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Resting-state functional magnetic resonance imaging study on cerebrovascular reactivity changes in the precuneus of Alzheimer's disease and mild cognitive impairment patients

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by memory impairment and cognitive decline, ultimately culminating in dementia. This study aims to evaluate cerebrovascular reactivity (CVR) and functional connectivity (FC) in patients with AD and mild cognitive impairment (MCI) using resting-state functional magnetic resonance imaging (rs-fMRI), bypassing the requirement for hypercapnia. The study cohort comprised 53 AD patients, 38 MCI patients, and 39 normal control (NC) subjects. CVR is derived by extracting signals within specific frequency bands of rs-fMRI. This study compares the differences in CVR and FC among the three groups, using the brain regions with CVR differences as region of interest (ROI) for FC analysis. The correlation between CVR and FC and cognitive scale score was discussed. Compared with NC subjects, AD patients exhibited a decrease in CVR in the PCUN.L, whereas MCI patients showed an increase in CVR in the PCUN.R. With PCUN.L as ROI, FC in PCUN.R decreased in AD patients, and FC in SFGmed.R and other brain regions increased in MCI patients compared with NC subjects. The results of the correlation analysis indicate that CVR in all patients, as well as FC with the PCUN.L as the ROI to the PCUN.R and SFGmed.R, show positive correlations with MMSE and MoCA scores. These results suggest that there are significant differences between CVR and FC with CVR differential brain regions as ROI among the AD, MCI, and NC groups, which may help to explain the hemodynamic mechanism. CVR obtained with rs-fMRI may be a potential biomarker for assessing cognitive impairment.

Keywords Alzheimer's disease, mild cognitive impairment, resting-state functional magnetic resonance imaging, cerebrovascular reactivity

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by memory impairment and cognitive decline[1], ultimately culminating in dementia[2]. In the article published in 2024, the National Institute on Aging and the Alzheimer's Association (NIA-AA) introduced the updated diagnostic criteria for AD. These criteria stratify the disease based on the degree of cognitive impairment, delineating seven clinical stages ranging from stage 0 (no impairment) to stage 6 (severe impairment)[3]. Mild cognitive impairment (MCI), classified as stage 3, is characterized by noticeable cognitive decline that does not yet satisfy the criteria for dementia. Annually, approximately 15.4–33.4% of individuals with MCI progress to AD[4]. It is estimated that, with the aging global population, the prevalence of dementia will exceed 150 million individuals by the mid-

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21st century[5], posing severe challenges to both socio-economic systems and healthcare infrastructures. Vascular dysfunction may play a significant role in the pathogenesis of AD and MCI[6,7]. Cerebrovascular Reactivity (CVR) may be an important indicator to evaluate the health of cerebral blood vessels, and its decrease may indicate the impairment of regulatory capacity, which may lead to cognitive decline. Therefore, as a potential biomarker, CVR is of great significance for the early diagnosis and treatment of AD and MCI.

CVR denotes the capacity of cerebral blood vessels to undergo dilation or constriction in response to provocations or interventions[8], representing a metric for assessing the vasodilatory capability of the cerebral vasculature[9]. In clinical research, the primary methodologies for acquiring CVR by MRI encompass a carbon dioxide inhalation test based on hypercapnia, a breath-holding test, and a hyperventilation test[8,10,11]. Inhalation of hypercapnic gas increases the concentration of CO_2 in the blood. CO_2, as an effective vasodilator, can dilate blood vessels, thereby increasing cerebral perfusion[12]. Consequently, CVR can be quantified through the changes in blood oxygen level-dependent (BOLD) signal associated with CO_2 inhalation. Existing literature has investigated CVR in elderly individuals with normal cognition, MCI, and AD patients. Some of these reports show significant differences between groups, mainly a decrease in CVR in AD patients compared with healthy controls (NC). Nevertheless, there remains a debate concerning the variability in CVR among MCI patients. The research by Sandeepa Sur et al. revealed that global CVR was reduced in the cognitive impairment group compared to the cognitively normal group[13]. Uma S. Yezhuvath et al.'s study demonstrated that CVR in brain regions such as the prefrontal cortex was decreased in AD patients compared to NC[14]. Other reports showed no differences between groups. Kenneth R. Holmes et al. showed that in the temporal cortex, the average CVR in the MCI group was the largest, followed by the AD group, and the NC group was the lowest. In the parietal cortex, the average CVR in the MCI group was the largest, followed by the NC group, and the lowest in the AD group. But there was no statistically significant difference between the three groups[15]. However, most of these studies measured CVR using MRI methods based on hypercapnia. The carbon dioxide inhalation experiment is regarded as the gold standard for assessing CVR, but it requires a gas delivery device, and all components of the device must be compatible with MRI[8]. Furthermore, the respiratory equipment must be sufficiently compact to accommodate the spatial limitations of the head coil, thereby minimizing patient discomfort[16]. This method usually requires training and practice before the experiment to ensure the accuracy of the measurement results, which consumes a lot of time[17]. The breath-holding test and hyperventilation test need the cooperation of patients, and these two methods may lead to head movement for the elderly or patients with cognitive impairment, thus reducing the success rate of the procedures[18].

Recent studies have demonstrated that rs-fMRI data can provide estimates of CVR without requiring subjects to perform CO_2-related tasks[9,19]. The method uses natural changes in respiration over time as an intrinsic vasoactivity stimulus to extract a proxy for arterial blood CO_2 fluctuations from a specific frequency band (0.02 - 0.04 Hz) in rs-fMRI data to represent CVR[9]. According to the latest literature, the CVR obtained by this method is defined as intrinsic CVR[9,20]. This method is non-invasive, does not bring additional discomfort to patients, has low requirements for patient cooperation, and is convenient for clinical promotion. In addition, the method has been used in CVR studies of cerebrovascular disease patients and healthy people, showing good feasibility and repeatability. Liu et al. pioneered this approach, validating it in healthy controls and patients with Moyamoya disease, confirming that the CVR obtained by the method was consistent with that obtained by hypercapnia (healthy controls: $r=0.88$, Moyamoya disease patients: $r=0.71\pm0.18$)[9]. Subsequently, Kamil Taneja and colleagues applied this method to stroke patients, demonstrating that rs-fMRI data could be used to repeatedly assess CVR in patients with cerebrovascular diseases[21]. Leonie Zerweck et al. compared this method with CVR obtained by fMRI with the breath-holding method and found a good correlation in Moyamoya disease patients ($r=0.71\pm0.13$)[22]. Ni and Yang et al. applied this method to patients with white matter hyperintensities (WMHs) and confirmed that the decrease of CVR in WMHs patients is related to cognitive impairment[23–25]. However, this approach has not yet been used in patients with AD and MCI.

Therefore, this study consists of two parts: First, we measured CVR in AD patients, MCI patients, and NC subjects using an rs-fMRI method that does not require hypercapnia, comparing changes in CVR among the three groups, and exploring the relationship between CVR and cognitive function in all patients. Second, the role of CVR differential brain regions in brain networks was further explored, and FC analysis was conducted by taking the brain regions with statistical differences in CVR among the three groups as ROI. Changes in FC between the three groups were compared, and the relationship between FC and cognitive function was assessed in all patients.

Materials and methods

Subjects

This study was approved by the Ethics Committee of Zhejiang Provincial People's Hospital (The People's Hospital of Hangzhou Medical College) (No. 2012KY002). All methods were conducted in accordance with the Declaration of Helsinki, and informed consent was obtained from all subjects with written consent documents signed by each participant. From September 2016 to February 2020, the study included a total of 63 AD patients and 45 MCI patients admitted to the psychiatric memory clinic at Zhejiang Provincial People's Hospital (The People's Hospital of Hangzhou Medical College), as well as 44 age-, sex-, and education-matched normal control (NC) patients recruited through the hospital's health promotion center. All subjects had not taken any anti-dementia medications, including piracetam, ginkgo biloba extract, cholinesterase inhibitors (donepezil, rivastigmine, galantamine), NMDA receptor antagonists (memantine), and combination formulations of cholinesterase inhibitors and NMDA receptor antagonists (memantine/donepezil combination). All were right-handed, and they all underwent medical history collection, laboratory examination, neuropsychological testing, physical examinations, and routine cranial MRI scans. Neuropsychological tests included the Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment (MoCA). AD patients were diagnosed

based on revised criteria from the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) for possible AD: MMSE score ≤ 24 and MoCA score ≤ 26[26]. The enrollment criteria for patients with MCI include self-reported memory dysfunction, lack of abnormal clinical manifestations, MMSE score > 24 but ≤ 27, and non-conformance to the diagnostic criteria for dementia as defined by the DSM-IV-R[27]. The inclusion criteria for the NC group were as follows: absence of hearing or vision impairments, other neurological deficits, or history of stroke, epilepsy, depression, or other neurological or psychiatric disorders; no evidence of cerebral infarction, hemorrhage, or tumor on routine cranial MRI; and MMSE scores ≥ 28. The exclusion criteria for both groups were stroke; brain trauma; brain tumors; Parkinson's disease; epilepsy; and other neurological diseases that cause cognitive impairment; severe anemia, hypertension, diabetes, and other systemic diseases; a history of mental illness or use of psychotropic drugs; and abnormal signals in the medial temporal lobe due to infection or vascular factors. Following these criteria, subjects with missing imaging data or excessive head motion (6 AD, 5 MCI, and 4 NC) (see preprocessing of data), as well as those lacking complete neuropsychological test results (4 AD, 2 MCI, and 1 NC), were excluded. Consequently, the final study cohort comprised 53 AD patients, 38 MCI patients, and 39 NC subjects (Fig. S1).

MRI data acquisition

The MRI data were acquired using a 3.0T magnetic resonance scanner (Discovery MR750; GE Healthcare, Wauke-sha, WI, USA) and an 8-channel phased array head coil. Initially, a routine cranial MRI scan was performed to exclude relevant brain pathologies. Subsequently, high-resolution three-dimensional T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) sagittal sequences were obtained with the following parameters: repetition time (TR) = 6.7 ms, echo time (TE) = 2.9 ms, inversion time (TI) = 450 ms, flip angle (FA) = 12°, field of view (FOV) = 256 × 256 mm^2, slice thickness = 1 mm, gap = 0 mm, in-plane resolution = 256 × 256, and 192 sagittal slices. Finally, the rs-fMRI images were acquired using the echo-planar imaging (EPI) sequence with parameters as follows: TR = 2000 ms, TE = 30 ms, FA = 90°, FOV = 220 × 220 mm^2, slice thickness = 3.2 mm, gap = 0 mm, in-plane resolution = 64 × 64, 210 volumes, and 44 slices. During the rs-fMRI data acquisition, subjects were asked to remain quiet and keep their eyes closed, but not to fall asleep. Sponge pads were placed on both sides of the head to minimize head movement, and subjects were equipped with built-in earplugs and external earmuffs to reduce noise interference and protect their hearing.

CVR calculation

Rs-fMRI data were analyzed using the MATLAB-based SPM12 toolbox, following the preprocessing steps described in previous studies[9,25], which included head motion correction, excluding subjects with head motion displacement > 3 mm, rotation > 3°, or mean frame displacement (FD) > 0.5; spatial normalization, aligning individual rs-fMRI images to individual 3D-T1WI images, then normalizing to MNI standard space and resampling to 3 × 3 × 3mm^3 voxels; spatial smoothing, using a 6 mm FWHM isotropic Gaussian kernel for spatial smoothing to reduce noise and improve the signal-to-noise ratio; linear detrending and bandpass filtering (0.02–0.04 Hz). As previous results from Liu et al. have shown[9], global BOLD signal fluctuations in this frequency range correlate best with natural arterial CO_2 variations during spontaneous respiration. Finally, a general linear model (GLM)-based voxel analysis method was used to obtain the CVR index of each voxel by taking the reference time course as the independent variable and the time course of each voxel as the dependent variable. (Fig. S2A)

FC calculation based on ROI

The rs-fMRI data were analyzed using the DPABI and SPM12 toolboxes based on MATLAB, with the preprocessing steps following those described in our previous studies[28]. It includes: (1) removing the first 10 volumes to ensure that the subjects adapt to the scanning environment and stabilization of MRI signals; (2) slice timing: selecting a standard slice collected at a certain time point and using the Sinc interpolation algorithm to align other slices to match the collection time of the standard slice; (3) head motion correction, removing subjects with head motion displacement > 3 mm, rotation > 3°, or mean framewise displacement (FD) > 0.5; (4) spatial normalization, registering individual rs-fMRI images to individual 3D-T1WI images, then normalizing to MNI standard space and resampling to 3 × 3 × 3 mm^3 voxels; (5) linear detrending; (6) detrending covariates, including 24 head motion parameters and white matter and cerebrospinal fluid signals; (7) bandpass filtering (0.01–0.10 Hz); (8) the brain regions with statistical differences in CVR among the three groups were used as ROI to extract the average time process from the pre-processed rs-fMRI data; (9) calculating the Pearson correlation coefficients between the average time course of each ROI and all other voxels in the brain to obtain a correlation map; (10) transforming the correlation map into a z-score map using Fisher's r-to-z transformation. (Fig. S2B)

Statistical analysis

Statistical analyses for clinical data and cognitive assessment were performed using SPSS version 26.0 (SPSS Inc., Chicago, IL, USA). Categorical data (gender) were represented by frequency counts and analyzed using the chi-square ($\chi 2$) test. The normality of continuous variables (age, education, and neuropsychological test scores) was assessed using the Shapiro-Wilk test. Data conforming to a normal distribution were expressed as mean ± standard deviation (mean ± SD) and analyzed using one-way analysis of variance (ANOVA). Data not conforming to a normal distribution were expressed as median (interquartile range) [M (IQR)] and analyzed using the Kruskal-Wallis test. If there was a statistical difference between the three groups, the Bonferroni method was used for multiple comparison correction, and $P < 0.05$ indicated that the difference was statistically significant.

Sample size	AD ($N=53$)	MCI ($N=38$)	NC ($N=39$)	P-value	Post hoc
Age (years)	66.83 ± 7.85	66.16 ± 8.52	65.56 ± 9.12	0.774[a]	–
Gender (male: female)	20:33	23:15	18:21	0.099[b]	–
Education (years)	6.00 (5.00)	6.00 (3.00)	7.00 (3.00)	0.960[c]	–
MMSE	20.00 (8.00)	27.00 (2.00)	29.00 (2.00)	<0.001[c]	NC> MCI> AD
MoCA	15.00 (8.00)	23.00 (3.00)	27.00 (2.00)	<0.001[c]	NC> MCI> AD

Table 1. Demographics and clinical characteristics of the participants. *AD* Alzheimer's disease, *MCI* mild cognitive impairment, *NC* normal controls, *MMSE* mini-mental state examination, *MoCA* montreal cognitive assessment. [a]*P*-value obtained by analysis of variance; [b]*P*-value obtained by the chi-square test.; [c]*P*-value obtained by the Kruskal-Wallis test. Post hoc testing obtained by Bonferroni correction.

Cluster	Brain regions	Peak MNI coordinates			Peak intensity	P-value
		X	Y	Z		
40	PCUN.R	9	− 57	45	9.945	<0.001
38	PCUN.L	− 9	− 45	39	9.051	<0.001

Table 2. Comparison of CVR among AD, MCI, and NC groups. *P*-value were obtained by analysis of covariance adjusting for age, sex, education and head motion. *CVR* cerebrovascular reactivity, *AD* Alzheimer's disease, *MCI* mild cognitive impairment, *NC* normal controls, *MNI* montreal neurological institute, *PCUN.R* right precuneus, *PCUN.L* left precuneus.

In the DPABI software of MATLAB, the whole brain template was used to remove cerebrospinal fluid and other disturbances; age, gender, years of education, and head movements were taken as covariables, and the CVR and FC of the three groups were analyzed and compared by voxel-based covariance analysis (ANCOVA). If there is a statistical difference between the three groups, then multiple comparison correction by Gaussian random field theory (GRF), voxel $P<0.005$, and cluster $P<0.05$ are considered statistically significant. To further validate our results, we grouped AD, MCI, and NC subjects by gender, with all males in one group and all females in another. We then conducted voxel-wise ANCOVA to compare CVR among the three groups.

Gender, age, and years of education were used as covariables to evaluate the correlation between CVR and FC and neuropsychological test scores (MMSE and MoCA) in all patients. $P<0.05/2$ ($P=0.025$, Bonferroni corrected) indicated that the difference was statistically significant[29].

Results

Demographics and neuropsychological tests

Table 1 summarizes demographic information and neuropsychological test scores for the AD, MCI, and NC groups. There were significant differences in MMSE and MoCA scores among the three groups (all $P<0.001$). The subsequent post hoc analysis showed that the overall cognition of the NC group was the best, the MCI group was medium, and the AD group was the worst. No statistically significant differences were identified with respect to age ($P=0.774$), gender ($P=0.099$), or education ($P=0.960$) among the three groups.

CVR between AD, MCI, and NC groups

ANCOVA results showed statistically significant differences (all $P<0.001$) in CVR in the right precuneus (PCUN.R) (peak MNI of 9, -57, and 45) and the left precuneus (PCUN.L) (peak MNI of -9, -45, and 39) between the three groups (Table 2; Fig. 1). The results of post hoc analyses adjusted for multiple comparisons showed that the CVR of the PCUN.L in AD patients was reduced compared with NC subjects, while those with MCI exhibited heightened CVR in the PCUN.R (Fig. 1). Comparatively, AD patients demonstrated a reduction in CVR in both the PCUN.R and PCUN.L when contrasted with MCI patients (Fig. 1). We found that the differential brain region trends in CVR between the AD, MCI, and NC groups were roughly the same between males and females.

FC between AD, MCI, and NC groups

The results of ANCOVA revealed two brain regions, PCUN.R and PCUN.L, where CVR changes were observed among AD, MCI, and NC groups. These regions were defined as ROI for FC analysis.

With PCUN.R as ROI, the ANCOVA results indicated significant statistical differences in FC among the three groups in the left crus II of the cerebellum (CC2.L) (peak MNI of -12, -87, and -33), the right dorsal cingulate gyrus and paracingulate gyrus (DCG.R) (peak MNI of 12, -42, and 36), the left angular gyrus (ANG.L) (peak MNI of -48, -66, and 24), the left middle frontal gyrus (MFG.L) (peak MNI of -27, 30, and 45), and the right middle frontal gyrus (MFG.R) (peak MNI of 33, 33, and 48) ($P<0.001$) (Table 3; Fig. 2A). Correction for multiple comparisons revealed that, compared to NC subjects, AD patients and MCI patients exhibited increased FC in all of the aforementioned brain regions (Fig. 2B).

With PCUN.L as ROI, the ANCOVA results revealed significant statistical differences in FC among the three groups in the left cerebellar lobule VIII (Cere8.L) (peak MNI of -42, -54, and -51), the right cerebellar lobule

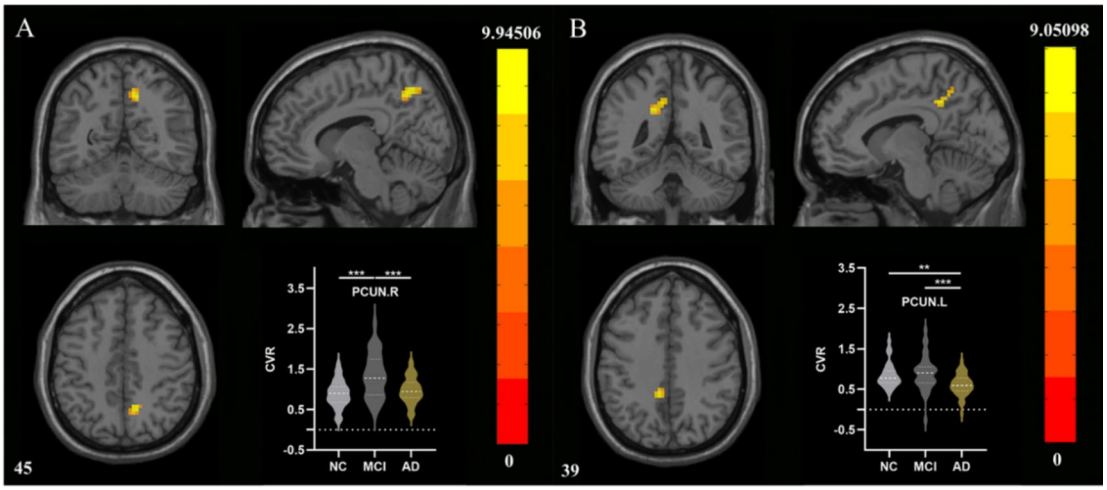

Fig. 1. CVR results among AD, MCI, and NC. Post hoc comparisons of analysis of covariance. The connection between two bars represents significant between-group differences (**denotes a significant level of $P<0.01$, and ***indicates a significant level of $P<0.001$, Bonferroni correction). (**A**) Right Precuneus. (**B**) Left Precuneus. *CVR* cerebrovascular reactivity, *PCUN.R* right precuneus, *PCUN.L* left precuneus, *NC* normal controls, *MCI* mild cognitive impairment, *AD* Alzheimer's disease.

ROI	Cluster	Brain regions	Peak MNI coordinates			Peak intensity	*P*-value
			X	Y	Z		
PCUN.R	844	CC2.L	− 12	− 87	− 33	15.595	<0.001
	783	DCG.R	12	− 42	36	17.1176	<0.001
	220	ANG.L	− 48	− 66	24	12.8245	<0.001
	178	MFG.L	− 27	30	45	15.5139	<0.001
	102	MFG.R	33	33	48	11.0085	<0.001
PCUN.L	317	Cere8.L	− 42	− 54	− 51	13.1563	<0.001
	87	Cere6.R	9	− 60	− 27	10.8644	<0.001
	344	PCUN.R	9	− 45	18	13.4664	<0.001
	168	SFGmed.R	3	42	39	11.2559	<0.001

Table 3. Comparison of FC among AD, MCI, and NC groups. *P*-value were obtained by analysis of covariance adjusting for age, sex, education and head motion. *FC* functional connectivity, *AD* Alzheimer's disease, *MCI* mild cognitive impairment, *NC* normal controls, *ROI* region of interest, *MNI* Montreal Neurological Institute, *PCUN.R* right precuneus, *PCUN.L* left precuneus, *CC2.L* left crus II of cerebellar hemisphere, *DCG.R* right middle cingulate and paracingulate gyri, *ANG.L* left angular gyrus, *MFG.L* left middle frontal gyrus, *MFG.R* right middle frontal gyrus, *Cere8.L* left lobule VIII of cerebellar hemisphere, *Cere6.R* right lobule VI of cerebellar hemisphere, *SFGmed.R* right Superior frontal gyrus, medial.

VI (Cere6.R) (peak MNI of 9, -60, and -27), the right precuneus (PCUN.R) (peak MNI of 9, -45, and 18), and the right medial superior frontal gyrus (SFGmed.R) (peak MNI of 3, 42, and 39) ($P<0.001$) (Table 3; Fig. 3A). Correction for multiple comparisons indicated that, compared to NC subjects, AD patients had increased FC in Cere8.L and Cere6.R but decreased FC in PCUN.R; MCI patients exhibited increased FC in Cere8.L, Cere6.R, and SFGmed.R (Fig. 3B). Compared to MCI patients, AD patients showed decreased FC in PCUN.R and SFGmed.R (Fig. 3B).

Correlation analysis of CVR abnormality and cognitive dysfunction

The results of partial correlation analysis showed that the CVR of the PCUN.R in all patients was positively correlated with MMSE ($r=0.326$, $P=0.002$) and MoCA ($r=0.325$, $P=0.002$) scores. Similarly, CVR in the PCUN.L was positively correlated with MMSE ($r=0.334$, $P=0.001$) and MoCA ($r=0.250$, $P=0.019$) scores (Table 4; Fig. 4).

Correlation analysis of FC abnormality and cognitive dysfunction

The results of partial correlation analysis showed that PCUN.R as ROI and FC in different brain regions were not significantly correlated with MMSE and MoCA in all patients (all $P>0.05/2$) (Table 5). The FC with PCUN.L as

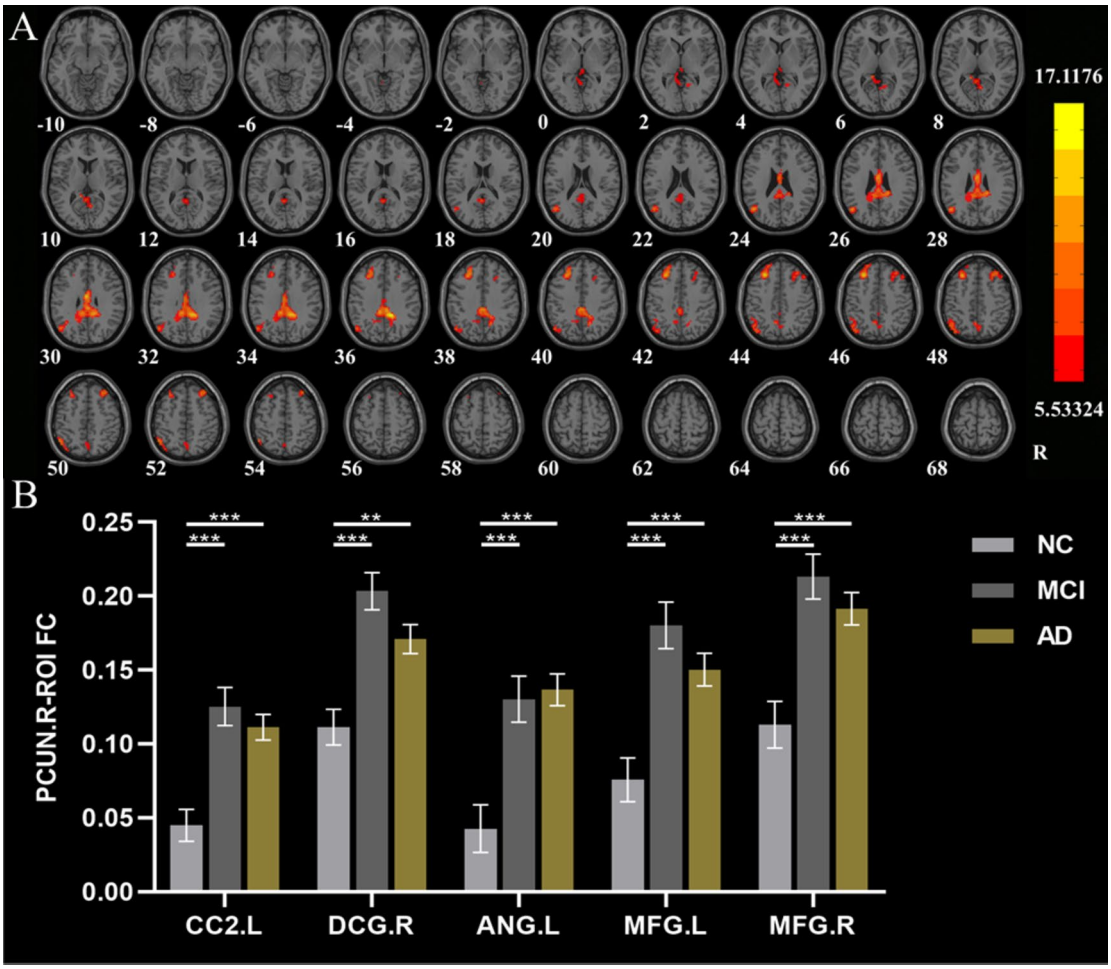

Fig. 2. PCUN.R-ROI FC results among AD, MCI, and NC. Post hoc comparisons of analysis of covariance. The connection between two bars represents significant between-group differences (** denotes a significant level of $P < 0.01$, and *** indicates a significant level of $P < 0.001$, Bonferroni correction). (**A**) Differences among the AD, MCI, and NC; (**B**) Post hoc comparisons between AD, MCI, and NC. *PCUN.R* right precuneus, *ROI* region of interest, *FC* functional connectivity, *CC2.L* left crus II of cerebellar hemisphere, *DCG.R* right middle cingulate and paracingulate gyri, *ANG.L* left angular gyrus, *MFG.L* left middle frontal gyrus, *MFG.R* right middle frontal gyrus, *NC* normal controls, *MCI* mild cognitive impairment, *AD* Alzheimer's disease.

ROI and PCUN.R were positively correlated with MMSE ($r = 0.311$, $P = 0.003$) and MoCA ($r = 0.311$, $P = 0.003$) scores (Table 5; Fig. 5). The FC with PCUN.L as ROI and SFGmed. R were positively correlated with MMSE ($r = 0.341$, $P = 0.001$) and MoCA ($r = 0.313$, $P = 0.003$) scores (Table 5; Fig. 5).

Discussion

In this study, rs-fMRI technology without hypercapnia was used to evaluate the CVR in patients with AD and MCI, and the CVR differential brain region was used as ROI for FC analysis and explored the relationship between CVR and FC with cognitive function in all patients. The results showed that, compared with NC subjects, the CVR of the PCUN.L in AD patients was decreased and the CVR of the PCUN.R in MCI patients was increased. CVR was reduced in both the PCUN.R and PCUN.L in patients with AD compared to patients with MCI. With PCUN.R as ROI, FC in CC2.L, DCG.R, and other brain regions increased in AD and MCI patients compared with NC subjects. With PCUN.L as ROI, FC in PCUN.R decreased in AD patients, and FC in SFGmed.R and other brain regions increased in MCI patients compared with NC subjects. CVR in all patients, as well as FC with PCUN.L as ROI to the PCUN.R and SFGmed.R, was positively correlated with MMSE and MoCA scores. These findings highlight the role of CVR in the maintenance of cognitive function and point to CVR as a potential biomarker for early diagnosis and treatment of AD and MCI.

Previous studies of CVR in patients with AD and MCI have mostly been based on hypercapnia, and the results are still controversial. Our results found that patients with AD had reduced CVR in both PCUN.R and PCUN.L, which is consistent with the findings of Sandeepa Sur and Andrew E. Beaudin et al.[13,30]. However, this is inconsistent with the findings of Kenneth R. Holmes et al.[15], who noted that there was no statistically significant difference in CVR in the AD group compared to the NC group. We speculate that the reason for this may be that the study had a relatively small sample size compared to ours, so the statistical power was relatively

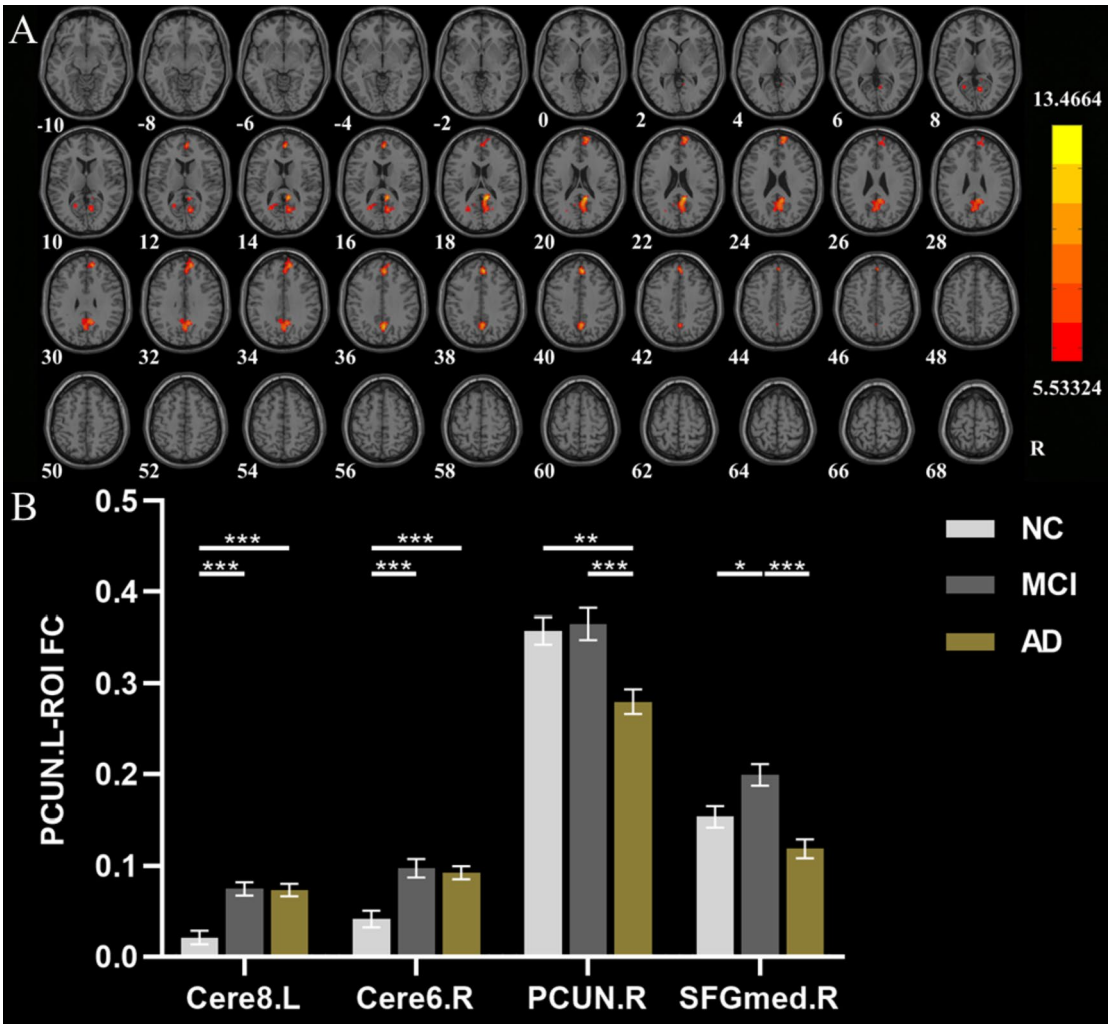

Fig. 3. PCUN.L-ROI FC results among AD, MCI, and NC. Post hoc comparisons of analysis of covariance. The connection between two bars represents significant between-group differences (*denotes a significant level of $P < 0.05$, **denotes a significant level of $P < 0.01$, and ***indicates a significant level of $P < 0.001$, Bonferroni correction). (**A**) Differences among the AD, MCI, and NC; (**B**) Post hoc comparisons between AD, MCI, and NC. *PCUN.L* left precuneus, *ROI* region of interest, *FC* functional connectivity, *Cere8.L* left lobule VIII of cerebellar hemisphere, *Cere6.R* right lobule VI of cerebellar hemisphere, *PCUN.R* right precuneus, *SFGmed.R* right superior frontal gyrus, medial, *NC* normal controls, *MCI* mild cognitive impairment, *AD* Alzheimer's disease.

weak. While the aforementioned studies directly assessed CVR, others have employed indirect measures such as BOLD response amplitude. A study using fMRI technology to evaluate CVR in patients with CAA, AD, and MCI during visual tasks showed that, compared with the NC group, the BOLD response amplitude was reduced in the CAA group, and there was no statistically significant difference between the AD and MCI groups[31]. This task-based study, which focused on regions strongly activated by the task, may ignore other brain areas. Our study mainly discusses the CVR based on the voxel level, which can detect changes in CVR in various brain regions more sensitively. Additionally, our study found that, compared to the NC group, the CVR of the PCUN.R in patients with MCI was increased. Previous MRI studies on CVR in MCI patients were few, and most recent studies showed that compared with NC, there was no significant statistical difference in CVR in MCI patients[15,30,31]. However, earlier studies have shown that CVR is reduced in patients with MCI compared with NC[14,32,33]. However, this is not consistent with our findings, and we speculate that CVR may have compensatory mechanisms in patients with MCI. Because we observed that CVR was elevated in patients with MCI compared with patients with AD, CVR showed a trend of first increasing and then decreasing in NC-MCI-AD. Previous literature has suggested that the vascular compensation mechanism may be explained by blood flow redistribution to compensate for reduced local neuronal function, or it can be understood as the result of the cerebrovascular system's efforts to adapt to a threatening cellular environment[34]. Alternatively, it may reflect an increase in neural activity designed to offset the decline in cognitive ability[35]. To further elucidate the specific mechanisms associated with increased CVR in MCI patients, future studies will require a larger sample size and more stringent inclusion and exclusion criteria to explore the changes in CVR, particularly at the voxel level.

Brain regions	MMSE		MoCA	
	r	*P*-value	*r*	*P*-value
PCUN.R	0.326	0.002	0.325	0.002
PCUN.L	0.334	0.001	0.250	0.019

Table 4. The correlation coefficients and the corresponding *P* values between CVR and cognitive scale scores. *P*-value were obtained by partial correlation analysis adjusting for age, sex, and education. $P < 0.05/2$ ($P < 0.025$, Bonferroni-corrected) is considered to be statistically significant. *CVR* cerebrovascular reactivity, *MMSE* mini-mental state examination, *MoCA* Montreal cognitive assessment, *PCUN.R* right precuneus, *PCUN.L* left precuneus.

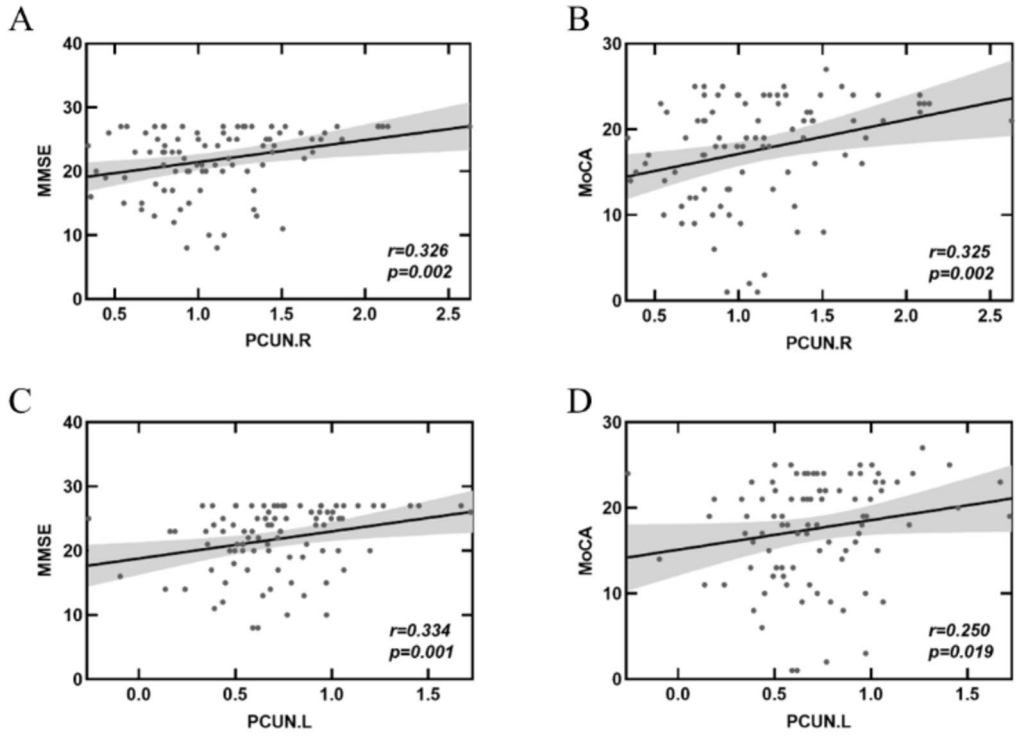

Fig. 4. Correlation between CVR values and MMSE/MoCA scales in patients with cognitive impairment (MCI + AD). (**A**) Positive correlation between CVR values of PCUN.R and MMSE scores; (**B**) Positive correlation between CVR values of PCUN.R and MoCA scores; (**C**) Positive correlation between CVR values of PCUN.L and MMSE scores; (**D**) Positive correlation between CVR values of PCUN.L and MoCA scores. *MMSE* mini-mental state examination, *MoCA* Montreal cognitive assessment, *PCUN.R* right precuneus, *PCUN.L* left precuneus, *CVR* cerebrovascular reactivity, *MCI* mild cognitive impairment, *AD* Alzheimer's disease.

The functional networks of the brain maintain cognitive functions through complex interconnections and dependencies. FC analysis using CVR differential brain regions among the three groups as ROI can more accurately reflect the network connectivity and activity of the human brain in the resting state, which provides an important basis for in-depth understanding of brain network changes in AD and MCI. Our results showed that PCUN.L was the ROI, and the FC of both PCUN.R and SFGmed.R were decreased in AD patients. This is consistent with the findings of most studies. TALWAR and ZHENG et al. have both found that FC in the brains of AD patients is impaired, with the default mode network (DMN) being the most severely affected[36,37]. It is widely accepted that the DMN primarily includes brain regions such as the posterior cingulate cortex, precuneus, and medial prefrontal cortex. Our study also found that, with the PCUN.R as the ROI, FC in the CC2.L and the DCG.R was increased in MCI patients; with the PCUN.L as the ROI, FC in the SFGmed.R was also increased in MCI patients. These findings are consistent with the majority of studies and our previous research results. Han et al.'s research indicates that preclinical AD patients exhibit increased FC in the frontal lobe[38]. MALOTAUX et al.'s study found that FC is increased in patients with mild MCI who progress to dementia compared to stable MCI[39], particularly in the DMN, where this change is more pronounced. This phenomenon of increased

ROI	Brain regions	MMSE		MoCA	
		r	*P*-value	r	*P*-value
PCUN.R	CC2.L	0.059	0.583	0.040	0.708
	DCG.R	0.147	0.171	0.183	0.088
	ANG.L	− 0.02	0.850	0.001	0.995
	MFG.L	0.223	0.037	0.192	0.073
	MFG.R	0.190	0.077	0.157	0.144
PCUN.L	Cere8.L	0.002	0.988	− 0.073	0.499
	Cere6.R	0.037	0.729	− 0.014	0.900
	PCUN.R	0.311	0.003	0.311	0.003
	SFGmed.R	0.341	0.001	0.313	0.003

Table 5. The correlation coefficients and the corresponding *P* values between FC and cognitive scale scores. *P*-value were obtained by partial correlation analysis adjusting for age, sex, and education. $P < 0.05/2$ ($P < 0.025$, Bonferroni-corrected) is considered to be statistically significant. *ROI*, region of interest, *MMSE* mini-mental state examination, *MoCA* Montreal cognitive assessment, *PCUN.R* right precuneus, *PCUN.L* left precuneus, *CC2.L* left crus II of cerebellar hemisphere, *DCG.R* right middle cingulate and paracingulate gyri, *ANG.L* left angular gyrus, *MFG.L* left middle frontal gyrus, *MFG.R* right middle frontal gyrus, *Cere8.L* left lobule VIII of cerebellar hemisphere, *Cere6.R* right lobule VI of cerebellar hemisphere, *SFGmed.R* right superior frontal gyrus, medial.

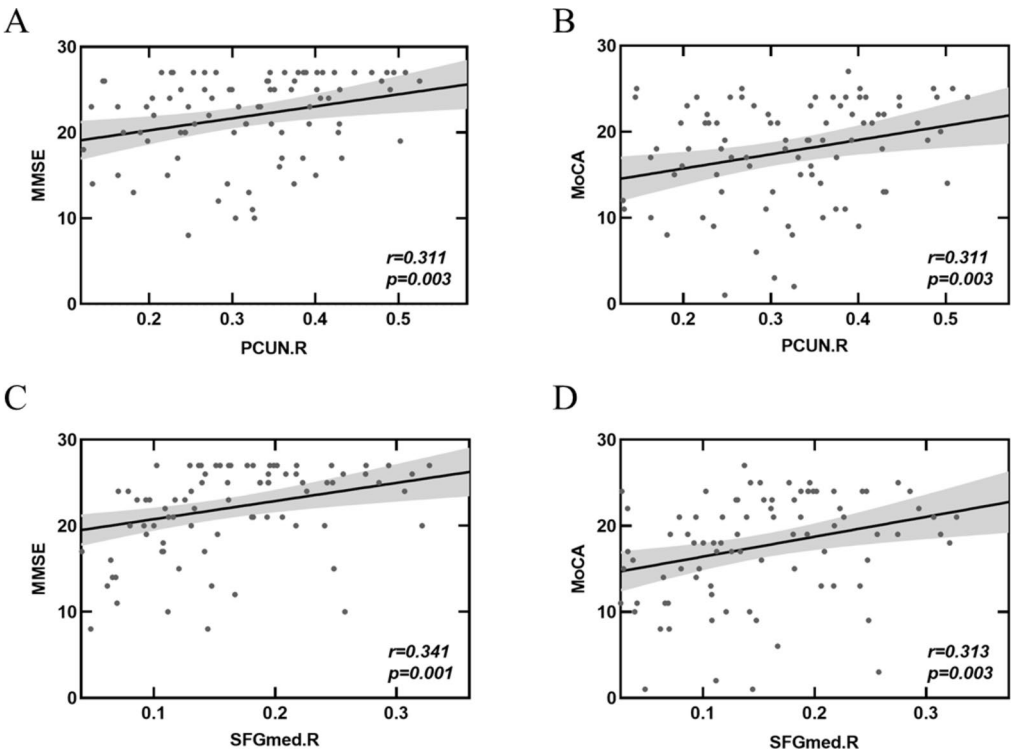

Fig. 5. Correlation between PCUN.L-ROI FC values and MMSE/MoCA scales in patients with cognitive impairment (MCI + AD). (**A**) Positive correlation between PCUN.L-ROI FC values of PCUN.R and MMSE scores; (**B**) Positive correlation between PCUN.L-ROI FC values of PCUN.R and MoCA scores; (**C**) Positive correlation between PCUN.L-ROI FC values of SFGmed.R and MMSE scores; (**D**) Positive correlation between PCUN.L-ROI FC values of SFGmed.R and MoCA scores. *MMSE* mini-mental state examination, *MoCA* Montreal cognitive assessment, *PCUN.R* right precuneus, *SFGmed.R* right superior frontal gyrus, medial, *PCUN.L* left precuneus, *ROI* region of interest, *FC* functional connectivity, *MCI* mild cognitive impairment, *AD* Alzheimer's disease.

FC is often interpreted as compensation for the decline in cognitive function in patients[28]. In addition to the aforementioned findings, our study results indicate that in AD, FC is increased in most brain regions with differential activity. Since the majority of AD patients we included are in the mild dementia stage {MMSE [20.00 (8.00)]}, they are likely to have progressed from the MCI patients mentioned in the aforementioned studies who are on the path to dementia. However, we can observe a trend of FC increasing and then decreasing across NC, MCI, and AD, suggesting that these AD patients would exhibit a decline in FC if they further progress, which is consistent with previous research findings.

The PCU, located on the medial surface of the parietal lobe, is associated with various higher cognitive functions, including episodic memory, self-referential information processing, and aspects of consciousness[40]. Studies of static ALFF have shown that patients with AD have a bilateral PCUN decrease in ALFF in both the classical, slow 4, and slow 5 bands[41]. Studies of cerebral blood flow have shown reduced CBF in PCUN.L in patients with AD[42]. SFGmed is located in the frontal lobe and is strongly associated with cognitive function, information encoding, retrieval, emotional thinking, and perceptual function[43]. Studies of static DC showed that AD patients exhibited a bilateral SFGmed DC reduction in the slow 5 frequency band[44]. Static FC showed that the FC of bilateral SFGmed was reduced in AD patients[28]. All of these studies show that PCUN and SFGmed are severely impaired in AD patients, both on the left and right sides, which is consistent with our findings. Additionally, the results of the correlation analysis showed that in all patients, CVR in the impaired brain regions and FC with the PCUN.L as the ROI to the PCUN.R and SFGmed.R were positively correlated with MMSE and MoCA scores. This suggests that the more severe the damage to the impaired brain regions, the more severe the overall cognitive impairment in patients. Most previous studies can provide strong evidence for this. The study by Donghoon Kim et al. indicates that in individuals at risk of cognitive decline, GM-CVR and WM-CVR are positively correlated with MMSE scores[16]. Additionally, two studies on CVR in AD have both found a positive correlation between CVR and MoCA scores[13,30]. Reduced CVR is thought to cause cognitive impairment by reducing oxygen and nutrient delivery to the brain. The above research results have given us some inspiration. Can we consider that when a reduction in CVR is observed in patients, it can be assumed that the patient's cognitive function has been impaired, and doctors can intervene in time for better treatment and prognosis. In addition, other studies have shown that CVR can observe the hemodynamic changes of AD patients earlier than CBF[14,45]. Therefore, we can consider CVR as a potential biomarker for assessing cognitive impairment.

Our study had several limitations. First, considering the specificity of our study involving dementia patients and prolonged MRI scans, in order to ensure the safety and comfort of subjects and the success rate of MRI scans, the rs-fMRI method used to measure the intrinsic CVR in AD subjects has not been validated by the clinically established prospective CO_2-targeted CVR method. However, this approach has been successfully conducted in arterial stenosis due to atherosclerosis, Moyamoya disease, white matter hyperintensities, and in healthy controls[21–25,46], all of which accurately reflect spatial variation in intersubject CVR. Of course, this is undeniably a limitation of our study. In future studies, we will comprehensively evaluate the overall situation of the patient and perform rs-fMRI scanning based on hypercapnia when the patient is awake and well-cooperated, so as to incorporate more mature CVR assessment methods, so as to enhance the robustness and feasibility of our study results. In addition, this study only collected demographic information such as age, gender, and years of education and only assessed overall cognitive function. In future studies, we should collect clinical data, including hypertension, diabetes, hyperlipidemia, BMI, and smoking, as well as single cognitive function tests such as episodic memory, executive function, language, and processing speed, so as to more comprehensively explore the pathophysiological mechanisms of AD and MCI patients. In addition, although our study cohort was carefully characterized, its size may limit the broad applicability of our findings. The sample size of this study is relatively small and cross-sectional, and in future studies, we will expand the sample size and continue to follow up with AD and MCI patients, including rs-fMRI scans and neuropsychological test scores, to observe changes in CVR over time and its association with cognitive decline, which will provide more in-depth insights into our research. Finally, this study used a global cognitive scale to distinguish between AD and MCI patients and did not use amyloid positron emission tomography, approved cerebrospinal fluid biomarkers, and accurate plasma biomarkers [especially phosphorylated tau 217][3], which may be subjective. Therefore, the inclusion of these biomarkers should be considered in future studies to make the findings more convincing.

Conclusion

The study's findings indicate statistical differences between CVR and FC with CVR differential brain regions as ROI among AD, MCI, and NC groups, which may elucidate the hemodynamic mechanisms underlying these conditions. Moreover, a positive correlation between CVR and FC with CVR differential brain regions as ROI and cognitive function scores suggests the potential of CVR, as measured by rs-fMRI, to serve as a non-invasive biomarker for assessing cognitive impairment. These insights offer a promising avenue for the non-invasive evaluation and monitoring of cerebrovascular diseases, although further research is warranted to confirm the clinical utility of CVR in these contexts.

Data availability

All data supporting the findings of this study are derived from Zhejiang Provincial People's Hospital (People's Hospital of Hangzhou Medical College). Due to privacy or ethical restrictions, the original neuroimaging data are not publicly available. However, with permission from Zhejiang Provincial People's Hospital (People's Hospital of Hangzhou Medical College), the data can be obtained from the corresponding authors Z.D. and X.X. upon reasonable request.

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Author contributions
XT: analysis of data, draft and revise; LW: conception, design and software; QF: acquisition of data; HH: analysis of data, draft and revise; YZ: analysis of data, draft; ZL: acquisition of data; ZD: conception, design and revise; XX: conception, design and revise.

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Declarations

Competing interests
The authors declare no competing interests.

Ethical approval
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study, and all experimental protocols were approved by the Ethics Committee of Zhejiang Provincial People's Hospital (The People's Hospital of Hangzhou Medical College) (No. 2012KY002).

Additional information
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