



## Arterial spin labelling reveals multi-regional cerebral hypoperfusion in patients with transient ischemic attack that are unrelated to ischemia location: A proof-of-concept study

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### ABSTRACT

**Background and Aims:** Patients with transient ischemic attack (TIA) have a substantially increased risk of early dementia. In this exploratory study, we aim to determine whether patients with TIA have 1) measurable regional cerebral hypoperfusion unrelated to the location of ischemia, and 2) determine the relationship of regional cerebral blood flow (rCBF) with their cognitive profiles.

**Methods:** Patients with TIA ( $N = 49$ ) and seventy-nine ( $N = 79$ ) age and sex matched controls underwent formal neuropsychological testing and MRI. Quantitative arterial spin labelling rCBF maps (mL/min/100 g) were registered to the corresponding high resolution T1-weighted image. Linear regression was used to determine the association between demographic, clinical and cognitive variables and rCBF.

**Results:** Patients with TIA had significantly ( $p < 0.05$ ) lower cognitive scores in the MMSE, MOCA, ACE-R, WAIS-IV DS Coding and Trail Making Tests A and B compared to controls. TIA patients had significantly lower rCBF in the left entorhinal cortex ( $p = 0.03$ ), right posterior cingulate ( $p = 0.04$ ), and right precuneus ( $p = 0.05$ ), after adjusting for age and sex, that were unrelated to the regional anatomical volume and DWI positivity. Regional hypoperfusion in the right posterior cingulate and right precuneus was associated with impaired visual memory (BVMT total,  $p = 0.05$  for both regions) and slower processing speed (TMT A,  $p = 0.04$  and  $p = 0.01$ ), respectively after adjusting for age and sex.

**Conclusions:** TIA patients have patterns of regional hypoperfusion in multiple cortical regions unrelated to the parcellated regional anatomical volume or the presence of a DWI lesion. Regional hypoperfusion in patients with TIA may be an early marker conferring risk of future cognitive decline that needs to be confirmed by future studies.

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## 1. Introduction

Dementia is poised to become a global health crisis if effective treatments are not developed soon [1]. It is now generally accepted that a significant contributor to the failure of therapeutic treatments is that the diagnosis can only be reliably established late in the disease course, when neuronal loss and neurosynaptic degeneration have taken hold, and years after irreversible damage to the brain has occurred. It is also appreciated, that stroke, including transient ischemic attack (TIA), is a distinct and important risk factor for dementia, doubling the risk of later cognitive decline independently of known vascular and lifestyle risk factors [2,3]. Therefore, the best future strategy appears to consist of early intervention in the preclinical phase, at a time when the brain is only minimally affected.

The identification of reliable neuroimaging markers for preclinical dementia may allow for the identification and implementation of therapeutic and pharmacological interventions in the earliest stages of disease. The AT (N) framework is an established biological construct of Alzheimer's disease (AD) [4], and an extension of the framework proposes the addition of biomarkers of vascular disease (labelled V) consisting of small vessel disease, cortical and sub-cortical stroke [5]. For instance, it has been proposed that late onset dementia relates to the regional convergence of Alzheimer disease (AD) pathologies that are modified by vascular pathologies [6], and the progression of disease may reflect regional vulnerability contributing to permanent alteration of connectivity involving the default mode, salience network and limbic network [7]. Further, reduced cerebral blood flow (CBF) is tightly coupled to brain metabolism, and therefore abnormalities in blood flow may indicate synaptic dysfunction and neuronal damage, preceding brain atrophy and cognitive symptoms [8].

In this exploratory study, we aimed to determine if there is regional subcortical or cortical hypoperfusion measured by magnetic resonance arterial spin labeling (ASL) sequence in patients with TIA compared to controls [3] similar to those reported in mild cognitive impairment (MCI) and AD [9,10], that is unrelated to the location of the ischemia and the volume of the anatomical region. Additionally, we aimed to assess the potential associations of regional hypoperfusion with cognitive performance.

## 2. Material and methods

### 2.1. Study population

This study was a prespecified substudy of the PREVENT VCI study, a prospective longitudinal cohort study of TIA and non-TIA controls [11]. The overarching aims of the PREVENT VCI study are to determine the relationship of MRI biomarkers, inclusive of cerebral perfusion, with cognitive outcome. Consecutive participants presenting with first documented episode of probable TIA [12] in the anterior circulation (aphasia, motor weakness) or posterior circulation (visual field deficit, ataxia/vertigo plus one or more of motor, diplopia, hemiataxia) and control volunteers between the ages of 50–75 years were prospectively recruited between May 2017 and December 2021 [11] following written informed consent. Ethics approval was provided by the University of Calgary Conjoint Health Research Ethics Board (REB13–0240). Patients with TIA presented to the Emergency Department at the Foothills Medical center or Calgary Stroke Prevention Clinic urgently. All patients had been investigated with non-contrast CT and CT angiography as part of their diagnostic workup, which is commonly performed in the emergency room before referral to the acute TIA clinic.

TIA was defined according to the National Institute of Neurological Disease and Stroke criteria [12]. There was a formal review of all cases by the study's senior neurologist (PAB) who confirmed the final diagnosis of TIA and inclusion into the study. All TIA patients were managed according to current stroke prevention guidelines. None of the patients underwent thrombectomy or thrombolysis. Control participants were

recruited from the community (e.g., hospital and community center advertisements) and spousal partners. Further inclusion criteria included: (1) absence of significant impairment in cognitive functioning or activities of daily living including Mini-Mental State Examination (MMSE) [13] scores between 24 and 30 and Clinical Dementia Rating (CDR) 0–0.5 [14]; (2) no depression (Geriatric Depression Scale Score <6 [15]); and (3) fluent in English. Exclusion criteria included: (1) previous symptomatic stroke; (2) chronic neurological disease (Parkinson's disease, dementia, seizure disorder, multiple sclerosis) or significant head trauma; (3) contraindications to MRI; (4) major depression, bipolar disorder, schizophrenia, generalized anxiety disorder as defined by DSM-5 criteria [16]; (5) alcohol or substance misuse according to DSM-5 criteria, aided by administration of the CAGE questionnaire [17]; and (6) unstable medical conditions that would make cognitive tests unreliable or impair follow-up. Patients considered to be mimics (migraine, transient global amnesia), which were defined clinically, were enrolled in the study as controls. TIA participants who had ASL images were matched for age ( $\pm 2$  years) and sex with up to two controls.

### 2.2. Clinical data collection

The study design included a medical evaluation (clinical characteristics, demographics, medical history and medications). Framingham risk score (FRS) [18], a common score used to estimate the 10-year cardiovascular disease (CVD) risk of individuals, was calculated using the Alberta Health Services FRS calculator, and a risk level of low (< 10% 10-year CVD risk), intermediate (10–19%), or high ( $\geq 20\%$ ), was coded as 1, 2, or 3 for each participant in order to conform to clinical practices of using the risk levels. A detailed standardized neuropsychological battery was administered as previously reported [11].

### 2.3. Image acquisition

Participants underwent MR imaging using a 3.0T Diagnostic MR Scanner (General Electric Discovery 750), using a standard multi-channel head coil. Patients with TIA had their imaging within 10 days of their presentation. A 3D fast-spin-echo pseudo-continuous arterial spin labeling sequence with background suppression was collected with the following parameters: post-labeling delay = 2025 ms, field of view (FOV) = 24.0 cm, repetition time = 4898 ms, echo time = 11.7 ms, slice-thickness = 4.0, thirty-six 4.0 mm slices with a 0-mm gap, and spiral acquisition with 660 points and six spiral arms. T1-weighted images were acquired using a 3D inversion recovery prepared spoiled gradient-echo sequence (3D; field of view (FOV) = 24 cm; one hundred and seventy-six 1.0-mm slices with a 0 mm gap; acquisition matrix = 256  $\times$  256; TE = 2.932 ms; TR = 6.7 ms; flip angle = 8°; inversion time (TI) = 650 ms; phase FOV = 85%; reconstructed voxel size = 1 mm isotropic). A 2D DWI sequence was acquired with an echo planar technique having an acquisition matrix size = 256  $\times$  256, FOV = 28 cm, twenty-three 3.0-mm slice with 0-mm gap; TE = 81.7 ms, TR = 8000 ms, flip angle = 90°. A 3D T2-weighted FLAIR sequence was acquired with an acquisition matrix size = 512  $\times$  512; FOV = 24 cm; thirty-eight 3.0-mm slice with a 0-mm gap; TE = 120 ms, TR = 8524 ms, TI = 2219 ms, flip angle = 90°.

### 2.4. Image processing

Quantitative CBF maps were automatically generated by the MR scanner from the ASL datasets in CBF units (mL/100 g/min). Quality control was performed visually to determine image quality (low signal-to-noise, or high levels of image artifact or image distortion). Adjudication of the image quality was graded according to neck and head movement, quality of registration and intensity inhomogeneities by two researchers (PAB, MR). ASL images were checked for arterial transit time heterogeneity by calculating the spatial coefficient of variation (CoV) [19]. All ASL scans were categorized as 'good' (CoV  $\leq 0.6$ ,

cerebral blood flow signal predominates artifacts) [19,20].

#### 2.4.1. ASL-T1 registration

Structural T1-weighted images and ASL-CBF images underwent BET brain extraction using FSL (BET – FSL v2.1) following standardized orientation. For quantitative analysis, the ASL-CBF images were then registered to the corresponding T1 images using a linear 12° FSL-FLIRT registration [21]. Regions of interest were segmented from the T1 images and subsequently masked to the corresponding ASL-CBF image in T1 space [Fig. 1] [21]. Whole brain white and gray matter of each hemisphere was segmented with FSL FAST [22]. Specific anatomical regions of interests were also segmented and investigated. Specifically, the hippocampus, thalamus and basal ganglia were segmented with FSL FIRST [23]. The anterior cingulate, entorhinal cortex, posterior cingulate and precuneus were segmented with the Desikan-Killiany atlas in FreeSurfer [24]. The volumes of these ROIs were also measured. These ROIs were chosen based on a literature review of cerebral blood flow imaging performed in MCI and AD [9,10]. Whole-brain volume, normalised for subject head size, was measured using SIENAX [25].

#### 2.4.2. White matter hyperintensities and diffusion lesions

White matter hyperintensities (WMHs) were segmented from FLAIR images using the Lesion Prediction Algorithm (LPA) in the Lesion Segmentation Toolbox implemented in SPM12 (<https://www.fil.ion.ucl.ac.uk/spm>). Diffusion lesions were segmented from the DWI images with ITK-SNAP (Version 3.6.0, [www.itksnap.org](http://www.itksnap.org)), a semiautomatic software application, by a trained researcher (MR) and underwent review by a stroke neurologist (PAB) [26].

#### 2.5. Statistical analyses

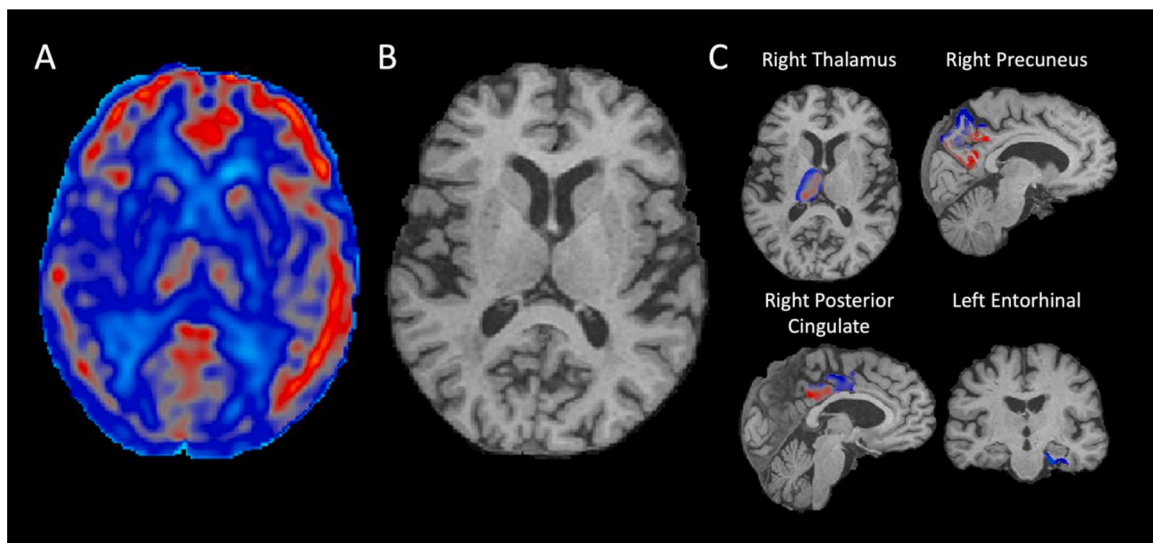
All statistical analysis was performed on STATA IC/15.0 (StataCorp. 2017. Stat Statistical Software: Release 15. College Station, TX: StatCorp LLC). Two-way two-sample *t*-tests ( $\alpha = 0.05$ ) and Chi square tests were used to compare risk factors (including demographic, medical, depression and premorbid intellect), cognitive profiles, and regional cerebral blood flow (rCBF) amongst the TIA and control participants. In our first objective, backwards stepwise multiple linear regression was used to analyze the associations between group status (TIA vs control) and rCBF after controlling for confounding variables, including age, sex, premorbid verbal intellect (NAART IQ), regional anatomical volume,

WMHs and FRS. Each region was tested in a separate model. To minimize multiple comparisons, it was predetermined to only perform the multiple linear regression analysis on regions that showed a significant group difference in CBF [Table 3]. For this objective, regression was performed to analyze relationships between vascular covariates (including DWI volume, DWI location, symptomatic hemisphere, vascular territory involved) and rCBF. In our second objective, a final regression analyzed the associations between regions of reduced CBF detected in objective 1 and cognitive scores while controlling for demographic variables, such as group status, age and sex. All data used in multiple linear regression met the basic required assumptions. Adjustments for multiple comparisons was not performed as objective 2 was exploratory in determining the potential relationships of regional CBF and cognitive performance. Dunn-Bonferroni non-parametric tests were used to compare rCBF between DWI-positive and DWI-negative in TIA patients and control subjects.

### 3. Results

Fifty-six (56) TIA patients and 84 control participants were initially selected. Seven (7) TIA and 5 control participants were subsequently excluded because they failed to meet image quality control standards (motion artifact and ASL quality), resulting in a studied sample of 49 TIA patients and 79 controls.

Demographic, clinical, and vascular factors for both the TIA and control population are summarized in Table 1. TIA patients had more extensive white matter hyperintensity (WMH) volume ( $p = 0.004$ ) and increased tobacco use ( $p = 0.023$ ) compared to controls. Of the TIA patients, 32 (65.3%) had left hemispheric symptoms, 38 (77.6%) had symptoms referable to the anterior circulation and 11 (22.4%) had posterior circulation symptoms. TIA patients had no identifiable vascular occlusion of the extracranial or intracranial vessels on the CT angiogram. Median time from symptom onset to MRI was 8 days (IQR = 5). DWI positive lesions (median 15.43mm<sup>3</sup>, IQR 5.74–42.00 mm<sup>3</sup>) were detected in fifteen patients (30.6%) and were located in the following vascular territories: middle cerebral artery ( $n = 11$ ), the posterior cerebral artery ( $n = 3$ ) and the anterior cerebral artery territory ( $n = 1$ ). Cognitive test scores in patients with TIA and controls are summarized in Table 2, revealing that patients with TIA had slightly lower scores on the MoCA and MMSE compared to controls, and slower processing speed than control subjects.



**Fig. 1.** Methodological overview of the analysis of regional cerebral blood flow. a) MR-ASL whole-brain CBF map was co-registered to (b) high resolution T1-weighted image and segmented anatomical regions and masked to ASL-CBF map shown in (c). Note: only some of the prespecified anatomical regions are shown (refer to methods section for full details).

**Table 1**

Demographic, clinical and imaging data of TIA and control participants compared with two-way two-sample *t*-tests (continuous variables), Wilcoxon rank-sum test (ordinal variables) and Chi-squared tests (proportional variables) ( $\alpha = 0.05$ ). Values reported are means and standard deviations unless otherwise stated.

Descriptive Variables	TIA (n = 49)	Control (n = 79)	p
Age, mean years	68.1 ± 7.4	67.2 ± 6.7	0.47
Sex, n women (%)	19 (38.8%)	42 (53.2%)	0.11
Education, years	14.4 ± 3.3	15.3 ± 2.9	0.12
Depression, raw CES-D	10.6 ± 8.1	9.4 ± 8.8	0.44
Premorbid Intellect, NAART	108.2 ± 10.2	110.6 ± 7.7	0.13
WMH/NBV, median (IQR)	3.1 (0.8–4.8)	2.4 (0.5–2.1)	0.00
NBV, cm <sup>3</sup>	1434 ± 88.0	1429 ± 110.3	0.79
Systolic BP, mmHg	134.2 ± 19.8	126.6 ± 17.1	0.03
FRS, raw score	15.67 ± 4.30)	13.68 ± (4.55)	0.02
DWI, n (%)	15 (30.6%)	0	0.00
DWI Volume, median mm <sup>3</sup> (IQR)	15.4 (5.7–42.0)	0	0.00
HDL Cholesterol, mmol/L	1.5 ± 0.09	1.5 ± 0.06	0.93
Diabetes, n (%)	4 (8.2%)	4 (5.1%)	0.48
Tobacco, n (%)	30 (61.2%)	32 (40.5%)	0.02

IQR = interquartile range, WMH = white matter hyperintensities, NBV = normalized brain volume, FRS = Framingham Risk Score, DWI = diffusion weighted image lesion.

**Table 2**

Cognitive test performance comparing TIA and control participants (median, interquartile ranges and Wilcoxon Rank-Sum tests ( $\alpha = 0.05$ )).

	TIA (n = 49)		Control (n = 79)		p
	Median	IQR	Median	IQR	
MMSE	29.0	27.0–30.0	30.0	29.0–30.0	0.01
MOCA	25.0	23.0–28.0	26.0	25.0–28.0	0.01
ACE-R	92.0	85.0–95.0	95.0	91.0–97.0	0.01
BVMT Total	20.0	16.0–26.0	22.0	17.0–27.0	0.22
BVMT Delayed	9.00	6.0–11.0	10.0	8.0–11.0	0.12
RAVLT	9.00	7.0–12.0	11.0	7.0–13.0	0.14
WAIS-IV DS Coding	53.0	47.5–61.0	62.0	51.0–76.0	0.00
TMT A	33.9	28.4–44.7	30.0	22.5–37.0	0.01
TMT B	87.4	61.3–99.1	66.7	55.0–91.0	0.03
Verbal Fluency, raw total from ACE-R	6.0	4.0–6.0	6.0	5.0–7.0	0.16

MMSE = Mini-Mental State Examination, ACE-R = Addenbrooke's Cognitive Examination-Revised, BVMT = Brief Visuospatial Memory Test, RAVLT = Rey Auditory Verbal Learning Test, DS Coding = Digit Symbol Coding, TMT = Trail Making Test, MOCA = Montreal Cognitive Assessment.

Whole brain CBF measurements in the deep white matter were not significantly different between TIA and control groups but there were differences in CBF in the cortical gray matter [Table 3]. Regional CBF (mL/min/100 g) between the TIA and control participants showed multiple regions of significant relative hypoperfusion in the TIA group [Table 3], that included the entorhinal cortex, precuneus and posterior cingulum, shown in Fig. 2. The volume of these anatomical regions did not significantly differ statistically between TIA and controls, apart from the left entorhinal cortex which was smaller in controls (median volume [IQR]: 2.04 mL [0.44]) than TIAs (2.24 mL [0.68];  $p = 0.03$ ). The presence of a DWI lesion did not have a significant effect on rCBF in the hypoperfused regions in the TIA cohort (left entorhinal  $p = 0.691$ , right precuneus  $p = 0.513$ , right posterior cingulate  $p = 0.791$ ) [Table S1].

Multiple linear regression analysis showed a significant relationship between TIA status and regional hypoperfusion in the left entorhinal cortex ( $\beta = -2.58 \pm 1.18$ ,  $p = 0.03$ ), right posterior cingulate ( $\beta = -3.53 \pm 1.71$ ,  $p = 0.04$ ), and right precuneus ( $\beta = -3.61 \pm 2.00$ ,  $p = 0.05$ ) [Table 4] that was independent of regional anatomical volume. There was no significant relationship between TIA status and CBF in the whole-brain cortical gray ( $p = 0.136$  and  $0.181$ ; for left and right respectively)

**Table 3**

Regional cerebral blood flow (mL/min/100 g) of TIA patients and control participants (median, interquartile range (IQR) and Wilcoxon Rank-Sum tests ( $\alpha = 0.05$ )).

		TIA (n = 49)		Control (n = 79)		p
		Median	IQR	Median	IQR	
White Matter	Left	37.3	31.1–42.1	37.0	31.9–43.4	0.91
	Right	35.2	29.1–41.3	32.9	29.3–41.4	0.95
Gray Matter	Left	43.5	34.1–48.8	47.4	38.4–55.1	0.04
	Right	41.3	32.9–48.3	45.3	36.2–51.7	0.05
Hippocampus	Left	39.8	34.1–42.7	41.1	35.3–45.6	0.13
	Right	38.6	31.8–43.0	40.6	34.1–46.4	0.08
Thalamus	Left	40.4	35.8–44.9	42.8	36.5–48.5	0.07
	Right	40.8	32.8–46.1	43.3	36.7–48.6	0.03
Anterior Cingulate	Left	49.4	44.6–56.6	52.6	47.8–60.3	0.07
	Right	48.4	43.1–54.9	51.7	45.0–57.7	0.10
Entorhinal Cortex	Left	35.2	31.1–37.5	38.9	33.4–44.4	0.01
	Right	34.2	31.4–41.7	37.4	32.6–42.1	0.16
Posterior Cingulate	Left	46.9	39.2–53.5	51.3	42.7–58.1	0.01
	Right	48.8	40.1–55.7	52.0	44.6–59.1	0.01
Precuneus	Left	41.7	33.1–51.2	46.9	38.4–56.5	0.03
	Right	42.2	35.2–53.1	48.5	39.3–58.9	0.01

or white matter ( $p = 0.891$  and  $0.840$ ; for left and right respectively) after adjusting for covariates. DWI volume, DWI location, side, vascular territory and etiology were not associated with rCBF [Table S2]. Hypoperfusion in the right posterior cingulate and the right precuneus were associated with a poorer outcome on the BVMT total score ( $\beta = 0.11 \pm 0.057$ ,  $p = 0.04$  and  $\beta = 0.096 \pm 0.048$ ,  $p = 0.05$ , respectively), and the Trail Making A Test ( $\beta = -0.25 \pm 0.12$ ,  $p = 0.04$  and  $\beta = -0.26 \pm 0.10$ ,  $p = 0.01$ , respectively). Regional CBF in the left entorhinal cortex was not associated with memory impairment [Table 5].

#### 4. Discussion

In this study we show that patients with TIA exhibit regional hypoperfusion in multiple areas of the brain that included the left entorhinal cortex, right posterior cingulate and right precuneus, and that hypoperfusion in these regions were independent of differences in regional anatomical volume in these cortical regions. Additionally, this regional hypoperfusion does not appear to be related to a whole-brain reduction in blood flow, since there was no difference in CBF in whole-brain gray or white matter between the two groups. In a cohort of TIA patients without high-grade intra- or extracranial stenosis, we found that regional hypoperfusion was not related to the presence of a DWI lesion (or its anatomical location). Our findings suggest that our cohort of TIA patients exhibit similar patterns of regional hypoperfusion that have been reported in AD [27], which may explain the increased risk of late-life cognitive decline in TIA and minor stroke patients, as these regions of hypoperfusion were not associated with differences in the regional anatomical volume of the regions or DWI lesions. Additionally, we examined the relationship of regional hypoperfusion with cognitive profiles and found that relative reduction of rCBF in the right posterior cingulate and right precuneus was significantly associated with poorer cognitive performance in the visual memory and processing speed domains. The pattern of regional hypoperfusion may have importance in identifying patients with TIA at greater risk of future cognitive decline. This hypothesis will need to be explored in the future by determining the relationship of ASL regional CBF measurements with cognitive decline and regional anatomical change in volume.

Lower global and regional CBF measured by ASL-MR have been reported in MCI and AD [10]. Furthermore, regional hypoperfusion in the precuneus and posterior cingulate, which was found in our TIA cohort, has predicted the conversion of MCI to AD [9]. It has been proposed that in the preclinical phase of AD dementia, regional accumulation of abeta

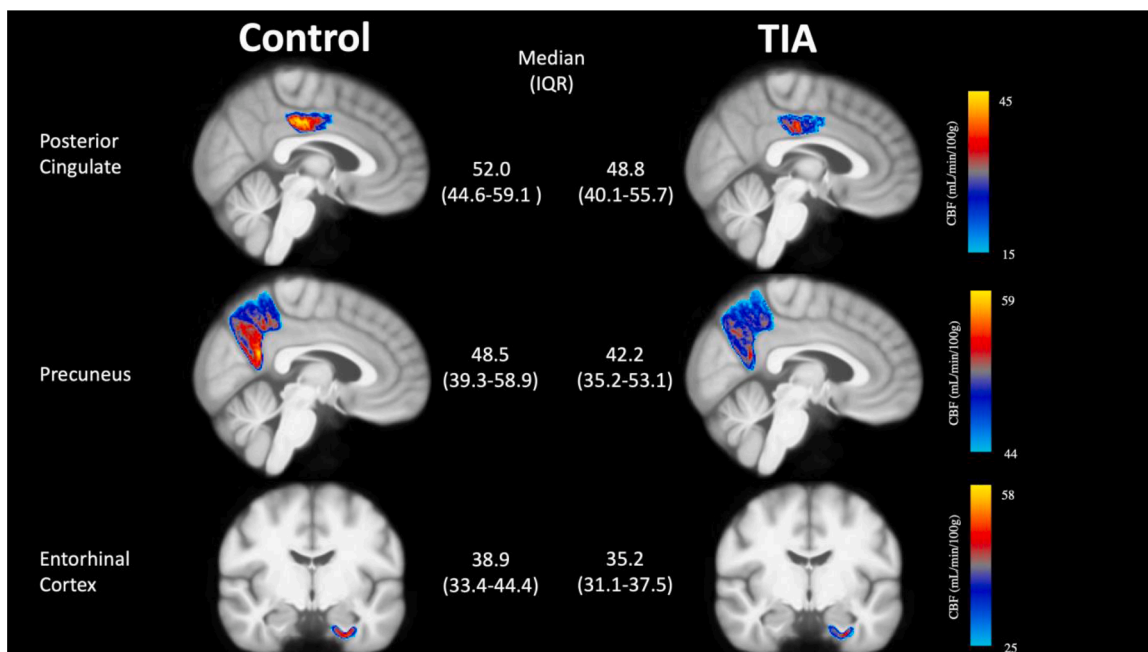


Fig. 2. Average cerebral blood flow (mL/min/100 g) acquired from ASL-MR overlaid on the anatomical T1-weighted MR image, showing regional hypoperfusion in the TIA cohort compared to control (age and sex matched) in the posterior cingulate gyrus, precuneus, and entorhinal cortex.

Table 4

Multiple linear regression analysis to test the associations between TIA group vs. control and regional cerebral blood flow (mL/min/100 g) after controlling for age, sex, premorbid verbal intelligence, regional anatomical volume, white matter hyperintensities, and Framingham Risk Score.

rCBF		Beta Coefficient	Standard Error	p
Entorhinal Cortex	Left	-2.575	1.181	0.03
	Right	-0.450	1.214	0.71
Posterior Cingulate	Left	-2.947	1.728	0.09
	Right	-3.529	1.713	0.04
Precuneus	Left	-2.877	2.022	0.16
	Right	-3.609	2.000	0.05

amyloid and tau occurs in vulnerable hypometabolic regions, occurring independently and modified by vascular disease and risk factors [6]. A large population-based study found that lower CBF was independently associated with increased risk of dementia seven years following the assessment [28]. Additionally, small vessel disease can contribute to subcortical damage disconnecting multiple brain regions [29]. This TIA cohort had a modest decrease on cognitive test scores compared to the controls, suggesting that this cohort of TIA patients may be in a pre-clinical dementia state.

This study has several potential limitations. It is possible that increased use of antihypertensive medications in the TIA group was partially responsible for a greater reduction in the CBF in TIA patients. However, the TIA cohort had marginally higher systolic BP than the controls which would not likely contribute to dysregulation of the cerebral perfusion pressure in the TIA subjects. In cases of significant systemic hypotension, the reduction in CBF would likely be global and not regional. Another potential limitation is the use of CBF maps generated directly from the MR scanner might have led to partial volume averaging. However, the effect of partial volume averaging was mitigated by obtaining CBF metrics from prespecified anatomical regions and adjusting for the regional anatomical volume in our multiple linear regression analyses. Furthermore, the post-labeling delay greater than 2 s used in this study might explain delayed arrival time in patients with chronic atherosclerosis. However, in this cohort, none of the patients showed significant intracranial stenosis and therefore, we do not think

Table 5

Multiple linear regression to analyze the associations between regional cerebral blood flow (mL/min/100 g) and cognitive scores while adjusting for group and demographics (control vs. TIA, age, and sex). Only regions that were found to be significantly associated with TIA status (Table 4) were analyzed. A) left entorhinal cortex, B) right posterior cingulate, C) right precuneus.

A) Left Entorhinal Cortex			
Cognitive Test	Beta Coefficient	Standard Error	p
BVMT Total	0.0902	0.0928	0.33
RAVLT	-0.0106	0.0471	0.82
TMT A	-0.247	0.180	0.17
TMT B	0.006	0.490	0.99
B) Right Posterior Cingulate			
Cognitive Test	Beta Coefficient	Standard Error	p
BVMT Total	0.113	0.0565	0.04
RAVLT	0.0474	0.0307	0.12
TMT A	-0.249	0.120	0.04
TMT B	-0.303	0.328	0.35
C) Right Precuneus			
Cognitive Test	Beta Coefficient	Standard Error	p
BVMT Total	0.0956	0.0482	0.05
RAVLT	0.0418	0.0270	0.13
TMT A	-0.258	0.0945	0.01
TMT B	-0.312	0.287	0.28

BVMT = Brief Visuospatial Memory Test, RAVLT = Rey Auditory Verbal Learning Test, TMT = Trail Making Test.

this is contributory to our findings. We also appreciate that our approach was exploratory with respect to evaluating the relationships between TIA, CBF and cognitive performance that was motivated to help inform future studies about the potential relationships.

### 5. Conclusions

In summary, in this exploratory study we show that having a TIA is associated with multi-regional hypoperfusion, measured by ASL-MRI, in the left entorhinal cortex, right posterior cingulate and right precuneus, suggesting the existence of vascular dysregulation in regions known to

be affected in AD. In this cohort of TIA patients without vascular stenosis, the regions of hypoperfusion were not associated with the regional anatomical volume of these regions or presence of a DWI. Our study demonstrates an association between regional hypoperfusion in the right posterior cingulate and right precuneus with memory and processing speed domains. The causes and implications of regional hypoperfusion in patients presenting with TIA is currently unknown but the clinical significance on the future risk of dementia will need to be explored.

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## CRediT authorship contribution statement

**Meaghan Reid:** Resources, Data curation. **George S. Tadros:** Data curation. **Connor C. McDougall:** Funding acquisition. **Noah Reaume:** Resources. **Brooklyn McDougall:** Resources. **Rani Gupta Sah:** Conceptualization, Resources, Project administration. **Meng Wang:** Project administration. **Eric E. Smith:** Data curation. **Richard Frayne:** Conceptualization, Formal analysis. **Shelagh Coutts:** Conceptualization. **Tolulope Sajobi:** Conceptualization, Project administration. **R. Stewart Longman:** Data curation, Formal analysis, Methodology. **Christopher D. d'Esterre:** Data curation, Formal analysis, Methodology. **Philip Barber:** Conceptualization, Data curation.

## Declaration of Competing Interest

The Authors declare that there is no conflict of interest.

## Data availability

The clinical and post-imaging analysis data is publicly accessible (10.5683/SP3/QC057T). Preprocessed imaging is available by contacting the corresponding author.

## References

- [1] S. Tariq, P.A. Barber, Dementia risk and prevention by targeting modifiable vascular risk factors, *J. Neurochem.* 144 (2018) 565–581, <https://doi.org/10.1111/jnc.14132>.
- [2] A. Ganesh, P.A. Barber, The cognitive sequelae of transient ischemic attacks—recent insights and future directions, *J. Clin. Med.* 11 (2022) 2637, <https://doi.org/10.3390/jcm11092637>.
- [3] S.T. Pendlebury, P.M. Rothwell, Incidence and prevalence of dementia associated with transient ischaemic attack and stroke: analysis of the population-based Oxford Vascular Study, *Lancet Neurol.* 18 (2019) 248–258, [https://doi.org/10.1016/S1474-4422\(18\)30442-3](https://doi.org/10.1016/S1474-4422(18)30442-3).
- [4] C.R. Jack, D.A. Bennett, K. Blennow, M.C. Carrillo, B. Dunn, S.B. Haerlein, et al., NIA-AA Research Framework: toward a biological definition of Alzheimer's disease, *Alzheimer's Dement.* 14 (2018) 535–562, <https://doi.org/10.1016/j.jalz.2018.02.018>.
- [5] S. Tariq, A. Tsang, M. Wang, N. Reaume, H. Carlson, T.T. Sajobi, et al., White matter tract microstructure and cognitive performance after transient ischemic attack, *PLoS One* 15 (2020), e0239116, <https://doi.org/10.1371/journal.pone.0239116>.
- [6] S. Villeneuve, M. Wirth, R La Joie, Are AD-typical regions the convergence point of multiple pathologies? *Front. Aging Neurosci.* 7 (2015) 42, <https://doi.org/10.3389/fnagi.2015.00042>.
- [7] A. Badhwar, A. Tam, C. Dansereau, P. Orban, F. Hoffstaedter, P. Bellec, Resting-state network dysfunction in Alzheimer's disease: a systematic review and meta-analysis, *Alzheimer's Dement.* 8 (2017) 73–85, <https://doi.org/10.1016/j.dadm.2017.03.007>.
- [8] Y. Chen, D.A. Wolk, J.S. Reddin, M. Korczykowski, P.M. Martinez, E.S. Musiek, et al., Voxel-level comparison of arterial spin-labeled perfusion MRI and FDG-PET in Alzheimer disease, *Neurology* 77 (2011) 1977–1985, <https://doi.org/10.1212/WNL.0b013e31823a0ef7>.
- [9] L.L. Chao, S.T. Buckley, J. Kornak, N. Schuff, C. Madison, K. Yaffe, et al., ASL perfusion MRI predicts cognitive decline and conversion from MCI to dementia, *Alzheimer Dis. Assoc. Disord.* 24 (2010) 19–27, <https://doi.org/10.1097/WAD.0b013e3181b4f736>.
- [10] A.E. Leeuwis, M.R. Benedictus, J.P.A. Kuijer, M.A.A. Binnewijzend, A. M. Hooghiemstra, S.C.J. Verfaillie, et al., Lower cerebral blood flow is associated with impairment in multiple cognitive domains in Alzheimer's disease, *Alzheimer's Dement.* 13 (2017) 531–540, <https://doi.org/10.1016/j.jalz.2016.08.013>.
- [11] S. Tariq, C.D. d'Esterre, T.T. Sajobi, E.E. Smith, R.S. Longman, R. Frayne, et al., A longitudinal magnetic resonance imaging study of neurodegenerative and small vessel disease, and clinical cognitive trajectories in non demented patients with transient ischemic attack: the PREVENT study, *BMC Geriatr.* 18 (2018) 163, <https://doi.org/10.1186/s12877-018-0858-4>.
- [12] A classification and outline of cerebrovascular diseases. II, *Stroke* 6 (1975) 564–616, <https://doi.org/10.1161/01.str.6.5.564>.
- [13] M.F. Folstein, S.E. Folstein, P.R. McHugh, Mini-mental state". A practical method for grading the cognitive state of patients for the clinician, *J. Psychiatr. Res.* 12 (1975) 189–198, [https://doi.org/10.1016/0022-3956\(75\)90026-6](https://doi.org/10.1016/0022-3956(75)90026-6).
- [14] C.P. Hughes, L. Berg, W.L. Danziger, L.A. Coben, R.L. Martin, A new clinical scale for the staging of dementia, *Br. J. Psychiatry* 140 (1982) 566–572, <https://doi.org/10.1192/bjp.140.6.566>.
- [15] T.L. Brink, J.A. Yesavage, O. Lum, P.H. Heersema, M. Adey, T.L. Rose, Screening tests for geriatric depression, *Clin. Gerontol.* 1 (1982) 37–43, [https://doi.org/10.1300/J018v01n01\\_06](https://doi.org/10.1300/J018v01n01_06).
- [16] D.A. Regier, E.A. Kuhl, D.J. Kupfer, The DSM-5: classification and criteria changes, *World Psychiatry* 12 (2013) 92–98, <https://doi.org/10.1002/wps.20050>.
- [17] M.W. Bernadt, J. Mumford, C. Taylor, B. Smith, R.M. Murray, Comparison of questionnaire and laboratory tests in the detection of excessive drinking and alcoholism, *Lancet* 1 (1982) 325–328, [https://doi.org/10.1016/s0140-6736\(82\)91579-3](https://doi.org/10.1016/s0140-6736(82)91579-3).
- [18] P.W. Wilson, R.B. D'Agostino, D. Levy, A.M. Belanger, H. Silbershatz, W.B. Kannel, Prediction of coronary heart disease using risk factor categories, *Circulation* 97 (1998) 1837–1847, <https://doi.org/10.1161/01.cir.97.18.1837>.
- [19] H.J. Mutsaerts, J. Petr, L. Václavů, J.W. van Dalen, A.D. Robertson, M.W. Caan, et al., The spatial coefficient of variation in arterial spin labeling cerebral blood flow images, *J. Cereb. Blood Flow Metab.* 37 (2017) 3184–3192, <https://doi.org/10.1177/0271678X16683690>.
- [20] H.J.M.M. Mutsaerts, S.S. Mirza, J. Petr, D.L. Thomas, D.M. Cash, M. Bocchetta, et al., Cerebral perfusion changes in presymptomatic genetic frontotemporal dementia: a GENFI study, *Brain* 142 (2019) 1108–1120, <https://doi.org/10.1093/brain/awz039>.
- [21] M. Jenkinson, C.F. Beckmann, T.E.J. Behrens, M.W. Woolrich, S.M. FSL Smith, *NeuroImage* 62 (2012) 782–790, <https://doi.org/10.1016/j.neuroimage.2011.09.015>.
- [22] Y. Zhang, M. Brady, S. Smith, Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm, *IEEE Trans. Med. Imaging* 20 (2001) 45–57, <https://doi.org/10.1109/42.906424>.
- [23] B. Patenaude, S.M. Smith, D.N. Kennedy, M. Jenkinson, A Bayesian model of shape and appearance for subcortical brain segmentation, *NeuroImage* 56 (2011) 907–922, <https://doi.org/10.1016/j.neuroimage.2011.02.046>.
- [24] R.S. Desikan, F. Ségonne, B. Fischl, B.T. Quinn, B.C. Dickerson, D. Blacker, et al., An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest, *NeuroImage* 31 (2006) 968–980, <https://doi.org/10.1016/j.neuroimage.2006.01.021>.
- [25] S.M. Smith, Y. Zhang, M. Jenkinson, J. Chen, P.M. Matthews, A. Federico, et al., Accurate, robust, and automated longitudinal and cross-sectional brain change analysis, *NeuroImage* 17 (2002) 479–489, <https://doi.org/10.1006/nimg.2002.1040>.
- [26] P.A. Yushkevich, J. Piven, H.C. Hazlett, R.G. Smith, S. Ho, J.C. Gee, et al., User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability, *NeuroImage* 31 (2006) 1116–1128, <https://doi.org/10.1016/j.neuroimage.2006.01.015>.
- [27] Y. Shi, M.J. Thrippleton, S.D. Makin, I. Marshall, M.I. Geerlings, A.J.M. de Craen, et al., Cerebral blood flow in small vessel disease: a systematic review and meta-analysis, *J. Cereb. Blood Flow Metab.* 36 (2016) 1653–1667, <https://doi.org/10.1177/0271678X16662891>.
- [28] F.J. Wolters, H.I. Zonneveld, A. Hofman, A. van der Lugt, P.J. Koudstaal, M. W. Vernooij, et al., Cerebral perfusion and the risk of dementia: a population-based study, *Circulation* 136 (2017) 719–728, <https://doi.org/10.1161/CIRCULATIONAHA.117.027448>.
- [29] A.M. Tuladhar, E. van Dijk, M.P. Zwiers, A.G.W. van Norden, K.F. de Laat, E. Shumskaya, et al., Structural network connectivity and cognition in cerebral small vessel disease, *Hum. Brain Mapp.* 37 (2016) 300–310, <https://doi.org/10.1002/hbm.23032>.