study was approved by the Ethics Committee of Beijing Tiantan Hospital, Capital Medical University.

Angiographic Findings

All 40 patients underwent DSA, including the bilateral ICA, external carotid artery (ECA) and vertebral artery to evaluate collateral flow. Two independent experienced investigators interpreted the images according to the following diagnostic criteria, they were blinded to the clinical data, and any differences in their results were resolved by consensus.

Suzuki's Vascular Criteria (1, 7)

Stage I: narrowing of ICA apex; stage II: dilatation of the intracerebral main arteries and initiation of the moyamoya; stage III, narrowing of the MCA and ACA and intensification of the moyamoya; stage IV: occlusion of the ICA extending to the junction of the posterior communicating artery and minimization of the moyamoya, resulting in enlargement of the collateral vessels from the external carotid artery; stage V: disappearance of all the main cerebral arteries and further minimization of the moyamoya; and stage VI: complete disappearance of the siphon of ICA, and disappearance of the moyamoya, resulting in cerebral blood flow supply from the external carotid artery and vertebrobasilar artery systems.

Grading Score of the Leptomeningeal System From the PCA Territory to The ACA and MCA Territory

According to the anatomy extent of pial collateral blood (10), the scores of the leptomeningeal system from the PCA territory to the ACA and MCA territory were the sum of the following 3 parts, a score of 0 was assigned if the leptomeningeal anastomoses were absent. (1) Retrograde flow from the parieto-occipital branch of the PCA or posterior pericallosal artery extending to the ACA territory: a score of 1 was assigned if the blood supply extended

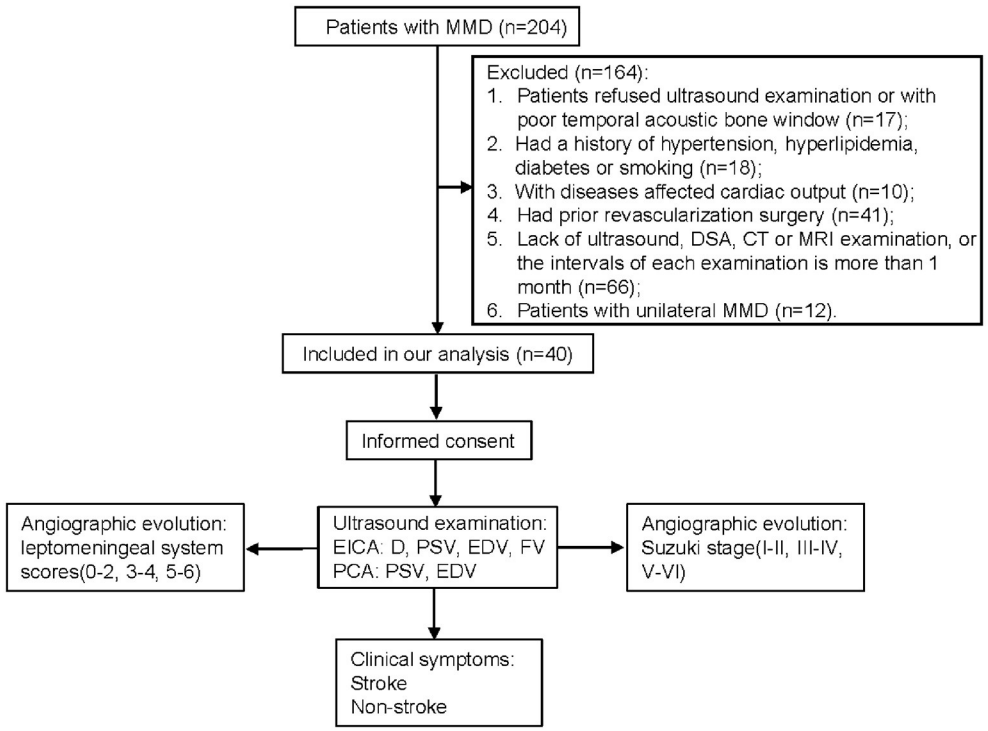

FIGURE 1 | Flow diagram of the study. MMD, moyamoya disease; DSA, digital subtraction angiography; CT, computed tomography; MRI, magnetic resonance imaging; EICA, extracranial internal carotid artery; D, diameter; PSV, peak systolic velocity; EDV, end diastolic velocity; FV, flow volume; PCA, posterior cerebral artery.

to the cortical border zone between the ACA and PCA territory; a score of 2 was assigned if the blood supply extended to the central sulcus. (2) A score of 1 was assigned if the anterior temporal branch of the PCA anastomoses to the temporal branch of the MCA. (3) The parieto-occipital branch of PCA anastomoses to the MCA: a score of 1 was assigned if the retrograde flow only extended to superficial vessels (M4 segment of MCA); a score of 2 was assigned if the retrograde flow extended into the Sylvian fissure (M3 segment of MCA); and a score of 3 was assigned if the flow extended to the reconstituted vessels at the distal end of the occlusion (M1 or proximal M2 segments of MCA).

Clinical Symptoms

According to clinical symptoms, patients were categorized into stroke group and non-stroke group by an experienced research neurologist. The stroke group included patients presented with ischemic stroke and hemorrhagic stroke. Ischemic stroke was defined as a new symptomatic neurologic deterioration lasting at least 24 h that was not caused by a non-ischemic cause, or a new symptomatic neurologic deterioration accompanied by a new cerebral infarction that was not caused by a non-ischemic cause, cerebral infarction was confirmed by MRI. Hemorrhagic stroke was defined as the acute extravasation of blood into the brain parenchyma, cerebral hemorrhage was confirmed by CT (19). The non-stroke group included patients presented with transient ischemic attack (TIA), headache, etc., with no evidence of cerebral infarction and hemorrhage on CT and MRI.

Ultrasound Examination

All subjects underwent ultrasound examination in the ultrasound department of our hospital by two experienced sonographers. Among them, 20 patients were randomly selected for the intra- and interrater reliability study, the second sonographer performed the same examination immediately after the first one, the first sonographer repeated the examination the next day, and the sonographers were blinded to the clinical data and radiographic findings.

Carotid Ultrasound

Carotid ultrasound was performed on a color-coded ultrasound system (EPIQ 7, Philips Medical Systems, Bothell, WA) with a 3–12 MHz linear array probe. The patient remained in a supine position with their head remaining dropped back and tilted to the opposite side slightly. The sonographer adjusted the gain, depth, pulse-repetition frequency and wall filter to the appropriate conditions, the size of the doppler sample volume was adjusted to 1/3–1/4 of the detected vessel, the doppler angle was adjusted to $\leq 60°$, and the parameters of the EICA were measured on the two-dimensional longitudinal section at 1–2 cm above the carotid bulb. The following parameters were measured: diameter (D), peak systolic velocity (PSV) and end-diastolic velocity (EDV). Then, the sonographer adjusted the doppler sample volume to the entire width of the vessel, when the signal was stable, the time-averaged mean velocity (TAMV) was measured over a minimum of three cardiac cycles, and the FV was calculated as the product

of TAMV and the cross-sectional area (A) of the circular vessel according to the formula FV=TAMV×A=TAMV×[(D/2)^2×π] (20, 21).

Transcranial Color-Coded Duplex Sonography

Transcranial color-coded duplex sonography was performed on a color-coded ultrasound system (EPIQ 7, Philips Medical Systems, Bothell, WA) with a 1.5–3.0 MHz phased array probe. The patient remained in a lateral position, the P2 segment of PCA was examined through a transtemporal window. The sonographer adjusted the gain, pulse-repetition frequency and wall filter to the appropriate conditions, the size of the doppler sample volume was adjusted to 3–5 mm, the depth of insonation for PCA was 60–70 mm, and the doppler angle was adjusted to $< 60^{\circ}$, when the signal was stable, the PSV and EDV of PCA were measured.

Statistical Analysis

Continuous variables were described as median (interquartile range), and categorical variables were described as percentages. The Mann-Whitney U test and Jonckheere-Terpstra test were used for continuous variables. Receiver operating characteristic (ROC) analysis was used to evaluate the discrimination performance of ultrasound parameters in distinguishing stroke from non-stroke patients with MMD. The intra-class correlation coefficient (ICC) was used to assess the intra- and interrater reliability of ultrasound parameters. Statistical analyses were performed using SPSS version 24.0 (IBM Corporation, Armonk, NY). All calculated *P*-values were 2-tailed, and a $P < 0.05$ was considered statistical significance.

RESULTS

Patient Characteristics

A total of 40 patients were included in our study, including 16 males and 24 females. All patients had bilateral MMD. The median age of the patients was 37 (28–44) years. According to clinical symptoms, there were 27 patients in the stroke group and 13 patients in the non-stroke group. Suzuki stages were as follows: stage I in 8 hemispheres, stage II in 19 hemispheres, stage III in 35 hemispheres, stage IV in 11 hemispheres, stage V in 6 hemispheres, and stage VI in 1 hemisphere. Grading score of the leptomeningeal system from the PCA territory to the ACA and MCA territory were score 0 in 5 hemispheres, score 1 in 2 hemispheres, score 2 in 12 hemispheres, score 3 in 9 hemispheres, score 4 in 28 hemispheres, score 5 in 21 hemispheres, score 6 in 3 hemispheres (**Table 1**).

TABLE 1 | Baseline characteristics of eligible patients.

Characteristics	Value
Age (y), median (interquartile range)	37 (28–44)
Sex, male (%)	16 (40.00)
Clinical symptoms (patients) (%)	
Stroke	27 (67.50)
Non-stroke	13 (32.50)
Suzuki stage (hemispheres) (%)	
I	8 (10.00)
II	19 (23.75)
III	35 (43.75)
IV	11 (13.75)
V	6 (7.50)
VI	1 (1.25)
Grading score of the leptomeningeal system (hemispheres) (%)	
0	5 (6.25)
1	2 (2.50)
2	12 (15.00)
3	9 (11.25)
4	28 (35.00)
5	21 (26.25)
6	3 (3.75)

Association Between Suzuki Stage and Ultrasound Parameters of the EICA in Patients With MMD

The association between Suzuki stage and ultrasound parameters of the EICA in MMD patients are shown in **Figure 2**. The D, PSV, EDV and FV of the EICA decreased as the Suzuki stage advanced (D: $P < 0.001$, **Figure 2A**; PSV: $P < 0.001$, **Figure 2B**; EDV: $P < 0.001$, **Figure 2C**; FV: $P < 0.001$, **Figure 2D**). Representative cases are shown in **Figure 3**.

Association Between Grading Score of the Leptomeningeal System From the PCA Territory to the ACA and MCA Territory and Ultrasound Parameters of the PCA in Patients With MMD

The association between grading score of the leptomeningeal system and ultrasound parameters of the PCA are shown in **Figure 4**. The PSV and EDV of PCA increased as the scores of the leptomeningeal system from the PCA territory to the ACA and MCA territory advanced (PSV: $P < 0.001$, **Figure 4A**; EDV: $P < 0.001$, **Figure 4B**). Representative cases are shown in **Figure 5**.

Comparison of Ultrasound Parameters Between Stroke and Non-Stroke Groups of Patients With MMD

The comparison of ultrasound parameters between stroke and non-stroke groups of patients with MMD are shown in **Table 2**. Compared with the stroke group, the D and FV of EICA were significantly higher in the non-stroke group (D: $P = 0.007$, PSV: $P = 0.014$), the PSV and EDV of PCA were also significantly higher in the non-stroke group (PSV: $P = 0.001$, EDV: $P = 0.008$).

Diagnostic Value of Ultrasound Parameters to Distinguish Stroke From Non-Stroke Patients With MMD

ROC analysis was performed based on the D and FV of EICA, the PSV and EDV of PCA and the combination of the four parameters to distinguish stroke from non-stroke patients with

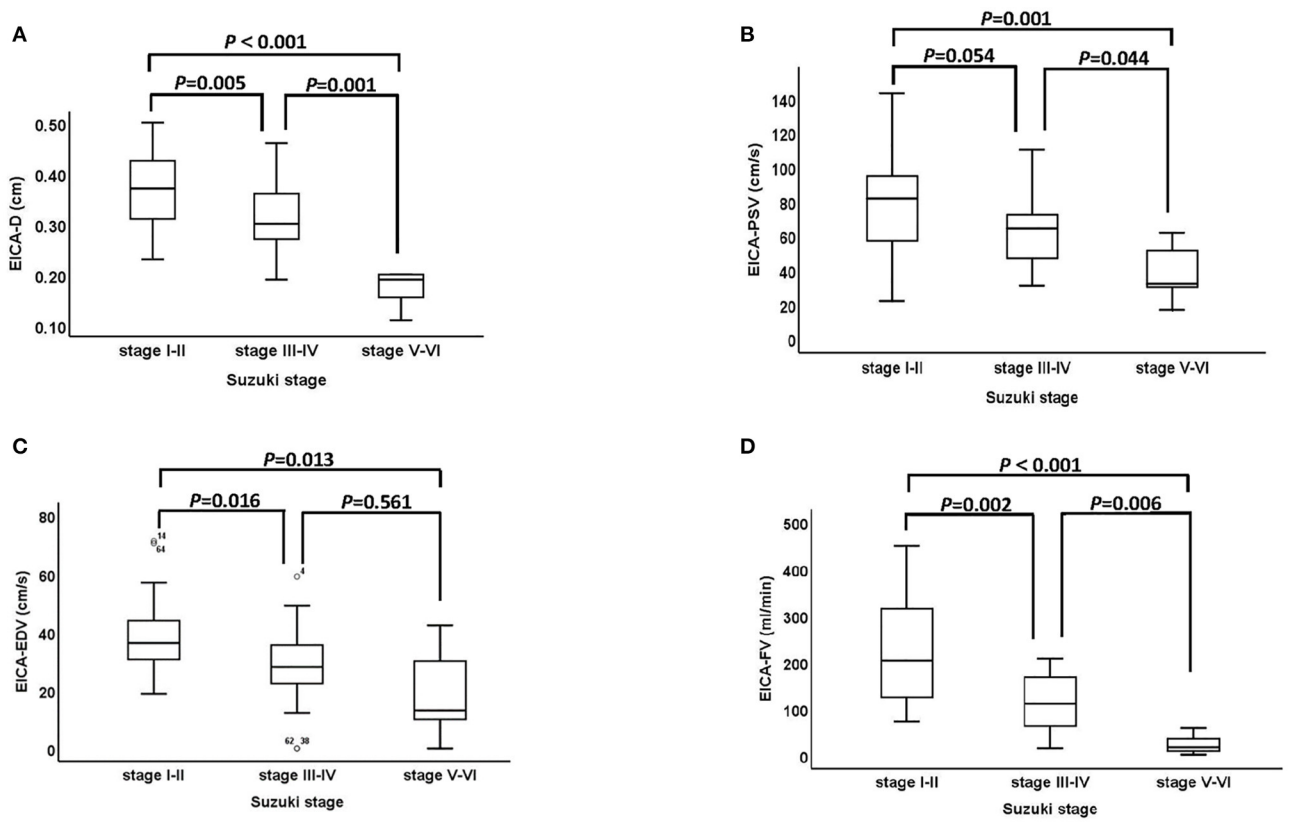

FIGURE 2 | The association between Suzuki stage and ultrasound parameters of the EICA. **(A–D)** The D, PSV, EDV, and FV of EICA between stage I-II, stage III-IV and stage V-VI groups were statistically significant (D: $P < 0.001$, PSV: $P < 0.001$, EDV: $P < 0.001$, FV: $P < 0.001$). EICA, extracranial internal carotid artery; D, diameter; PSV, peak systolic velocity; EDV, end diastolic velocity; FV, flow volume.

MMD (**Figure 6**). The area under the curves (AUCs) was 0.688 for the D of EICA, 0.670 for the FV of EICA, 0.727 for the PSV of PCA, 0.684 for the EDV of PCA and 0.772 for the combination of the four parameters, with sensitivity and specificity are 81.48% and 53.85%, 55.56% and 80.77%, 72.22% and 76.92%, 55.56% and 84.62%, 83.33% and 61.54%, respectively.

Intra- and Interrater Reliability of Ultrasound Parameters

We found excellent intrarater reliability for the D of EICA (ICC:0.973), PSV of EICA (ICC:0.939), EDV of EICA (ICC:0.923), FV of EICA (ICC:0.940), PSV of PCA (ICC:0.947) and EDV of PCA (ICC:0.944), and excellent interrater reliability for the D of EICA (ICC:0.971), PSV of EICA (ICC:0.920), EDV of EICA (ICC:0.917), FV of EICA (ICC:0.927), PSV of PCA (ICC:0.945) and EDV of PCA (ICC:0.941).

DISCUSSION

In this study, we investigated the hemodynamic changes of the EICA and PCA using ultrasound according to the DSA findings of Suzuki stage and grading score of leptomeningeal system from the PCA territory to the ACA and MCA territory in patients with MMD. The D, PSV, EDV, and FV of EICA decreased as the Suzuki stage advanced, the PSV and EDV of PCA increased as the leptomeningeal system scores advanced. MMD patients who presented with non-stroke symptoms were more likely to obtain higher D and FV of the EICA and higher PSV and EDV of the PCA than those who presented with stroke. Our results suggest that ultrasound parameters are related to DSA findings, and detection of ultrasound parameters might be useful in evaluating the clinical symptoms of patients with MMD.

At present, DSA is the gold standard for the diagnosis of MMD, however, DSA is invasive, radiative and can even cause serious complications. For people who cannot undergo DSA examination, MRI combined with magnetic resonance angiography can be used as an alternative method (17), but MRI combined with magnetic resonance angiography is time-consuming and expensive, and neither of these imaging methods can provide hemodynamic information. However, Clinicians expect to dynamically detect the hemodynamic changes in patients with MMD through simple and non-invasive means. The application of these methods in screening and long-term monitoring of MMD are limited. Ultrasonography is a non-invasive, economical, and repeatable technique, that has been used in the diagnosis of MMD, detecting the patency of bypass vessels, and evaluating postoperative hemodynamic changes (22–24).

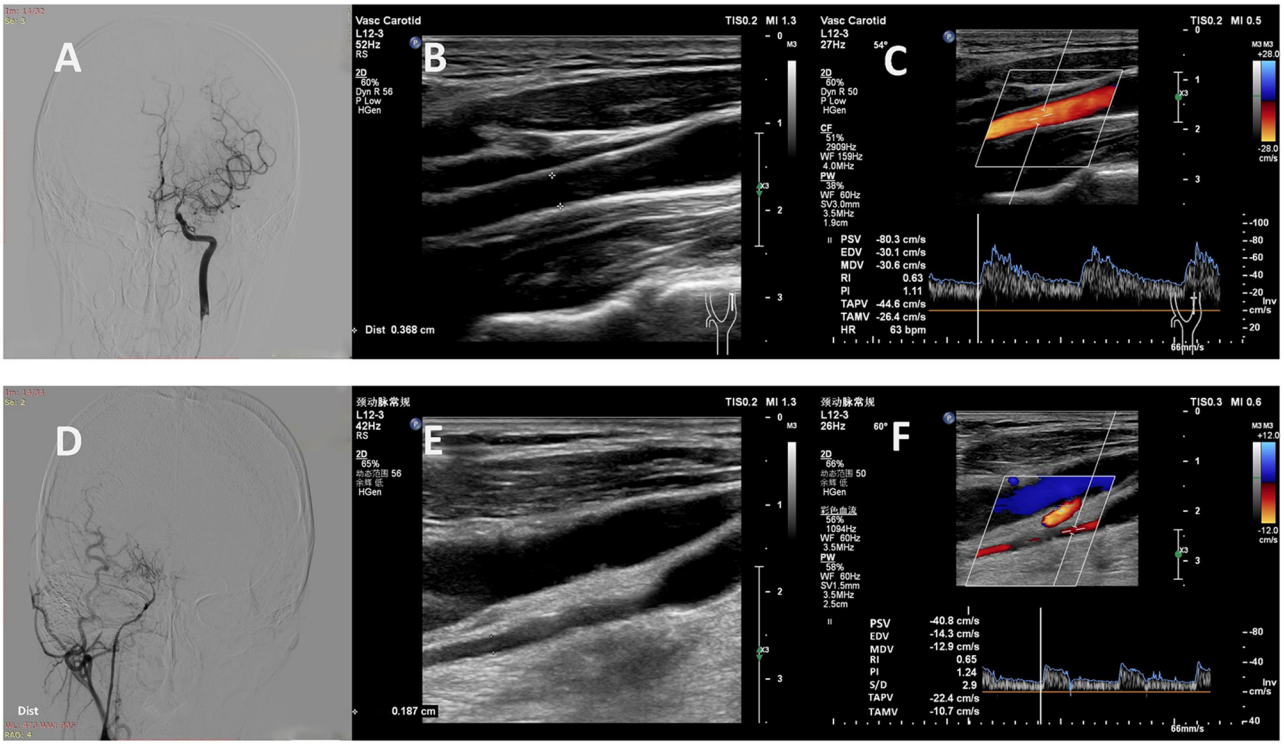

FIGURE 3 | Representative cases. **(A)** A 32-year-old man with MMD had a Suzuki stage II on the left. **(B, C)** Carotid ultrasound showed high values of D (0.37 cm), PSV (80 cm/s), EDV (30 cm/s), TAMV (26 cm/s) and FV (168 ml/min) in the left EICA. **(D)** A 37-year-old woman with MMD had a Suzuki stage V on the right. **(E, F)** Carotid ultrasound showed low values of D (0.19 cm), PSV (41 cm/s), EDV (14 cm/s), TAMV (11 cm/s) and FV (18 ml/min) in the right EICA. MMD, moyamoya disease; D, diameter; PSV, peak systolic velocity; EDV, end diastolic velocity; TAMV, time-averaged mean velocity; FV, flow volume; EICA, extracranial internal carotid artery.

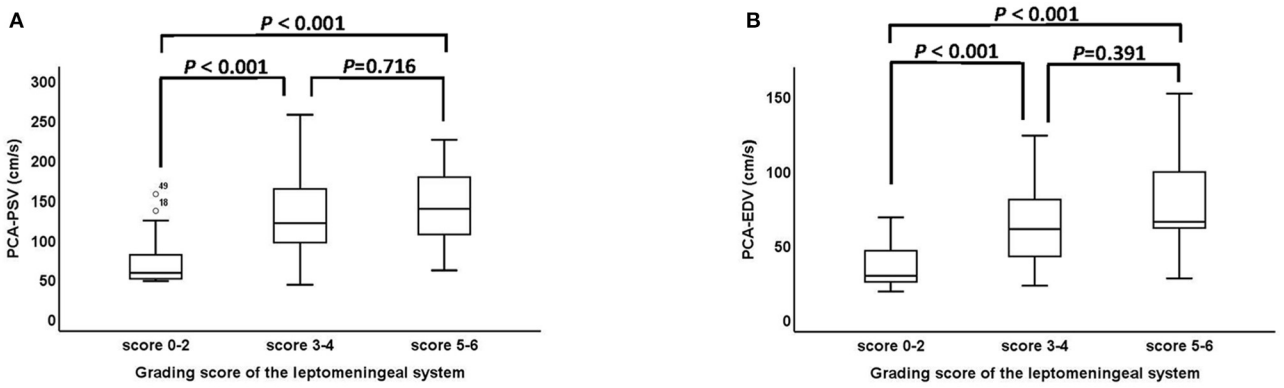

FIGURE 4 | The association between grading score of the leptomeningeal system from the PCA territory to the ACA and MCA territory and ultrasound parameters of the PCA. **(A,B)** The PSV and EDV of PCA between the score 0–2, score 3–4 and score 5–6 groups were statistically significant (PSV: $P < 0001$, EDV: $P < 0.001$). PCA, posterior cerebral artery; ACA, anterior cerebral artery; MCA, middle cerebral artery; PSV, peak systolic velocity; EDV, end diastolic velocity.

The histopathological change in the involved artery in MMD is eccentrically laminated thickening of the intracranial artery, as the disease advances, fibrocellular intimal thickening involves the EICA (25–27). Although most researchers have focused on the intracranial portions of the ICA and their branches in MMD, considering the histopathology aspects, MMD causes extracranial stenosis of the proximal portion of the ICA in some cases, the so-called bottleneck sign, which is a typical vascular finding of MMD (27). Yasuda et al. (15) reported that the bottleneck sign began to appear in patients with Suzuki stage III or higher. Our results demonstrated that as the Suzuki stage advanced, the D of EICA decreased. MMD is characterized by progressive stenosis

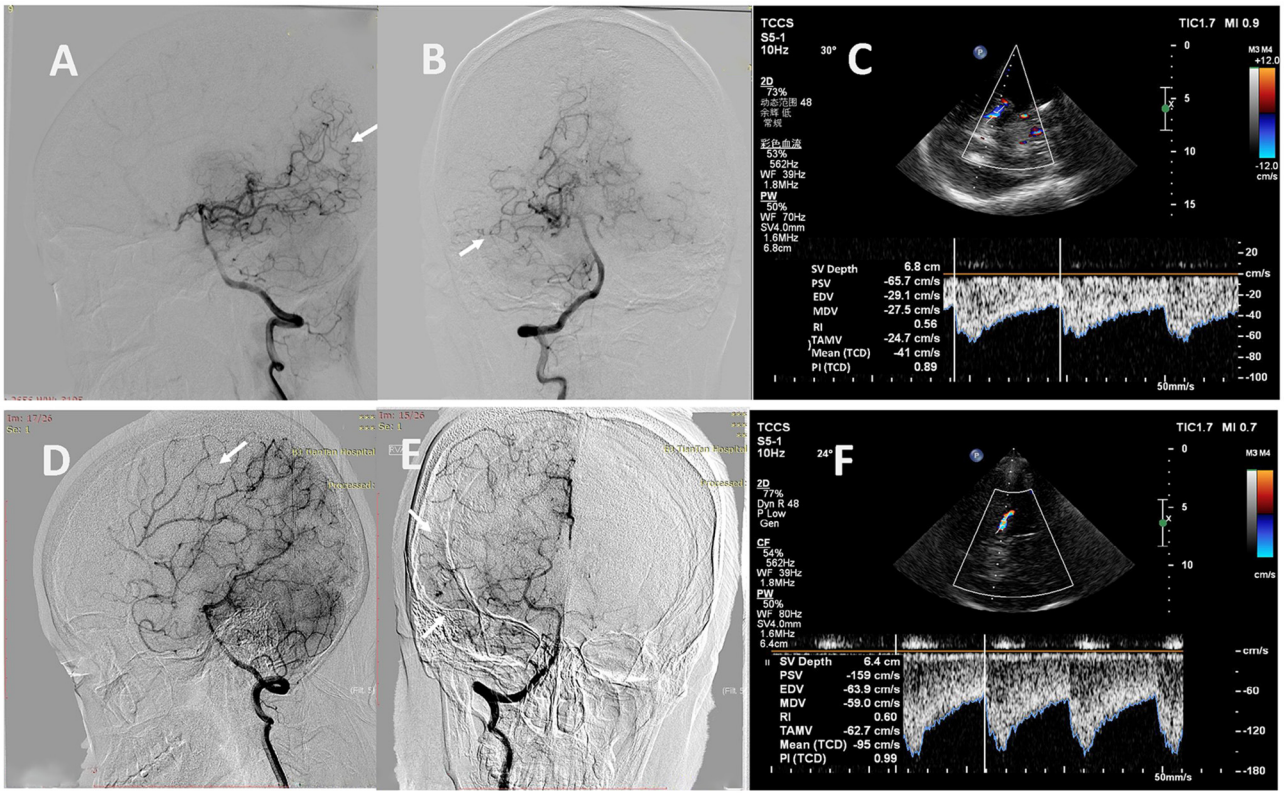

FIGURE 5 | Representative cases. **(A, B)** A 24-year-old man with MMD scored 2 points in the leptomeningeal system on the right (white arrows). **(C)** The TCCS showed low values of PSV (66 cm/s) and EDV (29 cm/s) in the right PCA. **(D, E)** A 33-year-old woman with MMD scored 5 points in the leptomeningeal system on the right (white arrows). **(F)** The TCCS showed high values of PSV (159 cm/s) and EDV (64 cm/s) in the right PCA. MMD, moyamoya disease; TCCS, transcranial color-coded duplex sonography; PSV, peak systolic velocity; EDV, end diastolic velocity; PCA, posterior cerebral artery.

TABLE 2 | Comparison of ultrasound parameters between the stroke group and the non-stroke group in patients with MMD.

Characteristics	Clinical Symptoms		*P*-Value
	stroke (n = 27 patients)	Non-stroke (n = 13 patients)	
EICA			
D(cm)	0.30(0.25–0.36)	0.37(0.30–0.41)	0.007
PSV (cm/s)	61.75(40.30–82.13)	65.75(59.43–73.83)	0.334
EDV (cm/s)	30.55(19.83–39.93)	31.70(26.33–36.20)	0.825
FV (ml/min)	103.00(52.82–177.93)	161.36(119.27–228.71)	0.014
PCA			
PSV (cm/s)	104.80(62.65–133.50)	150.85(118.75–200.35)	0.001
EDV (cm/s)	51.20(30.03–68.93)	65.50(58.20–97.05)	0.008

MMD, moyamoya disease; EICA, extracranial internal carotid artery; D, diameter, PSV, peak systolic velocity; EDV, end diastolic velocity; FV, flow volume; PCA, posterior cerebral artery.

of the bilateral terminal portions of internal carotid arteries, and their main branches, resulting in increased resistance in the distal vessel and decreased velocity and blood flow volume in the proximal vessel. Ruan et al. (28) showed that the time-averaged mean flow velocity of ICA in MMD patients was lower than that in normal controls, and the resistance index was higher than that in normal controls. Hong et al. (14) indicated that Suzuki's grade was inversely correlated with the FV of ICA. Our findings seemed to be consistent with previous studies, we found that as the Suzuki stage advanced, the PSV, EDV and FV of EICA decreased.

As MMD progresses, blood flow decreases in the anterior circulation, and patients may have TIA, headache even stroke. To sustain adequate cerebral perfusion, PCA could develop collateral branches to compensate for the ischemic brain, and the leptomeningeal system from the PCA plays an important role in supplying the ischemic cortex of the MCA and ACA territories. Liu et al. (10) proposed a new grading system for assessing the collateral circulation of MMD patients, according to the anatomic extent of collateral circulation from the PCA territory to the ACA and MCA territory, the grading score of the leptomeningeal system was scored from 0 to 6. As collateral circulation mainly comes from the P2 segment of PCA, in our study, we selected the P2 segment to measure ultrasound parameters. We found that the low-speed blood flow of the PCA was more common in low-score groups. In contrary, in high-score groups, high-speed blood flow of the PCA was more easily detected. The reason was that the high velocity could provide enough blood flow for the collateral circulation. When the P1 segment of PCA was involved, a low-velocity and low-resistance

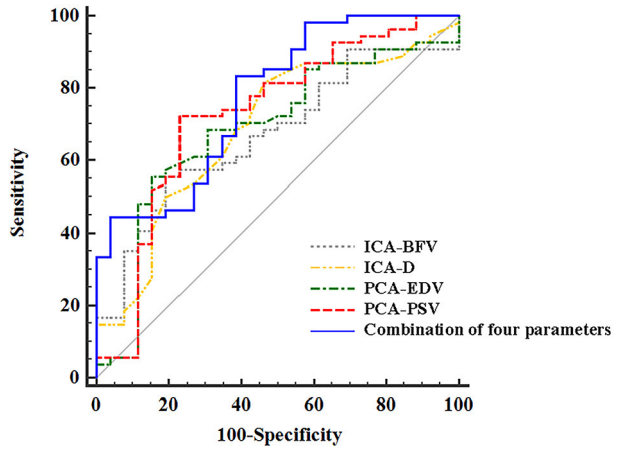

FIGURE 6 | The ROC curves of ultrasound parameters to distinguish stroke from non-stroke patients with MMD. The area under the ROC curves was 0.688 (95% CI, 0.575, 0.787) for the D of EICA, 0.670 (95% CI, 0.556, 0.771) for the FV of EICA, 0.727(95% CI, 0.616, 0.821) for the PSV of PCA, 0.684 (95% CI, 0.571, 0.784) for the EDV of PCA, and 0.772(95% CI, 0.665, 0.858) for the combination of the four parameters. ROC, receiver operating characteristic; MMD, moyamoya disease; EICA-D, diameter of the extracranial internal carotid artery; EICA-FV, flow volume of the extracranial internal carotid artery; PCA-PSV, peak systolic velocity of the posterior cerebral artery; PCA-EDV, end diastolic velocity of the posterior cerebral artery.

blood flow signal of the P2 segment was noted, and the low-velocity in P2 segment could not supply enough blood flow for the establishment of collateral circulation (16). Therefore, the more abundant collateral circulation formed by the PCA, the higher flow velocity of the P2 segment we detected.

The main symptoms of MMD are stroke and TIA. Hypoperfusion increases the susceptibility to ischemia, hemodynamic abnormalities are the main mechanism of ischemic stroke (10, 29). Hemorrhagic stroke is a deleterious consequence of compensatory mechanisms in response to ischemia (7). As progressive narrowing of the ICA, poor collateral circulation, rupture of fragile, dilated moyamoya vessels under unusually increased hemodynamic stress is the main cause of cerebral hemorrhage (30). For those who do not pay enough attention to TIA and pediatric patients who cannot accurately describe their TIA symptoms, delayed diagnosis and treatment could increase the risk of permanent disability due to stroke. The association between ultrasound parameters and clinical symptoms has rarely been reported. We assumed that the blood flow in the EICA reflects the blood supply of anterior circulation. Our findings have confirmed our hypothesis, a higher Suzuki stage represents a reduction blood flow in the ICA, indicating intracranial shrinkage of the anterior circulation, which is a risk factor for stroke. According to our study, the FV of EICA were significantly correlated with the clinical symptoms, patients who presented with stroke were more likely to have less FV in the EICA, but those who presented with non-stroke symptoms were more likely to have more FV in EICA. PCA is the main pathway of collateral circulation in patients with MMD, and plays an important role in the compensation of cerebral blood flow when principal conduits are insufficient. Successful compensatory collateralization is considered a preventive measure against stroke in patients with MMD (31). Wang et al. (16) indicated that as the velocity of the PCA decrease, the ischemic lesions spread to a wider range from the ICA to PCA territory and perfusion levels decreased from good to poor perfusion. Our study was coincident with the results of previous study. In our study, we explored the association between ultrasound parameters of the PCA and clinical symptoms of patients with MMD. We found that the increased velocity of PCA results in good collateral circulation could better prevent the occurrence of stroke. Our results showed that compared with the non-stroke group, patients in the stroke group presented a lower velocity of the PCA. In our study, we used ultrasound parameters of the EICA and PCA to assess stroke in patients with MMD. As a result, the combination of ICA and PCA parameters was found to be superior to each single parameter for evaluating stroke in patients with MMD. Our results indicated that ultrasound parameters are related to clinical symptoms.

Our study had some limitations. First, because of the relatively small sample size, patients were only divided into stroke and non-stroke groups according to their clinical symptoms. For patients with MMD, hemorrhagic stroke is a deleterious consequence of compensatory mechanisms in response to ischemia, so we didn't further classify the stroke group into ischemic stroke and hemorrhagic stroke groups. Second, in this study, we only investigated the hemodynamics of the EICA and PCA in patients with MMD, although PCA is the main pathway of collateral circulation in MMD and plays an important role in the compensation of cerebral blood flow, transdural collaterals from the ECA can also supply the ischemic brain. As ECA has many branches, the hemodynamic changes in one or two branches have little effect on the trunk, therefore, in this study, we did not study the parameters of the ECA (1, 32). Third, the present study was a single-center study with a small sample size, we did not perform ultrasound examination on patients with MMD before stroke. Therefore, prospective long-term follow-up studies with a large sample size are needed to confirm our findings.

CONCLUSIONS

Our results suggested that the DSA findings of Suzuki stage and scores of the leptomeningeal system from the PCA territory to the ACA and MCA territory are related to EICA and PCA ultrasound parameters, respectively. Ultrasonography can be used for preliminary screening and periodic monitoring of patients with MMD, detection of ultrasound parameters might be useful in predicting the occurrence of stroke in patients with MMD. Future prospective studies with large sample sizes and long-term follow-up are needed to confirm our findings.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board (IRB) of Beijing Tiantan Hospital, Capital Medical University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

SZ and PG: Conception and design. SZ, PG, ZS, JW, YL, and JZ: Acquisition of data. SZ and PG: Analysis and interpretation of data. SZ: Drafting the article. All authors: Critically revising the article and Reviewed submitted version of manuscript. WH and DZ: Approved the final version of the manuscript on behalf of all authors. TY, HZ, DZ, and WH: Study supervision.

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Surgical Management of Failed Revascularization in Moyamoya Vasculopathy

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Objectives: Moyamoya vasculopathy (MMV) is a rare stenoocclusive cerebrovascular disease associated with increased risk of ischemic and hemorrhagic stroke, which can be treated using surgical revascularization techniques. Despite well-established neurosurgical procedures performed in experienced centers, bypass failure associated with neurological symptoms can occur. The current study therefore aims at characterizing the cases of bypass failure and repeat revascularization at a single center.

Methods: A single-center retrospective analysis of all patients treated with revascularization surgery for MMV between January 2007 and December 2019 was performed. Angiographic data, cerebral blood flow analysis [H_2O PET or single-photon emission CT (SPECT)], MRI, and clinical/operative data including follow-up assessments were reviewed.

Results: We identified 308 MMV patients with 405 surgically treated hemispheres. Of the 405 hemispheres treated, 15 patients (3.7%) underwent repeat revascularization (median age 38, time to repeat revascularization in 60% of patients was within 1 year of first surgery). The most common cause of repeat revascularization was a symptomatic bypass occlusion (80%). New ischemic lesions were found in 13% of patients prior to repeat revascularization. Persistence of reduced or progressive worsening of cerebrovascular reserve capacity (CVRC) compared with preoperative status was observed in 85% of repeat revascularization cases. Intermediate-flow bypass using a radial artery graft was most commonly used for repeat revascularization (60%) followed by re-superficial temporal artery to middle cerebral artery (re-STA-MCA) bypass (26%). High-flow bypass using a saphenous vein graft and using an occipital artery to MCA bypass was each used once. Following repeat revascularization, no new ischemic events were recorded.

Conclusion: Overall, repeat revascularization is needed only in a small percentage of the cases in MMV. A rescue surgery should be considered in those with neurological symptoms and decreased CVRC. Intermediate-flow bypass using a radial artery graft is a reliable technique for patients requiring repeat revascularization. Based on our institutional experience, we propose an algorithm for guiding the decision process in cases of bypass failure.

Keywords: moyamoya disease, revascularization, failed revascularization, extra- intracranial bypass, surgical management

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INTRODUCTION

Moyamoya vasculopathy (MMV) is a rare stenoocclusive cerebrovascular disease affecting the basal cerebral arteries, which leads to the formation of fragile net-like vessels (1). As such, patients with MMV can present with intracranial hemorrhage from abnormal vessels as well as ischemic events (2, 3). To date, surgical revascularization remains the primary treatment for MMV, as it aims at restoring perfusion in order to stabilize cerebrovascular hemodynamics and reduce hemorrhagic and ischemic events (4, 5). Three primary surgical strategies can be utilized in the treatment of MMV: direct, indirect, and combined revascularization.

In the case of direct revascularization, anastomosis is performed between branches of the internal and external carotid arteries (ECAs). Most commonly, the superficial temporal artery (STA) is anastomosed with the middle cerebral artery (MCA) (STA-MCA bypass) (6). The anterior and posterior cerebral arteries (ACAs and PCAs) as recipient vessels or the occipital artery (OA) as a donor vessel may also be used to adapt revascularization to territories where it is most decreased (7). Indirect revascularization relies on neoangiogenesis between the cortical surface and a pedicled graft such as the dura mater [encephalodurosynangiosis (EDS)] or the temporalis muscle [encephalomyosynangiosis (EMS)] (8). Direct and indirect revascularization techniques can also be combined in an attempt to improve hemodynamic response over time (for example, STA-MCA bypass plus EDS or EMS) (9, 10). The decision to implement a particular bypass strategy for initial revascularization is made on an individual basis under consideration of patient risk profiles and the expected efficacy. To date, evidence points toward the use of direct or combined revascularization procedures (6–9).

Despite careful planning and initially successful surgical intervention with a high early bypass patency rate of up to 99% in MMV, some patients may continue to develop clinical symptoms of hemodynamic insufficiency (10, 11). Several diagnostic tools may be implemented to evaluate bypass insufficiency including angiographic studies; H_2O PET for analysis of cerebrovascular reserve capacity (CVRC) and detailed clinical assessment of the severity of the symptoms were described (12). Failed bypass surgery can be traced back to technical failures such as low flow delivery or secondary occlusion of a direct graft, which failed to take an indirect graft or insufficient collateralization. Disease progression in new vascular territories (i.e., posterior circulation) may also necessitate implementing a rescue strategy for revascularization. Whereas, selection of an appropriate rescue strategy should take the underlying cause of initial bypass failure into consideration, several techniques of direct or combined revascularization have been described to date as useful rescue strategies. These include radial artery grafts (radial artery grafts) from the ECA to the M2 or M3 segment of the MCA (RAG-ECA-MCA) (11), direct bypass of the OA to the PCA (OA-PCA) (13), or repeat STA-MCA bypass using an alternative branch of the STA (14).

Considering the variability of indications and surgical strategies for rescue revascularization in failed bypass surgery, we aimed at identifying the cases of repeat revascularization in our department in order to further characterize the causes of bypass failure, the indications for repeat surgery, and the surgical strategies that were implemented.

MATERIALS AND METHODS

Study Design

This retrospective analysis was approved by the local ethics committee and was conducted in accordance with the Declaration of Helsinki.

We retrospectively identified all moyamoya patients who were treated for failed revascularization in our department between January 2007 and December 2019 including those with unilateral moyamoya and moyamoya syndrome by using the International Classification of Diseases (ICD) code 167.5 (moyamoya). Moyamoya disease (MMD), moyamoya syndrome, and unilateral MMV were defined based on the guidelines established by the Research Committee for Moyamoya Disease; and patients with possible associated diseases were classified as moyamoya syndrome patients (2). Electronic medical records were used to gather data on patient demographics; time and type of initial surgery; follow-up time; angiographic, MRI, and hemodynamic diagnostics; and clinical outcome.

Statistical Analysis

Quantitative values are presented as median values with range. Group comparisons were performed using Fischer's exact test. Univariate Cox regression analysis was performed among all cases of surgically treated MMV using repeat revascularization as an outcome event. Multivariate regression analysis was conducted using repeat revascularization as a dependent variable when the univariate analysis delivered a $p < 0.015$. $p < 0.05$ were considered statistically significant. All analyses were done with Excel (version 14.7.7; Microsoft) and SPSS (version 24; IBM Corp.).

RESULTS

Patient Characteristics

We analyzed a total of 405 surgically treated hemispheres in 308 MMV patients. Of these patients, 68.5% had MMD, 8.1% had unilateral MMD (uMMD), and 25% had moyamoya syndrome. In our series, 19% of patients were juvenile. The median age at first surgical intervention was 38 years (range 2–63 years old, **Table 1**). Seventy-five percent of patients initially presented with ischemic events, and the remaining 25% presented with hemorrhagic lesions. Regarding gender distribution, 28% of patients were male and 72% were female. The majority of our cohort were of Caucasian background (87%), 8% were of Asian descent, and 5% were Middle Eastern descent.

A total of 20% of hemispheres each were classified as Suzuki stages 1, 2, and 3. Thirteen percent were Suzuki stage 4, and 27% were Suzuki stage 5 (**Table 1**). The Berlin Grading (15) could be calculated based on complete data available for 206 hemispheres (51%). Of these hemispheres, 13% of hemispheres were grade 1, 30% were grade 2, and 57% were grade 3.

TABLE 1 | Demographics and disease characteristics in revision cases and total cases.

	Revision	Non-revision	*p*
Total	15 (3.7%)	390	0.35
Age in years (median/range)	38/10–57	33/2–63	0.23
Suzuki Grade	*N* = 15	*N* = 390	1.000
1	3 (20%)	78 (20%)	1.000
2	3 (20%)	78 (20%)	1.000
3	3 (20%)	78 (20%)	0.440
4	3 (20%)	50 (13%)	0.533
5	3 (20%)	106 (27%)	
Berlin Grade	*N* = 15	*N* = 206	0.705
1	1 (7%)	26 (13%)	0.569
2	3 (20%)	62 (30%)	0.178
3	11 (73%)	118 (57%)	

Suzuki Classification was performed for all hemispheres. Data on Berlin Classification was available for 206 of 405 hemispheres (51%). Fischer's Exact Test. $p < 0.05$ is considered significant.

Cardiovascular comorbidities including hyperlipidemia and hypertension were found in 40 and 26% of patients requiring repeat revascularization, respectively.

First Revascularization Strategies

The most common initial revascularization strategy was STA-MCA bypass combined with EDS (44%), followed by STA-MCA bypass alone (20%) (**Table 2**). The median follow-up period after revascularization in unilateral cases was 12.7 months (range 3–47 months). In bilateral cases, median follow-up for the first hemisphere was 12.8 months (range 1–79 months) and for the second hemisphere 7.7 months (range 1–35 months). A total of 29 hemispheres did not receive follow-up on site after the revascularization (10 unilateral and 19 bilateral cases for the second hemisphere treated). Nine of these patients had traveled from abroad for surgery in our department and received a follow-up care in their home countries.

Demographics and Surgical Strategies for Initial Revascularization in Failed Cases

The most common initial surgical strategy used in patients was an STA-MCA bypass combined with EDS (73%). Of these cases, only 15 hemispheres (one hemisphere per patient) required surgical revision (3.7%). The median age of patients undergoing surgical revision was 38 years (range 10–57 years). A total of 40% of patients (6/15) received revision surgery within 1 year following the initial procedure. The majority of patients were female (13/15, 87%). The majority of the patients were diagnosed with MMD (12/15, 80%) with only 20% quasi-MMD. Most patients initially presented with ischemic symptoms before their initial surgery (12/15, 80%). The operated hemisphere in patients requiring revision surgery represented with 20% each of Suzuki grades 1–5. With the use of the Berlin Grading system, one hemisphere was grade 1 (6.6%), three hemispheres were grade 2 (20%), and 11 were grade 3 (73.3%).

TABLE 2 | Initial symptoms and surgical strategies for initial revascularization.

	Revision cases (*n* = 15)	Remaining Cases (*n* = 293)	*p*
Initial symptoms			
Ischemia	13 (87%)	231 (79%)	0.719
Hemorrhage	2 (13%)	62 (21%)	0.216
	Revision cases (*n* = 15)	**Remaining cases (*n* = 390)**	***p***
First surgical strategy			
STA/MCA + EDS	11 (73%)	173 (44%)	0.251
STA/MCA + EMS	2 (13%)	67 (17%)	0.329
STA/MCA + EDS/EMS	1 (67%)	50 (13%)	0.158
ECA/MCA	1 (67%)	4 (1%)	0.441
STA-MCA alone	0	78 (20%	–
EDGS	0	4 (1%)	–
EDS or EMS alone	0	14 (4%)	–

Calculations of initial symptoms are based on total patients (n = 308, 15 revision cases and 293 remaining) and surgical strategies on total hemispheres (n = 405). Fischer's exact test. $p < 0.05$ is considered significant.
STA-MCA, superficial temporal artery to middle cerebral artery; EDS, encephalodurosynangiosis; EMS, encephalomyosynangiosis; EDGS, Encephalodurogaleosynangiosis.

Regarding the first surgical strategy, 11/15 patients (73%) had been treated with a combined revascularization strategy using STA-MCA bypass and EDS and 2/15 (13%) with STA-MCA bypass and EMS. One patient (6.7%) was treated with combined STA-MCA plus EDS and EMS; and one patient received initial revascularization through an ECA-MCA bypass with radial artery graft. No statistical difference could be found in the proportions of initial surgical strategies used in revision cases vs. non-revision cases (**Table 2**).

Indications for Repeat Revascularization and Surgical Strategies

The most common cause of surgical revision was ischemic symptoms corresponding to bypass occlusion on digital subtraction angiography (DSA) (found in 12 of 15 cases, 80%). Of the remaining three cases, one procedure was performed in a symptomatic patient with a patent bypass, and one case was repeated due to secondary occlusion of the A. cerebri posterior (P2 segment). In a further case, the initial revascularization procedure had to be ended intraoperatively due to severe vasospasm (**Table 3**).

The most common symptom prior to repeat revascularization was transient ischemic attacks (TIAs) (8/15, 53%) followed by new paresthesias (4/15, 27%). One patient suffered a new hemorrhage prior to repeat revascularization, and one suffered new epileptic seizures (**Table 4**).

We also examined the CVRC as measured by PET or single-photon emission CT (SPECT) before surgical revision, compared with preoperative status. In two cases (13%), no additional

TABLE 3 | Surgical strategies, indication, and timing of repeat revascularization.

Second surgical strategy	
STA/MCA (frontal branch)	4 (26%)
ECA/MCA (RAG interposition)	9 (60%)
ECA/MCA (SV interposition)	1 (67%)
OA/MCA	1 (67%)
Reason for revision	
Symptomatic bypass occlusion on DSA	12 (80%)
Symptomatic patent bypass	1 (67%)
Intraoperative vasospasm (surgery not completed)	1 (67%)
Secondary P2 occlusion	1 (67%)
Time between initial surgery and revision	
0–1 Year	6 (40%)
1–3 Years	3 (20%)
3–6 Years	3 (20%)
>6 Years	3 (20%)

Summary of second surgical strategies, reason for repeat revascularization, and time between initial surgery and repeat revascularization.
STA-MCA, superficial temporal artery to middle cerebral artery; ECA-MCA, external carotid artery to middle cerebral artery; RAG, radial artery graft; SV, saphenous vein; OA-MCA, occipital artery to middle cerebral artery.

TABLE 4 | Imaging and clinical characteristics before and after repeat revascularization.

	Before revision	After revision (last follow-up)
CVRC		
Unchanged to preoperative status	11 (73%)	9 (60%)
Worsened to preoperative status	1 (67%)	0
Improved to preoperative status	0	2 (13%)
Data not available	2 (13%)	2 (13%)
New ischemic lesions		
Yes	2 (13%)	0
No	13 (87%)	15
Symptoms		
TIAs	6 (40%)	0
Hemorrhage	1 (67%)	0
Persisting paraestheisa	4 (33%)	0
Epileptic seizures	1 (6 7%)	0
Stable/No new clinical symptoms	1 (67%)	10 (66%)
Improved clinical status	0	5 (33%)

Summary of cerebral blood flow measurements (H_2O PET or SPECT), ischemic lesions on MRI, and neurological symptoms before and after repeat revascularization.
CVRC, cerebrovascular reserve capacity; TIA, transient ischemic attack; SPECT, single-photon emission CT.

measurement was performed before revision surgery (one case due to thrombus of the bypass after discontinuing ASS and one case following intraoperative vasospasm). In 85% of cases (11/13), the CVRC before surgical revision was unchanged to the status before the initial surgery, and two patients (15%) displayed worsening CVRC (**Table 4**).

The surgical revision strategy most commonly employed was the ECA-MCA using a radial artery graft (9/15, 60%) (**Figure 1**). In one case, the ECA-MCA bypass was completed using a saphenous vein (SV) graft due to hereditary systemic sclerosis of arterial vessels. Four cases (26%) were performed using a frontal STA branch (**Figures 2A–C**). One case was performed using an OA-MCA bypass (**Table 3**).

Follow-Up After Repeat Revascularization and Preoperative Predictors of Bypass Failure

The median observational period following repeat revascularization was 12 months (range 3–60 months). Following repeat revascularization, 10/15 patients (66%) maintained a stable neurological status and developed no new clinical symptoms, whereas 5/10 patients (33%) reported improved clinical status compared with before the repeat revascularization (**Table 4**). CVRC measurements were performed in 11 of 15 patients following repeat revascularization between 1 and 5 years after last surgery. Here, 82% (9/11) of patients showed unchanged CVRC, with 18% (2/11) having improved CVRC compared with preoperative status before the first revascularization attempt, and no patients showed worsening of CVRC following revision surgery. In 13% of patients (2/15), new ischemic lesions could be detected on MRI before repeat revascularization. No new ischemic lesions could be found at last observation in patients following revision surgery (**Table 4**).

We performed univariate Cox regression analysis to model the relationship of preoperative patient characteristics with the event of repeat revascularization considering gender, age (adult vs. child), ethnic background, symptoms at initial presentation, ischemic lesions on MRI, reduced CVRC, and Berlin Grading as independent variables. Univariate Cox regression analysis showed no significant increase in the proportional hazard ratio of the above-mentioned predictors on the event of repeat revascularization (**Table 5**).

Subsequent multivariate analysis included the variables "ischemic lesions on MRI prior to first surgery" as well as "symptoms at initial presentation." This analysis however displayed no significant relationship with the event of repeat revascularization, whereas "ischemic lesions" nearly reached significance at $p = 0.054$ (**Table 6**).

DISCUSSION

In this study, we reviewed our institutional series of cases requiring repeat revascularization in patients with MMV. We observed a low rate of bypass failure with only 3.7% in our patient cohort with good outcome after rescue operation. We did not identify any significant predictors for the bypass failure. The clear predominance of female patients in our series may be attributed to the overall female predominance in sporadic and familial MMD, especially in Caucasian patients (3, 25, 26). In contrast to previous series on repeat revascularization in which re-STA-MCA was most commonly utilized (17), the most commonly implemented technique in our cohort was the intermediate-flow bypass using a radial artery graft.

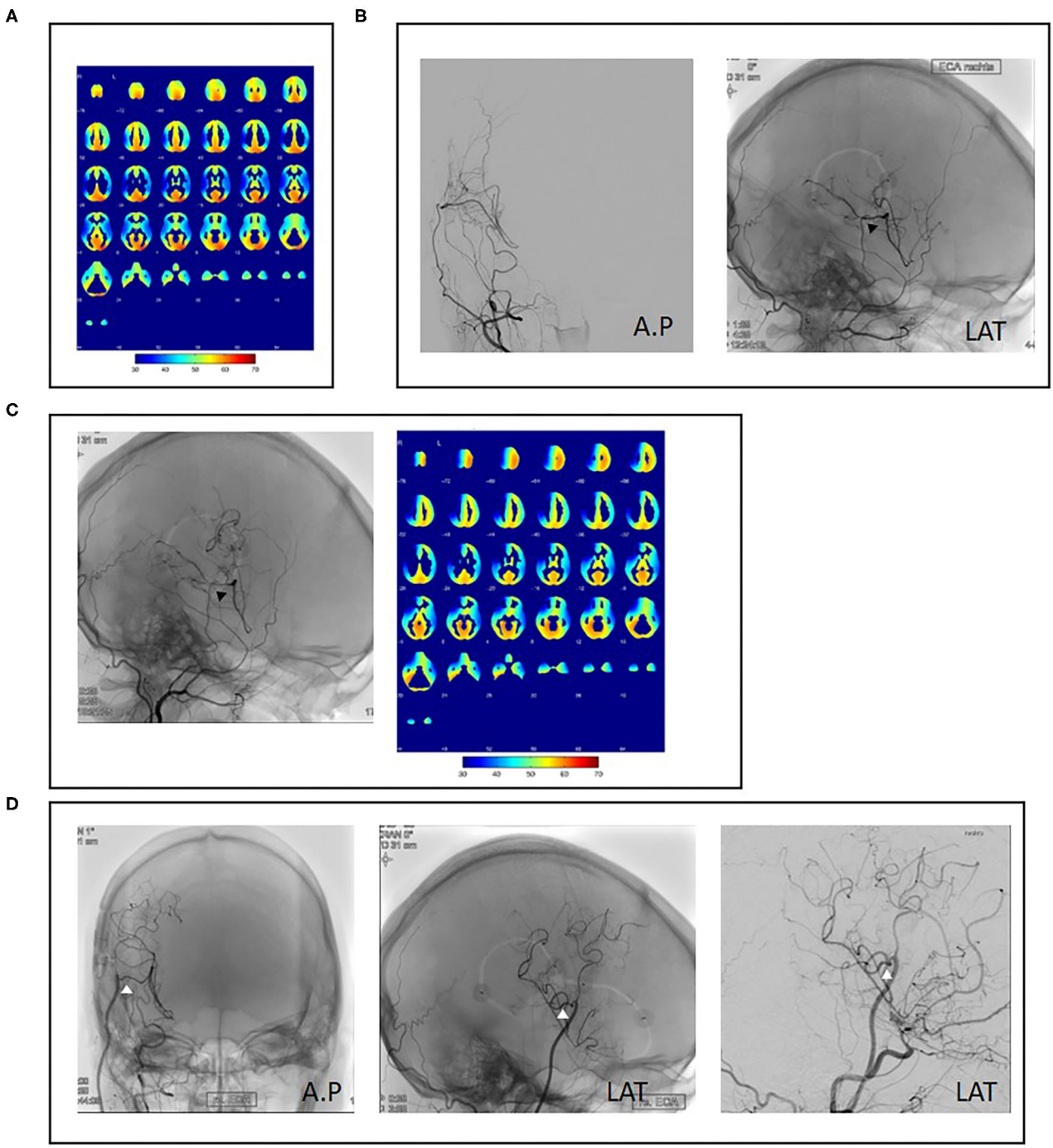

FIGURE 1 | Case example of repeat revascularization using a radial artery graft. A 34-year-old female patient presented with right hemispheric transient ischemic attacks (TIAs) and angiographic evidence of unilateral right-sided moyamoya disease as well as decreased cerebrovascular reserve capacity (CVRC) of the right hemisphere **(A)**. Revascularization using superficial temporal artery to middle cerebral artery (STA-MCA) plus encephalodurosynangiosis (EDS) was performed without complication (**B**, black arrowheads). Fourteen months after initial surgery, the patient reported new left-sided hemihypesthesia. Angiography showed decreased bypass patency and decreased CVRC of the anterior MCA territory (**C**, black arrowheads). As no frontal STA branch was available, repeat revascularization was performed with a radial artery graft (**D**, white arrowheads).

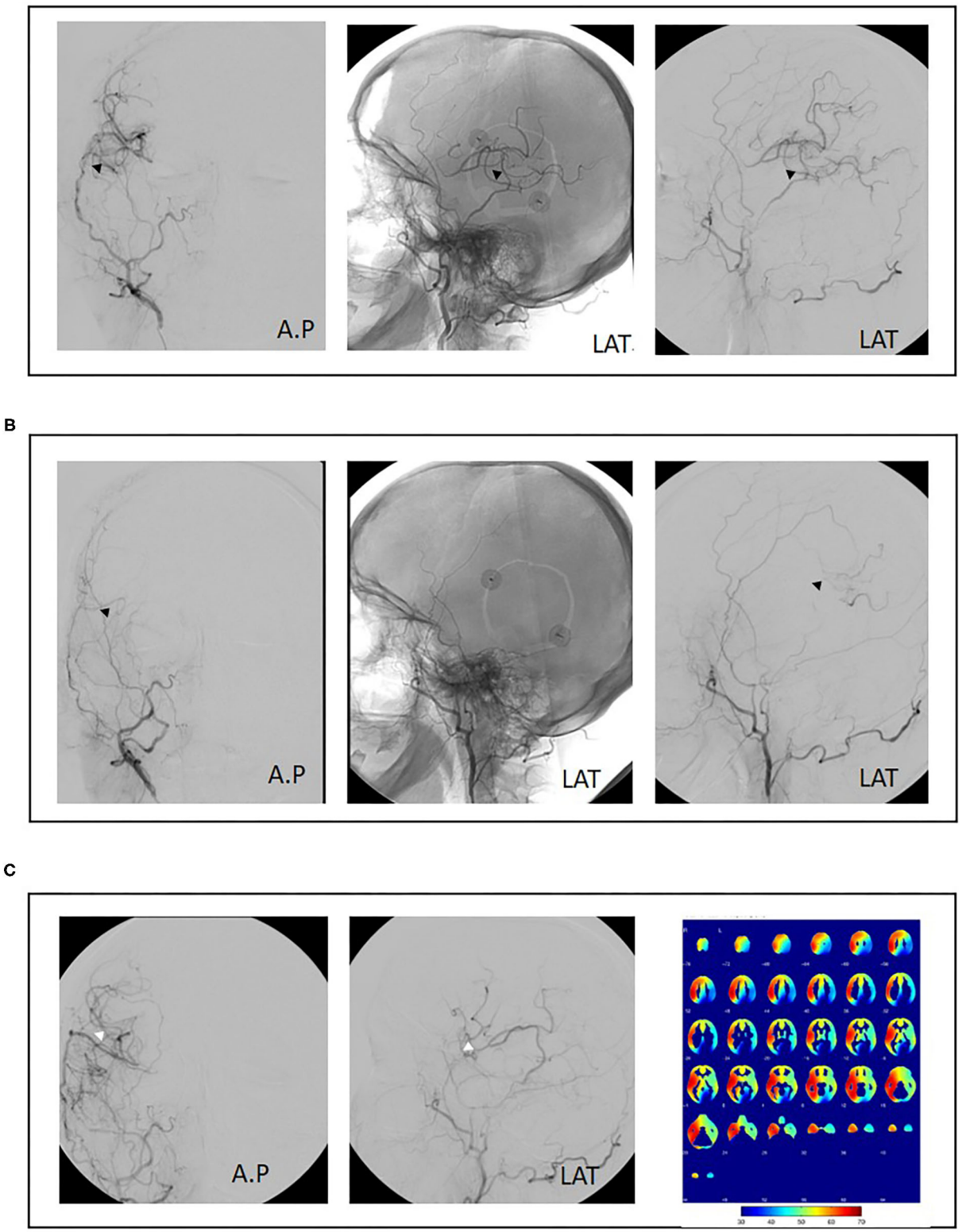

FIGURE 2 | Case example of repeat revascularization using superficial temporal artery to middle cerebral artery (re-STA-MCA). A 50-year-old female patient with bilateral moyamoya disease and right hemispheric transient ischemic attacks (TIAs) was treated with combined revascularization using STA-MCA (parietal branch) plus encephalodurosynangiosis (EDS) of the right hemisphere (**A**, black arrowheads). Postoperatively, no new neurological symptoms were observed. Eight months

(Continued)

FIGURE 2 | postoperatively, the patient suffered a fractured radius requiring orthopedic surgery. Aspirin was discontinued preoperatively and not resumed after surgery. The patient then presented with renewed right-sided TIAs. Angiography showed bypass occlusion (**B**, black arrowheads). Repeat revascularization was performed using a frontal branch of the STA plus EDS. Cerebral blood flow analysis 1 year after initial surgery shows adequate perfusion of the right hemisphere. Revascularization of the left hemisphere was performed consecutively (**C**, white arrowheads).

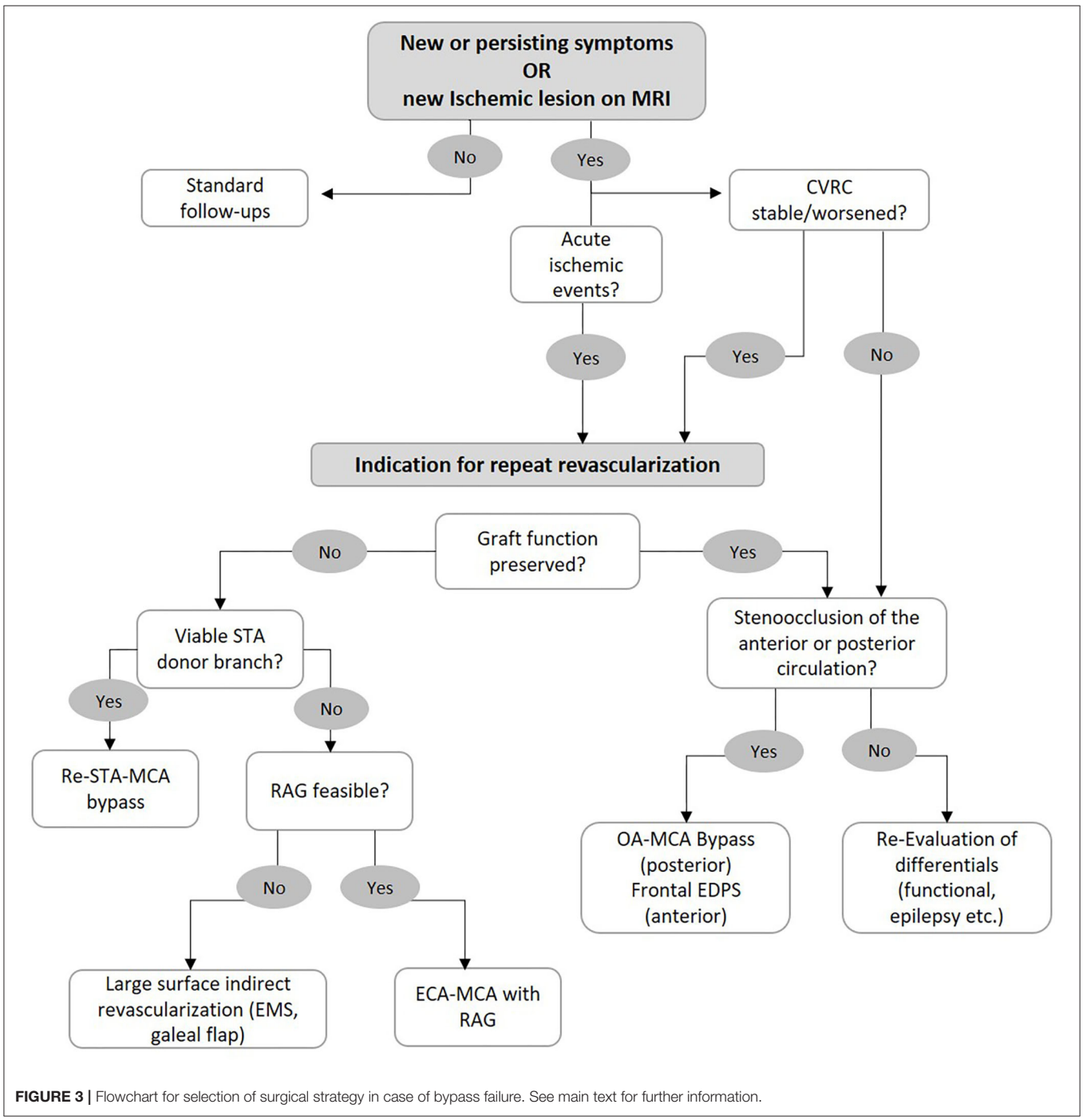

FIGURE 3 | Flowchart for selection of surgical strategy in case of bypass failure. See main text for further information.

Our analysis of 405 operated hemispheres showed an overall rate of repeat revascularization due to bypass failure of 3.7% similar to the previous large North American institutional series with 4% (14). In our previous analysis, the failure rate among 122 patients was similar at 3.2% (11). While there are very few studies specifically focusing on moyamoya patients requiring

repeat revascularization surgery, examining data on the rate of radiological STA-MCA bypass patency for MMV showed an early bypass patency rate of up to 99% at 1.5 years in the series of Guzman et al. (10), which is similar to the early bypass patency for other indications than moyamoya with up to 96% (16–22). Schick et al. reported a late graft failure (between 1.4 and 2.7 years following initial surgery) of approximately 10% in non-MMV patients within 5.6 years of follow-up (17). In our MMS cohort of 61 patients, the short- and long-term bypass patency was similar with 93 and 88% (within a mean follow-up period of 4.2 years), respectively (23). Importantly, a bypass occlusion does not always require a revision. A further theory regarding the etiology of bypass occlusion has pointed to a possible complementary role of indirect revascularization when combined with direct revascularization. Here, it has been proposed that the collaterals formed by indirect revascularization techniques may abrogate the demand placed on direct grafts, therefore allowing for their successive occlusion after combined procedures (16). However, in these cases, patients do not present with new symptoms and have maintained CVRC. Furthermore, the possible role of cardiovascular comorbidities in promoting bypass occlusion also has yet to be explored. The rates of hyperlipidemia (40%) and hypertension (26%) in our cohort of revised bypasses appear to differ from those of a previously reported German cohort including all entities of moyamoya (hyperlipidemia 15% and hypertension 50%) (24). Further analysis of whether or not these differences are pathophysiologically relevant or due to differences in cohort size and selection of bypass failure is necessary.

The lower proportion of patients requiring surgical intervention may underscore the importance of additional analysis of CVRC and clinical symptoms when considering a second surgical intervention. The review of our cases showed that iatrogenic acute graft occlusion or intraoperative complications account for only a minority of repeat revascularizations (2/15, 13%). One such case was due to symptomatic bypass occlusion during prolonged discontinuation of antiplatelet therapy following elective orthopedic surgery; the other was due to intraoperative vasospasm (in this case, the procedure was completed in a second session without complication). In the series of Teo et al. one patient underwent revascularization as a result of acute graft occlusion within 1 week following initial surgery (14). The remaining 12 cases (80%) in our series were performed due to symptomatic bypass occlusion. All patients showed reduced CVRC under acetazolamide challenge in H_2O PET or SPECT. In contrast, Teo et al. described only one case of graft occlusion following direct revascularization (STA-MCA), although 24/29 (83%) patients for whom cerebral blood flow studies were available showed reduced CVRC under acetazolamide challenge (14). These findings may indicate disease progression as a driver for repeat revascularization surgery in this cohort, whereas our series primarily received revascularization under conditions of correlation between bypass occlusion, neurological symptoms, and reduced CVRC and not bypass occlusion alone.

We did not identify any significant predictors for bypass failure; however, ischemic lesions in MRI prior to surgery seem to be the most relevant factor ($p = 0.054$). The Berlin classification was shown to correlate with symptoms and perioperative complications (15, 25, 26); however, it did not predict failed revascularization in our cohort most probably due to the low number of revision cases in our series. The majority of the revised cases (73%) were grade 3 according to the Berlin classification, indicating the severity of the disease in these patients.

TABLE 5 | Model summary of univariate Cox regression analysis.

Predictor	Hazard ratio	CI (95%)	*P*
Gender	0.944	0.614–1.455	0.796
Age (Adult vs. Child)	1.173	0.829–1.659	0.367
Ethnicity (Caucasian vs. Asian)	1.016	0.624–1.656	0.948
Clinical Presentation (Ischemia vs. Hemorrhage)	0.716	0.455–1.125	0.147
Ischemia on MRI	1.360	0.970–1.907	0.074
CVRC (Reduced vs. Preserved)	0.819	0.823–1.383	0.385
Berlin grade	0.917	0.346–2.433	0.863

CVRC, cerebrovascular reserve capacity.

Regarding the time points at which revascularization was performed, we found no clear distribution in our series. While 6/15 (40%) of patients underwent repeat revascularization within 1 year following initial intervention, this number includes the two unusual cases of discontinued antiplatelet therapy and intraoperative vasospasm. Observing these numbers without these two cases shows a nearly equal distribution of repeat revascularization at all time points (within 1 year after surgery; 1–3, 3–6, and over 6 years after surgery). These findings differ from those of Teo et al. in which the mean time of repeat revascularization surgery was 47 months (14). This discrepancy may be due to the difference of case numbers, as our series includes 15 (of 405) vs. 57 (of 1244) hemispheres in the North American series. An additional study regarding STA-MCA graft failure performed in patients suffering from atherosclerotic occlusion of the internal carotid artery bypass occlusion has shown rates of bypass failure in up to 10% of patients at an average of 38 months following initial surgery (17).

In our cohort, all patients were initially treated with direct or combined revascularization techniques. In contrast to our cohort in which only one patient (6.7%) showed new P2 stenoocclusion, the majority of patients in the North American series (11/20, 55%) received repeat surgery due to angiographic evidence of poor filling of another vascular territory and most commonly in the anterior MCA territory (14). This discrepancy may be due to the diagnostic criteria utilized to determine the indication for repeat vascularization (angiographic findings vs. cerebral blood flow studies). Regardless, these findings point toward the necessity of further characterization of disease progression in other vascular territories aside from the MCA, which is most commonly targeted by standard direct revascularization. Deciphering cases of insufficient leptomeningeal collateralization following MCA revascularization from separate progression of ACA-PCA insufficiency may help guide the choice of revascularization technique (re-STA-MCA vs. STA-ACA or OA-MCA).

TABLE 6 | Model summary of multivariate Cox regression analysis.

Predictor	Hazard ratio	CI (95%)	*P*
Clinical presentation (Ischemia vs. Hemorrhage)	1.461	0.000–4.667	0.965
Ischemia on MRI	0.301	0.089–1.020	0.054

In our study, no fatalities in relation to repeat revascularization were observed. The use of a high-flow SV graft as a repeat revascularization strategy has been previously reported to have led to fatal reperfusion hemorrhage in one case (14). Our series also included one patient who received SV graft under strict perioperative normotensive therapy without complications. In our experience, the SV graft when performed under consistent perioperative blood pressure monitoring and maintenance of normotension is a viable strategy and has been shown to demonstrate long-term patency (27).

Patients who are determined to require repeat revascularization surgery are therefore identified based on the presence of clinical symptoms in addition to reduced CVRC as measured by H_2O PET or SPECT under acetazolamide challenge. Based on our institutional experience, we have developed an algorithm to aid in our selection of the surgical strategy for patients with an indication for repeat revascularization surgery (**Figure 3**). Repeat STA-MCA bypass plus EMS or EDS is performed if a second branch of the STA is available for use in a re-STA-MCA, and the reduced CVRC can be adequately addressed with this bypass. We prefer a combined revascularization, as previous studies have shown the superiority of combined vs. indirect revascularization regarding hemodynamic normalization as well as the rate of secondary ischemic events (4, 6, 8). If no donor branch is available, the patient is assessed for feasibility of a RAG. This includes sonographic measurement of arterial diameter, flow, and functional determination of adequate collateral circulation of the hand (positive Allen's test). The presence of atherosclerosis of the ECA, stents, or other variables affecting its patency must also be assessed. We have already shown the efficacy and safety of RAG for rescue revascularization (11). If a RAG bypass is determined to not be safely feasible, large surface indirect revascularization (i.e., EMS and galeal flap) and high-flow grafts using the SV remain as alternative strategies.

If a patient meets the criteria for repeat revascularization although the graft patency and function in angiographic series is not found to be insufficient, we examine the progression of the posterior circulation. If new P2 stenoocclusive changes are found, we recommend an OA-MCA bypass. If the anterior circulation is affected and the ACA shows hypoperfusion, then frontal encephalo-duro-periosteal-synangiosis (EDPS) may be performed. If no new steno-occlusion is found in these patients, we recommend re-evaluation and consideration of differentials of clinical status such as functional lesions or epileptic seizures.

The major limitation of the current study is the retrospective design performed in a single institution; however, considering the rarity of MMV in Europe, this is the largest European series analyzed in respect to repeat revascularization. Furthermore, by conducting a time-to-event analysis in a retrospective cohort, possible modifiers associated with the necessity of bypass revision may not have been accounted for.

In conclusion, our study confirms the efficacy of the revascularization surgery in MMV with only a low rate of rescue revascularization. Nevertheless, we suggest the continuing review and critical analysis of failed bypass surgery over time in order to identify trends that may become visible as case numbers increase. Furthermore, examination of non-surgical factors such as relevant cardiovascular comorbidities or biochemical parameters should be considered. Ultimately, the practice of reviewing and analyzing failed revascularization cases should be considered an essential part of the ongoing management of patients with MMV.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Charité Universitätsmedizin Berlin. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

KL, GA, and PV conceptualized the study. NS and SG collected patient data. KL and GA analyzed the data and wrote the manuscript including figure and table production. PV reviewed and approved the final manuscript. All authors contributed to the manuscript and approved the final submitted version.

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REVIEW
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RNF213 and GUCY1A3 in Moyamoya Disease: Key Regulators of Metabolism, Inflammation, and Vascular Stability

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Moyamoya disease is an idiopathic chronically progressive cerebrovascular disease, which causes both ischemic and hemorrhagic stroke. Genetic studies identified *RNF213/Mysterin* and *GUCY1A3* as disease-causing genes. They were also known to be associated with non-moyamoya intracranial large artery disease, coronary artery disease and pulmonary artery hypertension. This review focused on these two molecules and their strong linker, calcineurin/NFAT signaling and caveolin to understand the pathophysiology of moyamoya disease and related vascular diseases. They are important regulators of lipid metabolism especially lipotoxicity, NF-κB mediated inflammation, and nitric oxide-mediated vascular protection. Although intimal thickening with fibrosis and damaged vascular smooth muscle cells are the distinguishing features of moyamoya disease, origin of the fibrous tissue and the mechanism of smooth muscle cell damages remains not fully elucidated. Endothelial cells and smooth muscle cells have long been a focus of interest, but other vascular components such as immune cells and extracellular matrix also need to be investigated in future studies. Molecular research on moyamoya disease would give us a clue to understand the mechanism preserving vascular stability.

Keywords: moyamoya disease, RNF213 mutation, GUCY1A3, CAV1 (caveolin-1), calcineurein/NFAT pathway, nitric oxide, inflammation, vascular

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INTRODUCTION

Moyamoya disease (MMD) is a progressive occlusive cerebrovascular disease of unknown etiology (1). Main treatment strategy is revascularization surgery to prevent cerebrovascular events, and antiplatelet therapy is another treatment option that has recently been reappraised (2, 3). However, there is no curative treatment that can prevent the progression of arterial stenosis. Understanding the molecular biology of MMD is needed to develop a new treatment strategy and proper biomarkers to evaluate therapeutic efficacy.

Genetics has contributed greatly to understanding the pathophysiology of MMD. In 2011, *RNF213* (also called as *Mysterin*) was identified as the major susceptibility gene for MMD and p.R4810K mutation was found as a founder mutation that increases the risk of MMD by ∼300 times (4). The mutation was detected in ∼80% of patients with MMD in Japan, ∼70% in Korea and ∼30% in China. Although the same mutation was not detected in European patients, other mutations such as p.D4013N were found in ∼10% (5). Thus, *RNF213* is a major susceptibility gene for MMD and it has been recognized as a key molecule to understand the pathophysiology of MMD.

It has been reported that patients with MMD have not only cerebrovascular lesions but also extracranial lesions including coronary artery disease and pulmonary artery stenosis. Importantly, these lesions have common pathological features (6). In agreement with this finding, the p.R4810K mutation in *RNF213* gene was also shown to be associated with coronary artery disease (7, 8), pulmonary artery hypertension (9), and renal artery stenosis (10). *GUCY1A3* mutations were first detected in patients with quasi-MMD (syndromic MMD) with achalasia (11), but some cases show only moyamoya arteriopathy in the absence of achalasia (12). Of note, *GUCY1A3* is also known to be associated with coronary artery disease (13) and pulmonary artery hypertension (14) as is the case with *RNF213*. Both *RNF213* and *GUCY1A3* are also associated with hypertension. These lines of evidence suggest that investigation of these two genes may give us some clues to elucidate the molecular pathogenesis of MMD and quasi-MMD.

In this narrative review, we will discuss characteristics of *RNF213* and *GUCY1A3* in terms of (1) genetics, (2) molecular structures and functions, (3) transcriptional regulation, (4) possible roles in vascular wall, (5) possible roles in vascular insults, and (6) other properties. Finally, we will propose a potential mechanism of MMD and future research directions.

GENETICS OF *RNF213* AND *GUCY1A3*

Susceptibility locus for MMD have been reported to reside on 3p, 8q23, and 17q25.3. Chromosome 17q locus was first identified by chromosome wide linkage analysis (15), and it was narrow down to the 3.5-Mb region at 17q25.3 (16) by linkage analysis of families with autosomal dominant inheritance with incomplete penetrance. In this locus, *RNF213* was identified as the major susceptibility gene for MMD (4). In East Asian patients, the p.R4810K mutation is most common, and it is also found in 2% of the general population, which is in agreement with low penetrance (1/100–1/200) of the gene. Intrudingly, genotypes of *RNF213* have impact on clinical features of MMD. The p.R4810K mutation was associated with severer form of the disease with higher frequency of bilateral involvement and posterior circulation involvement, and the homozygous p.R4810K mutation was associated with early-onset of the disease (17–19). The p.A4399T polymorphism, which is common in Chinese patients, is associated with hemorrhagic MMD (odds ratio = 2.8; 95% confidence interval, 1.2–6.5) (20). An intronic variant, rs9916351, was reported to be associated with early onset MMD in a Chinese population (21).

The p.R4810K mutation has been reported to be associated with various vascular phenotypes. First, it was shown to be associated with quasi-MMD (syndromic MMD) (22), moyamoya angiopathy with a known comorbidity such as Down syndrome or neurofibromatosis type I (NF1) (23). In addition, it was associated with other vascular diseases including non-moyamoya cerebral arteriopathy (24–26), coronary artery disease (7, 8) and pulmonary artery hypertension (9, 27). The p.R4810K mutation was also associated with hypertension in a dominant fashion (28), while rs9916351 was associated with hypertension in a recessive fashion (29).

Loci on 4q.32.1, 10q23.31, and Xp28 have been identified as loci for quasi-MMD, and *GUCY1A3*, *ACTA2,* and *BRCC3* was found to be a disease associated gene for each locus (11, 30, 31). Genetic analysis of three families with autosomal recessive mode of inheritance identified *GUCY1A3* as a cause of moyamoya angiopathy with achalasia, esophageal sphincter muscle contraction (32). Interestingly, some patients with moyamoya angiopathy associated with *GUCY1A3* mutation do not have achalasia (12), meaning that the patient can be diagnosed as MMD but not quasi-MMD. In *RNF213*, the p.R4810K mutation was common to various vascular phenotypes including MMD, coronary artery disease and pulmonary artery hypertension, whereas different mutations were found in each phenotype for *GUCY1A3*. In detail, nonsense mutation p.R349X, p.E391KfsX, and c.1086 +1G>A splice donor site mutation were associated with moyamoya with achalasia (11). *GUCY1A3* rs1842896 polymorphism was a risk factor for large artery arteriopathy stroke in a Southern Han Chinese population (33). Loss of function mutation p.L163FfsX was associated with an increased risk of myocardial infarction (13). Gain of function mutation p.A681T was associated with a reduced risk of pulmonary artery hypertension (14). A SNP, rs13139571, at the *GUCY1A3-GUCY1B3* locus was associated with high blood pressure (34). Thus, different mutations or SNPs exhibit different phenotypes, although hypertension is a common phenotype for most mutations and polymorphisms in *GUCY1A3*.

MOLECULAR STRUCTURE AND FUNCTIONS

Molecular Structure and Functions of RNF213/Mysterin

RNF213/Mysterin is a unique protein which has both functional AAA+ ATPase and E3 ligase. It is a large protein consisting of 5,207 amino acids with a size of 591-kDa (**Figure 1**). Isoform 3 which lacks exon 4 is the major isoform (NP_001243000.2) (4), and the p.R4810K mutation (rs112735431) is assigned as c.14429G>A. A recent cryo-EM analysis revealed the molecular structure of RNF213 (35, 36). It consists of three structural components including N-terminal structural motif (N-arm), AAA+ ATPase with Walker motif A and B, and E3 ligase with RING finger and NFX1-type zinc finger (**Figure 1**). Mutations associated with MMD are dominantly observed in E3 ligase.

E3 ligase plays a central role in post-translational modification by ubiquitination. It recruits an E2 ubiquitin-conjugating enzyme that has been loaded with ubiquitin, recognizes a protein substrate and assists or directly catalyzes the transfer of ubiquitin from the E2 to the protein substrate. Ubiquitin contains seven lysine (K) residues that, together with its amino terminus, provide eight attachment sites for further ubiquitin molecules (K0, K6, K11, K27, K29, K33 or K48, K63), thereby allowing the formation of polymeric chains (37). Variety of combination of branch sites and the length of polyubiquitin chain allow the system to be highly complex, and the system is called

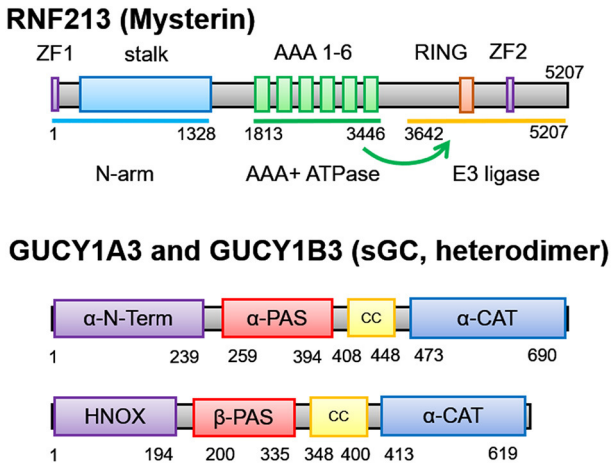

FIGURE 1 | Molecular structure of RNF213 and soluble guanylate cyclase (sGC) encoded by GUCY1A3. RNF213 consists of N-arm, AAA+ ATPase, and E3 ligase. E3 ligase contains two different types of zinc finger, RING and ZF2 (NFX1-type zinc finger). Not only mutations in the RING and ZF2, but also mutations in the AAA+ ATPase may disturb the function of E3 ligase. GUCY1A3 and GUCY1B3 encode the α1 and β1 subunits of soluble guanylate cyclase (sGC) to form a heterodimer.

"ubiquitin codes." Together with its various substrates, ubiquitin acts as a versatile cellular signal that controls a wide range of biological processes including protein degradation, DNA repair, endocytosis, autophagy, transcription, immunity, and inflammation. K63 linkages are known to regulate activation of the nuclear factor-kappa B (NF-κB) transcription factor, DNA repair, innate immune responses, clearance of damaged mitochondria, and protein sorting (38). RNF213 RING domain cooperates with Ubc13 E2 ubiquitin-conjugating enzyme to generate K63-linked polyubiquitin chains and induces NF-κB activation (**Figure 2**). MMD-associated mutations in the RING domain enhances NF-κB activation (39). Still, ubiquitination targets of RNF213 remain largely unknown, except for Nuclear factor of activated T-cells 1 (NFAT1) and filamin A (40).

The AAA+ superfamily of ATPases has diverse cellular functions including membrane fusion, proteolysis and DNA replication, and it works as molecular chaperon (41). RNF213 was considered to belong to the dynein ATPase family which has 6 AAA domains (35). It forms hexamer (42) and is similar to the resting states of dynein. However, it does not have motor activity along the microtubule but has trans-thiolation activity, which transfers ubiquitin from one substrate to another (36). This means that ATPase and E3 ligase work cooperatively. In fact, deletion or mutations of the ATPase domain reduced the function of E3 ligase (39, 43) (**Figure 1**). This may explain the reason why MMD is caused by both mutations in the ATPase and those in the E3 ligase.

Molecular Structure and Functions of Soluble Guanylate Cyclase Encoded by *GUCY1A3*

GUCY1A3 encodes the α1 subunit of soluble guanylate cyclase (sGC), which forms heterodimeric enzyme with ß1 subunit encoded by *GUCY1B3* (**Figure 1**), and it is the major receptor for nitric oxide (NO) (**Figures 2**, **3**). NO binds the heme iron of sGC to induce production of cyclic guanosine monophosphate (cGMP), which then activates the cGMP-dependent protein kinase (PKG) pathway (44). *Murine retrovirus integration site 1* (*MRVI1*) encodes Inositol 1,4,5-Triphosphate Receptor Associated 1 (IRAG1) and plays a role as NO/PRKG1-dependent regulator of IP3-induced calcium release. Phosphorylation of *MRVI1* by PRKG1 inhibits bradykinin and IP3-induced calcium release from intracellular stores, leading to inhibition of platelet activation and aggregation. It also mediates NO-dependent inhibition of calcium signaling, which contributes to NO-dependent relaxation of smooth muscle cells. Intrudingly, *MRVI1* mutation was found to increase the risk of developing moyamoya angiopathy in patients with NF1. Specifically, p.P186S substitution (rs35857561) in *MRVI1* was segregated with quasi-MMD in both the Italian and German NF1 families (45).

The p.C517Y mutation in *GUCY1A3* was found in patients with MMD without any evidence of achalasia (12). Sf9 cells expressing the *GUCY1A3* p.C517Y mutation showed lower basal cGMP-forming activity and lower maximal NO-induced activity. Thus, the p.C517Y missense mutation led to a significantly blunted response in NO signaling and decreased cGMP production. This was in consistent with previously published observations on rat sGC-containing nitrosylated C516 (mouse homolog to human C517) to resulted in desensitization of sGC to NO activation (46).

TRANSCRIPTIONAL REGULATION

Transcriptional Regulation of *RNF213*: A Sensor for Mitochondrial Damage and Inflammation

Various factors have been reported to upregulate *RNF213*, and all of them were related to inflammation caused by mitochondrial dysfunction and infection. In patients with MMD, morphological abnormalities of mitochondria with higher reactive oxygen species (ROS) as well as elevated Ca2+ levels and reduced mitochondrial reductase activity was detected in circulating endothelial colony-forming cells (47). Recent evidence suggests that mitochondrial dysfunction upregulates *RNF213* (**Figure 2**). Genetic ablation of several mitochondrial matrix factors such as the peptidase ClpP, the transcription factor Tfam increased *Rnf213* expression in various organs in mice (48). *RNF213* is also upregulated by poly(I:C), which triggers toll like receptor 3 (TLR3)-mediated responses to double-stranded RNA (dsRNA) toxicity (48) (**Figure 2**). Since dysfunctional mitochondria were recently reported to release immune-stimulatory dsRNA into the cytosol, RNA-dependent inflammation initiated by mitochondrial dysfunction or infections may increase the penetrance of patient mutations in *RNF213*. *SAMHD1* loss of function mutations is known to cause inflammatory vasculopathy including moyamoya angiopathy. It is noteworthy that SAMHD1 works as a sensor of dsRNA and dsDNA at replication fork (49), and dysfunction of this gene is associated with excess interferon (IFN) production and senescence associated secretory phenotype (SASP). Tocilizumab,

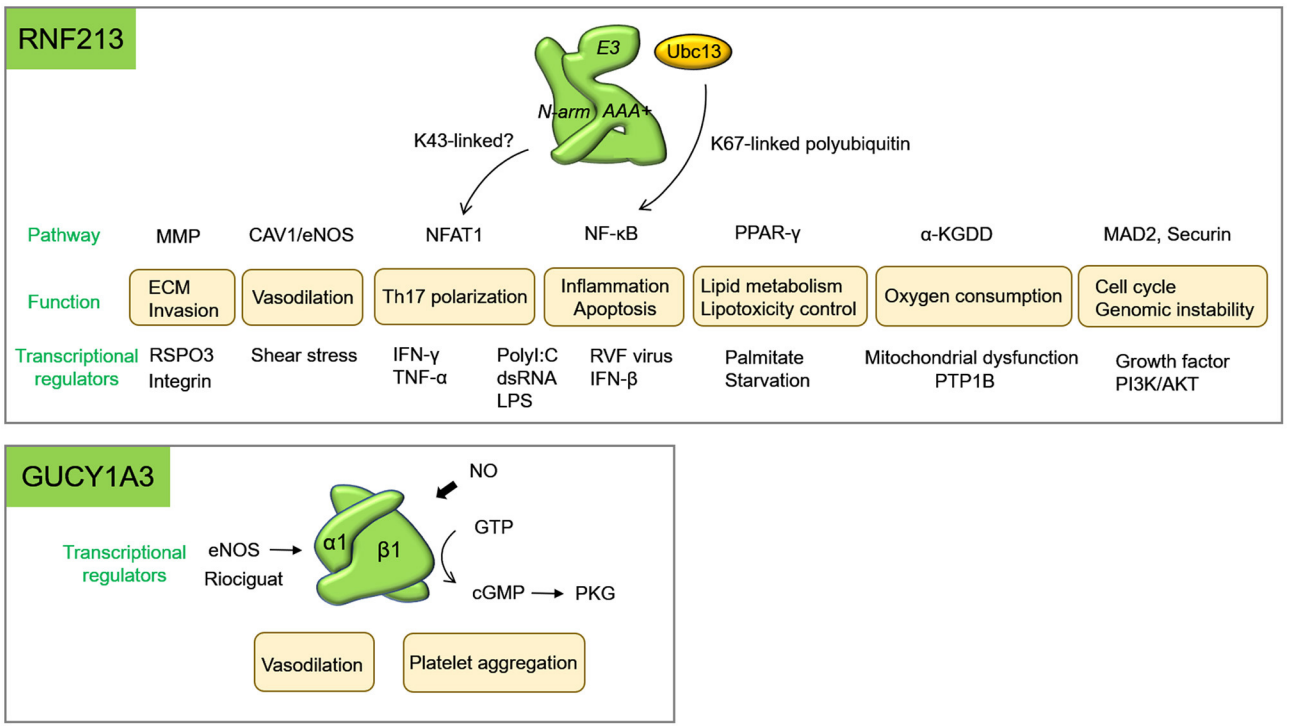

FIGURE 2 | Diverse biological functions of RNF213 and GUCY1A3. Biological functions and their related molecules were shown. Transcriptional regulators of RNF213 for each function were listed below. α-KGDDs represents α-Ketoglutarate-dependent dioxygenases; ECM, extracellular matrix; PDE, phosphodiesterase; RVF, Rift Valley fever.

an interleukin (IL)-6 antagonist, was effective to reverse cerebral vasculopathy in a patient with homozygous *SAMHD1* mutation (50). Therefore, regulation of inflammatory signals may be effective for *RNF213*-related disease as well.

In endothelial cells, *RNF213* is also upregulated by type I IFN (51), and the combination of IFN-γ and tumor necrosis factor (TNF)-α synergistically activated transcription of *RNF213* both *in vitro* and *in vivo* (52) (**Figure 2**). The transcription of *RNF213* was regulated by phosphatidylinositol-3 kinase (PI3K)/AKT and dsRNA-dependent protein kinase R (PKR) pathways. Key et al. also showed that PKR inhibitor inhibited *RNF213* expression; however, the effect was only seen in neuronal cell lines but not in fibroblast or HUVEC (48). These findings suggest that transcriptional regulation of *RNF213* seems to be cell type- and context-dependent. In macrophage or adipocyte, another inflammatory component, lipopolysaccharide (LPS), was shown to upregulate *RNF213*, while peroxisome proliferator-activated receptor γ (PPARγ), an anti-inflammatory signal, downregulated it (53). In adipocyte, PTP1B mediated higher expression of *RNF213* by TNF-α, while PTP1B suppresses E3 ligase activity of RNF213 in a breast cancer cell line under hypoxic condition (54) (**Supplementary Figure 1**). Again, regulation of *RNF213* was cell-type and context-dependent.

Transcriptional Regulation of *GUCY1A3*

As compared with *RNF213*, transcriptional regulation of *GUCY1A3* is not well-characterized. Investigation of the promoter activity of *GUCY1A3* identified several consensus sequences including NFAT and NF-κB (p50) (55). Because both NFAT and NFκB are master regulators of inflammation, *GUCY1A3* may also be regulated by inflammatory signals as is the case with *RNF213*. Another regulator of *GUCY1A3* is eNOS. It has been reported that *GUCY1A3* is upregulated by eNOS in the pulmonary vasculature (56). Upon chemical stimulation of sGC, Zinc finger E box-binding homeobox 1 transcription factor (ZEB1) binds to the promoter region of GUCY1A3 (57).

FUNCTIONS OF RNF213 AND SGC IN THE VASCULAR WALL AND CIRCULATION

Histopathological Features of MMD

Histopathological features of MMD were intimal fibrous thickening without significant inflammatory cell infiltration or lipids, the tortuous internal elastic lamina, and disrupted ECs (58, 59). VSMC proliferation was seen in the internal carotid artery, whereas it was atrophic in the distal part of the lenticulostriate arteries (59). Moth-eaten change of the cells with increased extracellular matrix (ECM) was observed in the media, suggesting irregular atrophy and damage of the smooth muscle cells. We will discuss a possible link between these histopathological characteristics and the molecular functions of RNF213 and sGC.

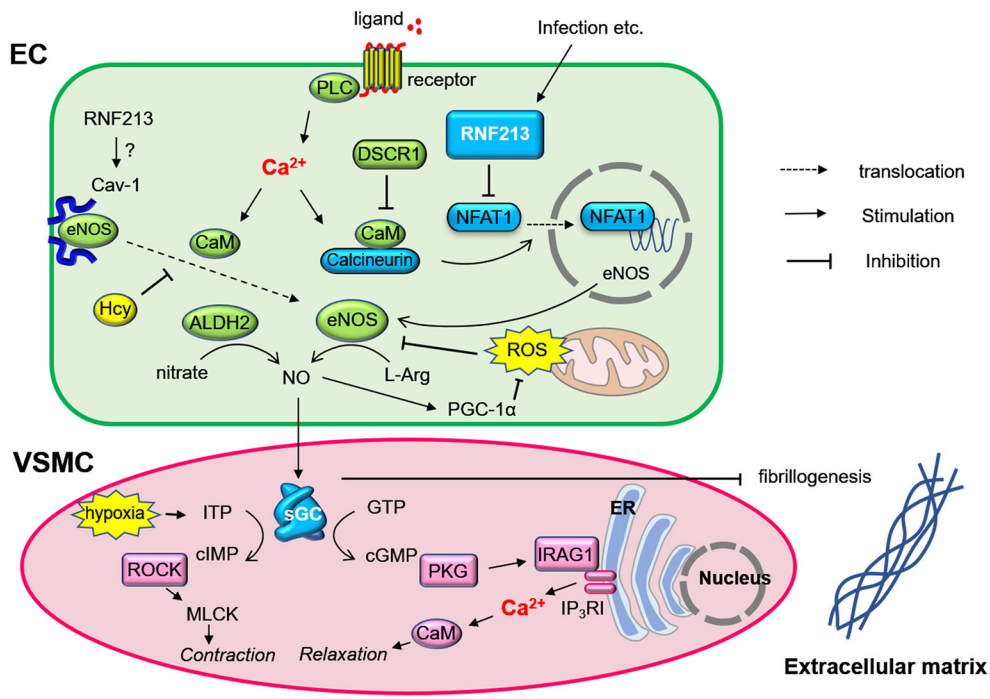

FIGURE 3 | Regulation of nitric oxide signaling by RNF213 (hypothetical role) and GUCY1A3 (sGC) in endothelial cells and vascular smooth muscle cells. Endothelial cell stimulation induces calcium-calmodulin-calcineurin signaling, which produces eNOS via NFAT1. RNF213 degrades NFAT1, which may regulate eNOS production. NO is synthesized from L-Arginin by eNOS and diffuses to vascular smooth muscle cells. NO is catalyzed by soluble guanylate cyclase (sGC), and cIMP or cGMP transmit signaling to induce vasodilatation. sGC also contributes to prohibiting fibrinogenesis. Many molecules known to be associated with moyamoya disease such as caveolin-1 (CAV1), Down Syndrome Critical Region 1 (DSCR1), inositol 1,4,5-Triphosphate Receptor Associated 1 (IRAG1) is involved in this molecular network. ALDH2, aldehyde dehydrogenase 2; CaM, calmodulin; cIMP, Inosine 3', 5'-cyclic monophosphosphate; EC, endothelial cell; Hcy, homocysteine; ITP, inosine triphosphate; L-Arg, L-Arginin; MLCK, myosin light-chain kinase; PKD, protein kinase G; PGC-1α, PPARγ-coactivator-1α; PLC, phospholipase C; ROCK, Rho kinase; ROS, reactive oxygen species; VSMC, vascular smooth muscle cell.

Functions of RNF213 in ECs and VSMCs

Effect of the p.R4180K mutation in endothelial cells were first reported by Hitomi et al. who compared iPS-derived ECs from patients with MMD and unaffected carrier with the p.R4810K mutation and control individuals with wild type *RNF213*. Angiogenic ability (tube formation) of iPS-ECs from patients and mutation carriers were lower than those from wild-type subjects (60). Gene expression profiles showed that Securin was down-regulated, and knock-down of Securin also impaired the angiogenic activity. *RNF213* p.D4013N, p.R4019C, and p.V4146A variants transfected into human umbilical vein endothelial cells had significantly decreased migratory abilities (61).

Effect of the p.R4810K in VSMCs were reported by Tokairin et al. By using VSMCs from iPS-derived neural crest stem cells, they showed that there was no significant difference in VSMC markers, cellular proliferation, migration, or contractile abilities between patients with the p.R4810K mutation and controls (62). Gene expression patterns of iPS-derived VSMCs were similar between patients and controls, whereas iPS-derived ECs displayed distinct patterns. Therefore, they speculated that pathological traits can be driven by naïve ECs predominantly and that VSMCs may require specific environmental factors. In agreement with this concept, RNF213 deficiency affected the cell growth of ECs but not VSMC or fibroblast (52). Likewise, peripheral blood-derived endothelial colony-forming cells but not smooth muscle progenitor cells were responsible for disturbed angiogenic activity when these cells from patients with MMD and healthy controls were co-cultured (63). Taken together, ECs is more likely to contributed to the pathogenesis of MMD, but this model cannot explain the damaged VSMCs as observed in patients with MMD. Therefore, there remains the possibility that even minimum changes in VSMCs caused by RNF213 mutation may affect the phenotype in long-term observation because MMD is a chronically progressive disease.

Although fibrous thickening is a distinguished pathological feature of affected arteries of patients with MMD, little is known about fibrosis in MMD. Hamauchi et al. showed that extracellular matrix (ECM) receptor-related genes, including integrin β3, were significantly downregulated in iPS-derived ECs from patients with the p.R4810K mutation (64). Masuo et al. investigated extracellular matrix secreted from ECs. They showed that iPS-derived ECs from patients with MMD produces less chondroitin sulfate as compared with those from controls (65). Because different types of chondroitin sulfate act differently on fibrillogenesis (e.g., versican enhances fibrillogenesis, while

aggrecan, decorin, and lumican have the opposite effect), it remains unclear whether the reduced amount of chondroitin sulfate is associated with increased fibrosis in patients with MMD. Perivascular adipose tissue (66–68) is a well-known source of fibrous tissue in vascular disease, but it is scarce in the intracranial arteries. Thus, VSMCs and their dedifferentiated form are postulated to be responsible for fibrosis, and ECs may have some role in regulating fibrillogenesis of VSMCs via secretion of MMPs and ECM.

Functions of sGC in ECs, VSMCs, and Platelets

In contrast to the major role of RNF213 in ECs, sGC plays an important role in VSMC relaxation and inhibition of platelet aggregation (69). NO-driven cGMP production exerts an anti-atherogenic effects, including vasodilatation, inhibition of vascular smooth muscle proliferation, blockade of leukocyte recruitment, and anti-platelet activity. Although functions of *GUCY1A3* mutation were not tested in VSMCs from patients with MMD, influence of sGC dysfunction in aortic or pulmonary smooth muscle cells have been well-studied. A single nucleotide polymorphism in *GUCY1A3*, rs7692387, was associated with coronary artery disease at genome-wide significance, and it interferes with binding of the transcription factor ZEB1 and impairs *GUCY1A3* expression, leading to lower sGC levels and lower sGC activity after stimulation (57). Accordingly, inhibitory effects of sGC stimulator on VSMC migration were abolished in homozygous risk allele carriers. In agreement with these human data, lower *Gucy1a3* expression correlated with more aortic atherosclerosis in a population of genetically diverse mice (70). Interestingly, sGC stimulation also has an anti-fibrotic effect (71). Therefore, dysfunctional mutations in *GUCY1A3* may be a cause of fibrous thickening of intima in moyamoya angiopathy. *In vitro* and *in vivo* models of moyamoya angiopathy having the *GUCY1A3* mutation should be developed and investigated.

NO and sGC are generally recognized as major regulators of platelet functions (72) (**Figure 2**). NO mediates inhibition of collagen-induced platelet aggregation and secretion via sGC. Hervé et al. compared platelet function between patients with moyamoya angiopathy with *GUCY1A3* mutation and control individuals. Bleeding time and platelet count was not different, but inhibition of collagen-induced platelet aggregation and secretion by NO donor (PROLI NONOate or sodium nitroprusside) was significantly impaired in platelets derived from patients (11). The risk allele of coronary artery disease, rs7692387 in *GUCY1A3*, also impaired an inhibitory effect of platelet aggregation by NO donor. In agreement with human data, loss of the α1 subunit of sGC in mice leads to enhanced thrombus formation (13).

Functions of sGC are mostly studied in VSMCs and platelets, but sGC also plays a role in ECs. Pyriochou et al. demonstrated that overexpression of sGC promotes EC proliferation, migration, and tube-like network formation (73). Interestingly, pharmacological stimulation of PKG, a downstream signaling molecule of sGC-cGMP, actually have effects on ECs, VSMCs and platelets. It reduces neointimal hyperplasia, inhibits platelet aggregation, and facilitates re- endothelialization (74).

FUNCTIONS OF RNF213 AND SGC IN VASCULAR INSULTS

Vascular Insults Associated With MMD

As discussed above in the section of transcriptional regulation, *RNF213* is upregulated by mitochondrial damage, LPS, poly I:C or type I IFNs, and *GUCY1A3* is regulated by NF-κB and NFAT. This suggests that vascular insults such as infection or hypoxia should be associated with moyamoya angiopathy. In fact, recent evidences suggest that dyslipidemia, an elevated level of homocysteine and viral or bacterial infection have an impact on the progression of MMD. Therefore, it is important to clarify the functions of RNF213 and sGC in association with these vascular insults.

Dyslipidemia and Lipotoxicity

Association of lipid metabolism and MMD has been reported very recently. Ge et al. reported that the HDL cholesterol level was inversely associated with the risk of MMD (75). Church et al. reported that dyslipidemia was a risk factor for contralateral progression in patients with unilateral MMD (76). Hirano et al. also reported that dyslipidemia was associated with symptomization of asymptomatic patients with MMD (77). These findings suggest that lipid metabolism may be involved in the pathogenesis of MMD, although neither study showed direct association between RNF213 mutations and dyslipidemia.

In this context, functions of RNF213 in lipid metabolism are of particular interest (**Supplementary Figure 1**). Strikingly, it has been reported that RNF213 accelerate triglyceride accumulation in lipid droplet (LD) by eliminating adipose triglyceride lipase (ATGL) from LD, one of the major lipolytic molecule of triglyceride (42). The authors showed that the ubiquitin ligase and ATPase activities of RNF213 were both important for its proper LD targeting and its fat-stabilizing activity. Specifically, localization of RNF213 on lipid droplet and its fat-stabilizing activity were disturbed when Caucasian cysteine/histidine mutations (e.g., p.C3997Y) in the RING finger domain or mutations in the AAA+ ATPase domain were introduced. Of note, the p.D4013N mutation in the RING domain and p.R4810K in the E3 core at the C-terminal region, which are mutations found in familial MMD, did not alter the functions of RNF213. This may partly explain the mechanism of low penetrance of the p.D4013N and p.R4810K mutations and high penetrance of the cysteine/histidine mutations.

Lipotoxicity represents toxic effects by saturated fatty acid such as palmitate or stearate, and lipotoxic effect of palmitate on β cells in pancreas can be a cause of diabetes mellitus. It has been reported that ablation of RNF213 decreased lipotoxicity by palmitate (78). Lipid droplet itself does not exert lipotoxicity (79), while it reduces lipotoxicity through incorporating saturated fatty acid inside (80). In fact, RNF213 deficiency increases the activity of SCD1, a key enzyme that promotes palmitate detoxification and storage into triglycerides (78) (**Supplementary Figure 1**). Consistent with the protective effect against lipotoxicity, ablation

of *Rnf213* recovered insulin levels in Akita mouse, a diabetes model with impaired insulin production, and it improved glucose tolerance by protecting islet β cells (81). In patients with MMD, the p.R4810K mutation was inversely associated with diabetes mellitus (odds ratio = 0.35; 95% confidence interval, 0.18–0.68) (82). Inverse association of p.R4810K with diabetes was also found in patients with coronary artery disease (odds ratio = 0.34; 95% confidence interval, 0.12–0.79) (7). In respect of protection from diabetes, the p.R4810K is considered to have loss-of-function or dominant negative properties.

Vascular Insults by Homocysteine

Hypehomocysteinuria has been recognized as a cause of quasi-MMD, and recent study showed that homocysteine level is associated with an increased risk of MMD, especially for unilateral MMD (75). Duan et al. showed that rs9651118 in *Methylenetetrahydrofolate reductase* (*MTHFR*) gene and rs9651118 in *Transcobalamin 2* (*TCN2*) gene was associated with a homocysteine level, and they were also associated with a risk of MMD (21). Alcohol intake or folate deficiency is associated with increased levels of homocysteine (**Supplementary Figure 1**), and it has recently been reported that daily alcohol drinking was an independent risk factor for contralateral progression of unilateral MMD and daily drinker with the p.R4810K mutation had significantly higher risk of progression (83). Homocysteine decreased the expression of *CAV1* in coronary artery ECs, which induced translocation of NO from caveolae to non-caveolae fractions (**Figure 3**). As a result, homocysteine impairs NO release from ECs (84), leading to vascular dysfunction. Homocysteine has been shown to induce ER stress and apoptosis in a variety of cell types. Jeong et al. demonstrate that pharmacological inhibition of sGC almost completely abolished the protective effects of NO and nitrite, whereas pharmacological elevation of cellular cGMP, mimicked the protective action of NO donors in neurons (85). Thus, NO donors inhibit homocysteine-induced ER stress and apoptosis via NO-sGC-cGMP signaling pathway. In relation to the signaling of RNF213, Hcy upregulates *PTP1B* (86).

Viral and Bacterial Infection

Infection has been postulated as a risk factor for MMD, although there is no solid evidence to prove it. Quasi-MMD associated with meningitis was reviewed by Mikami et al. It was estimated to be 2.2% of all the quasi-MMD (87), and it was caused by various pathogens. Among them, varicella zoster virus infection draws attention due to the high frequency of reversible arteriopathy called transient cerebral arteriopathy (TCA) or focal cerebral arteriopathy (FCA), which sometimes progresses to moyamoya (88). Pathological examination of postinfectious vasculopathy with progression to moyamoya angiopathy following Streptococcus pneumoniae meningitis was reported by Czartoski et al., and they showed that inflammation or atherogenic features were absent in the lesion. Due to the progressive course, elevated anti-β2-glicoprotein 1 IgG titers, and transient response to immunomodulatory therapy, they speculated that the vasculopathy was likely to be mediated by an autoimmune process (89).

Involvement of RNF213 in viral infection has just recently been reported. Homozygous deletion of *Rnf213* showed significantly shorter survival in C57BL/6 J mice lethally infected with Rift Valley fever virus (90), an enveloped negative single stranded RNA viruses. The mechanism of entry is dynamin-dependent, CAV1-mediated endocytosis (91). Because Rift Valley fever virus infection suppresses the response of IFN-β or other IFN-related molecules (92, 93), type-I IFN mediated upregulation of *Rnf213* is not likely to be the mechanism of action against the virus. More recently, it has been shown that RNF213 restricts the proliferation of cytosolic Salmonella and is essential for the generation of the bacterial ubiquitin coat, which initiates antibacterial autophagy (43). The ubiquitylation of LPS on Salmonella requires the AAA+ ATPase domain and newly identified NFX1-type zinc finger domain (ZF2 in **Figure 1**). Together with the fact that *RNF213* is upregulated by LPS, RNF213 would contribute to antibacterial immune reactions. However, no patient mutation tested in the study including p.R4810K mutation affected the ubiquitination of LPS.

OTHER PROPERTIES

Inflammation *via* NF-κB Signaling

RNF213 protects cells from ER stress and inflammation by lipotoxicity. Depletion of RNF213 stabilizes ER stress gene expression, normalizes the cellular lipidome, and blocks NF-κB pathway during palmitate exposure (78). Recent studies showed that RNF213 selectively cooperates with Ubc13 (E2 enzyme) to generate K63-linked polyubiquitin chains, but not K48-linked ones (39, 94). K63 linkages are known to regulate activation of the NF-κB transcription factor, DNA repair, innate immune responses, clearance of damaged mitochondria, and protein sorting (38). Interestingly, BRCC3, whose deletion was associated with X-linked syndromic moyamoya, is a E3 ligase that specifically cleaves K63-linked polyubiquitin chains (31). This molecule regulates the abundance of these polyubiquitin chains in chromatin and plays a role in the DNA damage response.

Importantly, most mutations in the RING domain found in patients with MMD reduced E3 ligase activity and many of them induced NF-κB activation (39). These mutations that induce NF-κB activation included not only Caucasian cysteine/histidine mutations but also proline mutations (p.P4007R in a Chinese patient and P4033L in a Caucasian patient). These mutations also induced apoptosis in a NF-κB dependent manner. However, p.D4013N mutation did not affect either E3 ligase activity or NF-κB activation. Importantly, critical point mutations in both the Walker A and B of the AAA domains, completely abrogated NF-κB activation by *RNF213* mutation in the RING domain. Thus, inflammation via NF-κB pathway was enhanced by patient mutations in *RNF213*, while it was suppressed in the absence of *RNF213*. In respect of NF-κB activation, *RNF213* mutations should have gain of function properties.

RNF213 also controls mitochondrial functions, cell cycle, or differentiation and maturation of immune cells (**Figure 2**, details are described in **Supplementary Material**). These properties will also play a role in the development of MMD.

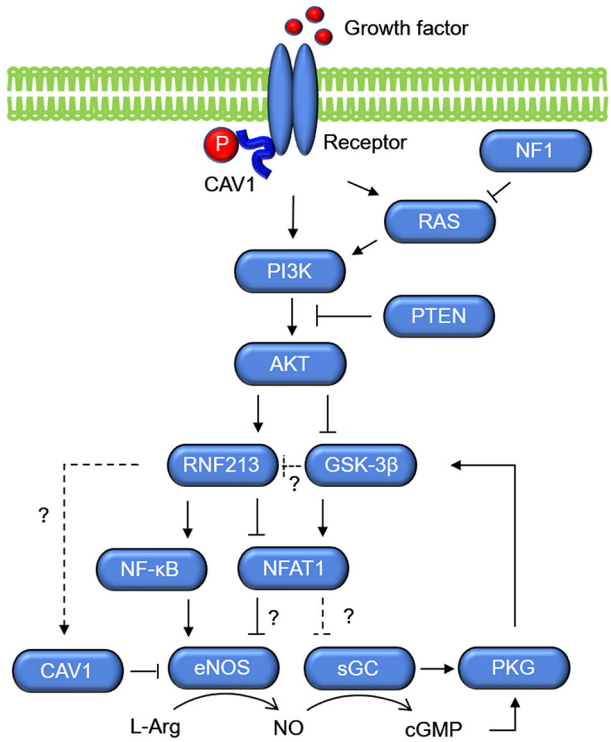

FIGURE 4 | Molecular networks of RNF213 and GUCY1A3 (sGC). Growth factors activate PI3K/AKT, which upregulate the expression of RNF213 and eNOS. Activation of PI3K/AKT leads to inactivation of GSK-3β, which increases degradation of NFAT1 by the proteasome possibly via ubiquitination by RNF213. As opposed to the Ca+/Calmodulin/NFAT1 signaling shown in **Figure 3**, NFAT1 is assumed to suppress eNOS expression in the context of PI3K/AKT. GSK-3β, glycogen synthase kinase-3β; PKG, protein kinase G; sGC, soluble guanylate cyclase.

POTENTIAL MECHANISM IN MMD

Possible Interaction of RNF213 and sGC

Molecular networks that potentially connects RNF213 and sGC are shown in **Figure 4**. The key molecule is NFAT1, which is a ubiquitin target of RNF213 downstream of non-canonical WNT/Ca^{2+} signaling (40). Activation of calcineurin/NFAT signaling by VEGF in human endothelial progenitor cells leads to increased eNOS protein expression and NO production (95). NFAT may also regulate the expression of sGC through binding of NFAT1 to the consensus sequence in GUCY1A3 (55). Activation of PI3K/AKT leads to inactivation of glycogen synthase kinase (GSK)-3β, which induces degradation of NFAT1 by the proteasome (96). Because PI3K/AKT was reported to be an upstream regulator of *RNF213* expression in ECs (52), the effect of PI3K/AKT on NFAT1 may be mediated by RNF213. Upregulation of *NFAT1* is also mediated by S-nitrosylation of RNF213 (97). S-nitrosylation is post-translational modification adding a nitrosyl group to the reactive thiol group of a cysteine to form S-nitrosothiol, which is a key mechanism in transferring NO-mediated signals. S-nitrosylation of ubiquitin ligase leads to its auto-ubiquitination and, as a consequence, increases its substrate levels. NFAT is, in turn, regulated by sGC (98) via activation of PKG, which phosphorylates GSK-3β.

Another key molecule that regulates NO signaling is caveolin. The integral membrane protein caveolin-1 (CAV1) is ∼21–24 kDa and is found primarily in 50–100-nm flask-shaped invaginations called plasma membrane caveolae, where it acts as a scaffold to organize multiple molecular complexes that regulate a variety of cellular events. CAV1 is regulated by a signal mediated through Ca^{2+}/calcineurin/NFAT (99). Importantly, CAV1 was reported to be associated not only with MMD, but also with coronary artery disease and pulmonary artery hypertension. The function of CAV1 is well-characterized in pulmonary artery hypertension (100–102). CAV1 level was decreased in patients with MMD as compared with either patients with intracranial atherosclerotic stroke or healthy subjects, and it was markedly decreased in *RNF213* R4810K variant carriers (103). However, it remains unclear whether RNF213 has direct, e.g., CAV1 is a target of ubiquitination by RNF213, or indirect association with CAV1. CAV1 is associated with eNOS release (**Figure 4**). NO is generated by eNOS release and it is metabolized by sGC. Direct binding of eNOS to the scaffolding domain of CAV1 is a well-accepted mechanism for inactivating eNOS (104). The absence of CAV1 is thought to promote eNOS dysfunction associated with cerebrovascular disease.

Potential Mechanism of MMD

Based on the molecular functions and networks of RNF213 and sGC encoded by GUCY1A3 as discussed above, we propose a potential mechanism in MMD as shown in **Figure 3**. Under the physiological condition, EC stimulation by growth factors or shear stress induces Ca^{2+}-calmodulin signaling. Calmodulin accelerates eNOS dissociation from CAV1 and upregulates eNOS production via Calcineurin/NAFT1. RNF213, which degrades NFAT1 through ubiquitin proteasome system, is not activated without pathological stimulations. eNOS then produces NO from L-Arginine, and NO diffuses into VSMCs. In VSMCs, NO activates sGC to produce cGMP. cGMP then activates PKG, which induces relaxation of VSMCs. Under the pathological condition where viral infection causes mitochondria destruction, *RNF213* is upregulated and prohibits eNOS production by degrading NFAT1. Patient mutations in *RNF213* may sustain the inflammation even after resolution of the infection, and it causes sustained cGMP signaling impairment. The same situation can be caused by *GUCY1A3* mutations. Impaired cGMP signaling leads to VSMC proliferation, impaired vasodilation, and fibrosis as well as endothelial dysfunction. These events will account for intimal hyperplasia with fibrous thickening, a typical pathological feature of MMD. Because impaired cGMP signaling can cause VSMC dedifferentiation and endothelial-to-mesenchymal transition, these cells can be potential sources of fibrosis.

Impaired protection from vascular insults is also a potential mechanism of arterial stenosis in MMD. As mentioned in the previous section, sGC acts protective against homocysteine, and RNF213 regulates lipotoxicity and has antiviral and antibacterial properties. Viral or bacterial infection causes type I IFN production and mitochondrial dysfunction,

and they increase the *RNF213* expression. When patient mutations induce dysfunction of RNF213 or sGC, vascular damage caused by homocysteine, dyslipidemia or infection may be amplified, leading to chronic inflammation. *RNF213* mutations also induce inflammation via NF-κB. This may cause damaged VSMCs, which is another typical pathological feature of MMD.

In terms of inflammation, autoimmune conditions with Th17+ T cell polarization seem to be associated with MMD as discussed in the **Supplementary Material**. However, the effect of *RNF213* and *GUCY1A3* mutations have been studied mostly in vascular cells (ECs and VSMCs) but not in immune cells. To understand the precise mechanism of MMD, investigation of functions of RNF213 and GUCY1A3 in immune cells especially T cell, B cell, neutrophil and dendritic cell will be needed.

Potential Therapeutic Strategy for MMD

Based on these potential mechanisms, we propose several therapeutic strategies that includes lipid and homocysteine regulation (Mediterranean or Japanese traditional diet, restriction of alcohol intake, and lipid/homocysteine lowering drugs), control of inflammation (avoidance of hypoxic condition and anti-inflammatory drugs), and pharmacological stimulation of eNOS-sGC-cGMP pathway. Candidates for pharmacological treatment of MMD would be anti-inflammatory drugs (e.g., COX-2 inhibitor or anti-IL-6 antibody), lipid lowering (e.g., statins or PCSK9 inhibitor) and homocysteine lowering drugs (e.g., folate or vitamin B12). Riociguat, a stimulator for sGC that is used to treat pulmonary artery hypertension, may be another option. However, there is not enough evidence that proves functionality and interaction of the candidate molecules, which account for the phenotypes of MMD. More precise molecular networks should be identified to develop a new treatment strategy.

CONCLUSIONS

In 10 years after identification of *RNF213* as a susceptibility gene for MMD, function of RNF213 has been gradually unraveled. It plays a key role in lipid metabolism, oxygen consumption, cell cycle control and inflammation, and it contributes to the maintenance of vascular cells. GUCY1A3 is a regulator of platelet function and VSMC contraction via NO-sGC-cGMP pathway. Both mutations in *RNF213* and *GUCY1A3* cause not only MMD, but also non-moyamoya intracranial arterial diseases, coronary artery disease, and pulmonary artery hypertension. They have significant interaction with CAV1 and NFAT1, both of which have diverse molecular functions including immune regulation and cell cycle control. Functions of RNF213 and GUCY1A3 in ECs and VSMCs have been well-studied, but a precise mechanism of intimal thickening, fibrosis and its origin remain unresolved. Involvement of other vascular components such as inflammatory cells, platelet and extracellular matrix need to be further investigated.

AUTHOR CONTRIBUTIONS

YM conceived and designed the study, performed the literature search, and drafted the paper. SM reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.687088/full#supplementary-material

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REVIEW
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Pathophysiology of Vascular Stenosis and Remodeling in Moyamoya Disease

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Moyamoya disease (MMD) and moyamoya syndrome (MMS) are progressive vascular pathologies unique to the cerebrovasculature that are important causes of stroke in both children and adults. The natural history of MMD is characterized by primary progressive stenosis of the supraclinoid internal carotid artery, followed by the formation of fragile collateral vascular networks. In MMS, stenosis and collateralization occur in patients with an associated disease or condition. The pathological features of the stenosis associated with MMD include neointimal hyperplasia, disruption of the internal elastic lamina, and medial attenuation, which ultimately lead to progressive decreases in both luminal and external arterial diameter. Several molecular pathways have been implicated in the pathophysiology of stenosis in MMD with functions in cellular proliferation and migration, extracellular matrix remodeling, apoptosis, and vascular inflammation. Importantly, several of these molecular pathways overlap with those known to contribute to diseases of systemic arterial stenosis, such as atherosclerosis and fibromuscular dysplasia (FMD). Despite these possible shared mechanisms of stenosis, the contrast of MMD with other stenotic pathologies highlights the central questions underlying its pathogenesis. These questions include why the stenosis that is associated with MMD occurs in such a specific and limited anatomic location and what process initiates this stenosis. Further investigation of these questions is critical to developing an understanding of MMD that may lead to disease-modifying medical therapies. This review may be of interest to scientists, neurosurgeons, and neurologists involved in both moyamoya research and treatment and provides a review of pathophysiologic processes relevant to diseases of arterial stenosis on a broader scale.

Keywords: angiopathy, cerebrovascular, moyamoya, stenosis, stroke

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INTRODUCTION

Moyamoya disease (MMD) and moyamoya syndrome (MMS) are cerebrovascular pathologies that are characterized by progressive stenosis and eventual occlusion of the intracranial internal carotid artery (ICA) and its proximal middle cerebral artery (MCA) and anterior cerebral artery (ACA) branches. This stenosis and occlusion cause ischemia and subsequent small vessel collateralization of the brain parenchyma that is supplied by the affected arterial segments. On cerebral angiography, these collateral vascular networks are visible as regions of contrast blush in the early arterial phase, and this distinctive appearance led to the name "moyamoya," which is Japanese for a puff of smoke (1).

When the term moyamoya is used alone, it refers to the characteristic pathologic findings of stenosis and collateralization. Moyamoya disease refers to primary moyamoya and includes this presentation in individuals who harbor the genetic risk alleles. Moyamoya syndrome refers to moyamoya that occurs in the setting of a known associated disease, condition, or exposure (2). Previously, unilateral moyamoya was classified as MMS, but this categorization has fallen out of favor due to mounting evidence that unilateral pathology frequently progresses to bilateral disease (3–8).

The most common presenting symptom of MMD and MMS is ischemic stroke, which occurs secondary to decreased perfusion downstream from moyamoya stenosis. Additionally, moyamoya frequently presents with transient ischemic attacks (TIAs), which are often recurrent, and less commonly presents with seizures or headaches (9). Intracranial hemorrhage also occurs in MMD and MMS due to the rupture of vessels in fragile collateral networks or associated aneurysms (10), and importantly, hemorrhage occurs as the presenting symptom more frequently as patient age increases (11). In 2018, Funaki et al. demonstrated that choroidal collaterals may be a bleeding source with a high risk for hemorrhagic recurrence and a predictor of recurrence in hemorrhagic MMD (12). Preliminary results obtained in 2019 by Funaki et al. (13) suggest that the presence of choroidal collaterals may affect the risk of *de novo* hemorrhage in nonhemorrhagic hemispheres; however, these results remain subject to verification in larger studies.

Moyamoya disease and MMS are relatively rare and occur with varying incidence in populations throughout the world. Incidence rates are highest in eastern Asia, and specifically Japan, where its incidence is reported at 0.35 per 100,000 individuals (14). By contrast, the overall incidence reported from the United States is 0.09 per 10,000 individuals, with the highest incidence occurring in Asian Americans (0.28/100,000), followed by African Americans (0.13/100,000), Caucasian Americans (0.06/100,000), and Hispanic Americans (0.03/100,000) (15). The age of onset occurs with a bimodal distribution, with an initial peak in the first decade of life and a second peak in the fifth decade, and studies suggest that the incidence in adults may be increasing (14, 16, 17).

Genetic risk factors may be involved in the development of MMD, and this idea is supported by differences in the incidence of MMD in different populations. Further support for inherited risk was provided by familial cases and a high concordance between monozygotic twins (18). However, incomplete penetrance and discordance have led some to suggest the necessity of an environmental "second hit" or possible epigenetic contribution (19, 20). Genetic linkage analysis from sequencing data identified MMD risk alleles and ultimately led to the identification of *RNF213* as the first known susceptibility gene for MMD (21, 22). Despite this progress, the definitive mechanisms through which mutant *RNF213* contributes to the initiation or progression of moyamoya pathology remain elusive. In 2012, Miyawaki et al. (23) performed a case-control study at a single hospital analyzing the *RNF213* variant c.14576G>A in patients with non-MMD intracranial major artery stenosis/occlusion (ICASO). The authors found an association between c.14576G>A and non-MMD ICASO. They therefore advocate screening for the c.14576G>A variant in ICASO patients. In 2013, this group performed a case–control study, in which they investigated the occurrence rate of the c.14576G>A variant in 323 ICASO patients (24). They hypothesized that a subset of patients with distinct ICASO phenotypes may have a common genetic variant, *RNF213* c.14576G>A, suggesting that the *RNF213* c.14576G>A variant may be a high-risk allele for ICASO. In 2015, this group performed a study in 78 MMD patients and nine non-atherosclerotic MMS patients demonstrating the absence of the *RNF213* c.14576G.A genetic variant in nonatherosclerotic MMS patients (25). The research group hypothesized that identification of this genetic variant could be used to help clarify the pathophysiology of vascular stenosis in the terminal ICA and associated moyamoya vessels.

Since MMD was first described in 1957, research efforts have significantly increased our understanding of its epidemiology and pathobiology. Despite this increased knowledge, medical treatments do not reverse or prevent progression of the primary disease process. In addition, definitive therapy with revascularization, although effective in reducing stroke risk and symptoms, has no effect on the progression of arterial stenosis (26, 27). Current moyamoya treatment is hindered by the lack of understanding of both the pathology process that initiates stenosis and the key signaling pathways that drive progression. In this review, we examine the unique arterial stenosis that occurs in moyamoya and explore the current state of knowledge related to its pathological characteristics, its molecular mechanisms, and the imaging methods utilized for its detection.

METHODS

References were identified by use of a comprehensive, systematic computerized literature search from January 1, 1957, through January 20, 2021, performed by the authors, using the PubMed, EMBASE, BIOSIS Previews, and Medline databases and various combinations of the key words "angiopathy," "cerebrovascular," "moyamoya," "stenosis," and "stroke." Relevant articles on MMD and supplemental basic science articles almost exclusively published in English were included. Reference lists of relevant articles were reviewed for additional references, and 165 articles were included in the final manuscript. As some aspects of MMD and MMS have been studied in greater detail compared to others, several topics receive additional attention. In spite of major progress in research in the field of MMD in recent years, the literature in great part remains descriptive. Continued collaborative clinical and basic research is fundamental to further elucidate the pathophysiology of MMD, which may lead to

Abbreviations: ACA, anterior cerebral artery; BOLD, blood oxygen level-dependent; HR-MRI, high-resolution magnetic resonance imaging; ICAD, intracranial atherosclerotic disease; MCA, middle cerebral artery; MMD, moyamoya disease; MMS, moyamoya syndrome; MRA, magnetic resonance angiography; MRI, magnetic resonance imaging; PCA, posterior cerebral artery. For a complete list of abbreviations and gene symbols, see **Supplemental Table 1**.

an increasingly differentiated diagnosis and disease-modifying treatment strategies.

PATHOLOGIC CHARACTERISTICS OF ARTERIAL STENOSIS IN MOYAMOYA

The arterial stenosis of moyamoya is the primary process that sets in motion the cascade of pathological adaptations and complications that encompass MMD and MMS. Isolated stenosis without evidence of collateral vessel formation occurs first and is defined as the first of six stages in the progression of the disease (1). In most moyamoya patients, stenosis occurs in the bilateral proximal portions of the anterior cerebral circulation and involves the terminal supraclinoid ICA and the proximal MCA and ACA (1, 28). The posterior circulation is spared in most moyamoya patients, despite possessing intracranial arteries with similar caliber and proximity to affected segments of the anterior circulation. Nevertheless, involvement of the posterior circulation can occur in moyamoya (29), and studies have demonstrated posterior circulation disease in approximately one-third of pediatric and adult patients (30–33). When present, stenosis of the posterior circulation typically affects the posterior communicating artery and the P2 segment of the posterior cerebral artery (PCA), while sparing the basilar artery and P1 segment of the PCA (34). Thus, posterior involvement is likely an extension of the stenotic process from the anterior circulation and only rarely arises independently from the proximal posterior circulation, as characterized by basilar artery and P1 segment involvement. The unique properties of posterior circulation moyamoya have led investigators to suggest an embryologic basis for susceptibility of the arterial segments to develop moyamoya (34, 35). Importantly, several lines of evidence suggest that the involvement of the posterior circulation in MMD is associated with a poor prognosis (36–38). Stenosis of extracranial vessels in association with intracranial moyamoya is uncommon, but several studies have demonstrated associated stenosis of the renal artery in a minority of patients (39, 40). In addition, a few studies have demonstrated associated stenosis of external carotid artery branches, coronary arteries, and abdominal arteries (41, 42). However, other studies have specifically looked for involvement of branches of the external carotid artery or intraabdominal arteries and have failed to demonstrate stenosis in these segments (40, 43). Despite these exceptions, the restriction of moyamoya stenosis to such a limited and specific anatomic region remains one of the most fundamental questions surrounding its pathology, as other forms of medium to large artery stenosis, such as atherosclerosis and fibromuscular dysplasia (FMD), are typically far more widespread throughout the arterial tree.

The histopathologic characteristics of stenosis in moyamoya represent a collection of pathologic processes that, in addition to the gross pathologic features described above, define moyamoya as a unique form of arterial stenosis. The primary cause of moyamoya stenosis is a concentric fibrocellular hyperplasia of the intima (44, 45). This hyperplasia is characterized by a proliferation of smooth muscle cells and extracellular matrix within the intima that leads to progressive intimal thickening (**Figure 1**) (46–49). In addition, the internal elastic lamina is altered such that it is frequently wavy, duplicated, and sometimes disrupted (45, 50–52). In contrast to the significant intimal thickening that occurs in moyamoya, the tunica media becomes progressively thinner as moyamoya progresses (46). This thinning occurs to the degree that, despite intimal expansion, the outer vessel diameter decreases in association with luminal stenosis (53, 54), and as moyamoya advances over time, the outer diameter progressively decreases (**Figure 1**) (55). In 2016, Yamamoto et al. suggested that MMS may comprise two distinct pathological subgroups, a constrictive subgroup and a nonconstrictive subgroup. Patients in the constrictive subgroup of MMS may show marked constrictive vascular changes and limited intimal hyperplasia. Patients in the non-constrictive subgroup of MMS may show profuse fibrosis (56). Luminal thrombi and microthrombi are frequently observed in autopsy specimens from moyamoya patients and are frequently associated with regions of intimal hyperplasia and stenosis (57). These thrombi contribute to arterial occlusion in moyamoya and are thought to be related to intimal pathology (2, 57).

Like moyamoya, atherosclerosis causes stenosis with intimal expansion and intimal smooth muscle cell hyperplasia. However, atherosclerosis is marked by characteristic inflammatory infiltration and lipid accumulation that do not typically occur in moyamoya (48). Furthermore, as luminal stenosis progresses in atherosclerosis, outward vascular remodeling occurs, with increased outer vessel diameter (58, 59), in contrast to the decreased outer diameter that occurs in moyamoya. Another form of arteriopathy, the intimal fibroplasia subtype of FMD, is characterized by intimal expansion in the absence of inflammatory cell infiltration or lipid accumulation (60, 61). Like moyamoya, it causes intimal extracellular matrix expansion and abnormalities of the internal elastic lamina, but the intimal fibroplasia subtype of FMD is not characterized by hyperplasia of the intima as is seen in moyamoya (62). However, pediatric patients with FMD can also present with stroke and renal artery stenosis (62, 63). These shared pathologic features highlight the need for future research to better understand the complex relationship between these two entities.

VASCULAR REMODELING IN MOYAMOYA DISEASE

The vascular wall pathology in MMD involves thickening of the intima without infiltration of inflammatory cells or considerable disruption of the internal elastic lamina (51, 64). Compared to typical findings in atherosclerosis, macrophage infiltration into the subintimal layer, and lipid pool accumulation are not characteristic features of moyamoya (64, 65). In contrast, a study by Masuda et al. (66) of autopsy specimens obtained from MMD patients showed proliferation of smooth muscle cells, the presence of T cells, and infiltration of macrophages into the vascular wall. In MMS associated with neurofibromatosis type I (NF-1), inflammatory cells may infiltrate the area surrounding the lesion of the arteriopathy, indicating that some types of

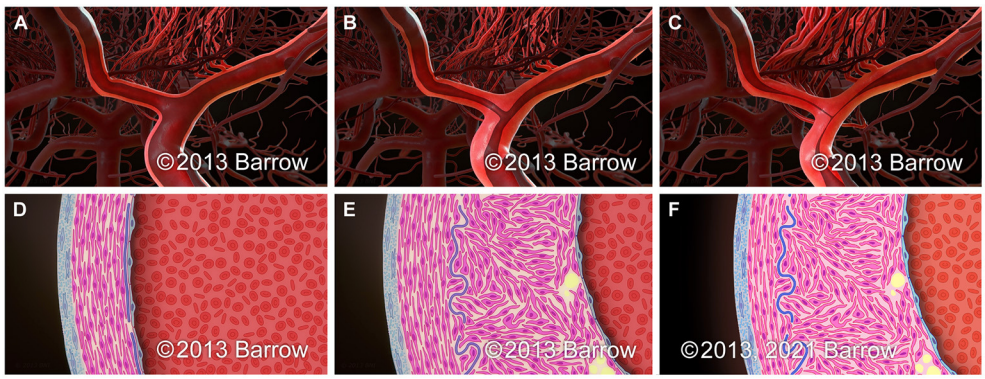

FIGURE 1 | Schematic representation of the paradigmatic progression pattern of arterial stenosis in moyamoya on the macroscopic **(A–C)** and microscopic **(D–F)** scale. **(A,D)** Normal terminal ICA. **(B,E)** Early in the progression of moyamoya, intimal hyperplasia leads to the development of luminal stenosis without a significant change in the outer diameter of the arterial segment. **(C,F)** As luminal stenosis continues to progress in moyamoya, attenuation of the medial layer of the artery leads to a reduction in the outer arterial diameter. *Used with permission from Barrow Neurological Institute, Phoenix, Arizona*.

MMS may be related to inflammation (64, 66). Kaku et al. (54) showed a reduction in both the inner and outer diameter of the ICA in the course of the moyamoya remodeling process, in contrast to the pathogenesis of atherosclerosis, which shows an outward remodeling pattern. These authors also found no reduction of the outer diameter of the circle of Willis in an MMS subgroup, and arterial shrinkage was insignificant (54, 64). Moyamoya vascular shrinkage may be detected specifically in the horizontal MCA (M1) section (64, 67). According to Yamamoto et al. (56), MMS pathology may include two distinct subgroups: a nonconstrictive subgroup as well as a constrictive subgroup. A portion of one subgroup of MMS may show excessive fibrosis. Another subgroup of MMS may show limited intimal hyperplasia and vascular constrictive changes (64). In 2015, Mikami et al. (67) showed an overall reduction in the size of the outer diameter of all vessels around C1 in MMD patients. Similar to a negative constrictive remodeling pattern, the MCA diameter was shown to be significantly smaller in patients with disease progression (67). In 2019, Choi et al. (68) used high-resolution MRI to compare plaque characteristics and the vascular remodeling pattern and hemodynamic changes related to intracranial plaques depending on the presence or absence of the *RNF213* p.Arg4810Lys variant in patients without moyamoya and demonstrated that, in these patients, the *RNF213* variant was associated with smaller outer vessel diameters in the distal ICA, proximal MCA, and basilar artery (68).

MOYAMOYA SYNDROME, PEDIATRIC MOYAMOYA SYMPTOMATOLOGY, AND ASSOCIATED MOLECULAR PATHWAYS

Moyamoya Syndrome

Moyamoya disease is idiopathic and occurs as a primary pathologic process. Conversely, MMS occurs in patients with a known associated condition, disease, or exposure that places the patient at significantly higher risk of developing moyamoya. The most frequent moyamoya-associated conditions are sickle cell disease, Down's syndrome, NF-1, and exposure to cranial radiotherapy, which are present in 10–20% of moyamoya cases (69). Less frequently associated conditions include: Alagille syndrome, Costello syndrome, microcephalic osteodysplastic primordial dwarfism type II, Noonan syndrome, Seckel syndrome, hyperthyroidism, and many others (2, 70–78). Importantly, moyamoya-associated conditions have the potential to inform the investigation into the mechanisms that may be important for the development of moyamoya. The genetic basis of many of these associated conditions is well-understood and, thus, provides important hints as to the importance of various signaling pathways in the development of moyamoya.

Moyamoya Syndrome and Associated Signaling Pathways

By identifying genes involved in MMD pathogenesis and several monogenic MMSs, investigators have associated various molecular pathways with MMD pathophysiology, including the Ras–Raf–mitogen-activated protein kinase (MEK)–extracellular-signal-related kinase (ERK) signaling pathway (NF-1, Noonan syndrome, Costello syndrome), the neurogenic locus notch homolog protein (Notch) signaling pathway (Alagille syndrome), the nitric oxide (NO)-soluble guanylyl cyclase (sGC) signaling pathway [Guanylate cyclase 1 soluble subunit alpha-3 (GUCY1A3)], and pathways involved in genomic stability [Lys-63-specific deubiquitinase BRCC36 [BRCC3], pericentrin [*PCNT*], and other genes involved in dwarfism] (20) (See the **Supplementary Table 1** for definitions of gene symbols, proteins, and additional terminology).

The Ras-Raf-MEK-ERK signaling pathway has been shown to be associated with NF-1 (79, 80). Loss-of-function mutations in *NF1* (17q11.2) lead to absent neurofibromin expression, which in turn may activate the Ras-Raf-MEK-ERK signaling pathway, leading to an increased mitogenic signaling (79, 80). In Noonan syndrome, gain-of-function mutations in *PTPN11* (12q24.13), *NRAS* (1p13.2), *SOS1* (2p22.1), *RAF1* (3p25.2), *BRAF* (7q34),

KRAS (12p12.1), and *MAP2K1* (15q22.31) genes may activate the Ras-Raf-MEK-ERK signaling pathway (20, 81–84).

Costello syndrome is encoded by *HRAS* (11p15.5) and *KRAS* (12p12.1), and gain-of-function mutations influencing p.Gly13 or p.Gly12 may cause germline activation of *HRAS*, and thereby lead to dysregulation of the Ras-Raf-MEK-ERK signaling pathway (20, 85, 86).

Defects in the Notch signaling pathway are associated with vasculopathy. Alagille syndrome is caused by loss-of-function mutations in either *JAG1* (20p12.2) or *NOTCH2* (1p12-p11) (20, 87–96).

Genomic stability signaling pathways are associated with congenital dwarfing syndromes. *PCNT* (21q22.3) loss-of-function mutations are linked to deficient pericentrin cell cycle progression in microcephalic osteodysplastic primordial dwarfism type II. In Seckel syndrome, *CEP63* (3q22.2), *ATR* (3q23), *CENPJ* (13q12.12), *NIN* (14q22.1), *CEP152* (15q21.1), and *RBBP8* (18q11.2) are associated with cycle cell progression, centrosomal function, and DNA repair (20, 71, 97–99).

BRCC3/MTCP1 is associated with MMD pathogenesis. *BRCC3* (Xq28) is potentially involved in DNA repair alteration. Absent BRCC3 expression inhibits angiogenesis of trunk intersegmental vessels in the zebrafish. MTCP1 may enhance phosphorylation and activation of *AKT1* and *AKT2* (20, 100–102).

The nitric oxide-soluble guanylyl cyclase-cyclic guanosine monophosphate (NO-sGC-cGMP) signaling pathway is a key signal transduction pathway associated with controlling vascular smooth-muscle relaxation, vascular tone, and vascular remodeling. It is hypothesized that homozygous loss-of-function mutations in *GUCY1A3* (4q32.1) may be involved in an alteration of the NO pathway in vascular smooth muscle cells (SMCs), potentially leading to aberrant vascular remodeling at bifurcation and curvature regions, such as the ICA bifurcation (20, 103).

Moyamoya Syndrome and Associated Autoimmune and Inflammatory Diseases

Moyamoya Syndrome in association with inflammation occurs with a frequency of 0.54–1.5% in pediatric MMD patients compared to a frequency of 1.7–4.7% in adult MMD patients (64, 104). In 1983, Suzuki et al. (105) analyzed the incidence of infections in MMD patients. They found that 61.6% of adult MMD patients and 82.6% of pediatric MMD patients suffered from infections of the face and head including both infections of unknown origin and maxillary sinusitis, otitis media, and tonsillitis (64). According to the Japanese national survey by Hayashi and colleagues (106), diseases other than atherosclerosis constitute 17.2% of all inflammatory diseases associated with MMS. For common autoimmune disorders related to MMS, meningitis constitutes 2.2%, hyperthyroidism constitutes 7.5%, and other autoimmune disorders constitute 17.2%. Less common autoimmune disorders associated with MMS may include polyarteritis nodosa, dermatomyositis, Addison's disease, Sjögren's syndrome, Kawasaki's disease, acute limbic encephalitis with anti-LGI1 antibody, antiphospholipid antibody syndrome, granulomatosis with polyangiitis, myasthenia gravis, systemic lupus erythematosus, multiple sclerosis, systemic sclerosis, primary systemic vasculitis, rheumatoid arthritis, polymyositis, and thyroiditis (64, 104, 106, 107). It may be difficult to distinguish the mechanisms associated with a distinct autoimmune disorder and from those associated with MMS because of the complexity of the differentiation between causality and correlation (64). In summary, a correlation between chronic systemic inflammation caused by an autoimmune response and MMS may be hypothesized (64).

Moyamoya Syndrome concomitant with hyperthyroidism is seen predominantly in females and may be associated with ischemia, a comparatively advanced age of disease onset, and faster disease progression compared to MMS (64, 108–111). Ischemia may worsen thyroid functioning. Ischemic symptoms caused by thyrotoxicosis were ameliorated after hyperthyroidism was controlled (64, 109). Increased thyroid hormone may cause MMS progression through an increase in vascular sensitivity to the sympathetic nervous system (64, 112). A T-cell-mediated autoimmune response may also advance MMS pathogenesis. Consequently, immunosuppressive treatment before surgery and/or treatment of a hormonal abnormality may be effective in MMS management related to autoimmune disorders (64, 113, 114). Tendler et al. (115) indicated that immunological stimulation in hyperthyroidism and vascular dysregulation and cellular proliferation in MMS may share a pathomechanism involving T-cell dysregulation. This pathomechanism mentioned above involves both vascular morphological alterations and vascular sensitivity (64, 116). In context with morphological alterations, surgical procedures including surgical revascularization may be utilized (64).

Moyamoya Syndrome caused by meningitis is rare, constituting approximately 2.2% of all disorders classified as MMS (64, 106). The associated infection may be caused by various pathogens, including *Hemophilus influenzae* (117, 118), *Streptococcus pneumoniae* (113, 119, 120), β-hemolytic group A *Streptococcus* (121), *Mycobacterium tuberculosis* (122–125), *Propionibacterium acnes* (126), *Leptospira* (127), *Mycoplasma pneumoniae* (128), *Neisseria meningitidis* with cytomegalovirus (129), neurosyphilis with human immunodeficiency virus (HIV) (130), HIV (131–133), Epstein–Barr virus (64, 129), varicella-zoster virus (134), and measles virus (135). Vascular complications related to meningitis usually become clinically manifest during the 2 weeks after disease onset (64, 136, 137). In MMS associated with meningitis, late-onset morphological alteration of the circle of Willis may be observed (113, 117–119, 123, 125). In addition, an increase in autoimmune antibodies may indicate an autoimmune trigger for the onset of MMS (64, 113). Liu et al. (138) found that the cerebrospinal fluid of MMS patients showed an immune response to leptospirosis and that MMS appears to be related to immune reactive vasculitis (64). In 2018, Takahashi et al. reported a case of MMS in familial MMD 9 years after the patient had nonherpetic acute limbic encephalitis (64, 139). The patient tested positive for anti-LGI1 antibodies, and the authors hypothesized that inflammation caused by an autoimmune disease process may have contributed to MMS progression. Consequently, chronic inflammation caused by an autoimmune disease process may lead to MMS in

the course of acute inflammation. In addition, acute systemic inflammation may be related to MMD pathogenesis (64).

Pediatric Moyamoya Symptomatology and Associated Mechanisms

Pediatric MMD patients presenting with headache or fatigue as the primary symptom may be misdiagnosed as having hypochondriasis or as experiencing a developmental deficit, or they may be simply dismissed as being "lazy." Lack of appropriate treatment in these patients may lead to severe ischemic stroke. General practitioners and physicians who do not routinely encounter MMD should be encouraged to consider a diagnosis of MMD in patients presenting with minor symptoms such as headache, fatigue, and school refusal.

Symptoms of MMD may be ascribed to changes in blood flow due to ICA stenosis. Two major etiologic categories of symptoms may be distinguished, those involving the consequences of compensatory mechanisms in response to ischemia, such as headache from dilated transdural collateral vessels and hemorrhage from fragile collateral vessels, and those caused by cerebral ischemia such as transient ischemic attacks, stroke, and seizures. Variations in the degree of arterial involvement, ischemic cortex areas, progression of stenosis, and response to a reduction in blood supply may help to clarify the broad range of symptoms in MMD (2). In Asian populations, 68% of children with MMD present with ischemic strokes or TIAs, and 2.8% present with hemorrhage (2, 140). Most children and adults with MMD in the United States present with symptoms of ischemia, with a rate of hemorrhage of 20.0% among adults compared to a rate of 2.8% among children (2, 140, 141). An increased frequency of completed strokes is evident in pediatric MMD. Presumably, children's inability to accurately report their symptoms may hinder a timely diagnosis and lead to an increased probability of completed strokes (2, 142). The symptoms of cerebral ischemia in patients with MMD are related to the areas of the brain whose blood flow is supplied by the ICAs and MCAs, specifically, the temporal, parietal, and frontal lobes. The predominant symptoms are cognitive impairment, aphasia, dysarthria, and hemiparesis (2, 140). Additional symptoms include syncope, visual deficits, seizures, and personality changes, the latter of which may be easily confused with a psychiatric disorder (2, 143). Common childhood actions, including crying with hyperventilation, may precipitate a stroke or TIA. Signs of cerebral ischemia may be temporary or permanent, and may be caused by induction of anesthesia for surgery or by exertion. A hypothesized mechanism precipitating strokes and TIAs in these patients is that the cortical vasculature, which is assumed to be fully dilated under the conditions of chronic ischemia, may constrict in response to a decrease in the partial pressure of carbon dioxide, this restriction may be caused by hyperventilation, eventually resulting in a reduction of cerebral perfusion (2, 144). Ischemic symptoms may also be precipitated by dehydration (2). Intracranial hemorrhage, which is frequently encountered in adult MMD patients, is equally evident in pediatric MMD patients (2, 140, 145). Intracranial hemorrhage occurs in the subarachnoid space, in the ventricles, and in the brain parenchyma, commonly in the basal ganglia area. In the past, intracranial hemorrhage has been ascribed to the rupture of moyamoya-associated fragile collateral vessels in the course of progressive ICA stenosis (2, 146, 147). An additional cause of hemorrhage in these patients may be alternating circulatory patterns at the base of the brain, which have been implicated in the emergence of intracranial aneurysms; these aneurysms are usually located at the apex of the basilar artery or in the PCA, which are common locations of increased shear stress in MMD (2, 148, 149). Headache is a predominant presenting symptom in MMD patients. In their 2005 review article, Seol at al. hypothesized that dilatation of leptomeningeal and meningeal collateral vessels may lead to the stimulation of dural nociceptors (2, 150). Characteristically, headache is refractory to therapy and resembles migraine in quality; despite effective surgical revascularization, headache may persist in as much as 63% of MMD patients (2, 150). Headache symptoms may abate within 1 year after surgery for MMD in some moyamoya patients, suggesting a potential regression of basal collaterals. Dilated moyamoya-related collateral vessels in the basal ganglia may be associated with the emergence of choreiform movements in pediatric MMD patients (2, 140, 151). In a study of pediatric MMD patients presenting with choreiform movements, 8 of 10 pediatric MMD patients experienced resolution of this symptom 1 year after revascularization surgery, suggesting that this surgical procedure appears to be effective in reducing the number of moyamoya-related collateral vessels in the basal ganglia (2). Another sign of MMD is the "morning glory disk," which is an optic disk enlargement associated with retinovascular aberrations; this sign may be evaluated by vascular imaging (2, 152).

Kawabori et al. (153) studied 29 pediatric MMD patients, hypothesizing that persistent disturbance of cerebral hemodynamics may be associated with severe headache in pediatric MMD. They found that encephalo-duro-myo-arteriopericranial synangiosis and STA-MCA anastomosis may be effective surgical procedures to causally treat headache by supplying collateral blood flow to the operated hemispheres. They advocate close follow-up because of the potential for the development of postoperative headaches in these patients (153). In 2019, Riordan et al. (154) studied 59 pediatric MMD patients who had undergone surgical revascularization by pial synangiosis more than 20 years previously and found that pial synangiosis may confer long-term protection against stroke in pediatric MMD. A history of cranial irradiation was ascertained in one patient with a late stroke and in four out of five deceased patients. The authors conclude that, in the absence of significant preoperative comorbidities and neurological deficits, revascularization surgery appears to be safe and effective (154). In 2019, Kazumata et al. (155) performed a prospective, observational, single-center study in 21 pediatric MMD patients [mean age 10 (3.0) years, range 5–14 years], hypothesizing mild cognitive dysfunction caused by cerebral hypoperfusion through the association of a reduced regional cerebral blood flow (rCBF) in the left dorsolateral prefrontal cortex (DLPFC) and medial frontal cortex with a reduced processing speed index (PSI), perceptual reasoning index (PRI), and full-scale intelligence quotient (FIQ). However, they found that average intellectual

ability was not reduced in pediatric MMD patients. They also found that the angiographic severity of the disease, ischemic symptoms, family history, and patient age at disease onset were not associated with inadequate cognitive performance (155). In 2019, Lee et al. (156) studied 131 surgically treated pediatric MMD patients to evaluate the risk factors and prevalence of hypertension. They demonstrated a high prevalence of hypertension in pediatric MMD patients. Consequently, they suggest that sufficient efforts should be made to achieve BP reduction in pediatric MMD patients to prevent subsequent vascular events (156).

MOYAMOYA IMAGING

From its discovery and definition, moyamoya has been a disease process that is diagnosed and classified based on vascular imaging studies. Recent advances in moyamoya imaging have furthered our understanding of the natural history of arterial stenosis in moyamoya and provide important insights into patient responses to therapeutic interventions.

Moyamoya Imaging Methods and Clinical Evaluation

In 2013, Kim et al. performed a high-resolution MRI (HR-MRI) study in patients with an MCA occlusion stroke that were angiographically confirmed to be 12 patients with MMD and 20 patients with intracranial atherosclerotic disease (ICAD). Consistent with previous pathological reports, the research group showed that MMD may be associated with smaller, concentric occlusive lesions that are rarely enhanced compared to symptomatic ICAD, suggesting that HR-MRI may assist in the evaluation of pathological changes in the vascular wall and in differentiating ICAD from MMD (157). In 2015, Kim et al. performed a case-control study in MMD patients and normal controls using magnetic resonance angiography (MRA) and computational fluid dynamics. The research group demonstrated significant morphological differences in the intracranial-extradural ICA of MMD patients. These differences may affect the hemodynamics around the ICA bifurcation. Whether these hemodynamic changes are the result or a cause of the ICA bifurcation stenosis remains to be elucidated (158).

Ladner et al. (159) proposed a scoring system for the degree of severity of moyamoya, Prior Infarcts, Reactivity, and Angiography in Moyamoya Disease (PIRAMD), which uses a combination of conventional angiography and non-invasive structural and hemodynamic 3 Tesla MRI parameters. These parameters potentially provide a measurement of the hemodynamic degree of severity of MMD, which has proved to correlate well with symptoms, and which may assist in evaluating intervention response and guiding management decisions. Limitations of this study, which was performed in 11 healthy control volunteers and 25 MMD patients with angiographically confirmed moyamoya, include its retrospective nature, and a small sample size, necessitating univariate analysis (159).

Han et al. (160) performed an HR-MRI study in 51 adult MMD patients, analyzing angiographic and HR-MRI findings as well as atherogenic risk factors. The research group suggested that HR-MRI may assist in a precise diagnosis of ICAD in MMD patients with risk factors for atherogenesis. Distinguishable symptoms observed in moyamoya patients in the presence or absence of atherosclerotic plaques may suggest a distinct pathophysiology, implying different treatment strategies (160).

Storey et al. performed a single-center study which included 204 MMD patients. Transdural collateral vessels were existent in nearly 50% of arteriograms done preoperatively on these MMD patients. Pial synangiosis was performed on these patients from 2005 to 2013. This group hypothesized that preoperative transdural collateral vessels may be utilized as radiographic biomarkers of MMD, that transdural collateral vessels may be associated with advanced moyamoya, that transdural collateral vessels may be related to stroke as a perioperative complication, and that transdural collateral vessels may be capable of inducing surgical collaterals after surgery, suggesting innovative methods to generate biomarkers that may be capable of predicting outcome and stratifying surgical candidates, and supporting the usefulness of preoperative diagnostic arteriography (161).

Song et al. performed a retrospective analysis in 90 pediatric MMD patients by MRI and digital subtraction angiography to assess the distribution of ischemic lesions and vascular abnormalities, including stenosis and occlusion, as well as diagnostic coherence between imaging modalities. Digital subtraction angiography and MRA were used to detect stenotic and/or occlusive changes of the bilateral ACA, MCA, and PCA. MRI demonstrated good diagnostic coherence regarding stenotic and/or occlusive changes in the bilateral PCA. However, it is important to note that in 6 to 11% of patients, an MRA evaluation may lead to misdiagnosis of moyamoya (162).

Zhao et al. (31) conducted a single-center study to compare the radiographic and clinical characteristics between adult and pediatric moyamoya patients, and also between moyamoya patients with and without PCA involvement. Records of 696 consecutive MMD patients from 2009 to 2015, including 574 angiograms, 434 of adult and 140 of pediatric MMD patients, were reviewed. Grading systems by Miyamoto and Suzuki were used to evaluate stenoses and/or occlusions of the anterior and posterior cerebral circulation. In both pediatric MMD and in adult MMD, occlusive and/or stenotic PCA lesions positively correlated with the ipsilateral ICA stage. In adult MMD patients, the degree of PCA involvement seemed to correlate with the frequentness of ipsilateral stroke. In comparison to adult MMD patients, pediatric MMD patients showed a tendency toward advanced PCA lesions. First, this study is limited by its retrospective nature. Second, this study may be limited by selection bias (31).

In their 2019 review article, Li et al. stated that the most acceptable and valuable imaging modality should be reliable and keep injury to the patient to a minimum. Doppler and quantitative MRI-based imaging modalities might be auspicious in establishing an accurate evaluation of moyamoya. Standardized protocols, including various imaging modalities, to evaluate the preoperative and postoperative status quo, may be advocated in future moyamoya research (163).

Hauser et al. performed a retrospective study in 20 consecutive patients with angiographically proven MMD and analyzed 160 vascular territories, indicating that CO_2-triggered blood oxygen level-dependent (BOLD) MRI may be a promising, easily implementable method for the evaluation of territorial hemodynamics in MMD patients with results comparable to those attained by $[^{15}O]H_2O$ positron emission tomography/computed tomography with acetazolamide challenge, suggesting that, after continued prospective evaluation, CO_2-triggered BOLD MRI may become a routine examination method in pre- and postoperative evaluation of MMD patients (164).

In their 2019 review article, Lehman et al. discuss novel MRI imaging achievements that may further improve the analysis of moyamoya, e.g., enhanced methods of cerebral perfusion and angiography, high-resolution characterization of the vessel wall, as well as artificial intelligence. In the clinical setting, contemporary and emerging MRI imaging methods may assist in the evaluation of MMD, and help guide therapy planning and response to treatment (165).

As vascular imaging methods advance in their potential to serve as research tools, our understanding of the pathophysiology of arterial stenosis in moyamoya is very likely to grow. These imaging advances are particularly important given the lack of an animal model that reproduces the spontaneous arterial stenosis of the proximal cerebrovasculature that is seen in moyamoya.

CONCLUSION

Considerable progress has been made in moyamoya research since it was first described, leading to greater epidemiologic and pathophysiological understanding, as well as advancements in clinical practice, encompassing imaging, diagnosis, and surgical treatment. However, the proximate mechanisms that underly stenosis and progression of stenosis remain elusive. As a result, there is no clear drug target to reverse the disease process or prevent progression, and there have been no disease-modifying medical therapies developed to accomplish these goals. Such a therapy is desperately needed, as current medical therapies do not prevent progression and current surgical therapies, while effective, are associated with significant risk of morbidity and mortality. Future research should continue to aggressively pursue novel hypotheses that attempt to unify the unique aspects of moyamoya anatomic localization, pathophysiology, disease associations, and genetic risk factors to explain why stenosis develops in moyamoya. Further, as the understanding of more common stenotic arteriopathies of the distal ICA, such as atherosclerosis and FMD, is advanced, newly discovered mechanisms should continue to be investigated in moyamoya given its overlapping pathological characteristics. The initiation of arterial stenosis in moyamoya represents the biggest mystery of this disease process, and until it is mechanistically explained, patient care in moyamoya will remain insufficient.

AUTHOR CONTRIBUTIONS

BF and KD contributed to developing the concept of the review, developing the figures, and writing and editing the manuscript. ML oversaw the project and contributed to developing the concept of the review. JW led oversight of the project and contributed to developing the concept of the review, developing the figures, and editing the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.661578/full#supplementary-material

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Platelet-to-Lymphocyte Ratio and Neutrophil-to-Lymphocyte Ratio in Patients With Newly Diagnosed Moyamoya Disease: A Cross-Sectional Study

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Inflammation has been proven to be one of the key factors in the pathogenesis of moyamoya disease (MMD). Platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR) are cheap and reliable biomarkers of inflammation. Nevertheless, evidence regarding the relationship among PLR and NLR in patients with MMD is limited. The focus of this subject was to explore the relationship between PLR and NLR in patients with newly diagnosed MMD.

Patients and methods: A cross-sectional study was performed including 261 patients with diagnosed MMD for the first time who were enrolled from our hospital, from 24 March 2013 to 24 December 2018. The clinical characteristics were collected for each patient. Univariate analysis, smooth curve fitting and multivariate piecewise linear regression were showed.

Results: The mean levels or median values (interquartile range) of PLR and NLR were 146.979 ± 51.203 and 2.241 (1.589–2.984), respectively. A significant positive correlation between PLR and NLR levels ($P < 0.001$) was showed by the univariate analysis. Furthermore, a non-linear relationship was detected between PLR and NLR by smooth curve fitting after adjusting for potential confounders. A multivariate piecewise linear regression model revealed a significant positive correlation between PLR and NLR when the PLR level was lower than 219.82 (β 0.012, 95% CI 0.005, 0.019; $P = 0.001$). PLR was also significantly positively associated with NLR when PLR concentrations were >219.82 (β 0.098, 95% CI 0.069, 0.128; $P < 0.001$).

Conclusion: There seemed to be a positive association between PLR and NLR in patients with MMD. This may help to further explain the role of inflammation in the occurrence and progress of MMD.

Keywords: moyamoya disease, inflammation, association, platelet-to-lymphocyte ratio, neutrophil-to-lymphocyte ratio

INTRODUCTION

Moyamoya disease (MMD) is a rare cerebrovascular disease characterized by progressive stenosis of large intracranial arteries and a hazy network of basal collaterals called moyamoya vessels (1). The disease may mainly lead to ischemic or hemorrhagic stroke. To date, the underlying mechanisms of MMD have remained to be fully demonstrated. With the deepening of research on MMD, systemic inflammation has been shown to play a critical role in the pathogenesis of MMD (2–4). A previous study reported a secure epidemiological association among MMD and certain diseases that have a segment of inflammation (5). These discoveries may demonstrate that in some patients with MMD, pathological vessel shapes may be a sequela of systemic inflammation (6). Currently, existing studies on MMD and inflammation have focused on inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-12 (IL-12), transforming growth factor-β1 (TGF-β1) and tumor necrosis factor-α (TNF-α) (5, 7–9). Nevertheless, evidence regarding the relationship between platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR) in patients with MMD is limited.

PLR and NLR are inexpensive and reproducible biomarkers of inflammation (10, 11). The relationship between PLR and NLR has been extensively studied in malignant tumors, systemic lupus erythematosus, rheumatoid arthritis and other diseases (12–15). PLR and NLR are reportedly associated with decreased overall survival or recurrence-free survival in patients with numerous cancers (16, 17). In addition, PLR and NLR have been identified as prognostic predictors of stroke (18). PLR is an integrated reflection of important opposite inflammatory pathways easily measured from a complete blood count. Platelet to lymphocyte ratio is a cheap tool and more predictive than either the platelet or the lymphocyte count alone (19). PLR originally served as a systemic inflammatory biomarker to predict the prediction of neoplastic diseases. The PLR was positively correlated with the standard of internal carotid artery stenosis (20). NLR serves as a secure prognostic index for patients suffering from various diseases. Neutrophil activation enhances the recruitment of a number of different cell types that are involved in acute and chronic inflammation (21). In addition, a high circulating NLR is a powerful biomarker of poor clinical event in numerous cancers (22). A previous study used 171 patients, which found that elevated NLR was independently associated with MMD (11).

The main purpose of this research is to clarify the relationship among PLR and NLR in patients with newly diagnosed MMD, which may help to further explain the role of inflammation in the occurrence and progress of MMD.

MATERIALS AND METHODS

Patients Population

According to the hospital electronic medical record system, this cross-sectional study involved 261 patients with newly diagnosed MMD who visited to Department of Neurosurgery, Affiliated Hospital of Jining Medical University, Jining, Shandong, China, between 24 March 2013 and 24 December 2018.

The clinical code for each participant was performed according to the Guidelines for Diagnosis and Treatment of Moyamoya Disease (Spontaneous Occlusion of the Circle of Willis) (2012 Edition) (23). The diagnostic standard were as follows: 1. Cerebral angiography must show at least the following findings: (1) Stenosis or occlusion of the terminal portion of the intracranial internal carotid artery (ICA) or proximal portions of the anterior cerebral artery (ACA) and/or the middle cerebral artery (MCA), (2) Abnormal vascular networks in the vicinity of the occlusive or stenotic lesions in the arterial phase, and (3) Bilaterality of the findings in (1) and (2). 2. The following situations must have been eliminated: (1) atherosclerosis, (2) autoimmune disease, (3) meningitis, (4) brain tumors, (5) Down's syndrome, (6) von Recklinghausen's disease, (7) head injury, (8) cerebrovascular lesions after head irradiation, and (9) others.

The selection criteria included patients hospitalized in our hospital who were diagnosed with MMD for the first time. The elimination criteria were: (1) patients with hematological diseases, malignancy, autoimmune diseases, metabolic diseases, or existing infections; (2) patients treated with glucocorticoid, permanent immunomodulatory drugs or anti-inflammatory drugs; (3) patients younger than 18 years old and (4) patients who have undergone MMD operation (24, 25).

The study was authorized by the Ethics Committee of Affiliated Hospital of Jining Medical University (protocol number 2020C034) and informed approve was abandoned due to the retrospective study design.

Data Collection

All patients' demographic and clinical information on admission were retrospectively gathered, including age; sex; body mass index (BMI); smoking status; alcohol consumption; diabetes; hypertension; blood routine index; type of onset; Suzuki stage. When patients were admitted to the hospital, obtained the blood routine index using the method in our previous study (26). PLR was calculated as platelet counts divided by those of lymphocyte and NLR was calculated as neutrophil counts divided by lymphocyte counts using the same blood samples drawn.

Statistical Analysis

We show continuous variables with a normal distribution as the mean ± standard deviation, and we gave continuous variables with skewed distributions as the medians (Q1–Q3). Categorical variables were gave as frequencies or percentages. First, we describe the demographic characteristics and blood routine index of the subjects. Next, a univariate analysis pattern was used to measure the significance of the association between PLR and NLR as well as the other separated variables. Then, to explore the non-linearity of PLR and NLR, we implemented a generalized additive model and smooth curve fitting. If non-linearity was identified, we first calculated the inflection point using the recursive algorithm and then created a two-piecewise linear regression on both sides of the inflection point. Finally, we divided MMD patients into patients with intracranial ischemia and intracranial hemorrhage according to the type of onset and investigated the relationship among PLR and NLR by smooth curve fitting after adjustment for possible confounders. All analyses were

TABLE 1 | Baseline characteristics of the participants.

Variables	All
Number	252
Age (years, mean ± sd)	48.9 ± 10.2
BMI (kg/m^2, mean ± sd)	25.57 ± 3.35
Platelet (10^9/L, mean ± sd)	244.673 ± 61.025
Neutrophil (10^9/L, median, Q1–Q3)	3.890 (2.910–5.000)
Lymphocyte (10^9/L, mean ± sd)	1.759 ± 0.577
NLR (median, Q1–Q3)	2.241 (1.589–2.984)
PLR (mean ± sd)	146.979 ± 51.203
Sex, *n* (%)	
Female	129 (51.2%)
Male	123 (48.8%)
Smoking status, *n* (%)	
No	178 (71.2%)
Yes	72 (28.8%)
Alcohol consumption, *n* (%)	
No	184 (73.6%)
Yes	66 (26.4%)
Diabetes, *n* (%)	
No	235 (93.3%)
Yes	17 (6.7%)
Hypertension, *n* (%)	
No	171 (67.9%)
Yes	81 (32.1%)
Type of onset, *n* (%)	
Intracranial ischemia	195(77.4%)
Intracranial hemorrhage	56 (22.2%)
Seizure	1 (0.4%)
Suzuki stage, *n* (%)	
Stage 1	1 (0.4%)
Stage 2	15 (5.9%)
Stage 3	160 (63.5%)
Stage 4	66 (26.2%)
Stage 5	10 (4%)

BMI, body mass index; PLR, platelet-to-lymphocyte ratio; NLR, neutrophil-to-lymphocyte ratio.

gave with the R statistical software packages (http://www.R-project.org, The R Foundation) and EmpowerStats (http://www.empowerstats.com, X&Y Solutions, Inc, Boston, MA). *P*-values < 0.05 (two-sided) were considered statistically significant.

RESULTS

Baseline Characteristics of the Selected Participants

The clinical characteristics of all the patients were described in **Table 1**. A total of 252 patients, consisting of 123 (48.8%) males and 129 (51.2%) females, were selected. The average age of the participants was 48.9 ± 10.2 years. The participants included 195 (77.4%) cases of intracranial ischemia, 56 (22.2%) cases of intracranial hemorrhage and 1 (0.4%) case of seizure. One (0.4%) patient was in Suzuki stage 1, 15 (5.9%) patients were in Suzuki stage 2, 160 (63.5%) patients were in Suzuki stage 3, 66 (26.2%) patients were in Suzuki stage 4, and 10 (4%) patients were in Suzuki stage 5. The average levels or median values (interquartile range) of PLR and NLR were 146.979 ± 51.203 and 2.241 (1.589–2.984), respectively.

TABLE 2 | Univariate analysis for NLR.

Covariate	Statistics	β (95% CI)	*P*-value
Sex			
Female	129 (51.2%)	Reference	
Male	123 (48.8%)	0.474 (−0.398, 1.346)	0.288
Age, years	48.9 ± 10.2	0.004 (−0.038, 0.047)	0.840
BMI, kg/m^2	25.57 ± 3.35	0.018 (−0.133, 0.170)	0.811
Smoking status			
No	178 (71.2%)	Reference	
Yes	72 (28.8%)	0.432 (−0.531, 1.395)	0.381
Alcohol consumption			
No	184 (73.6%)	Reference	
Yes	66 (26.4%)	0.902 (−0.082, 1.886)	0.074
Diabetes, *n* (%)			
No	235 (93.2%)	Reference	
Yes	17 (6.7%)	0.570 (−1.149, 2.288)	0.517
Hypertension, *n* (%)			
No	171 (67.9%)	Reference	
Yes	81 (32.1%)	−0.119 (−1.051, 0.813)	0.803
Platelet	244.673 ± 61.025	−0.001 (−0.008, 0.006)	0.773
Neutrophil	4.507 ± 2.703	1.033 (0.936, 1.130)	<0.001
Lymphocyte	1.759 ± 0.577	−2.845 (−3.515, −2.175)	<0.001
PLR	146.979 ± 51.203	0.023 (0.017, 0.028)	<0.001

BMI, body mass index; PLR, platelet-to-lymphocyte ratio.

Univariate Analysis for NLR

Univariate linear regression test was performed to measure the relationships between all tested variables and NLR. As shown in **Table 2**, for the unadjusted pattern, a significant positive relationship between NLR and PLR was observed ($P < 0.001$). We also observed a significant positive correlation between NLR and neutrophil ($P < 0.001$) and lymphocyte ($P < 0.001$) were significant negatively associated with NLR. No association was observed between NLR and sex, age, BMI, smoking status, alcohol consumption, diabetes, hypertension or platelet ($P > 0.05$).

Independent Correlation Between PLR and NLR by Multivariate Piecewise Linear Regression

As shown in **Figure 1**, smooth curve fitting was performed after adjusting for possible confounding factors, including sex, age, BMI, smoking status and alcohol consumption. The participants' NLR levels demonstrated an non-linear relationships with PLR. Specifically, the NLR levels displayed an increasing trend as the PLR increased. As shown in **Table 3**, we further analyzed the

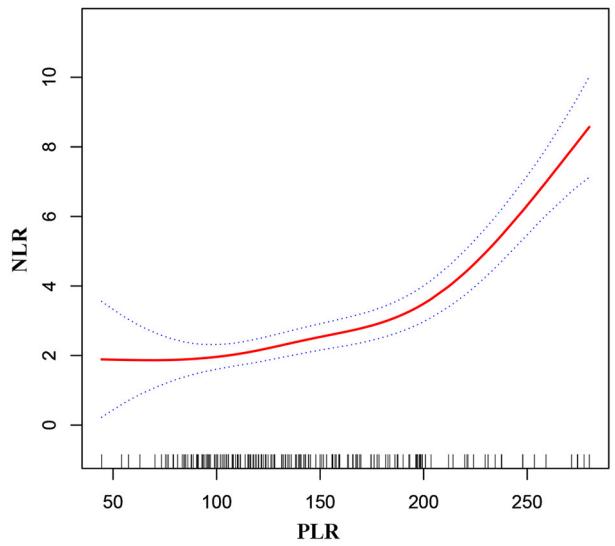

FIGURE 1 | Association between PLR and NLR in MMD patients. The solid red line stands for the smooth curve fit between the variables. Blue bands stand for the 95% confidence interval of the fit. The pattern was adjusted for sex, age, BMI, smoking status, and alcohol consumption.

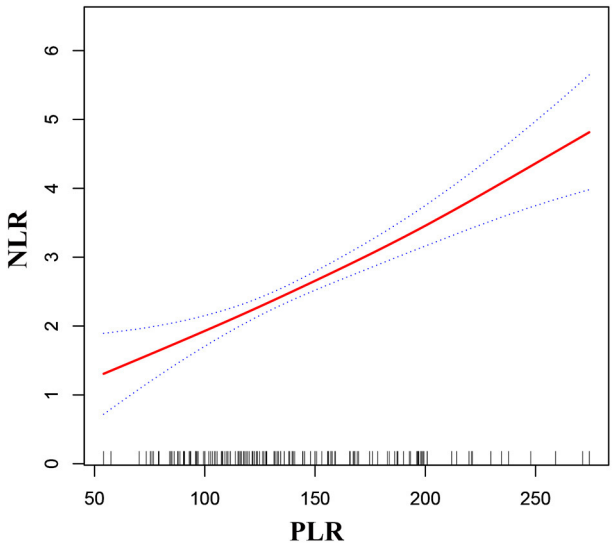

FIGURE 2 | Association between PLR and NLR in MMD patients with intracranial ischemia. The solid red line stands for the smooth curve fit between the variables. Blue bands stand for the 95% confidence interval of the fit. The pattern was adjusted for sex, age, BMI, smoking status, and alcohol consumption.

TABLE 3 | The result of two-piecewise linear regression model.

Inflection point of PLR	Effect size (β)	95% CI	*P*-value
<219.82	0.012	0.005, 0.019	0.001
≥219.82	0.098	0.069, 0.128	<0.001

PLR, platelet-to-lymphocyte ratio.

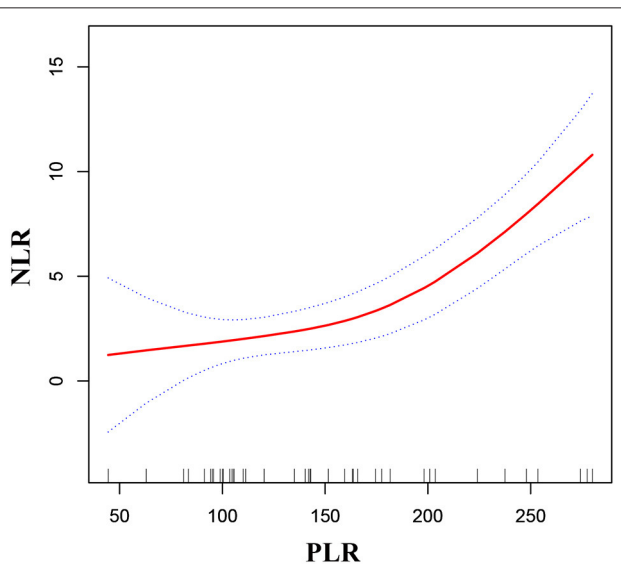

FIGURE 3 | Association between PLR and NLR in MMD patients with intracranial hemorrhage. The solid red line stands for the smooth curve fit between the variables. Blue bands stand for the 95% confidence interval of the fit. The pattern was adjusted for sex, age, BMI, smoking status, and alcohol consumption.

threshold effect based on curve fitting. We observed a significant positive correlation between PLR and NLR when the PLR level was lower than 219.82 (β 0.012, 95% CI 0.005, 0.019; $P = 0.001$). PLR was also significantly positively associated with NLR when PLR concentrations were >219.82 (β 0.098, 95% CI 0.069, 0.128; $P < 0.001$).

Independent Correlation Between PLR and NLR by Multivariate Piecewise Linear Regression in MMD Patients With Intracranial Ischemia or Intracranial Hemorrhage

As shown in **Figures 2**, **3**, smooth curve fitting was showed in MMD patients with intracranial ischemia or intracranial hemorrhage according to the method in **Figure 1**. We found that NLR levels displayed an increasing trend as PLR increased in MMD patients with intracranial ischemia and intracranial hemorrhage.

DISCUSSION

MMD was defined in 1969 by Suzuki and Takaku (27) as a distinct cerebrovascular disease that is characterized by progressive stenosis or occlusion of the bilateral internal carotid arteries with unknown etiology, and plentiful collateral vessels appear at the base of the brain (28). The disease generally occurs in East Asian populations, including pediatric and adult patients, and may bring about ischemic or hemorrhagic stroke, headaches,

epilepsy, or transient ischemic attack (29). For a long time, researchers have carried out in-depth research on the underlying mechanisms of MMD. Up to now, the mechanisms through which MMD occurs and evolves remain unidentified. Some researches manifest that MMD could be the result of vascular immune injury and inflammation response (2, 30). Recent studies of our team have found that the upregulated expressions of various plasma inflammatory factors, such as IL-1β, IL-12, and TNF-α, in patients with MMD suggested that inflammation might regulate the process of MMD (31). Inflammatory response ultimately leads to the hyperplasia of intimal vascular smooth muscle cells and neovascularization by proliferation of endothelial cells, that motivates lumen stenosis and collateral formation (32).

PLR and NLR are reliable marker of systemic inflammation. Although there have been extensive investigations on NLR and PLR, the normal ranges of NLR and PLR were less investigated. It was reported that the average NLR is 2.15 in the United States population (33) and 1.65 in South Korea (34), which suggested that NLR is race specific. A study investigated the reference range of NLR and PLR in Chinese Han population from Chaoshan region in South China. Researchers found that the 95% reference range of NLR in normal male and female are 0.43–2.75 and 0.37–2.87, PLR are 36.63–149.13 and 43.36–172.68, respectively (35).

The PLR and NLR have been regarded indicators of systemic inflammation in numerous present clinical studies, when patients have no evident infection (36). Therefore, the evaluation of such cheap and readily available prognostic indicator was essentially needed for large numbers of experiments. It is well-known that, while the number of neutrophils and platelets increase, lymphocytes decrease during an inflammation. As neutrophil and platelet counts increase, they secrete numerous kinds of inflammatory factors, which mean a stronger inflammatory response (12). Platelets play an dynamic role in inflammation. One of the most important mechanisms of platelets was their capacity to collect leukocytes to sites of inflammation. Platelets have capacity to create aggregates with neutrophils and monocytes, and also motivate an inflammatory monocyte phenotype (37). In addition, autopsy studies have demonstrated smooth muscle proliferation, infiltrating macrophages, and T lymphocyte in moyamoya vessel walls (6). The PLR and NLR can exhibit the inflammatory state and predict the prediction of the tumor (38). Recently, it has been reported that PLR and NLR can be managed to independently predict 90-day practical outcome in patients after primary brainstem hemorrhage (39). PLR has been shown as a novel indicator for major adverse outcomes in cardiovascular disorders (37). In patients with mitral annular calcification, there was a positive correlation between the PLR and NLR (40). Aortic stenosis was a progressive disease related with inflammation. PLR had significant positive correlation with NLR in patients with aortic stenosis (37). In our study, a non-linear positive relationship was observed between PLR and NLR in patients with newly diagnosed MMD. When we divided MMD patients into patients with intracranial ischemia and intracranial hemorrhage according to the type of onset, we found that NLR levels displayed an increasing trend as PLR increased in MMD patients with intracranial ischemia and intracranial hemorrhage. The clinical value of this study is as follows. (1) To the best of our knowledge, this is the first study to detect the independent association between PLR and NLR in patients with newly diagnosed MMD. (2) The relationship between PLR and NLR may help to further explain the role of inflammation in the occurrence and development of MMD. (3) Our study could enrich the role of PLR and NLR as inflammatory markers in various diseases.

There are some limitations in the current study. First, in this study, our consider subjects were patients with newly diagnosed MMD. Therefore, there is a definite deficiency in the universality and extrapolation of the study. Second, the dynamic changes of NLR and PLR are not explored because of unavoidable selection bias and assessment bias. Thirdly, because we excluded patients with hematological diseases, malignancy, autoimmune diseases, metabolic diseases or existing infections, patients treated with glucocorticoid, permanent immunomodulatory drugs or anti-inflammatory drugs and patients younger than 18 years old, the discoveries of this research cannot be generalized to these people. Lastly, the hospitalized patients with MMD did not used the modified Rankin Scale at the time. Only some patients have tested the cytokines. We will add these two parts in our follow-up research.

CONCLUSION

In conclusion, the present study showed a positive association between PLR and NLR among patients with newly diagnosed MMD. This may help to further explain the role of inflammation in the occurrence and progress of MMD.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The study was approved by the Ethics Committee of Affiliated Hospital of Jining Medical University (protocol number 2020C034) and informed consent was waived due to the retrospective study design.

AUTHOR CONTRIBUTIONS

WM, SF, GL, and GH were involved in the study design. JL, XQ, YH, MW, and LZ were responsible for the data collection. WM analyzed data and wrote the manuscript. FJ and CC modified and revised the manuscript. All authors have read and approved the final version of the manuscript.

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Physiological and pathophysiological mechanisms of the molecular and cellular biology of angiogenesis and inflammation in moyamoya angiopathy and related vascular diseases

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Rationale: The etiology and pathophysiological mechanisms of moyamoya angiopathy (MMA) remain largely unknown. MMA is a progressive, occlusive cerebrovascular disorder characterized by recurrent ischemic and hemorrhagic strokes; with compensatory formation of an abnormal network of perforating blood vessels that creates a collateral circulation; and by aberrant angiogenesis at the base of the brain. Imbalance of angiogenic and vasculogenic mechanisms has been proposed as a potential cause of MMA. Moyamoya vessels suggest that aberrant angiogenic, arteriogenic, and vasculogenic processes may be involved in the pathophysiology of MMA. Circulating endothelial progenitor cells have been hypothesized to contribute to vascular remodeling in MMA. MMA is associated with increased expression of angiogenic factors and proinflammatory molecules. Systemic inflammation may be related to MMA pathogenesis.

Objective: This literature review describes the molecular mechanisms associated with cerebrovascular dysfunction, aberrant angiogenesis, and inflammation in MMA and related cerebrovascular diseases along with treatment strategies and future research perspectives.

Methods and results: References were identified through a systematic computerized search of the medical literature from January 1, 1983, through July 29, 2022, using the PubMed, EMBASE, BIOSIS Previews, CNKI, ISI web of science, and Medline databases and various combinations of the keywords "moyamoya," "angiogenesis," "anastomotic network," "molecular mechanism," "physiology," "pathophysiology," "pathogenesis," "biomarker," "genetics," "signaling pathway," "blood-brain barrier," "endothelial progenitor cells," "endothelial function," "inflammation," "intracranial hemorrhage," and "stroke." Relevant articles and supplemental basic science articles almost exclusively published in English were included. Review of the reference lists of relevant publications for additional sources resulted in 350 publications which met the study inclusion criteria. Detection of growth factors, chemokines, and cytokines in MMA patients suggests the hypothesis of aberrant angiogenesis being involved in MMA pathogenesis. It remains to be ascertained whether these findings are consequences of MMA or are etiological factors of MMA.

Conclusions: MMA is a heterogeneous disorder, comprising various genotypes and phenotypes, with a complex pathophysiology. Additional research may advance our understanding of the pathophysiology involved in aberrant angiogenesis,

arterial stenosis, and the formation of moyamoya collaterals and anastomotic networks. Future research will benefit from researching molecular pathophysiologic mechanisms and the correlation of clinical and basic research results.

KEYWORDS

moyamoya angiopathy (MMA), molecular mechanism, pathophysiology, angiogenesis, inflammation, genetics, stroke, biomarker

Introduction

Moyamoya angiopathy (MMA) is an angiopathy unique to the cerebrovasculature that is characterized by chronically progredient stenosis of the bilateral intracranial internal carotid artery (ICA) and its proximal bifurcations and development of a network of aberrant collateral arteries to compensate for the stenosed vessels. MMA pathophysiology may include a consecutive secondary response of compensatory collateral circulation development by means of vasculogenesis and alteration of cerebral hemodynamics as a result of a primary narrowing of distinct intracranial vessels (1–6) (Figures 1–3) (see the Supplementary Table 1 for definitions of gene symbols, proteins, and additional terminology).

Comprehension of cellular signaling cascades linked to MMA may be essential for identifying diagnostic and therapeutic targets (5). Distinct monogenic moyamoya syndromes show radiological characteristics of MMA and may be related to various signaling pathways and genes associated with MMA pathogenesis (8). Through identification of genes involved in MMA pathogenesis and several monogenic moyamoya syndromes (MMS), researchers have associated various signaling pathways with MMA pathophysiology, including molecular signaling pathways [Rat sarcoma (Ras)–rat fibrosarcoma (Raf)–mitogen-activated protein kinase (MEK)–extracellular signal-related kinase (ERK) signaling pathway, nitric oxide (NO)–soluble guanylyl cyclase (sGC)–cyclic guanosine monophosphate (cGMP) signaling pathway], signaling pathways involved in inflammation [Phosphatidylinositol 3-kinase (PI3K)/and Akt1 (Akt)/mammalian target of rapamycin (mTOR) signaling pathway, hypoxia-inducible factor (HIF)-1/nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway, Caveolin-1/ERK signaling pathway, the wingless and Int-1 (Wnt)/(β-Catenin)/lymphoid enhancing factor (Lef)-1 signaling pathway, Calcineurein/nuclear factor of activated T-cells (NFAT) signaling pathway, mitogen-activated protein kinase (MAPK) signaling pathway, tumor necrosis factor alpha (TNFα)/protein tyrosine phosphatase 1B (PTP1B) and peroxisome proliferator activated receptor gamma (PPARγ) signaling pathway, toll-like receptor (TLR) signaling pathway], and signaling pathways involved in genomic stability [Ring finger protein 213 (RNF213) signaling pathway]. Genes encoding additional members of these pathways may themselves be involved in MMA pathogenesis (3, 9–11). Inflammatory proteins have been shown to be associated with MMA pathophysiology. However, inflammatory proteins have not been historically approved as causative agents of MMA (5, 12). Research into physiologic characteristics of angiogenesis, arteriogenesis, vasculogenesis, and associated signaling pathways may lead to a deeper understanding of moyamoya's complex pathophysiology (13–42).

The purpose of this review article is to describe the physiological and pathophysiological mechanisms of signaling pathways, cells, and genes relevant to angiogenesis and inflammation in MMA and MMS along with future moyamoya research perspectives and treatment strategies implemented into clinical practice (Figure 4). This article discusses if these mechanisms may be regarded as causative of the angiopathy or if they may be viewed as a consequence of ischemic processes observed in MMA. We also aim to further specify proposed therapeutic and diagnostic targets related to angiogenesis and inflammation in MMA, that may lead to disease-modifying treatment strategies (4, 6, 9, 43–46).

Methods

References were identified by use of a systematic, comprehensive computerized literature search from January 1, 1983, through July 29, 2022, performed by both authors, using the PubMed, Embase, BIOSIS Previews, CNKI, ISI Web of Science, and Medline databases and the key words "moyamoya," "angiogenesis," "anastomotic network," "moyamoya syndrome," "molecular mechanism," "signaling pathway," "genetics," "biomarker," "physiology," "pathophysiology" "blood-brain barrier," "endothelial function," "endothelial progenitor cells," "intracranial hemorrhage," "inflammation," and "stroke" in various combinations. Relevant articles on MMA and supplemental basic science articles almost exclusively published in English were included. References of included publications have been searched for supplementary sources, and 350 publications have consequently been cited in the manuscript. After being reviewed by a member of the panel, the manuscript has been reviewed by five expert peer reviewers. Even though several basic research results about physiologic characteristics of angiogenesis, arteriogenesis, vasculogenesis (13, 14), and associated signaling pathways (15–42) as well as knowledge regarding inflammation in pediatric ischemic stroke (47–56) have been included for the convenience of readers who may be unfamiliar with these topics, this article emphasizes MMA basic, laboratory and clinical research results, future research perspectives, treatment strategies, and their implementation in clinical practice. As several aspects of MMA have been studied in greater detail in comparison to others, distinct topics receive additional attention. Despite substantial progress in the MMA field of research in recent years, the literature in great part remains descriptive. Continued basic and clinical research is essential to further elucidate the pathogenesis of MMA, and to obtain significant results.

Abbreviations: MMA, moyamoya angiopathy.

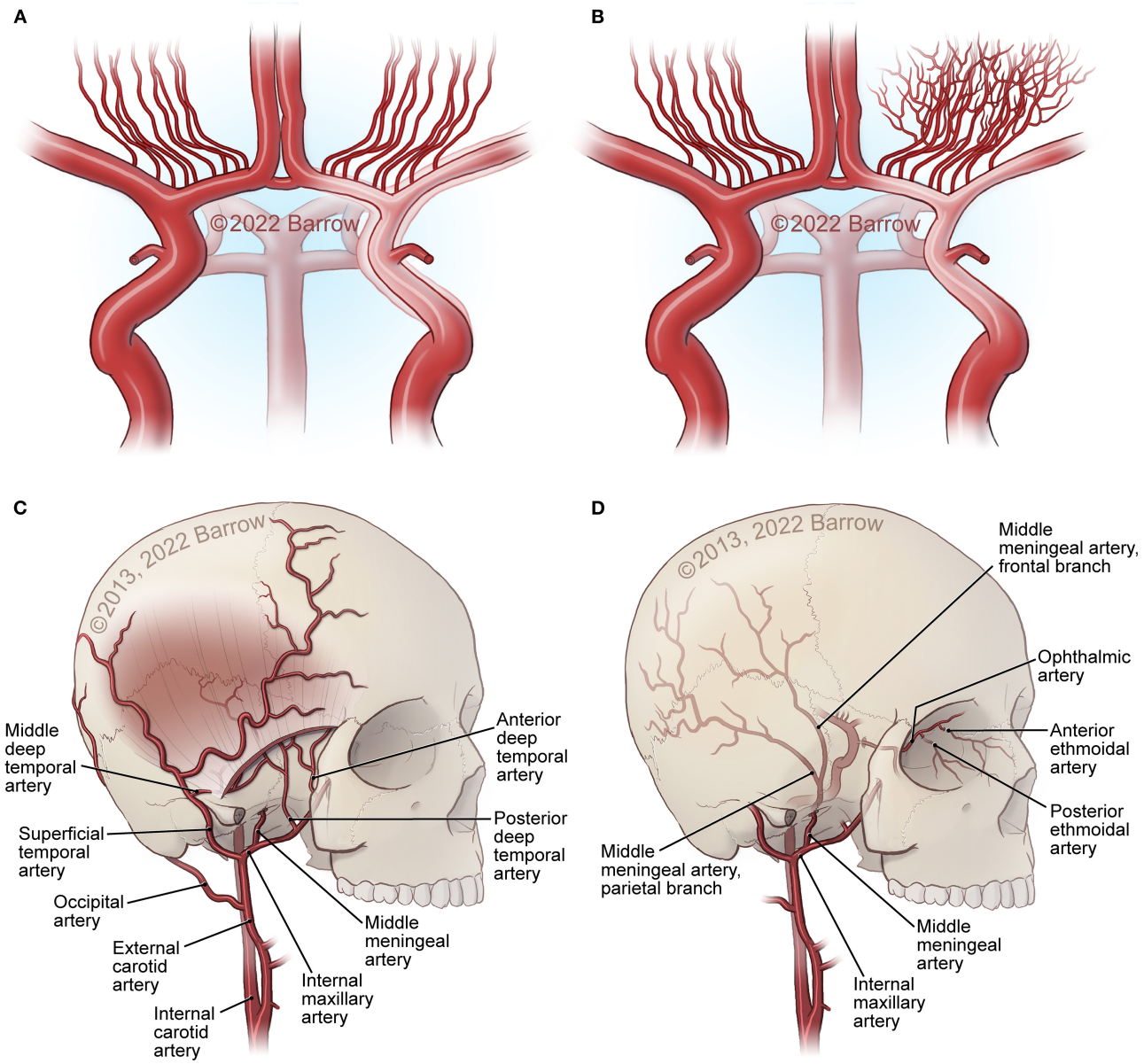

FIGURE 1
Major structural changes in MMA. **(A)** Carotid fork occlusion. **(B)** Development of moyamoya collaterals at the base of the brain. **(C, D)** External carotid artery (ECA) collaterals from the superficial temporal artery (STA), internal maxillary artery, ophthalmic artery ethmoidal artery and collaterals through foramina and sutures in the bone at the skull base and the surface of the skull, as well as middle meningeal artery connections from the dura into the middle cerebral artery (MCA) and anterior cerebral artery (ACA) surface branches. See Supplementary material. *Used with permission from Barrow Neurological Institute, Phoenix, Arizona.*

Pathologic characteristics of angiogenesis, inflammation, hemodynamics, vascular wall imaging, vascular regression, and hemorrhage in moyamoya angiopathy

Angiogenesis, inflammation, and hemodynamics in moyamoya angiopathy

Cerebrovascular diseases may present as a disruption and as aberrations of the intracranial vasculature, including cerebral blood supply (57). Initiation of the pathogenesis of various cerebrovascular diseases has been associated with the vascular wall (57). Stenotic changes in MMA involve the distal intracranial ICA. Disease progression involves the proximal anterior cerebral artery (ACA) (A1), the middle cerebral artery (MCA) (M1), and rarely the posterior circulation (5). MMA vascular wall pathology demonstrates fibrocellular intimal thickening with increased vascular smooth muscle cell (SMC) proliferation, fragmentation and tortuousness of the internal elastic lamina, media attenuation, microaneurysms, and fibrin deposits (5, 58, 59). Thrombosis, a consequence of vessel lumen collapse, may be demonstrated in moyamoya (5, 60). These MMA pathogenetic changes may cause hemorrhagic and ischemic

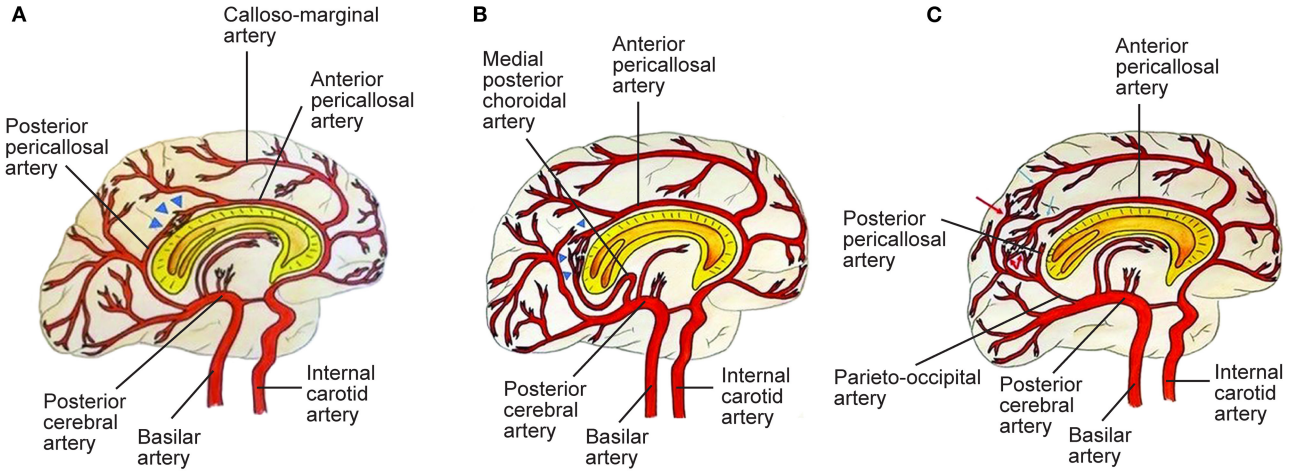

FIGURE 2
Three types of posterior cerebral artery (PCA)-anterior cerebral artery (ACA) collaterals. **(A)** Type I collaterals constitute the anastomosis between the anterior pericallosal artery (APA) and the posterior pericallosal artery (PPA) which is indicated by blue triangles. Pio-pial connections contribute to this anastomosis. **(B)** Type II collaterals between the APA and the medial posterior choroidal artery (MPChoA) are indicated by blue triangles. The MPChoA first turns anteriorly and then backwards around the splenium of the corpus callosum toward the APA. **(C)** Type III collaterals are leptomeningeal or pio-pial connections between cortical branches from the PCA, indicated by red arrows, and cortical branches from the ACA, indicated by light blue arrows. PPA-APA connections are present (7). See Supplementary material. *Source: Reprinted/adapted from Bonasia et al. (7) with permission from the AJNR, American Journal of Neuroradiology, American Society of Neuroradiology, and American Roentgen Ray Society*.

stroke (5). Masuda et al. demonstrated the infiltration of T cells and macrophages into vascular sections without stenosis, indicating that microthrombi may result from chronic inflammation instead of causing this process (5, 61). Presence of microthrombi may not be specific for MMA (5). Inflammation may cause hyperplasia of intimal SMCs and neovascularization through endothelial cell proliferation, leading to lumen stenosis and formation of collaterals (9). In 2006, Takagi et al. demonstrated that apoptosis, evidenced through activated caspase-3, may occur in the MCA media in MMA patients. Consequently, MCA specimens from MMA patients showed vascular wall/medial thinning compared to controls (62). In their 2008 study in 19 adult MMA patients, Kwag et al. suggested that linear and/or non-linear mean blood flow velocity (MBFV) changes in the posterior and anterior cerebral circulation, related to distinct intracranial vessels, may be helpful in both follow-up and initial evaluation of distinct angiographic Suzuki stages of MMA, and may provide results to further ascertain hemodynamic changes related to the disappearance of the bilateral anterior circulation. The research group stated that the MBFV in the ACA, terminal ICA, and the MCA showed a non-linear increase up to Suzuki stage III, and subsequently progressively decreased as far as Suzuki stage VI. Moreover, the ophthalmic artery showed non-linear changes of blood flow velocity, with an MBFV increase as far as Suzuki stage IV, followed by an MBFV decrease as far as Suzuki stage VI. The MBFV of the basilar artery showed a linear increase from a normal velocity at an early MMA stage to a stenotic velocity at a late MMA stage. No statistically significant regression model for the relationship between the angiographic Suzuki stage of MMA and the MBFV in the PCA was evident (63). In their 2011 study in 292 MMA or MMS patients, Lee et al. stated that, in response to superficial temporal artery (STA)–middle cerebral artery (MCA) bypass surgery, flow rates at the vascular anastomosis increased 5 fold to a mean of 22.2 $\pm$ 0.8 mL/min. In comparison to adult MMA or MMS patients (23.9 $\pm$ 1.0 mL/min; $P < 0.0001$), MCA flow rates were significantly decreased in pediatric MMA or MMS patients (16.2 $\pm$ 1.3 mL/min) (64). The research group hypothesized that increased local flow rates may be related to improvement of clinical symptoms. Persistent post-operative complications were low (<5%) (64). Also, the group suggested that eminently increased post-operative MCA flow rates, in comparison to controls, may be related to transient neurologic deficits (28.6 $\pm$ 5.6 mL/min; $P = 0.047$), hemorrhage (32.1 $\pm$ 10.2 mL/min; $P = 0.045$), and post-operative stroke (31.2 $\pm$ 6.8 mL/min; $P = 0.045$) (64). In their 2013 study in 13 MMA patients and 10 healthy, age-matched controls, Chen et al. ascertained the beginning of dynamic cerebral autoregulation impairment at an early MMA stage (65). Every autoregulatory parameter correlated well with the angiographic MMA stage (65). The research group suggested that cerebral autoregulation impairment may progress with MMA progression toward complete vascular occlusion (65). Due to an increased risk of intracranial hemorrhage and ischemia, blood pressure intervention may be warranted (65–67). In 2013, Schubert et al. referred to a characteristic proximal pattern of collaterals (68). In 2015, Baltsavias et al. stated that the previously imprecisely described "moyamoya abnormal network" in pediatric MMA may be specified as a composition of four anastomotic networks with a readily distinguishable vascular structure (69). Accordingly, in their 2015 retrospective study in newly diagnosed 14 pediatric MMA and 11 pediatric MMS patients, Baltsavias et al. described four types of anastomotic networks in pediatric MMA, two deep-parenchymal networks and two superficial-meningeal networks (69). As deep-parenchymal networks the research group detailed the previously undescribed subependymal network and the inner striatal and inner thalamic networks. The subependymal network may be fed by the intraventricular branches of the choroidal system and diencephalic perforators, which, at the level of the periventricular subependymal zone, anastomose with medullary-cortical arteries and also with striatal arteries (69). The inner striatal and thalamic networks may be comprised of intrastriatal connections among

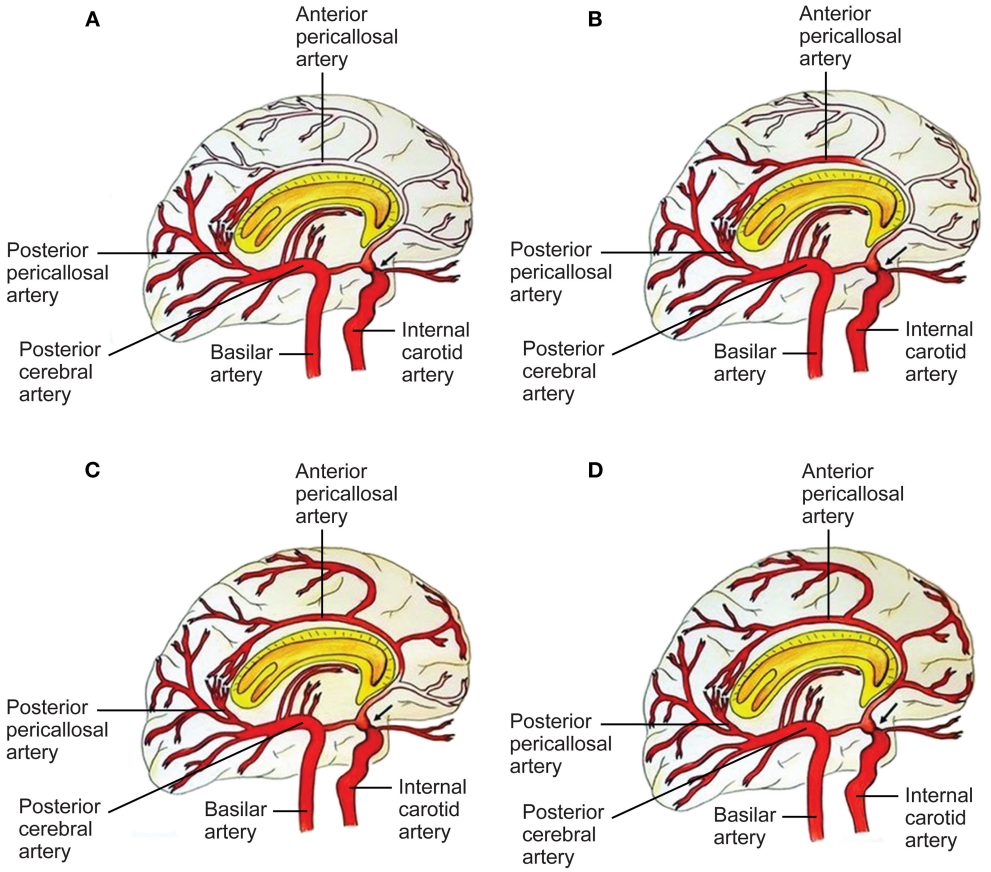

FIGURE 3
Capacity to compensate the ACA territory through posterior cerebral artery (PCA)-anterior cerebral artery (ACA) anastomoses in proximal ICA stenosis (black arrow). A four-grade classification. **(A)** In grade I, collaterals refill the first part of the ACA, without any cortical branches. Pio-pial connections and the posterior pericallosal artery contribute to refilling. **(B)** In grade II, the retrograde flow reaches a larger part of the ACA, including a cortical branch of the ACA. A contribution from the medial posterior choroidal artery and the pio-pial connection may be involved. **(C)** Grade III consists of retrograde refilling of three or two ACA branches, which may be strengthened by e.g., a medial posterior choroidal artery–anterior pericallosal artery anastomosis. **(D)** In grade IV, retrograde refilling reaches nearly the entire ACA territory. Major distinct connections may compensate the hypoperfusion of the ACA territory (7). See Supplementary material. *Source: Reprinted/adapted from Bonasia et al. (7) with permission from the AJNR, American Journal of Neuroradiology, American Society of Neuroradiology, and American Roentgen Ray Society.*

striatal arteries and intrathalamic connections among thalamic arteries when MMA compromises the origin of one or additional of their supply sources (69). As superficial-meningeal networks, the research group specified the leptomeningeal and the durocortical networks (69). Apart from the previously described leptomeningeal network observed in the convexial watershed zones, the group described the basal temporo-orbitofrontal leptomeningeal network. The second superficial-meningeal network was detailed as the durocortical network, with a calvarian or a basal location (69). In their 2015 study, Karunanithi et al., using computational fluid dynamics (CFD), evaluated 8 adult hemorrhagic MMA patients treated with encephaloduroarteriosynangiosis (EDAS) revascularization surgery, through analysis of pressure reduction in the right and left ICA before and after EDAS surgery, to ascertain how hemodynamic parameters including pressure reduction and flow rates may be the decisive factor for treatment outcome. The research group stated that pressure drop indicator (PDI), defined as the difference in pressure reduction in the ICA bilaterally, which, by use of patient-specific inflow rates, may be calculated post-operatively and at follow-up, may assist clinicians in reliable risk stratification of MMA patients regarding long-term follow-up (70). Also, PDI may further elucidate the hemodynamic mechanism associated with intracranial hemorrhage in MMA, including recurrent hemorrhage (70). In their 2016 retrospective, 1:2 matched case-control study in 180 MMA patients with or without Type 2 diabetes mellitus (T2DM), Ren et al. suggested that EDAS surgery may be an effective treatment for adult MMA, stating that T2DM patients may gain improvement of symptomatology as well as a more favorable collateral circulation post-operatively. Whereas T2DM was related to a favorable clinical outcome, PCA involvement and late post-operative stroke were identified as predictors of an unfavorable clinical outcome in both study groups (71). In 2016, Story et al. performed a study consisting of a single-institution case series of 204 MMA patients, with an average age at surgery of 9.5 years, who underwent pial synangiosis between 2005 and 2013. Transdural collaterals were present in almost half of all pre-operative arteriograms in MMA patients. These collaterals were demonstrated to be more common in advanced MMA, are associated with stroke as a perioperative complication, and may suggest an increased capacity to produce surgical collaterals post-operatively. Consequently, the research group supports the utility of pre-operative arteriography

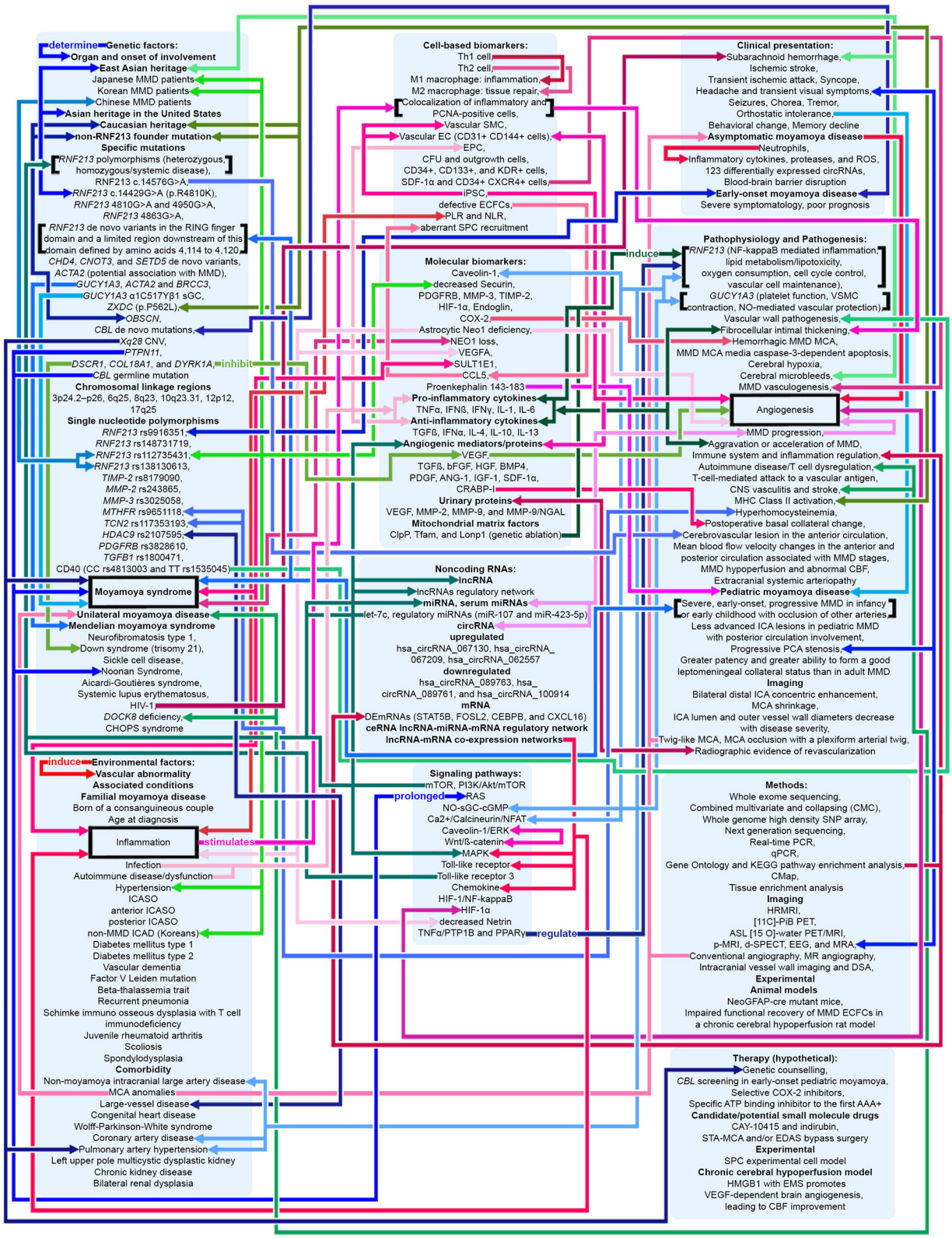

FIGURE 4
Potential pathophysiologic mechanisms in MMA as related to angiogenesis, inflammation, and genetics. Shades of red indicate that the mechanism relates to angiogenesis and inflammation. Shades of blue indicate that the mechanism relates to genetics. Shades of green indicate that the mechanism relates to both angiogenesis, inflammation, and genetics. Pointed arrows represent stimulatory regulation, double-ended arrows represent bidirectional regulation. See Supplementary material.

(72). In their 2016 letter to the editor, Wang et al. stated that, based on their study results, they have established microvascular density as a decisive factor contributing to the result of EDAS, and as a significant predictor of a favorable surgical outcome, potentially assisting to ascertain patients suitable for EDAS. Consequently, in case, during surgery, the cortex appears "white," the surgical procedure should be direct or combined anastomosis, not EDAS. On the contrary, if an increased number and diameter of vessels are observed which may lead to a "reddish" appearance of the cortex, the patient may be considered suitable for EDAS (73). Regarding their 2017 study results, Qiao et al. stated that blood oxygen level–dependent functional magnetic resonance imaging (BOLD-fMRI) may be an efficient imaging technique to evaluate hemodynamic change in MMA patients (74). In their 2017 comparative study in 41 MMA patients, Qiao et al. suggested that, in comparison to dynamic susceptibility contrast-magnetic resonance imaging (DSC-MRI), multiple inversion time arterial spin labeling (mTI-ASL) may effectively evaluate moyamoya cerebral hemodynamics and assess cerebral ischemia before surgical revascularization and reduction of ischemia after surgical revascularization. The research group indicated that mTI-ASL, not requiring contrast mediums, may be advantageous (75). In their 2018 review article, Yu et al. stated that, in case stenosis or occlusion occurs at the top of the ICA or the first segment of the ACA (A1), the first segment of the MCA (M1) or distal to the anterior choroidal artery (AChA), the AChA can be preserved. The AChA may play a decisive role in MMA (76). In 2019, Fan et al. suggest that their simultaneous hybrid positron emission tomography (PET)/magnetic resonance imaging (MRI) study may support the use of multidelay simultaneously acquired arterial spin labeling (ASL) MRI in clinical evaluation of MMA, in settings where nuclear medicine imaging is not available, and the application of a normative perfusion database to identify aberrant cerebral blood flow (CBF) in MMA patients (77). In 2019, Kronenburg et al. showed that the severity of MMA may be related to the presence of leptomeningeal collaterals and to cerebrovascular reactivity (Figures 1–3) (78). In 2019, Liu et al. proposed a new MMA collateral grading system, reflecting the intracranial collateral circulation status, which correlated well with therapeutic prognosis, hemodynamic status, and severity of symptomatology, which may help evaluate the severity of ischemic and hemorrhagic MMA, ascertain the applicable surgical indication, evaluate the surgical risk, and which may facilitate risk stratification and predict prognosis in MMA (79). In their 2019 retrospective study in 68 adult MMA patients, Zhang et al. showed that direct anastomoses of parasylvian cortical arteries with anterograde hemodynamic sources from the MCA may pose an increased risk of post-operative cerebral hyperperfusion in the course of STA-MCA bypass surgery in adult MMA patients (80). In their 2020 study in 16 MMA patients and 9 atherosclerotic cerebrovascular disease (ACVD) patients, using sodium fluorescein (NaFl) to evaluate blood-brain barrier (BBB) permeability *in vivo* intraoperatively, and using intraoperative indocyanine green (ICG) videoangiography, Lu et al. observed that BBB impairment in MMA may be of increased significance in comparison to ACVD. Regarding their study results, the research group stated that cortical perfusion may be significantly decreased in the cerebral cortex with BBB dysfunction in comparison to a cerebral cortex with an intact BBB in MMA patients. Moreover, the research group suggested that BBB dysfunction may lead to increased cortical perfusion after STA-MCA bypass surgery, subsequently contributing to an increased incidence of post-operative cerebral hyperperfusion syndrome (CHS), contributing to delayed intracranial hemorrhage or transient neurological deterioration in MMA patients (81). In their 2021 10-year follow-up study, Wang et al. determined potential predictors of neoangiogenesis and factors which may influence collateral circulation formation following EDAS (Figure 4) (82). The results of the prospective clinical trial between June 2017 and May 2018 in 106 MMA patients, conducted by Wang et al., suggested that atorvastatin administered at 20 mg per day may be effective and safe for post-operative collateral circulation formation induced by EDAS in MMA patients (45).

Moyamoya vascular wall imaging, moyamoya vascular regression, and hemorrhagic moyamoya angiopathy

Established perfusion and luminal imaging methods may not provide sufficient image resolution about progression, onset, and differentiation of cerebrovascular diseases (57). Intracranial High-resolution Magnetic Resonance Imaging (HRMRI) of the vascular wall proved to be an effective imaging method regarding evaluation and comprehension and of cerebrovascular diseases (57). Location and pattern of contrast enhancement in intracranial vascular wall imaging may allow novel insight into the etiology of inflammation in cerebrovascular diseases and may have the capability to anticipate treatment and diagnosis (57). Luminal imaging may not be capable of reliably distinguishing between MMS and MMA (57, 83). On vessel wall imaging, MMS, if accompanied by a vasculopathy, e.g., atherosclerosis, may demonstrate outward remodeling and focal eccentric lesion enhancement (57, 83). On the contrary, MMA-infested vascular segments may infrequently enhance without any outward remodeling (57, 83, 84). If MMA-infested vascular segments do enhance, they may show a slightly concentric, homogeneous pattern (57, 83, 84). In 2014, Ryoo et al. performed an HRMRI study in 32 MMA patients and 16 patients with ICAD-related strokes. In addition to evidence of MMA on imaging, MMA patients showed MCA shrinkage and bilateral distal ICA concentric enhancement (85). In 2015, Yuan et al. showed that HRMRI may detect different types of MCA stenosis. On HRMRI, moyamoya MCA segments were depicted through collaterals, homogeneous signal intensity, and concentric stenosis. MCA shrinkage may be associated with MMA progression (86). In their 2016 imaging study, Han et al. suggested that HRMRI may help diagnose intracranial atherosclerosis with increased precision in MMA patients with risk factors for atherosclerosis. A distinct symptomatology of MMA patients without an identifiable atherosclerotic plaque and MMA patients with an identifiable atherosclerotic plaque present may be indicative of distinct pathophysiologic mechanisms and consequently of potentially diverging treatment strategies (87).

In their 2016 retrospective imaging study in 148 consecutive vessel-wall MRI cases, Mossa-Basha et al. stated that vessel-wall MRI of the carotid artery territory may substantially improve differentiation of moyamoya vasculopathies, including MMA, atherosclerotic-MMS, vasculitic-MMS, and steno-occlusive

intracranial carotid disease, if combined with traditional imaging techniques (83). In their 2016 study in 20 consecutive MMA patients, using gradient echo T2* weighted imaging (WI) involving high-field MRI, Noshiro et al. suggested that cortical and subcortical vascular hypointensity (CSVH) on T2* WI may be a useful tool for both diagnosis and evaluation of the extent of MMA, demonstrating that MMA revascularization surgery may decrease CSVH (88). In 2017, Qiao et al. showed that cortical thickness in MMA may be multifactorial, including structural reorganization, cerebrovascular accident (CVA) lesions, collateral circulation, and major artery involvement, and may assist as a biological marker to evaluate MMA severity (89). Anomalies of the MCA occur less frequently than anomalies of other major intracranial arteries. MCA fenestration, a duplicated MCA origin, a duplicated MCA, and an accessory MCA may develop due to a fusion failure of the primitive arterial network. Clinically, it may be challenging to differentiate an unfused or twig-like MCA from unilateral MMA, in which stenotic change originates at the MCA. Although MCA anomalies may be asymptomatic, and may not require intervention, knowledge of this configuration of an anomalous MCA may be important in neuro-interventional and neurosurgical practice to perform safe endovascular or surgical interventions. If the twig-like MCA may be identically equal to the persistent fetal network of the primitive MCA remains to be ascertained (90). Regarding their 2019 imaging study results, using intracranial 3.0T vessel wall imaging (VWI) and digital subtraction angiography (DSA), Cogswell et al. suggested a decrease in supraclinoid ICA lumen and outer vessel wall diameters, but no significant change in vessel wall thickness, between 23 North American MMA patients and 23 age-matched controls. Furthermore, the research group showed that outer vessel wall diameters and the ICA lumen may decrease with MMA severity (91). In their 2019 study, using quantitative three-dimensional constructive interference in steady state (3D-CISS) imaging, including 8 hemispheres of 7 MMA patients whose Suzuki angiographic stage had progressed spontaneously during follow-up, Yamamoto et al. demonstrated that, in the course of spontaneous disease progression in early-stage MMA, stages 1–3, the outer diameter of respective arteries may serially decrease in parallel to luminal stenosis. The research group suggested that this mechanism may be associated with MMA pathogenesis (92). In their 2019 quantitative 3D-CISS imaging study, Yamamoto et al. showed that involvement of the P2 segment of the posterior cerebral artery (PCA) in MMA may demonstrate both arterial shrinkage and luminal stenosis. MMA progression in the PCA may additionally promote this vascular wall pathology. The research group hypothesized that, from an embryologic perspective, the pathophysiologic mechanism of MMA pathogenesis may be present in both the PCA and the carotid fork (93).

In their 2014 review article, Wan and Duan stated that hemorrhagic MMA may occur in adult patients of Asian populations, and many factors may contribute to the pathogenesis and the etiology of hemorrhagic MMA. Predominant imaging features of hemorrhagic MMA include aberrant branching and dilatation of the posterior communicating artery (PCoA) or anterior choroidal artery (AChA), as well as multiple microbleeds, potentially prognosticating subsequent intracranial hemorrhage (94). In their 2015 case series of 349 hemorrhagic MMA patients, Wan et al. stated that SAH may be a significant type of hemorrhage in MMA patients, ranking as the fourth most common type after intracerebral hemorrhage (ICH), intraventricular hemorrhage (IVH), and combined ICH and IVH. The research group suggested that SAH may predominantly occur in adult females, and rupture of the transdural anastomosis may be the main cause of this condition (95). In their 2016 Letter to the Editor, Duan et al. stated that revascularization surgery may not have the potential of fully preventing recurrent intracranial hemorrhage. Moreover, the research group stated that their research on the arterial vascular wall, using high-resolution magnetic resonance imaging (HRMRI), has demonstrated that ischemic MMA may have distinct features compared to hemorrhagic MMA, and that all episodes of intracranial hemorrhage may have appeared in MMA patients without plaques (96). In their 2016 case-control study, Liu et al. showed that, in comparison to cerebral hemispheres not affected by intracranial hemorrhage, cerebral hemispheres affected by intracranial hemorrhage may be more susceptible to recurrent intracranial hemorrhage. The study results of the research group demonstrated that dilation of the posterior communicating artery (PCoA) or the anterior choroidal artery (AChA), as well as posterior cerebral artery (PCA) involvement, may be related to initial hemorrhage in hemorrhagic MMA, but not to recurrent episodes of hemorrhage (97). In their 2018 retrospective study in 95 hemorrhagic MMA patients, Wang et al. showed that, through long-term follow-up, EDAS may result in a favorable outcome in hemorrhagic MMA patients. The research group suggested that anterior choroidal artery (AChA)-PCoA dilation may be related to initial intracranial hemorrhage in hemorrhagic MMA, and that recurrent episodes of hemorrhage may be age-related (98). According to their preliminary 2019 cohort study results, Funaki et al. indicated that presence of choroidal collaterals, an anastomosis between the medullary arteries and the posterior or anterior choroidal arteries, may affect the risk of recurrent intracranial hemorrhage in the non-hemorrhagic hemisphere of adult hemorrhagic MMA patients, registered in the Japan Adult Moyamoya (JAM) Trial and assigned to the non-surgical study arm (99). According to their 2019 cohort study results, Funaki et al. hypothesized that choroidal collaterals may be a bleeding spot with an increased risk for recurrent intracranial hemorrhage and a marker of recurrent hemorrhage in hemorrhagic MMA (100). In their 2019 retrospective study, Yu et al. stated that, in comparison to patients with acute idiopathic primary intraventricular hemorrhage (PIVH), patients with acute MMA-associated PIVH may exhibit a lower short-term mortality, be of a younger age, may have a more favorable renal function, and a lower admission blood pressure (101). In their 2020 study, Zhang et al. compared the five-year prognosis in 123 adult hemorrhagic MMA patients who underwent either combined superficial temporal artery to middle cerebral artery (STA-MCA) bypass and EDAS, or EDAS alone. The research group stated that both combined revascularization and EDAS alone may reduce the risk of recurrent hemorrhage in hemorrhagic MMA patients (102). Combined revascularization was found to be superior to EDAS alone regarding the prevention of recurrent hemorrhage (102). In Kaplan–Meier survival analysis, combined revascularization was demonstrated to have a more favorable prognosis compared to EDAS alone, and multivariate regression analysis demonstrated that the combined revascularization procedure may be related to a more favorable outcome (102). In 2021, Wu et al. stated that the choroidal anastomosis may be related to hemorrhagic adult MMA at an advanced stage, suggesting the validation of choroidal

anastomosis as an imaging biomarker of hemorrhagic MMA. HRMRI may provide detailed information on both aberrant collaterals and the anatomy in MMA, facilitating risk estimates of moyamoya hemorrhage (Figures 1–3) (103).

Pathophysiologic characteristics of inflammation in pediatric ischemic stroke

The significance of inflammation in pediatric stroke has become noticeably evident (47). Ischemia may trigger various cascades of inflammatory reactions, both alleviating and aggravating ischemia, including inhibition and activation of inflammation through chemokines, proteases, adhesion molecules, and cytokines (47, 48). Furthermore, it has been demonstrated that the pathophysiology of pediatric stroke may be associated with inflammation (47), as evident in transient cerebral arteriopathy (47, 49) and post-varicella angiopathy (47, 50). Consequently, in pediatric stroke, ischemia may cause inflammation, and inflammation may equally lead to ischemia (47). In comparison to the adult brain, significant differences are evident in the neonatal brain (47). In neonatal stroke, ischemia may be the predominant pathophysiologic mechanism, with inflammation and infection having a significant effect on the degree and course of tissue damage (47). In childhood, ischemia may be caused by an associated inflammatory pathophysiologic mechanism, as evident in MMA, sickle cell anemia, dissection, transient focal arteriopathy, and, increasingly generalized, in generalized vasculitis, meningitides, and genetic arteriopathies such as Deficiency of Adenosine deaminase 2 (DADA2) (47). Focal inflammation is prone to be located in the distal ICA or the proximal medial cerebral artery (MCA), whereas generalized inflammation predominantly affects small arteries (47).

Various genes may be associated with MMA (47, 51). Whether these genes are dysfunctional due to ischemia or inflammation or whether these genes are dysfunctional as such remains to be elucidated (47, 51). The *Ring finger protein 213* (*RNF213*) (17q25.3) genetic variant has been demonstrated to be expressed at an increased level in mature lymphocytes in comparison to lymphoid progenitor cells (47, 51). In MMA, blood levels of circulating endothelial progenitor cells (EPC) may be increased, suggesting that the *RNF213* genetic variant may alter the function of EPCs in the spleen (47, 51). C3, IgG, and IgM have been demonstrated in the vascular wall of MMA patients (47, 52). Fujimura et al. (47, 104) and Young et al. (5, 47) have reviewed signaling cascades and the histology associated with MMA, suggesting an increase in transforming growth factor (TGF), hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF) in MMA patients (47). These growth factors may be related to angiogenesis and inflammation (47). Extracellular inflammatory biomarkers including matrix metalloproteinase (MMP)-9, interleukin (IL)-8, and prostaglandin may be increased in MMA patients (47). If disease progression may be affected through stimulation or blockade of particular sequences of a signaling cascade remains to be ascertained (47). Blockade of several of such elements may reduce perioperative surgical risk (47, 53).

Cerebral ischemia may initiate an inflammatory signaling cascade causing cell death, which subsequently may initiate inflammation (47, 48). Hypoperfusion may initiate anaerobic glycolysis which may catalyze two main metabolic pathways causing inflammation: sodium-potassium pump failure may cause excitotoxic glutamate release and membrane destabilization. Activation of α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate and N-methyl-D-aspartate receptors or signaling pathways may lead to both necrosis, and, through intracellular increase of sodium and potassium, to inflammation, oxidative stress, and mitochondrial failure (47, 48). Through blood-brain barrier (BBB) disturbance and membrane degradation, anaerobic glycolysis may lead to inflammation, cyclooxygenase (COX) activation, leukocyte infiltration, and cell adhesion molecule expression. Inflammation may cause both necrosis and apoptosis (47, 48). Inflammation may cause the release of various distinct proteases, chemokines, cytokines, and adhesion molecules which may affect the inflammation process (47, 48). Inflammation may as well be related to coagulation, leading to a procoagulant state through its impact on fibrin formation (47, 48). Also, endothelial inflammation may affect coagulation and lead to blood clot formation (47, 48). The postischemic inflammation pathway in the adult is complex, yet increasingly ascertained (47). An extensive network of anti-inflammatory and pro-inflammatory chemokines, proteases, adhesion molecules, and cytokines exists (47, 48). Initial substrate release predominantly stimulates inflammation within the initial hours and minutes (47, 48). Subsequently, predominantly anti-inflammatory substrates, leading to angiogenesis and recovery, are released (47, 48). Post-ischemic necrotic neurons may release damage associated molecular patterns (DAMPs), thus activating macrophages (47, 48). Macrophages are associated with proinflammatory cytokine release, including IL-1β and TNF-α, which may induce inflammation (47, 48). Also, macrophage IL-23 release may cause T-cell recruitment which, through IL-17 release, may induce inflammation (47, 48). Such an inflammatory reaction may be induced within hours and minutes after ischemia onset (47, 48). Over weeks and days after ischemia, immune cells are associated with anti-inflammatory substance production, such as TGF, insulin-like growth factor (IGF), and IL-20 (47, 48). Purine release may assist in cleaning necrotic cells of debris, and VEGF release may lead to angiogenesis (47, 48). Secretion of anti-inflammatory and inflammatory biomarkers, such as proteases, chemokines, and cytokines, may be ascertainable in the cerebrospinal fluid (CSF) during acute stroke (47, 54, 55). These biomarkers may be related to stroke severity and stroke subtypes, and may further elucidate stroke pathogenesis (47, 54). Various research has been designed for further ascertainment of these distinctive signaling cascades, aiming at modifying factors relating to the post-ischemic disease process (47). Comparison of adult rodents to preterm and/or neonatal rodents demonstrated that, while signaling cascades may be similar, there may be differences between the adult system and the immature prenatal system (47). Various experimental models for age-related ischemia may be warranted to further ascertain these signaling cascades in addition to encourage research into interventions to improve patient outcome (47). The pathophysiology of pediatric stroke may be caused through inflammation, which may exert a specific effect on the inflammatory signaling cascade related to ischemia (47, 56).

Physiologic characteristics of angiogenesis, arteriogenesis, and vasculogenesis

Moyamoya vessels suggest that aberrant angiogenic, arteriogenic, and vasculogenic processes may be involved in the pathophysiology of the arteriopathy (105).

Physiologic angiogenesis comprises six steps. Step one includes vasodilation, endothelial permeability and periendothelial support. Vasodilation involves NO. VEGF increases vascular permeability and promotes angiogenesis. Angiopoietin (ANG)1 inhibits vascular permeability and stabilizes preexisting vessels. ANG2 removes vascular smooth muscle cells (SMC) and loosens the extracellular matrix (ECM) (13, 14). Matrix metalloproteinases (MMPs) degrade ECM molecules and activate VEGF, basic fibroblast growth factor (bFGF) and IGF-1 (13). Step two includes endothelial cell migration and proliferation. VEGF and VEGFR2 are involved in aberrant, embryonic and neonatal angiogenesis. VEGFR3 is involved in aberrant and embryonic angiogenesis (13). $VEGF_{120}$ initiates angiogenesis. VEGF-C may contribute to aberrant angiogenesis in the adult (13). ANG1 is chemotactic for endothelial cells, potentiates VEGF, and induces angiogenesis (13, 14). bFGF and platelet-derived growth factor (PDGF) affect angiogenesis by attracting inflammatory or mesenchymal cells (13). Markers involved in cell-matrix and/or cell-cell interactions, e.g., αvβ3 integrin, may facilitate endothelial spreading (13). EphrinB2 and platelet endothelial cell adhesion molecule (PECAM)-1 may be associated with aberrant angiogenesis (13). In ischemia, eNOS mediates aberrant VEGF-initiated angiogenesis (106). Step three comprises lumen formation. Endothelial cell thinning or intercalation and fusion of pre-existing vessels may increase vessel diameter and length (13). VEGF121, VEGF165 and their receptors increase lumen formation and vessel length. VEGF189 decreases the luminal diameter, VEGF in combination with ANG1 increases the luminal diameter (13). αvβ3 or α5 integrins influence lumen formation. Thrombospondin (TSP)-1 inhibits lumen formation (13). Step four comprises endothelial survival. Reduced endothelial cell survival causes vascular regression in the embryo (13). Shear stress is essential for vascular maintenance. Endothelial survival factors VEGF, ANG1, and αvβ3 activate p42/44 MAPK, survivin, and PI3K/Akt pathways (13). Step five comprises endothelial differentiation. Specialized endothelial cells are partly determined by their host tissue. Interaction between VEGF and the ECM, causes endothelial cells to become discontinuous and fenestrated (13). Step six comprises remodeling. Vessel replacement by matrix causes vessel branching. A morphogenetic function of VEGFR3, VEGF isoforms, Tie1, vascular cell adhesion molecule-1 (VCAM-1), Jagged, α4 integrin, Gα13 GTP-binding protein, chemokine receptor 4, and fibronectin may be suggested by gene inactivation studies (13). Aberrant angiogenesis is often induced by inflammation. In inflammation and wound healing, VEGF attracts monocytes/macrophages, mast cells, platelets and other leukocytes, which release arteriogenic as well as angiogenic factors, including TGF-β1, bFGF, VEGF, platelet-derived growth factor (PDGF), tumor necrosis factor (TNF)-α, monocyte chemoattractant protein-1 (MCP-1) and IL-8, causing recruitment of endothelial cells, SMCs, platelets, fibroblasts or leukocytes, leading to aberrant angiogenesis (13).

Physiologic arteriogenesis comprises three steps. Regarding step one, in SMC migration and growth, aberrant arteriogenesis causes enlargement of preexisting collaterals after occlusion of the supplying artery. Consequently, endothelial cells express MCP-1 as well as intercellular adhesion molecule 1 (ICAM-1). Vascular wall infiltration and media disruption by monocytes is associated with TNF-α and proteases. Subsequently, endothelial cells upregulate PDGF-BB, bFGF, and TGF-β1, thus inducing SMC growth and vessel enlargement (13). In step two, a lack of fibrillin-1, fibrillin-2, collagen and elastin causes vessel wall weakening and aneurysmal dilatation. Elastin decreases SMC growth, and thereby prevents intimal hyperplasia (13, 107). In atherosclerosis or restenosis SMCs dedifferentiate from a contractile to an embryonic, synthetic phenotype (13). Regarding step three, in remodeling a sustained imbalance between NO and endothelin-1 may induce vasospasms and progress to vascular loss (13).

Physiologic vasculogenesis refers to primitive network formation. VEGF, VEGFR2 and bFGF influence angioblast differentiation. VEGFR1 suppresses hemangioblasts. TGF-β1 and TGF-β receptor 2, α5 integrin and fibronectin affect vasculogenesis (13).

HIF-1β, HIF-1α, and HIF-2α induce angiogenesis and arteriogenesis through VEGFR2, VEGFR1, VEGF, ANG2, PDGF-BB, TGF-β1, Tie1, NOS, IL-8, endothelin-1 and cyclooxygenase (COX)2 expression. Hypoglycemia and a low pH induce vessel growth (13). Vasculogenesis is flow-independent, angiogenesis is flow-dependent and hypoxia regulated (13). bFGF affects vascular tone. NO and P-selectin influence vascular remodeling through shear-stress-induced gene transcription (13).

Physiologic characteristics of angiogenesis signaling pathways

Signaling pathways associated with a condition may provide an interface of genetic and environmental interaction. Integration and crosstalk between signaling pathways may occur at intracellular nodes where signaling cascades intersect (15), and also at the level of receptor activation (20).

ANG1-Tie receptor tyrosine kinases (Tie)2 binding leads to cross-phosphorylation of cytoplasmic Tie2 tyrosine residues, which recruit adaptor proteins that activate PI3K/Akt, MAPK, Erk, and docking protein 2 (Dok-R) signaling pathways. These pathways are involved in recruiting and sustaining periendothelial support cells (e.g., pericytes, SMCs) that relate to stabilization and maturation of newly formed vessels (18, 19). In quiescent vessels, ANG1 recruits Tie2 to cell-cell contacts, forming complexes with Tie2 from adjoining cells, thus activating PI3K/Akt signaling (16, 18, 19, 23). Migrating endothelial cells cause ANG1 to recruit Tie2 into contact with the ECM, which causes the formation of focal adhesion complexes and activation of PTK2/FAK, MAPK-1/ERK2, and MAPK3/ERK1; this, in turn, causes sprouting angiogenesis (16–22). Activation of Tie1-Tie2 heterodimers depends on β1 integrin (21). In ischemia, ANG1 causes synchronous activation of Tie2 and integrin signaling, which is related to angiogenic remodeling and tightening of endothelial cell junctions (Figure 5) (18). Tie1 deficiency impairs ANG1-induced Tie2 and Akt phosphorylation and FOXO1 inactivation, leading to FOXO1 nuclear translocation (21). Inflammation causes cleavage of the Tie1 ectodomain, which results in a switch of ANG2 from a Tie2 agonist to a Tie2 antagonist, linked to a positive feedback loop of FOXO1-driven ANG2 expression, causing endothelial cell destabilization *via* β1 integrin, vascular remodeling, and leakage

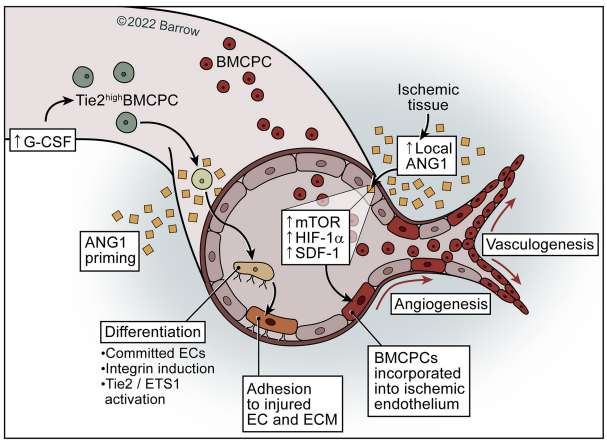

FIGURE 5
Model of ANG1 in neovascularization. ANG1 may activate and increase mTOR–HIF-1α-SDF-1 signaling in the ischemic endothelium and subsequently facilitate the incorporation of BMCPCs into the ischemic endothelium, leading to neovascularization in ischemic tissue. Priming of mobilized Tie2^{high} BMCPCs with ANG1 causes activation of Tie2/Ets-1, resulting in incorporation of BMCPCs into the endothelial linage and the induction of integrins, which leads to increased adhesion to endothelial cells and the extracellular matrix. See Supplementary material. *Used with permission from Barrow Neurological Institute, Phoenix, Arizona.*

(21, 24, 25). Autocrine secretion of ANG2 disrupts connections between endothelium and perivascular cells, causing cell death and vascular regression (16) that lead to impaired barrier properties of brain endothelial cells and intracranial hemorrhage and ischemia (16, 26). ANG2 and VEGF block ANG1-mediated stabilization and maturation, resulting in endothelial cell migration and proliferation and, then, angiogenic neovascularization (18). ANG1-Tie2 activation stimulates recruitment of ABIN-2 that, in turn, creates suppression of NF-κB, a pro-inflammatory transcription factor, and protection of endothelial cells from apoptosis. Truncated ABIN-2 inhibits ANG1 from preventing endothelial cell death (27).

The erythropoietin (Epo)/Epo receptor (EpoR) signaling pathway induces proliferation, migration, chemotaxis, and angiogenesis, and inhibits apoptosis (28, 29). EPO signaling potentially inhibits apoptotic pathways triggered by ischemia and may reduce hypoxic injury by promoting or facilitating angiogenesis (28). Cytokines and growth factors associated with hematopoiesis may also be involved in angiogenesis (31). Endothelial cells expressing EPO-R respond to EPO by differentiation into vascular structures, associated with JAK2 phosphorylation, cell proliferation, and MMP-2 production (30, 31).

The erythropoietin-producing human hepatocellular receptor (Eph)/Eph receptor-interacting protein (ephrin) signaling pathway is involved in vasculogenesis and tissue homeostasis (32, 35). Eph-ephrin bidirectional signaling affects both receptor- and ephrin-expressing cells and segregates Eph-expressing cells from ephrin-expressing cells (33–35). Eph and ephrin may contribute to vascular development by restricting arterial and venous endothelial mixing thus stimulating the production of capillary sprouts and also by stimulating mesenchymal differentiation into perivascular support cells (32). EphA receptor activation may be involved in VEGF-induced angiogenesis (32). Cooperation between ephrin-A1 and Slit2 regulates a balance between pro- and antiangiogenic functions of Slit2, suggesting Slit2 may differentially regulate angiogenesis in the context of ephrin-A1 (36). The Eph family transmembrane ligand ephrin-B2 marks arterial but not venous endothelial cells. The ephrin-B2 receptor Eph-B4 marks veins but not arteries. Differences between arteries and veins may be in part genetically determined, suggesting that reciprocal signaling between arterial and venous endothelial cells is essential for morphogenesis of the capillary beds (37). Interaction of ephrin-B2 on arterial endothelial cells and Eph-B3 and Eph-B4 on venous endothelial cells may define the boundary between arterial and venous domains (37). EphB2 and ephrin-B2 expression on mesenchymal cells suggests involvement in vessel wall development *via* endothelial-mesenchymal interaction (38). Absent ephrin-B2 expression in mice disrupts embryonic development of the vasculature due to a deficient restructuring of the primary network (39).

The Janus kinase-signal transducer and activator of transcription protein (JAK-STAT) signaling pathway includes cytoplasmic signal transducer and activator of transcription (STAT)1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. STATs are activated by tyrosine phosphorylation in response to external stimuli, including cytokines, growth factors, and hormones. Ischemia leads to estradiol-, IL-6-, EPO-, and G-CSF-mediated tyrosine phosphorylation and activation of STAT3. STAT3 dimerization and translocation to the nucleus stimulate binding of DNA regions in STAT-inducible elements, which leads to transcription of neuroprotective genes linked to estradiol-mediated neuroprotection and neuronal survival. Endothelial STAT3 activation causes endothelial cell migration and proliferation, leading to angiogenesis and ECM remodeling that are important in long-term post-stroke recovery (40–42).

Moyamoya angiopathy related angiogenesis and inflammation signaling pathways

The pathogenesis of MMA and MMS may be associated with infection and inflammation (5, 108). Imbalance of angiogenic and vasculogenic mechanisms has been suggested to be a potential cause of MMA (105). Aberrant expression of angiogenic factors, adhesion molecules, and mitogens, and/or an aberration of the cellular immune response to cytokines and growth factors may indicate an association of hematopoietic as well as inflammatory signaling pathways with cells of the vasculature, which has been hypothesized to constitute an essential pathophysiologic mechanism in MMA pathogenesis (Figure 4) (5, 109–113).

Phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) signaling pathway activation may occur due to Ras mutation, loss of phosphatase and tensin homolog, or increased expression of growth factor receptors. The PI3K signaling pathway is involved in blood vessel formation during embryogenesis. Embryos with a kinase-dead PI3K p110α catalytic subunit develop vascular defects. The PI3K/Akt signaling pathway modulates expression of angiogenic factors (e.g., NO, ANGs) (114). Activated Tie2 stimulates the PI3K signaling pathway, which activates the protein kinase B/Akt, eNOS, MAPK, and docking protein 2 (Dok-R)/cytoplasmic protein NCK1 (Nck)/p21-activating kinase (Pak) signaling pathways. These pathways affect endothelial cell survival and NO synthesis (115). VEGF stimulation

of endothelial cells activates the PI3K pathway and leads to cell migration. Endothelial activation of Akt1 is associated with structurally abnormal blood vessels. PI3K/Akt/mTOR pathway inhibition decreases VEGF and angiogenesis (114). Increases in caveolin-1 lead to decreases in ceramide synthesis, inhibiting the Akt signaling pathway, cell proliferation, migration, and invasion, thus inhibiting PI3K activity (116). Hypoxia-inducible factor (HIF)-1 expression is associated with the PI3K/Akt signaling pathway in MMA (9, 117). The PI3K/Akt signaling pathway in endothelial cells may lead to transcriptional activation of *Ring finger protein 213* (*RNF213*) (17q25.3). Inhibition of the PI3K/Akt signaling pathway has been demonstrated to decrease inflammation in autoimmune diseases (9, 118). MMA, if associated with inflammation, may be related to the PI3K/Akt pathway (9). Molecular networks may associate sGC with E3 ubiquitin-protein ligase RNF213 (RNF213) (119). Nuclear factor of activated T-cells 1 (NFAT1), a RNF213 ubiquitin target downstream of the non-canonical Wnt/$Ca2^{+}$ signaling pathway, is a pivotal molecule (119, 120). Calcineurin/NFAT signaling activation through VEGF in human EPCs may lead to increased expression of Nitric oxide synthase, endothelial (eNOS) and generation of NO (119, 121). NFAT may regulate sGC expression by NFAT1 binding to the Guanylate cyclase soluble subunit alpha-1 (GUCY1A3) consensus sequence (119, 122). PI3K/AKT activation may cause glycogen synthase kinase (GSK)-3β inactivation, leading to proteasomal degradation of NFAT1 (119, 123). The impact on NFAT1 by PI3K/AKT might be mediated through RNF213, as PI3K/AKT may be an upstream regulator of *RNF213* expression in endothelial cells (119, 124). Moreover, *Nuclear Factor Of Activated T Cells 1* (*NFAT1*) (18q23) upregulation may be mediated through RNF213 S-nitrosylation (119, 125). S-nitrosylation means posttranslational modification through addition of a nitrosyl group to the reactive thiol group of cysteine, forming S-nitrosothiol, which constitutes a pivotal mechanism in NO-mediated signal transfer (119). Ubiquitin ligase S-nitrosylation may lead to its auto-ubiquitination, thus increasing its substrate levels. NFAT, through cGMP-dependent protein kinase (PKG) activation, which leads to GSK-3β phosphorylation, may be regulated through sGC (119, 126). Furthermore, caveolin may be associated with NO signaling regulation (119).

Caveolin-1, an ∼ 21–24 kDa integral membrane protein, is present predominantly in plasma membrane caveolae, 50–100-nm flask-shaped invaginations, where it functions as a scaffold to arrange a multitude of molecular complexes which regulate diverse cellular functions. Caveolin-1 may be regulated through the $Ca2^{+}$/calcineurin/NFAT signaling cascade (119, 127). Caveolin-1 has been stated to be related to pulmonary arterial hypertension, coronary artery disease, and MMA (119). Caveolin-1 functioning may be sufficiently studied in pulmonary arterial hypertension (119, 128–130). In comparison to healthy controls or cerebral atherosclerotic stroke patients, caveolin-1 levels have been demonstrated to be decreased in MMA, and were shown to be distinctly decreased in patients with the *RNF213* R4810K genetic variant (119, 131). If RNF213 bears an indirect or a direct relation to caveolin-1, remains to be ascertained, e.g., caveolin-1 may be a target object for ubiquitination through RNF213. eNOS release is related to caveolin-1. eNOS release produces NO, which may be metabolized through sGC. eNOS binding to the caveolin-1 scaffolding domain has been associated with eNOS inactivation (119, 132). Caveolin-1 absence may lead to dysfunction of eNOS, which has been related to cerebrovascular diseases (119). NF-κB and HIF-1 are involved in inflammation regulation (9, 133). *RNF213* genetic variants may cause NF-κB-associated inflammation, leading to VSMC damage, which is characteristic of MMA pathophysiology (119). RNF213 may lead to lipotoxicity-mediated protection of cells against inflammation and endoplasmic reticulum (ER) stress (119, 134). RNF213 depletion may cause NF-κB pathway inhibition during exposure to palmitate, may reestablish the cellular lipidome, and may stabilize the expression of the ER stress gene (119, 134). Recent research has demonstrated that RNF213 may concur with Ubc13, the E2 enzyme, leading to K63-linked polyubiquitin chain generation (119, 135, 136). K63 linkages have been associated with protein sorting, removal of defective mitochondria, innate immune responses, DNA repair, and with regulation of NF-κB transcription factor activation (119, 137). Deletion of Lys-63-specific deubiquitinase BRCC36 (BRCC3), an E3 ligase cleaving K63-linked polyubiquitin chains specifically, has been related to X-linked MMS (119, 138). BRCC3 may be associated with DNA damage response, and may regulate an abundance of such polyubiquitin chains in chromatin (119). The majority of genetic changes in the *RNF213* RING finger domain proven in MMA patients may diminish E3 ligase activity, and various of these genetic changes may induce NF-κB activation (119, 136). Such genetic changes, which may lead to NF-κB activation, may include both Caucasian cysteine/histidine mutations and proline mutations, such as P4033L in a Caucasian patient as well as p.P4007R in a Chinese patient (119). In association with NF-κB, these genetic changes may lead to apoptosis (119). The p.D4013N genetic variant may neither affect NF-κB activation nor E3 ligase activity (119). Point mutations in both the Walker B and A motif of the AAA domains, may fully eliminate NF-κB activation through genetic changes in the *RNF213* RING finger domain (119). Consequently, NF-κB signaling pathway-mediated inflammation may be suppressed in absence of *RNF213*, while inflammation may be augmented through *RNF213* genetic variants in MMA patients (119). With respect to NF-κB activation, *RNF213* genetic variants may be associated with gain of function (119). RNF213 may as well regulate immune cell maturation and differentiation, the cell cycle, and mitochondrial function. These characteristics may be associated with MMA pathogenesis (119). Caveolin-1 is related to inflammation, and may be associated with MMA (9, 139, 140). Caveolin-1 serum levels were shown to be decreased in MMA, and demonstrated to be significantly decreased in MMA patients with the *RNF213* genetic variant (9, 131). Caveolin-1 has been associated with angiogenesis (9, 141, 142), along with a bidirectional interaction between the Caveolin-1/ERK and the Wnt/β-catenin pathway (9, 143).

In 2000, Galbiati et al. hypothesized that caveolin-1 expression may control Wnt/β-catenin/Lef-1 signaling through modulating the intracellular β-catenin localization (144). The Wnt signaling pathway may be related to angiogenesis (9). In 2016, Scholz et al. demonstrated that the endothelial RSPO3-driven non-canonical Wnt/$Ca2^{+}$/NFAT signaling pathway may be associated with vascular stability maintenance, providing insight into vascular remodeling mechanisms (120). Furthermore, the research group stated that *RNF213* in vascular endothelial cells may be associated with the Wnt signaling pathway and angiogenesis regulation (9, 120). Under physiologic conditions, stimulation of endothelial cells through shear stress or growth factors may induce the $Ca2^{+}$-calmodulin

signaling cascade (119). Calmodulin may accelerate dissociation of eNOS from caveolin-1 and may enhance eNOS generation through Calcineurin/NFAT1 (119) RNF213, which may degrade NFAT1 by means of the ubiquitin proteasome system, may not be activated in absence of a pathological stimulus (119). From L-Arginine, eNOS subsequently generates NO, which may diffuse into vascular smooth muscle cells (VSMCs) (119). In VSMCs, NO may induce sGC to generate cGMP. cGMP may subsequently activate the cGMP-dependent protein kinase (PKG), leading to VSMC relaxation (119). Under pathological conditions, in which a viral infection may lead to destruction of mitochondria, *RNF213* may be up-regulated and may inhibit the generation of eNOS through NFAT1 degradation (119). *RNF213* genetic variants in MMA patients may sustain an inflammation even after remission of the causative infection, which may lead to sustained impairment of the cGMP signaling pathway (119). *GUCY1A3* genetic variants may cause an identical environment (119). cGMP signaling pathway impairment may lead to endothelial dysfunction, fibrosis, impaired vasodilation, and proliferation of VSMCs (119). Such processes may lead to intimal hyperplasia with fibrous thickening, which has been associated with MMA pathogenesis (119). As cGMP signaling pathway impairment may lead to endothelial-to-mesenchymal transition and dedifferentiation of VSMCs, those cells may cause fibrosis (119). Lessened protection against stroke may constitute a pathophysiologic mechanism of MMA arterial stenosis (119). RNF213 may possess antibacterial and antiviral properties and may regulate lipotoxicity, whereas sGC may provide protection against homocysteine (119). Bacterial and viral infection may cause mitochondrial dysfunction and interferon (IFN) I generation, which may lead to increased *RNF213* expression (119). In case genetic variants in MMA patients cause a dysfunction of sGC or RNF213, vascular damage related to infection, dyslipidemia, and homocysteine may lead to chronic inflammation (119). Moreover, *RNF213* genetic variants may cause inflammation through NF-κB, leading to VSMC damage, a characteristic of MMA pathogenesis (119). In their 2016 study in 15 adult MMA patients, Gao et al. stated that expression patterns of long non-coding ribonucleic acids (lncRNAs) may differ between MMA patients and healthy controls (10). Various signaling pathways related to smooth muscle contraction, vasculogenesis, and immune response may be associated with the regulatory mechanism of lncRNAs (10). Mitogen-activated protein kinase (MAPK) signaling pathway was found to have a central function in this regulatory network of signaling pathways (10). In 2021, Sarkar and Thirumurugan demonstrated the regulation of *RNF213* through the TNFα/PTP1B signaling pathway and PPARγ, suggesting that *RNF213*, similar to TNFα, may constitute an additional connection between MMA, inflammation, insulin resistance, and obesity (11). Toll-like receptors (TLR) have been ascertained to be essential in activating the innate immunity through recognition of distinct patterns of microbial constituents. Toll-interleukin-1 receptor (TIR) homology domain-containing adapter protein Myeloid differentiation primary response 88 (MYD88) may be indispensable for the induction of pro-inflammatory cytokines induced by all TLRs (145). In 2020, Key et al. stated that in MMA, the low penetrance of *RNF213* mutations may be modified through dysfunctions in the TLR3 signaling pathway or the mitochondria (Figure 4) (146).

Due to infections or autoimmune diseases and induced by inflammatory cytokines, every signal transduction pathway involved in MMA may be reciprocally activated by *RNF213* (9).

Moyamoya angiopathy cell-based biomarkers

Derived from the bone marrow, circulating endothelial progenitor cells (EPC) are involved in postnatal physiological and pathological neovascularization (9, 147, 148). Circulating EPCs have become objects of moyamoya research, referring to the hypothesis that MMA is associated with constant vascular remodeling, involving both the subsequent angiogenesis from collateral development as well as the primary arteriopathy. SMC proliferation in the vascular wall of affected arteries has frequently been demonstrated in MMA (2, 61). Analysis of smooth muscle progenitor cells (SPCs) isolated from the blood of MMA patients demonstrated a differential expression exceeding 200 genes, including a decreased CD31 expression, and irregular tube formation in assays in comparison to matched controls (2, 149). Studies have indicated the migration of endothelial cells into the ICA intima in stenotic sections in moyamoya, hypothesizing that these cells might be involved in both distal collateral development and proximal arterial narrowing (2, 150). CD34+ cells, a subpopulation of endothelial progenitor cells, have been reported to be increased in the blood of MMA patients compared to healthy controls and also when compared to patients with non-MMA intracranial arterial stenosis (2, 151, 152). Inconsistent results have been obtained from research into CD34+ cells in pediatric MMA. Kim et al. performed a study in 28 pediatric MMA patients, demonstrating decreased levels as well as a defective function of CD34+ cells compared to 12 healthy volunteers (2, 9, 153). Rafat et al. performed a study in 20 adult MMA patients, demonstrating an enhancement of circulating EPCs. The research group suggested an involvement of circulating EPCs in angiogenesis and arteriogenesis in MMA (9, 154). A decrease in EPCs following revascularization surgery in MMA has also been reported (9, 155). EPCs secrete angiogenetic factors including ANG1, hepatocyte growth factor (HGF), VEGF, stromal-derived factor-1*a* (SDF-1*a*), bFGF, PDGF, and IGF-1 (9, 152, 154, 156–158). Tinelli et al. morphologically, phenotypically, and functionally characterized circulating EPCs from the peripheral blood of a homogeneous group of adult Caucasian, non-operated MMA patients and healthy controls, suggesting that a significantly reduced circulating EPC level may be a potential marker of MMA (105). Analyzing the function of circulating EPCs *in vitro*, as measured by assays of colony formation and tube formation, may indicate a significantly decreased function of these cells in MMA (2, 9, 159). Choi et al. suggested an impaired functional recovery of EPCs *in vivo* in moyamoya patients in comparison to controls (9, 160). In 2008, Jung et al. stated that distinct characteristics of circulating EPCs (CFU numbers and tube formation were found to be lower in advanced MMA cases than in those with early stage disease, and outgrowth cells were more frequently detected in those with early MMA and moyamoya vessels than in those with advanced MMA) may reflect mixed conditions of aberrant vasculogenesis and vasculars occlusion in MMA pathogenesis (159). Regarding their 2008 study results, Yoshihara et al. suggested that an increased level of CD34+ cells, related to ischemia, may be correlated with neovascularization of the human arterial cerebral circulation at sites of ischemic brain injury (151). In their 2010 study, Kim et al. demonstrated that pediatric MMA may be related to reduced expression of circulating EPCs, to proneness to senescence, defective tube formation, and

impaired differentiation. Such a limited capacity of EPCs may lead to insufficient cerebrovascular repair or aberrant vessel formation (153). In their 2011 study, Ni et al. suggested that binding of CXCR4 on CD34+ cells to mediate CD34+ cell migration may lead to an increased level of SDF-1α, hypothesizing that increased levels of circulating SDF-1α and CD34+ CXCR4+ cells in MMA patients may be associated with moyamoya vasculogenesis (152). In their 2011 study, using supraclinoid ICA specimens from two adult MMA patients, Sugiyama et al. stated that VEGFR2- and CD34-positive cells were abundantly demonstrated in the thickened intima of occlusive arterial lesions, clustered especially in the superficial layer of the thickened intima. Also, the research group demonstrated that CD34-positive cells expressed von Willebrand factor on the surface of the thickened intima and were positive for α-smooth muscle actin in the deeper layer, suggesting that circulating EPCs may be associated with occlusive arterial lesion development in MMA (150). In their 2018 study, Bao et al. researched circulating endothelial cells (CECs) in the plasma of 66 MMA patients compared to 81 healthy controls, showing that the amount of CECs was negatively correlated with concomitant disorders including coronary heart disease, diabetes mellitus, and hypertension in MMA patients (161). In their 2021 study, Matsuo et al. demonstrated that vulnerability to shear stress, caused through an aberrant peri-endothelial matrix, may be a predominant characteristic of MMA (162). The research group stated that the peri-endothelial extracellular matrix may be important regarding endothelial protection, cell adhesion and migration, and that an altered peri-endothelial matrix in MMA may contribute to endothelial vulnerability to vascular wall shear stress. Invading EPCs, which repair endothelial damage, may produce excessive hyaluronan and chondroitin sulfate in the intima, and may lead to vascular stenosis (162). In 2021, Wang et al. stated that collateral vessel formation in encephaloduroarteriosynangiosis (EDAS) surgery, a common method for indirect revascularization, may be associated with angiogenesis, and that the EPC count may be essential for facilitating collateral circulation formation. The research group hypothesized that EDAS may prove particularly advantageous for severe ischemic or younger MMA patients (163). In their 2021 study, Wang et al. performed comprehensive profiling of the protein profiles expressed in serum-derived exosomes (SDEs) of MMA patients performing Tandem Mass Tag-labeled quantitative proteomics, demonstrating disturbed actin dynamics in MMA patients, with actin-related protein 2/3 (ACTR2/3) and Cofilin-1 (CFL1) downregulation in SDEs. Distinct expression of immune-related proteins was shown in exosomes, suggesting an alteration of immune responses in hemorrhagic MMA patients. Also, the research group stated that exosomes in hemorrhagic MMA may lead to vascular endothelial cell (EC) proliferation, potentially by induction of mitochondrial dysfunction by means of oxidative phosphorylation and an aberrant electron transport chain (164).

In their 2022 review article, Xue et al. reviewed recent progress and pitfalls in MMA induced pluripotent stem cell (iPSC) research, providing a perspective of iPSC molecular mechanisms and novel MMA treatment strategies (165). In their 2016 study, Hamauchi et al. demonstrated that downregulation of ECM receptor-related genes may be related to impaired angiogenesis in iPSC-derived ECs of MMA patients. The research group stated that upregulation of splicing regulation-related proteins may imply varieties of splicing patterns between ECs of MMA patients and controls (166). In 2016, Cardano et al. described the establishment of an induced pluripotent stem cell (iPSC) line from an 8-year-old female patient with ischemic MMA (167). In 2016, Cardano et al. described the establishment of an induced pluripotent stem cell (iPSC) line from a 55-year-old male patient with hemorrhagic MMA (168). In 2020, Tokairin et al. performed a study in 3 MMA patients and 3 independent healthy controls, which determined vascular smooth muscle cells (VSMCs) from neural crest stem cells using patient-derived induced pluripotent stem cell (iPSC) lines to detail the transcriptome profile and the biological function of MMA VSMCs, suggesting that MMA pathology may be influenced by naive endothelial cells (EC), whereas MMA VSMCs may require specific environmental factors, thereby further elucidating MMA pathophysiology. The research group stated that, in addition to the existing iPSC derived EC model, their iPSC-derived VSMC model may further ascertain therapeutic and diagnostic objectives in MMA (169). In their 2021 study, Mao et al. demonstrated the generation of an induced pluripotent stem cell (iPSC) line HUSTTJi001-A from an MMA patient with a *RNF213* genetic variant. The research group stated that this iPSC line may show pluripotent biomarkers, may have the potential for *in vitro* differentiation into three germ layers, may be suitable for ascertainment of MMA cellular mechanisms, for the selection of therapeutic targets, and for drug development (170).

In 1993, Masuda et al. performed an autopsy study in 6 MMA patients, using immunohistochemical staining by cell-type-specific monoclonal antibodies, stating that SMCs in MMA may be proliferating in occlusive lesions of intracranial major arteries. Furthermore, the research group stated that colocalization of proliferating cell nuclear antigen (PCNA)-positive and inflammatory cells, including T cells and macrophages, may suggest that inflammation could induce proliferation of SMCs and thus contribute to formation of intracranial occlusive lesions in MMA (61). In their 1993 case report, Panegyres et al. suggested that the pathogenesis of unilateral MMA, associated with stroke and terminal ICA occlusion, subsequent to proliferation of subendothelial fibrous tissue and infiltration of mononuclear cells, T cells, into the carotid vascular wall, may be related to a T-cell-mediated response to a vascular antigen (171). Also, the research group stated that animal experiments on dogs may maintain this hypothesis (171, 172). In their 2014 study in 25 MMA patients and 22 healthy controls, Kang et al. showed that, through a suitable cell culture condition, circulating smooth-muscle progenitor cells (SPCs) may be established from the peripheral blood of MMA patients. In comparison to controls, SPCs obtained from MMA patients may demonstrate characteristic differentially expressed genes (DEGs) associated with vascular development, immune response, cell migration, and cell adhesion (149). The 2019 *in vivo* study results obtained by Choi et al. demonstrated impaired functional recovery of MMA endothelial colony-forming cells (ECFCs) in a chronic cerebral hypoperfusion rat model, in comparison to normal control ECFCs, which showed decreased apoptosis as well as increased neurogenesis and vasculogenesis, suggesting an involvement of ECFCs in MMA pathogenesis (160). In 2021, Ma et al. suggested a positive correlation between neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) biomarkers in MMA patients, which may further elucidate the pathology of inflammation in MMA pathogenesis (Figure 4) (173).

Moyamoya angiopathy molecular biomarkers

Treatment of an underlying inflammatory disease may lead to remission of MMA symptomatology (9). An immune response related to angiogenesis may be facilitated through M2 macrophages which may be induced through anti-inflammatory cytokines such as TGF-β, interferon (IFN)-α, IL-13, IL-10, and IL-4. Fujimura et al. demonstrated that CXCL5 and CD163 serum levels of MMA patients were significantly increased compared to controls, hypothesizing that MMA pathophysiology may be related to M2 macrophages (9, 174). Anti-inflammatory cytokines may induce angiogenetic markers. TGF-β of Treg/Th17 cells with distinct CD4+ T-helper cell subsets has been demonstrated to be associated with aberrant angiogenesis in MMA by means of VEGF signaling regulation (9, 175). Increase in angiogenetic markers including VEGF, HGF, PDGF, bFGF, cellular retinoic acid-binding protein-1 (CRABP-1), HIF-1 and MMPs may be associated with MMA (Figure 4) (9, 109, 154, 176–185). These markers have been hypothesized to be associated with proliferation of the intima as well as angiogenesis through their influence on endothelial cells, and with progression or initiation of MMA (9). Pro-inflammatory cytokines, including IL-6, IL-1, TNF-α, IFN-γ, and IFN-β, which may induce the pro-inflammatory, *RNF213*-dependent cytokine pathway, may have a different mechanism of action compared to cytokines involved in anti-inflammatory cytokine pathways (9). In 2016, Bang et al. demonstrated a correlation between caveolin-1, *RNF213*, and endothelial function in MMA (131). Also, caveolin-1 may be associated with negative arterial remodeling in MMA (186). VEGF levels have been demonstrated to be associated with caveolin-1 levels but not with MMA, suggesting that a change in plasma VEGF levels may not be a primary cause of MMA (131). In 2013, Hitomi et al. observed that iPSC-derived vascular endothelial cells (iPSECs) from MMA patients showed impaired angiogenic function. The *RNF213* R4810K genetic variant may become clinically manifest as aberrant angiogenesis through downregulation of Securin expression. The resulting defects in angiogenesis are considered MMA risk factors (187). In their 2013 high-throughput analysis of MMA autoantibodies, Sigdel et al. ascertained 6 MMA-related autoantibodies against EDIL3, ROR1, CTNNB1, STRA13, GPS1, and APP, providing important insight into the immune-mediated pathogenesis of MMA, and potentially advancing diagnostic tools for use in clinical practice (46). Accordingly, MMA-related autoantibodies against APP may be associated with an increased risk for hemorrhagic stroke (46, 96). In 2014, Jeon et al. demonstrated that increased CSF CRABP-I levels may be related to bilateral adult MMA. Also, the research group suggested that post-operative basal collateral vessel decrease could be associated with CRABP-I expression levels (188). In 2016, Zhang et al. demonstrated that COX-2 was up-regulated in the MMA MCA, predominantly in hemorrhagic MMA patients, hypothesizing that COX-2 may be associated with MMA pathogenesis, and potentially with hemorrhagic stroke in MMA (189). Based on their 2017 study result, Phi et al. hypothesized that in MMA, defective ECFCs may lead to aberrant recruitment of SPCs toward critical locations in the vasculature by means of chemokine (C-C motif) ligand 5 (CCL5) (190). In their 2018 prospective study in 11 MMA patients, Ishii et al. observed changes in biomarkers associated with tight junctions in the blood-brain barrier (BBB). The research group stated that their preliminary results may indicate that significant hemodynamic change and transient neurologic symptoms (TNS) in some patients may be related to BBB disruption after direct MMA bypass surgery (191). In 2018, Yokoyama et al. demonstrated that CSF proenkephalin 143–183 may be a useful diagnostic biomarker in pediatric MMA. The effect of enkephalin peptides by means of delta opioid receptor or opioid growth factor receptor may be related to MMA pathophysiology, suggesting an association between temporal changes in moyamoya collateral vessels and concentration of proenkephalin (192). In 2020, Surmak et al. showed that a [^{11}C]-PiB PET signal related to intracranial inflammation in MMA patients and a single relapsing-remitting multiple sclerosis (RRMS) patient may be corresponding to functional cerebral imaging of SULT1E1, suggesting that significant focal [^{11}C]-PiB PET signals may be received from the inflamed living human brain (193). In 2021, Han et al. suggested that elevated CSF and serum sortilin levels may be associated with MMA onset, and, in addition to levels of proinflammatory cytokines, may be effective markers in clinical practice. The research group hypothesized, that sortilin may break through a compromised blood brain barrier (BBB), may consecutively induce inflammation, and thus induce MMA (194). In their 2021 study, Ren et al. demonstrated that cortical astrocytic neogenin (NEO1) deficiency may be associated with MMA pathogenesis. NEO1, *a member of deleted in colorectal cancer* (*DCC*) *family netrin receptors*, was reduced in brain specimens of MMA patients. Astrocytic Neo1-loss resulted in an increase of small blood vessels, selectively in the cortex. These blood vessels were dysfunctional, with a leaky blood-brain barrier (BBB), thin arteries, and accelerated hyperplasia in veins and capillaries, resembling the symptomatology of a moyamoya disease-like vasculopathy. Additionally, the research group found that both MMA patients and Neo1 mutant mice exhibited altered gene expression in the cerebral cortex in proteins critical for both angiogenesis [e.g., an increase in vascular endothelial growth factor A (VEGFA)], and axon guidance (e.g., netrin family proteins) and inflammation. In aggregates, these results suggest a critical role of astrocytic NEO1-loss in the development of a moyamoya disease-like vasculopathy, providing a mouse model for investigating mechanisms of a moyamoya disease-like vasculopathy (195). In 2021, Sesen et al. described urinary biomarkers that may identify MMA presence to a high degree of accuracy and sensitivity. These markers may be detected from the CNS to the urine, and may correlate with response to treatment, such as radiographic verification of revascularization. Urinary MMP-2 showed an accuracy of 91.3%, a specificity of 100%, and a sensitivity of 87.5%. The research group hypothesized that urinary proteins may constitute a new, non-invasive device which may assist in treatment, follow-up, prognosis, and diagnosis of MMA (196). In their 2021 study, Dei Cas et al. carried out a complete lipidomic analysis of MMA patient plasma through mass spectrometry and measured inflammatory and angiogenic protein levels through enzyme-linked immunosorbent assay (ELISA). ELISA showed an MMP-9 decrease in MMA patient plasma. Lipidomic analysis demonstrated a cumulative depletion of lipid asset in MMA patient plasma in comparison to healthy controls. The research group noted a decrease in peripherally circulating membrane complex glycosphingolipids, observed in MMA patient plasma, compared to healthy controls, indicative of cerebral cellular recruitment. This quantitative targeted approach showed increased free sphingoid

bases, which may be related to aberrant angiogenesis. The results of the group may suggest that the lipid signature/plasma lipid profile of MMA patients may be closely associated with the condition and that a comprehensive biomarker profile may help to further elucidate the complexity of MMA pathogenesis (197). In their 2021 study, Lu et al., using plasma samples from 84 MMA patients, demonstrated that MMP-9 may serve as a biomarker for prediction of intracranial hemorrhage in MMA. The research group showed that a serum MMP-9 level >1,011 ng/ml may be an independent risk factor for hemorrhagic stroke in MMA. Also, the research group demonstrated that adult MMA patients, in comparison to pediatric MMA patients, showed an increased blood-brain barrier (BBB) permeability and MMP-9 serum level elevation. Furthermore, the group demonstrated that hemorrhagic MMA patients, in comparison to ischemic MMA patients, showed an increased BBB permeability and an elevated MMP-9 serum level (Figure 4) (198).

Moyamoya angiopathy genetic biomarkers and single nucleotide polymorphisms

In 2011, Kamada et al. established the *RNF213* genetic variant, c.14576G>A (p.R4810K, rs112735431) (17q25.3), as the first MMA susceptibility gene (9, 51, 199). MMA single-nucleotide polymorphism (SNP) studies have predominantly focused on the relation to atherosclerosis, the endothelium, on mechanical stress on the vasculature, vascular repair genes, and angiogenesis (200). In 2012, Liu et al. (201) screened for the *RNF213* p.R4810K polymorphism in the East and in Southeast Asian populations and stated that the prevalence proportion may differ depending on the country (9). Moreover, the research group hypothesized that additional factors, including immune response and inflammation, may be associated with MMA onset (9, 201). The frequency of the p.R4810K genetic variant has been demonstrated to be significantly increased in MMS compared to controls (9, 202, 203), suggesting an involvement of the *RNF213* p.R4810K genetic variant in MMS. In contrast, in 2015, Miyawaki et al., based on their results from a small sample size, stated that the *RNF213* c.14576G>A genetic variant may not be related to MMS (9, 204). Pro-inflammatory cytokines such as IFN-γ, IFN-β, and TNF-α may synergistically activate *RNF213* transcription both *in vivo* and *in vitro* (9, 124, 205). Pro-inflammatory cytokines may decrease angiogenic activity through *RNF213* induction (Figure 4) (9).

Various linkage analyses have demonstrated the involvement of inflammatory genes in MMA. Ikeda et al. demonstrated associations with vascular wall homeostasis as well as with loci 17q25, 12p12, 10q23.31, 8q23, 6q25, and 3p24.2–p26 (5, 200, 206). The chromosomal site 3p may be a major gene locus of genes associated with various signaling pathways, particularly the *Interleukin 5 Receptor Subunit Alpha* (*IL5RA*) (3p26.2), *Transforming Growth Factor Beta Receptor 2* (*TGFBR2*) (3p24.1), *Thyroid Hormone Receptor Beta* (*THRB*) (3p24.2), *Retinoic Acid Receptor Beta* (*RARB*) (3p24.2), and *Peroxisome Proliferator Activated Receptor Gamma* (*PPARG*) (3p25.2). These genes may be related to signaling pathways that are associated with inflammation as well as angiogenesis (5, 9). Changes in protein folding and gene transcription may be associated with aberrant expression of ICAM-1, VCAM-1 and E-selectin, induced through pro-inflammatory cytokines TNF-α and IL-1β, by means of NF-κB activation (5, 207). A variety of genes involved in MMA may be associated with inflammation. Whether these genes are causative factors of MMA or a result of MMA pathogenesis, remains to be elucidated (4, 5, 119, 205, 208–210).

In 2009, Shimojima and Yamamoto suggested that *Actin Alpha 2, Smooth Muscle* (*ACTA2*) (10q23.31) may not be a major MMA responsibility gene, especially in Japanese MMA patients. The researchers stated that, with no evidence of a co-existence of MMA and thoracic aortic aneurysms and dissections (TAAD), MMA may be an isolated disorder (211). MMA patients typically present with transient ischemic attacks (TIA) during the juvenile stage of MMA, suggesting that TAAD associated with *ACTA2* mutations may not be characteristic for MMA (211). In their 2010 case-control study in 208 MMA patients and 224 healthy controls, Li et al. demonstrated that the MMP-3 5A/6A functional polymorphism in the MMP-3 promoter may be related to both familial MMA and MMA in Chinese Hans (212). In 2010 Roder et al. stated that their study results may indicate potential genetic factors associated with MMA pathogenesis. The research group indicated that *Platelet Derived Growth Factor Receptor Beta* (*PDGFRB*) (5q32) and *Transforming Growth Factor Beta 1* (*TGFB1*) (19q13.2) may be related to vascular growth and transformation mechanisms which may be associated with MMA pathogenesis (213). In 2011, Roder et al. demonstrated a new mutation (R179H, heterozygous) in exon 6 of *ACTA2* in one central European MMA patient. The group was neither able to detect other previously described mutations nor did they establish any significant *ACTA2* sequence variations (214). In 2012, Liu et al. stated that no novel genetic variants were identified in their study of the first *TGFB1* (19q13.2) exon in European MMA patients. Moreover the research group hypothesized that, due to a negative association of rs1800471 and rs1800470 in Japanese MMA patients, an association of the first *TGFB1* exon with MMA pathogenesis may be doubtful (215). In their 2013 letter to the editor, Hu et al. referred to their replication study results in 55 Han Chinese MMA patients. The research group stated that no *ACTA2* mutation was detected through genomic sequencing, confirming that *ACTA2* may not have an important function in MMA pathogenesis (216). In 2013, Wang et al. demonstrated that *RNF213* rs148731719 and rs112735431 may have a significant influence on MMA pathogenesis. In comparison to these results, the influences of *PDGFRB* (5q32), *MMP-3* (11q22.2), and *TIMP-2* (17q25.3) on MMA may be unremarkable in the Chinese Han population. No significant interaction among these five polymorphisms may be evident in MMA pathogenesis (217). In 2014, Cecci et al. confirmed that *RNF213* alterations may predispose patients of various ethnicities to MMA, and that the p.R4810K genetic variant may predispose individuals of Asian descent in the United States to MMA (218). In their 2014 study, Han et al. stated that familial MMA patients may constitute an increased percentage among MMA patients than estimated before. Despite the absence of distinct symptoms, family members of MMA patients may also be affected by MMA (219). Based on their 2015 study results, Kobayashi et al. hypothesized that *RNF213* R4810K carriers may have a decreased angiogenic capacity, suggesting an increased susceptibility to cerebral hypoxia of these carriers. The research group demonstrated that inflammation, interferons, may function as an environmental factor. The group also stated that decreased angiogenesis may be causally related to the *RNF213* AAA+ function (205). In their 2016 study, Kim et al.

suggested that the c.14429G>A (p.R4810K) allele of *RNF213* may be related to Korean MMA patients. The homozygous c.14429G>A (p.R4810K) genetic variant may be associated with early-onset MMA, a poor prognosis, and severe symptomatology. The c.14429G>A (p.R4810K) homozygous genetic variant may provide a biomarker for early-onset MMA or unstable MMA with cerebral infarction in Korean MMA patients, requiring timely diagnosis and potentially revascularization surgery (220). In 2016, Shoemaker et al. performed a whole exome sequencing study in 125 MMA patients and 125 matched controls, establishing a non-*RNF213* founder mutation, an Asian, and a Caucasian subpopulation. Collapsing variant methodology ranked *OBSCN* (1q42.13), a gene associated with myofibrillogenesis, as most enriched in the non-*RNF213* founder mutation and among Caucasian cases. The most enriched variant in the non-*RNF213* founder mutation and among Caucasian cases was *ZXDC* (p.P562L) (3q21.3), associated with activation of MHC Class II. These results of the research group further support the East Asian origin of the *RNF213* (p.R4810K) variant and more exhaustively describe the genetic landscape of multiethnic MMA, detailing new, alternative genes and candidate variants which may be significant in MMA diagnosis, etiology, and the development of MMA models (209). In their 2016 meta-analysis, Sun et al. stated that *RNF213* rs112735431 may be related to an increased risk of MMA in the Japanese population, whereas combined screening with rs112735431 and rs138130613 may advance the detection rate for MMA in the Chinese population (221). *RNF213* is the major susceptibility gene of MMA patients in the Chinese population. The spectrum of rare genetic variants identified in Chinese MMA patients is diverse. Compared to MMA patients without rare *RNF213* genetic variants, p.R4810K heterozygous MMA patients were younger at diagnosis, had more familial cases, ischemia, and posterior cerebral artery involvement (222). In 2017, Guey et al. stated that *Cbl Proto-Oncogene* (*CBL*) (11q23.3) screening may be advocated in early-onset MMA, even in the absence of evident signs of a RASopathy. Identification of a pathogenic *CBL* mutation may raise questions concerning the hematological follow-up to be recommended to these patients (223). In 2017, Jang et al. suggested that, in their study cohort of 264 adult Korean MMA patients, *RNF213* p.Arg4810Lys may be the only genetic variant strongly related to MMA (224). In their 2017 meta-analysis, Liao et al. described the critical roles of *RNF213* p.R4810K in MMA, especially in familial MMA and intracranial major artery stenosis/occlusion (ICASO) in the Japanese, Korean, and Chinese population. Except for *RNF213* p.R4810K, MMA appears to be more complex in China. In addition to a distinct genetic background, other environmental or genetic factors may contribute to MMA (225). In 2017, Park et al. demonstrated that the *RNF213* rs112735431 polymorphism may be related to both non-MMA ICAD and MMA in the Korean population. Furthermore, the group hypothesized that the *RNF213* rs112735431 polymorphism may be associated with hypertension in MMA patients (226). In 2017, Park et al. confirmed that the *RNF213* 4950G>A and 4810G>A genetic variants may be related to both hemorrhagic and ischemic pediatric and adult MMA in the Korean population (227). In 2018, Duan et al. performed a two-stage genome-wide association study (GWAS) in 1,492 MMA patients and 5,084 controls, confirming an earlier demonstrated MMA risk locus on 17q25, and identifying 10 new MMA risk loci of genome-wide significance. The *RNF213* (17q25.3) rs9916351 single-nucleotide polymorphism (SNP) was demonstrated to have a more severe genetic impact on early-onset compared to late-onset MMA. An additional SNP related to MMA, *HDAC9* (7p21.1) rs2107595, had been associated with large vessel disease. Two new SNPs, *MTHFR* (1p36.22) rs9651118 and *TCN2* (22q12.2) rs117353193, were shown to be related to increased serum homocysteine levels in MMA patients. With a false discovery rate of <0.05, tissue enrichment analysis demonstrated genes of related loci to be exceedingly expressed in the immune system (208). In 2019, Tashiro et al. showed that, in contrast to MMA patients, the prevalence of the *RNF213* c.14576G>A polymorphism was significantly decreased in patients with an intracranial vertebral artery dissection. The *RNF213* gene polymorphism may preferentially be associated with cerebrovascular lesions in the anterior circulation, which originates from the primitive ICAs (228). In 2019, Peng et al. demonstrated that dysregulated genes in the peripheral blood of MMA patients may be associated with immune and inflammatory responses, and with ECM organization. In comparison to other vascular disorders, this gene dysregulation pattern may be specific for MMA. Moreover, resting natural killer cells, naive CD4 cells, and naive B cells were aberrantly disrupted in the peripheral blood of MMA patients (229). In their 2019 study, Pinard et al. analyzed exome sequencing results from 39 trios. With 12 altered genes predisposing to MMA, the research group demonstrated four *de novo* genetic variants in three genes, *SET Domain Containing 5* (*SETD5*) (3p25.3), *CCR4-NOT Transcription Complex Subunit 3* (*CNOT3*) (19q13.42), and *Chromodomain Helicase DNA Binding Protein 4* (*CHD4*) (12p13.31), which were previously regarded as unrelated to MMA. The aforementioned genes encode proteins involved in chromatin remodeling, and implicate disrupted chromatin remodeling as a molecular pathway predisposing to early-onset, large artery occlusive cerebrovascular disease. Moreover, these results may widen the spectrum of phenotypic pleiotropy because of alterations of *SETD5*, *CNOT3*, and *CHD4* extending beyond developmental disorders to late-onset cerebrovascular diseases, emphasizing the requirement to evaluate symptomatology up until adulthood for genes related to developmental disorders (230). In 2019, Shen et al. demonstrated two SNPs, related to CD40, to be associated with MMA (CC rs4813003 and TT rs1535045), which had been reported to be associated with Kawasaki disease. The research group proposed a correlation between an autoimmune disorder and MMA, hypothesizing that this genetic constitution may result in vascular wall pathogenesis (231). In 2020, Jee et al. performed a prospective computed tomography (CT) angiography study in 63 young adult MMA patients, suggesting that these patients may show a concomitant extracranial arteriopathy in distinct sites such as internal iliac, renal, celiac, superior mesenteric, and coronary artery stenosis. Also, the research group stated that MMA patients with an associated extracranial arteriopathy had an increased probability of occurrence of PCA involvement and diabetes mellitus. Furthermore, the group suggested that MMA patients carrying *RNF213* variants, particularly the homozygous *RNF213* p.Arg4810Lys variant, may benefit from screening for systemic arteriopathy (232). In 2020, Key et al. demonstrated that genetic ablation of several mitochondrial matrix factors, including the peptidase and AAA+ ATPase *Lonp1*, the transcription factor *Tfam*, and also the peptidase *ClpP*, may strongly induce *RNF213* transcript expression in several organs, along with other constituents of the innate immune system. Based on their results, the research group hypothesizes that

mysterin takes effect if infections or mitochondrial dysfunction have induced RNA-dependent inflammation. Therefore, MMA may resemble vasculopathies which comprise altered nucleotide processing, including systemic lupus erythematosus or Aicardi-Goutières syndrome (146). In their 2020 study in 1024 consecutive Korean individuals without MMA, using multivariate logistic regression analysis, Kim et al. examined associations between posterior and anterior intracranial major artery stenosis/occlusion (ICASO), the main cause of ischemic stroke, and *RNF213* genetic variants. The research group demonstrated that the genotype frequency of *RNF213* 4863G > A may differ significantly according to the presence of posterior ICASO. The GA genotype of *RNF213* 4950G > A and GA genotype of *RNF213* 4810G > A may be more frequent in individuals with anterior ICASO (233). In their 2020 case-control study including 1,385 Chinese MMA patients and 2,903 healthy controls, Wang et al. stated that the *RNF213* p.R4810K genetic variant may be associated with an increased susceptibility to MMA in the Chinese population and may be related to an increased severity of PCA involvement and early-onset MMA (234). In their 2020 meta-analysis, Wang et al. included 4,711 MMA cases and 8,704 controls of 24 studies, evaluating seven polymorphisms in six genes, demonstrating that *RNF213* rs148731719 and rs112735431 may be positively, and that *MMP-3* rs3025058, *MMP-2* rs243865, and *TIMP-2* rs8179090 may be inversely related to MMA. Furthermore, the research group identified genetic variants involved in various pathophysiologic mechanisms, including vascular SMC and vascular endothelial dynamics (235). In 2021, Mineharu and Miyamoto stated that *RNF213* may occupy a decisive role in inflammation, cell cycle control, oxygen consumption, and lipid metabolism, and may contribute to vascular cell maintenance. GUCY1A3 may be a regulator of VSMC contraction and platelet function through the NO-sGC-cGMP signaling pathway. Mutations in *Guanylate Cyclase 1 Soluble Subunit Alpha 1* (*GUCY1A1, GUCY1A3*) (4q32.1) and *RNF213* may cause both MMA, and non-moyamoya intracranial arterial disease, pulmonary arterial hypertension, and coronary artery disease. They have significant interaction with CAV1 and NFAT1, both of which may have diverse molecular functions involving cell cycle control and immune regulation (119). In their 2021 genetic association study in 24 non-East Asian sporadic MMA patients, 2 singletons and 22 trios, constituting the discovery cohort, and 84 probands, 55 singletons and 29 trios, constituting the validation cohort, Kundishora et al. stated that their results may provide the largest data gathering in non-East Asians with sporadic MMA harboring pathogenic variants in the identical gene until now, suggesting that *Diaphanous Related Formin 1* (*DIAPH1*) (5q31.3) may be a MMA candidate gene, which may impair vascular cell actin remodeling, and which may influence future treatment strategies and clinical diagnostics (236). In their 2021 study, Sarkar and Thirumurugan demonstrated RNF213 dynamicity and a potential mechanism causing MMA. The research group hypothesized that mutant RNF213 may lead to insulin resistance independent of TNFα (237). Also, the research group stated that insulin resistance may lead to pericyte death and that its absence may cause microaneurysms, an established MMA disease phenotype (237, 238). RNF213 located in the nuclear region may be associated with immune response, obesity, defense response, stress response, DNA repair, cancer, and ubiquitin-binding (237). In 2021, Wan et al. carried out an association analysis of the major histocompatibility complex region in 755 MMA patients and 2,031 controls by means of an HLA imputation method, stating that the genetic polymorphism of HLA-B and HLA-DQA2 may be a genetically predisposing factor for MMA in the Chinese Han population, providing potential evidence for additional HLA-related studies of MMA patients in Chinese Hans, and suggesting that MMA may be an immune-mediated disorder (Figure 4) (239). In their 2021 study, Zhao et al. demonstrated that in MMA patients, genes associated with vascular remodeling, such as *Wnt Family Member 5A* (*WNT5A*) (3p14.3) and its associated regulators, may be disrupted and aberrant. The research group indicated that their results may assist in the development of potential future therapeutic targets which may promote MMA angiogenesis (240). According to their 2022 study results, Jin and Duan, using bioinformatics analysis, demonstrated that aberrant expression of hub genes and the characteristics of immune cell infiltration into the cerebrovascular tissue of MMA patients may provide a novel insight into MMA progression. Jin and Duan established nine hub genes associated with neutrophil regulation, of which *Unc-13 Homolog D* (*UNC13D*) (17q25.3 or 17q25.1) may be a promising MMA biomarker candidate to ascertain the characteristics of neutrophil infiltration in MMA (241). Referring to their 2022 *in vitro* study, using the CRISPR-Cas9 genome editing technology, Roy et al. indicated that RNF213 may be associated with the regulation of cerebral endothelium integrity, whose disruption may be a pathophysiological mechanism associated with MMA. Also, this study of the research group may emphasize the significance of BBB integrity in MMA pathogenesis and additional RNF213-related diseases (242). Regarding their 2022 transcriptomic study results, Xu et al. highlighted that mitochondrial function and extracellular matrix (ECM) organization may be central molecular mechanisms associated with MMA, and have ascertained a sex difference in gene expression in intracranial arteries. The research group indicated that sex-specific DEGs, including *Nuclear Receptor Subfamily 4 Group A Member 1* (*NR4A1*) (12q13.13), *Superoxide dismutase 3* (*SOD3*) (4p15.2), and *Aquaporin-4* (*AQP4*) (18q11.2), may contribute to the sex difference in MMA (243). In their 2022 study, Zhang et al. suggested that *RNF213* loss of function may reorganize the vascular transcriptome and spliceosome, which may lead to disrupted angiogenesis and an aggravated vascular inflammatory response (244). The research group indicated that *RNF213* gene knockdown may sensitize endothelial cells to inflammation, leading to aberrant angiogenesis (244). The group ascertained significant associations between *RNF213* genetic variants and immune and inflammatory MMA inducers, as well as regarding the mechanism of action of the MMA epitranscriptome (244).

Moyamoya angiopathy non-coding ribonucleic acids

High-throughput sequencing has established a large quantity of distinct ribonucleic acids (RNAs) created from non-coding DNA (245, 246). Similar to protein-coding RNAs, non-coding RNAs appear to be linear molecules with 3′ and 5′ termini, which constitute defined end and start points of the RNA polymerase on the DNA template (245). Non-coding RNAs vary in length (245).

Long non-coding RNAs (lncRNAs) exceed 200 nucleotides, lack protein-coding capacity, and are associated with post-transcriptional

processing, transcriptional control, and chromatin remodeling (9, 247, 248). Regulation of lncRNAs may be associated with inflammation (9, 249, 250). Moreover, lncRNAs may be related to MMA pathophysiology by means of an inflammatory signaling cascade comprising the MAPK signaling pathway (9, 10, 251). In their 2017 study, Wang et al. demonstrated that an integrated analysis of lncRNA-mRNA coexpression networks may be associated with the MAPK signaling pathway, the Toll-like receptor signaling pathway, cytokine-cytokine receptor interaction, and inflammation. The research group indicated that differentially expressed genes may help to ascertain crucial components in MMA pathophysiology (251). In their 2020 study, Gu et al. carried out a bioinformatics analysis of candidate RNAs to identify a series of aberrant 2294 mRNAs, 3649 lncRNAs, and 94 miRNAs, differentially expressed between samples of MMA patients and controls. The research group established a synergistic ceRNA lncRNA-miRNA-mRNA regulatory network. Key mRNAs (CXCL16, CEBPB, FOSL2, and STAT5B) and essential regulatory miRNAs (miR-423-5p and miR-107) related to the ceRNA network were identified. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes pathway (KEGG) enrichment analyses employed with the DAVID database indicated that differentially expressed mRNAs (DEmRNAs) related to the ceRNA network may be associated with inflammation and immune system regulation in MMA. These research results may further elucidate the molecular MMA pathogenesis, and may contribute to constitute future treatment strategies (252). In 2020, Han et al. showed that metabolic adjustments by dysregulated lncRNAs in peripheral neutrophils may in part account for complete compensation of asymptomatic MMA patients (Figure 4) (253). In their 2021 study, Zhao et al. using KEGG and GO analysis, showed that lncRNAs and mRNAs may be differently expressed in the superficial temporal artery (STA) vascular wall in MMA patients. The research group proposed a list of altered lncRNAs and mRNAs associated with vascular remodeling, which may be possible targets of future exploration of MMA medication (240). In their 2022 study in 21 MMA patients and 11 controls, Mamiya et al. demonstrated that the lncRNA expression profile in MMA MCA microsamples differed from controls. The research group ascertained 308 differentially expressed lncRNAs (fold change > 2, $q < 0.05$), including 2 downregulated and 306 upregulated lncRNAs in the MCA of MMA patients. Gene Ontology (GO) analyses of potential protein-coding genes, the transcription of which may be regulated in cis through ascertained differentially expressed lncRNAs, indicated an association with branching related to blood vessel morphogenesis, positive regulation of cytokine production, the T-cell receptor signaling pathway, and antibacterial humoral response (254).

MicroRNAs (miRNAs) are endogenous, short non-coding ~23 nucleotide RNAs, which may regulate gene expression through pairing to the mRNAs of protein-coding genes to control their posttranscriptional repression or cleavage (9, 245, 255, 256). miRNAs may be of vital significance regarding the control of cell aging, differentiation, survival, and proliferation (9, 255). Additionally, miRNAs may be related to angiogenesis, neurogenesis, and inflammation (9, 257). miRNAs may regulate TLR signaling through reduction of inflammation, enhanced tissue repair, and regaining of homeostasis following tissue injury and infection (9, 258). MiR-126, miR-155, and miR-21 may be associated with inflammation and vascular disorders. To do research into the network involving miRNAs and their targets leading to a coordinated gene expression pattern may lead to results which may help establish new treatment strategies to approach both aberrant vascular remodeling and to induce neovascularization after ischemia (259). Increased expression of miRNA Let-7c and miRNA-196a2 may be used as MMA biomarkers (9, 260, 261). In their 2019 study, Lee et al. analyzed the impact of *RNF213* mutations and MMA on the profiles of cell-free miRNA and protein in patient plasma samples. Levels of selected MMA-affected miRNAs in EV-depleted plasma, extracellular vesicles (EVs), and whole plasma have been confirmed through real-time quantitative polymerase chain reaction (qPCR). The research group showed that EV-encapsulated miRNA may be utilized as non-invasive biomarkers to evaluate MMA progression (262). The changes of proteins and miRNAs ascertained may be related to signaling processes such as immune activation and angiogenesis which may further elucidate MMA pathogenesis (262). Ischemic conditioning may be used to decrease the stroke risk in asymptomatic intracranial atherosclerotic arterial stenosis (263, 264). Ischemic preconditioning involves inducing moderate ischemia to exert protective functions against following severe ischemic events. Epigenetics may be associated with the outcome and the pathophysiology of stroke. Recent research has demonstrated miRNA expression following ischemic preconditioning; miRNA profiling 3 h following ischemic preconditioning demonstrated upregulation of miRNA families miR-182 and miR-200 that have been associated with neuroprotective effects of the HIF-1 and prolyl hydroxylase 2 signaling pathways (264–266). Furthermore, ischemic preconditioning has been shown to promote anti-inflammatory mechanisms by modifying the expression of cytokines during ischemic insults, suggesting a critical role of the vasculature and endothelial cells during ischemic conditioning stimuli (264, 267). Also, ischemic post-conditioning may represent a promising neuroprotective strategy in ischemic insults by means of anti-inflammatory, anti-apoptotic, and CBF-based mechanisms (264, 268, 269). In 2014, Dai et al. using real-time PCR, identified a serum miRNA signature in MMA. The research group demonstrated in an independent MMA cohort that serum miR-125a-3p was significantly decreased, whereas serum miR-126, miR-130a, and miR-106b were significantly increased. Gene Ontology (GO) analysis demonstrated that differentially expressed serum miRNAs may be enriched in signal transduction, transcription, and metabolic processes. Pathway analysis demonstrated that the most enriched pathway may be the mTOR signaling pathway. Also, the research group demonstrated that 13 and 16 aberrant serum miRNAs coordinately inhibited BRCC3 and RNF213 protein expression at the posttranscriptional level, associated with MMA pathogenesis and aberrant angiogenesis (270). In 2015, Zhao et al. showed that increased serum miRNA let-7c expression in MMA patients may be associated with MMA pathogenesis through its influence on *RNF213*, suggesting that let-7c may be a potential biomarker of MMA (Figure 4) (260). In their 2018 MMA discordant monozygotic twin-based study, Uchino et al. confirmed a new circulating microRNA signature in MMA as a feasible diagnostic marker, which may be marginally confounded through genetic heterogeneity (271). The research group stated that this novel circulating microRNA signature may contribute to future functional microRNA analyses to ascertain novel therapeutic and diagnostic MMA targets (271).

In 2017, Zhao et al. demonstrated that various circRNAs may be involved in MMA pathogenesis, and may be associated with modulation of the MAPK signaling pathway. Besides providing a set of potential diagnostic biomarkers for MMA, the results of the research group suggest that therapeutic strategies targeting the MAPK signaling pathway or these circRNAs may be effective MMA treatment strategies (272). Recent research may provide evidence that regulatory RNAs including miRNAs or lncRNAs may be associated with MMA pathogenesis. In comparison with other kinds of miRNA sponges, circRNAs have higher expression levels and an increased amount of binding sites and, compared to linear RNAs, are viewed as more efficient regarding gene expression regulation and sequestering miRNAs (245, 273). CircRNAs have been associated with various disorders that involve various neurological disease, and are correlated with miRNA expression (273, 274). In 2017, Zhao et al. demonstrated that 146 circRNAs may be expressed in MMA patients, and these circRNAs may contribute to MMA pathogenesis (272, 273). Of these 146 circRNAs, 29 circRNAs were upregulated, and 117 circRNAs were downregulated (272, 273). Hsa_circRNA_067130, hsa_circRNA_067209, and hsa_circRNA_062557 were upregulated, while hsa_circRNA_089763, hsa_circRNA_089761, and hsa_circRNA_100914 were downregulated with highest fold variations, providing sufficient evidence to state that these circRNAs may be potential MMA biomarkers (272, 273). In their 2019 pilot study of neutrophil samples from asymptomatic MMA patients and an aberrant circRNA profile obtained through high-throughput microarray analysis, Ma et al. demonstrated a critical function of circRNAs and neutrophils in the differentiation of asymptomatic MMA patients compared to healthy controls, suggesting a relation of angiogenic and anti-inflammatory markers to asymptomatic MMA (264, 275). The research group carried out Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes pathway enrichment (KEGG) analyses to both predict functioning and for the annotation of differentially expressed circRNAs, stating that differentially expressed circRNAs may be associated with metabolism, angiogenesis and immune response in asymptomatic MMA. Also, the research group suggested an association of the HIF-1α signaling pathway with increased VEGF and angiogenesis in MMA pathogenesis (264, 275). Moreover, Ma et al. suggested that anti-inflammatory mechanisms and neutrophils may be associated with MMA progression (264, 275). Research results may indicate a HIF-1/VEGF mechanism associated with angiogenesis (264, 267). In their 2021 study, Li et al. conducted neutrophilic tsRNA profiling in asymptomatic MMA patients and healthy controls (276). Pathophysiological mechanisms, including immune response, angiogenesis, axon guidance, and metabolism adjustment, were highlighted through differentially expressed (DE)-tsRNAs and DE-mRNA in asymptomatic MMA patients, which may support the potential receptivity of asymptomatic MMA to medical therapeutics, such as immune-modifying drugs (Figure 4) (276).

Pediatric and adult moyamoya angiopathy

The distinct pathophysiology of pediatric MMA compared to adult MMA is evident both in angiographic findings and based on the symptomatology (Figure 5). In 2009, Czabanka et al. demonstrated that surgical revascularization by encephalomyosynangiosis (EMS) combined with extracranial-intracranial bypass (STA-MCA bypass) may lead to favorable clinical and angiographic results in both pediatric and adult MMA patients. Especially in pediatric MMA patients, EMS may constitute an appropriate alternative to STA-MCA bypass surgery in the European population (277). In their 2012 study, Bao et al. stated that the symptomatology of adult MMA patients in the Chinese population may be different from those in other Asian countries. EDAS surgery in adult MMA patients may carry a low risk, is effective at preventing future ischemic events, and improves the quality of life. The research group indicated that, despite the lack of prospective randomized trials to determine the efficacy of bypass surgery in MMA patients, the available data may support surgical treatment (278). In 2012, Kim et al. demonstrated that adult MMA is not a syndrome, rather a readily distinguishable disease entity including significant progression of unilateral MMA to bilateral MMA, stating that treatment strategies and diagnostics in adult MMA should be different from those in pediatric MMA (279). In 2013, Hishikawa et al. assessed the angiographic correlation between the posterior circulation and the anterior circulation in adult MMA patients and pediatric MMA patients, and evaluated the presence of steno-occlusive PCA lesions and the extent of steno-occlusive ICA lesions on angiography. The research group stated that less advanced ICA lesions may significantly complicate posterior circulation involvement in pediatric MMA patients (280). In 2014, Lee et al. stated that during follow-up of pediatric MMA patients, clinicians should be aware of potentially delayed PCA involvement and progressive PCA stenosis, if these patients report transient visual symptoms or headache, stating that indirect revascularization surgery may be effective in these patients (281). In their 2015 study, Acker at al. demonstrated that the percentage of hemorrhagic MMA in pediatric patients was slightly increased. In comparison to adult MMA patients, angiographic analysis showed that stenosis and/or occlusion within the posterior circulation may be increased in pediatric MMA patients (282). In their 2015 long-term survey, Bao et al. showed that the majority of surgically treated pediatric MMA patients maintained a favorable outcome. The research group suggested that both timely diagnosis and active intervention prior to establishment of irreversible hemodynamic change may be essential to obtain a favorable clinical outcome (283). In their 2015 article, Piao et al. state that, in comparison to adult MMA, prognosis and treatment of pediatric MMA may be of considerably larger clinical significance (284). Also, the group argued that, through adequate treatment, favorable results may be obtained, referring to the fact that a standard treatment plan for pediatric MMA is currently not implemented in clinical practice (4, 284). In their 2016 study, Liu et al. stated that EDAS surgery may effectively increase cerebral blood flow and establish a favorable outcome in pediatric MMA patients, which may lead to a decreased incidence of recurrent hemorrhage and to disappearance of intracranial aneurysms (285). In their 2016 histopathological study of the distal MCA in pediatric MMA and adult MMA, Takagi et al. demonstrated that MCA medial thinning occurred in both pediatric and adult MMA patients. Yet, MCA intimal thickening was demonstrated to be more prominent in adult MMA patients. Additional study of MMA MCA specimens may be warranted to further clarify MMA pathophysiology (286). In 2017, Mejia-Munne et al. stated that juvenile-onset autoimmune

disease and atherosclerosis were found to be associated with both adult and pediatric MMA. Adult-onset autoimmune disease was associated with pediatric MMA but not with adult MMA. The research group suggested that both adult and pediatric MMA may be associated with inflammation, hypothesizing that inflammation may be associated with MMA pathogenesis (287). In 2017, Uchino et al. stated that failure to notice non-focal physical symptoms, suggestive of orthostatic intolerance, including headache, motion sickness, difficulty getting out of bed, fatigue, and vertigo/dizziness, may significantly impair the quality of life in pediatric MMA patients up to 5 years after revascularization surgery, resulting in 57% of patients being unable to attend school. These symptoms, inversely associated with the number of years after surgery, may serve as independent clinical markers to monitor disease outcome (288). In their 2018 retrospective follow-up study, Bao et al. stated that EDAS surgery may be effective in a Chinese cohort of adult MMA patients. EDAS resulted in prevention of recurrent stroke and adequate long-term improvement of symptomatology. Hypertension may be a risk factor for ischemic stroke during follow-up (289). In their 2018 study, Elbers et al. suggested that lenticulostriate collaterals in children with unilateral intracranial arteriopathy may be a useful neuroimaging biomarker that may help stratify patients with distinct clinical features and patterns of vascular evolution (290). In their 2018 long-term follow-up study, Zhang et al. stated that EDAS surgery in pediatric MMA patients may be effective and safe, may improve the quality of life, and may diminish the risk of subsequent neurological events. The risk of ischemia-related complications was increased in younger patients, and older children showed more favorable outcomes. Compensation was greater with more prominent cerebral ischemia. The long-term clinical outcome largely depended on presence and extent of pre-operative stroke (291). In 2019, Lu et al. showed that the incidence of transient neurological events (TNE) was significantly increased in adult MMA patients compared to pediatric MMA patients (292). In their 2020 retrospective study in 131 adult MMA patients and 83 pediatric MMA patients, Liu et al. stated that pediatric MMA patients may show greater patency and an increased capability to establish a favorable leptomeningeal collateral status in comparison to adult MMA patients. The research group indicated that a poor leptomeningeal collateral status may correlate with an unfavorable post-operative outcome and severe symptomatology. The leptomeningeal collateral status may be associated with differences in prognosis and symptomatology between adult MMA patients and pediatric MMA patients (Figures 1–3) (293). In their 2020 letter to the editor, Yu et al. stated that the PCA-ACA/MCA anastomosis may increase the hemodynamic burden of the posterior circulation, increasing the risk of intracerebral hemorrhage (Figures 2, 3) (7, 294), which should be considered during the clinical management of pediatric MMA patients (294). Additional compensatory collaterals, including extracranial arterial collateral circulation anastomoses from the middle meningeal, maxillary and facial arteries to the ophthalmic artery, and dural arteriolar anastomoses from the occipital artery and middle meningeal artery through the parietal foramen and mastoid foramen, may as well correlate with the clinical outcome post-operatively (Figure 1) (294, 295). Most recently, the research group observed different hemodynamic sources of the recipient parasylvian continental arteries (PSCAs) among the parietal, temporal, and frontal PSCAs in MMA hemispheres (80, 294), suggesting that the recipient vessel in STA-MCA bypass surgery may not necessarily originate from the MCA (294). Consequently, neurosurgeons may be advised to rely predominantly on digital subtraction angiography (DSA) to ascertain the hemodynamic source of recipient vessels (294). In their 2021 retrospective validation and extension study on the function of the *RNF213* p.R4810K genetic variant in 2,877 Chinese MMA patients, Wang et al. stated that carrying rates and incidence of *RNF213* p.R4810K in various regions for Chinese MMA patients were obviously different. *RNF213* p.R4810K may have various predictive effects on the phenotypes of adult MMA patients and pediatric MMA patients (296). In their 2022 study in 15 pediatric MMA patients, Wang et al. stated that EDAS surgery may prevent ischemia/ischemic stroke, and may reduce aberrant collaterals and dilation of the anterior choroidal artery, potentially reducing the incidence of recurrent intracerebral hemorrhage of the posterior communicating artery or anterior choroidal artery during adulthood of these patients (297).

Moyamoya angiopathy, moyamoya syndrome, and inflammation

Two predominant pathways have been suggested to be associated with inflammation and initiation or progression of MMA. A pro-inflammatory cytokine pathway, leading to *RNF213* activation, as well as an anti-inflammatory cytokine pathway. The pro-inflammatory pathway is associated with increased inflammatory cytokines in inflammatory diseases which affect RNF213, leading to MMS onset. This hypothesis may be supported by the evidence of an increased frequency of the *RNF213* p.R4810K variant in MMS. Late-onset MMA may be associated with this variant (9, 298–301). Pro-inflammatory cytokines may be involved in fulminant MMA progression (9, 302). This particularly applies to MMS associated with hyperthyroidism (9, 303–306). The pro-inflammatory pathway may function as an initiator of MMA (9). The anti-inflammatory cytokine pathway involves anti-inflammatory mediators in the CSF or the blood that may affect acceleration or acute aggravation of MMS. Anti-inflammatory cytokines may be involved in autoregulation as well as vascular reactivity, leading to MMA progression (Figure 4) (9).

In their 2002 study, Soriano et al. using dual-antibody enzyme-linked immunoassays, demonstrated increased CSF levels of soluble endothelial adhesion molecules, vascular cell adhesion molecule Type 1 (VCAM-1), intercellular adhesion molecule Type 1 (ICAM-1), and E-selectin, suggesting that pediatric MMS patients may have persistent central nervous system inflammation, with marginal blood-brain barrier (BBB) impairment. The research group suggested that these soluble adhesion molecules may be of use in clinical practice as markers of this central nervous system inflammation process. Moreover, the research group stated that their results may not completely ascertain an association of these adhesion molecules with vascular pathological processes related to MMS, since cerebral ischemia as well may lead to expression of these adhesion molecules (110). In their 2011 case-control study in 114 pediatric MMA patients and 114 healthy controls, Li et al. stated that increased thyroid autoantibodies and elevated thyroid function may be related to MMA (307). In their 2013 retrospective study, Li et al. stated that MMA associated with Graves' disease may mainly be observed in adult female patients. Associated clinical symptoms may include ischemia and may be related to hyperthyroidism. MMA pathogenesis associated with Graves' disease may be

related to various immunologic and genetic influencing factors. Encephaloduroarteriosynangiosis (EDAS) surgery may diminish the likelihood of stroke recurrence and may lead to an effective collateral circulation (308). In their 2015 retrospective study, Han et al. stated that the radiographic and clinical characteristics of neurofibromatosis type I (NF-1) in MMS may be similar to MMA (309). In their 2015 case report, Hyakuna et al. described a rare case of *Cbl Proto-Oncogene (CBL)* (11q23.3) mutation related to MMA, hypothesizing that MMA could be induced through congenital dysregulation of cerebral angiogenesis associated with a RAS/MAPK pathway germline mutation. Furthermore, the research group hypothesized that prolonged RAS pathway signaling may lead to cerebrovascular development disruption (310). In their 2016 retrospective study, Acker et al. stated that their European Caucasian MMS cohort may show various disparities in comparison to a European Caucasian MMA cohort as well as to Asian MMS cohorts, hypothesizing that MMS may represent an independent disorder with a distinct ethnic symptomatology. Moreover, the research group stated that it may be important to standardize inclusion criteria and definition of MMS regarding associated disorders to optimally compare MMS results (311). In her 2016 case report about an 8-year-old female patient with a *Dedicator Of Cytokinesis 8* (*DOCK8*) (9p24.3) deletion, AlKhater stated that her patient was diagnosed with MMA (3, 312). DOCK8 deficiency may be associated with MMA (312, 313), potentially due to ischemia (312). Recent reports have described an underlying autoimmune disease mechanism related to T cell dysregulation in these patients, particularly in unilateral MMA, as evident in her patient (312, 314). Knowledge of therapy management and revascularization procedures for patients like the one described in this report remain to be ascertained (312). Prescribing antiplatelet drugs to patients affected with DOCK8 deficiency should be done with caution, due to hemorrhage being an ascertained symptomatology of MMA, particularly of hemorrhagic MMA (312, 315). In 2015, Chen et al. performed a retrospective study in 68 unilateral MMA patients and 316 bilateral MMA patients, suggesting a higher overall autoimmune disease prevalence in unilateral MMA compared to bilateral MMA. The research group hypothesized that, compared to bilateral MMA, unilateral MMA may be related to autoimmune disease to a greater extent. Moyamoya vessel formation in bilateral MMA and unilateral MMA may be associated with distinct pathogenetic mechanisms (314). Regarding their 2016 imaging study in 21 angiographically proven MMA patients, 14 MMS and 7 MMA patients, Yu et al. stated that differentiating MMS from MMA may be challenging, and high-resolution magnetic resonance imaging (HR-MRI) may help provide a more detailed comprehension of MMS and MMA, which may lead to a more precise diagnosis of increased reliability (316). In 2017, Zhang et al. showed that, in comparison to other MMA patients, the *RNF213* p.R4810K genetic variant may be related to autoimmune and atherosclerotic MMS in the Chinese population at a lower prevalence (202). In 2018, Yamanaka et al. hypothesized that HIV-associated vasculopathy, a cerebrovascular disease associated with HIV-1, caused through endothelial dysfunction, due to cytokine imbalances and inflammation related to HIV-1, may contribute to intracerebral hemorrhage and collateral vessel impairment, although the pathophysiologic mechanism of vascular damage in HIV-1 remains to be fully ascertained. Thus, adequate management of HIV-1 may be essential in MMS (317). In their 2019 moyamoya multicenter study, Bonasia et al. ascertained three types of anastomoses between the anterior and posterior cerebral circulation, consisting of collaterals from the posterior choroidal arteries (20%), the posterior callosal artery (20%), in addition to a potential pio-pial anastomosis between cortical collaterals of the posterior cerebral artery (PCA) and the anterior cerebral artery (ACA) (15%), with a distinct capacity for retrograde compensation of the anterior circulation. In advanced Suzuki stages from IV to VI in particular, collaterals are frequently observed in MMA. Collaterals may develop due to their ability to compensate the leptomeningeal anastomosis, duro-pial anastomosis, and the ophthalmic-ACA anastomosis collateral systems and due to a diminished blood supply to the ACA territory. The research group suggested a 4-grade classification based on the capability of the three types of PCA-ACA anastomoses to provide retrograde supply to the ACA territory (Figures 2, 3) (7). Based on their 2019 study in 48 MMS patients and 137 MMA patients, Feghali et al. stated that MMS patients and MMA patients may present with similar angiographic phenotypes and similar symptomatology, and may have an equally favorable outcome of surgical revascularization (318). Differentiation of MMS from MMA is important. Whereas the causes of MMS may be reversed by medication, MMA may require surgical revascularization (57). In 2020, Aloui et al. performed a rare *de novo* candidate copy number variant (CNV) screening in 13 MMA trios by use of whole genome high density single-nucleotide polymorphism (SNP) array data and whole exome sequencing (WES) reads depth data. WES and SNP array data of 115 unrelated MMA patients were used to detect recurrence of rare *de novo* CNVs, suggesting that recurrence of the Xq28 candidate CNV, its familial segregation in two additional families, and its *de novo* occurrence in one MMA patient may indicate pathogenicity. Relation of the Xq28 CNV to pulmonary hypertension and use of genetic counseling may be of relevance in clinical practice. The research group has demonstrated a new Xq28 CNV gain in both MMA and a novel MMS related to pulmonary vein stenosis, pulmonary hypertension, and other distinct systemic venous anomalies. These data may be relevant for clinical care and genetic counseling (319). MMA patients may present with a significantly increased rate of persistent carotid-vertebrobasilar anastomoses compared to controls (320), and may be 26 times more likely to suffer from Down's syndrome (321, 322). Accordingly, compared to controls, Down's syndrome patients may present with significantly increased stages of MMA, and may be more than 10 times as likely to show aberrations of the Circle of Willis (323), and vertebral arteries (322, 324). Several genes on chromosome 21 may be associated with angiogenesis, including *Down Syndrome Critical Region 10* (*DSCR10*) (21q22.13), *Dual Specificity Tyrosine Phosphorylation Regulated Kinase 1A* (*DYRK1A*) (21q22.13), and *Collagen Type XVIII Alpha 1 Chain* (*COL18A1*) (21q22.3), possibly by VEGF inhibition (322, 325, 326). In 2021, Kim et al. described a Korean female pediatric patient with CHOPS syndrome accompanied by systemic vasculopathy. The patient had been diagnosed with MMA at 6 years of age and had undergone three synangiosis surgeries. The infrarenal aorta and the renal arteries were diffusely narrowed. A new *AFF4* c.758C > T (p.Pro253Leu) missense variant was ascertained through whole exome sequencing. Except for the *RNF213* c.14429G > A (p.Arg4810Lys) genetic variant, inherited from an asymptomatic mother, no additional candidate variants associated with the vascular manifestation of this patient

were identified (327). In 2021, Pinard et al. suggested a new syndrome related to *RNF213* rare variants characterized by *de novo* mutations disrupting highly conserved amino acids in the RING domain as well as a discrete region distal to the RING domain delimited by amino acids 4,114–4,120, causing early-onset, severe MMA before 3 years of age and occlusion of additional arteries, e.g., the femoral artery, iliac artery, renal artery, and the abdominal aorta (328). In 2021, Sharina et al. demonstrated that decreased cGMP-forming activity of the rare *GUCY1A3* α1C517Yβ1 sGC genetic variant may be aggravated due to reduced protein stability and increased susceptibility to oxidative stress. Combination of these deficiencies may contribute to the severity of symptoms of achalasia and MMA evident in human carriers of the α1C517Yβ1 sGC variant (Figure 4) (329).

Conclusion, treatment strategies, and future research perspectives in moyamoya angiopathy

We have reviewed the physiological and pathophysiological mechanisms of signaling pathways, cells, and genes involved in MMA and MMS and their association with aberrant angiogenesis and inflammation (Figure 4). If mediators involved in these mechanisms are associated with signaling pathway activation or if they constitute downstream mediators remains to be elucidated (5). To do research into the effects of signaling molecules involved in MMA and the part of a signaling pathway they act, may be advocated (5). Moyamoya collateral vessel formation seems to be subsequent to ICA stenosis (5). Thus, prevention of the above-mentioned stenotic process may help avoid the subsequent formation of fragile moyamoya collaterals (5). Angiogenesis in MMA may be either decreased or facilitated (9). Research results indicate that aberrant angiogenesis, decreased or facilitated, may be associated with MMA pathogenesis. These findings seem to be validated by revascularization surgery for MMA, by which increased angiogenesis and an improved formation of a collateral circulation is achieved by restoration of blood flow to the brain (9). Despite a limitation of the number of cases involved, consensual evidence of inflammation in MMA appears to be present (5). Inflammation in pediatric stroke is critically important, both due to the inflammatory signaling cascades activated through ischemia and because of inflammatory baseline pathologies causing stroke (47). Reciprocal action of such fundamental pathophysiologic mechanisms may be of substantial importance, warranting further research (47). Focal pathophysiology may be associated with proximal vessels, such as the circulus arteriosus cerebri, the MCA (M1), ACA (A1), and the distal ICA, whereas generalized pathologies may affect small arteries or peripheral vessels (47, 330). Considerable differences in inflammatory signaling cascades in the neonatal and the adult brain are evident (47). Developmental trajectories of inflammatory signaling cascades from the neonate to the adult remain to be ascertained. Therapeutic interference with such an inflammatory pathology might be feasible by means of additional studies (47). Animal models are warranted to ascertain if these findings may be involved in MMA pathogenesis (5). Regardless whether these processes may induce MMA or result from the arteriopathy, there is growing evidence of a reversible inflammatory process being present in the vascular wall which may contribute to lumen stenosis (5). Inflammation, although not a direct cause of MMA and MMS, may influence *RNF213*, and thus result in aberrant angiogenesis (9).

Moyamoya angiopathy treatment strategies

Enhanced interaction between neurons and cells of the vasculature, increased angiogenic activity, induced curative angiogenesis, and increased formation of a collateral circulation may be Research Topics fundamental to establishing future treatment strategies (9).

In 2011, Li et al. suggested that monitoring of thyroid autoantibodies and thyroid function in MMA patients may be advocated to assist in continuing medical treatment (307). In their 2013 study of a BALB/C male mouse model of ischemic stroke, Rosell et al. researched if treatment with EPCs or their secreted factors may intensify neurogenesis and angiogenesis after persistent focal cerebral ischemia. The research group demonstrated that applying EPC-secreted factors may be an effective and safe cell-free potential future treatment strategy for stroke (158). In 2014, Han et al. suggested that routine screening may be warranted for all family members of familial MMA patients to increase the detection rate for this patient group. In MMA diagnostics, transcranial Doppler sonography may correlate well with magnetic resonance angiography (MRA). Being safe and cost-effective, transcranial Doppler sonography may be the favored screening modality (219). In their 2014 article, Wan and Duan suggested that in hemorrhagic MMA patients, quality of life and cognitive function should be assessed and integrated into evaluations of treatment strategy effectiveness. Also, the researchers indicated that revascularization surgery may be more favorable for hemorrhagic MMA patients, and that combined bypass may lead to a more favorable revascularization and AChA-PCoA extension improvement (94). In 2015, Han et al. suggested that routine vascular screening for NF-1 MMS patients may be advocated regarding early detection of MMS as well as of other cerebral arteriopathies. The research group stated that revascularization surgery may prevent progression of clinical symptoms and diminish the likelihood of subsequent stroke in NF-1 MMS patients (309). In 2015, Hyakuna et al. hypothesized that allogeneic hematopoietic stem cell transplantation may remedy MMA pathophysiology. Niemeyer et al. (331) established a relation between vasculitis and *CBL* germline mutation (310). Although the function of mutated *CBL* in MMA and vasculitis remains to be elucidated, hematopoietic stem cell transplantation may reduce the likelihood of vasculitis (310). In 2018, Duan et al. demonstrated various new MMA susceptibility genes to be associated with homocysteine metabolism. Furthermore, due to enrichment of the expression of these susceptibility genes in the immune system, the research group suggested that therapeutic interventions directed at those pathways could be efficient MMA treatment approaches (208). In 2018, Ishii et al. stated that, in case the post-operative serum level of matrix metalloproteinase (MMP)-9 and Occludin (OCLN) may be significantly elevated, systolic blood pressure should be continuously controlled to avoid post-operative intracranial hemorrhage and/or epilepsy. Particularly regarding MMP-9, the administration of minocycline may be considered (191, 332). In 2018, Wang et al. suggested that hemorrhagic MMA patients should undergo lifelong follow-up, even if their neurological status is excellent (98). In

2019, Nishihiro et al. demonstrated that High-mobility group box-1 (HMGB1) with encephalo-myo-synangiosis (EMS) in a chronic hypoperfusion model promoted cerebral angiogenesis in a VEGF-dependent manner, resulting in improvement of cerebral blood flow. This treatment may be an effective therapy for MMA patients (333). In 2020, Zhao et al. demonstrated that autogenous bone marrow stem cell mobilization combined with dexamethasone antiinflammation and anti-infection treatment after revascularization in MMA patients may accelerate recovery of nervous function and promote blood vessel formation. At the same time, this treatment approach may reduce inflammation and improve the quality of life of MMA patients (334). In 2020, Gu et al. stated that two potential small molecule drugs, indirubin and CAY-10415, were recognized as MMA candidate drugs through Connectivity Map (CMap) (252). In 2021, Mineharu and Miyamoto suggested various treatment strategies including pharmacological eNOS-sGC-cGMP pathway stimulation, inflammation control, avoidance of hypoxia, homocysteine control, and blood lipid control. Pharmacological treatment candidates of MMA may be homocysteine lowering drugs, such as vitamin B12 or folate, lipid lowering drugs, such as Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors and statins, and anti-inflammatory drugs, such as anti-IL-6 antibodies or COX-2 inhibitors. Soluble guanylate cyclase stimulator Riociguat, used for pulmonary arterial hypertension treatment, could be an alternative (119, 335). Yet, there is insufficient proof of both interaction and functionality of these candidate markers, which may be associated with MMA. Identification of detailed molecular networks may acquire novel therapeutic strategies (5, 119). In their 2022 review article, Zhang et al. stated that, in addition to various pre-existing MMA staging systems, which are based on medical imaging and symptomatology, a suitable MMA grading system, capable of ascertaining MMA disease progression, may be warranted (336). Referring to their 2022 study results, Wang et al. indicated that timely indirect surgery may be warranted in pediatric MMA patients. Even though the results of the research group did not directly prove efficacy in preventing recurrent intracerebral hemorrhage, the group stated that aberrant collaterals of the posterior choroidal artery had decreased post-operatively. All subjects had a favorable clinical outcome (297).

Moyamoya angiopathy future research perspectives

Recent MMA research may concentrate on the three main sectors therapy, prognosis, and diagnosis (6, 337). MMA therapeutic innovation research has remained behind the significant achievements in the diagnostic and prognostic area of MMA research (6, 337). Prognosis of MMA has been advanced through non-invasive biomarkers and new imaging methodologies (2, 6, 64, 196, 290, 338). MMA diagnosis has profited greatly from the latest advancements in molecular genetics, with significant progress in the identification of specific genetic variants related to clinical phenotypes and radiographic presentations (6, 51, 230, 236, 339).

Genetic analysis of familial MMA may help to ascertain the pathogenesis of MMA (4). In case of identification of relevant genes, development of novel gene therapies and prevention of MMA occurrence in genetically susceptible individuals may be possible (4). Also, the Japan Adult Moyamoya Trial (4, 99, 340–345) may contribute to ascertain the advantages of combined or direct bypass surgery for the prevention of recurrent hemorrhage in MMA (4). Additional follow-up and epidemiological studies are warranted to ascertain the pathogenesis of asymptomatic MMA (4). These results will be important to refine the guidelines for surgical and medical MMA treatment, in particular for asymptomatic or hemorrhagic MMA patients (4).

In 2002, Soriano et al. suggested that additional research may be warranted to ascertain if soluble endothelial adhesion molecules may be potential therapeutic or diagnostic markers for MMS therapeutic management (110). In 2008, Jung et al. suggested that further prospective studies may be warranted to ascertain if alterations in functioning and number of circulating EPCs may serve as prognostic or diagnostic biomarkers in MMA (159). In 2009, Shimojima et al. stated that further studies are required to identify a major disease-causing gene for MMA (211). In 2010, Kim et al. suggested that additional research may be warranted to ascertain the distinct functioning of EPCs in MMA pathogenesis (153). In 2011, Ni et al. indicated that further research may be warranted to ascertain the correlation between SDF-1α and CD34+ CXCR4+ cells in MMA (152). In 2012, Bao et al. stated that randomized clinical trials may be warranted to study the efficacy of revascularization procedures (278). In 2013, Chen et al. suggested that transfer function analysis derived phase shift and rate of recovery (RoRc) may be advantageous in clinical studies researching into hemodynamic compromise, as these may provide a both non-pharmacologic and non-invasive method with reliable sensitivity in correlation with angiography (65). In 2013, Hitomi et al. demonstrated that iPSECs may serve as an *in vitro* MMA model, expressing a useful benchmark phenotype for high throughput screening, which may be applied to drug development and used to ascertain MMA causative factors (187). In 2013, Hu et al. suggested that larger studies may be warranted to ascertain the potential association of *Actin Alpha 2, Smooth Muscle (ACTA2)* (10q23.31) and/or additional genes and MMA in different populations (216). In 2014, Dai et al. suggested that research into established angiogenesis-related genes may be a path to ascertain potentially unknown angiogenesis-associated miRNAs. Furthermore, adjustment of the pathophysiologic mechanism associated with the functioning of serum miRNAs in MMA may be a possible therapeutic strategy which may warrant additional research (270). In 2014, Kang et al. stated that their research into SPCs from the peripheral blood of MMA patients may supply a new experimental cell model for future MMA research (149). In 2014, Ryoo et al. suggested that distinct radiological findings may further elucidate MMA pathogenesis and distinguish ICAD from MMA (85). In 2014, Wan and Duan stated, that hemorrhagic MMA treatment strategies may not be standardized, and that randomized, prospective, large sample clinical trials may assist to ascertain the most favorable treatment approach (94). In 2015, Baltsavias et al. suggested that a more thorough understanding of the moyamoya collateral circulation and anastomotic networks may assist in the definition of a novel MMA staging system which may impact clinical practice (69). In 2015, Ganesan et al. suggested that addressing three distinct research areas may assist in further elucidating MMA pathogenesis (337). First, precise clinical and radiological phenotyping of distinct

MMA populations to encourage research into genetic and biological mechanisms (337). Second, development of new, standardized prognostic methods to instruct therapeutic decision making and stratify the risk of disease (337). Third, prospective analysis of the efficacy of MMA surgical revascularization, to contrast the risks of distinct treatment strategies, through application of standardized radiographic, neurocognitive, and clinical assessments, in order to unbiasedly assess the efficacy of various treatment strategies (337). In 2015, Karunanithi et al. suggested that their study results may warrant further research into pressure drop indicator (PDI) as a causal factor regarding post-operative complications in MMA patients (70). In 2015, Kobayashi et al. suggested that the pathology related to aberrant SMC proliferation and decreased angiogenesis, such as arterial stenosis in the Willis' circle or moyamoya vessel formation should be addressed in future studies. Furthermore, this group hypothesized, that research into the function of *RNF213* in maintenance and remodeling of the vascular system may help elucidate mechanisms of both cerebral artery stenosis in general and in MMA (205, 346). An ATP binding inhibitor specific to the Walker A motif in the first AAA+ may be an auspicious therapeutic candidate, potentially increasing CNS hypoxic tolerance in *RNF213* R4810K carriers (205). In 2016, Duan et al. stated that the specific function of the posterior communicating artery, anterior choroidal artery, and moyamoya collaterals need to be further investigated. Also, the research group suggested that additional research on cerebral microbleeds may be warranted to further elucidate the pathogenesis of pediatric hemorrhagic MMA. Furthermore, the group indicated that comprehensive, large-sample studies may be warranted to further ascertain pediatric hemorrhagic MMA (96). In 2016, Gao et al. suggested that doing research into the MAPK signaling pathway and lncRNAs, and their potential function as therapeutic targets, may be warranted (10). In 2016, Hamauchi et al. stated that additional research may be advocated to further elucidate the pathology of differentially expressed ECM receptor-related genes and splicing regulating proteins in MMA pathogenesis (166). In 2016, Liu et al. suggested that more detailed and longer angiographic and clinical follow-up study may be warranted to ascertain the pathophysiologic mechanism underlying recurrent intracranial hemorrhage in hemorrhagic MMA (97). In 2016, Mossa-Basha et al. suggested that, in case of confirmation in larger studies, the criteria for MMA and the moyamoya diagnostic algorithm may be revised according to improved diagnostic accuracy in addition to a potential limitation of invasive diagnostics (83). In 2016, Scholz et al. stated that the identification of an association between *RNF213* and NFAT1 may be another method for further research into the molecular pathogenesis of MMA (120). In 2017, Liao et al. suggest that studies identifying the ethnicity-specific factors and pathological role of *RNF213* genetic variants in MMA and intracranial major artery stenosis/occlusion (ICASO) may be warranted (225). In 2017, Mejia-Munne et al. suggested, that additional research may be warranted to ascertain the pathophysiology of MMA and inflammation (287). In 2017, Park et al. suggested that additional studies may be warranted to ascertain if the *RNF213* rs112735431 polymorphism may be related to hypertension in MMA patients and healthy controls in the Korean population. Definition of the relationship between the *RNF213* rs112735431 polymorphism and hypertension in MMA patients as well as determination of the specific biochemical function of *RNF213*, which may be involved in the pathogenesis of hypertension, may be advocated (226). In 2017, Qiao et al. suggested that studies to ascertain methods to evaluate the moyamoya collateral circulation by use of combined multimodality imaging techniques, such as perfusion imaging, structural MRA imaging and functional brain imaging, to assess cortical structural change as a consequence of revascularization surgery, may provide results which may assist in clinical decision making, including patient selection strategies for operative management of MMA patients (89). In 2017, Uchiyama stated that study of the *RNF213* genetic variant in twig-like MCA patients may clarify the twig-like MCA pathogenesis, enabling to establish a differential diagnosis of MMA (90). In 2017, Wang et al. suggested that future research may concentrate on the MAPK signaling pathway and on inflammation in MMA (251). In 2018, Ishii et al. suggested that histopathologic examination of the blood-brain barrier (BBB) and quantitative assessment of cerebral blood flow and cerebral blood volume may be warranted for validation of their hypothesis (191). In 2019, Liu et al. stated that longer follow-up studies and larger patient samples may be warranted to substantiate the value of their proposed new MMA collateral grading system (79). In 2019, Tashiro et al. suggested that the genetic background underlying intracranial vertebral artery dissection should be elucidated in future studies (228). In 2019, Corey and Luo suggested that research into the involvement of neutrophils in moyamoya progression could be an additional path for future research (264). According to their preliminary 2019 cohort study results, Funaki et al. suggested that verification of their present results in larger studies and additional research on the effect of choroidal collaterals on recurrent intracranial hemorrhage in hemorrhagic MMA may be warranted to ascertain the best possible treatment strategy for asymptomatic MMA patients and for non-hemorrhagic cerebral hemispheres in adult hemorrhagic MMA patients in the Japan Adult Moyamoya (JAM) Trial (99). In 2019, Shen et al. suggested continued future research to investigate if CD40 may function as a personalized MMA marker (231). In their 2019 article, Young et al. stated that further prospective studies may be warranted to evaluate the clinical utility of intracranial vessel wall imaging in differentiating MMS from MMA, and to predict hemorrhage and ischemia, which may help identify high-risk and low-risk patients and direct clinical management (57). In 2019, Bonasia et al. suggested that both an analysis of the three types of anastomoses between the anterior and posterior cerebral circulation by selective contrast injection into the PCA and an analysis of the ophthalmic-ACA anastomosis, the leptomeningeal anastomosis, and the duro-pial anastomosis collateral systems may be warranted to further elucidate MMA pathogenesis and to better identify patients who may benefit from bypass surgery (7). In 2020, Aloui et al. suggested that additional research may be warranted to further elucidate the mechanism associated with the Xq28 candidate copy number variant and the pathogenesis of vascular disease in patients affected with MMA and a novel MMS (319). In 2020, Han et al. suggested that platelet activation and renin secretion may help guide clinical management and may further elucidate the pathogenesis of asymptomatic MMA (253). In 2020, Kim et al. suggested that additional study of the molecular biology and functioning of *RNF213* may further ascertain the pathophysiology of cerebrovascular disease and ICASO (233). In 2020, Wang et al.

stated that larger cohorts, including different ethnicities, may be warranted to further clarify associations between *TGFB1* SNPs and MMA (235). Also, with both genetic and environmental factors being associated with MMA pathogenesis (347), multivariate analysis to adjust confounders, including biochemical, clinical, and behavioral factors, should be incorporated in future studies (235). In 2021, Byworth et al. suggested that further research may be warranted to ascertain whether additional copies of the *Down Syndrome Critical Region 10* (*DSCR10*) (21q22.13), *Dual Specificity Tyrosine Phosphorylation Regulated Kinase 1A* (*DYRK1A*) (21q22.13), and *Collagen Type XVIII Alpha 1 Chain* (*COL18A1*) (21q22.3) genes may be associated with aberrant vascular development in Down's syndrome, which may predispose to MMA (322). In 2021, Han et al. suggested that methods to alleviate inflammation and to restore the BBB may be efficient MMA treatment strategies (194). In 2021, Li et al. stated that additional studies may be warranted to help clarify the pathophysiologic mechanism associated with neutrophilic tsRNAs and their associated signaling pathways in asymptomatic MMA patients, to further elucidate MMA pathogenesis (276). In 2021, Lu et al. suggested that the serum levels of BBB-related proteins and MMP-9, in addition to their comparison between MMA subgroups, should be compared to healthy controls (198). Also, the research group indicated that the pharmaceutical significance of a strengthened impact of MMP-9 on surgery and the predictive value of intracranial hemorrhage prediction should be subject to validation in future research (198). Moreover, the group stated that additional research may be warranted to further ascertain the function of MMP-9 and BBB impairment in MMA pathophysiology (198). In 2021, Mineharu et al. stated that the functions of *GUCY1A3* and *RNF213* have been intensively studied in VSMCs and vascular ECs. However, with the distinct mechanism of fibrosis and intimal thickening in MMA remaining to be elucidated, research into the function of GUCY1A3 and RNF213 in immune cells, especially in dendritic cells, neutrophils, B cells, and T cells may be warranted. Additional vascular components, including the extracellular matrix (ECM), platelets, and inflammatory cells, should be subject of future research (119). In 2021, Sarkar et al. stated that further *RNF213* knockdown studies may be warranted to confirm both the function of *RNF213* in TNFα/PTP1B mediated obesity and insulin resistance and detailed pathophysiologic mechanisms related to this signaling pathway (11). In 2021, Sarkar et al. suggested that doing research on the effect of iron-binding in MMA and on the pathophysiologic mechanism of RNF213 in cancer and obesity may be warranted (237). In 2021, Wu et al. stated that additional prospective studies may be warranted to further ascertain the association between bleeding spots and aberrant MMA collaterals (103). Relating to their 2022 study results, Jin and Duan stated that, due to the complex functions and molecular genetic mechanisms, their bioinformatics results may warrant verification experiments. Jin and Duan hypothesized that the slow progression of MMA may be associated with a distinct gene expression at each MMA stage, that the genetics of adult MMA and pediatric MMA may be different, warranting additional clarification of such potential variations (241). Referring to the results of their 2022 transcriptome-wide analysis, Xu et al. suggested that the sex difference should be considered in future MMA research (243).

Continued research into MMA pathophysiology and associated signaling pathways may identify new treatment strategies, therapeutic applications, and mechanism-tailored interventions that may halt MMA progression. Research into EPCs, endothelial cells, and pericytes may further elucidate the function of vasculogenic, angiogenic, and anti-angiogenic markers and associated signaling pathways (3, 348). Reduction of interlaboratory variations and methodological differences may facilitate cooperation between laboratories (3, 348, 349). Constant evaluation of novel prognostic and diagnostic resources obtained through research may help to effectively and safely transfer research results into practice (2). Ongoing collaborative, prospective basic laboratory, and large-scale, large cohort clinical research on pathophysiologic mechanisms, a multi-professional, multi-center, international collaboration between vascular and stroke physicians, and clinician-scientists pursuing translational research are essential to establish large biorepository, imaging, and clinical data sets, which may be required if we are to better understand the complex etiology of MMA, potentially leading to increasingly differentiated diagnoses and disease-modifying treatment strategies (2, 6, 44, 92, 337, 350).

Author contributions

KD contributed to developing the concept of the review, developing the figures, and writing and editing the manuscript. JW oversaw the project and contributed to developing the concept of the review, developing the figures, and editing the manuscript. Both authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2023.661611/full#supplementary-material

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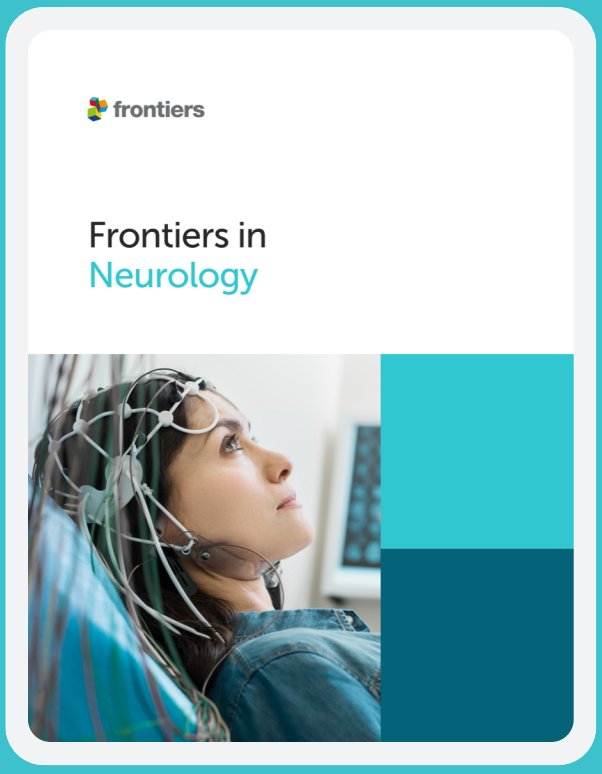

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