Cerebrovascular Reactivity Across the Entire Brain in Cerebral Amyloid Angiopathy

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Abstract

Background and Objectives
Reduced cerebrovascular reactivity is proposed to be a feature of cerebral amyloid angiopathy (CAA) but it has not been measured directly. Employing a global vasodilatory stimulus (hypercapnia) this study assessed the relationships between cerebrovascular reactivity and MRI markers of cerebral amyloid angiopathy and cognitive function.

Methods
In a cross-sectional study design, individuals with probable cerebral amyloid angiopathy, mild cognitive impairment, dementia due to Alzheimer disease, and healthy controls underwent neuropsychological testing and an MRI that included a 5% carbon dioxide challenge. Cerebrovascular reactivity was compared across groups controlling for age, sex and the presence of hypertension, and its associations with MRI markers of cerebral amyloid angiopathy in cerebral amyloid angiopathy participants and with cognition across all participants were determined using multivariable linear regression adjusting for group, age, sex, education and the presence of hypertension.

Results
Cerebrovascular reactivity data (mean±SD) were available for 26 participants with cerebral amyloid angiopathy (9 female; 74.4±7.7y), 19 participants with mild cognitive impairment (5 female; 72.1±8.5y), 12 participants with dementia due to Alzheimer disease (4 female; 69.4±6.6y) and 39 healthy controls (30 female; 68.8±5.4y). Grey and whiter matter reactivity averaged across the entire brain was lower in participants with cerebral amyloid angiopathy and Alzheimer disease dementia compared to healthy controls, with a predominantly posterior distribution of lower reactivity in both groups. Higher white matter hyperintensity volume was associated with lower white matter reactivity (standardized coefficient [β], 95% confidence
interval): -0.48, -0.90 to -0.01. Higher gray matter reactivity was associated with better global cognitive function (β: 0.19, 0.03-0.36), memory (β: 0.21, 0.07-0.36), executive function (β: 0.20, 0.02-0.39), and processing speed (β: 0.27, 0.10-0.45); and higher white matter reactivity was associated with higher memory (β=0.22, 0.08-0.36) and processing speed (β=0.23, 0.06-0.40).

Conclusions

Reduced cerebrovascular reactivity is a core feature of cerebral amyloid angiopathy, and its assessment may provide an additional biomarker for disease severity and cognitive impairment.

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Introduction

Cerebral amyloid angiopathy (CAA) is a small vessel disease caused by deposition of amyloid-beta (Aβ) in the media and adventitia of small blood vessels of the brain and leptomeninges. It causes 5-20% of intracerebral hemorrhages (ICH) and is a recognized cause of cerebral microbleeds (CMBs), white matter lesions of presumed vascular origin, cortical superficial siderosis (cSS), dilated perivascular spaces, and microinfarcts. CAA is also associated with cognitive decline and causes ~7% of dementia cases.

In patients with CAA, a greater burden of hemorrhagic (ICH, CMBs and cSS) and ischemic (white matter lesion and microinfarcts) consequences is associated with cognitive impairment. Another potential mechanism is reduced cerebrovascular reactivity (CVR), which limits the ability of brain regions to receive higher blood flow when needed. Reduced CVR has been observed in both transgenic mouse models of CAA and CAA patients, but its relationship with neuroimaging markers of CAA and cognitive function in CAA is poorly characterized. Furthermore, lower CVR in patients with CAA has only been observed as a reduced blood flow response through the posterior cerebral artery in response to a visual task, reduced increase in blood flow through the posterior and middle cerebral arteries during a standardized breath hold test and a lower response amplitude in blood oxygen level dependent (BOLD) functional MRI during a visual task. Currently, there are no data regarding the distribution of reduced CVR over the entire brain nor how grey and white matter CVR may relate to the hemorrhagic, ischemic, and cognitive consequences of CAA.

Alzheimer disease (AD) shares many pathophysiological links with CAA. Both are characterized by accumulation of Aβ, and AD pathology may coexist with CAA pathology on a spectrum from only vascular Aβ deposition characteristic of “pure” CAA to only parenchymal Aβ
deposition characteristic of “pure” AD. Some studies suggest that patients with AD have lower CVR compared to healthy controls. Therefore, concurrent AD pathology must be considered to potentially contribute to the reduced CVR and cognitive impairment observed in CAA.

The objectives of the current study were to assess CVR across the entire brain in CAA patients using a hypercapnic gas challenge, compare CVR between individuals with CAA, mild cognitive impairment and AD, and healthy controls, and to investigate the relationship between CVR and MRI markers of CAA and cognitive function.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

This study was performed according to the Declaration of Helsinki and approved by the Conjoint Health Research Ethics Board of the University of Calgary (REB15-0601) and the Health Research Ethics Board of the University of Alberta (Pro00067006). Participants were informed of study requirements prior to providing written informed consent.

Study population

Individuals ≥55 years of age without neurological or psychiatric disorders, or contraindications for MRI at 3T were recruited. Participants meeting the modified Boston criteria for probable CAA were recruited through stroke prevention and cognitive clinics in Calgary and Edmonton, Alberta, Canada. Participants presented with either ICH, transient focal neurological events (TFNE) or mild cognitive impairment (MCI). Acute effects of ICH were avoided by excluding participants with recent (<90 days) symptomatic stroke. Participants with
CAA-related inflammation were studied during remission when there was no evidence of cerebral edema on fluid-attenuated inversion recovery (FLAIR) MR images. Non-CAA participants with MCI were recruited from memory clinics and community advertisements, and reported a concern regarding a change in cognition, had a Clinical Dementia Rating score ≤0.5, ≥1 of the following - Logical Memory II (Delayed Recall) score <9 (16 years of education), <5 (8-15 years of education) or <3 (0-7 years of education), CERAD Word List Recall <6, Montreal Cognitive Assessment (MoCA) score ≤24 and a global CDR>0; and maintained activities of daily living (Lawton-Brody Instrumental Activities of Daily Living Scale (IADL) ≥15). Participants were considered to have non-CAA related MCI if they lacked multiple cortical, or cortical–subcortical hemorrhages, or the combination of a single lobar, cortical, or cortical–subcortical hemorrhage and cSS; MRI features consistent with the modified Boston criteria for probably CAA. Participants with a prior diagnosis of AD with dementia (“AD dementia”) were recruited from memory clinics. Diagnosis was based on the National Institute on Aging and the Alzheimer's Association (NIA-AA) clinical criteria for mild dementia due to Alzheimer’s disease and confirmed using the following criteria from the Canada-wide Comprehensive Assessment of Neurodegeneration and Dementia study - a gradual decline in memory and/or other cognitive functions over >6 months, had ≥1 of the cognitive impairments listed for MCI, reported changes in personality/behavior, and had impaired activities of daily living (Lawton-Brody IADL<15). AD dementia participants were included if they, similar to MCI participants, lacked MRI features indicative of probable CAA. Healthy controls (HCs) were recruited through community posters and newsletters and were screened by medical history and neuropsychological testing for the absence of stroke, MCI, or dementia.
**Study protocol**

All participants underwent a comprehensive neuropsychological exam by qualified personnel and an MRI that included a hypercapnic gas challenge to assess CVR across the entire brain.

Neuropsychological tests included the MoCA, Delis–Kaplan Executive Function System Verbal Fluency Test (VFT), Reitan Trail Making Test (TMT), Brief Visuospatial Memory Test-Revised (BVMT-R), the Rey Auditory Verbal Learning Test (RAVLT) and Wechsler Adult Intelligence Scale-3rd Edition Digit–Symbol Coding subtest (DSC). Raw test scores were converted into z-scores using normative data provided in test manuals and published literature. A memory z-score was derived by averaging the BVMT-R and RAVLT delayed recall z-scores; an executive function z-score was calculated as the mean of the TMT Part B and the VFT letter fluency z-scores; and a processing speed z-score was the mean of TMT Trail A and DSC z-scores.

In Calgary, MR imaging was performed on a 3T Discovery 750 (GE Healthcare, Waukesha, WI, USA) using a 32 channel receive-only coil (Nova Medical, Wilmington, MA, USA). In Edmonton, imaging was done on a 3T Prisma using a 64 or 20 channel receive only coil (Siemens Healthcare, Erlangen, Germany). Imaging at both centers included a 3D T1-weighted anatomical image, a T2-weighted FLAIR, a T2-star gradient recalled echo (T2* GRE), 30-direction diffusion-weighted echo planar imaging (EPI), and either a dual-echo pseudo-continuous arterial spin labelling sequence with a 2D EPI readout or a BOLD 2D EPI acquisition for the hypercapnic challenge. For the dual-echo sequence, BOLD images from echo 2 were used to quantify CVR to hypercapnia. MR acquisition parameters are provided in eTable 1.
The hypercapnic challenge consisted of breathing medical air for 6-min, a normoxic-hypercapnic gas mixture (5% CO₂, 21% O₂, balance N₂) for 2-min and then medical air for a final 2-min. Gases were delivered continuously at 20 L/min using an automated gas delivery system. The participant wore an anesthetic face mask (Quadralite, Intersurgical Ltd., Burlington, ON, Canada) connected to a non-rebreathing circuit with a gas reservoir open to room air on the gas delivery side. Respiratory gases were sampled continuously from a port ~2cm from the participant’s mouth and analyzed for the fraction of CO₂ and O₂ via fast responding gas analyzers (CO2100C and O2100C, BIOPAC Systems Inc., Goleta, CA, USA).

To compare CVR measures between sites, two healthy individuals (one female, 50y; and one male, 41y) were scanned at both sites with ~3 weeks between scans.

Radiological review

A single, experienced neuroradiologist performed all radiological reviews without knowledge of participant group assignment. The presence of CMBs, cSS and enlarged perivascular spaces was assessed on T2* GRE images and WMH burden was rated according the Fazekas Scale. A total CAA-related small vessel disease burden score (CAA SVD score; maximum=6) was calculated based upon the presence of lobar CMBs (2-4=1 point; ≥5=2 points), cSS (focal=1 point; disseminated=2 points), enlarged perivascular spaces within the centrum semiovale (>20=1 point) and moderate WMH burden (Fazekas ≥2=1 point). WMH volume was quantified on FLAIR images by qualified readers using a semi-automated seed-based 3D region growing algorithm (Cerebra-Lesion-Extractor v1.1.2, CIPAC, University of Calgary, Alberta, Canada).
MR image processing

Whole brain microstructural disruption of white matter was quantified via the peak width of skeletonized mean diffusivity (PSMD) calculated from diffusion-weighted images \(^{25}\) and tools from the FMRIB Software Library (FSL v6.0.0) \(^{26}\). Higher PSMD indicates greater white matter mean diffusivity variability; a measure reflecting greater disruption of white matter microarchitecture. \(^{6}\) Cortical thickness was measured from T1-weighted images with FreeSurfer (v6.0) \(^{27}\).

BOLD 4D images were cropped so that CVR was quantified using the final 6 minutes of the hypercapnic challenge (2 min air – 2 min hypercapnia – 2 min air). Images were motion-corrected and smoothed with a 5mm full-width at half-max gaussian kernel (SPM12, Wellcome Trust Centre for Neuroimaging, London, UK), and skull-stripped using FSL’s Brain Extraction Tool. \(^{28}\) The voxel-wise BOLD time course was modelled using FSL’s Expert Analysis Tool (FEAT) \(^{29}\) by convolving the 6 min hypercapnia stimulus paradigm with a gamma function (mean lag: 30 s; standard deviation: 15 s). The model also included a temporal derivative regressor to account for temporal delays in BOLD signal during the on- and off-hypercapnia transitions, and a linear drift nuisance regressor. For images acquired with the dual-echo sequence the model also included an ASL tag-control nuisance regressor. Voxel-wise BOLD percent signal change maps were created by dividing the modelled hypercapnic effect size by the estimated constant (baseline) term. \(^{22}\) Voxels with a signal increase ≥10% were removed as this magnitude of change is indicative of cerebral veins rather than parenchyma. \(^{30}\) Next, CVR maps were created by dividing the BOLD percent signal change maps by the change in partial pressure of end-tidal CO\(_2\) (PE\(_{\text{T,CO}_2}\)) from the 2 min air-breathing baseline to the last minute of hypercapnia. Thus, CVR was expressed as a \(\% \Delta \text{BOLD/mmHg}\) increase in PE\(_{\text{T,CO}_2}\).
Subcortical structures (putamen, caudate, globus pallidus, nucleus accumbens, thalamus, amygdala, hippocampus, amygdala, cerebellum and brainstem) were removed from CVR maps using an exclusion mask modified from FSL’s Brain Intensity AbNormality Classification Algorithm tool.\(^{31}\) Areas of ICH were removed using ICH masks created from FLAIR images registered to BOLD space.

To calculate grey matter (GM) and white matter (WM) CVR, T1-weighted images were skull-stripped and segmented into GM and WM using FSL’s FMRIB Automated Segmentation Tool (FAST). Resultant tissue partial volume estimate (PVE) maps were moved into the low-resolution BOLD space and, to account for partial volume effects, voxels with a PVE indicating <50% GM or WM were removed.\(^{32}\) Maps were then binarized to produce GM and WM masks which were merged to produce a global brain mask. Cortical GM, supratentorial WM and global CVR were quantified by multiplying whole brain CVR maps by the respective tissue mask and averaging all non-zero voxels within the resulting CVR maps.

CVR within the primary visual cortex (V1) and the middle temporal gyrus, posterior cingulate, precuneus and angular gyrus (principal regions affected by AD\(^ {33}\)) were quantified by creating a mask of each region in MNI152 space using the Juelich Histological Atlas (V1) or the Harvard-Oxford cortical structure atlas (principal regions affected by AD) in FSL. Masks were registered to participant low-resolution BOLD space and CVR was calculated by multiplying participant whole brain CVR maps by each region-of-interest mask and averaging all non-zero voxels within the resultant CVR maps. CVR within the middle temporal gyrus, posterior cingulate, precuneus and angular gyrus were averaged to create an AD regions specific CVR (AD\(_{regions}\)).
Statistical analyses

Sample size was determined \textit{a priori} based upon a two-tailed independent t-test comparison of CVR between CAA and HC participants. Twenty CAA and 26 HCs were determined to provide ~90\% power to detect a similar standardized difference of 1.0 (Cohen’s d) previously observed in vascular reactivity within V1 (percent increase in BOLD signal during visual task) between CAA and HC participants.\textsuperscript{9} This sample size was increased to 30 CAA and 40 HCs to provide ~80\% power to detect more moderate-sized differences (Cohen’s d=0.7) between CAA and HCs with either MCI or AD dementia participants, and for greater power to detect associations between CVR and cognition.

Participant characteristics were compared using a chi-square goodness-of-fit tests for categorical variables, 1-way ANOVAs for continuous, normally distributed variables, and Kruskal-Wallis tests for continuous, non-normally distributed variables. Post hoc group comparisons were corrected for multiple comparisons using the Dwass, Steel, Critchlow-Fligner analysis or a Tukey-Kramer correction, respectively.

Detection of CVR outliers was performed using the median absolute deviation (MAD) and all participant data. Participants were removed completely from analyses if their mean MAD score across all five CVR measures was >3\textsuperscript{34}; otherwise, participants were removed from individual comparisons if their MAD score was >3 for a specific CVR measure. CVR measures were compared between CAA participants with and without ICH, and across groups using ANCOVAs controlling for age, sex and the presence of hypertension, incorporating a Tukey-Kramer correction for multiple comparisons. Spatial differences in CVR between groups were determined on supratentorial CVR maps using non-parametric permutation-based threshold-free cluster enhancement analyses adjusted for age, sex and the presence of hypertension with 10,000
permutations via FSL’s Permutation Analysis of Linear Models controlling the false discovery rate and familywise error rate across all possible one-tailed group comparisons (n=12).\textsuperscript{35} Associations between MRI markers of CAA (CMBs, WMH volume and CAA SVD score), PSMD and cortical thickness and CVR were assessed within CAA participants using multivariable linear regression controlling for age, sex and the presence of hypertension. Lastly, relationships between cognitive scores and CVR were quantified using multivariable linear regression controlling for age, sex, education and the presence of hypertension. Statistical analyses were performed using Statistical Analysis System (SAS v9.4, Cary, North Carolina, USA) and an alpha ≤ 0.05 was considered significant.

Data availability

Anonymized data will be made available to other qualified researchers on request to the senior author (E.E.S).

Results

A total of 130 individuals were assessed for eligibility (Figure 1). Of these, CVR data were available for 96 - 39 HCs, 19 MCI (18 for V1), 12 AD dementia and 26 CAA participants. Participant characteristics are shown in Table 1. Of the 26 CAA participants with CVR data, 21 were recruited from stroke clinics and 5 were recruited from memory clinics, 11 presented with ICH, 10 with TFNE and five with MCI. CAA participants were older and more likely to be male than HCs and to have been diagnosed with hypertension. Cortical thickness was lower in AD dementia and CAA participants compared to HCs while all three patient groups performed worse on cognitive tests compared to HCs.
There were no differences in CVR across sites (GM, WM, Global and AD$_{regions}$: p≥0.076; V1: p=0.382) and the between site coefficient of variation across GM, WM, global, V1 and AD$_{regions}$ CVR for the travelling phantoms was 12.9±12.3% (mean±SD). There were no differences in GM (estimated mean difference (eMD), 95% CI: -0.01, -0.07-0.05), WM (eMD: -0.01, -0.03-0.04), global (eMD: 0.001, -0.05-0.05), V1 (eMD: -0.02, -0.10-0.05) and AD$_{regions}$ (eMD: 0.0002, -0.06-0.06) CVR between CAA participants who presented with ICH and those who did not (p≥0.536 for all comparisons adjusted for age, sex and the presence of hypertension). Therefore, remaining group comparisons did not include site as a covariate and included all 26 CAA participants.

CVR for each group is shown in Figure 2. Although there was overlap between groups, differences were observed in all CVR measures (GM, p=0.003; WM, p=0.010; global, p=0.003; V1, p=0.019; AD$_{regions}$, p=0.001) with AD and CAA participants having lower GM, WM, global and AD$_{regions}$ CVR compared to HCs. For V1, only CAA participants had a lower CVR compared to HCs.

Figure 3 shows the distribution of brain regions where CVR was lower in CAA and AD dementia participants compared to HCs. For both CAA and AD dementia participants, there was a predominantly posterior distribution of lower CVR compared to HCs that included the posterior cingulate, precuneus, tempororooccipital portion of the middle temporal gyrus and superior lateral occipital cortex. There were no regional differences in CVR between MCI and HCs.

Associations between CMB count, WMH volume, CAA SVD score, PSMD and cortical thickness with GM, WM, global and V1 CVR in CAA participants are shown in Table 2. Greater WMH volume was associated with lower WM (p=0.024) and global (p=0.045) CVR while
increased cortical thickness was associated with higher GM (p=0.047), WM (p=0.042) and global (p=0.031) CVR.

For multivariable linear regression analyses of cognitive scores and CVR, there was no interaction between participant group with GM and WM CVR for all cognitive scores (eTable 2). Therefore, associations between cognitive scores and CVR were quantified using all participants but controlling for group, age, sex, education (excluding MoCA) and the presence of hypertension. Figure 4 shows that a higher MoCA total score was associated with higher GM CVR (β, 95% CI: 0.19, 0.03 - 0.36, p=0.019) and there was a trend for a similar association with higher WM CVR (β, 95% CI: 0.15, -0.01-0.31, p=0.059). Figure 5 shows there were positive associations between GM CVR and memory (β, 95% CI: 0.21, 0.07-0.36, p=0.004), executive function (β, 95% CI: 0.20, 0.02-0.39, p=0.032) and processing speed (β, 95% CI: 0.27, 0.10-0.45, p=0.002) while higher WM CVR was associated with better memory (β, 95% CI: 0.22, 0.08-0.36, p=0.003) and processing speed scores (β, 95% CI: 0.23, 0.06-0.40, p=0.010).

Discussion

This study investigated whole brain CVR in CAA, AD dementia and MCI participants and its association with MRI markers of CAA and cognitive function. The main findings were:

1) GM, WM, global and AD regions CVR was lower in CAA and AD dementia participants compared to HCs whereas only CAA participants had a lower CVR within the primary visual cortex, 2) greater WMH volume was associated with lower WM CVR among CAA participants, and 3) MoCA, memory, executive function, and processing speed were positively associated with GM CVR while memory and processing speed were positively associated with WM CVR.
Mounting evidence implicates reduced CVR as a core feature of CAA. Studies using functional transcranial Doppler ultrasound have reported that patients with CAA have a lower visual-evoked blood flow response within the posterior cerebral artery in response to a flashing checkerboard\textsuperscript{12} and a smaller increase in middle cerebral artery blood velocity during a standardized breath hold challenge.\textsuperscript{13} CAA patients also have a reduced BOLD response amplitude during a visual task compared to HCs,\textsuperscript{8-11} with similar occipital visually-evoked electrical potential amplitudes.\textsuperscript{9,11} Provided visually-evoked electrical potentials reflect cortical metabolism\textsuperscript{36}, this finding provided indirect evidence that the lower BOLD response amplitude observed resulted from impaired vasodilation within V1. In contrast, the current study using a hypercapnic stimulus provides direct evidence that there is lower CVR in CAA and that it is not limited to V1 as it was also seen in the GM, WM, and AD regions. However, voxelwise comparisons revealed a predominantly posterior distribution of lower CVR (Figure 3) which is consistent with neuropathological evidence of the distribution of vascular amyloid in the brain.\textsuperscript{1} Unlike AD dementia CVR in V1 was lower in CAA compared to HCs, likely due to the greater posterior vascular amyloid burden in CAA reported via autopsy\textsuperscript{1} and amyloid-PET imaging\textsuperscript{37} compared to patients with AD dementia.

CVR to hypercapnia in MCI has been explored previously and has been found to be comparable to\textsuperscript{38} or lower than\textsuperscript{39,40} HCs. However, unlike the current study, these prior studies did not exclude participants whose MCI was associated with MRI evidence of CAA. Therefore, the similar CVR between our MCI participants and HCs may reflect the comparable degree of cerebrovascular disease between these participant cohorts (Table 1).

Whether CVR is reduced in AD is uncertain, with prior studies reporting either similar\textsuperscript{39,41} or lower\textsuperscript{42,43} CVR to hypercapnia compared to HCs. In contrast to this study the prior ones did
not screen AD participants for comorbid CAA, which is present in 20 to 30% of patients with AD and would affect CVR independent of the amount of AD pathology. In this study, we observed lower CVR in the AD dementia participants who did not have any MRI features indicative of probable CAA, suggesting that AD pathology may contribute to lower CVR even when the burden of vascular amyloid is minimal or absent.

In the current study, lower WM CVR was associated with higher WMH volume. This suggests CVR may decrease as CAA becomes more severe and is consistent with prior studies using functional hyperemia as a surrogate measure for CVR. Specifically, Smith et al. found that greater WMH volume was associated with smaller visual-evoked increases in blood flow through the posterior cerebral artery in response to a flashing checkerboard while Peca et al. and Switzer et al. found similar relationships between WMH volume and the BOLD response amplitude to a visual task. In contrast to prior studies, but consistent with this study, Dumas et al. found no relationship between CMB count and BOLD response amplitude. Rather, they found that greater WMH volume was associated with a longer time-to-peak BOLD response, thus still implicating vascular dysfunction in the pathogenesis of CAA-related WM changes. A reduced BOLD response amplitude to a visual task has also been reported in both symptomatic and pre-symptomatic individuals with hereditary CAA. Thus, the finding of a negative relationship between WMH volume and WM CVR in the current study, which used a vasodilatory stimulus independent of neuronal activation that acts directly upon the vascular endothelium and smooth muscle cells, suggests that CAA-related reduced WM CVR may contribute to the formation of WMH which are a common feature of CAA.

Reduced CVR has been hypothesized to contribute to cognitive impairment by reducing oxygen and nutrient delivery to the brain when needed. Associations between CVR and
cognitive function have been predominantly investigated in cohorts of HCs, MCI and/or AD patients with no reports in CAA participants. Our finding of a positive relationship between MoCA scores and GM CVR is comparable to that of Sur et al.\textsuperscript{45} who also found higher MoCA scores were associated with higher BOLD CVR responses to a 5% CO\textsubscript{2} challenge in a cohort of HCs, MCI and AD participants. Although we also observed a positive relationship between MoCA scores and WM CVR like Sur et al.\textsuperscript{45} it did not reach statistical significance (Figure 4B).

Few studies have investigated the relationships between individual cognitive domains and CVR using MRI in comparable patient groups. Sur et al.\textsuperscript{45} examined the relationship between GM, WM and global hypercapnic BOLD CVR with memory, executive function, and processing speed within their cohort of HCs, MCI and AD participants and found no relationship between CVR and these cognitive domains. This contrasts with our findings of positive associations between memory, executive function, and processing speed with GM CVR, and memory and processing speed with WM CVR. One potential reason for these differences is that the composite cognitive domain scores were created from different individual cognitive tests. The association of WM CVR with memory impairment was somewhat unexpected but may reflect dysfunction in white matter tracts involved in episodic memory. We assessed the associations between cognitive function and CVR using data from all participants, because there were no significant group-by-CVR measure interactions (eTable 2), indicating the relationships between each cognitive score and CVR measure were similar within each groups, including CAA participants.

In our analysis, we chose not to adjust for WMH volume or cortical thickness because we considered them to be mediating variables, not confounders. WMH are of presumed vascular origin\textsuperscript{46} and a reduced CVR to hypercapnia precedes the development of WMH.\textsuperscript{47} Thus, our
analyses were based on the hypothesis that CAA causes lower CVR which damages WM and GM leading to WMH and cortical thinning.

Another limitation of this study is the small sample size of AD participants. However, due to the robustness of the hypercapnic challenge used to quantify CVR, a difference in CVR between AD dementia participants and HCs was still observed, although confirmation of these findings in a larger sample size would be beneficial. A third limitation is that clinical criteria were used to define non-CAA related MCI and AD dementia rather than using cerebrospinal fluid and/or PET Aβ and tau biomarkers. This recruitment strategy was employed because cerebrospinal fluid Aβ is also altered in CAA\textsuperscript{11} and amyloid-PET tracers have limited diagnostic utility in differentiating between CAA and AD as they are non-specific for cerebrovascular or parenchyma amyloid.\textsuperscript{48} However, tau pathology is not expected in CAA and its presence would have indicated greater AD pathology. Therefore, future studies should examine associations between CVR and the presence of Aβ and tau brain deposits in both CAA and AD. In addition, MCI is a heterogenous syndrome with multiple causes of which AD is only one, and larger studies are required to distinguish differences in CVR between distinct MCI subtypes. Fourth, CVR was quantified using BOLD imaging and a fixed inspired fraction of CO\textsubscript{2}, which impose some limitations on the measurement accuracy and its interpretation. Though BOLD imaging is the most widely used MR acquisition for quantifying CVR, its signal represents a complex interaction of brain activity, cerebral oxygen metabolism and neurovascular factors that requires careful interpretation.\textsuperscript{49} Breathing a fixed inspired fraction of CO\textsubscript{2} (e.g., 5\%) does not produce the same increase in PETCO\textsubscript{2} across all individuals which can introduce greater variation in CVR across participant groups. Notwithstanding this greater variation and the overlap in CVR between groups (Figure 2), this study found clear differences between CAA and AD dementia
participants, and HCs. Nevertheless, more precise and accurate control of $\text{PET}_\text{CO}_2$ may have identified greater group differences and stronger associations between CVR and markers of CAA and cognition. Fifth, analyses assessing associations between CVR, and MRI markers of CAA and cognition were considered exploratory, and the precision of some estimates were low, resulting in wide confidence intervals. Therefore, the Type I error rate was not controlled for across the multivariable linear regression analyses and associations need to be confirmed by additional studies. Finally, this was a cross-sectional study and causal inferences between cognitive function and CVR cannot be made from our results.

In conclusion, a reduced CVR appears to be a core feature of CAA with areas of greater impairment mapping onto the posterior distribution of CAA-related pathology. While lower WM CVR was associated with greater WMH burden in CAA participants and a lower CVR was associated with worse cognitive function, the contribution of impaired CVR to these two sequelae remains to be investigated longitudinally. Assessment of CVR may prove to be a useful biomarker for the severity of its CAA and its effects on cognition.
References


Figure Legend

Figure 1 Participant flow through study.

A total of 130 individuals were assessed for eligibility. Of those, 119 were enrolled - 48 HC, 24 MCI, 16 AD dementia and 31 CAA. Six participants (4 HC, 1 AD, 1 CAA) declined the hypercapnic challenge, one CAA participant was excluded due to a medical contraindication (coronary angioplasty) and six participants could not fit the MR head coil with the CO₂ mask (3 HC, 2 MCI, 1 CAA). All remaining participants who underwent a hypercapnic CVR challenge completed the entire protocol. However, data from ten participants were excluded due to technical problems (n=5: 2 HC, 1 MCI, 1 AD, 1 CAA) or excessive motion (n=2: 1 MCI, 1 AD). One CAA participant was also excluded due to an artefact on all MR images. Two additional participants (1 MCI, 1 AD) were removed completely from CVR analyses because their mean MAD values across all CVR metrics were 3.6 and 4.5, respectively.
Figure 2  Cerebrovascular reactivity to hypercapnia (CVR) across groups. Boxplots of grey matter (GM), white matter (WM), global (GM+WM) and primary visual cortex (V1) CVR, and the mean CVR within principal brain regions impacted by Alzheimer’s disease (middle temporal gyrus, posterior cingulate, precuneus and angular gyrus; AD$\text{regions}$) for healthy controls (HC; n=39), mild cognitive impairment (MCI; n=19 except for V1 where n=18), Alzheimer’s disease dementia (AD; n=12) and cerebral amyloid angiopathy (CAA; n=26) participants. Post-hoc group comparison p-values adjusted using the Tukey-Kramer correction for multiple comparisons.
Figure 3  Areas of lower cerebrovascular reactivity to CO$_2$ (CVR) in CAA (A) and AD dementia (B) participants compared to healthy controls. Statistical maps showing brain regions within GM and WM (left) and on the cortical surface (right) where CVR is lower in participants with CAA (A) and AD dementia (B) compared to healthy controls. Clusters were determined by voxel-wise group comparisons performed using a non-parametric permutation-based threshold-free cluster enhancement analyses controlling for age, sex and the presence of hypertension with 10,000 permutations controlling the false discovery rate and familywise error rate across 12 pre-planned 1-tailed group comparisons.
Figure 4  Associations between MoCA total score and cerebrovascular reactivity to CO2 (CVR).  (A) Higher MoCA total scores were associated with higher grey matter (GM) CVR; (B) Higher MoCA total scores were not associated with white matter (WM) CVR. Standardized parameter estimates ($\beta$) and 95% confidence interval (CI) provided in plots reflect the relationships between MoCA total score and GM (A) and WM (B) CVR adjusting for group, age, sex and the presence of hypertension.
Figure 5  Associations between cognitive domains with grey (GM) and white matter (WM) cerebrovascular reactivity to CO2 (CVR).  
(A, B) better memory was associated with higher GM and WM CVR; (C, D) better executive function was associated with higher GM, but not WM CVR; (E, F) better processing speed was associated with higher GM and WM CVR.

Standardized parameter estimates ($\beta$) and 95% confidence interval (CI) provided in plots reflect the relationships between cognitive scores and GM (A, C, E) and WM (A, C, E) CVR adjusted for group, age, sex, education and the presence of hypertension.
Table 1  Participant characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HC</th>
<th>MCI</th>
<th>AD Dementia</th>
<th>CAA</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>39</td>
<td>19</td>
<td>12</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Female (%)</td>
<td>30 (76.9)</td>
<td>6 (31.6)</td>
<td>4 (33.3)</td>
<td>9 (34.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (y)</td>
<td>68.8±5.4</td>
<td>72.1±8.5</td>
<td>69.4±6.6</td>
<td>74.4±7.7</td>
<td>0.013</td>
</tr>
<tr>
<td>Education (y)</td>
<td>15.8±3.1</td>
<td>15.7±4.3</td>
<td>16.0±3.5</td>
<td>14.1±2.7</td>
<td>0.202</td>
</tr>
</tbody>
</table>

**Comorbidities**

- **Current Smoker (%)**: 0 (0.0) n=38
- **Past Smoker (%)**: 15 (39.5) 6 (31.6) 5 (41.7)
- **Never Smoked (%)**: 23 (60.5) 13 (68.4) 7 (58.3)
- **Hypertension (%)**: 6 (15.8) 5 (26.3) 4 (33.3)
- **Hypercholesterolemia (%)**: 16 (41.0) 11 (57.9) 3 (25.0)
- **Diabetes (%)**: 3 (7.7) 3 (15.8) 1 (8.3)
- **History of ICH (%)**: - - - 11 (42.3)

**MRI Markers of CAA**

- **Microbleeds (%)**: 5 (12.8) 6 (31.6) 0 (0.0) 24 (92.3)
- **cSS (%)**: 0 (0.0) 0 (0.0) 1 (8.3)
- **WMH Volume (mL)**: 2.9 (1.0-8.0) 3.4 (1.3-14.8) 6.6 (2.6-8.8) 21.7 (10.4-32.9)
- **WMH Volume (% ICV)**: 0.23 (0.06-0.54) 0.33 (0.10-1.05) 0.44 (0.18-0.53) 1.33 (0.95-2.27)
- **CAA SVD Score**: 0 (0) 0 (0.0) 1 (8.3) 4 (3-5)

**Additional MRI Findings**

- **PSMD (× 10^-4 mm^2/s)**: 2.5±0.46 3.0±0.99 2.9±0.64 4.5±1.3
- **Lacunes, basal ganglia (%)**: 2 (5.1) 0 (0.0) 0 (0.0) 2 (7.7)
- **Lacunes, centrum semiovale (%)**: 5 (12.8) 1 (5.3) 2 (16.7) 3 (11.5)
- **Cortical Thickness (mm)**: 2.4±0.09 2.3±0.09 2.6±0.19 2.8±0.15

**Cognitive Scores**

- **MoCA**: 27.2±1.6 23.4±2.8 18.1±4.1 21.2±5.5
- **Memory (z-score)**: 0.69±0.90 -1.00±1.16 -2.52±0.72 -1.56±1.11
- **Executive Function (z-score)**: 0.50±0.91 -0.72±1.19 -1.58±1.02 -1.31±1.20
- **Processing Speed (z-score)**: 0.86±0.79 -0.20±1.08 -1.55±1.24 -0.67±1.09

Number (%), categorical variables (p-value=χ^2); mean±SD, normally distributed continuous variables (p-value=t-test); median (IQR), non-normally distributed continuous variables (p-value=Mann-Whitney U).

< p<0.05 vs HC; = p<0.05 vs MCI; = p<0.05 vs AD; corrected using a Tukey-Kramer correction or the Dwass, Steel, Critchlow-Fligner analysis.

Abbreviations: ICH, intracerebral hemorrhage; CMBs, cerebral microbleeds; ICV, intracranial volume; cSS, cortical superficial siderosis; CAA, cerebral amyloid angiopathy; SVD, small vessel disease; PSMD, peak skeletonized white matter mean diffusivity; MoCA, Montreal cognitive assessment tool total score.
Table 2: Standardized parameter estimates ($\beta$ (95% CI)) for linear associations between MR markers of CAA with grey matter (GM), white matter (WM), global and primary visual cortex (V1) cerebrovascular reactivity to CO$_2$ (CVR) adjusted for age, sex and the presence of hypertension in CAA participants (n=26).

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>GM P-value</th>
<th>WM P-value</th>
<th>Global P-value</th>
<th>V1 P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log CMB (#)</td>
<td>-0.46 (-0.93 to 0.02)</td>
<td>0.057</td>
<td>-0.35 (-0.86 to 0.16)</td>
<td>0.170</td>
</tr>
<tr>
<td>Log WMH (%) ICV</td>
<td>-0.34 (-0.76 to 0.08)</td>
<td>0.110</td>
<td>-0.48 (-0.90 to -0.01)</td>
<td><strong>0.024</strong></td>
</tr>
<tr>
<td>CAA SVD Score</td>
<td>-0.40 (-0.86 to 0.06)</td>
<td>0.084</td>
<td>-0.46 (-0.93 to 0.004)</td>
<td>0.052</td>
</tr>
<tr>
<td>PSMD ($\times 10^{-4}$ mm$^2$/s)</td>
<td>0.06 (-0.45 to 0.57)</td>
<td>0.815</td>
<td>0.00 (-0.54 to 0.54)</td>
<td>0.999</td>
</tr>
<tr>
<td>Cortical Thickness (mm)</td>
<td>0.44 (0.01 to 0.87)</td>
<td><strong>0.047</strong></td>
<td>0.46 (0.02 to 0.90)</td>
<td><strong>0.042</strong></td>
</tr>
</tbody>
</table>

Abbreviations: CMB, cerebral microbleed; WMH, white matter hyperintensity; ICV, intracranial volume; SVD, small vessel disease; PSMD, peak width of skeletonized mean diffusivity.