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Whole-Brain Atrophy Rates, Regional Cerebral Blood Flow, and Cognitive Profiles of Transient Ischemic Attack Patients and Controls

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Whole-Brain Atrophy Rates, Regional Cerebral Blood Flow, and Cognitive Profiles of Transient Ischemic Attack Patients and Controls

by

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Abstract

Dementia is one of the most common causes of disability amongst the old and the prevalence is expected to double within the next twenty years. Recent prevention trials have failed to find a cure, likely due to inappropriate trial selection and a lack of reliable outcome measurements. Standardized clinical, demographic, imaging and neuropsychological biomarkers will improve selection criteria and therapeutic interventions. Transient ischemic attack (TIA) patients are at an increased risk of late-life cognitive decline due to their common vascular risk factors with dementia and underlying cerebrovascular pathology. We hypothesized that TIA patients would have increased longitudinal rates of cerebral atrophy as measured by T1 magnetic resonance (MR) imaging compared to non-TIA controls over 1 year and that increased cerebral atrophy rates would be associated with poorer cognitive outcomes. Secondly, we hypothesized that at baseline TIA patients would have lower regional cerebral blood flow (CBF) as measured by arterial spin labelled (ASL) MR imaging compared to non-TIA controls, and that CBF would be associated with cognition. Our results suggest that TIA patients show almost double the cerebral atrophy rates of non-TIA controls over 1-year, and in the absence of demonstrated change in cognition, supports that these subjects with TIA are in a preclinical stage of cognitive decline. Our results also show that TIA patients have reduced CBF in the left entorhinal cortex, the posterior cingulate bilaterally and the right precuneus which was associated with poorer memory outcomes. These predictors of early neurodegeneration and vascular changes show that TIA patients are a high-risk population for dementia and could improve inclusion criteria for clinical trials to *prevent* dementia in the future.

Preface

This thesis begins with an introductory chapter highlighting the concepts that will be discussed in manuscript style Chapters 2 and 3. Chapter 4 provides a conclusion of the thesis and highlights the important contributions of this thesis to the literature of late-life cognitive decline and prevention.

Chapter 1 is original, unpublished, independent work by the author, M. Reid. Chapter 2 examines the associations between longitudinal cerebral atrophy rates and cognition function between TIA patients and non-TIA controls. Chapter 2 uses longitudinal data collected from the PREVENT Study. Chapter 2 has been submitted to *Neurology*. Chapter 3 examines the relationship between cross-sectional regional cerebral blood flow and cognition in TIA patients and non-TIA controls. Chapter 3 uses cross-sectional data collected from the PREVENT Study.

Chapters 2 and 3 begin with a preface to outline the roles of each co-author in the research.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

$A\beta_{1-42}$	Amyloid-beta 1-42
ACE-R	Addenbrooke's Cognitive Assessment-Revised
AD	Alzheimer's Disease
ADNI	Alzheimer's Disease Neuroimaging Initiative
AH	Antihypertensive
APOE	Apolipoprotein E
ASL	Arterial Spin Labelling
AUC	Area Under Curve
BP	Blood Pressure
BL	Baseline
BVMT-R	Brief Visuospatial Memory Test-Revised
CBF	Cerebral Blood Flow
CES-D	Center for Epidemiologic Studies Depression Scale
CoV	Spatial Coefficient of Variation
CSF	Cerebrospinal fluid
CVD	Cerebrovascular disease
DS	Digit Symbol Coding
DSM	Diagnostic and Statistical Manual of Mental Disorders
DTI	Diffusion Tensor Imaging
DWI	Diffusion Weighted Imaging
ECD	Ethyl Cysteinate Dimer
FDG	^{18}F-fluorodeoxyglucose
FINGER	Finnish Geriatric Interventions to Prevent Cognitive Impairment and Disability
FLAIR	Fluid-Attenuated Inversion Recovery
FOV	Field of View
FU	Follow-Up
GM	Grey Matter
HATICE	Healthy Aging Through Internet Counselling in the Elderly
HYVET-COG	Hypertension in the Very Elderly Trial
IQR	Interquartile Range
MAPT	Multidomain Alzheimer Prevention Trial
MCI	Mild Cognitive Impairment
MMSE	Mini-Mental State Examination
MOCA	Montreal Cognitive Assessment
MR	Magnetic Resonance
MRC CFAS	Medical Research Council Cognitive Function and Ageing Study
NAART	National American Adult Reading Test
NBV	Normalized Brain Volume
NTB	Neuropsychological Test Battery
PET	Positron Emission Tomography
PiB	Pittsburg Compound B
PreDIVA	Prevention of Dementia by Intensive Vascular care

PREVENT	Predementia Neuroimaging of Transient Ischemic Attack Study
PROGRESS	Perindopril Protection Against Recurrent Stroke Study
P-tau	Phosphor-tau
QSM	Quantitative Susceptibility Mapping
RCTs	Randomized Controlled Trials
ROI	Region of Interest
SCOPE	Study on Cognition and Prognosis in the Elderly
SD	Standard Deviation
SPECT	Single-Photon Emission Computed Tomography
SYS-EUR	Systolic Hypertension in Europe trial
TE	Echo Time
TI	Inversion Time
TIA	Transient Ischemic Attack
TR	Repetition Time
TMT	Trail Making Test
T-tau	Total-tau
VaD	Vascular Dementia
VCI	Vascular Cognitive Impairment
RAVLT	Rey Auditory Verbal Learning Test
WAIS	Wechsler Adult Intelligence Scale
WMH	White Matter Hyperintensities

CHAPTER ONE: THE RELATIONSHIP BETWEEN VASCULAR RISK FACTORS AND DEMENTIA

1.1 Preface

Approximately 500 000 Canadians are living with dementia and the prevalence is expected to double in the next 20 years, which will increase social, personal and economic burden. Dementia related to Alzheimer's Disease (AD) is caused by progressive neuronal loss leading to brain atrophy, which presents clinically as progressive disruption in various cognitive domains, including memory. The progressive pre-clinical phase of dementia may span more than twenty years, and is related to the co-existence of vascular and life-style risk factors in mid-life. In the absence of a cure, it is crucial to identify high risk persons and implement precision dementia prevention strategies. Fundamentally, the aim should be to reduce microscopic brain tissue loss through the modification of vascular and lifestyle risk factors in mid-life and therefore optimize the opportunity to prevent dementia in the future.

1.2 Introduction

Dementia is a persistent progressive disease related to well described pathological neurodegenerative or vascular disorders which can be associated with cognitive impairment involving memory, executive function, language, behavior, personality and sensorimotor functions (1). Dementia is a major cause of disability and dependency in the elderly and is associated with personal, social and economic burden. Currently, it costs $10.4 billion (CAD) annually to take care of the 500 000 Canadians living with dementia (2). The cost for people with dementia is estimated to be almost six times greater than for healthy individuals of the same demographics (2). As the mean age of our population continues to increase, the prevalence of dementia is expected to double within 15 years (2). Over $800 billion per year globally is spent on caring with those with

dementia, rising to $2 trillion by 2030 (3). Health-care systems are in danger of becoming overwhelmed by the future costs of caring for those with dementia (4). While complete prevention of all dementias is not feasible, pushing back the age of delay in onset of 1 year could prevent more than 9 million cases of dementia globally by 2050 and delaying onset by 5 years could more than halve the prevalence of global dementia (5, 6). Until the past two decades, delaying or preventing dementia was deemed impossible (7, 8). A promising new era in dementia prevention has arrived as we now have begun to understand the complex pathological processes and associated risk factors of the disease.

The progressive pre-clinical phase of dementia may span more than twenty years, and in the absence of a cure, prevention in mid-life is a promising target to counteract the dementia epidemic (9,10). Late life cognitive impairment is commonly caused by progressive neuronal loss related to two dominant disease entities, AD and small vessel cerebrovascular disease (CVD). The early identification of people at risk for cognitive impairment is potentially the single most important factor to implement mid-life lifestyle and vascular risk reduction strategies for the prevention or postponement of dementia (9, 11). Traditionally, the dementia syndrome is subdivided in late-life into the neurodegenerative types, including AD and vascular dementia (VaD), but the commonest cause is mixed dementia (AD with vascular disease). Although there is vast and ongoing literature exploring the dichotomy between AD and VaD, they frequently co-exist and share common interactions related to the cardiovascular risk factors supporting the concept that they are closely related disorders (12–17).

1.3 Pathogenesis of Dementias

Dementia is a complex progressive manifestation of symptoms that involves two distinct disease processes, Alzheimer's Disease (AD) and cerebrovascular disease (CVD), which

contribute to cerebral atrophy years before clinical symptoms are detected (1, 9, 18–23). The DSM-5 has redefined dementia as major/minor Neurocognitive Disorder and differentiates between vascular and AD types (24). The use of brain imaging and specific disease related biomarkers have the capacity to not only support the diagnosis, but also help predict progression, and can potentially monitor disease and therapeutic response to secondary prevention approaches (24).

1.3.1 Alzheimer's Disease

Alzheimer's disease is a progressive neurodegenerative disease that is characterized pathologically by the accumulation of amyloid plaques and neurofibrillary tangles consisting of phosphorylated Tau protein, a neuronal marker of degeneration (12). There are several differing hypotheses regarding the possible pathogenesis of the disease, the most prominent being the amyloid cascade hypothesis in which there is abnormal accumulation of the misfolded amyloid beta$_{1\text{-}42}$ (A$\beta_{1\text{-}42}$) which leads to neuronal loss and depletion of neurotransmitters causing cognitive symptoms (25, 26). The selective toxicity of the amyloid protein initially affects the neurons in the hippocampus and entorhinal cortex, eventually spreading to the neocortex contributing to global brain atrophy (27, 28)

The neuropathology of AD is complex, but it has been established that AD patients have significant medial temporal lobe atrophy, including early disproportionate atrophy of the hippocampi and progressive neuronal loss compared to age-matched controls using quantitative structural magnetic resonance imaging (MR) imaging (29–32). This is consistent with the clinical presentation of the disease, as declarative episodic memory depends heavily on the hippocampus and other medial temporal lobe structures. Patients with AD present with a combination of insidious memory loss and focal cognitive dysfunction, while motor skills are relatively persevered

(13). A clinical diagnosis of probable AD requires evidence of memory loss (with episodic memory being the most affected), and impairment of at least one other cognitive domain (13). Furthermore, whole brain cerebral blood flow is reduced 20-40% in AD patients (33, 34). Hypoperfusion is consistently observed in the posterior cingulate, precuneus, inferior parietal and lateral prefrontal cortices in early AD (35–37). The posterior cingulate is integral to the limbic system and the precuneus is involved with episodic memory and visuospatial processing, which are consistent with our understanding of AD pathology. Decreased regional cerebral blood flow (CBF) and glucose metabolism are tightly coupled, are both associated with amyloid deposition, and have proven to identify similar regional abnormalities in AD patients (16, 38, 39). While researchers continue to debate the causes and progression of AD, it has been established that cerebrovascular disease is a frequent comorbidity.

Mild cognitive impairment (MCI) is a poorly standardized syndrome (not a diagnosis) defined by objective cognitive disturbance but not fulfilling criteria for dementia, and are considered at higher risk of developing AD. Persons with MCI may experience memory, attention and concentration problems at a higher level than is expected for their age and education, however their symptoms do not obviously interfere with daily life. Thus, it may be difficult to distinguish MCI from common mood-related disorders. It is estimated that 20-50% of those with MCI go on to develop AD within 5 years (40). A limitation with 'MCI' is that while the term has existed for three decades, it is not standardized and about 40% of individuals either do not progress to AD, or have cognitive symptoms that improve (40). Therefore, MCI is heterogeneous and has provided a useful construct for prodromal dementia but the non-standardized operational criteria makes it potentially unreliable and impractical for discriminating individuals at higher risk of developing Alzheimer's disease.

1.3.2 Vascular Dementia

Vascular dementia (VaD) can present as an abrupt deterioration of cognitive function following a stroke(s) or extensive periventricular ischemic white-matter disease (13). VaD is defined as cognitive impairment in two or more areas of cognitive domain with impaired activities of daily living (41–43). Vascular cognitive impairment (VCI) is also caused or associated with CVD and is defined as cognitive deficits in at least one cognitive domain without impaired activities of daily living (42, 43). Similar to MCI, VCI can eventually progress to AD/VaD (44). Strategic infarction can cause ischemic damage in strategic regions (e.g. thalamus) that can disrupt brain circuits that are essential for memory and cognition and cause significant cognitive deficits (45). These neurological events often produce a specific cognitive loss (e.g. mild aphasia after left parietal infarct) and repeated events cause progressive incapacitation (46). Multi-infarct dementia is characterized by multiple cortical infarcts in the cortex or subcortical areas (45). The reduction of functional brain capacity in multi-infarct VaD varies significantly depending on the size, location and number of ischemic events. A third subtype of VaD, cerebral small vessel disease, comprises of white matter lesions from lacunar infarcts, demyelination and gliosis (46). White matter lesions are common in elderly individuals and a higher burden of lesions can produce abnormal cognitive changes as the neuronal connections in the basal ganglia and ascending brainstem pathways become disrupted (46). Subsequently, this results in slowed mental processes, problems with decision making and apathy, amongst other symptoms (46). The different subtypes of VaD are frequently heterogeneous and complex; thus, the exact frequencies and mechanisms of sub-type VaD pathologies are unclear. Pure VaD is rare and estimated to account for only <5% of dementias (45, 47). As such, it is difficult to find a single definition for VaD that is operationally relevant, as well as specific, and it is unhelpful to classify a clear distinction between AD and VaD

in most cases.

1.3.3 Mixed Dementia

Mixed dementia recognizes the heterogeneity of dementias, and is classified as the coexistence of AD and CVD (1). While it is difficult to estimate, it is proposed that approximately 40-60% of dementias may be classified as mixed dementia, making it the commonest form of dementia (13). Population based autopsies suggest that vascular and neurodegenerative pathology frequently co-exist in older people with dementia, likely due to the relationship between CVD and AD (48). The prospective incidence OxVasc Study (2019) found that the 1-year standardized morbidity ratio for the incidence of dementia was 47.3 for major stroke, 5.8 for minor stroke and 4.0 for transient ischemic attack (TIA) (49). The Nun Study demonstrated that in participants with AD, those with microinfarcts had a 20-fold increase in developing dementia and poorer cognition compared to those without clinical infarcts (50).

White matter hyperintensities (WMH) are a consequence of cerebral small vessel disease and increase in severity and prevalence with age, arterial hypertension and cardiovascular disease (51–54). These lesions have multiple histopathological correlates, such as ependymal loss, cerebral ischemia, demyelination, microcystic infarcts, and gliosis (55, 56). They are considered a hallmark of CVD (VaD) and are also associated with a higher risk of progression to dementia (48, 57). Barnes et al. (2013) found that increased WMH burden and decreased cerebral spinal fluid $A\beta_{1\text{-}42}$ levels were independently associated with higher longitudinal brain volume loss in healthy control subjects, but not MCI or AD patients, likely due to different phases of disease progression (58). Therefore, the burden of vascular and AD-type pathologies are likely to be interdependent and the leading causes of dementia in the elderly (44, 59). Small-vessel disease, infarcts, and the presence

of more than one vascular pathological change are associated with dementia in the younger old (60). AD pathology, including neuritic plaques in the hippocampus/neocortex and neurofibrillary tangles in the hippocampus, is less predictive of the cognitive profile of the very old (60). Therefore, vascular treatments may be best targeted for younger cohorts as they may be less effective in the oldest old (60). Further, the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS) showed that cortical atrophy was a better correlate of dementia than classical AD-type lesions (61). Consequently, neuroimaging markers of disease progression may be more sensitive than classic AD pathology.

There are numerous theories on the mechanisms responsible for the association between CVD and AD. The Nun study postulates that the combined AD/CVD pathology accelerates the manifestation of dementia due to a reduction of a pathological threshold (50). It should be noted that post-stroke dementia could also be the co-existing result of an acute vascular insult and pre-existing AD (62). There are also preliminary reports that incipient vascular disease abnormalities may initiate early neurodegenerative disease in mid-life (63). Hypertension is recognized as the most consistent risk factor for both stroke and dementia (64–69). High blood pressure is associated with increased neurocognitive loss approximately 15-20 years later in life (70). Hypertension has also been found to contribute to brain volume loss, as systolic blood pressure and pulse pressure are associated with medial temporal atrophy in individuals with late-onset dementia (71) It is hypothesized that chronic hypertension may dysregulate the CBF system and lead to cerebral hypoperfusion (64). Cerebral hypoperfusion caused by CVD may result in a reduced capability to eliminate the amyloid protein, increase hypoxia leading to inflammation and white matter damage, and limit mechanisms of angiogenesis (17, 72). Hypoperfusion may also accelerate hyperphosphylation of tau resulting in accelerated neurofibrillary plaque accumulation (72).

Hypertension-related cerebral hypoperfusion can diminish cerebral metabolism and has even been shown to have direct negative cognitive effects in a rat model (73–75). Reduced cerebral blood flow is linked to deranged cerebral glucose homeostasis which is a hallmark of AD (74–77). Furthermore, oxidative stress may lead to mitochondrial dysfunction and hippocampal neurodegeneration and plasticity loss (72).

1.3.4 Transient Ischemic Attack and Cognitive Decline

TIA is a common medical emergency and is defined clinically temporary episode of neurological dysfunction due to an obstruction in the carotid artery, vertebral artery or cerebral small vessels causing transient cerebral ischemia (78). A TIA is currently defined by a combination of radiological and clinical criteria; transient focal brain hypoperfusion that is spontaneously resolved, no/very mild infarction, with disrupted cerebral function lasting <24 hours (79). TIAs are frequently misdiagnosed or not medically identified because patients and physicians alike fail to recognize the severity of the transient symptoms. TIAs should be treated as a medical emergency as approximately 15% of diagnosed strokes are preceded by them, and up to 80% of strokes after TIA are preventable (79, 80).

Levine et al. (2015) studied over 23 000 participants in a prospective study over 6 years and found that incident stroke was associated with acute decline in cognitive function and also accelerated and persistent cognitive decline (81). Furthermore, within 3-months of TIA symptoms, a third of patients have documented impairment of at least one cognitive domain, including working memory, attention, and information processing speed, which is not fully explained by clinical brain infarcts (82). Sachdev et al. (2014) concluded that TIA/stroke patients have rates of incident dementia of 5.9% per year compared to 0.4% in healthy controls (83). However, the

population criteria for stroke/TIA and DWI volumes were not described (83). The Oxford Vascular Study found that TIA patients have a 4-fold increased risk of late-life dementia, however the relationship between TIA and late-life cognitive impairment remains poorly understood (49, 84). We do know that cognitive decline and stroke share many common risk factors, and in a population attribution risk calculation, 8.4% of dementia incidence could be eliminated by preventing stroke and TIA (1). In a recent prospective observation study (Bivard et al., 2018), patients with TIA experienced a significant reduction in global gray matter and structural atrophy related to the area of ischemia) after 3 months, and increasing atrophy resulted in a proportional decline in cognitive performance measured by the Montreal Cognitive Assessment test (MOCA) (85). However, 28% of the 'TIA' participants had significantly large computed tomography perfusion deficits and DWI lesion volume was not reported (85). Further, the location of atrophy was partly dependent on the acutely affected circulation (85). Thus, in this study the detection of significant atrophy following only a period of 3-months in this study was likely due to infarction caused acute atrophy following a minor stroke. Walters et al. (2003) found that patients with TIA had more than twice the rate of global brain atrophy as age matched controls over the subsequent year suggesting atrophy is a possible early marker of the development of cognitive impairment over a longer follow-up (86). In a recent three-year longitudinal prospective study (Munir et al. 2019), TIA and minor stroke patients experienced a significantly higher rate of whole brain atrophy, although there were only modest associations with brain atrophy rates and episodic memory (87). If neurodegenerative changes precede cognitive symptoms, this study supports that TIA patients are an optimal population to investigate in mid-life in the pursuit of an early therapeutic window that would aim to prevent late-life cognitive decline (87).

TIA patients are an excellent population to observe with advanced neuroimaging in a longitudinal cognitive study because they have vascular risk factors that are common with dementias and do not suffer from disability following their ischemic event. TIA patients are generally younger (49, 88) and in better physical condition than stroke patients, facilitating the acquisition of detailed serial cognitive tests and avoid high attrition rates caused by stroke related co-morbidity and frailty (83, 89). Longitudinal prospective studies following TIA patients, such as the Predementia Neuroimaging of Transient Ischemic Attack (PREVENT), are urgently needed to identify microstructural and functional cerebrovascular damage through neuroimaging to understand the cause of cognitive decline. If successful, we will have the capability to understand modifiable risk factors in a TIA population and we will have the potential to reduce their risk of cognitive decline in the future.

1.4 Risk Factors for Late-Life Cognitive Decline

It is well established that an interaction between genetic and environmental factors contribute to the pathological consequences that lead to cognitive decline. For example, the detection of AD neuropathology is present in approximately one third of elderly people without dementia and cognitive impairment (90). Furthermore, an atrophy study on a population over ninety years of age demonstrated that half of people with dementia did not have sufficient neuropathology to account for their cognitive decline (91). These participants may show less impairment than expected due to protective compensatory factors, including higher education and cardiovascular health. Genetic factors are estimated to only account for 50% of the risk of developing AD (29). Individuals with two ApoE μ4 alleles are 3-7x more likely to develop AD compared to those with ApoE μ3 alleles (92). Worldwide, the highest estimated population attributable risk for AD is low education attainment (19.1%) and physical inactivity (20.0%) (93).

Moreover, a third of late-life dementia may be attributed to seven modifiable risk factors: low education, mid-life hypertension, mid-life obesity, diabetes, physical inactivity and depression (15). A reduction of these risk factors by 10-20% per decade is estimated to reduce worldwide prevalence of AD by 8-50% in 2050 (14). Chronic exposure to vascular risk factors can contribute to endothelial dysfunction, vessel wall thickening and perivascular drainage that can potentially lead to chronic hypoperfusion and oxidative stress, accelerating the hyperphosphylation of tau (48, 90). However, placebo controlled trials on the effect of antihypertensive treatment on dementia have had largely mixed results (94–99) as summarized in Table 1-1. For example. the Perindopril Protection Against Recurrent Stroke Study (PROGRESS), aimed to analyze the effect of an ACE inhibitor antihypertensive on longitudinal cognitive decline and dementia in a population with CVD (98). However, they found no clear effect of treatment on the overall risk of dementia (98). Reportedly, the risk of dementia related to recurrent stroke was reduced by one third which suggests that the benefits of treatment were primarily the consequences of stroke prevention, rather than a direct effect on cognition (98).

Table 1-1. Summary of data from placebo controlled trials on the effect of antihypertensive (AH) treatment on dementia. (short-CARE: short-Comprehensive Assessment and Referral Evaluation; PALT: Paired Associate Learning Test; TMT: Trail Making Test A; MHIS - Modified Hachinski Ischemic Score; WMH: White Matter Hyperintensities). Adapted with permission from Sana Tariq.

Trials	SHEP (94)	MRC Hypertension Study (95)	SYST-EUR (96)	HYVET-COG (97)	PROGRESS (98)	SCOPE (99)
Sample Size	4736	2584	2418	3336	6105	4964
Age	≥60	65-74	≥60	≥80	≥64	70-89
Follow-Up Period	5 years	4.5 years	2 years	2.2 years	3.9 years	3.7 years
Design	Double-blind	Single-blind	Double-blind	Double-blind	Double-blind	Double-blind
Cognitive Assessment	shore-CARE	PALT and TMT tests	MMSE	MMSE	MMSE	MMSE
Biomarkers	None	None	Modified ischemic score with brain imaging of MHIS	CT Scan and MHIS	WMH Imaging	None
Outcome	Incidence of dementia	Cognitive impairment	AD, VaD or mixed dementia	AD, VaD	Secondary outcome: dementia and cognitive decline	Secondary outcome: dementia and cognitive decline
Result	Non-significant reduction in dementia in treatment group by 16%	Non-significant effect on cognitive function	Reduced dementia risk by 50% (7.7 to 3.8) cases per 1000 patient-years; p=0.05	Non-significant reduction in incidence of dementia	Reduction in cognitive decline by 19% (95% CI 4-32%; p=0.01); reduction of dementia with recurrent stroke of 34% (95% CI 3-55%; p=0.03)	Non-significant effect on cognitive decline

Recent studies have attempted to target multiple vascular risk factors, including diet, exercise, cognitive training and vascular risk monitoring to improve or maintain cognitive function in at risk elderly people from the general population (94–99) as summarized in Table 1-2. The Finnish Geriatric Interventions to Prevent Cognitive Impairment and Disability (FINGER) study, enrolled individuals between the ages of 60-77 in a double-blind randomized controlled trial to analyze the effects of modifiable vascular and life-style risk therapies to prevent cognitive decline in at-risk elderly people (100). A two-year multidomain intervention was measured by the primary outcome; change in cognition through the comprehensive neuropsychological test battery (NTB). The NTB included the Wechsler Memory Scale visual immediate, the Wechsler Memory Scale verbal immediate, the Rey Auditory Verbal Learning Test (RAVLT) immediate, the Wechsler Memory Digit Span, the Controlled Word Association Test, the Category Fluency Test, the Wechsler Memory Scale visual delayed, the Wechsler Memory Scale verbal delayed, and RAVLT delayed (101). The FINGER Study found that this multidomain approach improved/maintained cognitive functioning in the elderly over 2 years (100). While the improvement was significantly greater in the intervention group, the control group also demonstrated cognitive improvement which may be a result of practice effects of repeated cognitive testing (102). However, further large randomized placebo controlled trials, such as the Prevention of Dementia by Intensive Vascular Care (pre-DIVA) failed to find an effect, calling attention to several methodological issues within current dementia trials (103).

Table 1-2. Summary of past and current multi-domain studies, and their outcomes and results. (FDG-PET: ^{18}F-fluorodeoxyglucose Positron Emission Tomography; ALDS: Academic medical center Linear Disability Score; APOE: Apolipoprotein E). Adapted with permission from Sana Tariq.

Trials	FINGER (100)	MAPT (104)	preDIVA (103)	HATICE (105)
Sample Size	1260	1680	3526	4600
Age	60-77	≥70	70-78	≥65
Follow-Up Period	2 years	3 years	6 years	1.5 years
Design	Double-blind placebo controlled	Placebo controlled	Open-label cluster-randomized controlled	Double-blind placebo controlled
Biomarkers	None	FDG PET scan (cerebral metabolism), MRI (atrophy rate), AV45 PET scan (brain amyloid deposit)	APOE genotyping	None
Outcome	Cognitive impairment with NTB(101)	Memory impairment with FCRST(106), battery	Dementia and disability score with ALDS (107)	1°: systolic blood pressure, LDL, BMI 2°: includes cognitive functioning with STROOP, AVLT, verbal fluency
Result	Multidomain approach could improve or maintain cognitive function in at-risk elderly	Multidomain approach leads to slowing of cognitive decline in older adults	Multidomain intervention did not reduce incidence of all-cause dementia in older adults	Pre-results: relationship with online cardiovascular coach qualitatively enhanced cardiovascular self-management (108)

Randomized controlled trials (RCTs) have failed to prove a single drug approach is effective in the prevention of dementia. Anti-hypertensive drugs represent the only exception, and

single life-style related interventions (e.g. physical activity and cognitive training) have shown only modest or short-term positive results (109, 110). RCTs must overcome ethical considerations as true double-blinded experiments would leave dangerous vascular risk factors untreated for many individuals. RCTs must also consider the importance of intervention timing in relation to disease onset, age and duration of the intervention (109). For example, in long-term population-based observation studies, hypotension is often observed in the years preceding AD whereas hypertension in mid-life has been linked to an increase of AD 20-30 years later (111). Most of these studies have overlooked fundamental challenges of attempting to address the efficacy of vascular reduction treatments on cognitive decline; special consideration needs to be given to identifying patients at highest risk of dementia since these patients are potentially most likely to benefit from vascular risk reduction, and the timing and type of intervention for optimal effectiveness (103). Further, detailed neuropsychological assessments and dementia biomarkers were limited or not included whatsoever. Future trials should continue to improve study methodology with large, longitudinal studies that include biomarkers and multi-domain interventions. Effects should be easily measurable and have a set definition; measuring changes in cognitive performance may capture subtle decline and therefore might be a more sensitive outcome than conversion from MCI to dementia. Furthermore, individuals at a high risk of dementia in the crucial pre-clinical window should be targeted. This study is crucial to facilitate optimal RCT design in the future.

It has become apparent that disease-modifying drugs and interventional therapies have been unsuccessful, likely because the brain has already suffered irreversible neuronal loss by the clinical stage of dementia. The exploration of the protracted prodromal phase of dementia by

quantitative neuroimaging is essential to understand, and subsequently, to guide precision therapies in mid-life that will *prevent* dementia in late-life.

1.5 Preclinical Dementia Biomarkers

Despite a global effort to find therapies that delay or prevent onset of dementia, treatments have shown only modest benefit at best. This may reflect limitations of the treatments themselves or, more likely, inappropriate trial populations. Large trials have traditionally recruited patients with mild to moderate cognitive symptoms, in which the disease processes may be too advanced to realistically modify with intervention. Through the identification of subjects at-risk of dementia, secondary prevention may be feasible. Preclinical dementia refers to the stage of dementia in which the molecular pathology of AD is already present in the brain but is not yet clinically expressed. Individuals are asymptomatic by definition and cognitively normal (112). Research criteria propose the use of biomarkers to define pre-clinical dementia, even in the absence of cognitive impairment (113–117). Biomarkers for preclinical dementia must be highly reproducible, standardized, sensitive to change over time, capable of detecting treatment effects, and should be operationally straightforward to deploy in multi-centre studies. Further, biomarkers should have the capability to capture sensitive treatment effects and monitor disease progression over time (118).

1.5.1 Structural Imaging

Structural MR imaging provides in-vivo measurements of global and regional brain volumes. Cerebral atrophy describes the loss of neurons and neuronal connectivity in the brain. Increased rates of cerebral atrophy are expected in a healthy ageing population; however, many neurodegenerative diseases can cause abnormally high rates of atrophy. For example, cerebral

volume loss is estimated to be 2-3% in AD patients annually, compared to 1.1 % in MCI patients and 0.2-0.5% in healthy controls (119–123). MCI subjects who convert to AD have twice the annualized atrophy rates than that of MCI subjects who do not progress, 1.6% and 0.9%, respectively (124). Each additional percent of annualized whole-brain atrophy rates in MCI patients is associated with a 1.3x higher odds of disease progression (125). It is therefore possible to differentiate persons with dementia and those without dementia by measuring rapid rates of atrophy (126). Rates of brain atrophy are integral to biological pathways linked to preclinical progression of vascular disease and AD (27, 126–129). Rate of clinical progression has been shown to parallel whole brain atrophy, and as such, whole brain atrophy is frequently used as a disease biomarker for clinical trials. Brain atrophy rates are also highly sensitive at predicting cognitive decline and can be measured more precisely than neuropsychological outcomes, since cognitive tests can be confounded by many factors, including baseline cognitive performance, co-morbid factors, pharmacological treatments, ceiling and floor effects, and learning effects (130–134). Atrophy can be generalized (global) or focal; affecting only a limited area of brain resulting in specific functional losses.

Cerebral atrophy can be measured by multiple neuroimaging modalities, including magnetic resonance (MR) imaging and computed tomography (CT). High resolution T1-weighted MR imaging sequences are often used to detect cerebral atrophy because of the high structural contrast and high signal-to-noise per unit time of scanning. T1 refers to longitudinal relaxation time, which is the time constant that determines the rate at which excited protons return to equilibrium. T1-weighted images are produced by using short time to echo (TE) and repetition time (TR) times and create images with bright white matter and dark cerebrospinal fluid (CSF). 3D-T1 weighted MR images have been consistently verified across multiple datasets for

longitudinal atrophy studies (135). T2-weighted (transverse-relaxation time) MR sequences are created with longer TE and TR times to create images with bright CSF and dark fat (white matter). T2-weighted MR images have also been used in atrophy studies, but are especially useful for scoring gray matter, subcortical lesions and infratentorial lesions (135). T2-wighted images may outperform T1-weighted MR for hippocampal subfield volumetry because the acquisition parameters can be set to maximize the contrast in this region of interest (136). Further MR scans, including T2-weighted fluid-attenuated inversion recovery (FLAIR) images can also be used to measure atrophy – although this use is not frequent in the literature. In the 1980s, serial CT was one of the first neuroimaging modalities to show abnormally large ventricular and sulcar enlargement in AD patients. CT uses x-rays scans from a multitude of angles to create a computed 3D reconstruction. There are few modern CT studies that investigate cerebral atrophy because the emphasis was put on MR due to better soft tissue contrast (137). CT does have many advantages over MR images, including lower cost, faster acquisition (100x), availability, higher spatial resolution and fewer contraindications. The disadvantages of CT include lower contrast to noise ratio and exposure of the patient to ionized radiation – which a concern with longitudinal studies. CT has been found to reliability detect accelerated cerebral volume loss in AD patients and may be used more frequently for whole-brain atrophy studies in the future (137–139).

AD patients in the early stages of disease show short-term memory loss and disorientation which is associated with hippocampal neuronal loss (140). The hippocampus has been studied extensively in AD because it plays an important role in declarative memory consolidation. Further, reduced hippocampal volume results in amnestic syndrome, a core feature of AD (114). In fact, damage to the hippocampus is often incorporated into the pathological features required for a diagnosis, including neurofibrillary tangles and Aβ deposition (141). Hippocampal atrophy rates

in an age matched population (65-75 years) are estimated to be about 2% annually for healthy controls, 3.8% for MCI patients and 4% for AD patients (142). In population based longitudinal studies (5-10 years) hippocampal volume and atrophy rates have been shown to predict future cognitive decline in cognitively normal subjects (143–145). The combination of low hippocampal volume and high atrophy rate have been found to strongly predict progression from MCI to AD (142). Two longitudinal studies found no association with hippocampal atrophy and $A\beta_{1\text{-}42}$ and tau (121, 146). A third protein, the TAR DNA binding protein of 43kDA (TDP-43) has been found in patients with AD and is associated with increased hippocampal and amygdala neuronal loss. TDP-43 should be considered a potential factor related to increased rates of regional atrophy in early AD and further research to detect TDP-43 in-vivo is necessary (147). It estimated that the selection of subjects with smaller hippocampi will likely reduce the overall cost of a multidomain trial in amnestic MCI (148).

In all age groups, AD patients with generalized (rather than focal hippocampal) atrophy and earlier disease onset are at a higher risk of cognitive decline (149). Several unbiased whole-brain voxel studies have found a pattern in atrophied regions that mirror the pathological progression of AD in cognitively normal subjects up to a decade prior to onset of dementia (150, 151). In patients with AD, white-matter hyperintensities ($p=0.003$) and $A\ß_{1\text{-}42}$ ($p=0.001$) were independently associated with whole brain atrophy (58). Global cerebral atrophy, which reflects the neuronal cell loss typical of dementia, can be measured accurately from structural MR imaging sequences, even prior to cognitive impairment in the sensitive prodromal phase (152). In patients with suspected MCI, $A\ß_{1\text{-}42}$ ($p<0.001$) and tau ($p=0.04$) were associated with whole brain atrophy (58). In a study of 102 MCI subjects it was found that each additional percent of annualized whole brain atrophy rate was associated with a higher odds of disease progression of 1.3 (125). In a study

of patients with AD, MCI and controls, a higher whole-brain atrophy rate was associated with decreased Mini Mental State Examination scores, however change in CSF biomarkers were not (125). Schott et al. (2008) found that increased annualized atrophy rates were significantly associated with rate of change in several non-memory based neuropsychological scores, providing further evidence that cerebral atrophy is a clinically relevant marker of AD progression (153).

Vascular and white matter changes in mid-adulthood can lead to a more severe white matter burden and a higher atrophy rate, increasing the risk of dementia in late-life (135, 154–159). The possible mechanisms may include chronic cerebral hypoperfusion caused by CVD that increases hypoxia and leads to inflammation and white matter damage, or oxidative stress that could lead to mitochondrial dysfunction and neuronal loss (17, 72). Limited longitudinal studies have investigated the relationship between vascular risk factors and whole-brain atrophy in a TIA/minor stroke cohort, and have found that age, diabetes, diastolic blood pressure and white-matter hyperintensities are associated with higher atrophy (86, 87). Munir et al. (2019) and Walters et al. (2003) demonstrated that annualized whole brain atrophy rates of TIA/minor stroke patients were approximately 0.85% compared to 0.4% in healthy age-matched controls (86, 87). Whole-brain atrophy rates were not associated with cognitive changes, perhaps highlighting that this population is experiencing neuro-pathological degeneration prior to cognitive decline (87). However, methodological inclusion criteria, imaging modalities, and cognitive outcomes have been inconsistent in these few studies. Preventative researchers are keen to identify a population in the crucial pre-clinical window to improve efficiency and effectiveness of future trials. Methodologically consistent measurements of brain atrophy rates may guide prevention approaches and the efficacy of vascular treatment interventions.

1.5.2 Cerebral Blood Flow and Glucose Metabolism

Cerebral blood flow (CBF) is a measure of the movement of blood through the cerebral arteries and veins. Blood vessels in the brain deliver essential nutrients to and remove metabolic waste products from the central nervous system (160). While the human brain accounts for only 2% of total body mass, it consumes 20% of the body's oxygen and glucose supply (161). Dysregulation of cerebral blood flow by vascular damage is frequently associated with and contributes to AD (160–165). AD patients show significant hypoperfusion in the right inferior parietal lobe extending into the bilateral posterior cingulate gyri and middle frontal gyri compared to healthy controls (166). An increased severity of AD, as measured by the Mini-Mental State Examination, correlates with posterior parietal and posterior cingulate decreases in perfusion, but not with temporal lobe flow (167). The two-hit vascular hypothesis of AD states that vascular risk factors and genetic factors lead to CVD, which can initiate neurodegeneration and subsequently promote the accumulation of $A\beta_{1\text{-}42}$ protein in the brain (160, 168–172). Mild chronic hypoperfusion can also impair synaptic plasticity, leading to hypoxia which can inhibit action potentials and lead to imbalances in pH, electrolytes, edema, white matter lesions and lead to neuronal death (160, 161, 173).

Moderate intensity exercise has been shown to result in acute augmentation of blood flow to the brain and thus has been implicated as a potential lifestyle intervention that could help reduce the incidence of dementia and AD (174). In a prospective study with 163000 nondemented participants, it was found that physical activity reduces the risk of dementia and AD by 28% and 45%, respectively (175). Smith et al. (2010) found in a large systematic review of RCTs that exercise groups show improvements in attention, processing speed and executive function, however the effects on memory were not consistent (176). A recent review did not find any

evidence of benefit from exercise in any cognitive domain, however they did include studies with minor fitness interventions (e.g. stretching) that would not be expected to increase cerebral blood flow (177). While interventions to improve cerebral blood flow are promising, RCTs must again better identify an at-risk population who will benefit strongly from intervention.

CBF can be measured in a fast and non-invasive way by arterial spin labelling (ASL) MR imaging by applying a 180^{o} inversion radiofrequency pulse to the patient's neck slice. A second image is then acquired in the region of interest (brain) after the inversion delay (tag image). The experiment is repeated without applying a radiofrequency pulse to the neck and a control image is acquired and subsequently subtracted. Magnetically labelled water protons from the blood of the excited neck slice act as a contrast agent. Quantitative cerebral blood flow measurements (mL/min/100g) from the whole-brain and regions of interest can then be measured. MR perfusion and CT perfusion can also be used to measure cerebral blood flow with the injection of a contrast agent. While contrast imaging provides a higher signal to noise ratio than ASL-MR imaging, it also has the disadvantage of a contrast injection which can cause a serious allergic reaction in some participants. Studies have investigated the prognostic value of ASL-MR imaging in prodromal AD cohorts. In a meta-analysis of regions of interest, patients with MCI have significant hypoperfusion ($p<0.001$) compared to controls, and CBF was significantly correlated with the Clinical Dementia Rating scale ($r=-0.32$, $p=0.001$) (178). Specifically, lower perfusion has been found in the parietal lobes, the middle temporal areas, the left middle occipital lobe and the precuneus in MCI patients compared to healthy controls (179–182). Hypoperfusion in the right precuneus and cuneus correlated with poorer Mini-Mental State Examination and the Rey Auditory Verbal Learning Test scores in MCI subjects (182). Finally, in a single longitudinal study with ASL-MR imaging, significant regional hypoperfusion in the right inferior parietal cortex,

middle frontal cortex, and precuneus was observed in 13 patients who converted from mild cognitive impairment to dementia (181).

Regional CBF and glucose metabolism are generally tightly coupled and have proven to identify similar regional abnormalities with a comparable diagnostic accuracy in AD patients (38, 39). Therefore, metabolic neuroimaging techniques can also be compared to conventional CBF measurements. Positron emission tomography (PET) has also been used for atrophy studies and functions by injecting small amounts of a radiotracer which localizes to a specific pathological protein/molecule or metabolic process in the brain, allowing for its precise quantitation and localization. ^{18}F-fluorodeoxyglucose (FDG) is a PET radiotracer that is a measure of cerebral glucose metabolism and is therefore useful in AD to detect characteristic regional hypometabolism (183). FDG-PET can distinguish AD subjects from normal controls with 99% sensitivity and 98% specificity (184). Specifically, bilateral tempero-parietal hypometabolism is the characteristic pattern associated with pathologically confirmed AD (185). Four studies with small sample sizes have found overlap in the hypometabolic regions associated with conversion from MCI to AD with FDG-PET – inferior parietal cortex, inferior parietal and medial temporal cortex (186–189). FDG-PET in combination with other proven biomarkers of dementia, namely cerebrospinal fluid samples, has successfully predicted the prognosis of AD (183).

Comparing 17 AD patients and 19 age-matched controls, qualitative similarities of the regional distribution of decreased CBF (by ASL-MR imaging) and diminished FDG-PET uptake shows many parallels with visual inspection, with classically affected regions (including the temporal and parietal lobes) showing deficits in both regions (39). A discrepancy was found in the frontal lobe, which showed significant hypometabolism with FDG-PET, however did not reach statistical significance on ASL-MR imaging (39). This may be due to image artifact, perfusion-

metabolism mismatch, or reader inexperience with ASL. True perfusion-metabolism mismatch may occur because ASL is susceptible to artifact due to vascular compromise, such as arterial stenosis. Qualitative analysis yielded similar sensitivity and specificity for AD detection with both modalities, with area under curve (AUC) of approximately 90% for both imaging techniques (39). Both FDG-PET and ASL-MR imaging show potential to be used as a marker for trial inclusion or stratification, although changes in cognitively normal subjects are extremely small, and thresholds for abnormality should be established (118).

Single-photon emission computed tomography (SPECT) is a nuclear medicine tomographic imaging technique that utilizes a specific marker radioisotope. A high concentration of the ligand can be seen in three dimensions by a gamma camera. Ethyl cysteinate dimer (ECD) SPECT can measure cerebral perfusion and is considered to be a reliable marker reflecting neuronal injury (114). There is vast literature on the utility of SPECT in the diagnosis of AD, as it was used before FDG-PET became widely available. When comparing ASL-MR imaging and SPECT, ASL shows significantly lower relative cerebral blood flow values (190). However, the reported levels of agreement among cortical blood flow between ASL-MR imaging and SPECT perfusion remains highly variable (191, 192) . The limitations of ECD-SPECT include low spatial resolution and lack of absolute values for quantification (193).

CBF changes in mid-life are a promising biomarker for future dementia risk, as regional hypoperfusion may precede neuronal atrophy and cognitive decline, according to the two-hit vascular hypothesis.

1.5.3 Molecular Biomarkers

The measurement and quantification of beta amyloid ($A\beta_{1\text{-}42}$) and tau levels can be useful to monitor the AD pathology. Cerebral spinal fluid (CSF) biomarkers, measured by lumbar puncture, are valuable to detect neurodegenerative disease in *vivo* because the brain interstitial fluid is in direct with the CSF by unrestricted bi-directional flow of proteins (194). Three core CSF biomarkers for early AD diagnosis, B-amyloid ($A\beta_{1\text{-}42}$), total tau (T-tau) and phospho-tau (P-tau), have been assessed in numerous studies (195). These CSF biomarkers reflect key aspects of disease pathogenesis, including neuronal and axonal degeneration, hyperphosphorylation of tau with tangle formation, and aggregation and deposition of the Aβ42 peptide into plaques, respectively (196). As plaque formation captures Aβ in the brain, a reduction of CSF Aβ reflects cerebral amyloid deposition and shows a very high concordance with amyloid PET (197). An increase in CSF T-tau reflects the intensity of neurodegeneration in AD, however can also be found in other neurodegenerative diseases and acute stroke (197). High CSF P-tau is specific to phosphorylated-tau found only in AD (197). Extended longitudinal studies have investigated the diagnostic accuracy of core AD CSF biomarkers for prodromal AD and found very high sensitivity and specificity to identify MCI conversion to AD (198–201). Variability in measurements between clinical laboratories, and over time between batches of reagents remains a problem with CSF biomarkers (202). Furthermore, lumbar punctures are often regarded as invasive, time-consuming and complicated for clinicians. In the future, blood biomarkers may replace CSF biomarkers. Preliminary plasma assays for $A\beta_{1\text{-}42}$ suggest high performance in predicting cerebral amyloid burden, making it a promising minimally invasive pre-clinical biomarker, although currently it is still difficult to measure the molecular mechanisms of AD at these significantly lower concentrations (203, 204).

The radiotracer N-methyl-[^{11}C]2-(4'-methylaminophenyl)-6-hydroxybenothiazole, commonly referred to as Pittsburgh Compound B (PiB), was the first PET radiotracer to investigate amyloid imaging in 2004 (205). PiB is a radioligand that readily crosses the blood-brain barrier and binds to amyloid-beta 40 and 42 (206). Several studies have compared PiB-PET to post-mortem histopathology which confirmed a direct correlation between amyloid levels in the frontal cortex and confirmed AD diagnosis (207, 208). Studies have shown a good concordance of CSF $A\beta_{1-42}$ and amyloid PET measures, although these markers may represent different pools of amyloid in the brain (209). Amyloid pathology measured with PET is an established prognostic marker in subjects with MCI and is associated with an increased risk of cognitive decline and progression to dementia in cognitively normal subjects (187, 210, 219, 220, 211–218). A ten-year longitudinal study found that increased amyloid burden in MCI patients significantly correlated with declines in verbal learning and memory, but not visual memory (213). However, further research investigating the ability of PiB-PET to predict conversion from MCI to AD has found conflicting results, thus highlighting the need for sufficiently long follow-up and large data sets to establish the exact relationship (183, 219–222). Unfortunately, the positive predictive value of PiB-PET is low, given that up to 35% of cognitively normal older people have positive amyloid PET results (223, 224). It has been suggested that the rate at which amyloid positivity and later cognitive decline occur may be dependent on the presence of neurodegeneration (225, 226). Furthermore, PET is difficult to implement because of the limited access to infrastructure, the brief half-life of many radiotracers, invasiveness, expense, and time requirements.

In a recent study, a combination of demographic information, apolipoprotein E4 genotyping and neuropsychological changes over 2 years had a positive predictive value 60% higher in detecting pre-clinical amyloid-positive subjects than CSF amyloid alone (227).

Therefore, a step-wise approach to subject inclusion, including clinical information and structural MR imaging should be implemented to screen patients for increased risk of amyloid pathology (118). Amyloid pathology is necessary to determine whether an individual will show cognitive decline due to AD pathology, but is not sufficient to stage disease progress or predict the rate of decline (118).

1.6 Cognitive Predictors of Dementia

The long preclinical period prior to dementia, where pathology precedes cognitive impairment, constitutes a new window for both risk factor reduction and secondary prevention of AD. A clinical diagnosis of MCI/AD is centered on the establishment of cognitive impairment, rather than the prodromal neuropathological changes (228). Further, cognitive tests have been largely derived by comparing those with AD to those without (228). Therefore, the methodology of secondary prevention trials have been handicapped by the lack of proximal cognitive markers (228). While a cognitive profile capable of tracking subtle cognitive changes is not yet well established, early decrements in episodic and semantic memory measured by specific cognitive tests have been identified consistently (229, 230). Some studies have found that general standard cognitive screening tests, such as the Mini-Mental State Examination (MMSE), can detect those who show later-life cognitive decline (231). However, the MMSE does not examine executive functions, and as with many cognitive tests, performance is affected by age, ethnicity, co-morbid treatments, and education level (amongst other factors) (232). It has also been shown that neuropsychological testing is susceptible to false-positive diagnostic errors in patients with suspected MCI (233, 234). The relationship between cognitive tests and biomarkers in early AD remains discordant, likely due to varied methodologies, few large longitudinal studies, and heterogeneity in the definition of MCI/at-risk populations. The combination of neuroimaging

biomarkers and specific cognitive tests is promising for better prediction of early disease progression (235). Neuroimaging outcomes also have the advantage over standard cognitive testing due to statistical efficiency, which would ultimately lead to efficiency and power within clinical trials (236). As specific cognitive tests for subtle cognitive changes continue to be validated, a combination of neuroimaging biomarkers and neuropsychological examinations are necessary to create a meaningful profile of those at highest risk of developing late-life cognitive decline.

1.7 Conclusion

Currently, there is no cure for dementia and preventative strategies are urgently needed. Advanced neuroimaging diagnostics, such as magnetic resonance (MR) imaging may be able to detect early cerebral abnormalities that are predictive of late-life cognitive decline and subsequently provide an opportunity for early intervention. Patients with TIA have a 4-fold increased risk in developing late-life cognitive decline, likely due to their common modifiable risk factors with dementias, and are thus a suitable group for the study of pre-clinical dementia. The Predementia Neuroimaging of Transient Ischemic Attack (PREVENT) Study is unique in that it aims to identify late-life cognitive decline risk prior to clinical symptoms in a TIA population.

1.8 Thesis Rationale and Experimental Approach

Patients with TIA and healthy controls will be recruited from the on-going Predementia Neuroimaging of Transient Ischemic Attack (PREVENT) Study, which encompasses baseline, 1-year, 3-year and 5-year follow-up appointments. Each appointment consists of clinical brain MR imaging, clinical data collection, a blood test, a cognitive battery, and an optional lumbar puncture.

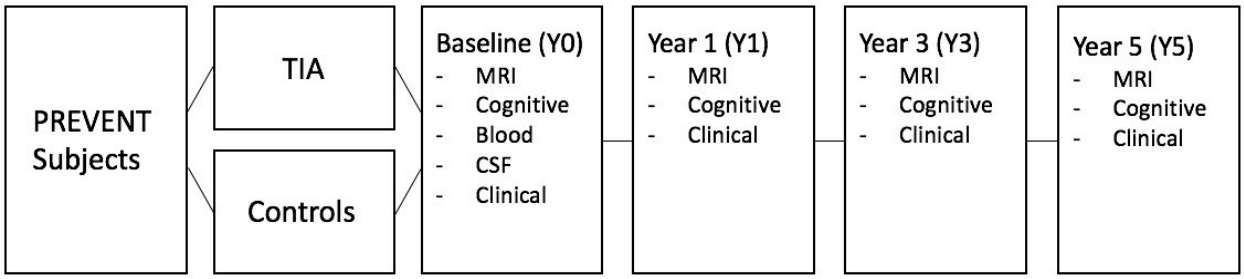

Figure 1-1. The PREVENT Study flowchart.

The PREVENT study started recruitment in April 2015 and is very close to reaching the target sample size of 360 subjects at Y0 and anticipate that subject recruitment will be complete by 2019. Currently, we are performing Y1 follow-up examinations. As expected, there has been an attrition rate of 10% for TIA participants and 5% for controls

This thesis aims to:

1. a. Determine whether TIA patients have abnormal rates of whole brain atrophy rate (mL/year) using high-resolution T1-weighted MR imaging over a 1-year period.
   b. Identify clinical and vascular risk factors that are associated with increased rates of whole brain atrophy rates over a 1-year period.
   c. Determine whether increase rates of whole brain atrophy are associated with a change in cognition over a 1-year period.

2. a. Determine whether TIA patients have abnormal cross-sectional regional cerebral blood flow measured by ASL-MR imaging (cerebral blood flow) (mL/min/100g).
   b. Identify clinical and vascular risk factors that are associated with regional cerebral blood flow.

c. Determine whether decreased regional cerebral blood flow is associated with cognitive outcomes.

In aim 1a and b, we modeled whole-brain atrophy rates as the outcome variable and aimed to determine if the presence of TIA and vascular risk factors are associated with a change in the response. In aim 1c, we modeled longitudinal cognitive change as the outcome variable in effort to determine the effect of higher rates of whole-brain atrophy. In aim 2 a and b, we modeled cerebral blood flow as the outcome variable and sought to determine if TIA status and vascular risk factors are associated with regional CBF decreases. In aim 2c, we used cross-sectional cognitive scores as outcome variables and aimed to determine if the presence of regional CBF decreases predicted cognition. In both models, we adjusted for the effects of covariates such as age, sex, depression, normalized brain volume, white matter hyperintensities, diffusion weighted lesion volume, and premorbid intellect.

The thesis aims to characterize the preclinical stages of cognitive decline in TIA by using whole-brain atrophy rates and cerebral blood flow measured by MR imaging. Moreover, the project aims to study how vascular risk factors impact our imaging outcome variables. Finally, we aim to understand the relationship of our neuroimaging biomarkers and sensitive cognitive measures. It is crucial to identify and understand the prodromal phase of dementia to implement therapeutic strategies to prevent dementia. We aim to demonstrate that TIA participants are the optimal group for targeting preventative strategies with evidence of macroscopic neuronal loss (whole brain atrophy) and regional decreased CBF. These correlations will clarify the relationship between vascular risk factors and early cerebral pathological changes preceding clinical dementia.

Early identification through neuroimaging and cognitive measures will help advance scientific knowledge to better identify those at greatest risk of late-life cognitive decline. Subsequently, this research will inform us about future clinical dementia prevention trial designs and sample size calculations to optimize therapies and promote healthy brain aging.

CHAPTER TWO: LONGITUDINAL STUDY OF COGNITION AND BRAIN ATROPHY RATES IN TRANSIENT ISCHEMIC ATTACK PATIENTS

Meaghan Reid, Noaah Reaume, Pauline Wu, Alex Pan, Arooj Aftab, Rani Gupta Sah, Meng Wang, Richard Frayne, Shelagh B. Coutts, Tolulope T. Sajobi, Richard Stewart Longman, Christopher D. d'Esterre, Philip A. Barber (2019). Longitudinal study of cognition and brain atrophy rates in transient ischemic attack patients. *Neurology.* In submission.

2.1 Preface

Excerpts from this chapter form the basis of an article in submission to *Neurology.* TTS, RSL, RF, SBC and PAB have made substantial contributions to the conception and design of this study. NR, PW, AP, AA and MR were involved in coordinating the study, recruitment, administering neuropsychological tests and data collection. MW and TTS provided significant statistical guidance for this project. RSL conceptualized the neuropsychological test battery and oversaw research assistant training for administration. RF provided guidance related to imaging modalities and the conceptual framework for analysis. RGS provided expertise in image processing. MR, CDD and PAB wrote the draft of the manuscript and all authors were involved in revising the manuscript critically for important intellectual content. All authors have given full approval of the version to be published.

2.2 Introduction

It is projected that worldwide, 50 million people are living with dementia in 2018 and this number will more than triple to 152 million by 2050 (237). Dementia is caused by progressive neuronal loss leading to brain atrophy and depletion of neurotransmitters (9). It is estimated that a progressive pre-clinical phase of dementia may span more than twenty years and is related to the co-existence of vascular risk factors (e.g., hypertension, diabetes) and lifestyle risk factors in mid-life (e.g., smoking, obesity) (109, 238). Transient ischemic attack (TIA) is a common medical emergency and characterized by temporary stroke symptoms. Within 3 months of TIA symptoms,

a third of patients have documented impairment of at least one cognitive domain, including working memory, attention, and information processing speed, which is not fully explained by clinical brain infarcts (82). Additionally, following TIA there is an increased risk of dementia; age and sex-matched adjusted incidence of post-event dementia at 1 year for TIA patients is associated with a 4-fold increase within 12 months and a 10% incidence of dementia over five years (49, 84). Nevertheless, the relationship between TIA and late-life cognitive impairment remains poorly understood (49). Studying longitudinal changes in cognitive measures and magnetic resonance (MR) data following TIA data may help determine the biomarkers that identify an individual's imminent risk of cognitive decline in the future. TIA patients generally can cooperate better with neuropsychological assessments than stroke patients, who are more likely to be older and have more severe disability (88) thereby avoiding ascertainment bias and high attrition rates caused by stroke-related co-morbidity and frailty (89).

Higher macroscopic brain loss (longitudinal cerebral atrophy) measured by MR imaging is an established neuroimaging biomarker associated with increased cognitive decline in mild cognitive impairment (MCI) and Alzheimer's disease (AD) patients, and predicts MCI progression to AD (58, 125). To date, there are few longitudinal studies that have investigated the relationship between vascular risk factors and cerebral atrophy rates in TIA patients with neuropsychological outcomes (86, 87).

In this longitudinal cohort study of cognitively normal TIA patients, we aim to determine the cerebral atrophy rates (mL/year) measured with repeated high-resolution T1-MR imaging in TIA participants and age and education-matched controls. We also aim to identify clinical and vascular risk factors that are associated with increased rates of annualized cerebral atrophy rates.

Finally, we aim to determine the associations between cerebral atrophy rates and cognitive changes over 1 year.

2.3 Materials and Methods

2.3.1 Study Population

Consecutive patients presenting with first documented TIA and non-TIA control volunteers (ages 45-80) were prospectively recruited to the Predementia Neuroimaging of Transient Ischemic Attack (PREVENT) study approved by the University of Calgary Research Ethics Board between April 2015 and December 2019 (239). TIA participants were recruited through the Emergency Department at the Foothills Medical Centre or Calgary Stroke Prevention Clinic within 48 hours of initial symptoms, and the diagnosis of TIA was confirmed by a stroke neurologist. Transient ischemic attack was defined according to the National Institute of Neurological Disease and Stroke Criteria (240). There was a formal review of all cases by a senior neurologist (PAB) who confirmed the final diagnosis of TIA and inclusion into the study. Volunteer control participants were recruited to the PREVENT Study through the community (e.g., hospital and community centre advertisements) and spousal partners. All participants were included if they had no clinical symptoms of cognitive impairment or dementia as defined by the National Institute of Aging – Alzheimer Association Criteria (241), Mini-Mental State Examination (MMSE) (242) scores between 24-30, had no contraindication to MR imaging, and were fluent in English. TIA or control subjects were excluded if there was: significant other neurologic disease (Parkinson's disease, multi-infarct dementia, seizure disorder, MS) or history of significant head trauma; psychiatric disorders (major depression, bipolar disorder or schizophrenia) as defined by DSM-5 criteria (24); alcohol or substance abuse or dependence within the past 2 years; any significant systemic illness or unstable medical condition; and/or current use of specific psychoactive medications (e.g.,

certain antidepressants, neuroleptics, chronic anxiolytics, or sedative hypnotics, etc.). All TIA participants had an MR scan within 14 days of their initial symptoms. All subjects provided written informed consent.

2.3.2 Clinical Data Collection

All study procedures were performed at baseline (BL) examination and approximately 1-year follow-up (FU) after TIA. Procedures included a medical evaluation, which was comprised of clinical characteristics, demographics, medical history and medication profiles that were collected through patient self-reporting and corroborated when possible by medical charts and pharmacological profiles. An average of two blood pressure readings was measured at baseline. Alcohol use was defined by self-report of >2 alcohol drinks per day; tobacco risk was defined by past or previous regular use of tobacco. All TIA patients were managed according to current stroke prevention guidelines.

A standardized neuropsychological battery was administered by a trained researcher at both time points that closely aligned with that of the Alzheimer's Disease Network Initiative (ADNI) (243), supplemented with tests for visual memory and frontal executive function. The memory domain was tested with the Brief Visuospatial Memory Test-Revised (BVMT), total and delayed recall (244) and the Rey Auditory Verbal Learning Test, total trial 6 (245). The processing speed domain was tested by the WAIS-IV Digit Symbol Coding test (246). The executive functioning domain was tested with the Trail Making Test B (247). Other tests included: the mean ordinal raw score of the Addenbrooke's Cognitive Examination Revised (ACE-R) categorical verbal fluency and total score (248, 249); the Montreal Cognitive Assessment (MOCA) (250); the premorbid intelligence calculated from the North American Adult Reading Test (NAART) (251); and

depressive symptoms from the Center for Epidemiologic Studies Depression Scale (CES-D), total (252). Different versions of the BVMT-R, MOCA, RAVLT and ACE-R were used at follow-up to minimize learning effects.

2.3.3 Image Acquisition

Participants underwent MR imaging at baseline (BL) and 1 year follow-up (FU) using a 3.0T Diagnostic MR scanner (General Electric Discovery 750), using a 12-channel head and neck coil. T1-weighted images, T2-weighted fluid-attenuated recovery (FLAIR) sequences, and diffusion weighted imaging (DWI) sequences were analyzed. The 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 slice with a 0 mm gap; acquisition matrix = 256 x 256; TE = 2.932 ms; TR = 6.66 ms; flip angle = 8°; inversion time (TI) = 650 ms; phase FOV = 85%; reconstructed voxel size = 5392 mm isotropic). The 2D DWI sequence was acquired with an acquisition matrix size = 256 x 256, FOV = 28 cm, twenty-three 3.0 mm slice with 0 mm gap; TE = 81.7 ms, TR = 8000 ms, flip angle = 90°. The 3D T2-weighted FLAIR sequence was acquired with an acquisition matrix size = 512 × 512; FOV = 24 cm; thirty-eight 3.0 mm slice with a 0 mm gap; TE = 120.0 ms, TR = 8524 ms, TI = 2219 ms, flip angle = 90°. Quality assurance to detect poor image quality (low signal-to-noise, or high levels of image artifact or distortion) was manually performed to ensure image quality.

2.3.4 Image Processing

All image data was anonymized and reading/processing of scans was performed blinded to all demographic and outcome data. Each imaging modality was processed independently.

Specifically, formal adjudication of the image quality was graded according to neck and head movement, quality of registration and intensity inhomogeneities by two trained individuals (PAB, MR).

2.3.5 Percentage Brain Volume Change

Brain tissue volume, normalized for subject head size, was measured with SIENAX (253, 254). Longitudinal cerebral atrophy (two-time point percentage brain volume change) was measured with SIENA (254, 255), part of FSL (254). SIENA extracted brain and skull images from the BL and FU time points for the whole-head input data (256). The two brain images were then aligned to each other (257, 258) (using the skull images to constrain the registration scaling); both brain images were resampled into the space halfway between the two image volumes. Next, tissue-type segmentation was carried out (259) in order to find brain/non-brain edge points, and then perpendicular edge displacement (between the two-time points) was estimated at these edge points. Finally, the mean edge displacement was converted into a (global) estimate of percentage brain volume change between the two-time points. Each processed MR image underwent quality control assessment, which included ensuring proper brain extraction results that did not include non-brain tissue, proper registration to MNI-152 template and accurate segmentation of the whole brain. Cerebral atrophy rates (mL/year) were calculated from normalized baseline brain volume and percentage brain volume change.

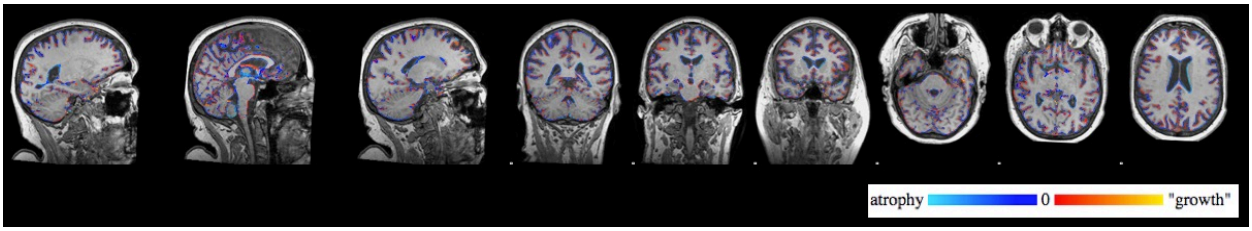

Figure 2-1. Example of FSL SIENA final brain edge movement image.

2.3.6 White Matter Hyperintensities and Diffusion Lesions

White matter hyperintensities (WMH) at baseline were segmented from FLAIR images using Cerebra-WML software, a semi-automated software measuring the volume (mL) of white matter lesions based on the global threshold and contrast between the different cerebral anatomical regions (260). DWI lesions were defined by clinical radiologists by the presence of hyperintense lesion on the DWI map with corresponding hypointensity on the apparent diffusion coefficient map. Acute diffusion lesions were segmented from the DWI images with ITK-SNAP (Version 3.6.0, www.itksnap.org), a semi-automatic software application used to segment structures in 3D medical images by a trained researcher (MR) and underwent quality control by a stroke neurologist (PAB) (261). The apparent diffusion coefficient decline associated with acute ischemia persists for 10 days and then normalizes, but the T2 component of the DWI lesion persists much longer for up to several weeks. Therefore, the <14 days window should identify acute DWI lesions with confidence and addresses the practical constraint of obtaining acute MR imaging for TIA patients.

2.3.7 Statistical Analysis

All statistical analysis was performed on STATA IC/15.0 (StataCorp. 2017. Stat Statistical Software: Release 15. College Station, TX: StataCorp LLC). Descriptive statistics were used to compare the mean (median for non-parametric data), standard deviations (interquartile ranges) and

frequency distributions of all variables. Two-way two-sample *t*-tests and Chi square tests ($\propto = 0.05$) were used to compare patients' demographic, clinical, and cognitive characteristics between the TIA and control participants. Wilcoxon-Rank Sum tests were ($\propto = 0.05$) used to compare WMH burden and DWI volumes at baseline and 1 year between TIA and control participants. Published cognitive normative data was compared to our control population (age and education-matched) using calculated two-way two-sample independent *t*-tests ($\propto = 0.05$). Pairwise-correlations and Wilcoxon-Signed rank tests were used to analyze changes from baseline to follow-up cognition.

All data for multiple linear regression fit the basic required assumptions including a linear relationship between the outcome variable and the independent variables, normally distributed residuals, no multicollinearity and homoscedasticity. The associations between baseline risk factors (including demographic, medical, WMH burden, WMH change, depression, MMSE, and premorbid intellect) and longitudinal rates of cerebral atrophy were tested using a backwards stepwise multiple linear regression. Variables with the highest p-values were removed until the adjusted R-square value no longer improved. Multiple linear regression was performed to analyze the associations between cerebral atrophy rates (mL/year) and cognitive profiles (baseline and 1-year change) while controlling for TIA status, age, sex, premorbid intellect, WMH and NBV.

2.4 Results

2.4.1 Demographics

Seventy (70) TIA patients and 39 control participants were initially included in the study following inclusion criteria, complete clinical/demographic information at both time points and complete baseline and follow-up MR imaging sequences. Eleven (11) TIA and 5 control participants were excluded because of poor image quality. Poor registration observed in these 16

cases was due to primarily due to motion artifact (4), intensity inhomogeneities (9) and poor head positioning (3). A total of 59 TIA patients and 34 control participants were included in the analysis. Demographic, clinical and vascular baseline factors for both the TIA and control population are summarized in Table 2-1.

Table 2-1. Demographic information, medical information and two-way two-sample *t*-tests of significance and Chi-square tests for demographic and clinical data of TIA and control participants ($\propto = 0.05$). IQR = interquartile range, WMH = white matter hyperintensities normalized by brain volume, NBV = normalized brain volume, DWI = diffusion weighted image lesion, BL = baseline, FU = follow-up.

Characteristics	**TIA (n=59)**	**Control (n=34)**	**p**
Age, *years*	66.31 ± 8.51	63.85 ± 8.31	0.183
Sex, *n women* (%)	26 (44.1%)	21 (61.8%)	0.102
Education, *years*	13.93 ± 2.34	14.60 ± 3.00	0.226
Baseline to Follow-Up, *days*	459.1 ± 124	484.3 ± 106	0.324
Depression, *raw CES-D*	11.27 ± 8.31	10.00 ± 8.30	0.479
Premorbid Intellect,	109.5 ± 9.70	112.2 ± 7.59	0.165
WMH BL, *median (IQR)**	2.141 (1.1-3.7)	1.170 (0.79-3.0)	**0.079**
WMH FU, *median (IQR)**	2.214 (1.4-4.6)	1.650 (1.0-3.2)	**0.074**
NBV, cm^3	1448 ± 74.1	1473 ± 80.9	0.118
Systolic BP, *mmHG*	138.0 ± 19.3	130.1 ± 17.2	0.052
DWI, *n participants (%)*	19.00 (32.2)	0	**0.000**
DWI Volume BL, *median* cm^3*(IQR)**	26.89 (10.4-48.5)	0	**0.000**
DWI Volume FU, *median* mm^3*(IQR)*	0.000	0.000	1.000
Statin Treatment, *n (%)*	27 (45.8%)	6 (17.6%)	**0.006**
Hypertension Treatment, *n (%)*	28 (47.5%)	10 (29.4%)	0.087
Diabetes, *n (%)*	4 (6.70%)	1 (2.90%)	0.431
Tobacco, *n (%)*	30 (50.8%)	15 (44.1%)	0.534
Alcohol, *n (%)*	4 (6.80%)	0	0.123

*Wilcoxon-Rank Sum tests used.

The TIA and control population did not significantly differ in sex, age, education, and all other factors excluding statin treatment (p=0.006), DWI (p<0.001) and white matter

hyperintensities normalized by brain volume at baseline and follow-up (p=0.079 and p=0.074, respectively).

The cognitive profiles were compared between the TIA and control participants using independent Wilcoxon Rank-Sum test at baseline and 1 year follow-up [Table 2-2].

Table 2-2. Wilcoxon Rank-Sum tests of cognitive domains comparing TIA and control participants ($\propto = 0.05$). 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. A) Baseline. B) 1 year follow-up.

A) Baseline

	TIA (n=59)		**Control (n=34)**		***p***
	Median	IQR	Median	IQR	
MMSE	30.0	29.0-30.0	30.0	29.0-30.0	0.521
MOCA	26.0	23.0-28.0	27.5	25.0-29.0	**0.046**
ACE-R	92.0	88.0-97.0	95.0	91.0-98.0	0.051
BVMT Total	22.0	17.0-28.0	25.0	19.0-30.0	0.242
BVMT Delayed	10.0	7.00-11.0	11.0	8.00-12.0	0.194
RAVLT	10.0	7.00-12.0	11.5	9.00-14.0	0.114
WAIS-IV DS Coding	56.0	49.0-65.0	65.0	53.0-73.0	**0.016**
TMT A	33.0	27.3-44.0	28.4	22.7-33.6	**0.002**
TMT B	71.0	59.0-94.0	63.2	48.0-79.1	**0.024**
Verbal Fluency, *raw total from ACE-R*	15.5	12.0-15.5	15.5	12.0-18.0	0.297

B) Follow-Up

	TIA (n=59)		**Control (n=34)**		***p***
	Median	IQR	Median	IQR	
MOCA	26.0	24.0-29.0	28.0	26.0-29.0	0.055
ACE-R	93.0	89.0-96.0	95.5	91.0-98.0	**0.034**
BVMT Total	26.0	20.0-30.0	25.0	20.0-31.0	0.607
BVMT Delayed	10.0	8.00-12.0	10.0	8.00-12.0	0.929
RAVLT	10.0	6.00-12.0	12.0	9.00-14.0	**0.021**
WAIS-IV DS Coding	67.5	57.0-82.0	68.5	57.0-79.0	0.836
TMT A	31.7	27.1-38.6	27.3	25.0-37.0	0.074
TMT B	69.3	56.2-98.1	66.4	47.9-81.4	0.134
Verbal Fluency, *raw total from ACE-R*	15.5	11.0-18.0	15.5	12.0-18.0	0.417

At baseline, the control participants had significantly higher MOCA scores (p=0.046), WAIS-IV DS Coding (p=0.016), TMT A (p=0.002) and B scores (p=0.024). In the 1 year follow-up, control participants had significantly higher ACE-R scores (p=0.034) and RAVLT scores (p=0.021) compared to TIA patients.

Wilcoxon Rank-Sum tests found that TIA participants had significantly higher cerebral atrophy rates (mL/year) compared to the control population (-11.79mL/year [IQR = (-14.89) - (-5.73)] and -7.80mL/year [IQR = (-14.9) - (-5.727)], respectively; p=0.037), although the baseline normalized brain volume (cm^3) was not significantly different in the two populations [Figure 2-2].

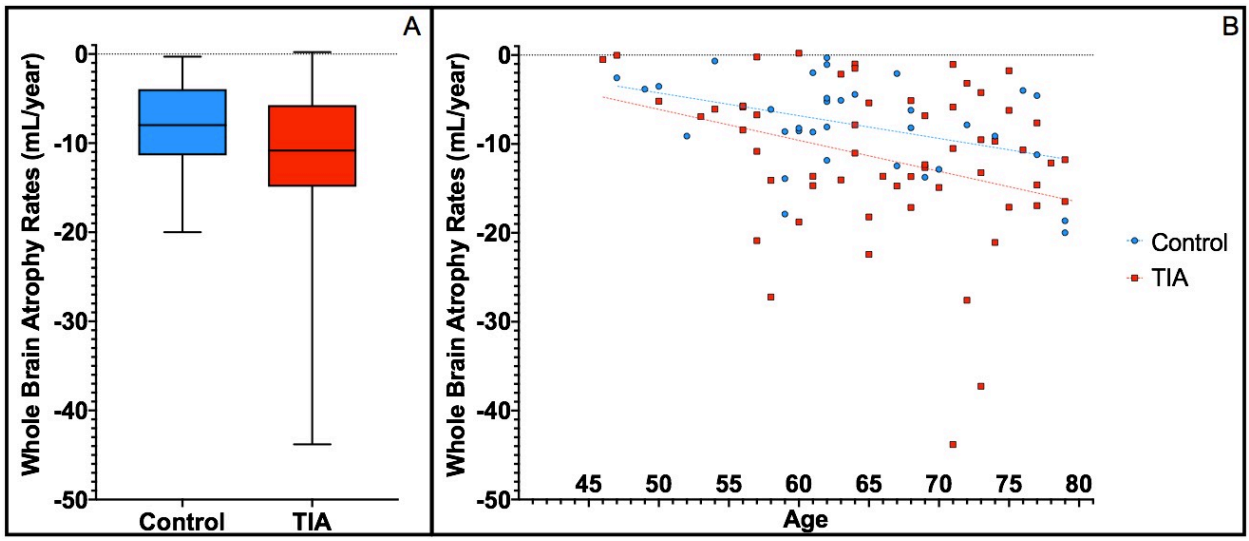

Figure 2-2. The cerebral atrophy rates (mL/year) of the TIA (n=59) and control (n=34) participants. **A**) The box plots of cerebral atrophy rates (mL/year). **B**) The independent cerebral atrophy rates (mL/year) and age of the TIA and control participants.

Wilcoxon-Signed Rank tests and pairwise correlations coefficients were calculated to determine the change in cognitive scores over 1 year [Table 2-3 A and B]. TIA participants significantly improved over 1 year in BVMT total (p=0.006), BVMT Delayed (p=0.045), and the

WAIS-IV Digit Symbol coding (p=0.000). Control participants significantly decreased in the BVMT delayed (p=0.035), and significantly improved in the BVMT total (p=0.007) and WAIS-IV DS Coding (p=0.000).

Table 2-3. Wilcoxon-Signed Rank tests and pairwise correlation coefficients (r) of cognitive domains comparing baseline and 1 year follow-up scores ($\propto = 0.05$). 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. A) TIA patients. B) Control subjects.

A) TIA Patients

	Baseline		**Follow-Up**		***p***	***r***
	Median	IQR	Median	IQR		
MOCA	26.0	23.0-28.0	26.0	24.0-29.0	0.220	0.363
ACE-R	92.0	88.0-97.0	93.0	89.0-96.0	0.922	0.670
BVMT Total	22.0	17.0-28.0	26.0	20.0-30.0	**0.006**	0.612
BVMT Delayed	10.0	7.00-11.0	10.0	8.00-12.0	**0.045**	0.691
RAVLT	10.0	7.00-12.0	10.0	6.00-12.0	0.255	0.763
WAIS-IV DS Coding	56.0	49.0-65.0	67.5	57.0-82.0	**0.000**	0.486
TMT A	33.0	27.3-44.0	31.7	27.1-38.6	0.314	0.701
TMT B	71.0	59.0-94.0	69.3	56.2-98.1	0.251	0.714
Verbal Fluency, *raw total from ACE-R*	7.00	6.00-7.00	7.00	6.00-8.00	0.582	0.493

B) Control Subjects

	Baseline		**Follow-Up**		***p***	***r***
	Median	IQR	Median	IQR		
MOCA	27.5	25.0-29.0	28.0	26.0-29.0	0.264	0.476
ACE-R	95.0	91.0-98.0	95.5	91.0-98.0	0.951	0.742
BVMT Total	25.0	19.0-30.0	25.0	20.0-31.0	**0.007**	0.770
BVMT Delayed	11.0	8.00-12.0	10.0	8.00-12.0	**0.035**	0.658
RAVLT	11.5	9.00-14.0	12.0	9.00-14.0	0.201	0.773
WAIS-IV DS Coding	65.0	53.0-73.0	68.5	57.0-79.0	**0.000**	0.522
TMT A	28.4	22.7-33.6	27.3	25.0-37.0	0.236	0.549
TMT B	63.2	48.0-79.1	66.4	47.9-81.4	0.229	0.613
Verbal Fluency, *raw total from ACE-R*	7.00	6.00-8.00	7.00	6.00-8.00	0.421	0.522

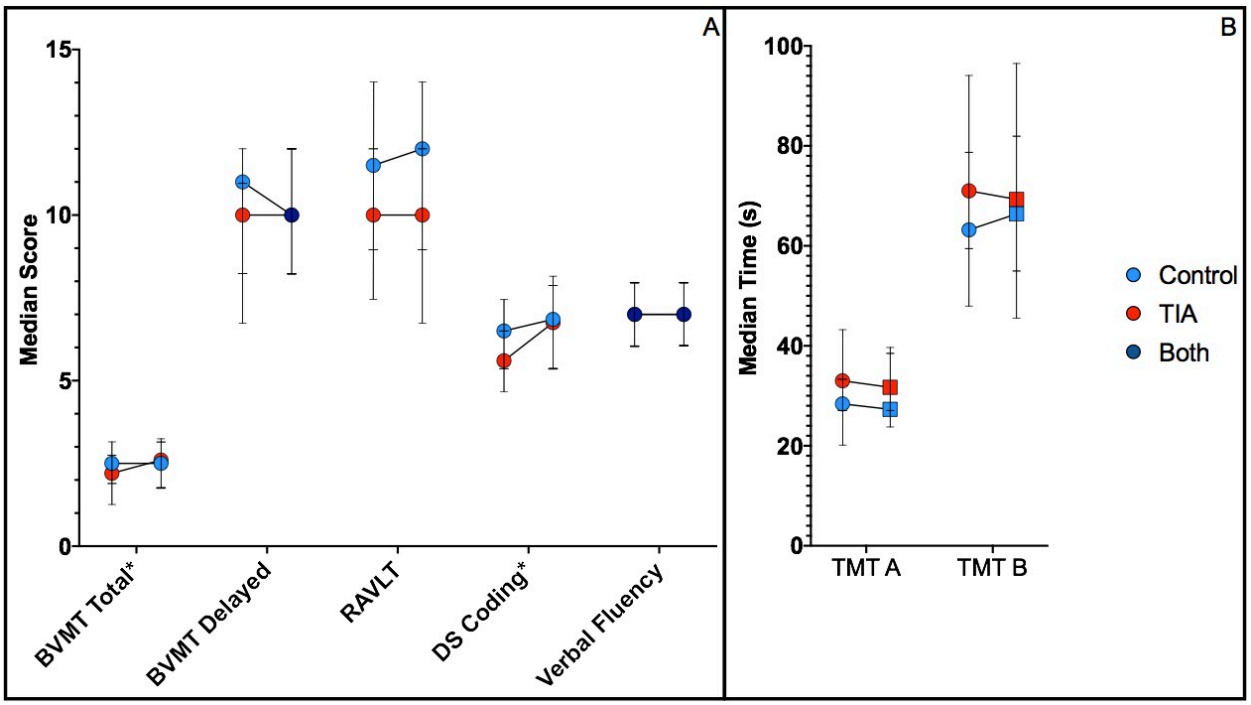

Figure 2-3. Median cognitive scores of TIA (*n*=59) and control (*n*=34) participants from baseline to 1 year follow up with interquartile range. **A**) Median raw scores. **B**) Median time (s) of TMT scores. * = Raw score divided by 10, BVMT = Brief Visuospatial Memory Test, RAVLT = Rey Auditory Verbal Learning Test, DS Coding = Digit Symbol Coding, TMT = Trail Making Test.

Normative neuropsychological literature was then compared to our control data, age and education-matched when possible in Table 2-4. The control participants in our study had a significantly better BVMT Delayed score and shorter time (s) to complete the TMT-A test, although it should be noted that the BVMT demographics were slightly inconsistent between the two groups.

Table 2-4. Two-way two-sample *t*-tests comparing neuropsychological data between published normative results and our control population ($\propto = 0.05$). Our control population had an average age of 63.85 $\pm$ 8.43 and education of 14.60 $\pm$ 2.90 years. MMSE = Mini-Mental State Examination, BVMT = Brief Visuospatial Memory Test, RAVLT = Rey Auditory Verbal Learning Test, DS Coding = Digit Symbol Coding, TMT = Trail Making Test, MOCA = Montreal Cognitive Assessment.

	Normative Values from Literature				**Control (n=34)**	**p**
	n	**Age [sd]**	**Education [sd]**	**Mean [sd]**	**Mean [sd]**	
MMSE (262)	358	65-69	>12	29.0 [1.300]	29.3 [0.221]	0.256
BVMT Total (244)	51	61.5 [4.5]	13.9 [2.5]	22.2 [5.500]	24.0 [7.574]	0.201
BVMT Delayed (244)	51	61.5 [4.5]	13.9 [2.5]	8.40 [2.100]	9.88 [2.240]	**0.003**
RAVLT (263)	56	50-64	>12	10.0 [2.600]	10.8 [3.514]	0.223
WAIS-IV DS Coding (246)	100	55-64	13-15	60.0 [15.25]	64.0 [13.20]	0.175
TMT A (264)	31	61.9 [15]	15.4 [1.2]	33.2 [9.100]	28.4 [6.487]	**0.016**
TMT B (264)	31	61.9 [15]	15.4 [1.2]	64.6 [18.59]	65.3 [19.96]	0.879
MOCA (265)	95	65-75	University Degree	27.1 [1.700]	26.7 [2.745]	0.369

2.4.2 Multiple Linear Regression

Backwards stepwise multiple linear regression was used to analyze the associations between risk factors (control vs. TIA, age, sex, education, normalized brain volume, premorbid intellect, depression, white matter hyperintensities/normalized brain volume, change in WMH burden, DWI volume, systolic blood pressure, hyperlipidemia, hypertensive, diabetes, tobacco, and MMSE screening) and annualized cerebral atrophy (mL/year). Higher age and systolic blood pressure were both significantly associated with increased cerebral atrophy rates (mL/year) [Table 2-5].

Table 2-5. Final backwards stepwise multiple linear regression analyzing the associations between risk factors and cerebral atrophy rates (mL/year). DWI = Diffusion weighted imaging lesion, MMSE = Mini-Mental State Examination.

		Adjusted *R*-squared = 0.2210
		Model *p* value = 0.0000
	***β* Coefficient**	***p* value**
TIA Status	-0.125	0.290
Age	-0.021	**0.002**
Premorbid Intellect	0.008	0.186
DWI Volume	-0.000	0.253
Systolic Blood Pressure	-0.006	**0.050**
Hypertensive Status	-0.146	0.209
Alcohol	0.305	0.263
Baseline MMSE	-0.049	0.263

Multiple linear regression was performed to analyze the associations between cerebral atrophy rates (mL/year) and baseline cognitive profiles over 1 year while controlling for TIA status, age, sex, premorbid intellect, WMH and NBV [Table 2-6]. No significant relationships with longitudinal cognition and atrophy rates were found.

Table 2-6. Multiple linear regression analyzing the associations between cerebral atrophy rates (mL/year) and baseline cognitive scores. This model is controlling for TIA status, age, sex, premorbid intellect, white matter hyperintensities and normalized brain volume. BVMT = Brief Visuospatial Memory Test, RAVLT = Rey Auditory Verbal Learning Test, DS Coding = Digit Symbol Coding, TMT = Trail Making Test.

	Cerebral Atrophy Rate (mL/y)		**Model**
BL Cognitive Score	β-Coefficient	p value	Adj R-squared
BVMT-Total	0.006	0.453	0.158
BVMT-Delayed	0.008	0.707	0.154
RAVLT	0.116	0.509	0.157
TMT A	-0.006	0.381	0.160
TMT B	-0.001	0.442	0.159
Verbal Fluency	0.033	0.973	0.153

Multiple linear regression was performed to analyze the associations between cerebral atrophy rates (mL/year) and cognitive changes over 1 year while controlling for TIA status, age, sex, premorbid intellect, WMH and NBV [Table 2-7]. No significant relationships with longitudinal cognition and cerebral atrophy rates were found.

Table 2-7. Multiple linear regression analyzing the associations between cerebral atrophy rates (mL/year) and change in cognition over a year. This model is controlling for TIA status, age, sex, premorbid intellect, white matter hyperintensities and normalized brain volume. BVMT = Brief Visuospatial Memory Test, RAVLT = Rey Auditory Verbal Learning Test, DS Coding = Digit Symbol Coding, TMT = Trail Making Test.

	Cerebral Atrophy Rate (mL/y)		**Model**
Change in Cognitive Score	β-Coefficient	p value	Adj R-squared
BVMT-Total	-0.530	0.664	0.033
BVMT-Delayed	-0.232	0.619	0.002
RAVLT	0.353	0.494	0.085
TMT A	-3.134	0.080	0.040
TMT B	-0.881	0.905	-0.046
Verbal Fluency	-0.092	0.696	0.099

2.5 Discussion

This study demonstrates that TIA patients have a higher cerebral rate of atrophy over 1 year compared to an age and education-matched control cohort. Increasing age and systolic blood pressure are associated with higher cerebral atrophy rates in both TIA and control participants. The TIA cohort has significantly higher WMH volumes, DWI lesions and cholesterol treatment than the control cohort, yet we found no relationship of these covariates with cerebral atrophy rates. Further, we did not find a significant association with hypertension treatment, diabetes, DWI lesion volumes, alcohol or smoking with increased cerebral atrophy rates. The absence of a relationship with cerebral atrophy rates and DWI lesions might support our hypothesis that the changes in brain volume are related to chronic progressive incipient neurodegenerative or vascular mechanisms. Further longitudinal data will be required to confirm whether these whole brain volume changes persist or even progress over time, and explore potential clinical, cognitive and biological predictors of increased whole brain atrophy rates. Nonetheless, our findings corroborate previous

studies that show higher longitudinal rates of cerebral atrophy in TIA/minor stroke participants (86, 87).

TIA participants in our study had a mean percentage annualized cerebral atrophy rate of -0.82% versus -0.53% for the control group. Rates of cerebral atrophy have been shown to parallel clinical and pre-clinical dementia progression, and therefore are frequently used as a disease biomarker for clinical dementia trials (121). Cerebral atrophy rates are estimated to be -1.1% in patients with suspected mild cognitive impairment (MCI) and between -2% and -3% in established AD (58, 124). Each additional percent of annualized cerebral atrophy rates in MCI patients is associated with a 1.3 x higher odds of disease progression to AD (125). TIA patients have a lower atrophy rate than MCI patients and therefore may be at an earlier stage of prodromal dementia. This study provides further evidence that cerebral atrophy rate, as measured by cerebral volume loss, may predispose TIA patients to a heightened risk of cognitive decline in the future (266). Our rates of cerebral atrophy in controls are consistent with the literature [(-0.2)-(-0.5%)] (267), however, are higher than the mean annualized atrophy rate of -0.3% reported by Walters et al. (2003) (86).

Higher systolic blood pressure was found to be significantly associated with cerebral atrophy in our cohort. Although the exact cause of dementia remains unknown, the modifiable vascular risk factors have emerged as an important risk factor for cognitive decline and AD. Hypertension is recognized as the most consistent risk factor for both stroke and dementia (65, 68). Also, hypertension has been found to be associated with increased brain atrophy in cross-sectional (268) and longitudinal studies (86, 87). Hypertension in midlife has been shown to have a direct relation to brain atrophy at autopsy and with increased AD pathology, namely increased accumulation of amyloid plaques and neurofibrillary tangles (269). The mechanisms by which

hypertension affects cognitive function remain uncertain, but it is conceivable that high blood pressure may dysregulate the cerebral blood flow system and lead to cerebral hypoperfusion (64). Additionally, approximately 60-90% of AD autopsy reports exhibit cerebrovascular disease pathology, supporting that neurodegenerative disease and vascular disease commonly co-exists (64).

Unlike previous TIA/minor stroke studies, we did not find an association with WMH and cerebral atrophy rates (86, 87). Barnes et al. (2013) found that increased WMH burden and decreased cerebral spinal fluid $A\beta_{1-42}$ levels were independently associated with higher longitudinal brain volume loss in healthy control subjects, but not MCI or AD patients, possibly because they represent a more severe stage of disease (58). These results suggest that other sensitive markers of vascular disease should be measured to determine the relation of small vessel disease with brain atrophy rates, such as quantitative cerebral blood flow or white matter diffusion tensor imaging.

Normative cognitive profiles of healthy adults are consistent with our control population, excluding the BVMT Delayed score and the Trail Making A test, which is not found to be important variables in our statistical analysis (244–246, 262–265). At baseline, the cognitive profiles of the TIA population show significantly lower MOCA, WAIS-IV Digit Symbol coding and Trail Making Tests (A and B) scores compared to our control population. At 1 year follow-up, our controls have significantly higher ACE-R and RAVLT total scores compared to the TIA patients. Control participants show a significant decrease in the BVMT Delayed domain over 1 year and an increase in the BVMT total and WAIS-IV DS Coding tests. TIA participants, conversely, showed a significant increase in the BVMT Total, BVMT Delayed and WAIS-IV Digit Symbol coding tests over 1 year. We find no significant relationship with baseline cognition or

longitudinal changes in cognition with cerebral atrophy rates, strongly suggesting that changes in brain volume are likely preceding cognitive decline in TIA subjects (86, 87). TIA patients have frequently been shown to have mild cognitive deficits following the incident event, but it is unclear whether these cognitive findings preceded the event or were a direct result of the TIA. Also, studies to date have not clarified whether the cognitive abnormalities progress over time. What these studies have shown is considerable heterogeneity of the cognitive profiles that appear unrelated to the presence of acute DWI lesions (270, 271). Some of the cognitive abnormalities might partially be explained by the increased incidence of neuro-affective symptoms; TIA participants have been shown to have higher subjective complaints of fatigue, anxiety and depressive symptoms following their TIA which could impact on their baseline cognitive scores (271).

Notwithstanding these observations, the reliability neuropsychological assessments of preclinical disease have limitations because performance is commonly affected by age, education, ethnicity, co-morbid treatments, and ceiling/floor effects and learning effects, which can limit our ability to detect change over time within a subject. We minimized learning effects by administering different versions of cognitive tests at baseline and 1 year. Compared to brain atrophy measurements, standard cognitive testing has a higher inter-individual variability, up to a 10-fold decrease in clinical trial power and fails to provide objective support for disease-modifying effects (236, 272). However, cerebral atrophy also remains a global, non-specific marker of diffuse neurodegenerative processes and does not highlight the regional neuropathological changes that occur during the progression of AD. Further research should focus on atrophy rates in focal regions of interest in a TIA population, such as the hippocampus, which has been studied extensively in AD and MCI (144). Disease progression must be analyzed over several time points to validate the reliability and sensitivity of brain atrophy rate measurements. This is of importance in pre-clinical

dementia trials, where current clinical outcomes have a limited ability to detect treatment effects within the typical time frame of a clinical trial – whereas structural MR imaging has been shown to detect change over 12 months. Longitudinal neuroimaging studies will also provide insight into the ideal inclusion criteria for clinical trials by identifying patients at the highest risk of cognitive decline. It is these patients that are potentially most likely to benefit from vascular risk-reduction therapies if the timing and type of intervention are adjusted for optimal effectiveness.

Our data in TIA patients show an almost two-fold increase in rates of cerebral atrophy measured by MR imaging compared to non-TIA controls, suggesting the existence of a pre-clinical dementia state in this understudied group of patients. Future studies will need to determine what biomarkers are able to determine an individual's imminent risk of cognitive decline and what we should measure to monitor the effect of preventative treatment, such as intensive blood pressure control, perhaps even beyond current clinical guidelines. Predictors of early neurodegeneration, such as whole brain atrophy rates are promising pre-clinical biomarkers that could monitor vascular risk management in the short-term, and ultimately improve subject selection strategies for future clinical trials.

CHAPTER THREE: THE ASSOCIATIONS BETWEEN ASL-MRI CEREBRAL BLOOD FLOW IN TRANSIENT ISCHEMIC ATTACK PATIENTS AND COGNITION

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3.1 Preface

Excerpts from this chapter for the basis of an article in submission. TTS, RSL, RF, SBC and PAB have made substantial contributions to the conception and design of this study. NR and MR were involved in coordinating the study, recruitment, administering neuropsychological tests and data collection. MW and TTS provided significant statistical guidance for this project. RSL conceptualized the neuropsychological test battery and oversaw research assistant training for administration. RF provided guidance related to imaging modalities and the conceptual framework for analysis. RGS provided expertise in image processing. CM provided significant guidance with data acquisition through MatLab. MR, CDD and PAB wrote the draft of the manuscript and all authors were involved in revising the manuscript critically for important intellectual content. All authors have given full approval of the version to be published.

3.2 Introduction

Dementia is a major cause of disability and dependency in the elderly and is associated with personal, social and economic burden. (4). Neurodegenerative or vascular processes associated with late life cognitive decline may span more than twenty years before symptom onset, providing a crucial pre-clinical window to identify those at the highest risk of cognitive decline. The identification of reliable neuroimaging markers for prodromal dementia will allow for the identification and implementation of therapeutic and pharmacological interventions in the earliest

stages of disease. Many neurodegenerative dementias have a vascular component (ranging from 40-60%) which increases with age (13). Common vascular modifiable risk factors of AD include cerebrovascular disease, hypertension and smoking, and can be directly attributed to approximately 25% of dementias (14, 15, 273). Transient ischemic attack (TIA) is common medical emergency and is characterized by stroke symptoms (lasting no longer than 24 hours) caused by transient ischemia. TIA patients have a 4-fold increased risk in developing late-life cognitive decline, likely due to their common modifiable risk factors with AD, and are thus a suitable group for the study of pre-clinical dementia (84, 274, 275).

The association between cerebrovascular disease and ischemic damage leading to cognitive decline is uncertain, however data suggests that the modifiable vascular and lifestyle risk factors, especially chronic hypertension, can lead to cerebral hypoperfusion and microvascular damage that may increase the susceptibility of the brain to both the formation of AD-type pathology (44) and small vessel disease. Regional cerebral blood flow (CBF) can be quantitatively measured with magnetic resonance (MR) imaging arterial spin labelling (ASL), which is a non-invasive technique that magnetically labels water protons to be used as a blood flow tracer. ASL-MR imaging has several advantages over nuclear medicine and contrast imaging techniques: it is non-invasive, does not require an intravenous contrast agent, is fast, easy to acquire and does not expose patients to ionizing radiation. Additionally, the anatomical images can be acquired at the same time as the perfusion images.

In this prospective cross-sectional study, we aim to determine regional CBF differences (mL/min/100g) measured by ASL-MR imaging in TIA and non-TIA controls. We also aim to determine demographic and vascular risk factors that are associated with CBF decreases. Finally,

we aim to determine whether ASL-MR imaging can discriminate cognitive profiles in TIA patients compared to non-TIA control subjects.

3.3 Materials and Methods

3.3.1 Study Population

Consecutive participants presenting with first documented TIA and non-TIA control volunteers between the ages of 45-80 were prospectively consented to the Predementia Neuroimaging of Transient Ischemic Attack (PREVENT) Study approved by the University of Calgary Research Ethics Board between May 2017 and December 2019 (239). TIA participants were recruited through the Emergency Department at the Foothills Medical Centre or Calgary Stroke Prevention Clinic within 48 hours of initial symptoms, and the diagnosis of TIA was confirmed by a stroke neurologist. Transient ischemic attack was defined according to the National Institute of Neurological Disease and Stroke Criteria (240). 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. Volunteer control participants were recruited to the PREVENT Study through the community (e.g. hospital and community centre advertisements) and spousal partners. All participants were included if they had no clinical symptoms of cognitive impairment or dementia as defined by the National Institute of Aging – Alzheimer Association Criteria (241), Mini-Mental State Examination (MMSE) (242) scores between 24-30, had no contraindication to MR imaging, and were fluent in English. TIA or control subjects were excluded if there was: significant neurologic disease (Parkinson's disease, multi-infarct dementia, seizure disorder, MS) or history of significant head trauma; psychiatric disorders (major depression, bipolar disorder or schizophrenia) as defined by DSM-5 criteria (24); alcohol or substance abuse or dependence within the past 2 years; any significant systemic illness or unstable medical condition; and/or current use

of specific psychoactive medications (e.g., certain antidepressants, neuroleptics, chronic anxiolytics, or sedative hypnotics, etc.). All TIA participants had T1-weighted MR imaging and ASL-MR imaging within 14-days of their initial symptoms. All subjects provided written informed consent.

3.3.2 Clinical Data Collection

The study procedures included a medical evaluation (clinical characteristics, demographics, medical history and medications) collected through patient self-report and corroborated when possible by medical charts. Alcohol use was defined by self-report of >2 alcohol drinks per day; tobacco risk was defined by past or previous regular use of tobacco. All TIA patients were managed according to current stroke prevention guidelines.

A standardized neuropsychological battery was administered by a trained researcher at both time points that closely aligned with that of the Alzheimer's Disease Network Initiative (243), supplemented with tests for visual memory and frontal executive function. The memory domain was tested with the Brief Visuospatial Memory Test – Revised (BVMT), total and delayed recall (244) and the Rey Auditory Verbal Learning Test, total trial 6 (245, 263). The processing speed domain was tested by the WAIS-IV Digit Symbol Coding test (246). The executive functioning domain was tested with the Trail Making Test B (247, 264). Other tests included the mean ordinal raw score (1-7) of the Addenbrooke's Cognitive Examination Revised (ACE-R) categorical verbal fluency and total score (248, 249); the Montreal Cognitive Assessment (MOCA) (250); the premorbid intelligence calculated from the North American Adult Reading test (NAART) (251); and depressive symptoms included the mean raw score from the Center for Epidemiologic Studies Depression Scale (CES-D), total (252).

3.3.3 Image Acquisition Protocols

Participants underwent MR imaging using a 3.0T Diagnostic MR Scanner (General Electric Discovery 750), using a standard head coil. ASL images, T1-weighted images, T2-weighted fluid-attenuated recovery (FLAIR) sequences, and diffusion weighted imaging (DWI) sequences were analyzed. The 3D fast-spin-echo Pseudo-Continuous Arterial Spin Labelling (pCASL) with a background suppression had the following parameters: post-labelling delay = 2025 ms, field of view (FOV) = 22.9cm, repetition time= 4898-4985 ms, echo time = 10.80-11.72 ms, seventy-four 3.5-4.0 mm slice with a 4 mm gap, and spiral acquisition with 1024 points and eight arms. Quantitative CBF maps were generated by the MR imaging scanner automatically from the ASL datasets in CBF units (mL/100g/min) using the following formula:

$$CBF = 6000\left(\frac{\left(\left(\lambda - e^{-\frac{ST}{T1T}}\right)e^{\frac{PLD}{T1B}}\right)}{(2T1B(s))\left(1-e^{\frac{LT}{T1}}\right)\varepsilon \times NEX}\right)\left(\frac{\Delta M}{SF \times PDref}\right) \quad (276)$$

where T1B and T1T represent blood and tissue T1, values (1.6s at 3T), respectively, λ is the partial coefficient set to 0.9, ε is the efficiency and is set to 0.80 x 0.75, ΔM is the difference between tag and no tag images, PD_{REF} is the reference proton density images, NEX is the number of excitations, SF is a scaling factor of 45, and PLD is the post-labelling delay (277). The 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 slice with a 0 mm gap; acquisition matrix = 256 x 256; TE = 2.932 ms; TR = 6.66 ms; flip angle = 8°; inversion time (TI) = 650 ms; phase FOV = 85%; reconstructed voxel size = 5392 mm isotropic). The 2D DWI sequence was acquired with an acquisition matrix size = 256 x 256, FOV = 28 cm, twenty-three 3.0 mm slice

with 0 mm gap; TE = 81.7 ms, TR = 8000 ms, flip angle = 90°. The 3D T2-weighted FLAIR sequence was acquired with an acquisition matrix size = 512 × 512; FOV = 24 cm; thirty-eight 3.0 mm slice with a 0 mm gap; TE = 120.0 ms, TR = 8524 ms, TI = 2219 ms, flip angle = 90°. Quality assurance to detect poor image quality (low signal-to-noise, or high levels of image artifact or distortion) was manually performed to ensure image quality.

3.3.4 Image Processing

All image data was anonymized and reading/processing of scans was performed blinded to all demographic and outcome data. Each imaging modality was processed independently. Specifically, formal adjudication of the image quality was graded according to neck and head movement, quality of registration and intensity inhomogeneities by two trained individuals (PAB, MR). ASL images were checked for arterial transit time heterogeneity by calculating the spatial coefficient of variation (CoV) (278). All scans were categorized as 'good' (CoV $\leq$ 0.6, cerebral blood flow signal predominates artefacts) (278, 279).

3.3.5 ASL-T1 Registration

Structural T1-weighted images and ASL-CBF images underwent BET brain extraction from 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 degree FSL-FIRST registration (280). Regions of interest were extracted from the T1 images with FSL and subsequently masked to the corresponding ASL-CBF image in T1 space (280). Whole brain white and grey matter of each hemisphere was segmented with FSL FAST (259). The hippocampus, thalamus and basal ganglia was segmented with FSL FIRST (281). The anterior

cingulate, entorhinal cortex, posterior cingulate and precuneus were segmented with the Desikan-Killiany atlas from FreeSurfer (282). The mean cerebral blood flow (mL/min/100g) for each region of interest was generated using MatLab 2017. The image processing steps were quality controlled by visual inspection for each participant. FSL brain tissue volume, normalised for subject head size, was estimated with SIENAX, part of FSL (253, 283).

3.3.6 White Matter Hyperintensities and Diffusion Lesions

White matter hyperintensities (WMH) were segmented from FLAIR images using Cerebra-WML software, a semi-automated software measuring the volume (mL) of white matter lesions based on the global threshold and contrast between the different anatomical regions of our brain (260). Diffusion lesions were segmented from the DWI images with ITK-SNAP (Version 3.6.0, www.itksnap.org), a semi-automatic software application used to segment structures in 3D medical images by a trained researcher (MR) and underwent quality control by a stroke neurologist (PAB) (261). The apparent diffusion coefficient decline associated with acute ischemia persists for 10 days and then normalizes, but the T2 component of the DWI lesion persists much longer for up to several weeks. Therefore, the <14 days window should identify acute DWI lesions with confidence and addresses the practical constraint of obtaining acute MR imaging for TIA patients.

3.3.7 Statistical Analyses

All statistical analysis was performed on STATA IC/15.0 (StataCorp. 2017. Stat Statistical Software: Release 15. College Station, TX: StataCorp LLC). Statistical analysis included the use of descriptive statistics to compare the mean (median), standard deviation (interquartile ranges) and frequency distributions of all variables. Two-way two-sample *t*-tests ($\propto = 0.05$) and Chi

square tests were used to compare risk factors (including demographic, medical, depression and premorbid intellect), cognitive profiles, and regional cerebral blood flow amongst the TIA and control participants. Wilcoxon Rank-Sum Tests were used to compare non-parametric DWI lesion volume and WHM burden between TIA and non-TIA controls. All data for multiple linear regression fit the basic required assumptions including a linear relationship between the outcome variable and the independent variables, normally distributed residuals, no multicollinearity and homoscedasticity. The associations between risk factors (including demographic, medical, WMH burden, depression, MMSE, and premorbid intellect, side of TIA) and regional CBF was tested using a backwards stepwise multiple linear regression. Variables with the highest p-values were removed until the adjusted R-square value no longer improved. Multiple linear regression was performed to analyze the associations between regional CBF and cognitive profiles while controlling for TIA status, age, sex, hemisphere of TIA, premorbid intellect, WMH and NBV.

3.4 Results

3.4.1 Demographics

Forty-five (45) TIA patients and 60 control participants were initially included into the study following inclusion criteria, complete clinical/demographic information and complete MR imaging sequences. Seven (7) TIA and 5 control participants were excluded upon imaging quality control. Poor registration in the 12 cases were due to motion artifact (2) and ASL quality (10) (e.g. artefact, uneven head angle, issues with pre-processing). A total of 38 TIA patients and 55 control patients were included in the analysis.

Demographic, clinical and vascular factors for both the TIA and control population are summarized in Table 3-1. TIA and control populations did not significantly differ in sex, age, education and all other factors, with the exception of DWI (p<0.001) and cholesterol treatment

(p=0.004). Of the TIA patients, 25 (65.8%) had a left side TIA and 13 (34.2%) had a right side TIA. Further, the regions of DWI lesions were mostly supplied by the middle cerebral artery (n=11, 73.3%), with 20% (n=3) supplied by the posterior cerebral artery and 7.0% (n=1) from the anterior cerebral artery.

Table 3-1. Demographic information and medical information of TIA and control participants compared with two-way two-sample *t*-tests and Chi square tests ($\propto = 0.05$). IQR = interquartile range, WMH = white matter hyperintensities normalized by brain volume, NBV = normalized brain volume, DWI = diffusion weighted image lesion.

Descriptive Variables	**TIA (n=38)**	**Control (n=55)**	**p**
Age, *years*	68.08 ± 7.5	66.87 ± 7.0	0.430
Sex, *n women* (%)	17 (44.7%)	31 (56.4%)	0.275
Education, *years*	13.84 ± 3.6	14.65 ± 2.4	0.201
Depression, *raw CES-D*	10.55 ± 7.9	9.02 ± 7.7	0.352
Premorbid Intellect, *NAART*	107.4 ± 10.1	109.6 ± 9.0	0.282
WMH, *median* (IQR)*	2.62 (1.0-4.7)	1.38 (0.8-3.9)	0.128
NBV, cm^3	1439 ± 90.9	1458 ± 91.1	0.302
Systolic BP, *mmHg*	133.8 ± 21.7	129.1 ± 16.8	0.243
DWI, *n (%)*	15 (39.5%)	0	**0.000**
DWI Volume, *median* mm^3*(IQR)*	154.3 (57.4-420.0)	0	**0.000**
Cholesterol Treatment, *n (%)*	21 (55.3%)	17 (30.9%)	**0.019**
Hypertension Treatment, *n (%)*	17 (44.7%)	16 (29.1%)	0.124
Diabetes, *n (%)*	4 (10.5%)	4 (7.27%)	0.587
Tobacco, *n (%)*	20 (52.6%)	21 (38.2%)	0.171
Alcohol, *n (%)*	4 (10.5%)	3 (5.45%)	0.368

*Wilcoxon-Rank Sum tests.

The formal neuropsychological tests for TIA and control are summarized in Table 3-2. We found control participants had significantly higher MMSE scores (p=0.03), ACE-R scores (p=0.008), WAIS Digit Symbol coding scores (p=0.002) and Trail Making Scores (p=0.004 and 0.008, respectively) than non-TIA controls.

Table 3-2. Median, interquartile ranges and Wilcoxon Rank-Sum tests of cognitive domains comparing TIA and control participants ($\propto = 0.05$). 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.

	TIA (n=38)		**Control (n=55)**		**p**
	Median	IQR	Median	IQR	
MMSE	29.0	27.0-30.0	30.0	29.0-30.0	**0.003**
MOCA	25.0	23.0-28.0	26.0	25.0-28.0	0.149
ACE-R	91.0	85.0-95.0	95.0	91.0-97.0	**0.008**
BVMT Total	19.5	16.0-25.0	23.0	17.0-28.0	0.080
BVMT Delayed	9.00	6.00-11.0	10.0	8.00-11.0	0.122
RAVLT	8.00	6.00-12.0	10.0	7.00-12.0	0.277
WAIS-IV DS Coding	51.0	45.0-60.0	64.0	51.0-76.0	**0.002**
TMT A	37.0	28.9-46.8	30.0	24.0-37.1	**0.004**
TMT B	88.0	65.0-99.1	67.4	55.0-88.0	**0.008**
Verbal Fluency, *raw total from ACE-R*	7.00	5.00-7.00	7.00	6.00-8.00	0.469

The cross-sectional regional cerebral blood flow (ASL-CBF) (mL/min/100g) were compared between the TIA and control participants using independent Wilcoxon Rank-Sum tests [Table 3-3]. The median white matter and grey matter CBF were not significantly different in TIA and control populations. Compared to control subjects, patients with TIA showed lower regional CBF in the left entorhinal cortex (p=0.001), both the left and right posterior cingulate (p=0.035 and 0.038, respectively) and the right precuneus (p=0.030). There were no significant differences between the regional CBF in the TIA and non-TIA controls in the white matter, grey matter, hippocampus, thalamus, basal ganglia, anterior cingulate, right entorhinal cortex and left precuneus.

Table 3-3. Median, interquartile range and Wilcoxon Rank-Sum tests of regional cerebral blood flow (mL/min/100g) comparing TIA and control participants ($\propto = 0.05$).

		TIA (n=38)		**Control (n=55)**		***p***
		Median	IQR	Median	IQR	
White Matter	Left	37.6	31.1-45.8	39.9	33.6-47.2	0.470
	Right	36.4	29.1-45.1	39.4	30.9-44.1	0.226
Grey Matter	Left	42.1	31.7-50.0	44.7	35.9-52.5	0.419
	Right	40.5	30.2-48.3	44.1	33.1-50.0	0.272
Hippocampus	Left	40.2	34.1-42.8	42.0	34.1-46.2	0.206
	Right	38.8	31.8-44.3	40.6	34.5-46.5	0.238
Thalamus	Left	40.3	35.6-46.8	43.2	36.3-48.5	0.201
	Right	40.6	32.8-46.5	43.6	36.7-48.5	0.111
Basal Ganglia	Left	37.9	34.2-42.4	38.0	35.5-42.0	0.708
	Right	36.8	33.6-41.7	37.8	34.1-41.0	0.684
Anterior Cingulate	Left	52.0	44.9-59.7	56.2	50.2-66.4	0.067
	Right	49.0	43.8-56.1	53.7	46.7-61.1	0.063
Entorhinal Cortex	Left	36.0	29.5-38.2	40.3	35.2-44.6	**0.001**
	Right	35.1	30.7-41.7	37.6	31.6-41.6	0.333
Posterior Cingulate	Left	46.5	39.1-55.9	52.5	42.6-61.5	**0.035**
	Right	49.7	40.1-56.5	53.6	43.7-62.6	**0.038**
Precuneus	Left	41.5	36.2-52.4	47.0	38.4-59.6	0.074
	Right	41.8	34.7-55.9	50.1	39.3-62.2	**0.030**

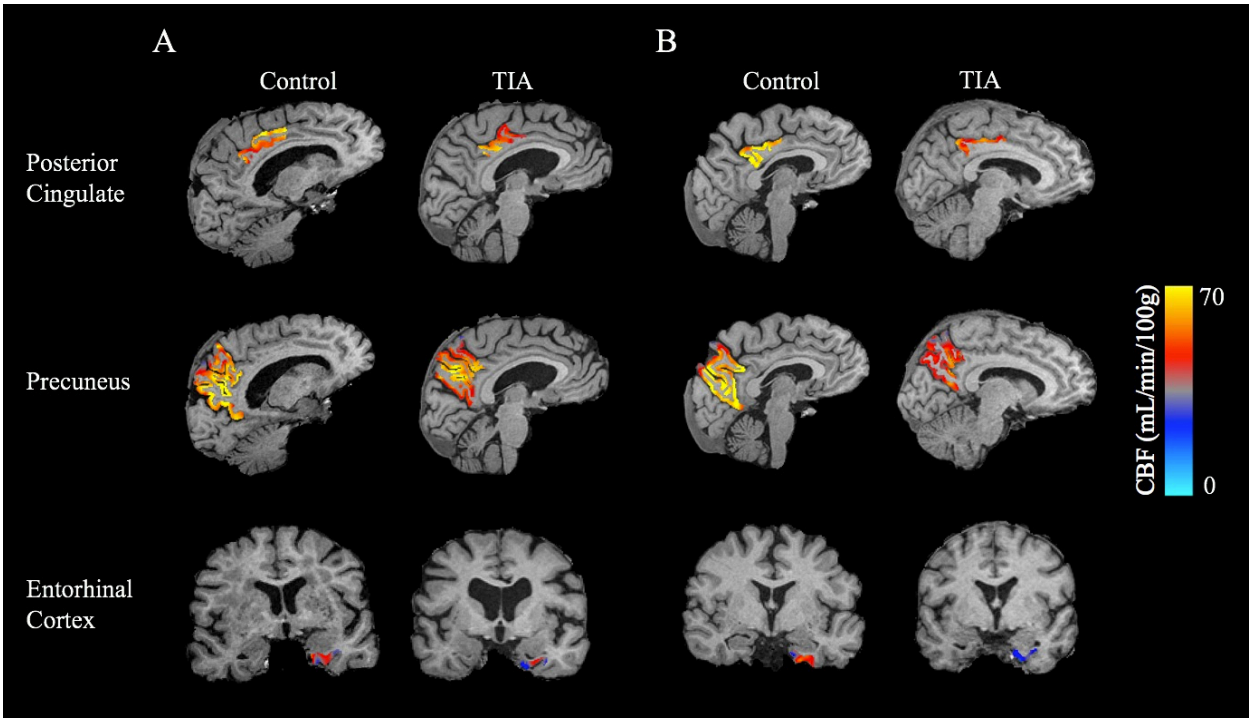

Figure 3-1. A comparison of quantitated cerebral blood flow (mL/min/100g) of the posterior cingulate, precuneus and entorhinal cortex acquired by ASL-MR overlaid on the anatomical T1-weighed MR image of age, sex and education matched TIA patients and controls. **A)** Female, aged 77, 16 years of education. **B)** Male, aged 68, 12 years of education.

3.4.2 Multiple Linear Regression

Increased education was associated with increased cerebral blood flow in both hemispheres of the white and grey matter. Further, age was significantly associated with right white matter hemispheric cerebral blood flow and depression was significantly associated with left grey matter cerebral blood flow. Decreased CBF in the hippocampus was associated significantly with males, higher NBV, high WMH volumes (right) and high systolic blood pressure (left). Reduced CBF in the entorhinal cortex was associated with age, hemispheric side of TIA (left) and increase in systolic blood pressure. In the posterior cingulate, CBF was associated with males and high

systolic blood pressure. In the precuneus, CBF was associated with males, high WMH volumes (left) and side of TIA (right). Overall, 6/12 regions of interest were significantly associated with males and 4/12 were associated with high systolic blood pressure. Control/TIA status, premorbid intellect, DWI lesion volume, cholesterol treatment, antihypertensive treatment, diabetes and tobacco use were not found to be significant in any model and were not included in the table [Table 3-4].

Table 3-4. Backwards stepwise multiple linear regression ($\propto = 0.05$) was used to analyze the associations between risk factors (control vs. TIA, age, sex, education, normalized brain volume, premorbid intellect, depression, white matter hyperintensities/normalized brain volume, side of TIA, DWI volume, systolic blood pressure, cholesterol treatment, antihypertensive, diabetes and tobacco) and regional cerebral blood flow (mL/min/100g). CESD = depression, NBV= normalized brain volume, WMH = white matter hyperintensities normalized by brain volume, DWI = diffusion weighted imaging lesion volume, BP = blood pressure, Adj = adjusted, L = left, R = right.

		Age	**Sex**	**Education**	**CESD**	**NBV**	**WMH**	**Side of TIA**	**Systolic BP**	**Adj R Square**
White Matter	L			0.003						0.146
	R	0.032		0.017						0.101
Grey Matter	L			0.002	0.026					0.159
	R			0.006						0.126
Hippocampus	L		0.007			0.020				0.142
	R		0.008			0.031	0.033			0.166
Entorhinal Cortex	L	0.011						0.028	0.008	0.219
	R	0.020							0.007	0.195
Posterior Cingulate	L		0.006						0.042	0.200
	R		0.001						0.017	0.233
Precuneus	L		0.000				0.033			0.251
	R		0.000				0.041	0.029		0.273

Decreased cerebral blood flow (mL/min/100g) in both hemispheres of the hippocampus, the posterior cingulate and precuneus were significantly associated with lower RAVLT scores.

Decreased CBF in the right entorhinal cortex was also significantly associated with poorer RAVLT scores [Table 3-5].

Table 3-5. Multiple linear regression analyzing the associations between regional cerebral blood flow (mL/min/100g) and cognitive scores. This model is controlling for TIA status, age, sex, premorbid intellect, side of TIA, white matter hyperintensities and normalized brain volume. Hip = hippocampus, E.C. = entorhinal cortex, P.C. = posterior cortex, Pre = precuneus, L= left, R = right, BVMT = Brief Visuospatial Memory Test, RAVLT = Rey Auditory Verbal Learning Test, DS Coding = Digit Symbol Coding, TMT = Trail Making Test, P = p-value, R^2=adjusted R-squared.

		BVMT Total		BVMT Delayed		RAVLT		TMT A		TMT B		Verbal Fluency	
		P	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2
Hip.	L	0.124	0.137	0.755	0.042	**0.011**	0.158	0.085	0.133	0.664	0.241	0.169	0.026
	R	0.247	0.126	0.697	0.042	**0.019**	0.147	0.283	0.113	0.797	0.240	0.684	0.005
E.C.	L	0.285	0.124	0.719	0.042	0.141	0.113	0.760	0.102	0.627	0.242	0.475	0.009
	R	0.056	0.150	0.278	0.054	**0.032**	0.138	0.175	0.121	0.855	0.240	0.379	0.013
P.C.	L	0.101	0.140	0.509	0.046	**0.019**	0.148	0.270	0.114	0.763	0.240	0.241	0.020
	R	0.137	0.135	0.486	0.046	**0.029**	0.140	0.192	0.119	0.983	0.239	0.312	0.015
Pre.	L	0.073	0.145	0.313	0.052	**0.012**	0.156	0.309	0.112	0.563	0.242	0.483	0.009
	R	0.071	0.146	0.337	0.051	**0.009**	0.162	0.193	0.119	0.476	0.244	0.337	0.014

3.5 Discussion

Our study can be summarized by the main findings: 1) TIA patients have significantly lower regional CBF in the left entorhinal cortex, both the left and right posterior cingulate and the right precuneus than non-TIA controls, 2) Lower CBF in the hippocampus, left entorhinal cortex, posterior cortex and precuneus is significantly association with poorer RAVLT scores, which is a marker of short-term auditory verbal memory 3) Lower CBF is related to sex and hypertension. Further, we confirm the finding of mild cognitive deficits in TIA patients (82) and demonstrate the

potential association of CBF with sex and hypertension. Reports have measured lower global and regional CBF measured by ASL in subjects with mild cognitive impairment (MCI) and AD when compared to control subjects (17, 34, 286–289, 62, 72, 166, 179, 181, 182, 284, 285). Lower CBF measured by ASL-MR imaging has also been shown to distinguish participants with MCI compared to those with AD, and regional hypoperfusion has been used to predict the conversion of MCI to AD (180, 181, 287, 290, 291). Most consistently, MCI patients who convert to AD show decreased precuneus and/or posterior cingulate blood flow (with atrophy correction) (180, 181, 287, 290, 291). We did not find an increase in hippocampal CBF, which is frequently found in MCI patients and is hypothesized to represent a compensatory mechanism at early stage of disease progression (180). Further longitudinal studies will provide insight into how lower CBF and regional CBF changes might predict cognitive decline.

Our study shows that six out of twelve regions show an association with males and reduced CBF, and four out of twelve regions show higher systolic blood pressure is associated with reduced CBF. Higher age is significantly associated with CBF decreases in both sides of the entorhinal cortex and right white matter. WMH burden is significantly associated with reduced CBF in the right hippocampus, and left precuneus, supporting an association of remote regional and cortical CBF changes with small vessel disease. The side of TIA is significantly associated with CBF in the left entorhinal cortex and right precuneus. This is likely not due to ischemia as the medial temporal structures are perfused by the posterior cerebral artery and most cases have symptoms in the anterior circulation, as 65.8% of our TIA patients have left hemisphere TIAs. Higher education is significantly associated with higher cerebral blood flow in both hemispheres of the white and grey matter, but not for any other regions of interest. We find no association with CBF and

control/TIA status, premorbid intellect, DWI lesion volume, cholesterol treatment, antihypertensive treatment, diabetes or tobacco use.

It is hypothesized that high blood pressure may dysregulate the cerebral blood flow system and lead to cerebral hypoperfusion (64). In combination with endothelial dysfunction in the small cerebral vessel, this dysregulation may result in chronic cerebral hypoxia (64). Alternatively, hypoperfusion may accelerate the hyperphosphylation of tau protein, contributing to accelerated neurofibrillary plaque accumulation. Medial temporal lobe neurofibrillary tangles have been related to cognitive function in patients with mild cognitive impairment (292, 293), and AD. In post-stroke patients, reduced CBF in the parietal lobe has been shown to accurately predict the onset of post-stroke dementia (294). Further, low CBF, rather than hippocampal volume predicted a lower Cambridge Cognition Examination – Revised score (CAMCOG), in those going onto develop post stroke dementia, which is in accordance with the suggestion that vascular damage as a result of stroke might be in some way responsible to the increased susceptibility of the brain to dementia (294). Regardless of the mechanisms, regions of interest with substantially decreased CBF in the hypertension group may be representative of functional decline over time (72, 295). Functional decline in these regions are far reaching, as decreases in the entorhinal cortex and posterior cingulate may impact spatial memory, memory formation and memory consolidation (296, 297). This finding of hypoperfusion in these anatomical regions of interest and the relationship of low CBF with hypertension in this cohort further support the need for rigorous blood pressure control with anti-hypertensive medication, even beyond current recommended guidelines on individualized basis (298).

Our study reveals that decreased CBF in the hippocampus, posterior cingulate, precuneus and left entorhinal cortex is associated with poorer performance on tests of verbal memory. In MCI

patients, hypoperfusion in the right middle frontal cortex has been shown to predict subsequent episodic memory decline from the California Verbal Learning Test (181). In amnestic MCI subjects, significant hypoperfusion in the right precuneus, cuneus and posterior cingulate correlate with lower RAVLT scores during a memory-task with functional ASL-MR imaging (182). These results indicate that mild memory impairment is related to the hypoperfusion in these regions of interest, suggesting that perfusion deficits in these brain regions are consistent with the symptomatology of AD. Still unknown is whether the observed hypoperfusion is contributing to the cognitive deficit, or is an epiphenomenon (i.e. the hyoperfusion is secondary to underlying neurodegenerative process).

ASL-MR imaging may be an important biomarker to predict preclinical disease and identify potential therapeutic techniques or treatment. In a prospective study with 163000 nondemented participants, it was found that physical activity reduces the risk of dementia and AD by 28% and 45%, respectively (175). Smith et al. (2010) found in a large systematic review of RCTs, that subjects attending organized exercise groups show improvement in attention, processing speed and executive function, however the effects on memory were not consistent (176). Regional cerebral blood flow is a promising pre-clinical biomarker and may allow for randomized control trials to effectively identify an appropriate population for which multiple interventions – including exercise or antihypertensive treatments - may show the greatest effect in the future.

In summary, in this study we show that TIA patients have significant decreases in regional CBF measured by ASL-MR imaging compared to non-TIA controls, suggesting the existence of vascular dysregulation. High systolic blood pressure is also associated with many regional CBF decreases. Our study shows significant association with regional CBF in hippocampus, posterior

cingulate and precuneus and verbal memory. The causes and implications of regional hypoperfusion in pre-clinical dementia are largely unknown and cannot be answered by this study. Future longitudinal studies will need to explore the potential clinical, cognitive, genetic and biological causes of CBF decreases. Specifically, investigations should explore what biomarkers are able to determine patients at the highest risk of cognitive decline and what modalities are sensitive to measure the effects of preventative treatments. The PREVENT study intends to determine the collective and cumulative effects of cerebral blood flow changes, white matter damage (small vessel disease), and molecular markers of in vivo AD or other age-related neurodegenerative processes that lead to increased rates of whole brain and hippocampal atrophy that can be measured by structural MR imaging over a five-year period following the original presentation of TIA. Longitudinal data of pre-clinical neuroimaging biomarkers, including regional CBF, will refine inclusion criteria and optimize the success of secondary prevention randomized control trials in the goal of preventing late-life cognitive decline.

CHAPTER FOUR: THESIS CONCLUSIONS

4.1 Overall Conclusions

TIA patients are at a high risk of late-life dementia and represent an ideal population to study the progressive pre-clinical phase of dementia that spans decades prior to clinical signs of cognitive decline (9, 10, 49). To date, there are few published longitudinal studies that have explored potential neurodegenerative and vascular disease processes that may synergistically co-exist and may predict the future risk of cognitive decline (48, 59, 270). The aim of this thesis was to study longitudinal cerebral atrophy rates and cross-sectional regional cerebral blood flow changes in TIA patients and see whether such changes predicted cognitive changes in this cohort. In Chapter 2 we aimed to determine whether patients presenting with TIA had greater change of whole brain volume over 1 year after TIA than volunteer controls even before a change in cognition could be detected. In Chapter 3 we explored one potential mechanism of cognitive impairment in TIA patients compared to controls by measuring regional CBF measurement using MR ASL in a cross-sectional analysis utilizing baseline data for participants in the PREVENT Study.

In Chapter 2, our study was consistent with others (86, 87) that showed that TIA patients experienced an almost doubled cerebral atrophy rate (ml/year) over one year compared to age and education matched controls. In both cohorts, age and higher systolic blood pressure were significantly associated with increased cerebral atrophy rates. In addition, detailed neuropsychological assessment revealed the TIA cohort exhibited worse cognitive outcomes in tests of memory, processing speed and executive function at baseline compared to controls, and cognitive performance did not deteriorate significantly over one year. Nor did we see a relationship with whole brain atrophy rates and change in cognitive performance, supporting the concept that this cohort of TIA patients are potentially at risk of cognitive impairment, but as yet have no objective deterioration in cognition. Longitudinal examinations are necessary to associate neuronal

loss in this population with cognitive decline, which will establish whether TIA patients represent an early stage of dementia. Future methodology should continue the investigations beyond one year and should include at least three observational time points to establish trajectories of cerebral atrophy. These future investigations will allow us to determine the demographic and life-style risk factors that are associated with increased neurodegeneration and subsequently determine an individual's future risk of cognitive decline – and the most robust multidomain interventions to prevent late-life dementia.

In Chapter 3, we showed that TIA patients exhibited decreased cerebral blood flow in multiple anatomical regions, namely, the left entorhinal cortex, the posterior cingulate gyri and the right precuneus compared to non-TIA controls. This is consistent with MCI literature, that shows those who convert to AD frequently have regional CBF deficits in the precuneus and posterior cingulate blood flow (180, 181, 287, 290, 291). This is observation is potentially important because hypoperfusion in these regions have been postulated to accelerate the hyperphosphylation of tau protein contributing to accelerated neurofibrillary tangle accumulation, and plaques in the medial temporal lobe are related to early cognitive dysfunction in patients with MCI and AD (292, 293). Conversely, chronic cerebral hypoperfuion may exacerbate AD pathology by increasing the production of amyloid-beta (cerebral amyloid angiopathy) (299) or interfering with autoregulation and neurovascular coupling (300, 301). Males and higher systolic blood pressure were commonly associated with decreased cerebral blood flow in both cohorts. This is consistent with research that suggests that high blood pressure may dysregulate microcirculatory control of blood flow in the brain and lead to cerebral hypoperfusion (64), which could in turn lead to chronic cerebral hypoxia or accelerate tau plaque formation early in the disease process. We showed decreased cerebral blood flow in the hippocampus, entorhinal cortex, posterior cingulum and precuneus is associated

with poorer performance in the RAVLT verbal memory test. Longitudinal CBF measurements exploring the relationship with cognitive decline and whole and regional brain atrophy will help strengthen our understanding of the vascular risk factors and dementia progression. ASL-MR imaging is a promising biomarker, however concerns over standardization and clinical cut-off values of normality remain valid, so more data is required. This study described in Chapter 3 is consistent with the cognitive data in Chapter 2, with TIA patients demonstrating poorer cognitive performance at baseline than non-TIA controls. Further, we showed that TIA patients have higher cerebral atrophy rates and regional cerebral blood flow deficits when compared to non-TIA controls at a stage where they are not clinically demented. This suggests that CBF measured by ASL MR imaging may be helpful in identifying underlying incipient disease processes in TIA patients, a population at increased risk of dementia.

4.2 Limitations and Future Directions

As with any case-control study, we were wary of 1) demographic matching between TIA and controls 2) self-selection bias in controls 3) longitudinal study attrition. Convenience sampling of cognitively normal participants is vulnerable to self-selection bias, because these participants tend to be more motivated than randomly recruited controls (302). Those willing to participate in dementia-related studies tend to be younger (303–305), better educated (303–306) and have a family history of Alzheimer disease (305), thus, carrying the APOE E4 allele more frequently (302). Volunteers are significantly more likely to be women (304), and furthermore women with TIA and stroke are underrepresented in research as they may be less likely to have stroke in mid-life (307, 308), are less aware of the symptoms, may have atypical TIA symptoms, and do not always benefit equally from treatment and care (309). Our studies matched age and education of our community and spousal controls to our TIA patients. While our groups were not significantly

different for age, education and sex, our controls remained younger, more highly educated and a higher percentage of women. Future studies with larger pools of controls would provide an opportunity for better matching between the clinical and control populations. However, we believe our control group is performing at a similar level as published cognitive norms, and therefore we have some confidence that our controls represent a 'normal population'.

Attrition bias in dementia research can arise in both the clinical group, especially if the risk factors under study are associated with attrition (310). Currently, our longitudinal analysis spanned only 1-year, however, we should be wary of "drop out" and mortality of older and sicker participants and include sensitivity analyses to illuminate the robustness of our findings in the future (310). Compared to stroke patients, TIA patients have reduced attrition rates caused by stroke-related co-morbidity and frailty (89, 311). Currently, the PREVENT study has experienced attrition by 10% of TIAs and 5% of controls within the 1-year follow-up, and this is well within our estimated attrition of 20%.

The reliability of neuropsychological assessments of preclinical disease have limitations because performance is commonly affected by age, education, ethnicity, co-morbid treatments (232), and ceiling/floor effects and learning effects, which can limit our ability to detect change over time within a subject (130–134). We minimized learning effects by administering different versions of cognitive tests at baseline and one-year. Compared to brain atrophy measurements, standard cognitive testing has a higher inter-individual variability, up to a 10-fold decrease in clinical trial power and fails to provide objective support for disease-modifying effects (236, 272). Therefore, clinical trials in predementia stages of disease will have to employ biomarkers. These biomarkers will need to have the following characteristics: 1) accurate 2) highly reproducible 3)

standardized 4) sensitive to change over time 5) capable of detecting treatment effects 6) operationally straightforward.

Rates of cerebral atrophy are integral to biological pathways associated with preclinical progression of vascular disease and AD, and as such, are frequently used as an accurate and reliable disease biomarker for clinical trials (27, 126–129). Brain atrophy rates are highly sensitive at predicting cognitive decline and can be measured more accurately than neuropsychological outcomes, which can be confounded by many factors (130–134). However, cerebral atrophy remains a global, non-specific marker of diffuse neurodegenerative processes and does not highlight the regional neuropathological changes that occur during the progression of AD. To get a more precise understanding of the disease processes, future research should investigate atrophy rates in focal regions of interest in a TIA population, such as the hippocampus, which has been studied extensively in AD and MCI (142–144). Disease progression must be analyzed over several time points to validate the reliability and sensitivity of brain atrophy rate measurements. This is of importance in pre-clinical dementia trials, where current clinical outcomes have a limited ability to detect treatment effects within the typical time frame of a clinical trial – whereas structural MR imaging has been shown to detect change over 12 months.

A longitudinal study of further MR imaging modalities is warranted to fully understand the neuropathological changes that precede cognitive decline in the TIA cohort. The PREVENT study aims to measure atrophy (hippocampal and whole-brain), white matter tissue integrity and whole brain connectivity (diffusion tensor imaging), iron accumulation (quantitative susceptibility mapping) and cerebral blood flow (arterial spin labelling) over 5-years. In combination with CSF and blood biomarkers for AD, clinical and cognitive data, this neuroimaging data will piece together the poorly understood timeline of the preclinical prodromal phase of dementia and

discover what parameters predict increased rates of atrophy and decreases in cognition. In the short-term, these predictors of early neurodegeneration can inform vascular risk management. Longitudinal neuroimaging studies will also provide insight into the ideal inclusion criteria for clinical trials by identifying patients at the highest risk of cognitive decline. It has become apparent that disease-modifying drugs and interventional therapies must be administered in the earliest and possibly pre-symptomatic stages of cognitive decline, before the disease process becomes too advanced. It is these patients that are potentially most likely to benefit from vascular risk-reduction therapies if the timing and type of intervention are adjusted for optimal effectiveness (103).

Despite a costly effort to find therapies to treat established AD dementia, treatments to modify the disease have failed (94–100, 103–105). This is perhaps reflective of the treatments not reaching the biological target, the treatment being administered when the disease is too advanced (end stage), or inappropriate patient selection. Large trials have traditionally recruited patients with mild to moderate cognitive symptoms in which the disease processes may be too advanced to modify with interventions. Therefore, the focus has changed from treated established disease to preventing disease, which is the subject of secondary prevention. As highlighted in this thesis, an alternative approach is prevention in subjects with vascular risk factors who are at a high-risk of late life cognitive decline, such as TIA patients.

Large scale multidomain prevention RCTs, including FINGER and MAPT, focus on intervention involving diet, exercise, cognitive training, and vascular risk monitoring to improve or maintain cognitive function in at-risk elderly people from the general population (100, 103, 104). These RCTs have struggled to provide conclusive evidence of therapeutic interventions because they are often conducted over short periods of time on patients with mild-moderate dementia and have a focus on cognitive screening as an outcome (100, 103, 104). In secondary

prevention trials targeting predementia AD and vascular disease, it may be challenging to meet clinical end-points with reasonable sample sizes in the context of the typical length of a RCT over 2-4 years, especially in the pre-clinical phase (312). Therefore, there is an urgent need for biomarkers that reflect slowing of disease progression. The data presented in this thesis supports the first critical step in determining the risk of dementia in TIA patients using cerebral atrophy and regional cerebral blood flow as a surrogate of incipient and potentially modifiable disease before cognitive decline occurs. Future studies will investigate the neurobiological basis of these changes in this cohort.

In summary, our data supports the hypothesis that, over 1 year, TIA patients experience almost double the cerebral atrophy rates compared to non-TIA volunteer controls. Additionally, at baseline, TIA patients demonstrate decreased regional cerebral blood flow which is associated with poorer memory scores. High systolic blood pressure appears to be a co-morbid vascular risk factor for both cerebral atrophy rates and reduced regional cerebral blood flow. Therefore, hypertension should continue to be targeted in RCTs. Future studies could also investigate diet and exercise as vascular risk factors in TIA populations. RCTs have remained inconclusive despite the established link between vascular risk factors, stroke, and late-life cognitive decline. Our study design includes demographic, clinical, neuropsychological and neuroimaging markers in a TIA population to provide novel insight into the complex, multiple, co-existing pathologies of AD and CVD. This research adds to the growing literature of neuroimaging biomarkers capable of improving inclusion criteria in secondary prevention trials and subsequently monitoring therapeutic interventions in the goal of preventing neurodegeneration, cognitive decline and dementia.

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APPENDIX A: COPYRIGHT PERMISSION

Chapter 2:

As a co-author of the manuscript entitled "Longitudinal Study of Cognition and Brain Atrophy Rates in Transient Ischemic Attack Patients", I permit Meaghan Reid to use this manuscript in her final thesis.

Signed via e-mail: Noaah Reaume, Pauline Wu, Alex Pan, Arooj Aftab, Rani Gupta Sah, Meng Wang, Richard Frayne, Shelagh B. Coutts, Tolulope T. Sajobi, Richard Stewart Longman, Christopher D. d'Esterre, Philip A. Barber.

Chapter 3:

As a co-author of the manuscript entitled "The Association of Arterial Spin Labelling-MRI Cerebral Blood Flow in Transient Ischemic Attack Patients and Cognition", I permit Meaghan Reid to use this manuscript in her final thesis.

Signed via e-mail: Connor McDougall, Noaah Reaume, Rani Gupta Sah, Meng Wang, Richard Frayne, Shelagh B. Coutts, Tolulope T. Sajobi, Richard Stewart Longman, Christopher D. d'Esterre, Philip A. Barber.

Sana Tariq provided email consent to use modified Table 1-1 and 1-2.