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FEATURED ARTICLE

Brain perfusion, cognition, and plasma Alzheimer's biomarkers in moyamoya disease

Xiang Zou[1,2,3,4,5] | **Yujun Liao**[1,2,3,4,5] | **Conglin Jiang**[1,2,3,4,5] | **Yifan Yuan**[1,5] | **Fan Zhao**[1,2,3,4,5] | **Ding Ding**[5,6,9] | **Liang Chen**[1,2,3,4,5,8] | **Bin Xu**[1,2,3,4,5] | **Ying Mao**[1,2,3,4,5,7,8]

[1]Department of Neurosurgery, Huashan Hospital, Fudan University, Shanghai, China

[2]Neurosurgical Institute of Fudan University, Shanghai, China

[3]Shanghai Clinical Medical Center of Neurosurgery, Shanghai, China

[4]Shanghai Key Laboratory of Brain Function and Restoration and Neural Regeneration, Shanghai, China

[5]National Center for Neurological Disorders, Shanghai, China

[6]National Clinical Research Center for Aging and Medicine, Huashan Hospital, Fudan University, Shanghai, China

[7]State Key Laboratory of Medical Neurobiology and MOE Frontiers Center for Brain Science, School of Basic Medical Sciences and Institutes of Brain Science, Fudan University, Shanghai, China

[8]Tianqiao and Chrissy Chen International Institute for Brain Diseases, Shanghai, China

[9]Institute of Neurology, Huashan Hospital, Fudan University, Shanghai, China

Correspondence
Ying Mao, Department of Neurosurgery, Huashan Hospital, Fudan University, No. 12 WulumuqiZhong Road, Shanghai 200040, China.
E-mail: hs_maoying@vip.126.com

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Abstract

Introduction: Because growing interest has been focusing on cerebral blood flow (CBF) to predict, prevent, and treat Alzheimer's disease (AD), it is important to clarify the role of CBF in AD pathology and cognitive decline.

Methods: In a moyamoya disease (MMD) cohort, we examined CBF, specific cognitive domains, and plasma AD biomarkers, as well as correlations among these variables.

Results: CBF was significantly reduced in newly diagnosed MMD patients, while plasma phosphorylated tau181 was elevated and positively correlated with hypoperfusion accumulation. MMD patients scored significantly lower than controls in multiple cognitive tests. Revascularization increased CBF to the recipient brain territories as well as cognitive performance but produced no significant change in AD biomarker levels.

Discussion: These data suggest a link between accumulated reductions in CBF and cognitive decline, as well as a possible role of AD-like pathological burden. Further studies in MMD will provide opportunities to explore new treatment strategies.

Xiang Zou, Yujun Liao, and Conglin Jiang contributed equally to this work.

KEYWORDS
Alzheimer's disease, biomarkers, cerebral blood perfusion, cognitive impairment, moyamoya disease

1 | INTRODUCTION

Vascular changes or pathologies are the earliest precursors for subsequent events in neurodegenerative processes, increasing the risk of vascular cognitive impairment and dementia, or even the risk of Alzheimer's disease (AD).[1–3] Normal brain functioning requires constant cerebral blood flow (CBF) to ensure adequate delivery of oxygen, energy metabolites, and nutrients, along with the removal of carbon dioxide and metabolic waste.[4] Chronic brain hypoperfusion (CBH), defined as long-term cerebral perfusion that is not commensurate with brain neurometabolic demands, may participate in the pathogenesis of dementia.[5,6] In most cross-sectional studies, CBF reduction is correlated with increased risk for dementia in normal aging, and also linked with higher amyloid beta (Aβ) deposition levels with more severe white matter hyperintensities and brain atrophy in AD patients.[7–10] However, some longitudinal studies failed to demonstrate any significant CBF decrease in patients with dementia.[11,12] Because growing interest in the role of CBF in the prediction, prevention, and treatment of AD has been emerging, it is important to trace hypoperfusion from initiation to progression. However, the decades-long and occult preclinical course of AD accompanied by various comorbid geriatric diseases make it difficult to clarify the role of CBF.

Previously, we hypothesized that moyamoya disease (MMD), a rare cerebrovascular disease characterized by chronic cerebral artery stenosis and cerebral hypoperfusion, is an ideal human model linking CBH to dementia.[13,14] Until now, no direct casual evidence between CBH and AD in human subjects has been proved, as chronic cerebrovascular disease and AD share the same risk factors, for example, aging, smoking, diabetes, and atherosclerosis. Additionally, vascular and neurodegenerative pathologies interact and are synergistic, which makes it challenging to clarify whether CBH can be the earliest AD pathogenesis or contribute to accelerating AD. Moreover, age-related degeneration also raises the question of the exact point in time when AD pathology does start developing.[15] Therefore, a human hypoperfusion model with pure etiology is needed to investigate the causal mechanism from CBH to AD, as well as the possibility of a revascularization strategy for prevention and the intervention of AD. Instead of the aging population studied in conventional AD research, most MMD patients are young to middle-aged and have a more independent CBH etiology. Hopefully, we may be able to study the period just after the onset of the disease. Herein, we hypothesize that CBH may be an independent factor that will induce AD-like hallmarks or even preclinical AD. We first investigated brain perfusion, neuropsychological performance, and AD biomarkers in an MMD cohort. We aimed to present the baseline of those factors in pure CBH populations and reveal their correlations, which may pose an intriguing approach for understanding the contribution of CBH to chronic brain degeneration, such as AD.

2 | METHODS

2.1 | Participants

This study was approved by the ethics committee of Huashan Hospital, Fudan University (KY2015-256), and informed consent was obtained from the participants. This work was also carried out in accordance with the Declaration of Helsinki for experiments involving humans. We included 90 MMD patients and 20 controls (age range, 14–65 years) diagnosed with intracranial aneurysms and hemangioma. The general exclusion criteria included previous neurosurgery history or evidence of intracranial hemorrhage or infarction on magnetic resonance imaging (MRI). Finally, 66 MMD patients were included (Figure S1 in supporting information). Among these, 43 were newly diagnosed with MMD, while 23 had already received unilateral bypass surgery with a follow-up period of 3 to 18 months. The main procedure of bypass surgery is to dissect superficial temporal artery (STA) as donor and choose a branch of the middle cerebral artery (MCA) with similar diameter as recipient vessel. Vascular anastomosis will then be performed as end-to-side, followed by encephalo-dura-myo-synangiosis (EDMS). The participants underwent an MRI scan, neuropsychological testing, and analyses of plasma Aβ42 and phosphorylated tau181 (p-tau181) levels. Apolipoprotein E (*APOE*) ε4 genotyping was also performed for 20 newly diagnosed MMD patients and 4 controls. Plasma lipid levels, including triglycerides (TG), cholesterol (TCHO), and high- and low-density lipoprotein (HDL, LDL), were recorded from routine laboratory reports. Blood pressure (BP), including systolic blood pressure (SBP), diastolic blood pressure (DBP), mean artery pressure (MAP), and pulse pressure (PP) were averaged from measurements taken on the first three mornings after admission. PP = SBP−DBP, while MAP = DBP + 1/3 PP.

2.2 | Neuropsychological assessment

A battery of neuropsychological tests was used to evaluate the cognitive function of each participant. The test battery comprised the Mini-Mental State Examination (MMSE), Auditory Verbal Learning Test (AVLT), Trail Making Test (TMT), Rey-O complex figures (ROCF), Boston Naming Test (BNT), Verbal Fluency Test (VFT), Clock Drawing Test (CDT), Similarity Test, and Digit Symbol Substitution Test (DSST). This comprehensive test battery covered global cognition, executive function, spatial construction function, memory, language, and attention. Although originally developed in Western countries, the tests were translated, adapted, validated, and harmonized with Chinese culture. The test battery was administered in Chinese by certified study psychometrists within a period of 90 minutes.

RESEARCH IN CONTEXT

1. **Systematic Review**: We carefully reviewed the relationship among cerebral blood flow (CBF), cognition, and Alzheimer's disease (AD)–like pathology, as well as the comprehensive studies about moyamoya disease (MMD)–related brain damage and cognitive impairment.
2. **Interpretation**: This study provided evidence that cognition can be impaired by chronic hypoperfusion independent of AD-like pathology, and can be improved by perfusion recovery without AD-like pathology changes. Moreover, the plasma phosphorylated tau181 level is closely associated with the duration and severity of brain hypoperfusion. These data suggest a link between accumulated reductions in CBF and cognitive decline, as well as a possible role of AD-like pathological burden.
3. **Future Directions**: Further large-sample longitudinal studies are needed to confirm dementia-related pathological changes in MMD and the changes in AD-like pathology after global revascularization. Studies including early-stage AD subjects can also help clarify the relationship among brain perfusion, cognition, and AD pathology.

2.3 | Neuroimaging assessment

Angiographic evaluation was based on digital subtraction angiography (DSA). The Suzuki stage of each hemisphere was assessed by two independent neurosurgeons blinded to the medical history of the participants. All MRI scans were performed on a 3.0 Tesla scanner (GE Healthcare) using a 32-channel intraoperative head coil. Cerebral infarction was recognized on T2-weighted image (T2WI) fluid-attenuated inversion recovery (FLAIR) MR images and evaluated by two independent neurosurgeons. A 3D stack spiral fast spin echo sequence was used to obtain arterial spin-labeling (ASL) perfusion maps, and the following parameters were adopted: labeling duration 1450 ms, post-labeling delay 2025 ms, field of view (FOV) 200 × 200 mm, slice thickness 4.0 mm, repetition time 4762 ms, and time to echo 10.6 ms. Data was preprocessed using DPABI and AFNI software.[16,17] After skull-stripping, the CBF map of each subject was spatially normalized to the standard Montreal Neurological Institute space with a re-slicing resolution of $2 \times 2 \times 2$ mm^3. The normalized CBF maps were smoothed with 8 mm full width at half-maximum (FWHM) isotropic Gaussian kernel and were normalized by the average of voxels in gray matter. Regions of interest (ROIs) were defined by Automated Anatomical Labelling (AAL) Atlas and quantitative ROI signal was extracted to do analysis of variance (ANOVA) with GRETNA. Statistics was done with SPM12. All whole-brain analyses assessed statistical significance based ofn cluster mass, with the voxel-defining threshold set to $P < 0.001$ and cluster-defining threshold set to $P < 0.05$. Only the clusters with the largest region area ratio were reported. We first extracted the mean CBF from frontal, parietal, temporal, and occipital lobes of each hemisphere for ROI-based analysis according to the AAL template. To eliminate physiological variations and scan inconsistency, the CBF value was further standardized with a cerebellum standardization method to obtain a relative CBF (rCBF) value.

2.4 | Brain aging factor

To verify the hypothesis that CBH accelerates brain aging, we built an integral model named "Brain Aging factor" that takes into consideration both the length and severity of the hypoperfusion. It has been reported that the Suzuki stage in MMD is related to severity of vasculopathy and the course of the disease.[18] In addition, each level of Suzuki stage (from I to V/IV) progress has been calculated to be equivalent to about 20 years of normal brain aging that will impair CBF.[19] Moreover, the value of rCBF also has significant contribution to brain aging.[20] Taken together, we can get the following formula:

$$BA = \ln \frac{(\mathrm{Su} \times 20 + \mathrm{Age})}{\mathrm{rCBF}} \quad (1)$$

In this equation, BA is brain aging factor and Su is the Suzuki stage, whose coefficient equals 20, which means the accelerated aging on brain as previously mentioned. For controls, the Suzuki stage is equal to zero. As a factor, we further reduce the absolute value by logarithmic function to facilitate calculation. This model predicts a positive correlation with hypoperfusion course and natural age, but a negative correlation with rCBF.

2.5 | Plasma biomarkers

Non-fasting blood was drawn from study participants and processed for plasma extraction before storage at −80°C until use. All biomarkers were measured by evaluators blinded to clinical information at the Huashan Hospital on the Simoa HD-X platform (Quanterix). Measurement of plasma p-tau181 with the AT270 mouse monoclonal antibody (MN1050; Invitrogen) specific for the threonine-181 phosphorylation site was based on an ultrasensitive Simoa immunoassay with minimal cross-reactivity and high reproducibility. Plasma Aβ42 was measured using the Neurology 3-plex A assay kit from Quanterix.

2.6 | Statistical analysis

Demographic characteristics were analyzed by the chi-square test (for categorical variables) and either the Mann–Whitney U test or Student's *t* test (for continuous variables). For plasma AD biomarkers and cognition, the comparisons were conducted by the *t* test or Mann–Whitney U test. For lobular rCBF, one-way ANOVA was applied and corrected by the Tukey post hoc test. To analyze the

association among plasma AD biomarkers, cognition, and rCBF, we first conducted Pearson's correlation analysis to examine the association between rCBF and cognition and corrected by false discovery rate (FDR). Because of the potential overlap among cognition domains, we then performed principal component analysis (PCA) for all the cognition scales, followed by stepwise regression analysis with lobular rCBF. The association of cognition with either rCBF or plasma AD biomarkers was further verified in multiple linear regression (MLR) models, after adjusting for covariates. AD biomarkers in the regression analyses were standardized to *z* scores to facilitate comparison between models. MLR models were calculated in two models adjusting for different covariates (Model 1: adjusting for age, sex, and *APOE* ε4 status; Model 2: adding more covariates, including BP, diabetes, and serum lipid). Taking both hypoperfusion severity and time course into consideration, the associations of brain perfusion with plasma AD biomarkers (log transformed) were analyzed using an integral model named "Brain Aging factor" (BA) described previously. Multicollinearity was assessed using tolerance, variance inflation factor (VIF), and Pearson's correlation coefficients. The VIF value was less than five in each model of this study. Statistical analyses were performed using SPSS version 17 (SPSS Inc.). *P*-values < 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Participant characteristics

Participant characteristics are presented in Table 1. The age difference between MMD patients and controls was not significant (43.86 ± 11.21 vs. 41.15 ± 14.06). Although serum lipid levels were significantly different between the two groups, the mean value in MMD patients was within normal limits or borderline high. Pulse pressure in MMD patients was remarkably higher than in controls (49.51 ± 7.875 vs. 41.89 ± 9.113, $P = 0.0015$), suggesting reduced blood vessel elasticity in MMD patients. Twenty percent of MMD patients were *APOE* ε4 carriers.

3.2 | Associations among brain perfusion, cognition, and plasma AD biomarkers

For plasma AD biomarkers, the MMD group had a higher p-tau181 level compared to that in controls (10.25 ± 20.46 vs. 1.57 ± 1.56 pg/mL, $P < 0.05$), while no significant difference was found between groups in Aβ42 and the p-tau181/Aβ42 ratio. For cognitive levels, the MMSE, DSST, BNT, TMT, and VFT scores in MMD patients were all significantly lower than in controls (Table 1).

3.2.1 | Brain perfusion and cognition

Generally, cerebral perfusion in newly diagnosed MMD patients was widely impaired compared to that in controls (Figure 1A). Bilateral frontal and parietal lobes were more likely to be involved in MMD. Correlations between mean rCBF from each lobular ROI and index scores of cognition scales were analyzed by Pearson's correlation. We found that cognition performance is widely correlated with lobular rCBF. Even after correcting for multiple comparisons, the CDT score was significantly associated with the left frontal lobe ($r = 0.37$). VFT scores were remarkably related to rCBF in the left parietal lobe ($r = 0.39$ and 0.37, Figure 1B). Considering the potential overlap among cognition domains, PCA was performed and showed there is one principal component that can represent 67.48% cognitive score (Figure 2A). Further stepwise regression analysis with lobular rCBF confirmed its significant positive correlation with left parietal lobe ($F = 4.25$, $P = 0.0447$, $R^2 = 0.08$, $\beta = 0.285$, Figure 2B).

3.2.2 | Brain perfusion and plasma AD biomarkers

Associations between brain perfusion and plasma AD biomarkers were further investigated by MLR models. After adjustments for covariates (Model 1), rCBF showed no association with Aβ42, p-tau181, or the p-tau181/Aβ42 ratio ($P = 0.089$, 0.731, and 0.812). When additional variables were included (Model 2), we found that plasma Aβ42 was negatively associated with the BA, but not that significant ($\beta = -0.209$, $P = 0.0608$, Figure 2C). Although rCBF has no significant contribution to plasma p-tau181 and p-tau181/Aβ42 ratios, TG and TCHO were significantly associated with their level by sex stratification (Table S1 in supporting information). When taking the accumulation of hypoperfusion into consideration, we find that BA factor is significantly associated with higher plasma p-tau181 level by sex stratification (female: $\beta = 0.703$, $P = 0.0347$; male: $\beta = 0.06$, $P = 0.853$, total: $\beta = 0.521$, $P = 0.0329$, Figure 2D).

3.2.3 | Cognition and plasma AD biomarkers

Because the plasma AD biomarkers and neuropsychological performance were remarkably different between MMD patients and controls, we further investigated their correlations. In newly diagnosed MMD patients and controls, no correlation was found between cognition and plasma AD biomarkers. However, when post-surgical follow-up MMD patients were included, elevated plasma Aβ42 level was positively correlated with the DSST score (β coefficient = 0.08561, $P = 0.049$, Table 2).

3.3 | Effect of revascularization

Given the close relationship between CBH and cognitive impairment, as well as plasma AD biomarkers, we further investigated the effect of revascularization for MMD. Remarkably, rCBF was increased in all the recipient territories (frontal, temporal, and parietal lobe, Figure 3A). Although brain perfusion was partially improved, Aβ42 and p-tau181 trended toward deteriorated plasma levels. Neuropsychological

TABLE 1 Participant characteristics

		MMD total	Control	*P*
N		66	20	
Age, year, mean (SD)		43.86 (11.21)	41.15 (14.06)	0.3748
Sex, female, *n* (%)		43 (65.15)	12 (60.00)	0.7914
Type 2 diabetes, *n* (%)		6 (10)	0 (0)	0.3286
APOE ε4 allele, positive, fraction		4/20	0/4	
Plasma biomarkers, mean (SD)				
Aβ42 (ng/mL)		8.823 (12.72)	6.283 (3.640)	0.4908
P-tau181 (pg/mL)		10.25 (20.46)	1.572 (1.564)	0.0357[a]
P-tau181/Aβ42 ratio		2.218 (4.919)	0.2936 (0.3216)	0.0574
Serum lipid, mean (SD)				
TG		4.136 (0.8927)	4.563 (0.8621)	0.0737
TCHO		1.943 (1.603)	1.123 (0.5015)	0.0368[a]
HDL		1.194 (0.3065)	1.406 (0.3505)	0.0146[b]
LDL		2.366 (0.7318)	2.769 (0.6950)	0.0408[b]
Blood pressure, mean (SD)				
SBP (mmHg)		124.1 (11.70)	119.4 (12.35)	0.1657
DBP (mmHg)		74.6 (7.813)	77.49 (7.085)	0.1922
MAP (mmHg)		91.1 (8.515)	91.45 (8.117)	0.8844
PP (mmHg)		49.51 (7.875)	41.89 (9.113)	0.0015[a]
Education, year, mean (SD)		10.71 (4.141)	15.95 (8.703)	0.0641
Neuropsychological assessment				
MMSE		25.70 (8.506)	28.62 (1.261)	0.0314[a]
AVLT		47.21 (20.12)	54 (13.61)	0.1125
DSST	Pre-90s	37.54 (17.09)	46.67 (16.28)	0.0351[b]
	Accidental memory	2.846 (2.879)	4.333 (3.109)	0.044[b]
CDT		18.94 (9.076)	22.87 (6.479)	0.0618
BNT		21.1 (6.809)	24.73 (4.317)	0.0276[b]
TMT	TMT-A	58.13 (32.00)	41.93 (15.19)	0.0072[a]
	TMT-B	129.9 (58.89)	103.9 (35.97)	0.0316[a]
	TMT-B-1 min	15.75 (23.69)	15.33 (3.155)	0.0163[a]
VFT	Animal	17.21 (23.66)	17.6 (4.085)	0.0134[a]
	City vegetables	10.81 (4.602)	12.67 (3.222)	0.0744
	Alternation	11.21 (5.169)	14.2 (3.406)	0.0196[b]
	ROCF	29.59 (8.808)	31.57 (4.052)	0.4979

Abbreviations: Aβ, amyloid beta; *APOE*, apolipoprotein E; AVLT, Auditory Verbal Learning Test; BNT, Boston Naming Test; CDT, Clock Drawing Test; DBP, diastolic blood pressure; DSST, Digit Symbol Substitution Test; HDL, high density lipoprotein; LDL, low density lipoprotein; MAP, mean arterial pressure; MMD, moyamoya disease; MMSE, Mini-Mental State Examination; PP, pulse pressure; p-tau, phosphorylated tau; ROCF: Rey-O complex figures; SBP, systolic blood pressure; SD, standard deviation; TCHO, total cholesterol; TG, triglyceride; TMT, Trail Making Test; VFT, Verbal Fluency Test.

[a]By *t* test.

[b]By Mann–Whitney U test.

performance after bypass surgery generally improved in most scales (Table 3); however, only DSST ($P = 0.0192$) and TMT were significant (part B, $P = 0.0362$). Furthermore, we investigated the rCBF changes in a very detailed AAL template that divided the brain into 116 areas. We found that rCBF in the orbital part of the right middle frontal gyrus (ORBmid.R, Figure 3B) and right angular gyrus (ANG.R, Figure 3B) were significantly impaired in newly diagnosed MMD compared to the normal controls (0.914 ± 0.151 vs. 1.009 ± 0.059, $P = 0.041$; 0.902 ± 0.134 vs. 0.985 ± 0.044, $P = 0.045$, Figure 3C), while the hypoperfusion was remarkably increased in these two areas during follow-up after revascularization (0.985 ± 0.092 and 0.978 ± 0.054, $P = 0.035$ and 0.037, Figure 3C).

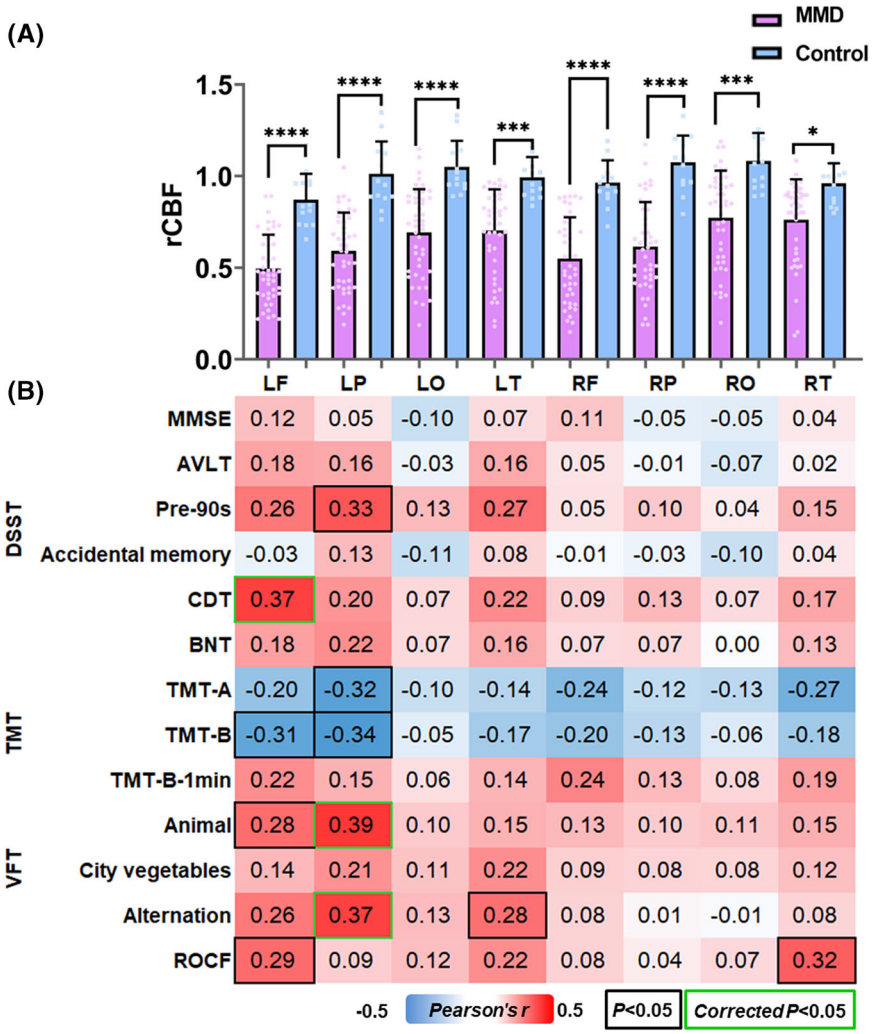

FIGURE 1 Lobular rCBF reduction in MMD patients and correlation with cognitive impairment. A, Lobular rCBF in MMD patients and normal controls. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$ compared to control. B, Pearson's correlation analysis between lobular rCBF and index scores of cognition scales, presented with the *r* value. Correlations with statistical significance are emphasized with black frames before multiple comparisons corrections, while emphasized with green frames after multiple comparisons corrections. AVLT, AVLT, Auditory Verbal Learning Test; BNT, Boston Naming Test; CDT, Clock Drawing Test; DSST, Digit Symbol Substitution Test; F, frontal; L, left; MMD, moyamoya disease; MMSE, Mini-Mental State Examination; O, occipital; P, parietal; R, right; rCBF, relative cerebral blood flow; ROCF, Rey-O complex figures; T, temporal; TMT, Trail Making Test; VFT, Verbal Fluency Test

4 | DISCUSSION

4.1 | Brain perfusion, cognition, and AD-like pathological burden

According to our results, CBF in newly diagnosed MMD is significantly lower than normal, both globally and regionally. Previous reports revealed that cognitive decline could occur in 30% to 40% of MMD patients without presentation of cerebral infarction and hemorrhage.[21] Notably, unlike typical vascular dementia (VaD) caused by ischemic or hemorrhagic stroke,[22] subjects in our study are with normal-appearing MR images.[23,24] As previously mentioned, MMD patients are mostly young to middle-aged, and have relatively independent etiology for CBH, which highlights MMD as an alternative model for narrowing the gap between CBH and AD pathogenesis, especially for mimicking the preclinical period of AD. The cognitive impairment in MMD patients can involve miscellaneous domains such as executive function, orientation, comprehension, calculation, and memory.[21,23] In line with existing reports, in this study, the proportion of mild, moderate, and severe cognitive impairment was 15.1%, 9.1%, and 4.5%, respectively. Further examination of detailed cognitive scales revealed notable correlations between specific cognitive domains and lobular perfusion. This suggests that regional CBF disturbances directly resulted in definite domain impairment. However, whether concomitant AD-like pathologic burdens will emerge as a characteristic of CBH or even play a role as a mediator of CBH-related cognitive impairment remains unclear. As a new screening strategy, plasma AD biomarkers are reported to have comparable diagnostic efficacy with gold standard methods.[25–31] In a previous study, plasma Aβ42 showed an increasing trend in normal to subjective cognitive decline before decreasing in mild cognitive impairment (MCI) and AD.[32] This reported biphasic change is similar to our results, in which plasma Aβ42 was slightly elevated but not significant in newly diagnosed MMD patients. The plasma p-tau181 level, by contrast, was significantly higher than normal. Although these markers are reported to have the ability to predict non-demented or MCI patients progressing to AD,[26,33] our results revealed no correlation with neuropsychological performance. In summary, our current results suggest that disturbances in regional CBF contribute to specific cognitive domain deficits independently of AD-like pathology, at least in the observed CBH populations.

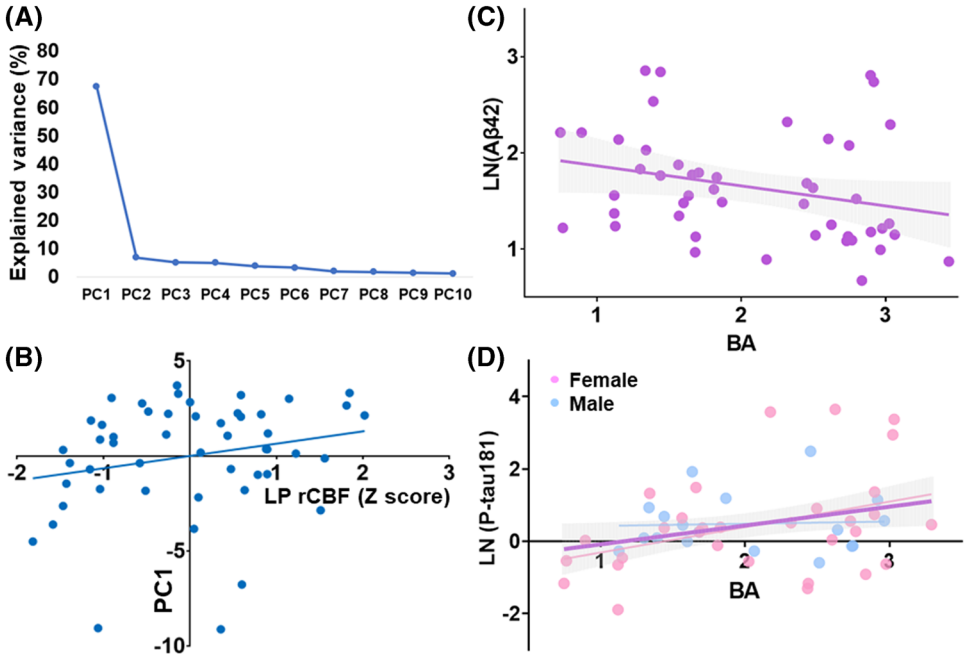

FIGURE 2 Association among brain chronic hypoperfusion, cognition scales, and plasma AD biomarkers. A, Explained variance proportion of each component for the whole cognition scales. B, Stepwise regression analysis with left parietal rCBF (z-score). C, Scatter plot between BA and logarithmic Aβ42, adjusting for age, sex, *APOE* ε4 status, BP, diabetes, and serum lipid. D, Scatter plot between BA and logarithmic p-tau181, adjusting for age, sex, and *APOE* ε4 status, BP, diabetes, and serum lipid. Aβ, amyloid beta; AD, Alzheimer's disease; *APOE*, apolipoprotein E; BA, brain aging factor; BP, blood pressure; PC, principal component; p-tau181, phosphorylated tau; rCBF, relative cerebral blood flow

TABLE 2 Associations between individual plasma biomarkers and neuropsychological assessment (β coefficient)

		Newly diagnosed MMD and control ($n = 36$)			Newly diagnosed, follow-up MMD and control ($n = 45$)		
		Aβ42	P-tau181	P-tau181/Aβ42 ratio	Aβ42	P-tau181	P-tau181/Aβ42 ratio
MMSE		0.04253	0.05588	0.2119	0.04596	−0.01754	−0.1171
AVLT		0.192	0.1997	1.028	0.2006	−0.04299	−0.2516
DSST	Pre-90s	0.1182	0.1502	0.4728	0.1217	0.07862	0.1744
	Accidental memory	0.07926	0.01366	0.0148	0.08561[a]	−0.01324	−0.0907
CDT		0.1243	−0.1305	−0.7874	0.1281	−0.07743	−0.5268
BNT		0.08845	0.04829	0.1948	0.08548	−0.01647	−0.1297
TMT	TMT-A	−0.1811	−0.3666	−1.496	−0.1684	−0.1283	−0.565
	TMT-B	−0.3973	−0.6221	−2.418	−0.3594	−0.7871	−3.957
	TMT-B-1 min	0.1004	0.1	0.3377	0.1021	0.02254	0.05759
VFT	Animal	−0.01885	0.01845	0.1801	−0.006786	−0.008147	−0.0194
	City vegetables	0.0185	0.0359	0.1582	0.02035	0.02369	0.1091
	Alternation	0.01136	−0.03213	−0.214	0.01467	−0.01575	−0.1058
ROCF		0.07152	0.09653	0.4001	0.06881	−0.01535	−0.137

Abbreviations: Aβ, amyloid beta; AVLT, Auditory Verbal Learning Test; BNT, Boston Naming Test; CDT, Clock Drawing Test; DSST, Digit Symbol Substitution Test; MMD, moyamoya disease; MMSE, Mini-Mental State Examination; p-tau, phosphorylated tau; ROCF, Rey-O complex figures; TMT, Trail Making Test; VFT, Verbal Fluency Test.

[a]Significantly different (simple linear regression $P < 0.05$).

Considering the lengthy and continuous injury from CBH, one challenge is to estimate the duration of brain hypoperfusion. Although identifying CBH patients during long-term follow-up seems to be difficult, especially in a non-demented population, the Suzuki stage system in MMD can provide clues in this duration.[14] In the present study, we developed an integrated model combining normal aging, impaired CBF, and hypoperfusion duration, which reflects the accumulation of CBH damage. Remarkably, this integration is positively associated with the plasma p-tau181 level, which is also considered an indicator of neurodegeneration,[34] and in accordance with the accumulation

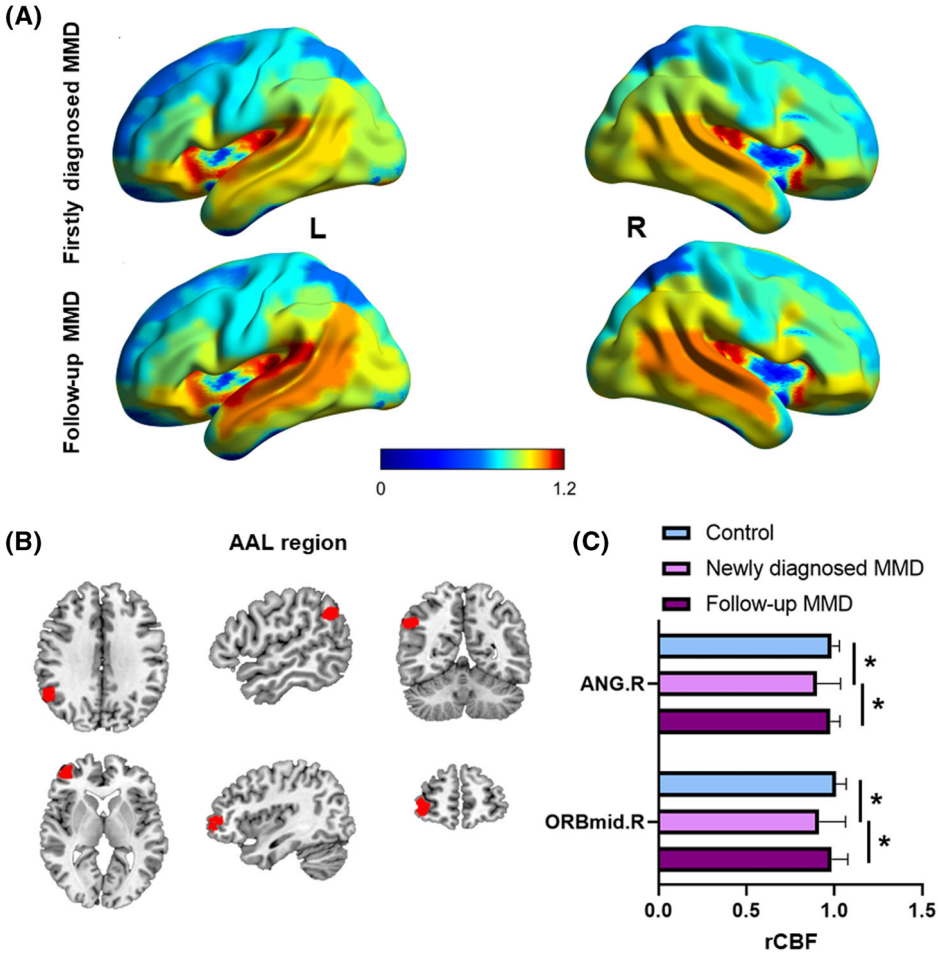

FIGURE 3 rCBF changes in newly diagnosed MMD patients and follow-up subjects after revascularization by AAL template. A, Whole brain view of rCBF. B, Significant rCBF changes are labeled in ORBmid.R and ANG.R, by horizontal, sagittal, and coronal view in standard brain MRI. C, rCBF value in normal controls, newly diagnosed, and follow-up MMD subjects. *$P < 0.05$ by post hoc test. AAL, Automated Anatomical Labelling; ANG.R: right angular gyrus; MMD, moyamoya disease; MRI, magnetic resonance imaging; ORBmid.R, orbital part of right middle frontal gyrus; rCBF, relative cerebral blood flow

damage effect suggested in the "calcium hypothesis" of AD and brain aging.[35] In addition, more evidence has proven that tau pathology may be an initiating factor in sporadic AD. P-tau will drive Aβ production and be phosphorylated by Aβ, setting up a vicious cycle.[36] Although the plasma Aβ42 level has a negative relationship with the accumulated CBH injury, the significance is not strong enough. Based on these findings, we hypothesized that CBH may be an independent factor that will induce AD-like hallmarks or even preclinical AD.

4.2 | Effect of revascularization

The most effective treatment for MMD to date is neurosurgical bypass.[37] This surgical procedure can help in augmenting or restoring CBF into the hypoperfusion territory, and effectively reduces 5-year recurrent stroke risk by 25% compared to medicinal treatment.[38] In agreement with other reports,[39] our investigation in follow-up of patients confirmed increased rCBF in the recipient territories and better neuropsychological performance after unilateral (more severe side) surgical intervention. A trend toward improvement could be identified in all the cognitive domains, although only DSST and TMT showed significance. Interestingly, we found ORBmid.R and ANG.R may contribute to these cognitive domains. It is reported that ANG.R plays a causal role in optimize visual search performance.[40] Notably, plasma AD biomarkers did not recover after unilateral revascularization during follow-up. A reasonable explanation for this is that CBH occurs with bilateral onset in most MMD patients, such that hypoperfusion still progresses on the other side of the brain receiving no surgical intervention. This result is in line with our previous conclusion that CBH-induced cognitive decline is not mediated by AD-like pathology, and also agrees with the opinion that higher AD biomarker levels may not be the only factors that reflect disease progression.[41]

4.3 | Interpretation

In summary, our observations highlight the strength of vascular disease in dementia. The cognitive impairment in MMD was discovered

TABLE 3 Brain perfusion, cognition, and plasma Alzheimer's disease biomarkers before and after revascularization

		Newly diagnosed MMD	Follow-up MMD	*P*
N		43	23	
rCBF, mean (SD)				
Left	Frontal	0.493 (0.1860)	0.6772 (0.1855)	0.0016[a]
	Parietal	0.5896 (0.2155)	0.7482 (0.1921)	0.011[a]
	Occipital	0.6998 (0.2314)	0.9227 (0.1804)	0.0013[a]
	Temporal	0.7059 (0.2262)	0.8418 (0.1054)	0.048[b]
Right	Frontal	0.559 (0.2322)	0.6976 (0.1951)	0.0291[a]
	Parietal	0.6223 (0.2532)	0.8242 (0.2695)	0.0087[a]
	Occipital	0.7782 (0.2614)	0.916 (0.2482)	0.0505
	Temporal	0.7666 (0.2244)	0.8403 (0.1614)	0.14
Plasma biomarkers, mean (SD)				
Aβ42 (ng/mL)		9.884 (15.41)	6.841 (4.298)	0.9337
P-tau181 (pg/mL)		7.091 (13.71)	16.14 (28.66)	0.9281
P-tau181/Aβ42 ratio		1.472 (3.597)	3.614 (6.691)	0.3998
Neuropsychological assessment				
MMSE		25.62 (8.952)	26.33 (3.391)	0.3675
AVLT		47.1 (21.33)	51.11 (18.09)	0.3011
DSST	Pre-90s	35.79 (17.72)	49.11 (13.10)	0.0192[a]
	Accidental memory	2.619 (2.740)	4 (3.000)	0.0916
CDT		18.55 (9.155)	22.22 (7.120)	0.132
BNT		20.83 (7.078)	23.67 (5.050)	0.1307
TMT	TMT-A	60.6 (34.74)	44.11 (13.40)	0.0611
	TMT-B	135.6 (61.51)	96.11 (40.48)	0.0362[b]
	TMT-B-1 min	16.19 (26.36)	14.89 (3.756)	0.0953
VFT	Animal	17.93 (26.26)	14.89 (4.400)	0.4976
	City vegetables	10.48 (4.645)	13.33 (4.848)	0.0514
	Alternation	10.69 (5.390)	13.44 (3.941)	0.0771
ROCF		28.71 (10.37)	31.56 (4.851)	0.4679

Abbreviations: Aβ, amyloid beta; AVLT, Auditory Verbal Learning Test; BNT, Boston Naming Test; CDT, Clock Drawing Test; DSST, Digit Symbol Substitution Test; MMD, moyamoya disease; MMSE, Mini-Mental State Examination; p-tau, phosphorylated tau; rCBF, relative cerebral blood flow; ROCF, Rey-O complex figures; SD, standard deviation; TMT, Trail Making Test; VFT, Verbal Fluency Test.

[a]By *t* test.

[b]By Mann–Whitney U test.

more than a decade ago, but whether there is AD-like pathology (Aβ and tau) in MMD is unclear. We specifically investigated patients with pure CBH etiology and examined the link between accumulated reductions in CBF and cognitive decline, as well as the possible role of AD-like pathological burden. Unlike typical vascular dementia caused by ischemic or hemorrhagic stroke, or small vessel disease, we enrolled MMD patients without evidence of stroke. Besides, unlike the onset of ischemic stroke, which was often in senile patients, MMD patients are mostly young to middle-aged, which means they have fewer risk factors for CBH like hypertension, diabetes, or hyperlipemia. This model can extend the missing timeline in hypoperfusion-related dementia. Although previous studies have shown that CBF is reduced very early in the development of AD and the aggravated vascular pathology contributes to the acceleration of regional neuropathology and neurovascular unit dysfunction,[42] the current data reveal that AD biomarkers may be less significant in the early stages of disease. Instead, we propose that those factors play a role mostly in the late stages of disease that mediate the cascade reaction. Over the span of decades, AD pathology likely takes away the dominant role from vascular factors, accelerating brain death. Prognosis after CBH intervention was reported in this study. Herein we found improvement of CBF and cognitive performance after revascularization but not AD biomarkers. Nevertheless, longer follow-up is still needed to prove the changes of AD-like pathology. Meanwhile, studies showing the effect of omental

transposition in AD patients should not be ignored.[43] Revascularization surgery in MMD provides the opportunity to explore this new treatment strategy.

4.4 | Limitations and future directions

To our knowledge, this is the first cohort study that investigated AD biomarkers, as well as their association with brain perfusion and cognition in MMD. Still, there are several limitations in this study. Although cerebrospinal fluid (CSF) analysis and amyloid positron emission tomography are gold standard methods of detecting AD biomarkers,[44–46] plasma ultra-sensitive immunoassays (e.g., single molecule array [Simoa]) are becoming promising alternatives. Due to the non-invasive and accessible nature of plasma AD biomarker analysis, no current radiotracer imaging or analysis of CSF biomarkers was performed in this research. Numerous risk factors for dementia and AD have been reported, including advanced age, carrying the *APOE* ε4 allele, sex, and family history. Many factors that increase the risk of cardiovascular disease are also associated with cerebral hypoperfusion as well as a higher risk of dementia, such as smoking, diabetes, hypertension, and serum lipid level.[5,45,47,48] These factors should be taken into consideration when investigating the pathogenesis of dementia and AD. It has been reported that incremental changes in blood TG and TCHO increase the risk of AD and that higher serum lipid levels are associated with plasma AD biomarkers.[49,50] In our study, there was a difference in serum lipid levels between MMD patients and controls; however, the differences were small and most likely are not clinically meaningful. Enrolling patients with pure CBH etiology is difficult due to disease rarity. Because of the tedious process of cognition assessment, the number of normal control subjects is much smaller despite the demographic similarity between the two groups. Meanwhile, relatively small sample size also results in lower β confidence. Nevertheless, further large-sample longitudinal studies are still needed to confirm dementia-related pathological changes in MMD and the changes in AD-like pathology after global revascularization.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest. Author disclosures are available in the supporting information.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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