MONASH University

A Study of Mechanisms linking Type 2 Diabetes Mellitus and Dementia

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Abstract

Background: Dementia is highly prevalent in older age, accounts for a significant proportion of age-related disability, and is one of the most expensive disorders affecting older Australians. T2DM affects about 85% of all people with diabetes and occurs more commonly in older age. T2DM increases the risk of vascular dementia and Alzheimer's dementia (AD) although there may be substantial overlap of the two pathologies. The underlying pathways between T2DM and dementia may involve neurodegeneration, vascular disease, or both, with several common intermediary mechanisms.

Aims & methods: The broad aim of this thesis was to study the disease pathways that underlie the association between T2DM and dementia. The majority of the research presented was conducted within the Cognition and Type 2 Diabetes in Older Tasmanians (CDOT) study. A further study was conducted in a second sample, derived from the United States' Alzheimer's disease Neuroimaging Initiative (ADNI).

Results: The main novel results of my thesis are summarised below:

1. Brain atrophy is a key mediator of T2DM-related cognitive impairment and the regional distribution of brain atrophy seen in T2DM appears similar to that seen in early AD.

2. Tissue advanced glycation is associated with brain atrophy in T2DM (and in those without T2DM) and may partially mediate the association between T2DM and brain atrophy.

3. T2DM is associated with excess production of CSF phosphorylated tau, and this partially mediates the association between T2DM and reduced cortical thickness, providing the first in-vivo evidence mechanistically linking T2DM with neurodegenerative AD-type pathology.

4. Retinal vascular architecture and retinopathy (subclinical markers of small cerebral vessel disease) were not associated with MRI biomarkers of T2DM-related brain disease, raising speculation about the relative importance of vascular pathways leading to brain disease in people with T2DM receiving good glycaemic and vascular risk control.

Conclusions: Brain atrophy is a key mediator of diabetes-related cognitive impairment and mechanisms similar to that seen in AD may play a role in T2DM-related cognitive impairment. These findings do not exclude the possibility that cerebrovascular disease or other non-AD-type processes contribute to T2DM-related cognitive impairment. A greater understanding of the mechanisms linking T2DM and dementia may facilitate development of new avenues for treatment of dementia.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Thesis including published works General Declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes 4 original papers published in peer reviewed journals and 2 unpublished publications. The core theme of the thesis is A Study of Mechanisms linking Type 2 Diabetes Mellitus and Dementia. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the Stroke and Ageing Research Centre under the supervision of Associate Professor Velandai Srikanth and Professor Thanh Phan.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of Chapters 1, 4, 5, 6, and 7 my contribution to the work involved the following:

Thesis chapter	Publication title	Publication status*	Nature and extent (%) of students contribution
1	Cerebral small vessel disease: a review of clinical, radiological, and histopathological phenotypes	Published	Performed literature review, wrote and revised first draft of and generated figures in manuscript.
4	Brain atrophy in type 2 diabetes: regional distribution and influence on cognition	Published	Developed study questions, collated appropriate data, performed data analysis, wrote and revised first draft of manuscript.
5	Type 2 diabetes, skin autofluorescence, and brain atrophy	Published	Developed study questions, collated appropriate data, performed data analysis, wrote and revised first draft of manuscript.
6	Type 2 diabetes, retinal vascular disease and brain atrophy	Under submission	Developed study questions, collated appropriate data, performed data analysis, wrote and revised first draft of manuscript.
7	Type 2 Diabetes Mellitus and Biomarkers of Neurodegeneration.	Accepted	Developed study questions, collated appropriate data, performed data analysis, wrote and revised first draft of manuscript.

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis

Student signature: [redacted] **Date:** 26/6/15

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student and co-authors' contributions to this work

Main Supervisor signature: [redacted] **Date:** 26-6-2015

Dedicated to Emma, Maureen and Patrick

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I would like to thank my two supervisors Associate Professor Velandai Srikanth and Professor Thanh Phan for their unwavering support and guidance. I would also like to thank Dr Richard Beare and Jian Chen for their advice and guidance regarding neuroimaging analysis and Associate Professor Leigh Blizzard for his statistical advice and patience. Professor Alun Hughes and Dr Nicholas Witt were invaluable in providing advice regarding retinal image analysis. Professor David Bruce and Dr Timothy Greenaway were equally helpful with advice on diabetes related issues.

I would especially like to thank all the participants and staff of the Cognition and Diabetes in Older Tasmanians study and the Alzheimer's Disease Neuroimaging Initiative.

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2. **Moran C**, Phan TG, Chen J, Blizzard L, Beare R, Venn A, Muench G, Wood A, Forbes J, Greenaway T, Pearson S, Srikanth VK. Brain atrophy in Type 2 Diabetes – regional distribution and influence on cognition. *Diabetes Care*, 2013;36:4036-42. (*Received Editorial).*

3. **Moran C**, Münch G, Beare R, Blizzard L, Venn A, Phan TG, Forbes, J, Srikanth V. Type 2 diabetes mellitus and cerebral atrophy – the role of tissue advanced glycation end-products. *Diabetes* 2015;64:279-83.

4. **Moran C,** Beare R, Phan TG, DG. Bruce, Callisaya M. Srikanth V. Type 2 diabetes mellitus, brain atrophy and brain biomarkers in the Alzheimer's Disease Neuroimaging Initiative (ADNI). *Neurology* (accepted 3rd June 2015)

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Papers

1. Callisaya M *****, **Moran C ***, Srikanth V. Type 2 Diabetes Mellitus as a causal factor for dementia – is there sufficient evidence from interventional studies? *Australasian Epidemiologist*, 2013:20:26-28. (*****Joint first author)

2. Mok V, Srikanth V, Phan G, **Moran C,** et al. Ethnicity and Cerebral Small Vessel Disease – Comparison between Chinese and White Populations. *International journal of Stroke*, 2014;9 Suppl A100:36-42.

3. **Moran C**, Kipen E, Chan P, Niggemeyer L, Scharf S, Hunter P, Fitzgerald M, Gruen R. Understanding Post-Hospital Morbidity Associated with Immobilisation of Cervical Spine Fractures in Older People Using Geriatric Medicine Assessment Techniques: A Pilot Study. *Injury* 2013;44:1838-42.

Book Chapters

1. Stroke Epidemiology, Prevention and Management. Brocklehurst's Textbook of Geriatric Medicine (In press, 2015)

2. Stroke: Clinical Presentation, Management and Organisation of Services. Brocklehurst's Textbook of Geriatric Medicine (In press, 2015)

Awards and Scholarships

- Alzheimer's Australia Dementia Research Foundation Scholarship (2012-2015)
- Monash Health, Clinical Academic Teaching Fellowship (2012-2014)
- Career Investigator Award - Australian and New Zealand Society of Geriatric Medicine (ANZSGM) Conference, 2013
- RM Gibson Young Investigator Award – Australian and New Zealand Society of Geriatric Medicine (ANZSGM) Conference, 2012
- Peter Bladin New Investigator Award - Stroke Society Australasia Conference, 2012
- Best New Investigator Prize, Southern Health Research Week, 2012

Published abstracts

1. Climie R, **Moran C**, Callisaya M, Blizzard L, Sharman J, Venn A, Phan T, Beare R, Srikanth V. Abdominal obesity modifies the association between Type 2 diabetes mellitus and brain atrophy. Australasian Journal on Ageing 2014;33:S1:14:40

2. Srikanth V, Blackburn N, Charlesworth J, Callisaya M, Thomson R, **Moran C**, Marthick J, Dickinson J. Diabetes Mellitus (DM) modifies the association between mean relative telomere length and white matter hyperintensity (WMH) volume. Australasian Journal on Ageing 2014;33:S1:14:40

3. **Moran C**, Phan TG, Chen J, Blizzard L, Beare R, Venn A, Münch G, Wood A, Forbes J, Greenaway T, Pearson S, Srikanth VK. Mechanisms of cognitive impairment in Type 2 Diabetes Mellitus. In press, Diabetes (supplement) July 2013

4. **Moran C**, Münch G, Beare R, Blizzard L, Venn A, Phan TG, Forbes, J, Srikanth V. Type 2 Diabetes Mellitus and Brain Atrophy – The role of Advanced Glycation End-Products. Australasian Journal on Ageing 2013;32:S1:6-33

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6. **Moran C**, Phan TG, Chen J, Blizzard L, Beare R, Venn A, Muench G, Wood A, Forbes J, Greenaway T, Pearson S, Srikanth VK. Type 2 Diabetes Mellitus (T2DM) and Cognitive Impairment – the role of Cerebrovascular Disease. International Journal of Stroke 2012;7:S1:9

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8. **Moran C**, Phan TG, Chen J, Blizzard L, Beare R, Venn A, Muench G, Wood A, Forbes J, Greenaway T, Pearson S, Srikanth VK. Type 2 diabetes mellitus (T2DM) and cognition – A cross sectional study. Australasian Journal on Ageing 2012;31:S1:16-33

9. **Moran C**, Phan TG, Chen J, Blizzard L, Beare R, Venn A, Muench G, Wood A, Forbes J, Greenaway T, Pearson S, Srikanth VK. Regional effects of Type 2 Diabetes Mellitus (T2DM) on Grey Matter Atrophy. Australasian Journal on Ageing 2012;31:S1:34-60

Conference presentations

1. Type 2 Diabetes Mellitus, Brain Atrophy and Cerebrospinal Fluid (CSF) biomarkers of Alzheimer's disease, 2015, Australia and New Zealand Society of Geriatric Medicine Conference, Perth, Australia.

2. Type 2 Diabetes Mellitus and Brain Atrophy – The role of Advanced Glycation End-Products, 2013, Australia and New Zealand Society of Geriatric Medicine Conference, Adelaide, Australia.

3. Type 2 Diabetes Mellitus (T2DM) and Cognitive Impairment – the role of Cerebrovascular Disease, 2012, Stroke Society of Australasia, Sydney, Australia.

4. Regional effects of Type 2 Diabetes Mellitus (T2DM) on Neurodegeneration, 2012, Stroke Society of Australasia, Sydney, Australia.

5. Type 2 Diabetes Mellitus (T2DM) and Cognition-A Cross Sectional Study, 2012, Australia and New Zealand Society Geriatric Medicine Conference, Sydney, Australia.

6. Type 2 diabetes mellitus (T2DM) and cognitive impairment – the roles of cerebrovascular disease and neurodegeneration, 2012, European Stroke Congress, Lisbon, Portugal.

7. Type 2 diabetes mellitus and cerebral atrophy – the role of advanced glycation end-products. International Diabetes Federation World Congress, 2013 Melbourne, Australia.

Organization of thesis

The chapters of the thesis are ordered as follows:

Chapter 1: Dementia, Alzheimer's Disease & Vascular Cognitive Impairment

This chapter will introduce dementia, its sub-types, epidemiology, pathophysiology, brain imaging, biomarkers, and pathological characteristics. It will include a discussion on cerebral small vessel disease and will be based on my paper "Cerebral small vessel disease: a review of clinical, radiological, and histopathological phenotypes." published in *International Journal of Stroke*. It will describe the main phenotypes of cerebral small vessel disease, which I have measured and analysed in my studies of T2DM

Chapter 2: Type 2 Diabetes Mellitus and Dementia – a review

An introduction to the association between T2DM and dementia, risk factors explaining the association, potential vascular and neurodegenerative mechanisms, a brief overview of basic and pathological research in the field.

Chapter 3: Study framework

This chapter will provide an overview of the different studies conducted below.

Chapter 4: Brain atrophy in type 2 diabetes: regional distribution and influence on cognition

This chapter will be based on my paper "Brain atrophy in Type 2 Diabetes – regional distribution and influence on cognition" published in *Diabetes Care*. It describes the cross-sectional associations found between T2DM and cognitive impairment, and how grey matter atrophy mediates this association.

Chapter 5: T2DM, skin autofluorescence and brain atrophy

This chapter will be based on my paper "Type 2 diabetes mellitus and cerebral atrophy – the role of advanced glycation end-products." published in *Diabetes*. It describes the role of AGEs, a measure of long-term tissue collagen cross-linking, in mediating the relationship between T2DM and brain atrophy.

Chapter 6: T2DM, retinal microvascular disease and brain atrophy

This chapter will be based on a cross-sectional analysis relating retinal vascular geometry to T2DM and brain atrophy.

Chapter 7: T2DM and Biomarkers of Neurodegeneration

This chapter will be based on my paper "Type 2 diabetes mellitus, brain atrophy and brain biomarkers in the Alzheimer's Disease Neuroimaging Initiative (ADNI)" using a United States-based cohort of people with varying degrees of cognitive impairment and dementia, that has been accepted for publication in *Neurology* on 3rd June 2015. It describes the association of T2DM with cortical thinning and measures of tau but not markers of β amyloid.

Chapter 8: Summary and Future Directions

This chapter will summarize the previous findings, placing them in the context of current knowledge and how they guide future research directions.

A Study of Mechanisms linking Type 2 Diabetes Mellitus and Dementia

Monash University

Declaration for Thesis Chapter 1

Declaration by candidate

In the case of Chapter 1, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Performed literature review, developed figures and wrote draft of included publication	70

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Thanh Phan	Reviewed draft	
Velandai Srikanth	Reviewed draft	

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	**Date** 26/6/15
Main Supervisor's Signature	[redacted]	**Date** 26.6.2015

*Note: Where the responsible author is not the candidate's main supervisor, the main supervisor should consult with the responsible author to agree on the respective contributions of the authors.

Dementia, Alzheimer's Disease & Vascular Cognitive Impairment

1.1Definition

Dementia is described by the Diagnostic and Statistical Manual of Mental Disorders –fifth edition (DSM-5) as a neurocognitive disorder (minor or major depending on the standard deviation from norms in cognitive scores) characterised by cognitive decline from a previous level of performance in one or more cognitive domains (complex attention, executive function, learning and memory, language, perceptual-motor, or social cognition) that interferes with independence in everyday activities (American Psychiatric Association, 2013). **Table 1** presents the essential clinical criteria to fulfil the diagnosis of any dementia.

Table 1.1 DSM-5 clinical criteria for diagnosis of dementia (Major or minor neurocognitive disorder)

The presence of cognitive impairments that:

1. Interfere with ability to function at work or at usual activities
2. Are a decline from previous level of function
3. Not explained by delirium or major psychiatric disorder
4. Cognitive or behavioural impairment involves at least 2 of the domains below:
    a. Ability to acquire and remember new information
    b. Impaired reasoning and poor judgement
    c. Impaired visuospatial abilities
    d. Impaired language
    e. Changes in personality or behaviour

People often present with concerns regarding a change in cognition that do not meet the above criteria for a formal diagnosis of dementia. If such people demonstrate impairment in one or more cognitive domains but remain independent with regards to their functional abilities, they are considered to have Mild Cognitive Impairment (MCI) (Albert et al., 2011). People with MCI are at an increased risk of the development of dementia in the future (Albert et al., 2011).

1.2 Epidemiology and burden of dementia

The world-wide estimate of dementia prevalence in those over 60 years of age is reported to be 3.9% (Ferri et al., 2005). The global incidence of dementia is reported to be around 7.5/1000 in the general population (Ferri et al., 2005). Data from administrative sources suggest that there are currently approximately 245,000 people living with dementia in Australia (AccessEconomics, 2009). Dementia has been highlighted as a societal health priority in the G8 nations (Norton et al., 2014) and in 2012 was recognized as the ninth Australian National Health Priority Area (Welfare, 2012).

The burden of dementia disproportionately affects older people, with prevalence and incidence rates increasing with age. For example, the estimated prevalence of dementia increases from approximately 6% of those age 75-79 years to approximately 34% of those aged 90-94 years (AccessEconomics, 2009). Incidence estimates for new cases of dementia diagnosed per year are similarly distributed with increasing age. The estimated global incidence of dementia is 4.6 million cases per year and, in Australia, 7.0 per 1000 per year (Ferri et al., 2005). In Australians aged 75-79 years, the annual incidence is reported to be approximately 1.5%, whereas in those aged 90-94 years the annual incidence rate is approximately 19.5% (AccessEconomics, 2009). In Australia, dementia is the second leading

cause of death (Statistics, 2015b), and in the USA, dementia due to Alzheimer's disease (AD) was the sixth leading cause of death in 2010 (National Vital Statistics Reports, 2013). Dementia is currently the leading single cause of disability in people over 65 years of age (AccessEconomics, 2011). Using 2003-2004 estimates, Australian dementia-related health and residential aged care costs were 4.5% of the total health and ageing care budget (AccessEconomics, 2009). It is a major burden on survivors of the disease and on the care provider from both psychological and financial perspectives. This may include care provided in the home environment as well as the potential requirement for a person's care needs to be met in a structured residential care setting.

The demographic distribution of the world population is changing. Older people are likely to represent a greater proportion of the whole population in the future. This increase in numbers of older people means a greater burden of dementia in the community that will require management. The global prevalence of dementia in 2010 was estimated to be 35.6 million people (Prince et al., 2013). The prevalence of dementia in Australia is predicted to increase four-fold from 245,400 people in 2003 to an estimated 1.13 million people in 2050 (AccessEconomics, 2011). The incidence of dementia is predicted to increase accordingly from 69,600 new cases per year in 2003 to 385,000 in 2050 (AccessEconomics, 2011). The associated health and residential care costs associated with this increase in burden are compelling. Using 2003 cost estimates, dementia care costs are predicted to increase from $3,847,000 to $82,703,000 by 2062-2063, potentially becoming the top health care expenditure in Australia (AccessEconomics, 2009). A greater understanding of the risk factors and pathogenesis of dementia may lead to interventions to prevent, treat or delay the onset of dementia, and hopefully lead to a reduction in the future burden of the disorder.

1.3 Sub-types of dementia

There are several subtypes of dementia, and the ensuing section will deal with the two major subtypes of dementia relevant to this thesis, namely Alzheimer's disease (AD) and Vascular Dementia (VaD). Other subtypes of dementia including Dementia with Lewy Bodies, Dementia with Parkinson's disease and frontotemporal dementia are much less common (Kalaria et al., 2008) and are beyond the scope of this thesis. AD is the most common cause of dementia, accounting for approximately 60% of clinically diagnosed cases globally (Kalaria et al., 2008). Dementia due to vascular causes account for between 6 and 12 clinically diagnosed cases per 1,000 persons per year aged 70 years and over (Hebert et al., 2000). The term vascular cognitive impairment (VCI) includes those with VaD, as well as those with cerebrovascular disease and cognitive impairment not fulfilling the diagnostic criteria for dementia. Although AD and vascular disease in theory seem distinct diseases, many older people with dementia may have both underlying pathological processes. In a recent autopsy study of 4,629 people diagnosed in life with AD, 79.9% had evidence of vascular pathology (Toledo et al., 2013) reflecting the large number of people with dementia having a "mixed" dementia (Schneider et al., 2007, Schneider et al., 2009). The rest of this chapter will focus on aspects of AD and VCI as they encompass the likely pathways through which Type 2 diabetes mellitus (T2DM) could contribute to dementia.

1.3.1 Dementia due to Alzheimer's disease (AD)

1.3.1.1 Diagnostic criteria

The National Institute on Ageing and the Alzheimer's Association (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) first developed guidelines for the diagnosis of AD in 1984 (McKhann et al., 1984), and further revised these in 2011. There is no gold standard for the diagnosis of AD, and the use of neuropathological findings

(Hyman et al., 1997) combined with clinical and cognitive criteria described in **Table 1.2** below are considered to be the most accurate. Clinically, the diagnosis of probable AD is based on an insidious decline in cognition (most often memory-based) without a clear contribution of vascular causes established, either based on a temporal relation to a stroke or on the presence of substantial cerebrovascular disease. However, clinicopathological correlation of cognitive performance with neuropathological findings is important if an accurate diagnosis to be made (Nelson et al., 2012). There are two main neuropathological criteria by which a probabilistic likelihood of AD being the causative disease is assigned. Both the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (Mirra et al., 1991) and the Braak and Braak criteria (Braak et al., 1991) use the regional presence and density distribution of amyloid plaques and neurofibrillary tangles to determine likelihood of AD (Hyman et al., 1997). As neuropathology is rarely obtained, the degree of clinical diagnostic certainty ranges from "probable AD" through "possible AD" to "dementia unlikely to be due to AD" (McKhann et al., 2011). More recently, consensus criteria have been proposed for research purposes (Hyman et al., 2012) to try and characterise AD neuropathology that may assist in early diagnosis, recognising that brain changes are likely to develop far earlier than cognitive symptoms. The NINCDS-ADRDA core clinical criteria required for the application of probable AD are below (McKhann et al., 2011).

Table 1.2 Clinical criteria for diagnosis of "probable AD"

Meets criteria for dementia as described in **Table 1.1**

a. Insidious onset
b. Clear cut history of worsening of cognition
c. The initial and most prominent cognitive deficits are either:
    i. Amnestic (most commonly effected domain)
    ii. Non-amnestic:
        1. Language
        2. Visuospatial
        3. Executive
d. Probable AD should not be applied if:
        1. Evidence of substantial cerebrovascular disease
        2. Stroke temporally related to onset of cognitive impairment
        3. Presence of extensive infarcts or white matter hyperintensities
        4. Core features of another potential causative process

1.3.1.2 Epidemiology of AD

Frequency of disease

AD is the most common dementia subtype (Kalaria et al., 2008). Globally, the prevalence of dementia in 2010 was estimated to be 35.6 million people (Prince et al., 2013), and it is estimated that AD represents approximately 60% of this. AD is thought to be present in approximately 3% of those between the ages of 65-74 and 50% of those >80 years of age (Sosa-Ortiz et al., 2012). However, as discussed in the previous section, neuropathological studies suggest mixed AD and vascular pathology to be common (Kalaria et al., 2008).The incidence of AD in North America and Europe ranges from 15-21/1000 people/year in those

> 80 years of age (Sosa-Ortiz et al., 2012). Australian-specific incidence data are not available but are assumed to be similar to North American and European rates (AccessEconomics, 2011).

Risk factors

Non-modifiable risk factors

Increasing age is the strongest risk factor for the development of AD (Gorelick, 2004). Immediate family history and a number of genetic risk factors also increase the risk of AD (Goedert et al., 2006), particularly younger onset AD. Despite advances in genome-wide association studies continuing to find new genetic loci associated with AD and its biomarkers, the main genetic risk factor for the development of late onset, sporadic and familial AD is the ε4 polymorphism of the Apolipoprotein E (ApoE) gene (Querfurth et al., 2010, Bertram, 2011). ApoE is one of the major cholesterol and lipid carrying proteins and is as the only lipid transporter in the brain as well as a transporter for β-amyloid (Aβ). Of the 3 main polymorphisms of ApoE, ε2 is uncommon; ε4 is present in approximately 20%, with the rest having the ε3 polymorphism (Petersen et al., 1995). Individuals who are homozygous for the ε4 allele represent approximately 50% of those who develop AD in their mid-60s (Petersen et al., 1995, Raber et al., 2004). Those individuals heterozygous for the ε4 allele represent approximately 50% of those who develop AD in their mid to late 70s (Raber et al., 2004). Prior history of severe head injury may be associated with an increased risk of AD, with a further increased risk in those who are positive for ApoE ε4 but variation in the criteria used to define the significance of head trauma make this relationship still controversial (Jellinger, 2004). Female sex may also be a risk factor for the development of AD, even with adjustment for women's greater survival at older ages (Shumaker et al., 2003). The mechanisms underlying this increased risk are unclear. Although post-menopausal oestrogen

deficiency was considered a potential reason, oestrogen replacement in the Women's Health Initiative Memory Study was associated with an increased risk of AD (Shumaker et al., 2003).

Modifiable risk factors

Most modifiable risk factors for AD are related to lifestyle and vascular health. Although these may also be related to the risk of VaD and VCI, some of their relationships with dementia may be due to overlapping of the two dementia subtypes. Greater levels of education may have a protective effect on the risk of development of AD, presumably by increasing cognitive reserve (Stern et al., 1994, Unverzagt et al., 1998). Greater physical activity may also play a protective role in the development of AD, possibly by influencing neuroinflammation and/or neuroplasticity (Rovio et al., 2005, Lautenschlager et al., 2008). Mid-life obesity (also discussed in the later section on VCI in this chapter) may be an important risk factor for dementia (Anstey et al., 2011). While less extensively studied, diet might also affect AD risk, with some investigators reporting that a Mediterranean diet may protect against AD (Scarmeas et al., 2006), possibly due to reduced oxidative stress. According to the results of a systematic review and meta-analysis, current smoking may also increase the risk of AD (Anstey et al., 2007, Peters et al., 2008b). Meanwhile, light to moderate alcohol consumption may be protective against all-cause dementia (Peters et al., 2008a). The presence of hypertension is also a risk factor for the development of AD (Luchsinger et al., 2005), possibly through damage to the vessel wall and endothelial dysfunction, resulting in chronic cerebral hypoperfusion (Leszek et al., 2012). Depression may also be a risk factor for the development of AD (Speck et al., 1995, Geerlings et al., 2000, Diniz et al., 2013) with the results of a recent study comparing 191 people with a first major depressive episode to 282 healthy controls showing depression is associated with

hippocampal atrophy (Cole et al., 2011). T2DM is a well-recognized risk factor for the development of AD. The results of a meta-analysis of 19 longitudinal studies comparing a total of 6,184 people with T2DM to 38,530 without T2DM showed that those with T2DM had a relative risk (RR) of the development of AD of 1.46 (95%CI:1.20-1.77) (Cheng et al., 2012). As the link between T2DM and dementia is the focus of this thesis, the relevant studies linking T2DM with cognitive impairment and dementia, and the mechanisms involved will be dealt with in more detail in Chapter 2.

1.3.1.3 Pathophysiology of AD

The predominant cerebral pathology in AD is the accumulation of misfolded proteins resulting in neuroinflammation (Heneka et al., 2015) and oxidative stress, leading to synaptic failure and neuronal loss (Querfurth et al., 2010). The two main proteins implicated in the pathogenesis of AD are Aβ and tau (Querfurth et al., 2010). Aβ is derived from the amyloid precursor protein (APP) which is broken down by the sequential action of the β secretase, beta-site amyloid precursor protein-cleaving enzyme 1 (BACE-1), followed by the action of γ-secretase (Querfurth et al., 2010). This process generates Aβ peptides (monomers) that range from 36-43 amino acids.

Among the Aβ monomers, $A\beta_{40}$ is far more common than the more toxic $A\beta_{42.}$ The latter forms insoluble extracellular pleated sheets and eventually the amyloid plaques seen in AD. Oligomers of 2-6 peptides are also formed from the degradation of APP. These soluble oligomers are much more toxic than the plaques formed by $A\beta_{42}$, and, in addition to being directly toxic to neuronal synapses, also form intermediate assemblies that are directly neurotoxic (Walsh et al., 2007). In normal physiology, it is postulated that Aβ peptides can help prevent neuronal hyperactivity. Aβ monomers are cleared by the action of insulin

degrading enzyme, the enzyme also responsible for the clearance of insulin as well as the enzyme neprilysin which assists in the clearance of both Aβ monomers and oligomers (Querfurth et al., 2010). **Figure 1.1** summarizes the putative mechanisms involved in toxic Aβ generation and clearance. In the amyloid hypothesis, it is postulated that an imbalance of production and clearance leads to an accumulation of toxic Aβ (Selkoe, 2001). Although insoluble Aβ plaques are the most recognized pathology of AD, the cognitive deficits seen in AD correlate more closely with soluble oligomer burden than total Aβ load (Lue et al., 1999).

Tau is a soluble neuronal protein that encourages the assembly and stability of the microtubules supporting neuronal health, and also contributes to vesicle transport (Querfurth et al., 2010). A number of different enzymes regulate tau hyperphosphorylation. When hyperphosphorylated, tau loses its affinity for microtubules, and these destabilized microtubules cause impaired axonal transport. Hyperphosphorylated tau self-associates, forming intermediate aggregates such as paired helical fragments that are cytotoxic (Lee et al., 2001, Khlistunova et al., 2006). These paired helical fragments can then go on to develop neurofibrillary tangles that, while a marker of severity of AD (Querfurth et al., 2010) and are correlated with neuronal loss (Gomez-Isla et al., 1997) might also be neuroprotective by sequestering the more toxic, soluble tau intermediates in insoluble neurofibrillary tangles (Querfurth et al., 2010). There is likely substantial cross-over between tau and amyloid pathways. In mouse models, Aβ accumulation occurs before, and may drive tau aggregation (Gotz et al., 2001, Lewis et al., 2001, Oddo et al., 2003). Meanwhile, other mouse models suggest that the cognitive deficits seen due to Aβ-related neurodegeneration are dependent upon the availability of endogenous tau (Rapoport et al., 2002, Roberson et al., 2007).

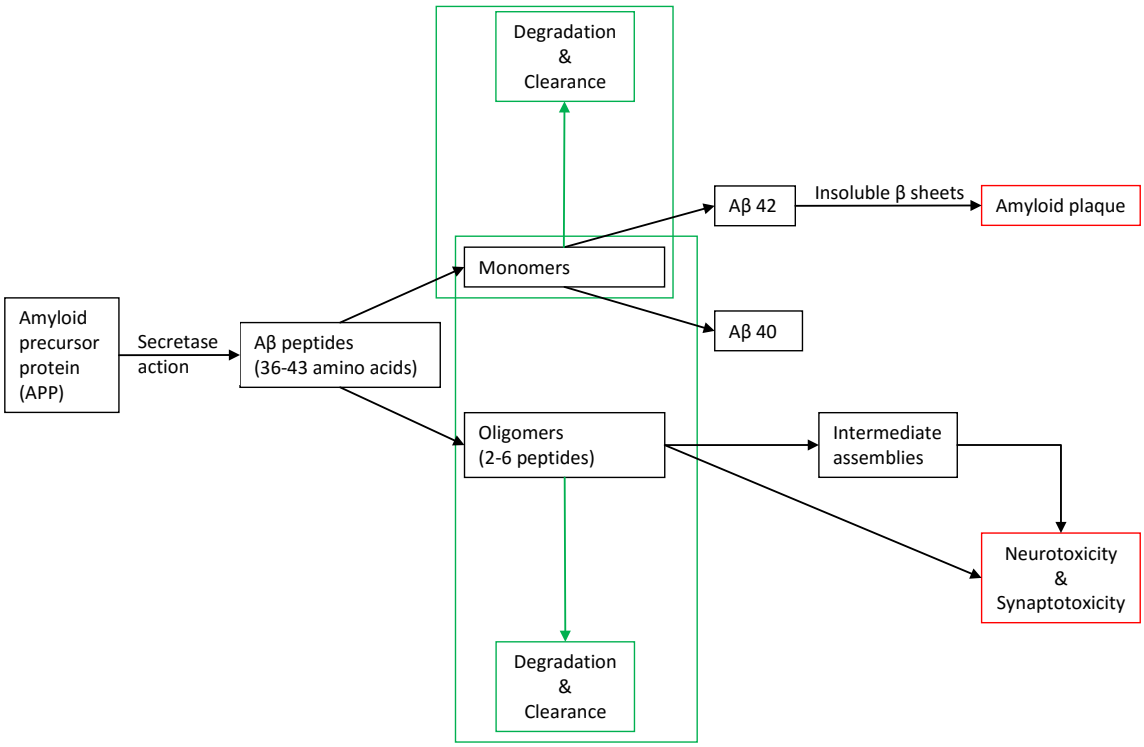

Figure 1.1. Aβ generation and clearance

1.3.1.4 Biomarkers of AD

Biomarkers of AD may assist in clinical diagnosis or serve as surrogate measures of disease pathophysiology for the purpose of research (Jack et al., 2011). For example, brain Magnetic Resonance Imaging (MRI) is commonly used to assist in clinical diagnosis by excluding other causes of dementia and is also extremely useful to track brain atrophy and cerebrovascular disease progression in research settings. Positron Emission Tomography (PET), cerebrospinal fluid (CSF) markers and blood biomarkers are presently most useful in the research setting.

<u>Brain MRI</u>

The poor tissue contrast obtained from computerized tomography (CT) limits its clinical application. Brain MRI on the other hand, is much more useful to rule out potentially

treatable abnormalities such as tumours or subdural haematomas, to evaluate cerebrovascular pathology (a more detailed exposition of MRI cerebrovascular lesions is presented in the following sections on VCI), and to evaluate the distribution of cortical atrophy (Reiman et al., 2012). Patients with AD typically have reduced cortical volumes in the hippocampus, entorhinal cortex, precuneus, posterior cingulate, parietal and temporal cortex (Dickerson et al., 2009, Jack et al., 2009). Furthermore, cortical atrophy as measured by MRI correlates well with memory decline (Dickerson et al., 2001, Jack et al., 2004) and conversion from MCI to mild AD (Dickerson et al., 2001, Jack et al., 2004, Bakkour et al., 2009). MRI is also useful in detecting white matter loss, and specialised sequences such as diffusion tensor imaging (DTI) and the use of high-field MRI (3Tesla, 7Tesla) lend themselves to finer evaluation of white matter loss, integrity and connectivity – these biomarkers are mainly used in the research arena. In the very early stages of AD, regional cortical atrophy may be too subtle to be clinically detectable, and computational methods involving algorithms to calculate exact volumes or cortical thickness may be required to identify tissue loss for research purposes.

Cerebral PET

Fluorodeoxyglucose PET (FDG PET) measures the cerebral metabolic rate for glucose (CMRgl) as a surrogate marker of neuronal activity. In patients with AD, FDG PET detects consistently lower CMRgl in the precuneus, posterior cingulate, parietal and temporal cortex (Minoshima et al., 1997, Hoffman et al., 2000, Mosconi et al., 2009). Additionally, in some studies, FDG PET has been found to predict cognitive deterioration or neuropathological diagnosis of AD (Hoffman et al., 2000, Silverman et al., 2001, Alexander et al., 2002). Clinically, FDG PET may be useful to help differentiate AD from frontotemporal dementia due to the large differences in the regions affected (Foster et al., 2007). FDG PET has much

more research promise because CMRgl reductions have been detected in asymptomatic people at risk for AD (Reiman et al., 2012), with consequent interest in its ability to predict the future development of AD at the individual level.

A number of PET radioligands are now available for research to visualize Aβ plaque deposition. The most well-known ligand is the [11C]-labelled Pittsburgh Compound B(PiB) (Klunk et al., 2004). There is a high correlation between PiB retention and Aβ pathology on autopsy specimens, as well as between PiB retention and low CSF Aβ (indicating reduced clearance) (Jack et al., 2013). Similar to autopsy studies, approximately 30% of cognitively normal older people, 60% of those with MCI and ~90% of those with AD have globally increased PiB retention (Jack et al., 2013). However, PiB retention only addresses current cerebral amyloid burden, not tau mediated pathology or neuronal damage. The correlation between amyloid burden and cognitive function is not particularly robust (Jack et al., 2013). The short half-life of PiB (20 minutes) requires the capacity to make the compound on-site, limiting its widespread adoption. Ligands with longer half-life based on ^{18}Fluorothymidine that are able to be transported to other facilities are currently becoming more available (Rowe et al., 2008, Choi et al., 2009).

Developing a ligand for tau imaging is challenging for a number of reasons (Villemagne et al., 2012). Tau aggregates are predominately intracellular thus requiring that any successful ligand must cross the blood brain barrier and the cell membrane (Villemagne et al., 2012). Furthermore, tau aggregates exist in six different isoforms and undergo multiple post-translational modifications limiting the ability of a single agent to target all of the relevant polymorphisms (Villemagne et al., 2012). Ligands targeting the more stable, paired helical filament tau are currently being developed with research ongoing (Villemagne et al., 2012).

CSF biomarkers

Elevated CSF levels of total tau and phosphorylated tau (p-tau) provide evidence of downstream neuronal degeneration (Hampel et al., 2008). Soluble tau is cytotoxic (Khlistunova et al., 2006) and increased levels in the CSF have been found to be associated with impaired cognition (Santacruz et al., 2005, Oddo et al., 2006). Increased CSF p-tau and total tau, by virtue of their strong correlation with poor cognition (Wallin et al., 2006) have the potential to improve the accuracy of identifying those with MCI who may be at increased risk of progressing to clinical AD (Mattsson et al., 2009). A reduction in CSF $A\beta_{42}$ is seen in about 50% of people with AD compared to controls without AD (Hampel et al., 2008). The reason why CSF $A\beta_{42}$ concentrations decrease in AD is unclear. There is good but not perfect correlation between CSF $A\beta_{42}$ concentration and PiB-PET measures of brain amyloid (Mattsson et al., 2009, Landau et al., 2013). In one study, CSF $A\beta_{42}$ concentration had a diagnostic sensitivity and specificity of differentiating AD from healthy subjects of between 80% and 90% (Blennow et al., 2003). CSF biomarkers are extremely useful in the research setting as surrogate measures of neurodegeneration. However, they are not recommended for routine use in the clinical setting due to limitations in measurement standardization between laboratories, and the need for a better understanding of their additional contribution towards improving the accuracy of core clinical diagnostic criteria for AD (McKhann et al., 2011).

Blood biomarkers

Blood biomarkers would have the obvious advantages over CSF biomarkers of simplicity, patient convenience and being less invasive. A number of blood-based protein biomarker panels are in development to identify those at risk of AD. Some of these include panels consisting of cortisol, pancreatic polypeptide, insulin-like growth factor binding protein 2, β_2 microglobulin, vascular cell adhesion molecule 1, carcinoembryonic antigen, matrix

metalloprotein 2, CD40, macrophage inflammatory protein 1α, superoxide dismutase, homocysteine, ApoE, epidermal growth factor receptor, haemoglobin, calcium, zinc, interleukin 17, and albumin (Ray et al., 2007, O'Bryant et al., 2010, Laske et al., 2011, Doecke et al., 2012). These panels, ranging in size from 8- 30 proteins, show some value in differentiating AD from healthy controls (Doecke et al., 2012) but further research is required to understand their role in predicting the conversion from healthy to MCI or from MCI to AD. Furthermore, it is unclear whether these panels reflect the pathogenic process of AD (Laske, 2013). Research into developing blood based biomarkers of the AD process is ongoing (Henriksen et al., 2014).

1.3.2 Vascular cognitive impairment (VCI) and vascular dementia (VaD)

1.3.2.1 Definition

There has been considerable controversy in the past regarding the role of vascular disease and its contribution to cognitive impairment (Moorhouse et al., 2008). Previously used terms to describe different aspects of cognitive impairment related to cerebrovascular disease include VaD, multi-infarct dementia and post-stroke dementia (Stewart, 2002). Neuropathological studies and increasingly sophisticated neuroimaging techniques have highlighted the large degree of heterogeneity in cerebrovascular pathology, as well as the considerable co-existence of AD neuropathology with such vascular pathology in patients with dementia (Moorhouse et al., 2008). A large number of different diagnostic criteria have been proposed, designed to exclude different non-vascular neuropathological processes to increase diagnostic certainty that the symptoms are attributable to cerebrovascular disease (Moorhouse et al., 2008). Some of these criteria used the nature of the cognitive impairment (executive dysfunction) (World Health Organization, 1992), recent history of stroke (Roman et al.,

1993), location of stroke on neuroimaging (Roman et al., 1993) or even the presence of vascular risk factors to support a vascular cause for dementia (Hachinski et al., 1975). Overall, it is uncommon for those with dementia to exclusively have cerebrovascular disease, with the brains of most people dying with dementia demonstrating a mix of degenerative and vascular changes (Hulette et al., 1997, Snowdon et al., 1997, Nolan et al., 1998, Lim et al., 1999, Xuereb et al., 2000, Barker et al., 2002). Post-mortem studies of those clinically diagnosed with VaD have found a high proportion with AD-type changes (del Ser et al., 1990, Kosunen et al., 1996). The understanding that these processes act synergistically (Snowdon et al., 1997, Lim et al., 1999), and a desire to include those with all degrees of cognitive impairment led to the creation of the umbrella term “Vascular cognitive impairment” (O'Brien et al., 2003, Moorhouse et al., 2008). This term now encompasses patients with various types of diagnoses previously used commonly, such as post-stroke dementia, multi-infarct dementia, subcortical ischaemic VaD, strategic-infarct dementia, hypoperfusion dementia, haemorrhagic dementia, dementia caused by specific arteriopathies, mixed AD and VaD, and vascular mild cognitive impairment (O'Brien et al., 2003).

1.3.2.2. Epidemiology of VCI

Frequency of disease

The differences in criteria used to diagnose VCI limits a good understanding of the true estimates of its incidence and prevalence (Jorm et al., 1987). Prevalence rates for VaD vary greatly depending on the population studied, even after adjustment for age and sex (Hebert et al., 1995). Depending upon the diagnostic criteria used, VaD accounts for approximately 26% of dementia (Kalaria et al., 2008). The incidence of VaD is estimated at between 6 and 12 cases per 1,000 persons per year aged 70 years and over (Hebert et al., 2000).

Risk Factors

Older age, male sex, low education, and several vascular factors (hypertension, T2DM, obesity, lifestyle) related to the risk of cerebrovascular disease are all implicated in the development of VCI and VaD (Gorelick, 2004). The most relevant factors are discussed below.

Stroke

Stroke is recognized as a risk factor for the development of dementia. Estimates of post stroke dementia depend upon the population studied but a recent systematic review and meta-analysis reported the prevalence of post-stroke dementia to range from 7% in population based studies to approximately 40% in hospital-based studies where pre-stroke dementia was not included (Pendlebury et al., 2009). Haemorrhagic stroke, recurrent stroke, location and infarct volume are all important factors in modifying post-stroke dementia risk (Pendlebury et al., 2009). The risk of dementia related to haemorrhagic stroke may be in part explained by co-existent amyloid angiopathy (Ellis et al., 1996). Recurrent stroke is an important determinant of the expression of cognitive decline and dementia, probably by virtue of greater burden of neuronal destruction (Srikanth et al., 2006), raising the possibility that stroke prevention may reduce dementia risk. Strokes occurring in strategic locations such as the mesial temporal lobe and thalamus may cause widespread cognitive impairments consistent with a dementia, often referred to as “strategic infarct dementia” (Leys et al., 1999). The volume of infarction is clearly a factor influencing cognitive decline, but a large number (and therefore summed volume) of small subcortical infarcts (lacunes) are also associated with the presence of dementia (Vermeer et al., 2003b).

Hypertension

Globally, hypertension is the most common modifiable risk factor for stroke (Tu, 2010) and is recognized as a risk factor for the development of cognitive impairment and dementia (Sahathevan et al., 2012). The results of two large cohort studies, the Honolulu-Asia Aging Study (HAAS) and the Rotterdam study, support the potential role of hypertension as an independent risk factor for the development of dementia. In the HAAS study, 3,703 Japanese-American men were followed for approximately 30 years with those who developed hypertension in mid-life at the highest risk of cognitive impairment (Launer et al., 2000). In the Rotterdam study, 6,249 men and women were followed for approximately 15 years (Ruitenberg et al., 2001). Participants who had used antihypertensive medications were at a lower risk of the development of VCI than those who had high blood pressure and had never used an antihypertensive. There have been several trials of blood pressure lowering to reduce the risk of dementia. The results of a recent meta-analysis suggested that, in those who were stroke-free, blood pressure lowering does not decrease the incidence of dementia (McGuinness et al., 2008). However, the beneficial effect of blood pressure lowering to prevent dementia may depend upon other baseline risk factors. In the observational Rotterdam study, the size of the beneficial effect of antihypertensive therapy on lowering dementia risk was doubled (8% risk reduction) in those <75 years of age compared with those >75 years of age (4% risk reduction) (Haag et al., 2009). In The Perindopril Protection Against Recurrent Stroke (PROGRESS) study, a randomised placebo controlled trial to assess the benefit of the blood pressure lowering for secondary stroke prevention, a reduction in risk of dementia was observed only among those with recurrent strokes (Tzourio et al., 2003).

Depression

There is a strong association between vascular risk factors, cardiovascular events and depression (Musselman et al., 1998, Joynt et al., 2003). There is an association between depression and an increased risk of the development of VaD in later life. The results of a meta-analysis of 23 community-based prospective cohort studies including 14,901 people with VaD (1,801 with late life depression) reported that depression was associated with an increased risk of VaD (pooled odds ratio 2.52 (95%CI 1.77-3.59)) (Diniz et al., 2013).

Type 2 diabetes mellitus (T2DM)

T2DM is a well-recognized risk factor for the development of VCI (Biessels et al., 2008). The results of a meta-analysis of 19 longitudinal studies comparing a total of 6,184 people with T2DM to 38,530 without T2DM showed that those with T2DM had a relative risk of the development of VaD of 2.48 (95%CI: 2.08-2.96) (Cheng et al., 2012). Further discussion of the relationship between T2DM and dementia will be focus of Chapter 2.

Obesity

Although obesity is traditionally considered a “vascular” risk factor, it may contribute to mechanisms other than vascular disease, such as chronic inflammation, and hence may be a common risk factor for VCI and AD. Being overweight (Body Mass Index (BMI) 25-30) or being obese (BMI>30) is a risk factor for the development of cognitive impairment, but this risk may be dependent upon the period of life in which obesity develops. This issue has been evaluated in a recent meta-analysis of 15 prospective studies in 25,624 participants with incident Alzheimer’s disease (AD), 15,435 participants with incident VaD and 30,470 participants with incident overall dementia (Anstey et al., 2011). Being overweight in midlife was associated with a 33% increased risk of VaD and, interestingly, a 35% increased risk of

AD and 26% increased risk of any dementia. The results from a Swedish study of 8,534 twins aged over 65 years further confirmed this association between midlife obesity and dementia (Power et al., 2011). Being overweight or obese in later life may not carry the same risk, with one study of 12,047 men ≥ 65 years of age reporting a lower dementia risk in those who were overweight or had a waist-hip ratio ≥0.9 (Power et al., 2011). The reason behind this is unclear but results from the HAAS study suggest that weight loss may precede the development of dementia by several years (Stewart et al., 2005). In the late life examinations in the final 6 years of HAAS, those who would go on to develop dementia lost weight at a greater rate than those who did not (Stewart et al., 2005). The mechanisms underlying the link between obesity and cognitive decline may not be dissimilar to those linking T2DM and dementia. In addition to the risk of cardiovascular disease in obese people, several studies have confirmed an association between obesity and brain atrophy, supporting a role for obesity-related chronic inflammation and neuroinflammation (Debette et al., 2011, Karlsson et al., 2013).

Lifestyle factors

Physical activity is well established as being protective against vascular disease, and is becoming increasingly recognized as protective for brain health. (Thompson et al., 2003). The results from a recent meta-analysis of 29 randomized controlled trials support a role for exercise in improving attention, processing speed and executive function (Smith et al., 2010). There is also some evidence to support the beneficial effect of exercise on dementia risk (Lautenschlager et al., 2008) but whether this is specific to VCI is unclear. Dietary habits are also important for vascular risk reduction. In a 2010 meta-analysis of 7 prospective studies, a Mediterranean diet was associated with lower risk of cardiovascular events as well as dementia (Sofi et al., 2010). Smoking is clearly a risk factor for vascular disease, and

contributes to an increased risk of all-cause dementia and AD, but the evidence to support its contributory role in VCI is less clear (Anstey et al., 2007, Peters et al., 2008b). Light to moderate alcohol consumption may have a protective role, similar to its known cardiovascular benefits (Ruitenberg et al., 2002). Overall, a healthy lifestyle addressing activity, diet, and habits such as smoking and alcohol consumption may protect against vascular disease, and indirectly against dementia (Ngandu et al., 2015). To support this concept, the results of a recent trial demonstrate a beneficial effect of a multi-domain intervention combining vascular risk reduction and monitoring, dietary modification, exercise and cognitive training (Ngandu et al., 2015).

1.3.2.3 Pathophysiological substrates of VCI

Stroke due to large vessel disease and cardioembolism

Ischaemic stroke commonly occurs as a result of large artery-to-artery embolism and cardioembolism. Such infarcts contribute to the development of cognitive impairment and dementia either by affecting brain regions strategically important for cognition, or through a cumulative load of infarcts (multi-infarct dementia) (Moskowitz et al., 2010). Patients suffering from the cognitive effects of such acute strokes may be prone to static long-term cognitive impairment, or a worsening of pre-existent dementia. Although such infarcts are obviously important in the development of VCI and VaD, this thesis focuses largely on the manifestations of small cerebral vessel disease which are far more common in older people and play an important role in the expression of VCI and VaD.

Small cerebral vessel disease*

The contents of the remainder of this section regarding small vessel disease have been published in a review article in the International Journal of Stroke: Moran C, Phan TG, Srikanth VK. Cerebral small vessel disease: a review of clinical, radiological, and histopathological phenotypes. International journal of stroke : official journal of the International Stroke Society 2012;7:36-46.* ***For the full article, See Appendix A**.*

Diseases of small cerebral blood vessels have generated significant interest because of their insidious impact on brain function and the difficulty pinning down underlying disease mechanisms. The term "small vessel disease" or "SVD" has been used to reflect clinical, radiological or pathological phenomena attributed to disease of small perforating arteries and arterioles supplying deep brain structures. More recently, the roles of venules and capillaries are also beginning to be emphasized (Pantoni, 2010). During life, phenotypes of SVD may be identified clinically, radiologically or using both approaches in combination. As SVD is difficult to directly visualise, we rely predominantly on radiological phenotypes as surrogate markers of disease. The advent of newer imaging techniques has contributed significantly to our understanding of these phenotypes, in particular their effects on brain function and underlying risk factors. However, such recent descriptions also need to be carefully considered in the context of clinico-pathological studies prior to the advent of brain imaging (Fisher, 1965, Fisher, 1968, Fisher, 1971, Fisher, 1979). The principal SVD phenotypes of clinical interest are small deep brain infarcts, cerebral white matter lesions (WML), deep brain haemorrhages and cerebral microbleeds. The causes or mechanisms underlying these phenotypes are understood in varying degrees of detail, and further advances in high-field imaging, genetic studies and bench-based research have the capability to address the gaps in knowledge.

Deep brain infarcts

When using the term “deep brain infarcts” we refer to small subcortical infarcts (variously defined by researchers as between 3-20mm in diameter) identified on Computerised Topography (CT) or Magnetic Resonance Imaging (MRI). They may be associated with acute focal deficits (symptomatic infarcts), or be found incidentally on brain imaging in those without a history of clinical stroke (subclinical or “silent” infarcts). The terms “lacunar infarct” and “lacunar syndrome” are often used to refer to such infarcts. The latter refers to a set of clinical features that are observed commonly in the setting of acute infarcts due to deep perforator occlusion (Donnan et al., 2002). However, these clinical ‘lacunar syndromes’ may also occur in the setting of large artery atherosclerosis or cardioembolism (Mead et al., 2002, Potter et al., 2010). The use of the term “deep brain infarcts” avoids speculation as to what the actual underlying stroke mechanism may be.

Symptomatic deep brain infarcts account for between 20-30% of all stroke sub-types, with an incidence rate in developed countries of approximately 0.33/1000 per year in community-based studies of the general elderly population (Sudlow et al., 1997). The mortality rate for symptomatic deep brain infarcts is less than 1% at 1 month and ranges between 2.4% to 10% at 1 year (Bamford et al., 1987). The prevalence of subclinical deep brain infarcts ranges from 8-28% depending on the type of sample studied, being less common in the general population than in clinical samples (Vermeer et al., 2007). Their incidence rate in population-based studies is approximately 2-3% per year in older people living in developed countries (Vermeer et al., 2003a, Vermeer et al., 2007). These estimates of prevalence or incidence are subject to variability depending on whether CT or MRI is used for detection, the latter being more sensitive (Pantoni, 2010, Wardlaw, 2011).

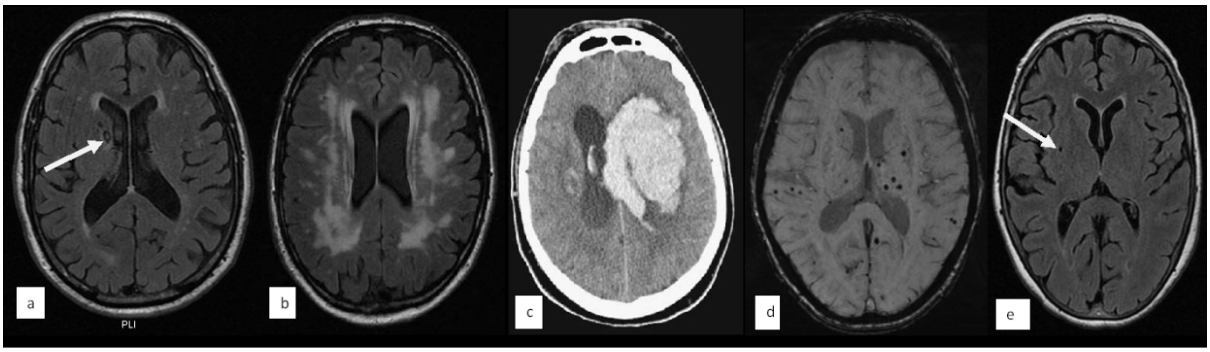

Figure 1.2 – Radiological Phenotypes of Cerebral SVD

a. Deep brains infarcts (arrows) on FLAIR
b. Severe WML
c. Large intracerebral haemorrhage with smaller right sided haemorrhage
d. Cerebral Microbleed on SWI imaging
e. Enlarged Perivascular Space (arrow) on FLAIR

Radiology and pathology of deep brain infarcts (Figure 1.2a)

Acute deep brain infarcts are best detected using Diffusion Weighted Imaging (DWI) MRI, and can appear hyperintense on Fluid Attenuated Inversion Recovery (FLAIR) MRI sequences. Chronic infarcts are hypointense on T1-weighted MRI and FLAIR (Patel et al., 2011), often with a hyperintense rim on FLAIR. They are commonly seen in the basal ganglia, internal capsule and pons (Norrving, 2009) and are considered the radiological manifestation of the "lacune", a term first used by Déchambre in 1838 to describe small cerebral cavities following resorption of necrotic tissue related to infarction (Arboix et al., 2009). The term "lacune" then expanded to encompass small round lesions seen in white and

grey matter on autopsy, as well as the scars of small residual infarcts (Arboix et al., 2009). Pierre Marie and Ferrand, at the turn of the 20th century, redefined "lacune" to its original meaning of deep cerebral infarction due to local small blood vessel occlusion (Arboix et al., 2009). However, the actual pathological process underlying the vessels related to "lacunes" remains poorly understood.

Histopathological studies of deep brain infarcts are limited by the often long time elapsed between occurrence and death (Lammie, 2002, Wardlaw et al., 2009). Irregular cavities are seen with surrounding gliosis, lipid-rich and haemosiderin-rich macrophages, and fragmented blood vessels. The significance of haemosiderin-rich macrophages is unclear, but may be explained by microhaemorrhage due to endothelial damage (Fisher, 1968). In an attempt to identify the causative lesion underlying such infarcts, Fisher meticulously performed serial dissection of the vascular supply proximal to the infarction of 50 "lacunes" derived from 4 patients reaching autopsy (Fisher, 1965). In all, 45/50 of these "lacunes" had total occlusion of the perforating artery supplying the infarct (Fisher, 1968). He observed that the majority (40/45) of these total occlusions were due to "segmental arterial disorganisation" (Fisher, 1968), a term describing patchy, asymmetrical focal vascular changes resulting in the loss of normal arterial wall architecture and thickening of very small vessels (40-200μm), similar to changes he observed in larger vessels of those with poorly controlled hypertension (Fisher, 1968). He also observed extravasation of plasma proteins into the arteriolar wall and the subsequent conversion to fibrin, which he termed "lipohyalinosis" (Fisher, 1971), and referred to by others as fibrinoid necrosis (Figure 1.3a) (Rosenblum, 2008). In a separate study, Fisher also showed that 9 of 11 patients with internal capsule infarcts had obstructive vascular lesions in the proximal larger vessel supplying the region, including plaque with superimposed thrombus and plaque causing severe stenosis, coupled with mural or occlusive

thrombi in the deep perforating artery (Figure 1.3b) (Fisher, 1965, Fisher, 1968, Fisher, 1979). It is uncertain whether or not such thrombi are primary events or terminal events in a prolonged process of intrinsic vasculopathy. Although such older pathological studies are the most detailed yet in the field, they were based on a small number of cases and set in a time when vascular risk factors were poorly controlled. It has been noted that deep brain infarcts still occur in those with well-controlled hypertension (Wardlaw, 2011) raising the possibility that more complex mechanisms may be at play. Advances in neuroimaging with the development of high-field MRI may provide more insight into other potential mechanisms.

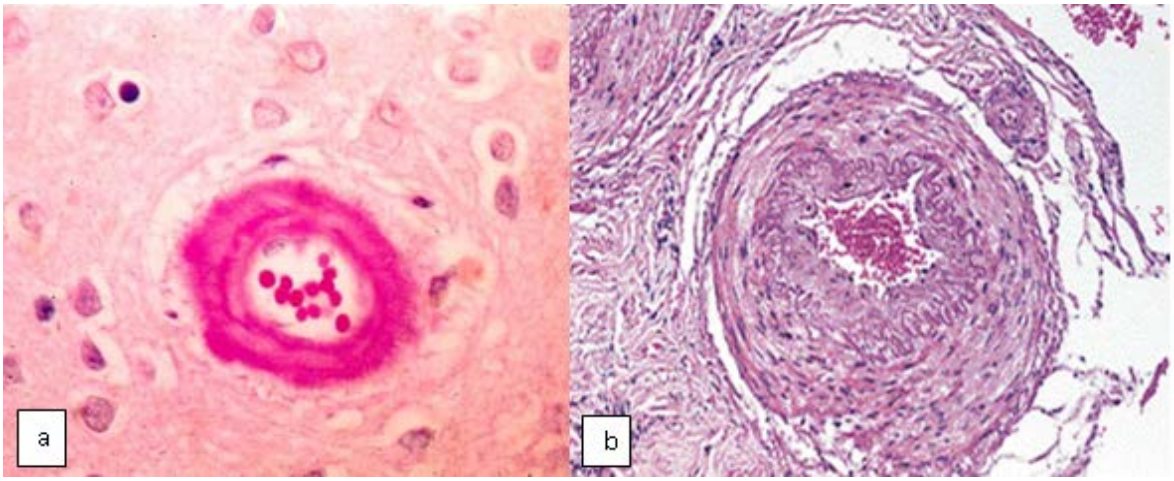

Figure 1.3 - Histopathology of fibrinoid necrosis and arteriosclerosis

a. Fibrinoid necrosis: Deposition of fibroid around entire circumference of vessel with loss of collagen

b. Arteriosclerosis: lipid-laden stenosis of perforating arteriole

Risk factors and mechanisms of deep brain infarcts

Risk factors for larger cortical strokes such as increasing age, hypertension, diabetes mellitus, smoking, prior stroke or transient ischaemic attack (TIA), excess alcohol consumption and raised cholesterol are also associated to varying degrees with an increased risk of developing deep brain infarcts, whether symptomatic or subclinical (Jackson et al., 2005, Das et al.,

2008, Jackson et al., 2010). A causal role for hypertension, although likely, is by no means the only contributing factor. Early data supporting a strong association with hypertension may also have been biased by the inclusion of the risk factor in the determination of stroke sub-type (Wardlaw, 2011), hence a possible circular bias. More recently, the association of hypertension and deep cerebral infarcts has been found to be less clear, with deep brain infarcts being seen in those with well controlled or without hypertension (Jackson et al., 2010). Less evidence is available to support the contribution of elevated lipid levels, smoking and diabetes to the development of deep cerebral infarcts (Jackson et al., 2005, Jackson et al., 2010). The relationship between carotid stenosis and deep brain infarcts is weak, with the severity of stenosis being similar within patients suffering a recent "lacunar" infarct in one study (Mead et al., 2002). Atrial fibrillation and ischaemic heart disease occur less frequently in those with the 'lacunar' syndrome than those with 'non-lacunar' syndrome (Jackson et al., 2005). Genetic studies may also provide clues to understanding the pathogenesis of deep brain infarcts (Jackson et al., 2005, Jackson et al., 2010). In a recent genome wide association study of subclinical cerebral infarcts in 9,401 participants, the presence and frequency of the single nucleotide polymorphism rs2208454 (in a region possibly regulating growth factor signalling and potentially angiogenesis and neurogenesis) was associated with a decreased odds of MRI infarction (Debette et al., 2010a).

The mechanism of intrinsic SVD, as compared with embolic vessel occlusion, has been suggested as the principal mechanism underlying deep brain infarcts (Wardlaw, 2011) with recent observational evidence supporting this in studies of retinal vessels (Arboix, 2009, Doubal et al., 2009b, Lindley et al., 2009, Thompson et al., 2009). Retinal vessels are like cerebral vessels in many ways including their functional similarity with the blood brain barrier, position as end arteries, embryological origin and calibre (Doubal et al., 2009b).

Patients with clinical deep brain infarcts identified on DWI-MRI are more likely to have a range of retinal microvascular signs (narrowed arterioles, arteriovenous nipping, and enhanced arteriolar light reflex) suggestive of arteriosclerosis than those without, and others have postulated that endothelial dysfunction may underlie such disease (Lindley et al., 2009, Thompson et al., 2009, Wardlaw, 2011). Ageing, and to some extent, hypertension, may also impair the integrity of the blood brain barrier (Wardlaw et al., 2009, Knottnerus et al.), and some have shown an increase in endothelial permeability measured with gadolinium leakage on MRI (Wardlaw et al., 2009) in people with clinical "lacunar" strokes. It is unclear whether this is an epiphenomenon or causal, and still leaves open the question regarding its antecedent mechanisms, with immune or cytokine-mediated damage remaining possible suspects (Knottnerus et al.). While intrinsic disease is a likely mechanism, a proximal embolic source cannot be completely excluded (Fisher, 1965, Fisher, 1968). Acute deep brain infarcts on occasion may be associated with the presence of a visible clot in a larger vessel such as the middle cerebral artery (Figure 1.4), suggesting embolism from a more proximal source (Wang et al., 2006). A study of non-human primates showed that the direct injection of emboli into the internal carotid artery resulted in deep brain infarcts in a small proportion of cases (< 6%) (Macdonald et al., 1995). Similarly, a proximal embolic source may be present in at least 10% of symptomatic deep brain infarcts, particularly in those with larger infarcts or those with multiple small subcortical lesions on DWI (Tegeler et al., 1991, Mead et al., 2002, Tejada et al., 2003, Seifert et al., 2005). The limited phenotyping in most prior studies of subcortical stroke argues for careful consideration of additional information (such as angiographic methods) before a small vessel mechanism can be assigned with reasonable confidence.

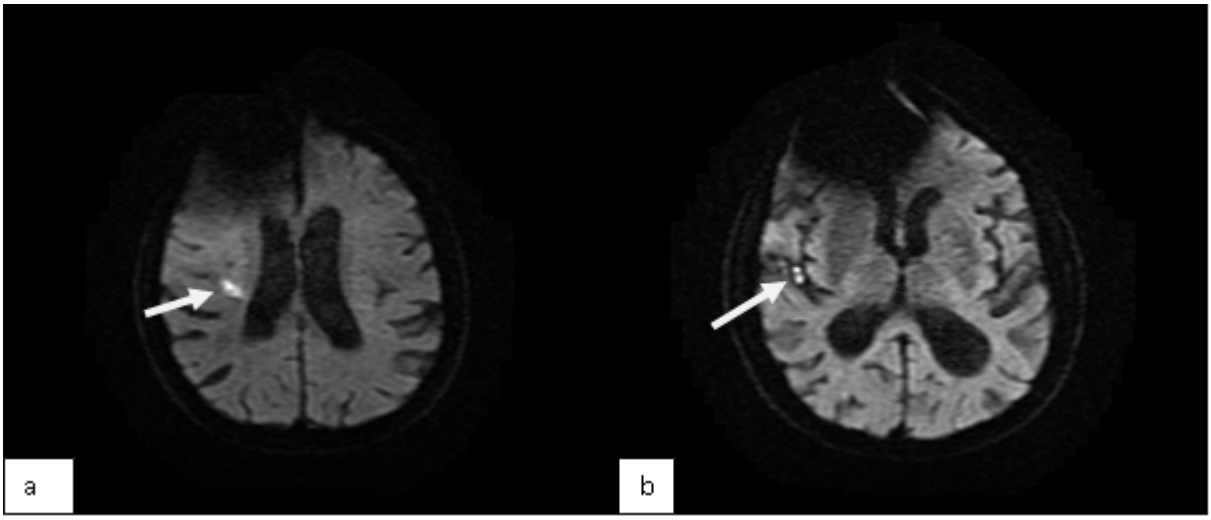

Figure 1.4 - Deep brain infarct with underlying large arterial clot occlusion

Acute subcortical infarcts (a) may be associated with the presence of a visible clot in a larger vessel such as the middle cerebral artery in the same patient (b), suggesting embolism. Right frontal lobe artefact present from previous surgery.

Clinical presentations, effects on function, and treatment of deep brain infarcts

Clinical syndromes (termed as “lacunar syndromes”) that are most commonly associated with acute deep brain infarcts include pure motor hemiplegia, pure sensory stroke, sensorimotor stroke, ataxic hemiparesis and dysarthria-clumsy hand syndrome (Donnan et al., 2002, Donnan et al., 2009), although several others have also been described (Donnan et al., 2002, Donnan et al., 2009). These clinical syndromes, although closely associated with radiological evidence of deep brain infarcts, are not perfectly predictive of type of lesion or location (Gan et al., 1997, Arboix et al., 2009, Arboix et al., 2010) and can therefore limit attempts to identify underlying mechanisms and guide appropriate secondary prevention. There is evidence from prior studies that approximately 20% of patients presenting with a clinical “lacunar syndrome” will have a non-small vessel mechanism underlying the stroke (Gan et al., 1997, Arboix et al., 2010). Conversely, in one study, around 7% of those with radiologically confirmed deep brain infarcts were shown to have presented with atypical

syndromes such as dysarthria facial paresis, isolated dysarthria and isolated hemi-ataxia (Arboix et al., 2006). Moreover, between 4-10% of patients with clinical "lacunar" syndromes' may actually have a deep intracerebral haemorrhage (Anzalone et al., 1989, Arboix et al., 2000). Thus, reliance on clinical syndromes alone may introduce bias in clinical studies (Wardlaw, 2011) and potentially lead to incorrect medical management.

Compared with larger and cortical strokes, acute deep brain infarcts are less severe and have better short term physical outcomes (Donnan et al., 2002, Wardlaw, 2011). However, they are associated with an important risk of recurrence, and affected patients have an increased risk of developing cognitive impairment, depression and functional impairment over time (Vermeer et al., 2007, Baezner et al., 2008, Arboix et al., 2009). A recent longitudinal study has shown that progression of deep brain infarcts has been associated with subtle decline in executive, speed and motor control functions (Jokinen et al., 2011). Pharmacological management of symptomatic deep brain infarcts lies in the use of standard therapies including antiplatelet agents (Das et al., 2008), blood pressure reduction (Schiffrin, 2002) and diabetes control (Seshadri et al., 2004). A sub-study of SPARCL (Stroke Prevention by Aggressive Reduction in Cholesterol Levels) (Lavallee et al., 2009) showed that cerebrovascular reactivity (a marker of endothelial function) in symptomatic patients with "lacunar" stroke was unaffected by Atorvastatin therapy, raising some doubt regarding the usefulness of lipid reduction. Although it is unclear whether carotid endarterectomy is effective in the secondary prevention of deep brain infarcts, previous major trials have not differentiated between types of infarcts (Tegeler et al., 1991, Rothwell et al., 1999, Mead et al., 2002, Tejada et al., 2003). A large randomized controlled trial is currently underway testing the efficacy of antiplatelet therapy and blood pressure lowering in reducing the recurrence of small subcortical stroke events and the incidence of cognitive dysfunction

(Secondary Prevention of Small Subcortical Strokes-SPS3) (Benavente et al., 2011). Subclinical deep brain infarcts also have important long-term cumulative effects, contributing to a greater risk of future dementia (Vermeer et al., 2007), clinical stroke, falls and mortality (Pantoni et al., 2005). The use of the antiplatelet agents Dilazep Hydrochloride and Cilostazole was associated with fewer incident subclinical infarcts in two small trials of Japanese patients with T2DM (Shinoda-Tagawa et al., 2002, Nakamura et al., 2005), suggesting that these may need further exploration. Clearly there is a need for larger scale trials of vascular therapies to prevent subclinical deep brain infarcts.

Age-related White Matter Lesions (WML)

WML are distinct from deep brain infarcts although they frequently co-exist (Pantoni, 2010). In the general population they occur in approximately 80% of Caucasians aged over 60 years (de Leeuw et al., 2001). They are also present in 2/3 of patients with dementia and 1/3 of those diagnosed with Alzheimer's disease (Steingart et al., 1987). They are seen as hyperintensities in white matter on T2 MRI sequences, and may occur around the ventricles (periventricular WML) or more peripherally (sometimes referred to as 'deep' subcortical WML). They occur more frequently in the frontal white matter (Srikanth et al., 2010), in women (de Leeuw et al., 2001), and tend to progress among those with a large lesion load (Enzinger et al., 2007) which occurs in approximately a third of the general older population (Enzinger et al., 2007). The results of pathological and epidemiological studies suggest that an ischaemic basis due to SVD may be responsible for at least this subset of people with severe WML (Enzinger et al., 2007). Age and hypertension remain the only consistently identified risk factors for WML in prospective studies (Godin et al., 2009), whereas the evidence supporting the effect of other vascular risk factors is weak. There is an association between increasing frequency of migraines and the burden of WML, but only in females

(Kruit et al., 2004). Although there is high heritability for WML, no convincing evidence has yet been obtained to support the associations of candidate genetic polymorphisms with these lesions (Paternoster et al., 2009), and future large genome wide association studies may shed more light on this matter.

Radiology and pathology of WML (Figure 1.2b)

Before the advent of MRI, WML were observed as diffuse areas of white matter attenuation on CT scans, and termed “leukoaraiosis” (Hachinski et al., 1987). However, FLAIR is now the preferred imaging modality to detect such lesions in clinical settings. It can miss small lesions in the thalamus and posterior fossa compared with high resolution T2 weighted sequences. A number of different visual rating scales have been developed in an attempt to allow further study of clinical correlates (Fazekas et al., 2005, van Straaten et al., 2006), but there are significant limitations with such scales, particularly with regards to intra-observer variability (O'Sullivan, 2008). Several methods of semi-automated or automated segmentation of WML currently exist to compute volume of WML, which may be a more sensitive measure for research purposes (Beare et al., 2009). Diffusion Tension Imaging (DTI) allows the measurement of fractional anisotropy (FA) and diffusivity (D) of white matter tracts, to further allow measurement of the integrity of axonal membranes in white matter lesions and in normal appearing white matter (de Laat et al.). Magnetisation Transfer Ratio (MTR) may provide another surrogate marker of axonal injury while studying WML (Fazekas et al., 2005, Bastin et al., 2009). Although there is increasing interest in the use of high-field MRI (7 Tesla and upwards) to study SVD (Kang et al., 2010), its ability to detect WML may be approximately equivalent to the ability of 1.5 Tesla MRI (Theysohn et al., 2011).

Pathological examination of WML reveal a spectrum of changes ranging from myelin pallor, enlarged perivascular spaces, tissue infarction, gliosis and axonal loss (Fazekas et al., 1993). As these lesions become more confluent, there is complete loss of the entire nerve fibre (Fazekas et al., 1993). Perivascular infiltration of foam cells, and pro-inflammatory mediators including ApoE, α2-macroglobulin (A2M) and immunoglobulin G have also been described (Lammie, 2002, Nag, 2003), as have reactive astrocytosis and microglial activation (Fazekas et al., 1993). Other evidence also points to a loss of integrity of small vessel endothelium or the blood brain barrier (Young et al., 2008). Venous collagenosis has also been observed in areas of WML, although its pathogenetic significance remains uncertain (Brown et al., 2002). More recently, molecular biological studies have suggested that alteration of RNA transcription may occur in multiple genes that are involved in immune regulation, cell cycle, apoptosis, and proteolysis among others, highlighting the complexity of the phenotype (Simpson et al., 2009).

Effects of WML on function and treatment

There is now little doubt that those with a large lesion load of WML clearly suffer physical, cognitive and psychological effects. When present in large amounts, they are associated with cognitive impairment, gait disorders, falls, mood disturbance and bladder instability (de Leeuw et al., 2001, Baezner et al., 2008). Their effects on cognition usually manifest as subtle impairments of executive function, set-shifting and processing speed (van Straaten et al., 2006, Jokinen et al., 2011). Cross-sectional associations between WML and depression have been demonstrated (Pantoni et al., 2005, Teodorczuk et al., 2007). Greater WML, particularly in periventricular frontal regions (Srikanth et al., 2010), are associated with poor gait (Srikanth et al., 2009) and a high risk of falls (Srikanth et al., 2009). Importantly, in a meta-analysis of 46 longitudinal studies, WML were associated with a greater risk of future

stroke, dementia and death, highlighting their potential significance as surrogate markers of major disease burden in the elderly (Debette et al., 2010b).

To date, there is no convincing evidence for the efficacy of pharmacological or lifestyle therapies for slowing the progression of WML. In small post-hoc analyses of data from the PROGRESS study (Dufouil et al., 2005) and the ROCAS study (Mok et al., 2009), the use of an angiotensin converting enzyme inhibitor or a statin were found to be associated with slower progression of WML on MRI, raising the possibility that their use may be beneficial. However, there need to be larger definitive trials in this field.

Deep Intracerebral Haemorrhage

Up to 50% of all intracerebral haemorrhages (ICH) are due to deep brain haemorrhage (Flaherty et al., 2005), the rest being lobar. They are a common but often less emphasised manifestation of SVD, but are associated with a significant mortality and morbidity rate, accounting for up to 15% of all deaths from stroke (Xi et al., 2006), with a higher fatality rate than ischaemic stroke (Broderick et al., 2007). Deep ICH is strongly associated with the presence of other SVD phenotypes such as deep brain infarcts (Tanaka et al., 1999) and WML (Rost et al., 2010). Hypertension continues to be a strong risk factor in the development of deep ICH along with increasing age (Fisher, 2003, Flaherty et al., 2005). Approximately 49% of ICH are deep cerebral haemorrhages (Flaherty et al., 2005). Putaminal and thalamic haemorrhage accounts for 19-53% and 4-26% of these respectively (Donnan et al., 2009). Caudate nucleus haemorrhage occurs in approximately 1-5% of ICH while haemorrhages in the pons or cerebellum account for between 2-10% each (Donnan et al., 2009).

Radiology and pathology of ICH (Figure 1.2c)

Non-contrast CT remains the preferred mode of diagnosis due to greater availability and lesser contraindications. MRI may be helpful in the acute phase in differentiating an infarct with haemorrhagic transformation from that of a primary haemorrhage, identifying small haemorrhages, and detecting underlying vascular abnormalities or lesions (Broderick et al., 2007).

On neuropathological study, there is a common finding of fibrinoid necrosis, lipohyalinosis, and medial degeneration of the vessel wall affecting deep penetrating arteries or arterioles (Rosenblum, 2008). Similar findings occur in relation to small deep infarcts, except for the prominence of fibrinoid necrosis, which may be driven by a local inflammatory process among those with hypertension (Rosenblum, 2003). It is as yet unclear why there would be a predisposition towards haemorrhage rather than infarction, given the similarity in vessel pathology. Previously, it has been postulated that such haemorrhages may occur due to the rupture of microaneurysm (Charcot-Bouchard microaneurysms) in the middle or distal portions at or near bifurcations of the penetrating artery (Fisher, 2003, Rosenblum, 2003). However, there is still uncertainty and controversy whether such microaneurysms are common, and whether or not they bear any association with ICH (Rosenblum, 2008). Deep brain infarcts have been found to underlie deep intracerebral haemorrhages in approximately 10% of cases, identifying a further potential contributor to the development of ICH (Mori et al., 1985).

Clinical effects and treatment of ICH

The clinical effects of deep cerebral haemorrhages are dependent on the volume of haemorrhage, location, and presence of mass effect. The acute management of deep

intracerebral haemorrhage as recommended by AHA/ASA guidelines can be viewed elsewhere (Broderick et al., 2007). Hypertension is the main target of the modifiable risk factors for secondary prevention (Broderick et al., 2007).

Cerebral Microbleeds (CMB)

The widespread use of MRI has resulted in the increased recognition of CMB (Greenberg et al., 2009). They are usually 2-5mm hypointense lesions on gradient echo (GRE-T2*) or susceptibility-weighted images (SWI) on MRI (Tanaka et al., 1999). In a systematic review, their prevalence was around 5% in healthy adults, 34% in people with ischaemic stroke, and 60% in people with non-traumatic ICH using GRE-T2* (Cordonnier et al., 2007). They are more prevalent in the setting of recurrent ischaemic strokes (44%) than first-ever ischaemic strokes (23%), and in those with recurrent ICH (83%) than in first-ever ICH (52%) (Cordonnier et al., 2007). There may be two subtypes of CMB as suggested by findings from the Rotterdam study (Vernooij et al., 2008). Those in deep subcortical regions (deep CMB) tend to be associated with cardiovascular risk factors and a greater load of deep brain infarcts and WML (Vernooij et al., 2008). Those in lobar regions (lobar CMB) have been reported to be associated with the presence of ApoEε4 genotype (Vernooij et al., 2008).

Radiology and pathology of CMB (Figure 1.2d)

Lesions seen on gradient echo MRI correlate with haemosiderin deposition from both old and more recent haemorrhage (Greenberg et al., 2009). More recent advances in imaging (SWI-Susceptibility Weighted Imaging) sequences have greater sensitivity in detecting CMB than GRE sequences (Greenberg et al., 2009), with the latter only identifying 33% of SWI lesions. In a post-mortem study of Alzheimer's disease patients, SWI hypointensities corresponded to acute microhaemorrhage, haemosiderin residua of old haemorrhages, and small lacunes

ringed by haemosiderin (Schrag et al., 2009). In lesions where the bleeding vessel could be identified, beta-amyloid was detected in the vessel wall, suggesting that amyloid angiopathy may play a role in the pathogenesis of CMB and partly explain the overlap between SVD and Alzheimer's disease (Grinberg et al.). However, CMB may occur independent of amyloid deposition in older people without a history of stroke or Alzheimer's disease (Grinberg et al., 2010). The strong association of CMB with confluent WML also suggests that they may have shared mechanisms (Greenberg et al., 2009).

Clinical effects of CMB

The clinical significance of microbleeds is a topic of current investigation, and there is reasonably strong evidence to support an association with symptomatic ICH (Lee et al., 2004). There is some evidence that the burden of CMB is associated with cognitive impairment (Werring et al., 2004) particularly in the presence of previous stroke suggesting a cumulative effect. At the present time, it is unclear whether CMB is a target for therapy, or is simply a surrogate marker for SVD.

Enlarged Perivascular Spaces

Enlarged perivascular or Virchow-Robin spaces deserve mention when discussing SVD, with increasing interest in whether they may be involved in the pathogenesis of WML and deep brain infarcts. They are common findings on brain MRI in older people, and may be a radiological mimic of deep brain infarcts (Wardlaw, 2011). These cerebrospinal fluid-filled cavities surround small penetrating cerebral arterioles and are visible as high signal areas on T2-weighted MRI, and low signal on T1 and FLAIR sequences, usually < 2mm in diameter, although they may be larger on occasion (figure 1.2e) (Doubal et al., 2010). Increasing age is

associated with an increased prevalence of enlarged perivascular spaces (Zhu et al., 2011). Although present in most adults, they are also more commonly seen in those diagnosed with dementia (Gouw et al.), and in association with greater volume of WML and deep brain infarcts among stroke patients (Doubal et al., 2010). Their association with deep brain infarcts appear stronger than with cortical infarcts, suggesting a possible involvement in SVD (Doubal et al., 2010). Mechanisms suggested in the formation of such spaces include altered permeability of the vessel wall due to inflammation or blood brain barrier breakdown, amyloid accumulation along the vessel wall and cerebral atrophy (Doubal et al., 2010). However, their potential mechanistic involvement in SVD is still speculative.

1.4 Conclusion

Dementia is a common condition, affecting individuals, carers and society that is likely to increase in the future. Of the number of dementia sub-types, AD and cerebrovascular mechanisms represent most of the burden of disease. There is considerable overlap between VCI and AD pathways and risk factors. A better understanding of the mechanisms underlying dementia may help guide preventive or therapeutic measures to reduce this burden. T2DM is a well-established risk factor for the development of all cause dementia, VCI and AD and may represent a model of accelerated ageing. Chapter 2 discusses the links between T2DM and dementia and the potential mechanisms through which this link may occur.

Type 2 Diabetes Mellitus and Dementia – a review

2.1 Introduction

The recognition that diabetes may be associated with cognitive impairment dates from as early as 1922 when Type 1 diabetes mellitus (T1DM) was reported to be associated with "neuromuscular slowing" and slowing in completing mental tasks (Miles et al., 1922). A more recent meta-analysis of 33 studies confirms that T1DM is associated with impairments particularly in the domains of intelligence and mental speed and flexibility (Brands et al., 2005). Given that the focus of this thesis is Type 2 diabetes mellitus (T2DM), the following sections will deal only with the relationship between T2DM and cognitive decline/dementia. Further discussion of T1DM is outside the scope of this thesis.

2.1.1 Definition, clinical features and complications of T2DM

2.1.1.1 Definition

T2DM is a chronic illness, defined by hyperglycaemia resulting from a progressive insulin secretory defect on the background of insulin resistance (American Diabetes, 2010). The American Diabetes Association 2011 guidelines recommend diabetes be diagnosed if fasting plasma glucose level ≥7.0 mmol/l (126 mg/dl) or HbA_{1c} ≥6.5% (7.7mmol/l) or 2-hour plasma glucose ≥ 11.1 mmol/l (200mg/dl) following a 75g oral glucose tolerance test (OGTT) or classic symptoms of hyperglycaemia (polyuria, polydipsia, weight loss) or hyperglycaemic crisis with a random plasma glucose ≥11.1 mmol/l (200mg/dl) (American Diabetes, 2010).

2.1.1.2 Epidemiology of T2DM

Diabetes is a global health concern with an estimated 285 million people between the ages of 20-79 years globally having the disease. This is projected to increase to a prevalence of 7.7% (439 million people) by 2030 (Shaw et al., 2010). The reason for this increase is likely secondary to increasing incidence due to obesity and sedentary lifestyles becoming more common. The rate of increase in incidence is greatest in developing countries, possibly due to an increase in urbanization, a surrogate marker of lifestyle change (Shaw et al., 2010).

2.1.1.3 Clinical features and pre-diabetes

Many people with T2DM are unaware they have the disease as the symptoms are relatively mild. Symptoms of T2DM include thirst, polydipsia and polyuria (American Diabetes, 2002). Given the mild nature of early symptoms, screening of people at risk of the development of T2DM is recommended and can also identify help those who have pre-diabetes (a state of increased risk of developing T2DM) identified by impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) (Colagiuri S, 2009, American Diabetes, 2010). IFG is defined as the presence of fasting glucose levels between 6.1-6.9 mmol/l and IGT as 2-hour plasma glucose between 7.8-11.1 mmol/l after OGTT (American Diabetes, 2010).

2.1.1.4 Complications of T2DM

T2DM is associated with serious long term complications including macrovascular and microvascular disease affecting several organs, depression, poor quality of life, cognitive impairment and dementia (American Diabetes, 2002, Bruce et al., 2003, Biessels et al., 2006b, American Diabetes, 2010, U.S. Department of Health and Human Services, 2011). Macrovascular complications include stroke and coronary artery disease whereas microvascular complications include retinopathy, nephropathy and neuropathy (American

Diabetes, 2002). A description of the full range of complications of T2DM is outside the scope of this chapter, and hence the following sections will focus mainly on its cognitive complications and disease pathways that may lead to such complications.

2.1.2 T2DM, cognitive decline and dementia/cognitive impairment

One of the earliest studies to specifically examine the associations between T2DM and cognitive impairment was published in 1984 (Perlmuter et al., 1984). This study compared 140 people with non-insulin dependent diabetes with 38 people without diabetes. Diabetes was associated with poorer retrieval memory but intact encoding memory (Perlmuter et al., 1984). Since 1984, the results of a large number of studies have shown T2DM to be cross-sectionally associated with cognitive dysfunction, with the most commonly affected domains being processing speed, executive function and memory (Brands et al., 2005, Reijmer et al., 2010). Several large longitudinal studies have also been performed to study this issue **(Table 2.1)**. The results of these generally show that older people with T2DM are more likely to experience a greater rate of cognitive decline than those without T2DM.

There has been increasing interest in the effect of the time of life during which T2DM develops on the future risk of cognitive decline. Four recent studies have examined this question. The results from all four studies showed that baseline diagnosis of T2DM was associated with increased rates of cognitive decline (Nooyens et al., 2010, Spauwen et al., 2013, Tuligenga et al., 2014, Mayeda et al., 2015). However, the impact of incident development of T2DM on cognitive decline may depend upon the age of entry into the study. Of these four studies, two did not demonstrate an association between the development of incident T2DM and an increased rate of cognitive decline (mean age at entry 54-70 years) (Tuligenga et al., 2014, Mayeda et al., 2015). However, in two other studies with younger samples(mean age 40-55 years), the development of T2DM during the study was associated

with an increased rate of subtle cognitive decline (Nooyens et al., 2010, Spauwen et al., 2013), suggesting that mid-life onset of T2DM may carry a greater risk of future cognitive decline than late-life onset.

Over 50 studies have examined the association between T2DM and the risk of dementia. These studies include cross-sectional and longitudinal designs and vary with regards to how dementia was diagnosed, or whether AD, vascular or mixed dementia subtypes were considered. In a systematic review published in 2006, T2DM was found to be associated with an increased risk of all-type dementia in 7 of 10 longitudinal studies examined (Biessels et al., 2006b). In a subsequent meta-analysis of 19 longitudinal studies comparing a total of 6,184 people with T2DM to 38,530 without T2DM, those with T2DM had ~50% increase in the risk of incident dementia (Relative Risk RR 1.51, 95% CI: 1.31-1.74) and incident AD (RR 1.46, 95%CI:1.20-1.77), and a much higher risk of incident VaD (RR 2.48, 95%CI: 2.08-2.96) (Cheng et al., 2012). **Table 2.2** summarizes key prospective, population-based, cohort studies that have shown an association between T2DM and an increased risk of incident dementia (Yoshitake et al., 1995, Leibson et al., 1997, Ott et al., 1999, MacKnight et al., 2002, Peila et al., 2002, Arvanitakis et al., 2004, Luchsinger et al., 2004, Schnaider Beeri et al., 2004, Xu et al., 2004, Borenstein et al., 2005, Luchsinger et al., 2005, Whitmer et al., 2005, Akomolafe et al., 2006, Hayden et al., 2006, Muller et al., 2007, Rastas et al., 2007, Irie et al., 2008). In a Swedish cross-sectional study of 210 twin participants >65 years of age discordant for dementia, T2DM was associated with an increased odds ratio of dementia of 1.89 (95%CI 1.51-2.38) (Xu et al., 2009). In this study, those who developed T2DM before the age of 65 years had a greater odds ratio of developing dementia (OR 2.41 (95%CI 1.05 to 5.51)) compared with those who developed T2DM after this age (OR 0.68 (95%CI 0.3 to 1.53)) These results support the previously discussed results that the time in life of T2DM onset may be an important effect modifier of the risk of developing dementia. Such a twin

study design is also useful as it allows for the control of maternal and early life risk factors for the development of dementia. Monozygotic (identical) twin pairs had greater odds ratio (OR) for the development of dementia (OR 1.87 95%CI: 0.30 to 11.79) compared with dizygotic (non-identical) twin pairs (OR 1.06, 95%CI: 0.48 to 2.34). The stronger association in monozygotic twin pairs may be due to tighter control of genetic and early life risk factors allowing for clearer visualization of the effect of early adult and midlife risk factors.

Table 2.1. Prospective, population-based longitudinal studies describing the association between T2DM and cognitive function

Author	Study	Year	Total number	Number with T2DM (%)	Length of follow up	Mean age or range	Main Results
Mayeda et al., 2015	Sacramento Area Latino Study on Aging (SALSA)	2015	1634	530 (32%) at baseline 258 (16%) new diagnosis	7.6 years	~70 years	Baseline T2DM associated with faster cognitive decline Rate of cognitive decline similar between newly diagnosed with T2DM and those without T2DM
Tuligenga et al., 2014	Whitehall II	2014	5563	187 (3%)	10 years	54.4 years	Baseline T2DM associated with 45% faster decline in memory; 29% faster decline in reasoning and 24% faster decline in global cognitive function Newly diagnosed T2DM and pre-diabetes: similar rates of decline as normal controls Poorer glycaemic control (HbA_{1c})associated with faster decline in memory
Spauwen et al., 2013	Maastricht Aging Study	2013	1290	68 (5%)	12 years	40-82 years	Baseline T2DM cross-sectionally associated with worse cognitive scores, and associated with greater decline in information processing, executive function and delayed word recall New development of T2DM associated with subtle decline in processing speed only

Nooyens et al., 2010	The Doetinchem Cohort Study	2010	2613	61 (2%) at baseline 78 (3%) Incident	5 years	55-60	Nearly 3-fold greater decline in global cognitive function decline in those >60 years of age, prevalent T2DM associated with 3.6-fold greater decline in cognitive flexibility. Incident T2DM associated with 2.5-fold greater decline in cognitive flexibility
Van den Berg et al., 2006	Leiden 85-plus Study	2006	599	96 (16%)	5 years	85-90 years	Cross-sectional associations with poorer psychomotor speed & executive function; but no increase in rate of cognitive decline
Kanaya et al., 2004	Rancho Bernado	2004	999	118 (12%)	4 years	73 years	No cross-sectional associations Women with T2DM had 4 fold increased risk of decline in verbal fluency
Arvanitakis et al., 2004	Religious Orders Study	2004	824	127 (15%)	5.5 years	75 years	Cross-sectional associations with lower global cognition, episodic memory, semantic memory, working memory and visuospatial ability Greater rate of decline in perceptual speed only
Yaffe et al., 2004	Multiple Outcomes of Raloxifene (MORE)	2004	7027	267 (4%)	4 years	66 years	Cross-sectional associations with poorer composite cognitive scores. Greater decline in executive function & composite cognitive battery score Increased risk of developing cognitive impairment (OR 1.64, 95%CI 1.03 – 2.61)

Hassing et al., 2004a	Origins of variance in old-old (OCTO)	2004	274	36 (13%)	6 years	82.8 years	No cross-sectional associations Increased rate of decline in MMSE, perceptual & processing speed, semantic & episodic memory
Wu et al., 2003	Sacramento Area Latino Study on Aging (SALSA)	2003	1789	585 (33%)	2 years	70 years	Baseline T2DM predicted "major cognitive impairment" (OR 1.68, 95CI% 1.21-2.34) and poorer short term semantic memory. No difference in rate of change of cognitive scores
Fontbonne et al., 2001	Epidemiology of vascular ageing study	2001	926	55 (6%)	4 years	65 years	No cross-sectional associations Greater decline in memory, psychomotor speed, attention & reasoning Greater risk of "severe worsening" in memory, psychomotor speed and attention
Knopman et al., 2001	Atherosclerosis Risk In Communities	2001	10,963	1349 (12%)	6 years	57 years	Greater decline in psychomotor speed & verbal fluency scores
Gregg et al., 2000	Study of Osteoporotic Fractures Research Group	2000	9679	682 (7%)	3-6 years	72 years	Women with T2DM lower cross-sectional scores on m-MMSE, Digit symbol and trails B test Greater decline in psychomotor speed &

							m-MMSE performance Greater odds of “major cognitive decline” (OR 1.74) Duration of T2DM >15 years associated with 57%-114% greater risk of cognitive decline compared to those without T2DM
Haan et al., 1999	Cardiovascular Health Study	1999	5888	~500 (8%)	5-7 years	73 years	Greater decline in psychomotor speed

MMSE: Mini Mental State Examination; OR: Odds ratio

Table 2.2. Prospective, population-based studies of the association between T2DM and incident dementia.

Author	Study	Year	Subjects	Subjects	Length of follow up (Years)	Mean age of entire group (years)	Dementia risk (by type)
			Total number	Number with T2DM (%)			
Irie et al., 2008	Cardiovascular Health Study	2008	2547	320 (12.6%)	5.4	~74	All cause: HR 1.4 AD: HR 1.6
Muller et al., 2007		2007	2259	483 (21%)	4.4		All cause: RR 1.6 AD:RR 1.4
Rastas et al., 2007		2007	339	91 (27%)	3.5	~88	All cause: RR1.3
Akomolafe et al., 2006	Framingham study	2006	2210	202 (9.1%)	12.7	70	AD: RR 3.0
Hayden et al., 2006	Cache County study	2006	3264	343 (10.5%)	3.2	74	All cause: HR 1.6 AD: 0.9
Luchsinger et al., 2005		2005	1138	~231 (20.3%)	5.5	76.2	AD:4.8
Borenstein et al., 2005	Kame project	2005	1859	~320 (17.2%)	6	~75	AD:HR 3.3
Whitmer et al 2005		2005	8845	~1000 (11.3%)	30	~75	All cause: HR1.5

Schnaider-Beeri et al., 2004	Israeli Ischemic Heart Disease study	2004	1892	~473 (2.5%)	35	~45	All cause: OR 2.8
Xu et al., 2004	Kungsholmen Project	2004	1301	~104 (8.8%)	6	81	All cause: 1.5 AD: 1.3
Luchsinger et al., 2004		2004	683	~152 (22.3%)	5.4	~76	All cause: HR 2.2 AD:HR 2.2
Arvanitakis et al., 2004	Religious orders study	2004	824	127 (15.4%)	9	~75	AD: HR 1.6
Macknight et al., 2002	Canadian Study of Health and Aging	2002	5574	503 (9.0%)	5	74	All cause: RR1.3 AD: RR1.3
Peila et al., 2002	Honolulu Asia Aging study	2002	2574	~900 (35%)	3	77	All cause: RR1.5 AD:1.8
Ott et al., 1999	Rotterdam study	1999	6370	~694 (10.9%)	2.1	~70	All cause: RR1.9 AD: 1.9
Leibson et al., 1997		1997	10970	1455	7	~81	All cause: RR1.6 AD:RR1.6
Yoshitake et al., 1995	Hisayama	1995	826	69 (8.4%)	7	~73	AD: RR 2.18

HR: Hazard Ratio; OR: Odds Ratio; RR: Relative risk

2.2 T2DM & dementia: risk factors, potential disease pathways and mechanisms

There is strong interest in identifying risk factors, causal mechanisms and pathways that are involved in the link between T2DM and dementia. **Figure 2.1** below displays a schema of important potential risk factors, molecular pathways and manifestations of brain disease that may be involved. The most likely final common pathways may be either neurodegeneration or cerebrovascular disease, or interactions between them.

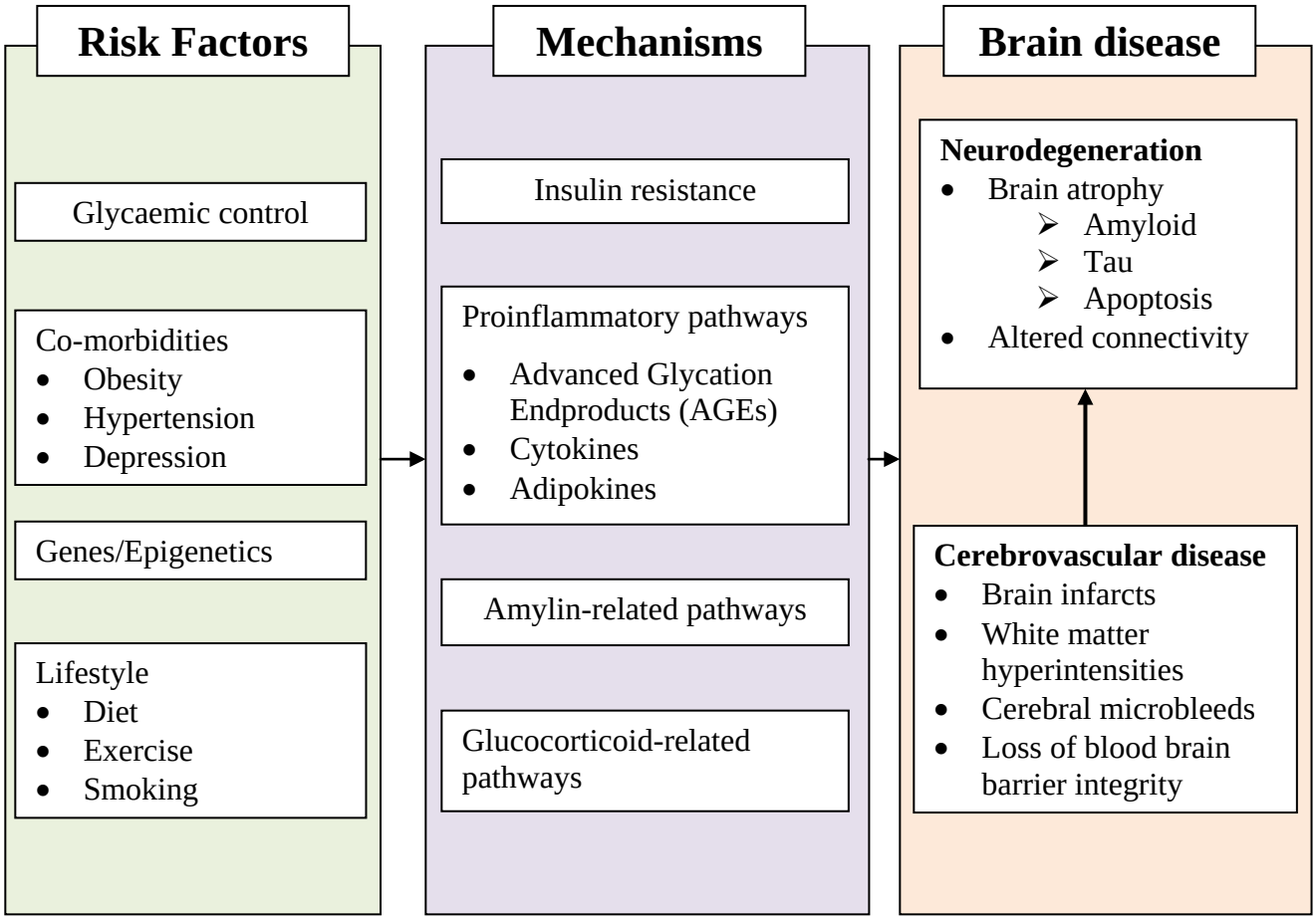

Figure 2.1. Schematic diagram of potential links between T2DM and dementia

2.2.1 Risk factors

2.2.1.1 Glycaemic control

The defining characteristic of diabetes mellitus is hyperglycaemia. In a study of 20 people with T2DM subjected to an hyperinsulinaemic glucose clamp, those with acute hyperglycaemia were found to exhibit impaired attention, processing speed and working memory (Sommerfield et al., 2004), but whether this has long term implications is unknown. A systematic review of 73 studies found that high concentration of HbA_{1c} (reflecting medium term control) was negatively associated with cognition in those without dementia, raising the possibility that glucose control may be beneficial in protecting against cognitive decline (Geijselaers et al., 2015). In the same review, the relationship between markers of glycaemic control (HbA_{1c} and fasting glucose) and radiological markers of cerebrovascular disease or dementia were inconclusive (Geijselaers et al., 2015). In a recent meta-analysis limited to randomized control trials, intensive glucose control was not associated with a slower rate of cognitive decline (Tuligenga, 2015). The Action to Control Cardiovascular Risk in Diabetes-Memory in Diabetes (ACCORD-MIND) measured the effects of intensive glucose control on Digit Symbol Substitution Test (DSST) performance in those with T2DM (Launer et al., 2011). In this study of ~1400 people per treatment group (intensive control compared to standard treatment), DSST scores were similar in those receiving intensive glucose control (achieved HbA_{1c}= 6.6%; 49 mmol/mol) compared with those receiving standard care (achieved HbA_{1c} = 7.5%; 58 mmol/mol) at 40 months. It may be that attempts to achieve intensive glucose control increase the incidence of hypoglycaemic episodes and these may have a detrimental effect on cognition (Launer et al., 2011, Geijselaers et al., 2015, Tuligenga, 2015). Overall, hyperglycaemia may play a role in explaining the link between T2DM and cognitive decline. Hyperglycaemia reflects insulin resistance and this may potentiate other pathways involving glucose transport and signalling, inflammation and

oxidative stress that will be discussed further below. It is unlikely, however, that hyperglycaemia per se explains all the cognitive complications of T2DM.

There is evidence to support an inverse association between the occurrence and frequency of hypoglycaemic events and cognitive function (Geijselaers et al., 2015). However, the results of these studies have to be interpreted with caution, paying attention to study design and being aware of the potential for reverse causality. In both the ACCORD-MIND and the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trials, the intensive glucose control arms had a higher incidence of hypoglycaemic episodes, but this did not lead to a greater rate of cognitive decline in these groups (de Galan et al., 2009, Launer et al., 2011). However, in these studies, the effect of hypoglycaemia on the rate of cognitive decline may have been offset by the benefits of better glucose control. In the Freemantle Diabetes Study, a history of hypoglycaemia was not associated with an increased risk of the development of dementia at 5 years but there were cross-sectional associations between hypoglycaemia, cognitive status and dementia with the presence of dementia being a strong risk factor for the development of a serious hypoglycaemic event (Hazard Ratio (HR) 3.0 (95%CI 1.06-8.48)) (Bruce et al., 2009). This potential for reverse causality is further supported by the results from the Health, Aging and Body Composition Study of 783 older people, followed over 12 years which reported a bidirectional association between dementia and hypoglycaemic events (Yaffe et al., 2013). In contrast, the Edinburgh Type 2 Diabetes Study reported that a history of severe hypoglycaemia was associated with greater decline in cognitive function from an estimated, baseline pre-morbid cognitive state (Feinkohl et al., 2014). Overall, the effect of hypoglycaemia on cognitive function is complex, but understanding its role will be essential in guiding the development of guidelines to prevent T2DM-related cognitive impairment (Biessels et al., 2014).

2.2.1.2 Obesity

Being overweight (Body Mass Index (BMI) 25-30) or being obese (BMI>30) is a risk factor for the development of dementia. This risk may also depend upon the period of life in which it develops. In a recent meta-analysis of 15 prospective studies (Anstey et al., 2011), being overweight in midlife was associated with an increased risk of AD (RR 1.35, 95%CI 1.19-1.54), VaD (RR 1.33, 95%CI 1.02-1.75) and any dementia type (RR 1.26, 95%CI 1.10-1.44). For those obese in mid-life, the RR of the development of AD, or any dementia compared to those with normal BMI was 2.04 (95%CI: 1.59 to 2.62) and 1.64 (95%CI: 1.34 to 2.00) respectively (Anstey et al., 2011). The results from a Swedish study of 8,534 twin individuals over 65 years of age also found midlife overweight or obesity to be associated with an increased risk of dementia (Power et al., 2011). Being overweight or obese in later life may not carry the same risk, with one study of 12,047 men ≥ 65 years of age reporting those who were overweight or had a waist-hip ratio ≥0.9 to have a lower risk of the development of dementia (Power et al., 2011). The reason behind this is unclear, but results from the HAAS study suggest that weight loss may precede the development of dementia by several years (Stewart et al., 2005). In the late life examinations in the final 6 years of HAAS, those who would go on to develop dementia, lost weight at a greater rate than those who did not develop dementia (Stewart et al., 2005). Although there is a close relationship between T2DM and obesity it is unclear whether obesity mediates the relationship between T2DM and cognitive decline. In the Freemantle Diabetes Study, those who had dementia or cognitive impairment were less likely to be overweight or obese at baseline (Bruce et al., 2008) suggesting a potentially protective effect. In contrast, in a study of 253 people with T2DM, measures of central adiposity (waist hip ratio and waist circumference), were cross-sectionally and negatively associated with MMSE score and a composite score of executive and attentional function (Abbatecola et al., 2010). Furthermore, those with T2DM in the greatest tertile of

total fat mass had a greater decline in both MMSE and the composite cognitive score than those in the lowest tertile. The relevance of central adiposity rather than obesity measures is also of interest, with a recent imaging study demonstrating that visceral fat accumulation was associated with reduced cortical thickness independent of BMI (Veit et al., 2014). Overall the relationship between obesity, T2DM and cognition remains to be clarified with increasing interest in the mechanism through which measures of adiposity might reflect underlying mechanisms such as inflammation and insulin resistance (Hotamisligil et al., 1993, Abbatecola et al., 2010).

2.2.1.3 Hypertension

Hypertension is a recognized risk factor for dementia and commonly co-exists with T2DM. Few studies have examined the role of hypertension in mediating or modifying the relationship between T2DM and cognitive impairment. In the OCTO-twin study of 258 participants (mean age 83 years) followed over approximately 6 years, there was no decline in cognition among those with hypertension alone (n=92) (Hassing et al., 2004). Although those with T2DM alone (n=16) showed significant cognitive decline, it was those with both T2DM and hypertension (n=22) that showed the steepest cognitive decline (Hassing et al., 2004). This suggests that the effect of T2DM may be exacerbated by the presence of hypertension. Consistent with this finding, a large retrospective study of the hospital records of 380,000 people reported that hypertension in addition to T2DM, increased 2 year dementia risk (Hazard ratio 1.08, 95%CI 1.03-1.14) (Johnson et al., 2012). Furthermore, this study suggested that this risk could be attenuated depending upon the choice of antihypertensive agent. The Fremantle Diabetes Study also reported hypertension to be an important predictor of 8 year AD risk (Bruce et al., 2008). The authors reported an increase of 5mmHg in diastolic blood pressure to be associated with an increased risk of AD (OR 1.32, 95%CI 1.04-1.69). In contrast, although not specifically examined, those who achieved greater blood

pressure reduction in the ACCORD-MIND study had similar rates of cognitive decline than those who did not. Overall further research is required to better understand the role of hypertension in mediating the relationship between T2DM and cognitive decline.

2.2.1.4 Depression

The association between depression and cognitive impairment and T2DM and depression is well established (de Groot et al., 2001, Katon et al., 2012). A few large studies suggest that depression contributes to T2DM-related cognitive impairment. In the Diabetes and Aging Study of 19,239 diabetes registry members, those with T2DM and depression (n=3,766) had a greater 3-5 year risk of developing dementia (Hazard ratio 2.02 (95%CI 1.73-2.35)) than those with T2DM alone (Katon et al., 2012). Similarly, in the ACCORD-MIND study, the combination of T2DM and depression was associated with a greater rate of decline compared to T2DM alone in all cognitive domains (Sullivan et al., 2013). From these studies it appears that co-morbid depression adds to the risk of cognitive decline in those with T2DM. It remains to be seen whether treating depression in T2DM reduces the risk of cognitive decline or dementia.

2.2.1.5 Genes and epigenetic factors

Approximately 25% of the general population over 55 years of age have a first degree relative with dementia (Slooter et al., 1998). However, a direct, inherited, single gene mutation causing dementia is uncommon, with a Mendelian single gene mutation being responsible for AD in about 500 families (Loy et al., 2014). ApoE status, a recognised risk factor for AD, may also interact with vascular risk factors in predicting dementia risk. In the HAAS study, those with T2DM and at least one ε4 allele had a greater risk of developing AD (RR 5.5) than those with T2DM alone (RR 1.8) (Peila et al., 2002). Similar to these results, those from the Kungsholmen project, in 1,301 community dwelling people aged over 75

followed over 6 years, an association was found between T2DM and AD only in those who possessed at least one ε4 allele (HR 2.4 compared to HR1.0) (Xu et al., 2004). These results suggest that there may be an additive effect of T2DM to the risk of dementia conferred by ApoE status.

In addition, epigenetic modification of chromatin and altered gene expression in the brain is beginning to be recognized as potentially important in AD pathogenesis, particularly Aβ metabolism (Bennett et al., 2015). In recent work, it is also just emerging as a potential mechanism linking T2DM with dementia (Wang et al., 2014). In animal models, these epigenetic changes are associated with an increased susceptibility to amyloid induced hippocampal dysfunction (Wang et al., 2014). Epigenetic changes are theoretically modifiable either pharmacologically (Wang et al., 2014) or through exercise (Gomez-Pinilla et al., 2011).

2.2.1.6 Lifestyle factors

There have been few studies of the role of diet or exercise in modulating the risk of dementia associated with T2DM (Reijmer et al., 2010). Interventions involving moderate and vigorous aerobic (Colcombe et al., 2006, Gons et al., 2013) or resistance training (Erickson et al., 2011) have been shown to preserve brain structure and function as well as improve glycaemic control in older individuals (Lindström et al., 2003). In one small study, the effect of 2-4 times per week exercise and 6 monthly healthy diet advice in 19 people newly diagnosed with T2DM and 36 with impaired glucose tolerance was compared to 74 normal controls who did not receive the lifestyle intervention (Yamamoto et al., 2009). At baseline, those with T2DM and impaired fasting glucose had lower Mini Mental State Examination (MMSE) score, revised Hasegawa Dementia Scale (HDSR) scores and delayed recall scores than the normal controls. After 2 years, those who received the lifestyle intervention had similar

MMSE and HDSR as the normal controls but did not have any improvement in delayed recall performance.

Although smoking is associated with an increased risk of dementia (Anstey et al., 2007) few studies have examined the role of smoking in T2DM-related cognitive decline. In the Zutphen Elderly Study, current smokers in the composite group of cardiovascular disease and/or diabetes had a greater 3 year decline in MMSE score than those who never smoked (Launer et al., 1996). Further studies are required to better understand the effect of lifestyle interventions such as smoking, physical activity and dietary modifications on dementia risk in those with T2DM.

2.2.2 Mechanistic pathways

2.2.2.1 Insulin resistance

Target cell insulin resistance and the subsequent hyperinsulinaemia is the key pathophysiological characteristic of T2DM (DeFronzo, 2004). Insulin resistance is defined as a smaller than expected response to a given dose of insulin (De Felice et al., 2014). This cellular resistance to the action of insulin may begin years before the development of T2DM, and not everyone with insulin resistance will go on to develop T2DM (Bonora et al., 1998). There is substantial interest in the roles of insulin resistance and signalling in mediating or modulating neurodegeneration in T2DM (Craft et al., 2004). Brain insulin resistance may lead to increased extracellular Aβ accumulation, decreased Aβ clearance and increased tau phosphorylation and neurofibrillary tangle formation (Craft et al., 2004). Insulin can cross the blood brain barrier (BBB) through a saturable mechanism and a small amount of insulin is made in the brain. Insulin plays an important role in normal memory formation, affecting

synaptic modulation in long-term potentiation (LTP) and long-term depression (TD) (Zhao et al., 2009, McNay et al., 2011). It does this by regulating pathways involving a-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), type-A Gamma-Aminobutryic Acid (GABA) and (N-methyl-D-aspartic acid) NMDA (Zhao et al., 2009). Insulin signaling also affects ATP-gated K potassium channels partially through phosphoinositide 3-kinase (PI3K) but also through mitogen-activated protein kinase (MAPK) and as yet unknown other pathways (McNay et al., 2011). Insulin receptors are distributed throughout the brain with a particular concentration in the hippocampus further supporting the role of insulin in memory (De Felice et al., 2014).

The results of a recent systematic review found an equivocal relationship between either fasting insulin levels or insulin resistance and cognitive outcomes (Geijselaers et al., 2015). Similarly, two recent autopsy-based studies reported conflicting results on the association of peripheral insulin resistance with AD pathology (Matsuzaki et al., 2010, Thambisetty et al., 2013). In a Japanese study of 135 autopsies, insulin resistance was associated with neuritic plaque formation but not neurofibrillary tangles (Matsuzaki et al., 2010). In contrast, a US-based study of 197 autopsies found no association between insulin resistance and brain markers of AD using ^{11}C-PiB-PET, or the Consortium to Establish A Registry for Alzheimer's disease (CERAD) or Braak score (Thambisetty et al., 2013). In a neuroimaging study using fludeoxyglucose F18–positron emission tomography (FDG-PET), greater insulin resistance was associated with more diffuse and extensive activation in a pattern similar to that seen in early AD suggesting insulin resistance may share common pathways with AD pathology (Baker et al., 2011). However, most of these studies used crude measures of insulin resistance such as the homeostasis model assessment technique and also differed with regards to sample selection. These contradictory results highlight the complexity of understanding the role of insulin resistance in contributing to dementia, probably best examined in basic

experimental models or complex physiological in-vivo studies, using, for example hyperinsulinaemic-euglycaemic clamps.

Brain resistance to the action of insulin is seen both in T2DM and in AD suggesting common disease mechanisms linked to the generation of amyloid and tau (Hoyer et al., 1988, De Felice et al., 2014). Abnormalities in insulin and insulin-like-growth-factor 1 signaling and receptors are seen in both the peripheral tissues of those with T2DM and in the brain of those with AD (Steen et al., 2005, Ma et al., 2009, Moloney et al., 2010, Talbot et al., 2012). There is increasing interest in the potential mediating role of soluble amyloid-β oligomers (AβOs) that are toxic to synapses and can cause insulin resistance (Craft, 2012, Talbot et al., 2012, De Felice et al., 2014). These oligomers can bind to synaptic dendrites and remove insulin receptors (Zhao et al., 2008, De Felice et al., 2014) as well as affecting the insulin/AKT pathway (Park, 2011). Impaired insulin signaling results in the reduced inhibition of glycogen synthase kinase-3 (GSK3), and the ensuing increased GSKα activity increases APPγ-secretase activity thus increasing $A\beta_{42}$ levels, while increased GSK3β increases the phosphorylation of tau which may also potentiate $A\beta_{42}$ generation (Phiel et al., 2003, Hooper et al., 2008). Increased GSK3β activity is associated with down-regulation of the tau associated protein O-GlcNAcylation, as well as increased tau phosphorylation (Deng et al., 2009, Liu et al., 2009). Further evidence to support the role of insulin in the pathogenesis of AD is found in the action of insulin degrading enzyme (IDE). IDE is the major catalytic enzyme of both insulin and Aβ (Park, 2011). In the setting of hyperinsulinaemia, more efficient binding of insulin to IDE, reduces the availability of IDE to breakdown toxic Aβ, exacerbating AD pathology (Park, 2011). In animal models, IDE deficiencies result in greater AD pathology and enhancement of IDE reverses this (Leissring et al., 2003).

Drugs that sensitize cells to insulin such as thiazolidinediones may also hold some promise in modifying AD pathology (Kummer et al., 2015). In a study of 42 patients with T2DM and

mild AD who were randomised to either pioglitazone or placebo, those in the intervention arm showed improved working memory and cerebral SPECT perfusion compared with the placebo group (Sato et al., 2011). In another study of older people without dementia but with T2DM (n =145) randomised to 24 weeks of either rosiglitazone or glyburide, improvement in working memory was observed equally in both arms (Ryan et al., 2006). Treatment not only improved insulin levels but also measures of glycaemic control, making it difficult to uncouple whether the effects were due to alteration in insulin, glucose or both. However, the use of thiazolidinediones to modify dementia risk may be limited by an increased associated risk of cardiovascular events and bladder carcinoma (Singh et al., 2007). More recently developed agents for T2DM that show potential promise in altering AD mechanisms (Kosaraju et al., 2013a, Kosaraju et al., 2013b, Xu et al., 2015) include glucagon-like peptide (GLP-1) receptor agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors. These agents work on the same mechanistic pathway (DPP-4 is the enzyme that degrades GLP-1) and have a number of modes of action that effect insulin signalling in the peripheral tissue (Drucker, 2003). Trials examining the potential benefit of intranasally-administered insulin are also showing promise and are ongoing (Craft et al., 1999, Craft et al., 2012).

2.2.2.1 Advanced Glycation End-products

Hyperglycaemia increases the formation of Advanced Glycation End-products (AGEs) (Bucala et al., 1992) as well as promoting other markers of oxidative stress and inflammation (Whitmer, 2007b). AGEs are products of non-enzymatic reactions between reactive carbonyl groups of compounds (such as glucose, fructose, methylglyoxal) with proteins, lipids or nucleic acids (Price et al., 2007). The initial reversible products of these reactions (such as Schiff's bases) may undergo further degradation/rearrangement to form several AGEs, which interact with the receptor for AGEs (RAGE) and induce a chronic inflammatory process known to contribute both to the pathogenesis of diabetes (Forbes et al., 2011, Uribarri et al.,

2011) as well as the vascular complications of diabetes (Forbes et al., 2013). Schiff bases can also react irreversibly with amino acid residues of proteins, leading to intracellular protein cross-linking resulting in cellular structural and functional changes (Gerrits et al., 2008). In addition, AGE-related protein cross-linking within the extracellular matrix of major blood vessels may contribute to arterial stiffening (Price et al., 2007, Lund et al., 2011).

In addition to mediating vascular disease in T2DM, there is a large body of evidence from in vitro model research supporting a mechanistic role for AGEs in neurodegeneration and AD (Srikanth et al., 2013). Intracellular AGEs may lead to direct neuronal toxicity, or modify tau and thus contribute to neurofibrillary tangle formation (Yan et al., 1995). Extracellular AGEs may play a role in accelerating beta-amyloid aggregation (Kuhla et al., 2007) or initiate pro-inflammatory processes in the brain (Takeda et al., 1998). Greater serum levels of AGEs are associated with cognitive decline (Yaffe et al., 2011) and lower grey matter volumes in older people (Srikanth et al., 2013). There is strong evidence from basic science research that tissue AGE accumulation plays a role in the pathogenesis of dementia in those with T2DM (Srikanth et al., 2011). Greater levels of AGEs have been found to be co-located with amyloid plaques (Smith et al., 1994, Vitek et al., 1994) and paired helical filament tau in sporadic AD (Yan et al., 1994) and may act by stabilising plaques and promoting fibrillation of tau through protein cross-linking (Luth et al., 2005, Li et al., 2012, Munch et al., 2012). AGEs may also be directly cytotoxic to neurons in culture (Takeuchi et al., 2000) and able to directly induce inflammation and oxidation (Srikanth et al., 2011) by binding with RAGE in mitochondria, generating free radicals and reducing clearance of pre-existing reactive oxygen species (Munch et al., 2012). Furthermore, RAGE interacts with serum β-amyloid, increasing the transport of β-amyloid across the BBB, activating pro-inflammatory cytokines and reducing cerebral blood flow (Deane et al., 2003).

2.2.2.3 Cytokines and Adipokines

Increasing age, T2DM and AD are all associated with chronic inflammation (Clark et al., 2012, Vitale et al., 2013, Ferreira et al., 2014). AGEs and hyperinsulinaemia increase inflammatory mediators such as tissue necrosis factor α, interleukin-1β and interleukin-6 (Park, 2011). Chronic inflammation, particularly involving these three cytokines is associated with dementia (Halliday et al., 2000, Strachan et al., 2011, De Felice et al., 2014). The results from a number of recent studies have supported the presence of an association between inflammatory mediators and cognitive impairment (Strachan et al., 2011). Although inflammatory mediators have the ability to cross the blood brain barrier and alter its permeability (Perry et al., 2010), it is unclear whether they act directly on the brain to cause neurodegeneration, act as simply markers of disease or contribute to vascular disease that may cause cognitive impairment (De Felice et al., 2014). Neuroinflammation is difficult to measure directly in-vivo and hence has not yet been studied in people with T2DM. It is becoming increasingly recognized that adipose tissue is metabolically active, producing adipokines that mediate metabolism and cytokines that mediate inflammation (Luchsinger et al., 2009). In addition, adipokines such as leptin, adiponectin and resistin are being investigated as possible mechanistic links between T2DM-related obesity and the increased risk of dementia (Luchsinger et al., 2009). Blood levels of leptin are directly related to adiposity and help regulate body weight in mammals (Friedman et al., 1998). Direct leptin administration improved memory in mice (Harvey et al., 2005) and in one human study (n=3) with a recessive ob gene that produces leptin deficiency and obesity, was associated with increased grey matter (Matochik et al., 2005). However, in contrast, another study of 32 young adults (mean age 32 years) reported that increased levels of leptin were associated with greater grey matter volume in some brain regions (left cerebellum and left inferior temporal

gyrus) but reduced grey matter volume in others (left inferior frontal operculum, left postcentral gyrus, and right putamen) (Pannacciulli et al., 2007).

Adiponectin is almost exclusively excreted by adipose tissue and regulates insulin sensitivity, glucose homeostasis and has a strong anti-inflammatory effect making it a potential mediator of obesity and T2DM-related cognitive impairment (Dzielinska et al., 2003, Chan et al., 2012, van Himbergen et al., 2012, Teixeira et al., 2013). In a study of 157 subjects, baseline levels of adiponectin were lower in those with mild cognitive impairment (MCI) and AD than in normal controls (Teixeira et al., 2013). In the Framingham Heart Study, greater and not lower levels of adiponectin were associated with an increased 13 year risk of all cause dementia and AD, possibly due to weight loss early in dementia (van Himbergen et al., 2012). Animal models suggest that adiponectin may have a neuroprotective effect related to improving insulin sensitivity but studies investigating its effect in those with T2DM are lacking (Ahima et al., 2006, Semple et al., 2007, Hivert et al., 2008, Chan et al., 2012). Another adipokine involved in insulin resistance, resistin, may also play a role but further research is required (Whitmer, 2007a, Anan et al., 2010, Kiliaan et al., 2014).

2.2.2.4 Amylin

Amylin, or islet amyloid polypeptide (IAPP) was first identified in the pancreas of people with T2DM (Cooper et al., 1987). Insoluble deposition of the misfolded protein in β pleated sheets is found in the brain as well as the pancreas of those with T2DM (Jackson et al., 2013). The full range of functions of amylin is unclear but includes being amyloidogenic and, in human but not rat models, neurotoxic (Fu et al., 2013). Soluble amylin appears to be similar in its neurotoxicity to Aβ (Lim et al., 2008, Lim et al., 2010), and may share similar mechanisms of toxicity (Fu et al., 2013). Amylin deposition has been found in blood vessels and parenchyma of people with AD without T2DM (Jackson et al., 2013) and in some cases,

were co-localized with Aβ in cerebral plaques (Jackson et al., 2013). Amylin is elevated in obesity and in pre-diabetes insulin resistance and may contribute to the oxidative and inflammatory stress seen in T2DM but further research to better understand its contribution to cognitive impairment is required (Despa et al., 2013).

2.2.2.5 Glucocorticoid-related mechanisms

Both T1DM and T2DM are associated with increased levels of circulating cortisol (McNay et al., 2011) which is negatively associated with cognitive impairment (Lupien et al., 1994). In animal models, increased levels of cortisol have been associated with reduced synaptic plasticity and neurogenesis, particularly in the hippocampus (Csernansky et al., 2006, Stranahan et al., 2008, Huang et al., 2009) and have been shown to be associated with reduced learning and poor memory (Stranahan et al., 2008). Similar findings have been reported in humans with increased circulating levels of cortisol found to be associated with hippocampal atrophy (Lupien et al., 1994) and impaired memory (Bruehl et al., 2007). However, the exact nature of the contribution of glucocorticoid dysfunction is unclear as there may be a number of confounders. Depression is strongly associated with T2DM and with increased levels of cortisol possibly confounding the role of glucocorticoid dysfunction (Strachan et al., 2011). Furthermore, although cortisol levels are elevated in both T1DM and T2DM, it is predominately those with T2DM that develop significant cognitive impairment (McNay et al., 2011) highlighting that further studies are required to extract the contributory role of glucocorticoid dysfunction in T2DM-related cognitive impairment.

2.2.3 Potential disease pathways in the brain

There are a number of pathways in the brain through which T2DM may contribute cognitive impairment. These include neurodegenerative and cardiovascular pathways. Both of these pathways likely contribute to T2DM-related cognitive impairment and understanding the relative contribution of these may help guide targeted interventions to either prevent or treat cognitive decline (Launer, 2009).

2.2.3.1 Neurodegeneration

Several of the postulated mechanistic factors described previously such as insulin resistance, advanced glycation and chronic low grade neuroinflammation may play important roles in leading to accelerated loss of neurons and connectivity in people with T2DM. In addition, it is also possible that vascular disease related to T2DM contributes to this process of neuronal loss.

Brain atrophy and altered connectivity

Brain atrophy is the shrinkage of brain regions secondary to the loss of neurons and their interconnections (Jobst et al., 1994). Brain imaging techniques using MRI allow the in-vivo measurement of brain atrophy as a biomarker of neurodegeneration. At the time of commencement of this thesis, a few brain imaging studies had been conducted demonstrating an association between T2DM and lower brain volumes, suggesting that T2DM may promote neurodegeneration (den Heijer et al., 2003b, Biessels et al., 2006a, van Harten et al., 2006, de Bresser et al., 2010, Kamiyama et al., 2010, van Elderen et al., 2010). In other cross-sectional studies, T2DM was found to be concomitantly associated with impairment of cognitive domains and lower brain volumes (Akisaki et al., 2006, Jongen et al., 2007, Kumar et al., 2008, Tiehuis et al., 2009, Hayashi et al., 2011), but these did not explore whether the link between T2DM and cognitive dysfunction was mediated by brain atrophy. Although it was

known that T2DM was associated with hippocampal atrophy and amygdalar atrophy (den Heijer et al., 2003a), there was no information in the literature regarding the regional distribution of atrophy on a whole-brain basis. Moreover, very few longitudinal studies have explored the relationship between T2DM and cognitive decline. In the Utrecht Diabetic Encephalopathy Study, the trajectory of cognitive decline was compared between 68 patients with T2DM and 38 controls without T2DM over 4 years (Reijmer et al., 2011). A total of 17/68 (25%) of those with T2DM demonstrated accelerated cognitive decline, and this decline was associated with a greater increase in ventricular volume, a surrogate marker for brain atrophy. In addition to overt atrophy, several studies using functional MRI techniques have demonstrated loss of functional connectivity in people with T2DM early in the course of disease even before clinical signs of cognitive dysfunction appear (Musen et al., 2012, Hoogenboom et al., 2014). Such loss of connectivity may indicate early neurodegeneration in vulnerable regions such as those involved in the default mode network (precuneus, cingulate cortex).

2.2.3.2 Cerebrovascular disease

Vascular disease is an established complication of T2DM related to the effects of chronic low grade inflammation, endothelial dysfunction and atherosclerosis. Given that the brain is highly susceptible to the effects of vascular disease, it is logical that such disease plays a role in T2DM-related brain dysfunction.

Brain infarcts

T2DM is a well-recognized risk factor for the development of acute cortical and subcortical infarcts (Mankovsky et al., 1996, Longstreth et al., 1998, Vermeer et al., 2002, Pendlebury et al., 2009, Pantoni, 2010, Savva et al., 2010). T2DM is also associated with an increased risk of “silent” or subclinical infarcts in radiological studies (Longstreth et al., 1998, Vermeer et al., 2002). Strokes, clinical (Srikanth et al., 2006) or subclinical (Vermeer et al., 2007), are

associated with an increased risk of dementia. In the Rotterdam study, the presence of subcortical stroke was found to double the risk of developing all cause dementia as well as AD (Vermeer et al., 2003b). But, it is not certain as to what role brain infarcts play in explaining the association between T2DM and dementia. In a longitudinal Japanese study of 67 patients with T2DM (Imamine et al., 2011), the number of silent brain infarcts on MRI at baseline (n=27) was a strong predictor of cognitive function at 3 year follow up suggesting a contributory role in T2DM-related cognitive decline. In contrast, in another Japanese study of 1,543, neurologically normal individuals (mean age ~62 years), the association between metabolic syndrome (central obesity and presence of two of: hypertension, diabetes or dysglycaemia) (n=186) and lower executive function was independent of silent brain infarcts (Bokura et al., 2010). Further studies are required to clarify the contribution of brain infarcts in T2DM-related cognitive impairment, in particular whether their role is independent of neurodegeneration or whether they interact with neurodegenerative processes to cause cognitive dysfunction.

Large and small vessel disease/dysfunction

T2DM is associated with macrovascular and microvascular complications and some markers of this vessel disease may provide evidence of the mechanistic pathways through which T2DM-related cognitive decline occurs.

Markers of large vessel disease

Non-invasive measurement of the carotid artery can provide clues of the health of large blood vessels. An increase in the distance between the lumen-intima junction and the media-adventia junction, carotid intima-media thickness (CIMT) is a sign of atherosclerosis and has been shown to be associated with an increased risk of cardiovascular events (Bots et al., 1997, Lorenz et al., 2007). There is not a consistent relationship between T2DM and CIMT,

with one study finding those with T2DM had greater CIMT (Brohall et al., 2006) while another did not find an association (Manschot et al., 2007). Although two longitudinal studies have shown increased CIMT to be associated with an increased risk of future cognitive decline (Komulainen et al., 2007, Sander et al., 2010), there have been no studies examining this relationship in those with T2DM. Similarly, carotid pulse wave velocity, a marker of arterial stiffness, has been shown to be associated with longitudinal cognitive decline (Waldstein et al., 2008, Elias et al., 2009) and, in the Age, Gene/Environment Susceptibility – Reykjavik study was associated with lower memory scores, subcortical infarcts and white matter hyperintensity (WMH) volume (Mitchell et al., 2011). In this study, the inclusion of MRI brain imaging markers attenuated the association between arterial stiffness and the cognitive scores suggesting the arterial stiffness may lie on a potential mechanistic pathway. Further studies are required to examine its role in T2DM-related cognitive impairment.

Markers of small vessel disease

The similar embryological, anatomical and functional properties of retinal and cerebral vessels combined with ease of visualization of retinal vessels make them a potentially useful surrogate of cerebral small vessel disease (Patton et al., 2005, Ikram et al., 2013c). This has led to recent investigators using retinal vascular imaging measures in studies of those with and without cerebrovascular disease (Kwa et al., 2002, Patton et al., 2005, Nguyen et al., 2006, Hughes, 2007), and such measures are associated with a greater risk of future stroke (Wong et al., 2001) and dementia (Wong et al., 2002, Baker et al., 2007, Lesage et al., 2009, de Jong et al., 2011, Ding et al., 2011, Haan et al., 2012). T2DM is associated with retinopathy (Klein et al., 1992) and retinal vascular changes including greater retinal arteriolar calibre (Nguyen et al., 2008), and greater arteriolar tortuosity (Sasongko et al., 2011). Therefore, it is possible that retinal vascular imaging may assist in establishing a small vessel basis for T2DM-related brain atrophy.

Nephropathy is also a marker of microvascular disease and can be measured by the presence and extent of microalbuminuria (Mogensen et al., 1985). In one study of 2,049 people, greater microalbuminuria was cross-sectionally associated with poorer psychomotor speed in those with peripheral artery disease (Kuo et al., 2007). In a study of 28,384 participants combined from two clinical trials, those with vascular disease or T2DM who had microalbuminuria had lower MMSE scores than those with normoalbuminuria (OR 1.26, 95%CI 1.11-1.44) (Barzilay et al., 2011). On five year follow up, those with microalbuminuria at baseline were more likely to exhibit a 3 point decline in MMSE (OR 1.22, 95%CI 1.07-1.38). Interestingly, the use of an angiotensin-converting enzyme inhibitor and/or an angiotensin receptor blocker appeared to attenuate some of the risk associated with microalbuminuria suggesting a therapeutic pathway for further exploration.

White matter hyperintensities

WMH, as described in Chapter 1, commonly coexist with stroke, are associated with increasing age and hypertension and have established effects on cognitive and motor function (Debette et al., 2010b). They are often referred to as having a “microvascular” cause, although this is by no means certain. The links between T2DM and the prevalence of WMH are less clear. A meta-analysis of 25 studies reported an inconsistent relationship between T2DM and WMH burden (van Harten et al., 2006). Differences in the use of ordinal visual scales or the use of volumetric measurements may have contributed to this lack of consistency. Of the nine studies that could be included in the meta-analysis, the OR of the association between T2DM and WMH, ranged from 1.8-2.4 (van Harten et al., 2006). A study of 2011 participants in the Dallas Heart study reported that the combination of hypertension, diabetes and greater body mass index was associated with greater WMH burden in those > 50 years of age (p=0.0008) (King et al., 2014). However, there was only a trend for those with T2DM alone (n=245) to have greater WMH burden (p=0.053) suggesting

that the effect of T2DM may be small and linked to other diseases that commonly co-exist with T2DM such as obesity and hypertension. In the same Japanese study of 67 patients with T2DM described above, the burden of WMH was associated with baseline performance in the Digit Symbol Substitution test (DSST) (a test of psychomotor speed and attention). WMH progression was associated with more rapid decline in DSST performance compared to those who had stable WMH burden over 3 years (Imamine et al., 2011). The anatomical distribution of WMH i.e. in periventricular or deep white matter, may also be an important factor. In a Dutch study of 92 patients with T2DM (mean age 73 years), an association was found between periventricular WMH and processing speed, but not between deep WMH and any cognitive domain (van Harten et al., 2007).

Cerebral microbleeds

Little is known about the prevalence of cerebral microbleeds in those with T2DM and, whether they play a mediating role in the T2DM-cognition relationship. In an Icelandic study of 4,218 participants, those with T2DM (n=469) and retinopathy (indicating greater vascular disease) were more likely to have cerebral microbleeds than those without T2DM and retinopathy (Qiu et al., 2008). Conversely, in a study of 523 patients with acute stroke, there was no association between T2DM and cerebral microbleeds (Kim et al., 2008). These inconsistencies may also be due to the different MRI sequences or resolution of scanner used. In a study using ultra-high field 7T MRI comparing 48 people with T2DM to 49 people without T2DM (mean age 70 years) (Brundel et al., 2014), there was no association between T2DM and the presence of cerebral microbleeds. Furthermore, the presence or number of microbleeds was not associated with cognitive function. Further studies are required for a greater understanding of the role of cerebral microbleeds in cognitive impairment and T2DM.

Blood brain barrier integrity (microvascular disease)

T2DM is associated with disruption of the BBB in animal models (Antonetti et al., 1998, Hawkins et al., 2007) but the association between T2DM and BBB disruption in humans autopsy studies is less consistent (Serlin et al., 2011). One of the theories regarding the pathogenesis of small vessel disease and its contribution to dementia is that it is related to endothelial dysfunction and disruption of the BBB, allowing leakage of plasma components into the vessel wall and damaging surrounding tissue (Wardlaw et al., 2003, Farrall et al., 2009, Erickson et al., 2013). Imaging of the integrity of the BBB in-vivo is challenging, with some researchers adopting MRI tracking of the gadolinium containing compound, gadopentetate dimeglumine diethylenetriaminepentaacetic acid (Gd-DTPA) as a potential method. In a very small study comparing BBB integrity using MRI Gd-DTPA between 10 men with T2DM >65 years of age and 10 non-T2DM controls, those with T2DM had greater BBB permeability (and therefore less integrity) and furthermore, this was more pronounced in those who had WMH (whether having T2DM or not) (Starr et al., 2003). More in vivo studies of BBB integrity are required to better understand its role in T2DM-related cognitive impairment.

2.3 Conclusion

The study of risk factors, mechanisms and disease pathways linking T2DM and dementia are of substantial interest. The relative contributions of neurodegeneration and cerebrovascular disease, and their interactions are not well understood. A better understanding of this area may lead to therapeutic avenues to reduce the risk of dementia in those with T2DM and in the general population. At the time of commencing this research, the associations between T2DM and the different radiological markers of brain disease were unclear. This was particularly the case when addressing whether these markers (and by extension, causative mechanisms)

mediated the relationship between T2DM and cognitive impairment. There are a large number of potential risk factors and mechanistic pathways which likely interact to explain the association between T2DM and dementia. The relative strength of contribution of each of these factors likely differs at the individual level. The studies presented in subsequent chapters of this thesis provide evidence that enable a better understanding of the contributions of cerebrovascular and neurodegenerative pathways to T2DM-related cognitive impairment, and provide some insights into potential mechanisms.

Study framework

3.1 Introduction

The broad aim of this thesis is to study the disease pathways that underlie the association between T2DM and dementia. The majority of the research presented (Chapters 4-6) was conducted within the Cognition and Type 2 Diabetes in Older Tasmanians (CDOT) study. A further study conducted in a second sample, derived from the United States' Alzheimer's disease Neuroimaging Initiative (ADNI), forms the basis of Chapter 7. In this current chapter, I will present the aims and hypotheses of my research, broadly introduce the samples and measurements used, and highlight my contribution to the research. Relevant details of background, design, analytical methods and discussion pertinent to each hypothesis are described in the relevant ensuing chapters.

3.2 Cognition and Type 2 Diabetes in Older Tasmanians (CDOT)

Aims and Hypotheses

Aim 1 *(Chapter 4):*

To define the regional distribution of brain atrophy in T2DM and to examine whether atrophy or cerebrovascular lesions are feasible links between T2DM and cognitive function.

Hypothesis 1:

Compared with a similarly-aged population-based reference sample without T2DM, older people with T2DM will have:

- Poorer cognitive function
- Greater cerebrovascular disease and brain atrophy
- These cognitive differences will be mediated by brain structural differences

Aim 2 *(Chapter 5):*

To study the association of tissue advanced glycation endproduct (AGE) accumulation with T2DM-related brain atrophy.

Hypothesis 2:

Compared with a similarly-aged population-based reference sample without T2DM, older people with T2DM will have:

- Greater tissue AGE levels
- Tissue AGEs will mediate the relationship between T2DM and brain atrophy

Aim 3 *(Chapter 6):*

To study the role of changes in retinal vascular architecture and clinical retinopathy, as surrogates for cerebral small vessel disease, in T2DM-related brain atrophy.

Hypothesis 3:

Compared with a similarly-aged population-based reference sample without T2DM, older people with T2DM will have:

- Greater retinopathy and changes in retinal vascular architecture
- Retinal measures will modify or mediate the relationship between T2DM and brain atrophy

Sampling

CDOT is a cohort study designed to study the mechanisms of cognitive decline and dementia in T2DM, with an emphasis on brain imaging markers of disease. Participants with T2DM were drawn from a database of people with T2DM registered in the National Diabetes Service Scheme (NDSS) and living in Southern Tasmania (postcodes 7000-7199). Tasmania is the southern island state of Australia with a population of 515,000 persons in 2015 (Statistics, 2015a). Southern Tasmania includes the capital city, Hobart with a population of

217,973 persons living in the greater Hobart area **(**see map, **Figure 3.1)**. The NDSS is a program of the Commonwealth Government, administered by Diabetes Australia Ltd. that provides equipment for monitoring and treatment of diabetes at subsidized prices to people who register for its benefits, with registration being voluntary. The NDSS is also available for research purposes, and those registered in the NDSS can be approached for studies if they have indicated a preference to be involved. A comparison sample of similarly aged people without T2DM was drawn from an existing randomly selected population-based sample accrued within the Tasmanian Study of Cognition and Gait (TASCOG) derived from the electoral roll within the same postcodes (7000-7199) (Srikanth et al., 2013). Recruitment and measurement of the sample spanned the period 2007 to 2011.

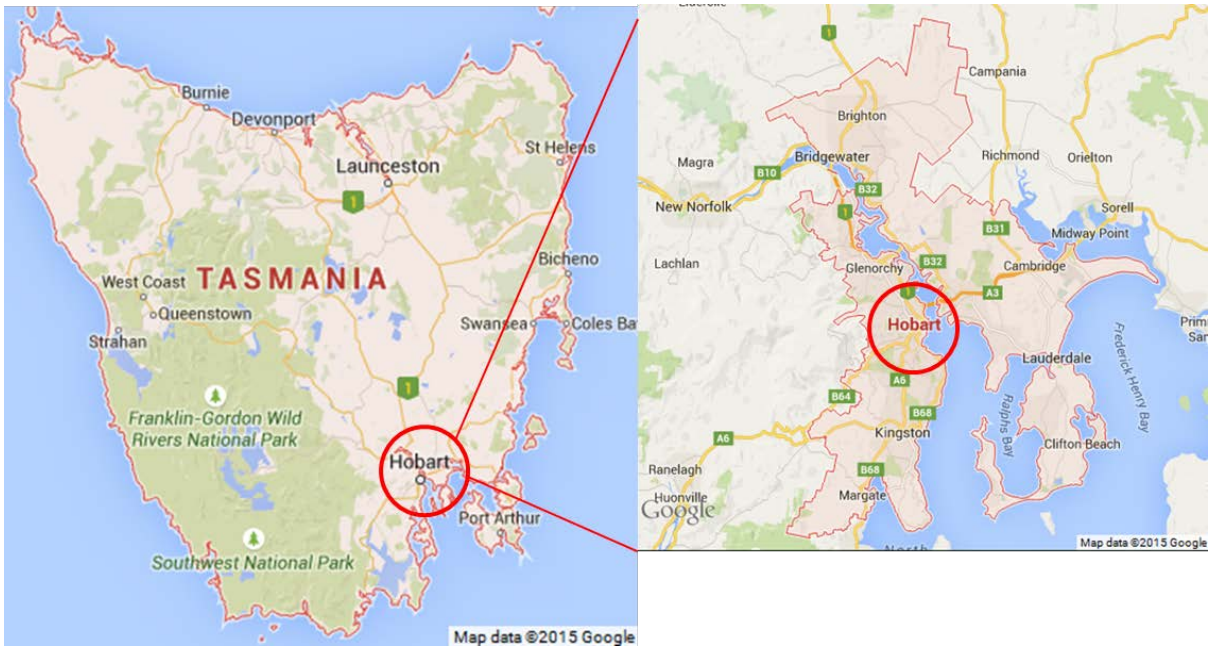

Figure 3.1 Hobart and Tasmania

Inclusion/Exclusion criteria

1. Participants with confirmed T2DM were included if >55 years of age, community dwelling, able to walk without the use of a gait aid and able to speak adequate English for cognitive testing.
2. The participants without T2DM drawn from TASCOG were included if >60 years of age, community dwelling, able to walk without the use of a gait aid and able to speak adequate English for cognitive testing.
3. Exclusion criteria, common to both samples were contraindications to Magnetic Resonance Imaging (MRI) or living in a residential aged care facility.

The diagnosis of T2DM was based on based on careful physician assessment by standard criteria (fasting plasma glucose ≥7.0 mmol/l, random plasma glucose ≥ 11.1 mmol/l or 2-h glucose ≥11.1 mmol/l after oral glucose tolerance test) (American Diabetes, 2010).

Funding

The studies were funded by project grants from the National Health and Medical Research Council of Australia (NHMRC), Application IDs 436797, 403000, 606503, and 491109.

Ethical approval

Written consent was obtained from all participants. The Southern Tasmanian Health and Medical Human Research Ethics Committee and the Monash University Health Research Ethics Committee provided approval for the studies.

Study Measurements

The collection of measurements in CDOT involved several methods including self-reporting using standardized questionnaires, direct interview and face-to-face measurements, cognitive testing, specialized vascular imaging and brain imaging, and collection of blood samples. Data collection was performed by a team involving the candidate, research nurse and research assistants at the premises of the Menzies Research Institute, Hobart. Brain MRI was performed at the Calvary Hospital, Hobart. Post processing of MRI scans and segmentation procedures were conducted at the imaging laboratory in the Stroke and Ageing Research Centre, Monash Medical Centre, Monash University. The specific details of methods and data collection relevant to the study hypotheses are provided within each ensuing chapter.

Broadly, the measurements included:

- Demographic data including education and employment history.
- Medical history with an emphasis on duration, treatment and complications of hypertension, cardiovascular and cerebrovascular disease, head injury, dementia, smoking and T2DM. In those with T2DM, further detail included duration of diagnosis, usual range of glycaemic control, frequency of hypoglycaemic events, glucose excursions requiring hospital admission, glucose lowering medication and insulin use, and presence and severity of microvascular and macrovascular complications.
- Prescribed and over-the-counter medications were checked and confirmed by a nurse.
- Physical activity was recorded using a self-report of length of time per day spent sitting, and performing light, moderate and vigorous physical activity. A pedometer was also worn by participants for 7 days to objectively measure the mean number of steps walked per day.

- Self-reported ability to complete activities of daily living was recorded and participants completed the Geriatric Depression Screening tool (Yesavage et al., 1982) to measure mood.
- Clinical measurements included height, weight, waist and hip circumference as well as blood pressure, measured as a mean of three seated measurements.
- Blood samples for ApoE4 status, fasting glucose, HbA_{1c} and insulin concentrations.
- A battery of cognitive tests was performed and described in detail in **Appendix B**.
- Skin AGE reader to measure tissue AGE levels.
- High-resolution 1.5 Tesla brain MRI for brain volume and cerebrovascular lesion measurement.
- Retinal photography.

Candidate's contribution

- Developing and refining study hypotheses based on existent literature.
- Contribution to design – particularly with respect to questionnaire design and data forms for the collection or T2DM-specific information (complications, treatment).
- Data collection - review of each participant's brain MRI, classifying the presence and location of cortical and sub-cortical infarcts and cerebral microbleeds using standardized methods. Individual brain segmentation of infarcts and post-processing voxel-based morphometric analyses under the guidance of imaging lab experts.
- Checking and clarification of T2DM phenotype status using self-report information, fasting glucose, HbA_{1c}, or use of glucose lowering medications as appropriate.
- Classification of participant's medication type into antihypertensive, lipid lowering, potentially effecting cognition (central nervous system active). In those with T2DM, glucose lowering medications classified into drug type. Developing summary score of

Geriatric Depression Scale score and creation of depression variable (score ≥5).

- Compilation of all demographic, medical history, medication, physical activity, mood, clinical and blood testing into complete dataset and data cleaning. Compilation of cognitive scores and summary measures using principal component analyses.
- Analysis of all data as reported within chapters and papers, generation of first draft of and revision of manuscripts.

3.3 Alzheimer's Disease Neuroimaging Initiative (ADNI)

The following aim was examined within the ADNI study:

Aim 4 *(Chapter 7):*

To explore the relationships between T2DM and biomarkers of neurodegeneration usually implicated in the development of AD.

Hypothesis 4*:*

When compared to a sample without T2DM, those with T2DM will have:

- Lower cortical thickness
- Greater levels of tau and lower levels of $A\beta_{42}$ in the cerebrospinal fluid (CSF)
- CSF biomarkers will mediate the relationship between T2DM and cortical thickness

Context and sampling

ADNI is a large multi-centered measurement-intensive study of AD and mild cognitive impairment (MCI) conducted in the United States of America (USA), from which data are made available for research access with permission. The primary goals of ADNI were to characterize clinical, cognitive, neuroimaging and genetic biomarkers of AD and MCI, to enable identification of those at risk of progression to dementia (Weiner et al., 2010). It had

the additional aims of establishing standardized methods for biomarker collection and analyses with the intent to inform their use in clinic trials (Weiner et al., 2010).

ADNI was funded by a public/private partnership (approximately 67% from the National Institute of Ageing). There are 58 recruitment sites and administration is divided into eight "cores" that manage: positron emission tomography; neuropathology; genetics; biomarkers; clinical; biostatistics; informatics and MRI (Weiner et al., 2010). Study protocol were reviewed by each site's hospital/university/institutional review board according to State and Federal requirements as described at www.adni-info.org. Written informed consent was obtained from all participants. The ADNI sampling targeted those between 55-90 years of age and was based on a clinical trial population referred from memory clinics combined with self–referral. Participants received a thorough cognitive examination and were clinically characterized as having AD, MCI or normal cognition. A key objective of ADNI was to create a data repository for academics for research (Weiner et al., 2010).

In order to follow up on initial findings in this thesis, the candidate requested permission to examine his hypothesis within the ADNI sample, and was granted access to the primary data. The role of diabetes in dementia was not a stated goal of ADNI, and hence the candidate undertook a rigorous effort to identify and phenotype people with T2DM for the purpose of this thesis. T2DM status was assigned based on fasting blood glucose $\geq$ 7.0mml/L as per ADA guidelines (American Diabetes, 2010) or the use of glucose-lowering agents.

Study Measurements

Specific data collected included:

- Demographic information on age, sex and educational attainment.
- Prescribed and over-the counter medications.
- Blood Pressure.
- Blood samples for fasting glucose but not HbA_{1c}, and ApoE4 status.
- Cognitive testing included the Mini-mental state examination (MMSE) and the Alzheimer's Disease Assessment Scale – cognitive subscale (ADAS-Cog). A study neurologist classified participants as having normal cognition, MCI or AD.
- High-resolution brain MRI was performed using (1.5 Tesla) scanners with rigorous quality control given multiple scanner sites. Measures of cortical thickness, hippocampal volume and white matter hyperintensity volume were generated, and raw scan data were also available for in-house analyses.
- Pittsburgh Compound B-^{11}C- Positron Emission Tomography (PiB-PET) imaging was performed in a subsample to quantify in-vivo cerebral amyloid deposition and raw scans were made available for in-house methods.
- CSF was drawn in a subsample and concentrations of total tau, phosphorylated tau and Aβ42 measured.

Candidate's contribution

- Developing and refining study hypothesis
- Data collection - Collation and cleaning of all demographic, medication, clinical, blood testing, brain imaging and CSF measures into complete dataset. This was followed by phenotyping of T2DM status for each individual using fasting glucose or

use of glucose lowering medications as appropriate. In those with T2DM, glucose lowering medications were classified into drug type.

- Design of analytical models for MRI, PET and CSF biomarkers for the purpose of this study, with assistance from supervisor and imaging analyst.
- Analysis of all data, generation of first draft, and revision of manuscript.

Monash University

Declaration for Thesis Chapter 4

Declaration by candidate

In the case of Chapter 4, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Developed and refined study hypotheses, classification of brain infarct and microbleeds, checking and clarification of T2DM phenotypes, statistical analysis, and interpretation and drafting of manuscript	70

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution
Thanh Phan	Image analysis, data interpretation, manuscript revision
Jian Chen	Image analysis, data interpretation, manuscript revision
Leigh Blizzard	Study concept & design, statistical analysis, manuscript revision
Richard Beare	Image analysis and manuscript revision
Alison Venn	Study design, data interpretation, manuscript revision
Gerald Munch	Study concept, data interpretation, manuscript revision
Amanda Wood	Study design, data interpretation, manuscript revision
Josephine Forbes	Study design, data interpretation, manuscript revision
Timothy Greenaway	Study design, manuscript revision
Susan Pearson	Study design, manuscript revision
Velandai Srikanth	Study concept, design analyses, data interpretation, manuscript revision

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	**Date** 26/6/15
Main Supervisor's Signature	[redacted]	**Date** 26.6.2015

*Note: Where the responsible author is not the candidate's main supervisor, the main supervisor should consult with the responsible author to agree on the respective contributions of the authors.

Brain atrophy in type 2 diabetes: regional distribution and influence on cognition

4.1 Introduction

This chapter describes the association between T2DM and MRI biomarkers of neurodegeneration and cerebrovascular disease, and attempts to study the mediating role of these biomarkers in explain T2DM-related cognitive dysfunction. At the time of commencement of this study, it was unclear whether T2DM contributes to cognitive impairment through predominantly neurodegenerative processes or cerebrovascular pathways, and the potential for mediation of effects had not been examined. Additionally, the regional distribution of brain atrophy in T2DM had not been described. The study described in this chapter addressed these issues, and was published in Diabetes Care (Moran et al., 2013) and received an editorial (Biessels, 2013). The chapter is presented as the published pdf version of the manuscript.

Brain Atrophy in Type 2 Diabetes

Regional distribution and influence on cognition

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OBJECTIVE—Type 2 diabetes (T2DM) is associated with brain atrophy and cerebrovascular disease. We aimed to define the regional distribution of brain atrophy in T2DM and to examine whether atrophy or cerebrovascular lesions are feasible links between T2DM and cognitive function.

RESEARCH DESIGN AND METHODS—This cross-sectional study used magnetic resonance imaging (MRI) scans and cognitive tests in 350 participants with T2DM and 363 participants without T2DM. With voxel-based morphometry, we studied the regional distribution of atrophy in T2DM. We measured cerebrovascular lesions (infarcts, microbleeds, and white matter hyperintensity [WMH] volume) and atrophy (gray matter, white matter, and hippocampal volumes) while blinded to T2DM status. With use of multivariable regression, we examined for mediation or effect modification of the association between T2DM and cognitive measures by MRI measures.

RESULTS—T2DM was associated with more cerebral infarcts and lower total gray, white, and hippocampal volumes (all $P < 0.05$) but not with microbleeds or WMH. T2DM-related gray matter loss was distributed mainly in medial temporal, anterior cingulate, and medial frontal lobes, and white matter loss was distributed in frontal and temporal regions. T2DM was associated with poorer visuospatial construction, planning, visual memory, and speed ($P \leq 0.05$) independent of age, sex, education, and vascular risk factors. The strength of these associations was attenuated by almost one-half when adjusted for hippocampal and total gray volumes but was unchanged by adjustment for cerebrovascular lesions or white matter volume.

CONCLUSIONS—Cortical atrophy in T2DM resembles patterns seen in preclinical Alzheimer disease. Neurodegeneration rather than cerebrovascular lesions may play a key role in T2DM-related cognitive impairment.

Type 2 diabetes (T2DM) is associated with an increased risk of incident cognitive impairment, dementia, and Alzheimer disease as a possible result of cerebrovascular and/or neurodegenerative disease (1–3). T2DM is associated with brain infarcts (4,5) on magnetic resonance imaging (MRI) and less consistently with cerebral white matter hyperintensities (WMHs) (6,7) and cerebral microbleeds (8,9). Lower hippocampal volume (10–12) and total brain volume (13), which are features of Alzheimer disease, are also more likely to occur in T2DM. However, few studies have clarified the regional distribution of brain atrophy attributable to T2DM (14–16). These studies were small, and only one compared people with and without T2DM, with the results suggesting that temporal lobe gray matter may be affected in T2DM (15). Understanding the pattern of brain atrophy in T2DM may provide clues toward the underlying neurodegenerative process. For example, gray matter atrophy occurs early in the temporal, parietal, and limbic cortices before spreading to involve frontal and occipital regions in Alzheimer disease (17). Moreover, although some studies demonstrated associations of T2DM with brain atrophy or cerebrovascular disease, no data describe how MRI measures of atrophy and cerebrovascular disease mediate the difference in cognitive function between those with and without T2DM. Manschot et al. (18) found an association between T2DM and more deep white matter lesions, cortical and subcortical atrophy, and infarcts as well as impaired cognitive performance. In subgroup analysis of only those with T2DM, they found that cognitive performance was inversely associated with deep white matter lesion volume, atrophy, and infarcts. In the current study, we examined the distribution of brain atrophy in older people with T2DM, predicting that MRI measures of brain atrophy and cerebrovascular disease would mediate or modify the association between T2DM and cognitive function.

RESEARCH DESIGN AND METHODS

Sampling

We used a cross-sectional study design, recruiting participants ≥55 years of age with T2DM who lived in Southern Tasmania and who were enrolled in the Cognition and Diabetes in Older Tasmanians (CDOT) study between January 2008 and January 2010. We used the National

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C.M., L.B., and V.S. contributed equally to the statistical analysis.

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Diabetes Service Scheme (NDSS) as a sampling frame. Diabetes Australia administers the NDSS, providing products, information, and support to people with diabetes who voluntarily enroll. The diagnosis of T2DM in the NDSS is based on careful physician assessment by standard criteria (fasting plasma glucose ≥7.0 mmol/L, random plasma glucose ≥ 11.1 mmol/L, or 2-h glucose ≥11.1 mmol/L after oral glucose tolerance test). Registrants indicated a willingness to participate in research. Approach letters were sent to all eligible people aged ≥55 years living in the postcodes 7000–7199. Exclusion criteria were people living in nursing homes, signifying severe frailty; those with insufficient English for cognitive testing; and contraindication to MRI. We derived the comparison group from a sample of people aged ≥60 years without T2DM who were recruited into the population-based Tasmanian Study of Cognition and Gait (TASCOG), which has been described previously (19). Approach letters were sent to residents randomly identified from the electoral roll who lived in the same postcodes as those in the CDOT study. Exclusion criteria were identical to the CDOT study. Absence of T2DM in the comparison group was defined as a fasting plasma glucose <7.0 mmol/L, random plasma glucose <11.1 mmol/L, and HbA_{1c} <6.5% (48 mmol/mol) in those without a history of T2DM. We calculated that 300 participants were needed in each group to detect a partial R^2 in the range of 2% (no covariates) to 3% (10 covariates). The Southern Tasmanian Health and Medical Human Research Ethics Committee and the Monash University Human Research Ethics Committee approved the study, and written informed consent was obtained.

Outcome measurements

MRI scans. MRI scans were obtained with a single 1.5-T General Electric scanner with the following sequences: high-resolution T1-weighted spoiled gradient echo (GRE) (repetition time [TR] 35 ms, echo time [TE] 7 ms, flip angle 35°, field of view 24 cm, 120 contiguous slices, isotropic voxel size 1 mm^3), T2-weighted fast spin echo (TR 4,300 ms, TE 120 ms, number of excitations 1, turbo factor 48, voxel size 0.90 × 0.90 × 3 mm); fluid attenuated inversion recovery (FLAIR) (TR 8,802 ms, TE 130 ms, inversion time 2,200 ms, voxel size 0.50 × 0.50 × 3 mm); GRE (TR 0.8 ms, TE 0.015, flip angle 30°, voxel size 0.9 × 0.9 × 7 mm).

Cerebrovascular lesions. Fully automated WMH segmentation was performed on FLAIR sequences using a validated method (20), and WMH volume was computed by a voxel counting algorithm. A single trained rater (C.M.) determined the presence of MRI infarct and microbleed with confirmation by consensus between two stroke experts (T.P., V.S.). Infarct was defined as a hypointensity ≥3 mm in diameter on three-dimensional T1-weighted and FLAIR images with a surrounding hyperintense rim on FLAIR (21). Microbleeds were defined as small, rounded, hypointense lesions with clear margins, ranging from 2–10 mm on GRE sequences. All measurements were done while blinded to group, age, sex, and outcome measures.

Brain Atrophy. Three-dimensional T1 and GRE sequences were registered in the standard Montreal Neurological Institute space using the Functional MRI of the Brain Linear Image Registration Tool (22). This process has the effect of normalizing the brain according to a standard template to take account of variation in brain size. A multispectral segmentation process was applied with the use of three-dimensional T1 and GRE sequences, and Statistical Parametric Mapping version 5 software (23) was used to produce tissue probability maps of gray and white matter. The images were modulated to correct for volume change induced by the normalization process. We also created maps of white matter unaffected by WMH by marking locations corresponding to WMH as empty in the tissue probability maps. Tissue maps were smoothed with an isotropic Gaussian kernel (full width at half maximum 8 mm) before voxel-based morphometric (VBM) analysis. With these tissue probability maps, we used a voxel counting algorithm to calculate gray, white, and WMH volumes. A single expert manually segmented both hippocampi by established methods known to have high test–retest reliability in our laboratory (intraclass correlation coefficient 0.97) (24). Tissue volumes of the segmented areas (total gray, normal-appearing white matter, and hippocampal) were calculated with voxel counting algorithms.

Cognitive testing. A standardized test battery was applied, which included the digit span, digit symbol coding, and symbol search subtests of the Wechsler Adult Intelligence Scale–Third Edition (25); the Hopkins Delayed Verbal Recall (26); the Controlled Oral Word Association Test (COWAT) (letter and animal categories) (26); the Victoria Stroop test (dot, color, and word) (26); and the Rey-Osterrieth Complex Figure (RCFT) copy and delayed recall tests (Supplementary Table 1) (26).

Other measurements. Fasting plasma glucose was recorded by a Roche cobas 6000 analyzer with hexokinase and HbA_{1C} determined with a Bio-Rad D-10 analyzer. We used standardized questionnaires to record demographic and clinical information about duration of T2DM; years of formal education; health and medical history, including vascular disease and risk factors; ever smoked; medication use; and alcohol use (grams per day). We measured weight, height, and waist and hip circumferences and calculated the BMI as weight in kilograms divided by height in meters squared. Habitual physical activity was calculated from the mean number of steps per day measured with a Yamax pedometer worn over a 7-day period. Mood was determined with the 15-item Geriatric Depression Scale (GDS) (27). Blood pressure (BP) measured with an Omron M4 sphygmomanometer while sitting was the average of three recordings from the right arm.

Data analysis

Student *t* and χ^2 tests were applied to compare mean scores and proportions of demographic, clinical, and cognitive variables between the T2DM and unaffected groups.

Voxel-based morphometry. VBM allows for unbiased, voxelwise comparison of local tissue volumes between groups. To identify the regions of brain atrophy attributable to T2DM, we used linear regression modeling to generate maps of gray and white matter atrophy associated with T2DM, including age, sex, education, and total intracranial volume as covariates, and a stringent false discovery rate ($P < 0.001$) to correct for multiple comparisons. Clusters of >100 statistically significant voxels were considered important and placed on a standard brain image to aid visualization. We used the Talairach atlas (28) to identify the anatomical locations of these clusters.

Multivariable regression. We studied the associations of T2DM with individual MRI measures, adjusting each regression for age, sex, and total intracranial volume. We used linear regression for continuous variables (total gray, total white, total WMH, and right and left hippocampal volumes) and logistic regression for infarcts and microbleeds (presence vs. absence). Similarly,

we performed linear regressions of T2DM against cognitive scores, adjusting for age, sex, education, and mood. For all these analyses, potential for confounding was examined for additional covariates and adjustments made if the addition of these terms changed the coefficient for T2DM by >10%. Covariates considered were hypertension (defined as mean BP >140/90 mmHg or previous diagnosis), hyperlipidemia (yes/no), alcohol use (grams per day), ever smoked (yes/no), mean steps per day, ischemic heart disease, stroke (except in the analysis of brain infarcts), psychoactive medication use, BMI, and waist–hip ratio.

To examine whether MRI measures mediated the associations detected between T2DM and cognition, we successively entered terms for MRI measures (total gray matter, total white matter, total WMH, right and left hippocampal volumes, cerebral infarcts, and cerebral microbleeds) into the models relating T2DM to relevant cognitive scores, adjusting for age, sex, and total intracranial volume. If the MRI measure introduced substantially attenuated the β coefficient for T2DM (>30%) and the coefficient of the MRI measure remained unchanged from its unadjusted value without T2DM in the model, it was considered a potential mediator. We also examined for two-way interactions between T2DM and MRI variables with a test of significance of product terms. We applied standard regression diagnostics to assess the adequacy of models. Statistical analyses were carried out with STATA 11.1 (StataCorp, College Station, TX) software.

RESULTS—There were 350 people in the T2DM group (mean age 67.8 [SD 6.9] years) and 363 in the non-T2DM comparison group (mean age 72.1 [7.2] years). Group characteristics and comparisons are presented in Table 1. Participants with T2DM reported a median disease duration of 7 years (interquartile range 4–12 years). T2DM participants had greater fasting blood glucose levels and HbA_{1C} values, higher BMI and waist–hip ratio, and greater GDS scores and were more likely to report a history of hypertension and hyperlipidemia, treatment with antihypertensive drugs and statins, and lower daily alcohol consumption.

Table 1—*Sample characteristics*

	T2DM (n = 350)	No T2DM (n = 363)	P value
Age (years)	67.8 (6.9)	72.1 (7.2)	<0.001
Female sex	140 (40)	168 (46)	0.09
Formal education (years)	11.3 (3.5)	10.9 (3.7)	0.24
Systolic BP (mmHg)	136.4 (19.1)	141.6 (22)	<0.001
Diastolic BP (mmHg)	76.2 (10.4)	80.4 (11.9)	<0.001
Self-reported history of hypertension or mean systolic BP >140 or diastolic BP >90 mmHg	252 (72)	163 (45)	<0.001
Use of BP-lowering medications	219 (62.6)	90 (25.7)	<0.001
Ischemic heart disease	82 (23.4)	69 (19.0)	0.15
TIA or stroke	37 (10.6)	24 (6.6)	0.06
Hyperlipidemia	167 (47.7)	32 (8.8)	<0.001
Statin use	218 (62.3)	89 (24.5)	<0.001
Ever smoked	191 (54.6)	179 (49.3)	0.15
Alcohol intake (g/day)	10.8 (16.3)	14.2 (17.5)	0.01
BMI (kg/m^2)	31.1 (8.6)	27.3 (4.3)	<0.0010
Overweight (BMI 25–30)	0 (0)	7 (2)	0.110
Obese (BMI >30)	129 (37)	183 (50)	0.004
Waist–hip ratio	0.96 (0.1)	0.90 (0.1)	<0.001
Steps per day	6,013 (3,605)	6,106 (3,185)	0.73
GDS score	2.5 (2.7)	1.9 (2.2)	0.002
Fasting blood glucose (mmol/L)	7.7 (2.3)	5.3 (0.6)	<0.001
HbA_{1c} (%)	7.2 (1.2)	5.6 (0.4)	<0.001
HbA_{1c} (mmol/mol)	55	38	
Age at diabetes diagnosis (years)	57.8 (12.0)	—	—
Median duration of T2DM (years) (IQR)	7 (4–12)	—	—
Insulin use	72 (20.6)	—	—
Cognitive scores (raw, unadjusted measures)*			
Hopkins immediate	23.7 (5.6)	21.8 (6.6)	<0.001
Hopkins recognition	10.2 (1.7)	9.9 (2.0)	0.08
Hopkins delayed	8.1 (2.9)	7.5 (3.1)	0.02
RCFT copy	28.1 (6.5)	31.6 (6.0)	<0.001
RCFT delay	12.7 (6.5)	14.6 (7.1)	<0.001
Digit symbol coding	52.1 (14.4)	49.6 (16.3)	0.03
Symbol search	24.5 (7.6)	22.5 (8.0)	<0.001
COWAT word	35.8 (12.9)	36.2 (13.0)	0.70
COWAT category	18.4 (4.8)	17.0 (5.1)	<0.001
Digit span	16.1 (4.0)	15.8 (3.9)	0.27
Stroop dot time	16.0 (5.1)	15.6 (5.4)	0.24
Stroop word time	20.2 (6.3)	21.6 (11.2)	0.07
Stroop color time	36.4 (15.4)	37.8 (23.5)	0.36

Data are mean (SD) or n (%) unless otherwise indicated. TIA, transient ischemic attack. *Cognitive score comparisons are unadjusted for age, sex, education, or mood.

T2DM and MRI measures

Unadjusted and adjusted comparisons of the MRI measures between groups are presented in Table 2. After adjusting for age, sex, and total intracranial volume, T2DM was associated with lower total gray, white, and hippocampal volumes ($P < 0.001$) and the presence of infarct ($P < 0.001$) but not with WMH volume or microbleeds. Restricting the analyses for only the highest quartiles of WMH volume did not alter the findings. Adjustment for other vascular risk factors did not appreciably attenuate the association of T2DM with the MRI measures (data not shown).

Results of VBM analysis of gray matter volume loss attributable to T2DM are shown in Fig. 1, and the anatomical regions of gray matter volume loss are listed in Supplementary Table 2. T2DM was associated with loss of cortical gray matter mainly in temporal, parahippocampal, cingulate, precuneus, insula, and medial frontal regions and with loss of subcortical gray matter in the caudate nucleus and putamen. The left hemisphere demonstrated more cortical gray matter loss

Table 2—*Associations between T2DM and MRI measures*

MRI measures	T2DM (n = 350)	No T2DM (n = 363)	Association of T2DM with MRI measures[1]	P value for regression
Gray matter volume (mL)	579.9 (66.9)	583.4 (63.1)	−13.1 (−18.7 to −7.6)	<0.001
Right hippocampal volume (mL)	2.32 (0.47)	2.77 (0.50)	−0.47 (−0.54 to –0.40)	<0.001
Left hippocampal volume (mL)	2.22 (0.44)	2.61 (0.48)	−0.41 (−0.48 to −0.34)	<0.001
Total hippocampal volume (mL)	4.54 (0.86)	5.38 (0.91)	−0.88 (−1.01 to −0.75)	<0.001
White matter volume (mL)	454.8 (62.1)	456.1 (55.5)	−6.14 (−11.9 to −0.42)	0.05
White matter lesion volume (mL)	6.04 (6.99)	7.10 (8.0)	0.59 (−0.54 to 1.71)	0.32
Infarct yes/no (%)[a,b]	75 (21)	58 (16)	0.62 (0.21 to 1.04)	0.001
Microbleed yes/no (%)[b]	14 (4)	22 (6)	−0.25 (−0.97 to 0.46)	0.41

Data are mean (SD) and β (95% CI). [1]Adjusted for age, sex, and total intracranial volume. [a]Not adjusted for total intracranial volume. [b]Not adjusted for stroke history.

than the right. T2DM was associated with white matter loss, mainly in frontal and temporal white matter (Supplementary Table 3). These associations were largely unchanged when further adjusted for gray matter volume.

T2DM and cognition

T2DM was independently associated with worse scores in RCFT copy ($P < 0.001$) and delayed recall ($P < 0.001$) and with a longer time to complete the Stroop dot test ($P = 0.004$) (Table 3). A longer duration of T2DM (≥15 years [n = 157]) was associated with poorer scores in RCFT copy ($P = 0.03$), digit symbol coding ($P = 0.001$), and symbol search ($P = 0.001$) than was <15 years disease duration (n = 183). Although there was a trend for poorer performance in other cognitive tests with longer duration of T2DM, these associations were not significant.

T2DM, MRI measures, and cognition

Supplementary Table 4 shows the magnitude of change in the β coefficients for T2DM against the cognitive scores caused by the stepwise addition of relevant MRI variables, wherein all models were adjusted for age, sex, education, mood, and total intracranial volume. The addition of total gray matter volume (including hippocampal volume) substantially attenuated the β coefficients of T2DM in RCFT copy (by 36.2%), RCFT delayed recall (by 54.9%), and Stroop dot (by 71.7%) scores. However, only very small additional changes in these coefficients were observed with the inclusion of terms for WMH volume (≤9.4%), white matter volume (≤1%), or infarcts (≤17.2%). We did not find biologically meaningful interactions between T2DM and MRI measures to explain cognitive performance.

CONCLUSIONS—This study provides novel voxel-based data from a large sample on the regional distribution of brain atrophy in older people with T2DM. In addition to the previously established association with hippocampal atrophy, T2DM was associated with temporal, frontal, and limbic gray matter atrophy and to a lesser extent with frontal and temporal white matter atrophy. To our knowledge, this study is the first to demonstrate that brain atrophy rather than cerebrovascular lesions may substantially mediate the relationship between T2DM and cognitive impairment, emphasizing the need to explore its underlying biological mechanisms.

Strengths of the study are the large sample size; careful definition of T2DM, the use of comprehensive MRI measures,

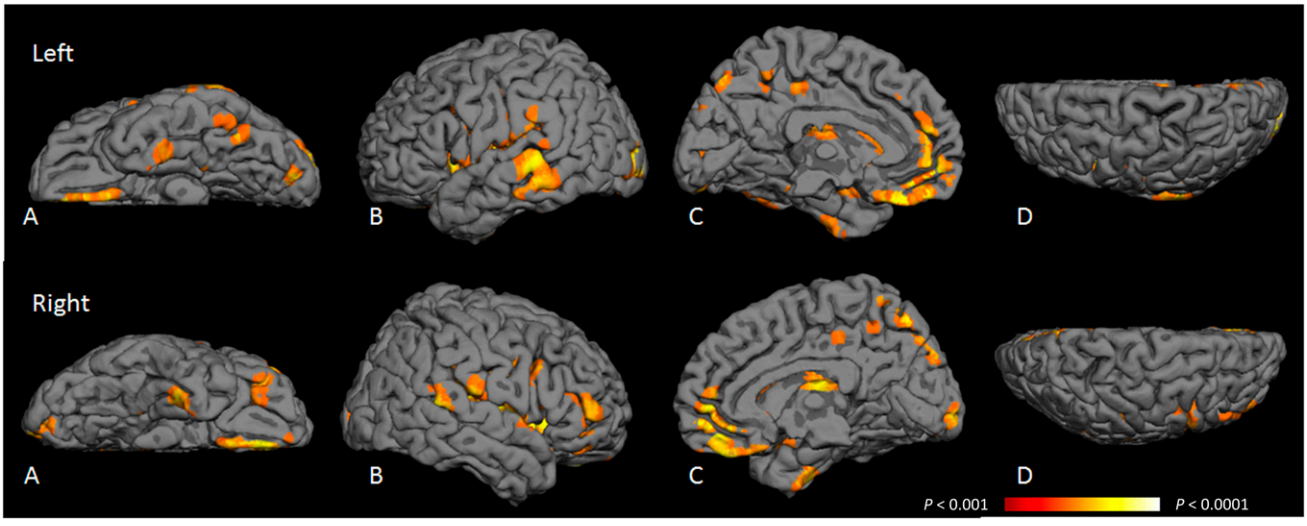

Figure 1—*Probability map of location of gray matter atrophy attributable to T2DM. VBM was used to create a probability map of areas of gray matter atrophy attributable to T2DM when adjusted for age, sex, education, and total intracranial volume. Voxels highlighted are those areas most likely to have gray matter atrophy attributable to T2DM, with a false discovery rate $P < 0.001$ (orange) to $P < 0.0001$ (yellow). These areas are detailed in Supplementary Table 2. A: Inferior region. B: Temporal region. C: Medial region. D: Superior region.*

Table 3—*Associations between T2DM and cognitive measures*

Cognitive variable	β (95% CI)	Standardized β	*P* value
Hopkins immediate	0.85 (0.02 to 1.68)	0.07	0.05
Hopkins recognition	−0.02 (−0.49 to 0.45)	−0.003	0.93
Hopkins delayed	0.09 (−0.34 to 0.52)	0.01	0.68
RCFT copy	−4.50 (−5.39 to –3.62)	−0.36	<0.001
RCFT delay	−3.28 (−4.26 to –2.29)	−0.24	<0.001
Digit symbol coding	−1.29 (−3.34 to 0.76)	−0.04	0.22
Symbol search	0.09 (−1.15 to 0.97)	−0.01	0.87
COWAT word	−1.34 (−3.31 to 0.60)	−0.05	0.17
COWAT category	0.27 (−0.45 to 1.00)	0.03	0.46
Digit span	0.20 (−0.80 to 0.39)	−0.03	0.50
Stroop dot time	1.13 (0.37 to 1.90)	0.11	0.004
Stroop word time	0.22 (−0.98 to 1.42)	0.01	0.72
Stroop color time	1.31 (−1.66 to 4.28)	0.03	0.39

β coefficient (95% CI) adjusted for age, sex, education, and GDS score. Standardized β coefficient of T2DM for regression against each cognitive measure.

fully automated brain segmentation, lesion detection while blinded to group status, a voxel-based whole-brain approach to study distribution of atrophy, and careful regression modeling to examine for mediation and effect modification. The study also has certain limitations. The cross-sectional design limits inference regarding causality, but the findings provide a strong basis for studying the global and regional effects of T2DM on brain atrophy longitudinally. Because we recruited T2DM participants from those indicating willingness to participate in research through their NDSS membership, participants at the healthier end of the spectrum of T2DM may have been overrepresented in the sample, explaining why cognitive differences were not more widespread. However, strong and consistent differences were found in more-sensitive brain MRI measures, suggesting that we may have captured people at an early stage of brain disease commensurate with less advanced T2DM. T2DM may be associated with changes in visual acuity, which may affect performance in visual cognitive tasks. However, if this were the case, we should expect that performance in all visual cognitive tasks would be confounded by vision. This is unlikely to be the case given that no association was found between those with T2DM and other complex visual attention/scanning tasks, such as digit symbol search, symbol coding, and Stroop word/color. In addition, we ensured that visual aids were used if required. Furthermore, visual acuity is unlikely to invalidate the mediation of cognitive differences by gray matter volume and hippocampal volume. Although we carefully adjusted for several important confounders, we cannot exclude the small possibility of residual confounding. Of note, the comparison sample was drawn from the same source population as those with T2DM, adding confidence to the observed results.

Until now, the distribution of brain atrophy in T2DM has been poorly defined. Results of few previous studies showed that T2DM is associated with total gray matter (16,29,30) and hippocampal volume loss (10–12). To our knowledge, only three were designed to examine regional gray matter loss associated with T2DM (14–16). A region of interest approach showed that gray matter volume is lower in the hippocampus in middle-aged people with T2DM (age range 60–64 years) (16). A study comparing 56 patients (mean age 68.1 years) with 30 control subjects found T2DM to be associated with lower cortical thickness in the middle temporal gyri (14). In the only published VBM study (n = 16 per group, mean age 61.2), T2DM was found to be associated with gray matter atrophy in the right temporal and precentral gyri (15). The present study, with the advantage of being much larger and having a substantial comparison group, demonstrates that T2DM is associated with gray matter in several bilateral regions of temporal, cingulate, and medial frontal cortices, with peak associations tending to be seen more in the left hemisphere. A notable parallel is that a similar distribution of cortical atrophy was described in early Alzheimer disease in neuropathological studies (31) and a longitudinal MRI study (17), where gray matter loss began in the temporal, entorhinal, and parietal lobes before progressing to orbitofrontal regions and more so in the left hemisphere. Increased insulin resistance has also been found to be associated with gray matter atrophy in a distribution similar to that found in Alzheimer disease (32). Although the study was not powered to assess the role of T2DM, the results suggested that glucose dysregulation may contribute to the pathophysiology of Alzheimer disease (32). The present results regarding white matter loss adds to findings of a single previous study (15) in which T2DM was found to be associated with temporal white matter volume loss. Although gray matter loss may lead to downstream white matter atrophy, the regional associations of white matter loss with T2DM were unchanged by the addition of gray matter volume in the model, suggesting a primary effect of T2DM on white matter. The association between T2DM and cerebral infarcts is well recognized (4,5) and likely to result from the proinflammatory vascular effects of T2DM as well as from other commonly coexistent risk factors, such as hypertension, smoking, and ischemic heart disease. We found no association between T2DM and WMH or microbleeds, which agrees with some studies in the field (16) but is inconsistent with others (8,29,33). It is possible that we may observe associations with these measures with longitudinal follow-up and accrual of more lesions. We were unable to estimate the association of T2DM with cerebral microvasculature, which is difficult to measure with current MRI techniques. Effects on blood-brain barrier integrity, neurovascular coupling, and cerebral microinfarcts are best estimated in basic models or pathological studies.

This study is the first in our knowledge to directly examine the mediating effect of MRI measures on the difference in cognitive performance between people with and without T2DM. Previous analyses were limited to within-T2DM groups alone (30,34). In one study, baseline total brain volume was correlated with a decline in the immediate Picture Learning Test (r = −0.292, P = 0.01) (30), and in another (34), periventricular WMH volume was associated with poor motor speed (β = −0.269, P = 0.04). In contrast, we were able to examine the mediation of the T2DM–cognition relationship by MRI measures in a large comparison group. In the present sample, T2DM was clearly associated with poorer function in visual construction, planning, visual memory,

and cognitive speed. Although infarcts and WMH were by themselves associated with poorer cognition (data not shown), the findings suggest that the predominant pathway linking T2DM and cognition (at least early in the course of disease) is brain atrophy. We also explored whether the presence of cerebrovascular lesions rendered the brain more susceptible to the effects of T2DM-related atrophy on cognitive function but were unable to demonstrate such an interaction. However, the study may have been underpowered to detect very small interactions, which may become apparent with a longer duration of T2DM and a greater load of cerebrovascular lesions, best demonstrable in longitudinal cohorts. Moreover, current modalities of brain imaging are not sensitive measures of blood-brain barrier integrity or microinfarcts, and thus, we cannot exclude a mediating role for microvascular disease.

The mechanisms underlying brain atrophy in T2DM may include endocrine, metabolic, and vascular pathways (35). T2DM is characterized by impaired glucose control and insulin resistance. Chronic hyperglycemia increases the formation of advanced glycation end products, which promote oxidative stress, cross-linking of amyloid fibrils, modification of cytoskeletal tau proteins, and inflammation (36). Insulin plays a major role in modulating cerebral glucose metabolism, and insulin receptors are selectively distributed in the hippocampus and cerebral cortex (37). Reduced insulin transport across the blood-brain barrier and cerebral insulin resistance in areas of high receptor concentrations may impair regional glucose metabolism (37) and contribute to preferential atrophy in these areas. Insulin and insulin-degrading enzyme also modulate intracellular β-amyloid release and extracellular clearance, potentially contributing to an Alzheimer-like neurodegeneration (32,38). Inflammatory cytokines associated with T2DM may also contribute, with interleukin-6, C-reactive protein, and homocysteine having been shown to be associated with cerebral atrophy (39). In addition, microvascular disease related to T2DM may be a cause of neuronal apoptosis and brain atrophy through impaired blood flow to the neurovascular unit.

In summary, gray matter atrophy associated with T2DM is widely and bilaterally distributed in hippocampi, temporal, frontal, and cingulate cortices and subcortical nuclei. It appears to be the primary driver of cognitive dysfunction in people with T2DM.

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C.M. contributed to the statistical analysis and data analysis and interpretation and drafted the manuscript. T.G.P. and J.C. contributed to the image analysis, data interpretation, and revision of the manuscript. L.B. contributed to the study concept and design, supervision of the statistical analysis, and revision of the manuscript. R.B. contributed to the development and supervision of the image analysis and revision of the manuscript. A.V., A.G.W., and J.F. contributed to the study design, data interpretation, and revision of the manuscript. G.M. contributed to the study concept, data interpretation, and revision of the manuscript. T.M.G. and S.P. contributed to the study design and revision of the manuscript. V.S. contributed to the study concept, design, and supervision; analyses; data interpretation; and revision of the manuscript and obtained the funding. V.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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SUPPLEMENTARY DATA

Supplementary Table 1. Cognitive domains and tests

Cognitive Domain	Tests
Memory factor	Hopkins Immediate Verbal Recall
	Hopkins Delayed Verbal Recall
	Hopkins Recognition
Visuospatial and planning factor	Rey Complex Figure Copy Task
	Rey Complex Figure Recall Task
Speed factor	Digit Symbol Coding
	Digit Symbol Search
Executive function	COWAT Word test
	COWAT Category Test
	Digit Span Recall
	Stroop Dot Time
	Stroop Word Time
	Stroop Color time

Supplementary Table 2. Stereotaxic coordinates and anatomical locations of gray matter loss in T2DM

Hemisphere	Stereotaxic voxel coordinates			Regions Involved
	X	Y	Z	
Left	-14	2	-16	Parahippocampal gyrus
Left	-64	-40	-10	Middle temporal gyrus
Left	-64	-24	-15	Inferior temporal gyrus
Right	44	-22	12	Transverse temporal gyrus
Left	-2	-35	39	Cingulate gyrus
Left	-5	-54	42	Precuneus
Right	8	48	2	Anterior cingulate
Left	-7	13	9	Caudate body
Left	-20	8	5	Putamen
Left	-40	-14	11	Insula
Left	-49	-9	6	Precentral gyrus
Left	-54	4	20	Inferior frontal gyrus
Left	-1	49	2	Medial frontal gyrus
Right	42	42	4	Middle frontal gyrus

SUPPLEMENTARY DATA

Supplementary Table 3. Stereotaxic coordinates and anatomical locations of white matter loss attributable to T2DM

Hemisphere	**Stereotaxic voxel coordinates**			**Regions Involved**
	X	Y	Z	
Right	6	12	52	Superior frontal lobe
Right	44	32	6	Inferior frontal lobe
Right	8	0	60	Medial frontal lobe
Left	-26	44	0	Middle frontal lobe
Right	21	-33	18	Cingulum
Right	32	-22	0	Corpus callosum
Right	38	-2	8	Insula
Left	-34	-2	8	Insula
Right	21	-45	2	Pulvinar nucleus
Right	28	-34	59	Parietal lobe

Supplementary Table 4. Magnitude of change in associations between T2DM and cognitive scores by step wise introduction of brain MRI measures

Brain MRI variable	RCFT copy		RCFT delay		Stroop dot time [a]	
	β (T2DM)	% change [b]	β (T2DM)	% change [b]	β (T2DM)	% change [b]
T2DM	-4.50		-3.28		1.13	
Total gray matter volume [c]	-2.87	36.2	-1.48	54.9	0.32	71.7
White matter volume	-2.90	1.0	-1.48	0	0.32	0
WMH volume	-2.89	0.3	-1.46	1.4	0.29	9.4
Infarct (Yes/No)	-2.85	1.4	-1.52	4.1	0.24	17.2

RCFT – Rey Complex Figure Task, β - beta-coefficient of T2DM

All models adjusted for age, sex, education, mood and total intracranial volume

[a] Higher scores in Stroop dot time indicate worse function

[b] Indicates % additional change in β coefficient of T2DM with introduction of each MRI variable

[c] Includes gray and hippocampal volume

4.3 Conclusion

The results from this paper showed that although T2DM was associated with cerebral infarcts, its association with brain atrophy mediated the relationship between T2DM and cognitive dysfunction. Furthermore the regional distribution of this grey matter atrophy was similar to that seen in AD. These results set the scene for the exploration of the mechanisms underlying T2DM-related brain atrophy as below:

- The hyperglycaemia associated with T2DM is a strong promoter of Advanced Glycation End-products (AGEs), promoters of oxidative stress, vascular stiffening and AD pathology (Srikanth et al., 2011). **Chapter 5** describes a study relating tissue accumulation of AGEs with brain atrophy in T2DM.
- To better understand the potential contribution of cerebrovascular disease to brain atrophy in T2DM, I examined the associations of retinal vessel architecture and retinopathy (surrogates of small cerebral vessel disease) with MRI biomarkers. The results of this analysis are presented in **Chapter 6.**
- The suggestion that T2DM may be related to an AD-type process led to analyses of the ADNI dataset presented in **Chapter 7**, firstly to replicate findings from this chapter in an independent dataset and secondly, to test whether imaging and CSF biomarkers of AD may play a role in T2DM-related brain atrophy.

Monash University

Declaration for Thesis Chapter 5

Declaration by candidate

In the case of Chapter 5, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Drafted and revised manuscript, statistical analyses and data interpretation	70

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution
Gerald Munch	Data interpretation and revision of manuscript
Josephine Forbes	Data interpretation and revision of manuscript
Richard Beare	Image analysis and data interpretation
Leigh Blizzard	Analysis and interpretation of data and revision of manuscript
Alison Venn	Interpretation of data and revision of manuscript
Thanh Phan	Image analysis, interpretation of data and revision of manuscript
Jian Chen	Image analysis and interpretation of data
Velandai Srikanth	Study concept and design, interpretation of data and manuscript revision

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	**Date** 26/6/15
Main Supervisor's Signature	[redacted]	**Date** 26.6.2015

*Note: Where the responsible author is not the candidate's main supervisor, the main supervisor should consult with the responsible author to agree on the respective contributions of the authors.

Chapter 5

T2DM, Skin Autofluorescence and Brain Atrophy

5.1 Introduction

Advanced Glycation End products (AGEs) are postulated to play an important mechanistic role in T2DM-related brain disease, possibly by promoting low grade inflammation, oxidative stress, promotion of brain tau and amyloid, and vascular stiffening due to protein cross linking (Srikanth et al., 2011). At the time of this paper's publication, serum greater AGE levels were known to be associated with cognitive decline and lower grey matter volume (Yaffe et al., 2011, Srikanth et al., 2013). However, no studies had examined whether AGEs influenced the relationship between T2DM and brain atrophy. This chapter describes the association between T2DM and skin autofluorescence (SAF), a surrogate marker of long term advanced glycation end-product (AGE) accumulation. This chapter was published in Diabetes (Moran et al., 2015) and is presented as the published pdf version of the manuscript.

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Type 2 Diabetes, Skin Autofluorescence, and Brain Atrophy

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Type 2 diabetes mellitus (T2DM) is associated with brain atrophy, but the mechanisms underlying this link are unknown. Advanced glycation end products (AGEs) accumulate in T2DM, resulting in inflammation, oxidative stress, and protein cross-linking, which are known contributors to neurodegeneration. We aimed to study whether tissue AGE accumulation is associated with T2DM-related brain atrophy. We performed brain magnetic resonance imaging, cognitive tests, and noninvasive skin autofluorescence (SAF; a measure of tissue AGE levels) on people aged >55 years with and without T2DM. Multivariable linear regression was used to study the relationships among T2DM, SAF, and gray matter volume (GMV). There were 486 people included in the study. T2DM was associated with greater SAF. Greater SAF, T2DM, and cognitive impairment were each associated with lower GMV independently of age, sex, and total intracranial volume. SAF partially mediated the association between T2DM and GMV. Longitudinal studies may help confirm whether tissue AGE accumulation is associated with brain atrophy in T2DM.

Type 2 diabetes mellitus (T2DM) is associated with an increased risk of incident cognitive impairment and dementia (1). Brain atrophy may be a key driver of T2DM-related cognitive dysfunction (2). T2DM is also associated with the excessive accumulation of advanced glycation end products (AGEs) in tissues (3). AGEs are products of nonenzymatic reactions between reactive carbonyl groups of compounds (such as glucose) with proteins, lipids, or nucleic acids (4). There is a large body of evidence from in vitro model research supporting a role for AGEs in neurodegeneration and Alzheimer disease (AD) (4). Greater serum levels of AGEs are associated with cognitive decline (5) and lower gray matter volume (GMV) in older people (4). Given these observations, it is possible that AGEs play a mechanistic role in T2DM-related brain atrophy. However, there have been no previous studies examining the role of AGEs in T2DM-related brain atrophy.

Tissue AGEs can be measured reproducibly and noninvasively in the skin by means of a specialized light emitter and detector. Skin autofluorescence (SAF) measured in this manner has been shown to be highly correlated with biopsy-derived skin AGE concentrations (6). We hypothesized that SAF levels would either mediate or modify the association between T2DM and brain atrophy.

RESEARCH DESIGN AND METHODS

Sampling

We used a cross-sectional study design, and sampling methods have been described previously (2). Participants

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were included from two studies: the Cognition and Diabetes in Older Tasmanians study (CDOT) and the Tasmanian Study of Cognition and Gait. Participants with T2DM aged ≥55 years were recruited into CDOT between January 2008 and January 2010 using the National Diabetes Service Scheme database as a sampling frame. The Tasmanian Study of Cognition and Gait sample was recruited by mailing approach letters to eligible registrants aged ≥55 years, living in the same Southern Tasmanian postcodes as those in the CDOT study, and has been described previously (4). The phenotype of T2DM was based on self-report and confirmed using a single plasma glucose level according to standard criteria (fasting plasma glucose ≥7.0 mmol/L, random plasma glucose ≥11.1 mmol/L, and HbA_{1c} >6.5% [48 mmol/mol]). People living in a nursing home, those with insufficient English for cognitive testing, or contraindication to magnetic resonance imaging (MRI) were excluded. The Southern Tasmanian Health and Medical Human Research Ethics Committee and the Monash University Human Research Ethics Committee approved the study, and we obtained written, informed consent.

Measurements

SAF

We used the AGE reader (DiagnOptics BV, Groningen, the Netherlands) to measure SAF. The spectrometer reader uses a light source to illuminate ~4 cm^2 of skin on the volar surface of the right arm 10 cm below the elbow fold. SAF is calculated as the ratio between the emission light and reflected excitation light, multiplied by 100 and expressed in arbitrary units. In our laboratory, the test-retest reliability for SAF was high (intraclass correlation coefficient 0.93; n = 11) when individuals were measured 5 days apart.

MRI Scans

MRI scans were obtained using a single 1.5T General Electric scanner with the following sequences: high-resolution T1-weighted spoiled gradient echo (GRE; TR 35 ms; TE 7 ms; flip angle 35°; field of view 24 cm; 120 contiguous slices; and isotropic voxel size 1 mm^3); T2-weighted fast spin echo (repetition time [TR], 4,300 ms; echo time [TE], 120 ms; number of excitations, 1; turbo factor, 48; and voxel size, 0.90 × 0.90 × 3 mm); fluid attenuated inversion recovery (TR, 8,802 ms; TE, 130 ms; TI, 2,200 ms; and voxel size, 0.50 × 0.50 × 3 mm); and GRE (TR, 0.8 ms; TE, 0.015 ms; flip angle, 30°; and voxel size, 0.9 × 0.9 × 7 mm).

Brain Volumes

Three-dimensional T1 and axial GRE sequences were registered into standard Montreal Neurological Institute space using Functional Magnetic Resonance Imaging of the Brain's Linear Image Registration Tool. A multispectral segmentation process was applied using three-dimensional T1 and GRE sequences using Statistical Parametric Mapping software version 5 (7) to produce tissue probability maps of gray and white matter. Tissue maps were smoothed using an isotropic 8-mm Gaussian kernel. A single expert manually segmented both hippocampi using established methods known to have high test-retest reliability in our laboratory (intraclass correlation coefficient 0.97) (8). Tissue volumes of the segmented areas (total gray, white matter, and hippocampal) were calculated using standard voxel-counting algorithms.

Other Measurements

Standardized questionnaires were used to record demographic and clinical information. Weight, height, waist and hip circumferences, habitual physical activity using a pedometer worn over 1 week, and blood pressure (BP) in a sitting position as an average of three recordings from the right arm were measured and BMI calculated. A standardized cognitive battery was used to test domains of memory, speed, and executive and visuospatial function (Supplementary Table 1) as described previously (2). Diagnosis of cognitive impairment was assigned, blinded to T2DM status, if function in any of the domains was <1.5 SDs from age-, sex-, and education-adjusted norms.

Data Analysis

The analyses were conducted on a complete dataset consisting of those in whom both measures of SAF and brain imaging were available.

Logistic regression was used to describe the associations of T2DM and brain atrophy with cognitive impairment. Linear regression was used to estimate the associations of SAF and T2DM with measures of brain atrophy. Covariates for age, sex, total intracranial volume (TICV), and other variables were added to the regression models for brain atrophy if their inclusion produced a statistically significant increase in model fit or changed the coefficient of the covariate for T2DM by >10%. Putative factors considered were hypertension (defined as mean BP >140/90 mmHg or previous diagnosis), ever smoked tobacco, creatinine, mean steps per day, history of ischemic heart disease, stroke, hyperlipidemia, BMI and waist-to-hip ratio, and the use of specific medications that have been shown to influence AGE levels (pravastatin, irbesartan, and metformin) (9–11). We then examined whether SAF mediated the associations estimated between T2DM and brain atrophy. For this, we entered SAF into the model relating T2DM to brain volume outcome measures adjusting for age, sex, smoking, serum creatinine, and TICV. If the introduction of SAF substantially attenuated the regression coefficient of the binary covariate for T2DM, and the coefficient of SAF remained largely unchanged from its value without T2DM in the model, it was considered a potential mediator. We also investigated any modifying effect (interaction) of SAF using a test of significance of the coefficient of a covariate formed as the product of the covariates for T2DM and SAF. Statistical analyses were carried out using STATA version 11.1 (StataCorp, College Station, TX).

RESULTS

There were 285 people with T2DM (mean age 67.5 years, SD 6.9) and 201 in the non-T2DM comparison group (mean age 73.4 years, SD 6.9) with SAF measures. A total of seven participants had inaccurate measures of SAF and were excluded from the analysis. Summary measures of the characteristics of each group are presented in Table 1. Comparisons of the characteristics of people with and those without T2DM are presented in Supplementary Table 2.

Associations of SAF With Study Factors

Greater SAF was associated with greater age (β = 0.014; $P < 0.001$), but not with sex (β = 0.07; $P = 0.26$). After adjustment for age and sex, T2DM was associated with greater SAF (β = 0.14; $P = 0.007$). In the whole sample (T2DM and non-T2DM), greater BMI ($P < 0.001$), less habitual physical activity ($P = 0.009$), and greater serum creatinine ($P = 0.006$) were individually associated with greater SAF. Among those with T2DM, greater SAF was associated with greater HbA_{1c} ($P < 0.001$) and longer duration of T2DM ($P = 0.016$). Among those without T2DM, there was no association between SAF and HbA_{1c}.

T2DM was associated with lower GMV (standardized β = −0.020; $P = 0.05$), but not with total hippocampal volume (standardized β = 0.03; $P = 0.51$) or white matter volume (standardized β = 0.002; $P = 0.90$). Lower GMV was significantly associated with the risk of any cognitive impairment (β = −0.03; CI −0.04 to −0.01; $P = 0.005$). In the whole sample, greater levels of SAF were significantly associated with the risk of any cognitive impairment (β = 0.41; CI 0.01–0.82; $P = 0.05$) and with lower GMV (standardized β = −0.036; $P < 0.001$), but not with hippocampal volume (standardized β = −0.046; $P = 0.258$) or white matter volume (standardized β = 0.019; $P = 0.096$) independent of age, sex, smoking, serum creatinine, and TICV. Addition of SAF attenuated the association between T2DM and GMV by 20%, rendering it nonsignificant (standardized β = −0.016; $P = 0.12$), whereas SAF remained independently associated with GMV in the model (standardized β = −0.034; $P < 0.001$). Additional adjustments for BMI, HbA_{1c}, or duration of T2DM did not change these relationships (data not shown). There was no interaction between T2DM and SAF in explaining GMV. Fig. 1 shows the scatter plots of the association between SAF and GMV stratified by T2DM status.

Table 1—Sample characteristics

	Mean (SD) or *N* (%) total (*n* = 486)
Age (years)	69.9 (7.5)
Female sex	208 (43)
Diabetes	285 (59)
Formal education (years)	11.3 (3.7)
Self-reported history of hypertension or mean systolic BP >140 or mean diastolic BP >90 mmHg	374 (77)
Use of BP-lowering medications	284 (58)
Statin use	223 (46)
Ischemic heart disease	81 (17)
TIA or stroke	32 (7)
Hyperlipidemia	153 (31)
Ever smoked	253 (52)
BMI (kg/m^2)	29.1 (5.0)
Normal (BMI 20–25)	86 (18)
Overweight (BMI 25–30)	216 (44)
Obese (BMI >30)	177 (36)
Mean steps per day	6,337 (3,372)
Serum creatinine (μmol/L)	78.5 (24.7)
Any cognitive impairment	146 (30%)
SAF (AUs)	2.05 (0.53)

AU, arbitrary unit; TIA, transient ischemic attack.

DISCUSSION

This is the first study examining the relationship among tissue AGE accumulation, T2DM, and GMV. T2DM was associated with greater accumulation of tissue AGEs (as measured by SAF) and with lower GMV. Consistent with our previous study of circulating AGEs (4), we found that greater SAF was independently and modestly associated with lower GMV, but additionally demonstrate that SAF may partially mediate the association between T2DM and lower GMV. The associations we describe are novel and provide a solid basis for further studying the relationship between tissue AGE accumulation and brain atrophy.

There is strong evidence from basic science research that tissue AGE accumulation plays a role in the pathogenesis of dementia (12). In the case of AD (the most common type of dementia), autopsy studies have shown the process of atrophy is due to the accumulation of extracellular amyloid plaque and intracellular tau neurofibrillary tangles (13). Greater levels of AGEs have been found to be colocated with amyloid plaques (14,15) and paired helical filament tau in sporadic AD (16) and may act by stabilizing plaques and promoting fibrillation of tau through protein cross-linking (17,18). We speculate that SAF may reflect AGE-mediated cross-linking of other cellular proteins, such as in neurons.

AGEs may also be directly cytotoxic to neurons in culture (19) and able to directly induce inflammation and oxidation (12) by binding with receptor for AGE (RAGE) in mitochondria, generating free radicals, and reducing clearance of pre-existing reactive oxygen species (17). Furthermore, RAGE interacts with serum β-amyloid, increasing the transport of β-amyloid across the blood–brain barrier, activating proinflammatory cytokines, and reducing cerebral blood flow (20).

Strengths of our study include the large sample size, reproducible and sensitive measures of tissue AGEs and

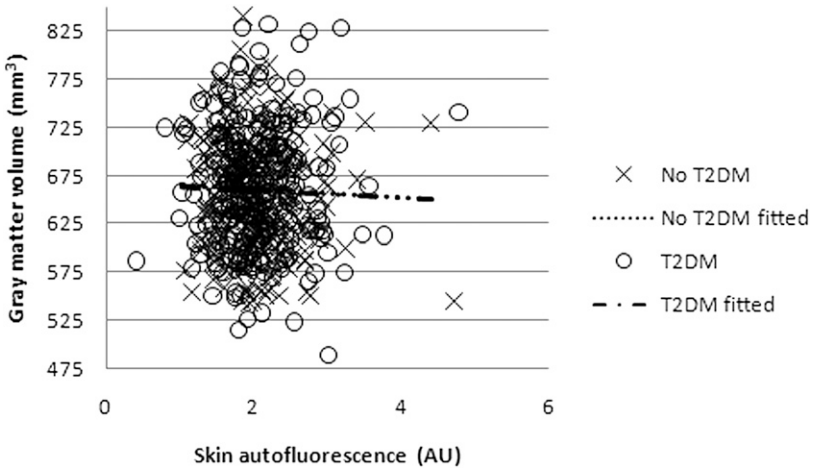

Figure 1—The association of SAF with GMV. The raw data points of the association of SAF and GMV stratified by T2DM. The fitted lines show the stratified associations between SAF and GMV adjusted for age, sex, smoking, serum creatinine, and TICV. In those without T2DM: SAF $\beta = -4.78$; standardized $\beta = -0.41$; $P = 0.003$; and adjusted $R^2 = 0.97$. In those with T2DM: SAF $\beta = -3.60$; standardized $\beta = -0.03$; $P = 0.02$; and adjusted $R^2 = 0.96$ .The high R^2 values reflect the adjustment for TICV (head size), which is collinear with GMV. The fitted lines for the two groups also overlap considerably, demonstrating a lack of interaction between T2DM and SAF in explaining GMV.

brain structure, clear definition of T2DM, and careful statistical modeling. We carefully adjusted as required for smoking, renal function, BMI, hypertension, and hyperlipidemia that may be related to both AGEs and brain or vascular health. Although medications used to treat these conditions (pravastatin and irbesartan) (9,10) and specific antidiabetes drugs such as metformin (11) have been postulated to have a protective effect against the effects of AGEs, adjusting for the use of these medications did not change our findings (data not shown). Our study has some limitations. Our study is cross-sectional, limiting inferences of causality, and needs to be confirmed in longitudinal analyses. The AGE reader does not measure AGEs that do not exhibit autofluorescence (nonfluorophores) and may also measure non-AGE fluorophores (6). However, the results of a number of earlier studies support the use of SAF as a surrogate marker of fluorescent and nonfluorescent AGE content in the skin (6,21).

Given the modest strength of β coefficients for SAF with GMV, it is likely that AGE accumulation is only one of a large number of pathways that contribute to the development of dementia in T2DM, explaining why SAF only partially mediated the T2DM–GMV relationship. The clinical relevance of these results is uncertain, but they support further research to understand the role of AGEs in the pathogenesis of dementia in relation to T2DM and overall. Prospective studies are needed to assess if tissue AGE accumulation is causally related to brain atrophy in T2DM and, subsequently, to study whether limiting AGE accumulation may slow neurodegeneration.

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Author Contributions. C.M. drafted and revised the manuscript and performed statistical analysis and data interpretation. G.M. and J.M.F. contributed to discussion and reviewed the manuscript. R.B. performed image analysis and interpreted data. L.B. contributed to analysis and interpretation of the data and reviewed the manuscript. A.J.V. contributed to interpretation of data and reviewed the manuscript. T.G.P. supervised image analysis, contributed to discussion, and reviewed the manuscript. J.C. performed image analysis and interpreted data. V.S. developed the study concept and design, performed analysis and interpretation of data, revised the manuscript, and obtained funding. V.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Supplementary Table 1. Cognitive domains and tests**.**

Cognitive Domain	Tests
Memory factor	Hopkins Immediate Verbal Recall
	Hopkins Delayed Verbal Recall
	Hopkins Recognition
Visuospatial and planning factor	Rey Complex Figure Copy Task
	Rey Complex Figure Recall Task
Speed factor	Digit Symbol Coding
	Digit Symbol Search
Executive function	COWAT Word test
	COWAT Category Test
	Digit Span Recall
	Stroop Dot Time
	Stroop Word Time
	Stroop Color time

Supplementary Table 2. Sample characteristics by diabetes status.

	T2DM n=285	No T2DM n=201	P value
Age (years)	67.5 (6.9)	73.4 (6.9)	<0.001
Female sex	41% (117/285)	45% (91/201)	0.35
Formal education (years)	11.5 (3.4)	11.2 (3.9)	0.40
Self reported history of hypertension or mean SBP >140 or mean DBP >90 mmHg	88% (251/285)	72% (144/201)	<0.001
Use of blood pressure lowering medications	70% (199/285)	43% (86/201)	<0.001
Statin use	62% (176/285)	23% (47/201)	<0.001
Ischemic Heart Disease	19% (54/285)	13% (27/201)	0.11
TIA or Stroke	8% (24/285)	4% (8/201)	0.053
Hyperlipidemia	48% (138/285)	7% (15/201)	<0.001
Ever smoked	53% (152/285)	51% (102/201)	0.60
BMI (kg/m^2) Normal (BMI 20-25) Overweight (BMI 25-30) Obese (BMI>30)	30.5 (5.1) 11% (31/285) 41% (117/285) 47% (135/285)	27.2 (4.2) 27% (55/201) 50% (100/201) 21% (42/201)	<0.001 <0.001 0.048 <0.001
Mean steps per day	6377 (3660)	6414 (3014)	0.91
Fasting blood glucose (mmol/l)	7.7 (2.3)	5.3 (0.5)	<0.001
HbA_{1c} (%) (mmol/mol)	7.2 (1.2) 55	5.6 (0.3) 38	<0.001
Serum creatinine (μmol/L)	78.7 (25)	78.4 (24)	0.91
Age at diabetes diagnosis (years)	57.5 (11.3)		
Median duration of T2DM (years)	7 (4-12)		
Use of oral glucose lowering medications	62% (176/285)		
Use of insulin	4% (10/285)		
Any cognitive impairment	107 (38)	39 (19)	<0.001
Skin autofluorescence (AU)	2.03 (0.54)	2.07 (0.51)	0.49

5.3 Conclusion

The results from this paper showed that T2DM was associated with greater skin autofluorescence (SAF) and lower grey matter volume. It showed for the first time that greater SAF was associated with lower grey matter volume and that SAF partially mediated the relationship between T2DM and lower grey matter volume. The results from basic science research support the role of AGEs in mediating this relationship and suggest that AGEs play an active role in driving AD-type pathology, particularly contributing to tau fibrillation and protein cross-linking, allowing for more stable neurofibrillary tangles (Yan et al., 1994, Li et al., 2012, Munch et al., 2012). Other studies suggest that AGEs may contribute to arterial stiffness by collagen cross-linking (Price et al., 2007, Lund et al., 2011). Stiffening of central blood vessels such as the aorta may contribute to abnormal blood flow and pressure-related damage to the microcirculation of the brain which is a high-flow low resistance vascular system (Mitchell et al., 2011), potentially damaging the neurovascular unit (Mitchell et al., 2011). Further longitudinal studies are required to clarify the causal relation of AGEs to brain atrophy.

Monash University

Declaration for Thesis Chapter 6

Declaration by candidate

In the case of Chapter 6, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Drafting the manuscript, statistical analysis, and interpretation of data	60

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution
Robyn Tapp	Revising the manuscript and interpretation of data.
Alun Hughes	Revising the manuscript, retinal image analysis and interpretation of data.
Costan Magnussen	Revising the manuscript and interpretation of data.
Leigh Blizzard	Revising the manuscript and supervision of statistical analysis
Thanh Phan	Revising the manuscript, interpretation of data, development and supervision of brain image analysis
Richard Beare	Revising the manuscript, development and supervision of brain image analysis
Nicholas Witt	Revising the manuscript, retinal image analysis and interpretation of data
Alison Venn	Revising the manuscript and interpretation of data
Gerald Munch	Revising the manuscript and interpretation of data.
Chathrie Amaratunge	Revising the manuscript and grading of retinal images
Velandai Srikanth	Revising the manuscript, study concept, design, analyses and interpretation of data

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	**Date** 26/6/15
Main Supervisor's Signature	[redacted]	**Date** 26.6.2015

*Note: Where the responsible author is not the candidate's main supervisor, the main supervisor should consult with the responsible author to agree on the respective contributions of the authors.

Chapter 6

Type 2 diabetes, retinal vascular disease and brain atrophy

This is a draft manuscript currently in submission.

6.1 Abstract

Objective: It is uncertain whether small vessel disease drives the relationship between Type 2 Diabetes Mellitus (T2DM) and brain atrophy. We aimed to study whether retinal vascular architecture, as a proxy for cerebral small vessel disease, may modify or mediate the associations of T2DM with brain atrophy.

Research Design and Methods: Cross-sectional study using Magnetic Resonance Imaging (MRI) scans and retinal photographs in 451 people with and without T2DM. We measured brain volumes, geometric measures of retinal vascular architecture [arteriolar and venular calibre, tortuosity, branching angle and parent-daughter vessel calibre relationships], clinical retinopathy, and MRI cerebrovascular lesions [infarcts, microbleeds, white matter hyperintensities]. Linear or logistic regression was used to study relationships between T2DM, brain MRI and retinal measures.

Results: There were 270 people with (mean age 67.3 years, SD 6.7; 59% males; HbA_{1c} 7.1 %, SD 1.2) and 181 without T2DM (mean age 72.9 years, SD 6.7; 54% males; HbA_{1c} 5.6%, SD 0.3). T2DM was associated with lower grey matter volume (β= -3.60, 95%CI -6.28 to -0.93, p=0.008) and the presence of MRI infarcts (β= 0.84, 95%CI 0.22 to 1.45, p=0.008). In univariable regression, T2DM was associated with greater arteriolar diameter (p=0.03) and optimality ratio (p = 0.04), but these associations did not survive adjustments for age and sex. Of the retinal measures, only optimality ratio was associated with lower grey matter volume (β = -22.5 95%CI -41.69 to -2.61, p=0.026). The inclusion of retinal measures in regression models did not affect the association of T2DM with grey matter volume.

Conclusions: In this sample of people with well controlled T2DM, the association of T2DM with lower brain volume was independent of retinal vascular architecture and clinical retinopathy. Measures of retinal vascular architecture and clinical retinopathy may not be sufficiently sensitive to confirm a microvascular basis for T2DM-related brain disease, but longitudinal studies are required.

6.2 Introduction

T2DM increases the risk of cognitive impairment and dementia (Ott et al., 1999, Biessels et al., 2006b). Brain atrophy mediates a substantial portion of the association between T2DM and cognitive dysfunction (Moran et al., 2013). Cerebrovascular disease, particularly disease of small cerebral vessels, has been postulated as a potential mechanism leading to T2DM-related brain atrophy, possibly through mechanisms including infarcts, ischaemia, inflammation and oxidative stress (Exalto et al., 2012), but this is yet to be clarified. Although T2DM is commonly associated with microvascular disease (Garcia et al., 1974), the associations between T2DM and brain MRI markers of small cerebral vessel disease such as white matter hyperintensities (WMH) or cerebral microbleeds are not strong, with contradictory findings in the literature (van Harten et al., 2006, Jongen et al., 2008, Qiu et al., 2014). The similar embryological, anatomical and functional properties of retinal and cerebral vessels combined with ease of visualization of retinal vessels make them a potentially useful surrogate of cerebral small vessel disease (Patton et al., 2005, Ikram et al., 2013c). This has led to recent investigators using retinal vascular imaging measures in studies of those with and without cerebrovascular disease (Kwa et al., 2002, Patton et al., 2005, Nguyen et al., 2006, Hughes, 2007), and such measures are associated with a greater risk of future stroke (Wong et al., 2001) and dementia (Wong et al., 2002, Baker et al., 2007, Lesage et al., 2009, de Jong et al., 2011, Ding et al., 2011, Haan et al., 2012). T2DM is associated with retinopathy (Klein et al., 1992) and retinal vascular changes including greater retinal arteriolar caliber (Nguyen et al., 2008), and greater arteriolar tortuosity (Sasongko et al., 2011). Therefore, it is possible that retinal vascular imaging may assist in establishing a small vessel basis for T2DM-related brain atrophy.

To explore this issue further, we aimed to use retinal imaging to examine the roles of retinal vessel abnormalities and clinical retinopathy in explaining the association between T2DM

and grey matter atrophy. We hypothesized that T2DM would be associated with measures of retinal vascular architecture and clinical retinopathy, and that these measures would mediate or modify the association between T2DM and brain volume.

6.3 Research Design and Methods

Sampling

We used a cross-sectional design. Our sampling methods have been described previously (Moran et al., 2013). In brief, we recruited participants with T2DM and aged ≥55 years who were living in specific postcodes (7000-7199) of Southern Tasmania into the Cognition and Diabetes in Older Tasmanians study (CDOT) between January 2008 and January 2010. The National Diabetes Service Scheme (NDSS) was used as a sampling frame. The NDSS is administered by Diabetes Australia, providing products, information, and support to patients with T2DM who voluntarily enrol. The diagnosis of T2DM in the NDSS is based on physician assessment using standard criteria (fasting plasma glucose ≥7.0mmol/L, random plasma glucose ≥11.1mmol/L or 2 hour glucose ≥11.1mmol/L post oral glucose tolerance test), with registrants indicating willingness to participate in research. Exclusion criteria were people living in a nursing home signifying severe frailty, those with insufficient English for cognitive testing, contraindication to Magnetic Resonance Imaging (MRI) and presence of cataracts (to enable successful retinal imaging). We derived our comparison group from a sample of people aged ≥60 years without T2DM, recruited into the concurrently conducted population-based Tasmanian Study of Cognition and Gait (TASCOG). TASCOG participants were randomly identified from the electoral roll from the same postcodes as those in CDOT. Absence of T2DM in our comparison group was assessed using fasting plasma glucose < 7.0mmol/L, random plasma glucose < 11.1mmol/L, and HbA_{1c} <6.5% (48mmol/mol) in those without a history of T2DM. Exclusion criteria were identical to the CDOT study. The

Southern Tasmanian Health and Medical Human Research Ethics Committee and the Monash University Human Research Ethics Committee approved the study and we obtained written informed consent.

Outcome Measurements

Retinal measurements - A 45° digital disc centred Canon CR-DGi non mydriatic retinal camera was used to photograph two fields per eye using a Canon RK-F1auto refractor to account for refractive errors. The digitised retinal images were graded by a single expert and processed using automated algorithms at the National Heart & Lung Institute, Imperial College, London using well-established methods (Witt et al., 2006). Colour photographs were converted to monochrome by extraction of the green layer. Using a custom written program in Matlab, vessel diameters were measured using a Sliding Linear Regression Filter (SLRF) (Chapman et al., 2001) which achieves sub-pixel accuracy, and vessel length was measured between bifurcations using automatic tracking. SLRF is based on fitting a line by linear regression and progressively moving this across the entire section of interest, thus significantly decreasing noise. Measurements were made from ≥7 informative vessel segments and ≥5 bifurcations in both arterial and venous vessels from each subject. An informative vessel segment was defined to be either linking 2 clearly visible bifurcations or else traversing a linear distance of ≥1.5 disc diameters (the width of an average optic disc in photographic images) from the optic disc boundary without bifurcating. The analysis was performed on a sequence of complete trees generally from a single eye, continuing on the second eye if necessary to achieve the required number of vessel segments and bifurcations. The following measures of retinal vessel architecture were obtained: arteriolar and venular diameters and length; length/diameter ratios (LDR) of arteriolar segments (corrects for refractive errors effecting measurements); arteriolar tortuosity; arteriolar bifurcation angles; arteriolar optimality ratio and optimality deviance. Tortuosity was calculated as the ratio of

arc length (l_a) of the vessel segment (measured by tracking) to the straight line length of the segment (chord length, l_c). The relationship of arteriolar diameters at bifurcations has been shown previously to relate to endothelial function (Griffith et al., 1987). Optimality ratio is the ratio of sum of 'daughter' arteriolar diameters (d_1) divided by the 'parent' arteriolar diameter (d_0) corrected for asymmetry (Witt et al., 2010). Departures away from a theoretically predicted optimum (Murray, 1926) is an indicator of endothelial dysfunction (Witt et al., 2006). Accuracy and reproducibility of our methods are high and have been previously described with SLRF repeatability coefficient of diameter measurement of 3.89 (Chapman et al., 2001).

Digital copies of the retinal photographs were also graded by a single clinical expert trained in ophthalmology for the presence/absence of features of clinical retinopathy blinded to diabetes status. These included the presence of optic disc disease, copper/silver wiring, general arteriolar narrowing, focal arteriolar narrowing, arteriovenous crossing abnormalities, microaneurysm, intraretinal haemorrhage, nerve fibre haemorrhage, hard exudate, new vessel formation, cotton wool spots, macular degeneration, intraretinal microvascular abnormalities, and evidence of photocoagulation therapy.

Brain MRI - MRI scans were obtained using a single 1.5T General Electric scanner with the following sequences: high-resolution T1 weighted spoiled gradient echo (SPGR) (TR35 ms, TE 7ms, flip angle 35°, field of view 24cm, 120 contiguous slices, isotropic voxel size $1mm^3$); T2 weighted fast spin echo (TR 4300ms; TE 120ms; NEX 1; turbo factor 48; voxel size 0.90×0.90×3mm); FLAIR (fluid attenuated inversion recovery) (TR = 8802ms, TE = 130ms, TI = 2200ms, voxel size 0.50×0.50×3mm); gradient echo (GRE, TR=0.8ms; TE=0.015; flip angle 30°; voxel size = 0.9x0.9x7mm).

Brain MRI segmentation –3D-T1 and gradient echo (GRE) sequences were registered into standard Montreal Neurological Institute (MNI) space using FMRIB's Linear Image Registration Tool (FLIRT) (Jenkinson et al., 2001). A multispectral segmentation process was then applied using both 3D-T1 and GRE sequences using Statistical Parametric Mapping software version 5 (SPM5) (Ashburner et al., 2005) to produce tissue probability maps of grey and white matter, while ensuring correct classification of subcortical grey matter. The images were modulated to correct for volume change induced by the normalization process. All tissue maps were smoothed using an isotropic Gaussian kernel, full width at half maximum (FWHM)=8mm. A single expert manually segmented both hippocampi using established methods known to have high test-retest reliability in our laboratory (ICC 0.97) (Wrench et al., 2009). Using the tissue maps generated by these methods, total grey matter, white matter, and hippocampal volumes were calculated with voxel counting algorithms. WMH volumes were obtained by automated segmentation as described previously (Moran et al., 2013). A single trained rater (C.M.) determined the presence of MRI infarct and microbleed with confirmation by consensus between 2 stroke experts. Infarct was defined as a hypointensity ≥3mm in diameter on 3D-T1-weighted and FLAIR images with a surrounding hyperintense rim on FLAIR (Moran et al., 2012). Microbleeds were defined as small, rounded hypointense lesions with clear margins, ranging from 2 to 10mm on GRE sequences. All measurements were blinded to group, age, sex and outcome measures.

Other Measurements - We used standardized questionnaires to record demographic and clinical information about duration of T2DM, years of formal education, health and medical history including vascular disease and risk factors, smoking, medication use and alcohol use (g/day). We measured weight, height, waist and hip circumferences, and calculated Body Mass Index (BMI) as weight(kg)/ height^2(m^2); habitual physical activity by calculating mean number of steps per day using a Yamax pedometer worn over a seven day period; mood using

the 15-item Geriatric Depression Scale (GDS) (Yesavage et al., 1982) and blood pressure (BP) using a Omron M4 sphygmomanometer in a sitting position as an average of 3 recordings from the right arm. Fasting plasma glucose was recorded using a Roche Cobas 6000 analyser with hexokinase determination and HbA_{1C} using a Bio-Rad D10 analyser.

Data analysis

Student's t test and Chi square tests were applied to compare mean scores and proportions of demographic and clinical variables between people with and without T2DM.

Multivariable regression: We first established the associations of T2DM with individual MRI measures adjusting in each regression for age, sex and additionally for total intracranial volume (except in the case of MRI infarcts and microbleeds). Linear regression was used for continuous outcome variables (total grey, total white, right and left hippocampal volumes) and logistic regression for binary outcomes (infarct or microbleed). For all above analyses, potential for confounding was examined for additional covariates and adjustments made if addition of these terms changed the coefficient for T2DM by >10%. Covariates considered were hypertension (defined as mean BP>140/90mmHg or previous diagnosis), use of blood pressure lowering medications, hyperlipidaemia (yes/no), alcohol use (g/day), HbA_{1c}, ever smoked(yes/no), mean steps per day, ischaemic heart disease, stroke, BMI and waist-hip ratio.

To examine whether retinal measures mediated the associations detected between T2DM and brain atrophy, we entered any retinal measure associated with T2DM into the model relating T2DM to brain volume measures adjusting for age, sex and total intracranial volume. If the introduction of the retinal measure substantially attenuated the β coefficient for T2DM, and the coefficient of the retinal measure remained unchanged from its unadjusted value without T2DM in the model, it was considered a potential mediator. We also examined for two-way

interactions between T2DM and retinal measures using a test of significance of product terms. We applied standard regression diagnostics to assess the adequacy of models. Statistical analyses were carried out using STATA version 11.1(StatCorp.College Station Tx.).

6.4 Results

Retinal photographs and MRI scans were available for 451 people, 270 with T2DM (mean age 67.3 years, SD 6.7) and 181 (mean age 72.9 years, SD 6.7) without T2DM. Sample characteristics and comparisons between those with and without T2DM are presented in **Table 6.1**. Participants with T2DM reported median disease duration of 6 years (interquartile range 4-11 years). Those with T2DM had greater fasting blood glucose and HbA_{1c} levels, higher BMI and waist-hip ratio, greater GDS score, were more likely to report a history of ischaemic heart disease, hypertension, hyperlipidaemia, and receive treatment with blood pressure lowering drugs and statins (all $p<0.05$). A total of 53 participants used insulin.

Retinal photographs could not be graded accurately in 15 people (10 with T2DM, 5 without T2DM) because of poor image quality. Those with non-gradeable retinal photographs were similar in age, mean fasting blood glucose and HbA_{1c} levels, BMI waist-hip ratio, GDS score, history of hypertension and hyperlipidaemia, and treatment with blood pressure lowering drugs and statins to those with gradeable retinal photographs (data not shown).

Table 6.1. Sample characteristics

	T2DM Mean (SD) or n(%) n=270	No T2DM Mean (SD) or n(%) n=181	*p* value
Age (years)	67.3 (6.7)	72.9 (6.7)	<0.001
Male sex	159 (59)	97 (54)	0.27
Formal Education (years)	11.2 (3.6)	11.0 (3.9)	0.61
Systolic blood pressure (mmHg)	135 (18.9)	137 (19.0)	0.23
Diastolic blood pressure (mmHg)	76 (10.3)	78 (10.8)	0.01
Self reported history of hypertension or mean SBP >140 or mean DBP >90 mmHg	223 (83)	123 (68)	<0.001
Use of blood pressure lowering medications	190 (70)	85 (47)	<0.001
Ischaemic Heart Disease	57 (21)	27 (15)	0.01
TIA or Stroke	22 (8)	9 (5)	0.19
Hyperlipidaemia	127 (47)	10 (6)	<0.001
Statin use	163 (60)	45 (25)	0.005
Ever smoked	144 (53)	89 (49)	0.36
Alcohol intake (g/day)	12 (17)	17 (20)	0.01
BMI (kg/m^2)	30.7 (5.1)	27.6 (4.3)	<0.001
Waist-hip ratio	0.96 (0.09)	0.90 (0.09)	<0.001
Mean steps per day	6416 (3619)	6640 (3101)	0.52
GDS score	2.4 (2.6)	1.5 (1.6)	<0.001
Fasting blood glucose (mmol/l)	7.7 (2.1)	5.3 (0.6)	<0.001
HbA_{1c} (%)	7.1 (1.2)	5.6 (0.3)	<0.001
Age at diabetes diagnosis[1]	57.6 (10.9)	NA	
Median duration of T2DM (years) [1] (IQR)	6 (4-11)	NA	
Insulin use[1]	53 (20)	NA	

[1]In those with T2DM; NA: Not applicable; TIA: Transient Ischaemic attack; BMI: Body Mass Index; GDS: Geriatric Depression Scale; IQR: Interquartile range

The comparisons of raw scores of retinal vascular measures are presented in **Supplementary Tables 6.1 and 6.2**. The associations of T2DM with retinal measures are displayed in **Table 6.2**, first unadjusted, followed by additional adjustment for age and sex, and further for other covariates as required.

Table 6.2. Association between Type 2 Diabetes Mellitus and retinal measurements[1]

Retinal variable	Model 1 β (95% CI)	Model 2 β (95% CI)	Model 3 β (95% CI)
Arteriolar measures			
Length	14.38 (-36.27 to 65.04)	13.01 (-42.00 to 68.01)	31.15 (-37.76 to 94.06)
Diameter	**0.48** **(0.05 to 0.91)[a]**	0.34 (-0.13 to 0.80)	0.01 (-0.52 to 0.54)
Length/diameter ratio	0.08 (-2.11 to 2.26)	0.12 (-2.24 to 2.49)	1.24 (-1.46 to 3.95)
Simple tortuosity	0.007 (-0.001 to 0.02)	0.006 (-0.003 to 0.01)	0.006 (-0.003 to 0.02)
Internal Angle	2.89 (-0.34 to 6.12)	2.83 (-0.66 to 6.33)	2.63 (-1.50 to 6.76)
Optimality Ratio	**0.01** **(0.001 to 0.02)[a]**	0.01 (-0.01 to 0.02)	0.004 (-0.01 to 0.02)
Venular measures			
Length	3.87 (-31.40 to 39.14)	21.30 (-16.43 to 50.02)	8.30 (-35.99 to 52.59)
Diameter	0.08 (-0.61 to 0.77)	-0.21 (-0.95 to 0.54)	-0.45 (-1.32 to 0.43)
Length/diameter ratio	0.18 (-1.12 to 1.47)	0.95 (-0.44 to 2.33)	0.65 (-0.98 to 2.27)
Simple tortuosity	0.001 (-0.001 to 0.003)	0.002 (-0.001 to 0.004)	0.001 (-0.002 to 0.004)
Retinopathy			
Any retinopathy	0.001 (-0.38 to 0.38)	0.39 (-0.04 to 0.81)	0.34 (-0.16 to 0.84)

[1]Including non-symmetrical second order vessels for all bifurcations.

Model 1 unadjusted

Model 2 adjusted for age, sex

Model 3 adjusted for age, sex, vascular risk factors (history of smoking, Hypertension, Systolic and Diastolic blood pressure, BMI and history of stroke, ischaemic heart disease and hyperlipidaemia)

[a]p value < 0.05;[b] p value <0.01; [c] p value <0.001

Although T2DM was associated with larger arteriolar diameter (p=0.03) and optimality ratio (p=0.04) in univariable regression, these associations did not survive further adjustment. T2DM was not associated with any other measure of retinal vascular architecture. With respect to clinical retinopathy, a greater proportion of those with T2DM had copper/silver wiring, generalized and focal narrowing, arterio-venous crossing abnormalities, presence of microaneurysm, intraretinal haemorrhage, hard exudates and macular degeneration (all $p<0.05$).

In the overall sample, only greater optimality ratio was associated with lower grey matter volume (β= -22.15 95%CI -41.69 to -2.61, p= 0.03) when adjusted for age, sex and total intracranial volume. Adjusting for vascular risk factors only slightly weakened this rendering it not statistically significant (β= -19.13 95%CI -38.85 to 0.59, p=0.06). Greater optimality ratio was also associated with a decreased risk of infarct (β= -4.97 95%CI -9.62 to -0.33, p= 0.04). Adjusting for vascular risk factors weakened this association rendering it not statistically significant (β= -4.44 95%CI -9.41 to 0.53, p=0.08). No associations were found between optimality ratio and any other MRI measure, nor between other retinal and MRI measures. These associations did not differ in magnitude between those with and without T2DM, and there were no statistical interactions (**Supplementary Table 6.3**). **Table 6.3** shows the effect of adding arteriolar diameter and optimality ratio to regression models of T2DM with brain MRI measures. Introduction of these two measures did not meaningfully change the association between T2DM and grey matter volume and other MRI measures in fully adjusted models.

Table 6.3. Retinal arteriolar diameter, optimality ratio, T2DM and MRI measures

	Model 1 (T2DM)	Model 2 Addition of arteriolar diameter (β of T2DM)	Model 3 Addition of optimality ratio (β of T2DM)
Grey matter volume	**-3.60** **(-6.28 to -0.93)[b]**	**-3.70** **(-6.39 to -1.01)[b]**	**-3.68** **(-6.36 to -1.01)[b]**
Right hippocampal volume	0.07 (-0.006 to 0.15)	0.07 (-0.005 to 0.15)	0.07 (-0.005 to 0.15)
Left hippocampal volume	0.02 (-0.05 to 0.10)	0.02 (-0.05 to 0.10)	0.02 (-0.05 to 0.10)
Total hippocampal volume	0.08 (-0.05 to 0.22)	0.08 (-0.05 to 0.22)	0.08 (-0.05 to 0.22)
White matter volume	0.15 (-2.54 to 2.84)	0.24 (-2.47 to 2.94)	0.23 (-2.48 to 2.93)
White matter hyperintensity volume	-0.06 (-1.46 to 1.34)	-0.06 (-1.47 to 1.36)	-0.05 (-1.47 to 1.36)
Infarct present	**0.84** **(0.22 to 1.45)[b]**	**0.91** **(0.28 to 1.53)[b]**	**0.93** **(0.30 to 1.57)[b]**
Microbleed present	0.75 (-1.76 to 0.25)	-0.69 (-1.71 to 0.33)	-0.69 (-1.71 to 0.34)

T2DM: Type 2 diabetes; MRI: Magnetic Resonance Imaging

Model 1, adjusted for age, sex and intra-cranial volume;

Model 2 β of T2DM when adjusted for arteriolar diameter, age, sex and intra-cranial volume;

Model 3 β of T2DM when adjusted for arteriolar diameter, optimality ratio, age, sex and intra-cranial volume

[a]p value < 0.05;[b] p value <0.01; [c] p value <0.001

6.5 Discussion

We examined the role of retinal vascular measures and retinopathy in mediating the association between T2DM and MRI imaging biomarkers of brain disease. We found that T2DM was associated with increased retinal arteriolar diameter and optimality ratio, and, as expected, with several measures of clinical retinopathy. Only greater retinal arteriolar optimality ratio was associated with lower grey matter volume. The association between T2DM and lower grey matter volume appeared to be independent of retinal vascular architecture and clinical retinopathy. Although this suggests that brain atrophy in T2DM may have neurodegenerative mechanisms independent of small vessel disease, these findings require confirmation with longitudinal follow-up. The possibility of true cerebral microvascular disease (loss of blood brain barrier integrity) playing a role cannot be excluded.

T2DM is associated with several non-proliferative retinal vessel abnormalities before progressing to proliferative disease (Fong et al., 2004). As expected, we found that those with T2DM had a greater burden of traditional measures of non-proliferative diabetic retinopathy (Fong et al., 2004) than those without T2DM. Measures of retinal vessel architecture have also been reported to be abnormal in T2DM probably as a result of chronic inflammation or endothelial dysfunction (Guerci et al., 2001, Park, 2011, Van Doornum et al., 2011). Consistent with previous studies we found T2DM to be associated with larger retinal arteriolar diameter (Nguyen et al., 2008, Ikram et al., 2013a), and, for the first time demonstrate a relationship with optimality ratio, but not other measures of retinal architecture. It is possible that the lack of associations of T2DM with other retinal vascular measures could be explained by the tight glycaemic and cardiovascular risk control in our sample, as would be expected in those registering in the NDSS. Those with T2DM had relatively good glycaemic control (mean HbA_{1C} 7.1%), short duration of disease (median 6

years), and a high proportion of participants with T2DM on blood pressure lowering medications including renin-angiotensin blockers (70%). However, controlling for the use of these medications did not significantly change the observed relationships.

Although the associations between retinal markers and the incidence of dementia (Ikram et al., 2012) and clinical cerebrovascular disease (stroke) (Doubal et al., 2009a) are well established, their associations with subclinical brain biomarkers of cerebrovascular disease and neurodegeneration are less clear, and may vary according to the study sample, type of retinal measure, or the competing influences of diabetes or hypertension. Previous investigators have examined the relationship between retinal markers and neurodegeneration, but with conflicting results (Wong, 2004, Sharrett, 2007, Cheung et al., 2012, Ikram et al., 2013a, Ikram et al., 2013b, Hilal et al., 2014). The presence of clinical retinopathy was associated with larger and increasing ventricular size (a surrogate marker of brain atrophy) in a population-based sample of relatively healthy people (Wong et al., 2003, Kawasaki et al., 2010). In a sample of people with acute stroke, clinical retinopathy was associated with subcortical atrophy but not cortical atrophy (Baker et al., 2010). The Rotterdam study (Ikram et al., 2013a), using a similar automated method to our own, reported that greater venular and narrower arteriolar diameters were associated with white matter but not grey matter atrophy. In contrast, the Women's Health Initiative did not report an association between retinopathy and brain atrophy (Haan et al., 2012). Similar to our study, retinal vessel diameters were cross-sectionally unrelated to the severity of WMH and lacunar infarcts in the Rotterdam study (Ikram et al., 2006) and in a Chinese sample (Hilal et al., 2014), although in the latter sample a positive association was found for cerebral microbleeds. In contrast, stronger associations have been shown in the follow-up of the Rotterdam sample (Ikram et al., 2006), and in the Atherosclerosis Risk in Communities Study (ARIC) study (Cheung et al., 2010). In ARIC, retinal arteriovenous nicking (a sign of hypertensive arteriopathy) predicted incident

brain infarcts and WMH more strongly in those with hypertension, whereas overall retinopathy predicted infarcts more strongly in those with diabetes (Cheung et al., 2010). This suggests that the two disorders may affect retinal vasculature differently, and indeed, arteriolar narrowing is commonly seen in hypertension rather than the dilatation seen in T2DM (Witt et al., 2006). The common co-existence of these two conditions in our sample, and the tight control of cardiovascular risk factors may partly explain the lack of associations seen. It is a traditionally held view that retinal hypoxia caused by vascular disease may lead to loss of blood-retinal barrier integrity (Baker et al., 2008, Kaur et al., 2008). While this may be so, measures of retinal architecture may not be sufficiently sensitive as markers of early disease of the blood brain barrier (BBB), particularly in well treated individuals. (Liew et al., 2008).

Strengths of our study include large sample size, choice of a comparison group from the same source population as those with T2DM, careful definition of T2DM, the use of fully automated brains segmentation, comprehensive measures of retinal vessel architecture and retinopathy, measurements blinded to diabetes status, and careful regression modelling to examine for mediation and effect modification. Importantly, our comparison sample was drawn from the same source population as those with T2DM. Our study has certain limitations. The cross-sectional design limits inference regarding causality. As mentioned previously, people at the healthier end of the spectrum of T2DM may have been over-represented in our sample. However, the prevalence of markers of retinopathy in our study (~35%) is higher than that seen in other large community based studies (~10%) (Klein et al., 1992, Tapp et al., 2003) suggesting this alone is unlikely to explain the lack of associations. One possibility is that the retinal photographs of those with the most severe retinal changes may have been excluded due to poor quality, but those excluded did not appreciably differ from those included. Given that previous studies have encountered similar differences

between their cross-sectional and follow-up results, this study warrants further longitudinal analyses before firm conclusions can be drawn. We examined a large number of associations between retinal measures (eleven) and structural brain measures (six). Multiple comparison testing increases the chance of finding an association where none exists, and this may explain the associations we found between optimality ratio and grey matter volume and presence of stroke. Although we carefully adjusted for several important confounders, we cannot exclude the small possibility of residual confounding by unmeasured factors. Participants on average were not very old, and hence less likely to demonstrate substantial variation in MRI biomarkers.

In summary, although we found associations between T2DM and a number of different retinal measures, the relationships between T2DM and brain markers of cerebrovascular or neurodegenerative disease appeared independent of the retinal measures.

Supplementary Table 6.1 Mean retinal measures[1]

	T2DM Mean (SD) N=270	No T2DM Mean (SD) N=181	P value
Arteriolar measures			
Arteriolar length	651 (264)	637 (274)	0.58
Arteriolar diameter	24.3 (2.3)	23.8 (2.2)	0.03
Length/diameter ratio	27.0 (11.3)	27.0 (12)	0.95
Simple tortuosity	0.05 (0.04)	0.04 (0.04)	0.07
Internal Angle	75.5 (18.2)	72.6 (14.6)	0.08
Optimality ratio	0.83 (0.06)	0.82 (0.06)	0.04
Venous Measures			
Venous length	508 (180)	504 (202)	0.83
Venous diameter	29.4 (3.7)	29.3 (3.7)	0.81
Venous length/diameter ratio	17.7 (6.8)	17.5 (7.2)	0.79
Venous simple tortuosity	0.02 (0.01)	0.02 (0.01)	0.43

T2DM: Type 2 diabetes; SD: Standard Deviation

[1]Including non-symmetrical second order vessels for all bifurcations.

Supplementary Table 6.2. Descriptive characteristics of retinopathy changes

	T2DM N(%) n=283	No T2DM N(%) n=187	p value
Disc disease	8 (3)	11 (6)	0.10
Copper/silver wiring	47 (17)	16 (9)	0.01
Generalised narrowing	31 (11)	34 (18)	0.03
Focal narrowing	4 (1)	13 (7)	0.002
A-V crossing	17 (6)	24 (13)	0.01
Microaneurysm	22 (8)	1 (1)	<0.001
Venous calibre change	5 (2)	1 (1)	0.24
Intraretinal haemorrhage	25 (9)	2 (1)	<0.001
Nerve fibre haemorrhage	10 (4)	3 (2)	0.21
Hard exudate	10 (4)	0	0.009
New vessel formation	0	0	
IRMA	0	0	
Cotton wool spot	3 (1)	2 (2)	0.99
Macular degeneration	8 (3)	16 (9)	0.006
Photocoagulation Treatment	11 (4)	0	0.006
Any retinopathy	106 (37)	70 (37)	1.0

A-V- Arteriovenous crossing; IRMA – intraretinal microvascular abnormalities; NA – not applicable

[a] p value < 0.05;[b] p value <0.01; [c] p value <0.001

Supplementary Table 6.3 Associations between retinal measurements and brain measurements

Retinal variable	Grey matter volume (ml) β (95% CI)	Total hippocampal volume (ml) β (95% CI)	White matter volume (ml) β (95% CI)	WMH volume (ml) β (95% CI)	Infarct present[1,2] β (95% CI)	Microbleed present[1,2] β (95% CI)
Arteriolar measures						
Length						
WG	0.002(-0.003-0.006)	-0.0002(-0.0004-0.00001)	0.002(-0.002 to 0.006)	0.0002(-0.002 to 0.003)	-0.0003 (-0.001 to 0.001)	-0.001(-0.003 to 0.001)
T2DM	0.002 (-0.003-0.008)	-0.0001(-0.0004-0.0002)	0.004 (-0.002-0.01)	0.0005(-0.002-0.003)	-0.001(-0.002-0.0004)	-0.002(-0.005-0.001)
No T2DM	0.002 (-0.005-0.080)	-0.0002(-0.001-0.0001)	-0.001 (-0.01 -0.01)	-0.0003(-0.004-0.004)	0.0004(-0.001-0.002)	-0.001(-0.003-0.002)
Diameter						
WG	0.10(-0.42 to 0.62)	0.004 (-0.03 to 0.03)	-0.22(-0.74 to 0.31)	0.006(-0.27 to 0.28)	-0.07(-0.19 to 0.05)	-0.12(-0.33 to 0.10)
T2DM	0.49 (-0.18 to 1.17)	0.005 (-0.03 to 0.04)	-0.20 (-0.88 to 0.47)	-0.06 (-0.39 to 0.26)	-0.10 (-0.24 to 0.05)	-0.30 (-0.65 to 0.05)
No T2DM	-0.31 (-1.12 to 0.51)	-0.005 (-0.04 to 0.04)	-0.26 (-1.11 to 0.58)	0.11 (-0.38 to 0.61)	-0.10 (-0.31 to 0.12)	0.05 (-0.23 to 0.33)
LDR						
WG	0.04(-0.06 to 0.14)	-0.004 (-0.01 to 0.001)	0.05(-0.05 to 0.16)	-0.003(-0.06 to 0.05)	-0.01(-0.03 to 0.02)	-0.02(-0.06 to 0.02)
T2DM	0.04 (-0.09 to 0.18)	-0.004 (-0.01 to 0.003)	0.09 (-0.04 to 0.22)	0.005 (-0.06 to 0.07)	-0.02 (-0.04 to 0.01)	-0.02 (-0.09 to 0.05)
No T2DM	0.04 (-0.11 to 0.20)	-0.005 (-0.01 to 0.003)	-0.005 (-0.17 to 0.16)	-0.02 (-0.11 to 0.08)	0.01 (-0.03 to 0.05)	-0.02 (-0.07 to 0.04)
Simple tortuosity						
WG	4.28(-24.77 to 33.32)	-0.58 (-2.06 to 0.89)	2.75(-26.29 to 31.80)	1.55(-13.61 to 16.70)	-1.04(-7.49 to 5.42)	2.42(-8.53 to 13.37)
T2DM	3.39 (-32.73 to 39.51)	-1.12 (-3.00 to 0.76)	6.16 (-29.92 to 42.24)	-0.07 (-17.59 to 17.44)	-1.26 (-9.00 to 6.47)	1.65 (-14.72 to 18.02)
No T2DM	17.33 (-32.06 to 66.71)	-0.04 (-2.48 to 2.41)	-15.51 (-66.14 to 35.12)	1.86 (-27.83 to 31.54)	-1.14 (-14.21 to 11.94)	5.88 (-9.54 to 21.29)
Internal Angle						
WG	-0.05(-0.12 to 0.02)	-0.0001 (-0.004 to 0.003)	0.05(-0.02 to 0.12)	-0.01(-0.05 to 0.02)	0.003(-0.01 to 0.02)	-0.02(-0.05 to 0.01)
T2DM	-0.05 (-0.13 to 0.03)	-0.001 (-0.006 to 0.003)	0.06 (-0.02 to 0.14)	-0.01 (-0.05 to 0.03)	0.002 (-0.01 to 0.02)	-0.01 (-0.05 to 0.03)
No T2DM	-0.03 (-0.16 to 0.11)	0.002 (-0.004 to 0.008)	-0.01 (-0.15 to 0.13)	-0.01 (-0.09 to 0.07)	-0.004 (-0.04 to 0.03)	-0.02 (-0.07 to 0.03)
Optimality Ratio	**-22.15(-41.69- -2.61)[a]**	-0.54(-1.52 to 0.45)	13.97(-5.63 to 33.56)	3.70(-13.95 to 6.54)	**-4.97(-9.62 to -0.33)[a]**	-5.52(-14.10 to 3.07)
WG	-17.63 (-43.87 to 8.61)	-0.03 (-1.38 to 1.33)	7.75 (-18.25 to 33.76)	-0.88 (-13.48 to 11.71)	**-6.57 (-12.56 to -0.58)[a]**	-5.31 (-17.62 to 7.00)
T2DM	-26.00 (-54.94 to 2.95)	-1.30 (-2.71 to 0.10)	20.86 (-9.10 to 50.83)	-7.77 (-25.41 to 9.87)	-3.79 (-11.95 to 4.38)	-5.21 (-17.07 to 6.65)
No T2DM						

Venular measures						
Length						
WG	-0.001(-0.008-0.005)	-0.0001(-0.0004 to 0.0002)	-0.006(-0.01 to 0.0001)	0.001(-0.002 to 0.004)	0.0003(-0.001 to 0.002)	-0.002(-0.005 to 0.001)
T2DM	0.001 (-0.01 to 0.01)	-0.0002 (-0.001 to 0.0003)	-0.006 (-0.01 to 0.002)	-0.0005 (-0.005 to 0.004)	0.001 (-0.001 to 0.002)	0.001 (-0.003 to 0.004)
No T2DM	-0.003 (-0.01 to 0.01)	-0.00004(-0.0005 to 0.0004)	-0.005 (-0.01 to 0.005)	0.002 (-0.003 to 0.008)	-0.001 (-0.003 to 0.002)	-0.004 (-0.01 to 0.001)
Diameter						
WG	0.29(-0.03 to 0.60)	0.001(-0.01 to 0.02)	0.004(-0.31 to 0.32)	-0.04(-0.21 to 0.12)	-0.04(-0.12 to 0.03)	0.02(-0.11 to 0.14)
T2DM	0.36 (-0.04 to 0.76)	-0.001 (-0.02 to 0.02)	-0.10 (-0.50 to 0.30)	-0.16 (-0.35 to 0.04)	-0.05 (-0.13 to 0.04)	-0.01 (-0.19 to 0.17)
No T2DM	0.10 (-0.40 to 0.61)	0.01 (-0.02 to 0.03)	0.20 (-0.32 to 0.72)	0.15 (-0.15 to 0.45)	-0.04 (-0.17 to 0.09)	0.03 (-0.13 to 0.19)
LDR						
WG	-0.08(-0.25 to 0.09)	-0.003(-0.01 to 0.006)	-0.13(-0.30 to 0.04)	0.03(-0.06 to 0.12)	0.02(-0.02 to 0.05)	-0.04(-0.12 to 0.03)
T2DM	-0.04 (-0.26 to 0.18)	-0.005 (-0.02 to 0.01)	-0.11 (-0.33 to 0.11)	0.03 (-0.08 to 0.14)	0.03 (-0.01 to 0.07)	0.02 (-0.07 to 0.11)
No T2DM	-0.08 (-0.34 to 0.18)	-0.002 (-0.02 to 0.01)	-0.18 (-0.44 to 0.09)	0.03 (-0.12 to 0.18)	-0.02 (-0.09 to 0.05)	-0.11 (-0.24 to 0.01)
Simple tortuosity	-25.58(-114.88 to 63.72)	-0.53(-5.14 to 4.08)	-51.25(-140.99 to 38.49)	19.73(-28.64 to 64.51)	8.98(-10.32 to 28.28)	-23.50(-66.46 to 19.45)
WG	18.58 (-98.88 to 136.0)	-1.63 (-7.87 to 4.61)	-26.16 (-143.66 to 91.34)	37.00 (-20.10 to 94.10)	12.92 (-11.31 to 37.15)	-1.93 (-56.08 to 52.23)
T2DM	-58.92(-197.13to 79.3)	-0.15 (-7.07 to 6.77)	-106.9 (-248.80 to 35.00)	-13.00 (-94.69 to 68.71)	-4.47 (-43.23 to 34.29)	-50.56 (-129.74 to 28.62)
No T2DM						

LDR: length/diameter ratio; WG: whole group combined; T2DM: Type 2 diabetes; WMH: White matter hyperintensity

Model 1, adjusted for age, sex and intra-cranial volume

[1] Not adjusted for history of stroke

[2] Not adjusted for intra-cranial volume

[a]p value < 0.05;[b] p value <0.01; [c] p value <0.001

Monash University

Declaration for Thesis Chapter 7

Declaration by candidate

In the case of Chapter 7, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Study concept, conducted the analysis and wrote the original draft of the manuscript	70

The following co-authors contributed to the work. If co-authors are students at Monash University, the extent of their contribution in percentage terms must be stated:

Name	Nature of contribution
Richard Beare	Conducted the analysis and contributed to the writing of the manuscript
Thanh Phan	Conducted the analysis and contributed to the writing of the manuscript
David Bruce	Contributed to the writing of the manuscript
Michele Callisaya	Contributed to the writing of the manuscript
Velandai Srikanth	Conceived the idea for the study, conducted the analysis and contributed to the writing of the manuscript

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work*.

Candidate's Signature	[redacted]	**Date** 26/6/15
Main Supervisor's Signature	[redacted]	**Date** 26.6.2015

*Note: Where the responsible author is not the candidate's main supervisor, the main supervisor should consult with the responsible author to agree on the respective contributions of the authors.

Chapter 7

Type 2 Diabetes Mellitus and Biomarkers of Neurodegeneration

7.1 Introduction

As described in the previous chapters, the regional distribution of grey matter atrophy associated T2DM is similar to that seen in AD and may be due to AD-type processes. To investigate this further, I obtained access to the Alzheimer's Disease Neuroimaging Initiative (ADNI) with the intent to investigate the association between T2DM and biomarkers of AD, namely neuroimaging and cerebrospinal fluid markers of amyloid and tau. In addition, I aimed to examine whether these biomarkers have a mediating role in T2DM and brain atrophy. This manuscript was accepted for publication in *Neurology* on 3rd June 2015 and is presented below.

Type 2 Diabetes Mellitus and Biomarkers of Neurodegeneration

This manuscript was accepted for publication in "Neurology" on 3rd June 2015

Abstract

Objective: Our objective was to investigate whether Type 2 Diabetes Mellitus (T2DM) influences neurodegeneration in a manner similar to Alzheimer's disease (AD), by promoting brain amyloid beta (Aβ) or tau.

Methods: We studied the cross-sectional associations of T2DM with cortical thickness, brain Aβ load, and cerebrospinal fluid (CSF) levels of Aβ and tau, in a sample of people from the Alzheimer's Disease Neuroimaging Initiative with diagnoses of AD dementia, mild cognitive impairment, and normal cognition. All (n=816) received magnetic resonance imaging, and a subsample underwent brain amyloid imaging (n=102), and CSF Aβ and tau measurements (n=415). Analyses were performed across and within cognitive diagnostic strata.

Results: There were 124 people with T2DM (mean age 75.5 years) and 692 without T2DM (mean age 74.1 years). After adjusting for age, sex, total intracranial volume, ApoE4 status and cognitive diagnosis, T2DM was associated with lower bilateral frontal and parietal cortical thickness (ml) (β= -0.03, p= 0.01). T2DM was not associated with ^{11}C PiB Standardized Uptake Value Ratio (AU) in any brain region or with CSF Aβ42 levels (pg/ml). T2DM was associated with greater CSF total tau (pg/ml) (β=16.06, p=0.04) and phosphorylated (p)-tau (β=5.84, p=0.02). The association between T2DM and cortical thickness was attenuated by 15% by the inclusion of p-tau.

Conclusions: T2DM may promote neurodegeneration independent of AD dementia diagnosis, and its effect may be driven by tau phosphorylation. The mechanisms through which T2DM may promote tau phosphorylation deserve further study.

Introduction

Type 2 diabetes mellitus (T2DM) is associated with a nearly two-fold increased incident risk of dementia and Alzheimer's disease (AD) dementia (Peila et al., 2002, Biessels et al., 2006b). The possible mechanisms underlying this association include both cerebrovascular disease and neurodegeneration (Biessels et al., 2006b). T2DM is also associated with neurodegenerative imaging biomarkers, namely hippocampal (den Heijer et al., 2003b) and whole brain atrophy (Knopman et al., 2011). Recently, we demonstrated that the association between T2DM and cognitive impairment in older age may primarily be driven by brain atrophy rather than cerebrovascular brain lesions, and that this atrophy occurs in cortical regions similar to those affected in AD dementia (Moran et al., 2013). However, there are limited data from in-vivo studies whether T2DM contributes to the accumulation of AD pathology (Tomita et al., 2013).

In AD, two main pathological processes occur to promote neurodegeneration, involving amyloid-beta(Aβ) and neuronal tau (Querfurth et al., 2010). Abnormal cleavage of Aβ creates the non-soluble Aβ42 oligomers which forms extracellular amyloid plaques that contribute to neurodegeneration (Reitz, 2012). Brain Aβ42 load can be measured in vivo either by using Positron Emission Tomography (PET) neuroimaging with special ligands (e.g. Pittsburgh Compound B-^{11}C PiB), or estimated by detecting low Aβ42 levels in the cerebrospinal fluid (CSF), possibly reflecting Aβ sequestration within cerebral plaques (Apostolova et al., 2010). Additionally, tau-related pathology is commonly seen in AD. Intracellular tau proteins stabilize neuronal microtubules, a process which is important for neuronal health. In AD, there is hyperphosphorylation of tau (p-tau) resulting in the accumulation of neurofibrillary tangles and subsequent neuronal death (Querfurth et al., 2010). Elevated CSF levels of tau and p-tau are also in-vivo markers of tauopathy in AD and correlate well with intracerebral AD pathology (Braak et al., 1991, Apostolova et al., 2010).

The aim of this study was to explore the relationships between T2DM and biomarkers of neurodegeneration usually implicated in the development of AD. We examined the relationships between T2DM, brain atrophy, and in-vivo brain and CSF biomarkers of Aβ and tau in people with AD dementia, its precursor, amnestic Mild Cognitive Impairment (MCI), and in normal controls.

Methods

The data used for this analysis were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (https://ida.loni.usc.edu) (Apostolova et al., 2010). ADNI was launched in 2003 with the primary aim of identifying magnetic resonance imaging (MRI), positron emission tomography (PET), cerebrospinal fluid, biochemical, clinical, and neuropsychological biomarkers of potential progression of mild cognitive impairment (MCI) to early AD dementia (Apostolova et al., 2010). ADNI aimed to recruit 800 adults between the ages of 55-90 years with 400 people with MCI, 200 people with early probable AD dementia and 200 normal controls (NC). These cognitive diagnoses were based on the National Institute of Neurological and Communicative Disorders and Stroke/ AD and Related Disorders Associations (McKhann et al., 1984). Those with MCI had memory complaints but no significant functional impairment based on Clinical Dementia Rating. Subjects were excluded if they had Hachinski ischemic score (Hachinski et al., 1975) >4 (a high risk of cerebrovascular disease contributing to cognitive impairment), were unable to undergo MRI imaging, had other neurological disorders, active depression, history of psychiatric diagnosis, alcohol or substance dependence in the last two years, had less than six years of education or were not fluent in English or Spanish.

Standard Protocol Approvals, Registrations, and Patient Consents

Written informed consent was obtained from all participants. Full details of ethics approval, study design, participant recruitment and clinical testing have been published previously and are available at www.adni-info.org.

Clinical and genetic data

Data on demographic information, medical history, Apolipoprotein ε4 (ApoE4) genotype, baseline cognitive diagnosis, fasting venous blood glucose levels, and medication use were downloaded from the ADNI clinical data database in August 2013. Cognitive diagnosis at baseline was used for grouping of participants. We assigned diabetes status based on fasting blood glucose ≥ 7.0mml/L as per American Diabetes Association guidelines (American Diabetes, 2010) or the use of glucose-lowering agents. ApoE4 genotypying was performed on venous blood derived DNA at the ADNI Biomarker Core Laboratory, (University of Pennsylvania) and subjects deemed ApoE4 positive if they carried at least one ApoE4 allele.

MRI scans and image processing

The process for MRI acquisition has been described previously in ADNI publications (Jack et al., 2008, Risacher et al., 2009). In brief, all ADNI subjects had a 1.5T MRI performed at either screening or baseline visits between August 2005 and October 2007. The ADNI project offers scans that have been preprocessed (gradient warping, scaling, B1 correction and N3 inhomogeneity correction) to correct for different scanners across sites (ADNI, 2013a). In our imaging laboratory we used FreeSurfer v5.3 (http://surfer.nmr.mgh.harvard.edu/) to parcellate the cortex in these scans into 74 regions per hemisphere, based on the Destrieux atlas (Destrieux et al., 2010). Quality control of FreeSurfer parcellation was carried out by testing for outliers, using Bonferoni p-values for Studentized residuals, in a regression model

predicting regional cortical thickness from age, sex and cognitive diagnosis. Any scan in which one or more regions were detected as an outlier was inspected for processing errors. From ADNI databases, the following measures were also obtained: hippocampal volume measures(ml) using an automated tissue classifier (AdaBoost) (Apostolova et al., 2010); WMH volume(ml) using fully automated segmentation (Schwarz et al., 2009) and the presence of MRI infarcts as identified by a specially trained physician (DeCarli et al., 2013).

PiB PET scans

PiB PET scans were performed at 12 ADNI sites. Participants were injected with 15 ±1.5mCi PiB and images were acquired 50-70 minutes following injection. Further details of PiB PET acquisition and the region of interest protocol has been summarised previously (Apostolova et al., 2010). ADNI provides PiB PET scans that have been pre-processed (coregistered, averaged, standardized image and voxel size, uniform resolution) to account for different scanners across sites (ADNI, 2013b).

We adopted three methods to investigate group differences in PiB uptake/binding. For the first method, we used the regional standardized uptake value ratios (SUVR, AU) derived in ADNI relative to the cerebellum for 13 regions using an automated region of interest template (Jagust et al., 2010). The second and third analyses were conducted in our imaging laboratory. For our second form of analysis, we co-registered PiB PET scans with the corresponding T1-weighted MRI scan using the co-registration facility of SPM (Statistical Parametric Methods). The T1 scan was automatically parcellated into 168 regions of interest using FreeSurfer (version 5.3) and the mean PiB intensities computed using FreeSurfer's mri_segstats command. For the third method, we used voxel based morphometry (VBM) to compare differences in SUVR uptake between those with and without T2DM at the voxel level.

CSF data

CSF collection and procedural protocols have been described previously (Shaw et al., 2009). Briefly, fasting CSF was collected and analysed using a Luminex platform (Luminex Corporation, Austin, TX) with an Innogenetics immunoassay kit (INNO-BIA AlzBio3; Ghent, Belgium) that included monoclonal antibodies for A-Beta142, t-tau and p-tau181(pg/ml).

Data analysis

Student's t test and Chi square tests were applied to compare demographic, clinical and cognitive variables between T2DM and non-T2DM groups.

We studied the associations of T2DM with individual global brain MRI measures (cortical thickness, hippocampal volume, WMH, infarcts) adjusting in each regression for age, sex, cognitive diagnosis, ApoE4 and total intracranial volume (analyses with infarcts were not adjusted for total intracranial volume). Linear regression was used for continuous variables and logistic regression for categorical variables.

Differences in regional thickness between T2DM and non-T2DM were tested in 148 regions of interest using a regression model with thickness as the dependent variable and T2DM status, age, sex, cognitive diagnosis, and total intracranial volume as independent variables. Significance of the T2DM status term was examined after correction for multiple comparisons using a false discovery rate FDR ($p<0.05$).

For PiB PET analyses, first we compared PiB SUVR between cognitive diagnostic groups irrespective of T2DM status to ensure consistency with previous reports from ADNI (Jagust et al., 2010). Differences in PiB SUVR between T2DM and those without T2DM were then tested in four regions of interest (Jagust et al., 2010) using both ADNI-provided data and our

own laboratory method, with SUVR as the dependent variable and T2DM status, age, sex and cognitive diagnosis as independent variables. For the VBM component of the PiB PET analysis, we used a FDR ($p<0.05$) to correct for multiple comparisons.

The associations of T2DM with individual CSF measures and CSF Aβ/tau ratios were analysed using linear regression modelling adjusting for age, sex, ApoE4 and cognitive diagnosis. We examined for two-way interactions between ApoE4 and T2DM and cognition and T2DM with a test of significance of product terms. We additionally conducted sensitivity analyses by reclassifying the presence of T2DM using fasting glucose alone, or the combination of fasting glucose and the use glucose-lowering medication, but not metformin (as this is sometimes used in those without T2DM). Further sensitivity analyses were performed using only participants in whom fasting glucose levels were available (n=736). All the above analyses were repeated after stratifying by cognitive diagnosis.

Results

There were 124 people with T2DM (mean age 75.5 years, SD 6.2) and 692 without (mean age 74.1 years, SD 7.0). The numbers and proportions of T2DM in each cognitive diagnostic group were: 38/228 (17%) among cognitively normal controls, 59/397 (15%) among those with MCI, and 27/191 (14%) among those with AD dementia. Group characteristics and comparisons are presented in **Table 1**. Participants with T2DM were more likely to be male, have greater fasting blood glucose levels and body mass index than those without T2DM. A total of 75 participants used oral hypoglycaemic agents to control their T2DM and ten used insulin (five of whom were also on oral agents).

Table 1. Participant characteristics

	T2DM N(%) or mean (sd)	No T2DM N(%) or mean (sd)	p value
N	124 (15)	692 (85)	
Age	75.5 (6.2)	74.1 (7.0)	0.58
Male sex	85 (69)	389 (56)	0.01
Fasting glucose	7.3(2.3)	5·3 (0.7)	<0.001
Average SBP	137 (17)	135 (18)	0.23
Average DBP	74 (10)	75 (10)	0.86
Weight (Kg)	80.7 (17)	74.0 (14.0)	<0.0001
BMI (Kg/m^2)	28.0 (4.8)	26.3 (4.2)	0.0001
MRI infarct	11 (9)	55 (8)	0.73
Smoker	42 (34)	279 (40)	0.18
MMSE	26.2 (3.4)	26.2 (3.6)	0.98
ADAS-Cog	11.7 (6.5)	11.7 (6.4)	0.94
Oral diabetes medications	75 (60)		
Insulin use Insulin & oral agent	10 (8) 5 (4)		
Cognitive diagnoses			
Normal control (n=228)	38	190	
MCI (n=397)	59	338	0.75[1]
AD (n=191)	27	164	

T2DM, Type 2 diabetes mellitus; SBP, systolic blood pressure, BMI; Body Mass Index, MRI, magnetic resonance imaging; MMSE, mini mental state examination score; ADAS-Cog, Alzheimer's disease Assessment Scale –Cognitive subscale; MCI, mild cognitive impairment; AD, Alzheimer's disease dementia.[1] Chi square test for trend of proportion of T2DM across cognitive groups.

T2DM and MRI biomarkers

Cortical thickness measures were available in 816 participants (228 NC, 397 MCI, 191 AD dementia). As expected, mean cortical thickness was greatest in NC, followed by those with MCI and then those with AD dementia (**Fig 1**). When adjusted for age, sex, total intracranial volume, ApoE4 status and cognitive diagnosis, T2DM was associated with lower total cortical thickness (mm) (β= -0.03, 95%CI -0.05 to -0.006,p= 0.01) but not with hippocampal volume (ß= -70.90, 95%CI -248.00 to 106.19,p=0.43), presence of infarct on MRI (ß=0.13, 95%CI -0.56 to 0.82,p=0.71, Odds Ratio, OR 1.14, 95%CI 0.57 to 2.27 or white matter hyperintensity volume(ml) (ß= -0.12, 95%CI -0.61 to 0.38,p=0.64). Regions of cortical thinning attributable to T2DM included bilateral sub-central gyri and sulci, right inferior pre-central sulcus, rectus gyrus, front and middle sulcus (frontal lobe), and inferior parietal gyrus (**Fig 1**). When stratified by cognitive diagnosis, T2DM was associated with lower cortical thickness in those NC and MCI (both p=0.04) but not in those with AD dementia (p=0.77). T2DM was associated with lower hippocampal volume in those with MCI (p=0.05) but not in those NC or with AD dementia. There was no association between T2DM and either WMH or infarcts in any of the cognitive diagnostic groups.

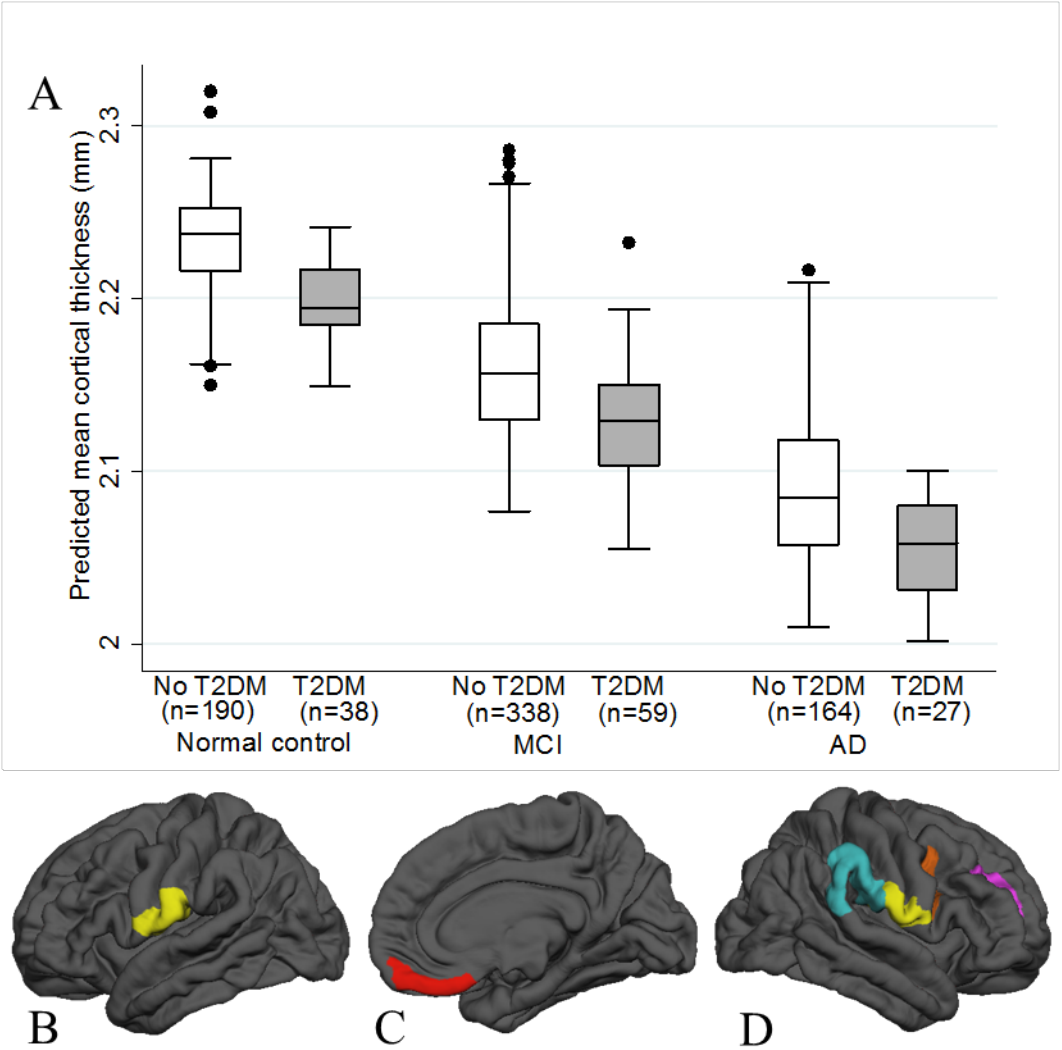

Figure 1. Association of T2DM with cortical thickness (n = 816).

A. Association of T2DM with cortical thickness stratified by cognitive diagnosis [1].
B-D. Regions of cortical thinning associated with T2DM [2].
B Left hemisphere: Lateral view
C Right hemisphere: Medial view
D Right hemisphere: Lateral view

Regions
Yellow: Subcentral gyrus and sulcus
Red: Rectus gyrus
Orange: Inferior precentral sulcus
Pink: Front and middle sulcus
Blue: Inferior parietal gyrus

[1] Adjusted for age, sex, total intracranial volume and ApoE4 status.
[2] Adjusted for age, sex, total intracranial volume, ApoE4 status and cognitive diagnosis.

T2DM: Type 2 Diabetes Mellitus, CI : confidence interval, MCI : Mild Cognitive Impairment, AD : Alzheimer's Disease dementia; ApoE4: Apolipoprotein ε4;

T2DM and ^{11}CPiB PET uptake

^{11}CPiB PET scans were available for 102 participants (19 NC, 64 MCI, 19 AD), of whom 19 had T2DM. As expected, SUVR increased across groups from NC through MCI to AD dementia (**Fig 2**). We did not find a statistically significant association between T2DM and ^{11}CPiB SUVR when adjusting for age, sex, ApoE4 status and cognitive diagnosis using either ADNI-derived data or our in-house Freesurfer and VBM methods. Similarly, no associations were detected between T2DM and regional SUVR when the analyses were stratified by cognitive diagnosis.

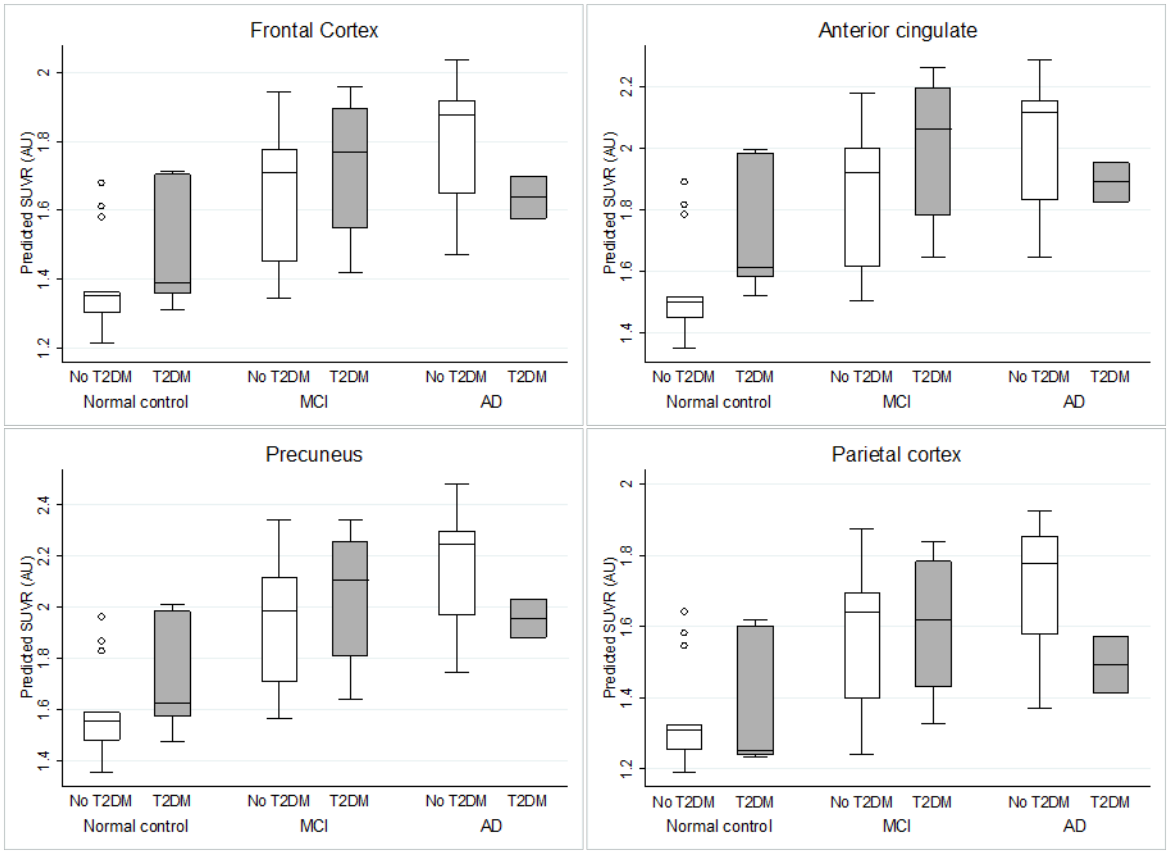

Figure 2. Regional associations of T2DM with PiB-SUVR

Adjusted for age, sex, cerebellum PiB uptake, ApoE4 status and cognitive diagnosis.T2DM: Type 2 Diabetes Mellitus, CI : confidence interval, MCI : Mild Cognitive Impairment, AD : Alzheimer's Disease dementia; ApoE4: Apolipoprotein ε4; SUVR – Standardised uptake value ratio.
Normal control: n=19, T2DM n=6; MCI: n=64, T2DM, n=11; AD: n=19, T2DM n=2.

CSF measures of amyloid and tau

CSF measurements were available for 415 participants (n=114 NC, 199 MCI, 102 AD dementia), of whom 56 had T2DM (**Supplementary Table 1**). T2DM was associated with greater CSF total tau (β=16.06, 95%CI 1.10 to 31.02,p=0.035) and p-tau181 (β=5.84, 95%CI 0.95 to 10.73,p=0.02) when adjusted for age, sex, ApoE4 and cognitive diagnosis. There was no association found between T2DM and CSF Aβ42 levels (ß= -6.90 95%CI -20.27 to 6.48,p=0.31). When stratified by cognitive diagnosis **(Fig 3**), T2DM was associated with greater CSF p-tau181 only among those with MCI, but was not associated with CSF total tau or Aβ42in any of the cognitive diagnostic groups.

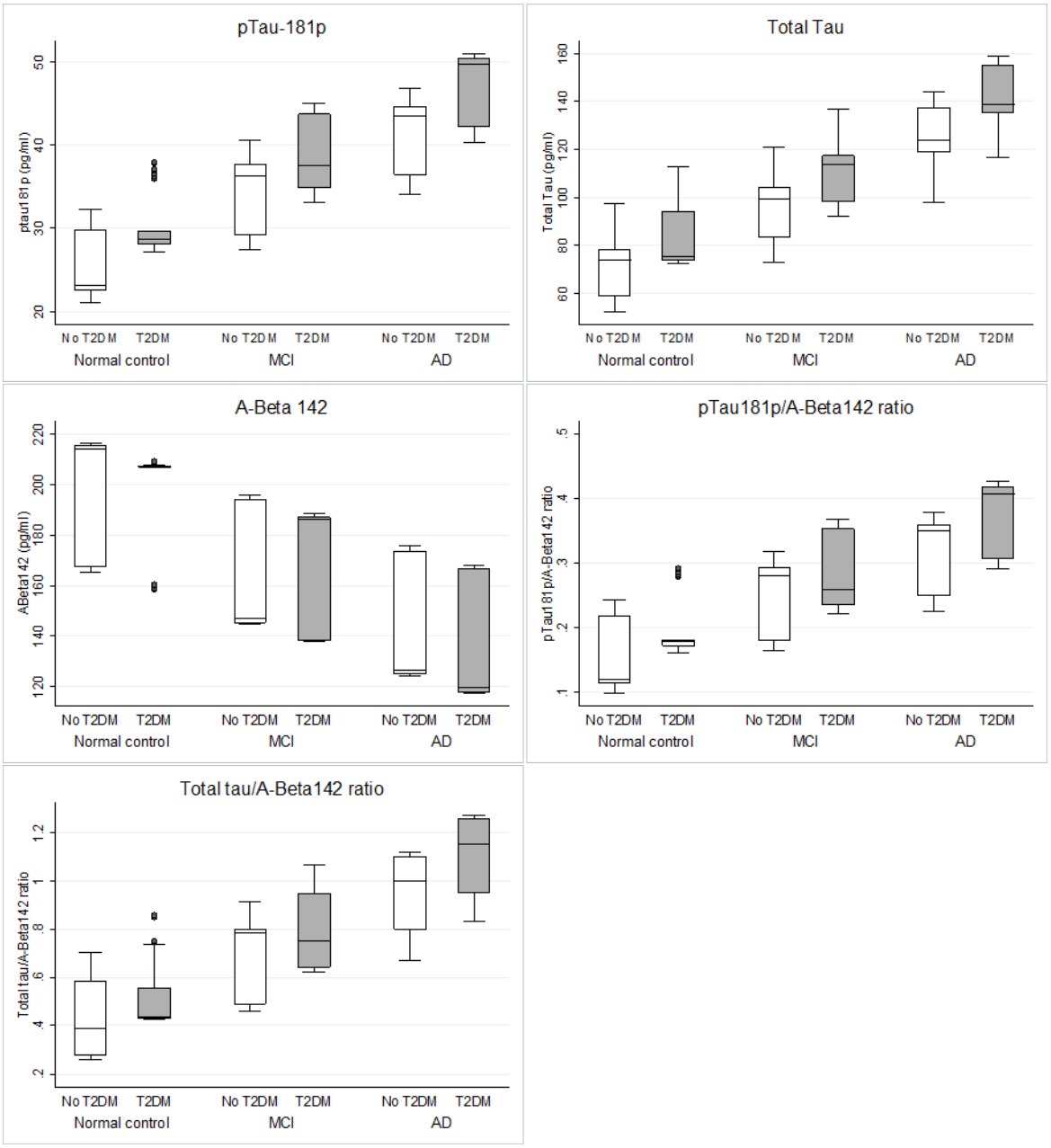

Figure 3. T2DM and Cerebrospinal Fluid (CSF) biomarker levels [1]

[1] Adjusted for age, sex and ApoE4 status.

T2DM: Type 2 diabetes mellitus, MCI: Mild Cognitive Impairment, AD: Alzheimer's Disease dementia

Normal control: n=114, T2DM n=17; MCI: n=199, T2DM, n=25; AD: n=102, T2DM n=14

Among those who had both cortical thickness and CSF measures available (n=407), the addition of total tau as a term in the regression of T2DM with cortical thickness attenuated the beta coefficient of T2DM by 15% (from β=-0.039 to β=-0.033). The addition of p-tau181 attenuated the T2DM-cortical thickness association by 15% (from β=-0.039 to β=-0.033) while the addition of Aβ42 attenuated the association only by 4% (from β=-0.039 to β=-0.037). We did not find any interaction between ApoE4 and T2DM or cognitive diagnosis and T2DM status in predicting brain imaging or CSF biomarker measures. Sensitivity analysis using alternative definitions of T2DM did not result in variation from the above results, particularly in relation to the association of T2DM with cortical thickness and CSF-tau, for which the strength of the associations remained unchanged, and for the absence of associations between T2DM and Aβ42 measures.

Discussion

In this study, we examined the relationships between T2DM and in-vivo mechanistic biomarkers of neurodegeneration in a sample enriched with patients with AD dementia and MCI. In addition to confirming prior findings that T2DM is associated with brain atrophy (Brundel et al., 2010, Moran et al., 2013), we now demonstrate for the first time, a strong relationship between T2DM and the amount of phosphorylated tau in the CSF. We did not find evidence of a significant relationship between T2DM and brain or CSF Aβ levels. Regression analyses suggest that the association of T2DM with cortical thickness may be partially mediated by CSF phosphorylated tau levels irrespective of cognitive diagnosis or ApoE4 status. Cortical thinning related to T2DM was observed in frontal and parietal cortices rather than the mesial temporal predilection for AD-related atrophy. In all, our findings

suggest that the neurodegenerative effects of T2DM may be independent and possibly additive to those of AD, and driven by pathways that promote neuronal tau more than Aβ.

Our results are consistent with animal histopathological data showing that T2DM is associated with hyperphosphorylation of neuronal tau (Jung et al., 2013). By contrast, our results are inconsistent with data from human post-mortem studies relating T2DM with AD pathology. Results from such studies suggest that the cerebral load of tau-related neurofibrillary tangles or amyloid plaques are either similar (Heitner et al., 1997, Arvanitakis et al., 2006, Thambisetty et al., 2013, Tomita et al., 2013) or lower (Beeri et al., 2005, Nelson et al., 2009, Ahtiluoto et al., 2010) in those with T2DM than in those without T2DM. The difference in results may largely be explained by factors related to study design. Previous human pathological studies were either performed in samples from single centres (Heitner et al., 1997, Beeri et al., 2005, Nelson et al., 2009), or with retrospective ascertainment of diabetes status (Heitner et al., 1997, Beeri et al., 2005, Nelson et al., 2009), in people whose mean age at death was >80 years (Beeri et al., 2005, Arvanitakis et al., 2006, Nelson et al., 2009, Ahtiluoto et al., 2010), suggesting survivor-bias, or in samples who were highly educated (Arvanitakis et al., 2006, Nelson et al., 2009) and with healthy lifestyles (Arvanitakis et al., 2006, Thambisetty et al., 2013). In the Honolulu-Asia Aging Study (Peila et al., 2002), a prospective study using rigorous phenotyping of diabetes (mean age 77 years), the risk of AD pathology was greater in people with T2DM, but only among those positive for the ApoE4 allele. In contrast, we did not find effect modification by ApoE4 status, but the power to assess this interaction in our study may have been low.

There are several pathways through which T2DM may contribute to increased levels of phosphorylated tau in the brain. Chronic hyperglycemia is associated with increased production in tissues of Advanced Glycation Endproducts (AGEs) which may promote protein cross-linking and stabilisation of the paired helical filament tau (Munch et al., 2012).

Furthermore, hyperglycemia leads to abnormal brain glucose transport that may also contribute greater levels of phosphorylated tau (Liu et al., 2009). Impaired insulin signalling, a hallmark of T2DM, may also contribute to increased cerebral phosphorylated tau possibly through insulin receptor substrate 1 (IRS-1), extracellular signal–related kinase/mitogen activated protein kinase (ERK/MAPK), and PI3 kinase/Akt (PI3K/AKT) pathways (Yarchoan et al., 2014). Brain insulin resistance is seen in addition to the peripheral insulin resistance that characterises T2DM (Deng et al., 2009). The down-regulation of cerebral insulin receptors leads to the over activation of the important tau phosphorylation-regulator GSK-3ß. In a rat model, the administration of intranasal insulin normalised cerebral GSK-3 levels and reduced CSF p-tau levels (Yang et al., 2013), suggesting that cerebral insulin levels play a modulatory role in tau phosphorylation.

Our study had certain limitations. The low probability of cerebrovascular disease (low Hachinski score) in the ADNI selection criteria do not allow exploration of potential vascular mechanisms (Biessels et al., 2014). While this is in a sense a limitation, it is also a strength, because it facilitates better control for the confounding effects of vascular disease. The exclusion of those with a high Hachinski score may also explain the lower prevalence of T2DM (17% in cognitive normal controls) than would be expected in a US sample with mean age 75 years (around 25%) as per the National Diabetes Statistics Report 2014 (Promotion, 2014). This selection bias is likely to limit the generalisability of our results. The samples of people with T2DM and PiB-PET (n=19) or CSF studies (n=56) were small, and hence our study may have been limited in its power to detect statistically significant associations of T2DM with brain or CSF amyloid. Thus we cannot completely exclude the possibility that T2DM is associated with greater amyloid accumulation. However, the absence of an association between T2DM and cerebral amyloid load has also been reported in another study examining human in vivo PET PiB uptake (Thambisetty et al., 2013, Roberts et

al., 2014) and a few reports from human post-mortem data (Arvanitakis et al., 2006, Alafuzoff et al., 2009). We used 3 different approaches to PiB image analyses to increase the confidence in our null association. Furthermore, the expected increasing gradient of PiB SUVR was observed going from normal cognition, to MCI and AD dementia, making it unlikely that that measurement error could explain the null association for T2DM. In addition, we found an absence of an association between T2DM and CSF amyloid levels, consistent with the imaging findings. The absence of an association between T2DM and hippocampal or white matter hyperintensity volume, in contrast to results described in other studies (den Heijer et al., 2003b, Jeerakathil et al., 2004, Knopman et al., 2011), may be attributable to the exclusion of those with a large burden of cerebrovascular disease as well as the larger proportions of those with MCI and AD dementia in ADNI than the other population-based cohorts.

Our analysis was cross-sectional, therefore limiting inferences of causality, raising the possibility that the reported brain changes may precede the development of T2DM. Further longitudinal analyses will assist in establishing whether the associations support causality. As the primary objectives of ADNI were not related to the study of T2DM, information regarding prior diagnosis of T2DM, details of duration of T2DM and effectiveness of glucose control were unavailable, and these would have provided extra information in exploring our hypotheses. The development of T2DM in mid-life may be a greater risk factor for the subsequent development of dementia (Biessels et al., 2014). The lack of data on disease duration and the older age of ADNI participants may therefore partly explain some of the null associations we report.

Acknowledgements

*Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at:

http://adni.loni.usc.edu/wpcontent/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

Supplementary Table 1. Associations of T2DM with CSF measures (n=415)

	Whole sample[1] (n=415) **β (95% CI)** **p value**	**Normal Controls[2] (n=114)** **β (95% CI)** **p value**	**MCI [2] (n=199)** **β (95% CI)** **p value**	**ADd [2] (n=102)** **β (95% CI)** **p value**
pTau-181p (pg/ml)	5.84 (0.95 to 10.73) 0.02	6.01 (-1.27 to 13.30) 0.11	7.73 (0.46 to 15.00) 0.04	2.65 (-8.32 to 13.62) 0.63
Total Tau (pg/ml)	16.06 (1.10 to 31.02) 0.04	1.12 (-14.78 to 17.02) 0.89	23.64 (-1.04 to 48.31) 0.06	18.49 (-15.19 to 52.17) 0.28
A-Beta 142 (pg/ml)	-6.90 (-20.27 to 6.48) 0.31	-3.79 (-29.77 to 22.19) 0.77	-18.09 (-39.39 to 3.21) 0.10	7.09 (-13.0 to 27.38) 0.49
pTau-181p/A-Beta 142 ratio	0.06 (0.01 to 0.10) 0.01	0.05 (-0.01 to 0.11) 0.12	0.10 (0.03 to 0.17) 0.007	0.002 (-0.10 to 0.10) 0.97
Total tau/A-Beta 142 ratio	0.15 (0.01 to 0.29) 0.03	0.01 (-0.12 to 0.14) 0.91	0.31 (0.07 to 0.56) 0.012	0.03 (-0.26 to 0.31) 0.85

[1] Adjusted for age, sex, ApoE4 and cognitive diagnosis
[2] Adjusted for age, sex, ApoE4
T2DM: Type 2 Diabetes Mellitus, CI : confidence interval, MCI : Mild Cognitive Impairment, AD: Alzheimer's Disease dementia; ApoE4 : Apolipoprotein

7.3 Conclusion

This study confirmed the finding reported in Chapter 2 and *Diabetes Care* (Moran et al., 2013) of a cross-sectional association between T2DM and brain atrophy. The results demonstrated for the first time that there was a strong relationship between T2DM and CSF measures of phosphorylated tau. I did not find evidence of a significant relationship between T2DM and brain or CSF levels of Aβ. The independence of associations from diagnosis of AD, MCI or normal cognition suggests that the neurodegenerative effects of T2DM on brain atrophy may be additive to AD, possibly driven more by pathways promoting tau rather than Aβ. Further studies are required to explore this further, including replicating these finding longitudinally.

Summary and future direction.

8.1 Summary of thesis

Dementia is highly prevalent in older age, accounts for a significant proportion of age-related disability, and is one of the most expensive disorders affecting older Australians (AccessEconomics, 2011). Australia, as does most of the world, has an ageing population with the proportion of people aged > 65 years expected to rise above 20% by the year 2056 (Statistics, 2012). T2DM is a complex chronic disease and is also an Australian National Health Priority. T2DM affects about 85% of all people with diabetes (Zimmet, 2003), occurs more commonly in older age with a prevalence of 12-25% in people >65 years, and is characterised by cellular insulin resistance, several metabolic abnormalities and chronic inflammation. T2DM causes accelerated ageing of most organ systems (Morley, 2008) leading to premature morbidity and mortality. Its effects on the brain till recently were largely under-recognised. T2DM increases the long term risk of dementia by nearly 2-fold (Biessels et al., 2014), and one in ten cases of dementia in the world population may be attributable to the effects of T2DM (Biessels et al., 2014). Models of disease projection suggest that attention to modifiable risk factors (such as T2DM) may delay the onset of dementia, and doing so by even 1 year may reduce the worldwide burden of cases in people over 60 years by ~10% (Brookmeyer et al., 2007). T2DM increases the risk of vascular dementia (VaD, Relative Risk, RR~2.5) and Alzheimer's dementia (AD, RR~1.5) (Biessels et al., 2006b, Xu et al., 2009), although there may be substantial overlap of the two pathologies. The underlying pathways between T2DM and dementia may involve neurodegeneration, vascular disease, or both, with several common intermediary mechanisms. Such mechanisms may

include insulin signalling abnormalities, neuroinflammation, advanced glycation and oxidative stress– all with potential for modification to treat dementia overall.

The main novel results of my thesis are summarised below:

1. Brain atrophy is a key mediator of T2DM-related cognitive impairment and the regional distribution of brain atrophy seen in T2DM appears similar to that seen in early AD (Moran et al., 2013). (Chapter 4)

2. Tissue advanced glycation is associated with brain atrophy in T2DM (and in those without T2DM) and may partially mediate the association between T2DM and brain atrophy (Moran et al., 2015). (Chapter 5)

3. T2DM is associated with excess production of CSF phosphorylated tau, and this partially mediates the association between T2DM and reduced cortical thickness, providing the first in-vivo evidence mechanistically linking T2DM with neurodegenerative AD-type pathology. (Chapter 7)

4. Retinal vascular architecture and retinopathy (subclinical markers of small cerebral vessel disease) were not associated with MRI biomarkers of T2DM-related brain disease, raising speculation about the relative importance of vascular pathways leading to brain disease in people with T2DM receiving good glycaemic and vascular risk control. (Chapter 6)

8.2 Future directions

The combined influence of progression of vascular and neurodegenerative changes in leading to accelerated cognitive decline in T2DM has not yet been shown. The interaction between T2DM and CSF markers of AD pathology (beta-amyloid and tau) on the progression of brain atrophy and cognitive decline is unknown.The research presented in this thesis creates opportunities for future research possibilities:

- Studying the longitudinal relationships between biomarkers of brain disease and cognitive function in T2DM.
    - The work presented in this thesis is based on my cross-sectional analyses of the CDOT, TASCOG and ADNI data. Longitudinal study of the relative contributions of cerebral atrophy and vascular lesions in the expression of cognitive decline would provide greater insight into causality and disease pathogenesis.

- Studying the longitudinal relationships of modifiable risk factors and mechanistic factors with cognitive decline and biomarkers of brain disease in T2DM in CDOT and TASCOG. Several modifiable risk factors or molecular mechanisms related to T2DM may be relevant for the pathogenesis of cognitive decline such as:
    - ***Obesity***, a promoter of inflammation and insulin resistance, is an important risk factor for cognitive decline and brain atrophy (Jagust et al., 2005). Cross-sectional work I have done in the CDOT study that does not form part of this thesis suggests that abdominal adiposity substantially mediates the association between T2DM and reduced brain volumes. Longitudinal examination of this hypothesis will be informative.

- Understanding the effect of ***longer term glycaemic control*** may provide better insights into the impact of glucose control on cognitive decline than single measures reflecting only medium term control, and allow teasing out of the competing effects of hyperglycaemia and hypoglycaemia.
- T2DM is associated with chronic inflammation, and inflammatory cytokines are implicated in the creation of ***insulin resistance*** which is being increasingly recognized as a promoter of AD pathology (Yarchoan et al., 2014). Further work is required within these studies to shed more light on the role of insulin resistance as a mediator of T2DM-related cognitive impairment.
- ***Advanced Glycation End-products (AGE)*** and their receptor RAGE are of great interest as therapeutically modifiable factors in the development of AD as described in Chapter 5. Longitudinal relationships of tissue and circulating AGEs with brain measures in T2DM are yet to be studied.

8.3 Conclusion

The mechanisms underlying the association between T2DM and cognitive impairment are multifactorial and include both neurodegenerative and cerebrovascular pathways that overlap. Better understanding of the molecules that drive these processes and the time of life in which they and their associated risk factors occur will be essential in offsetting these risks. Intervention trials that address more than one of these mechanistic pathways at the same time, such as drugs to improve insulin sensitivity or lifestyle modifications such as exercise, show promise and warrant further exploration. Hopefully with improved understanding of these mechanistic pathways we can prevent or even treat T2DM related cognitive impairment.

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Appendix A

Cerebral small vessel disease: a review of clinical, radiological, and histopathological phenotypes

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Cerebral small vessel disease is difficult to directly visualize *in vivo*. Therefore, we rely on radiological phenotypes as surrogate markers of disease. The principal phenotypes of clinical interest are small, deep brain infarcts, cerebral white matter lesions, deep brain haemorrhages, and cerebral microbleeds. The causes or mechanisms underlying these phenotypes are understood in varying degrees of detail. This review aims to summarize recent knowledge regarding these phenotypes and place it in context with classical clinicopathological observations to provide mechanistic, clinical, and therapeutic insights into small vessel disease.

Key words: cerebral small vessel disease, lacune, stroke

Introduction

Diseases of small cerebral blood vessels have generated significant interest because of their insidious impact on brain function and the difficulty pinning down underlying disease mechanisms. The term 'small vessel disease' or 'SVD' has been used to reflect clinical, radiological, or pathological phenomena attributed to disease of small perforating arteries and arterioles supplying deep brain structures. More recently, the roles of venules and capillaries are also beginning to be emphasized (1). During life, phenotypes of SVD may be identified clinically, radiologically, or using both approaches. As SVD is difficult to directly visualize, we rely predominantly on radiological phenotypes as surrogate markers of disease. The advent of newer imaging techniques has contributed significantly to our understanding of these phenotypes, in particular, their effects on brain function and underlying risk factors. However, such recent descriptions also need to be carefully considered in the context of clinicopathological studies prior to the advent of brain imaging (2–5). The principal SVD phenotypes of clinical interest are small deep brain infarcts, cerebral white matter lesions (WMLs), deep brain haemorrhages, and cerebral microbleeds (CMBs). The causes or mechanisms underlying these phenotypes are understood in varying degrees of detail, and further advances in high-field imaging, genetic studies, and bench-based research have the capability to address the gaps in knowledge. This review aims to summarize recent knowledge regarding sporadic SVD and cerebral autosomal dominant arteriopathy with sub-cortical infarcts and leukoencephalopathy (CADASIL), the major genetic phenotype, and place it in context with clinicopathological observations (2–5).

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Sporadic SVD

We use the term 'sporadic SVD' while referring to a heterogeneous group of phenotypes based on brain imaging, but not caused by single gene disorders. Acquired, rare small vessel vasculopathies such as Susac's syndrome, Cogan syndrome, and Sneddon's syndrome/antiphospholipid syndrome are beyond the scope of this article. The phenotypes discussed in this section are deep brain infarcts (clinical and sub-clinical), age-related WMLs, deep intracerebral haemorrhages (ICHs), and CMBs, together with a consideration of enlarged perivascular spaces.

Deep brain infarcts

When using the term 'deep brain infarcts', we refer to small sub-cortical infarcts (variously defined by researchers as between 3 mm and 20 mm in diameter) identified on computerized tomography (CT) or magnetic resonance imaging (MRI). They may be associated with acute focal deficits (symptomatic infarcts), or found incidentally on brain imaging in those without a history of clinical stroke (sub-clinical or 'silent' infarcts). The terms 'lacunar infarct' and 'lacunar syndrome' are often used to refer to such infarcts. The latter refers to a set of clinical features that are observed commonly in the setting of acute infarcts due to deep perforator occlusion (6). However, these clinical 'lacunar syndromes' may also occur in the setting of large artery atherosclerosis or

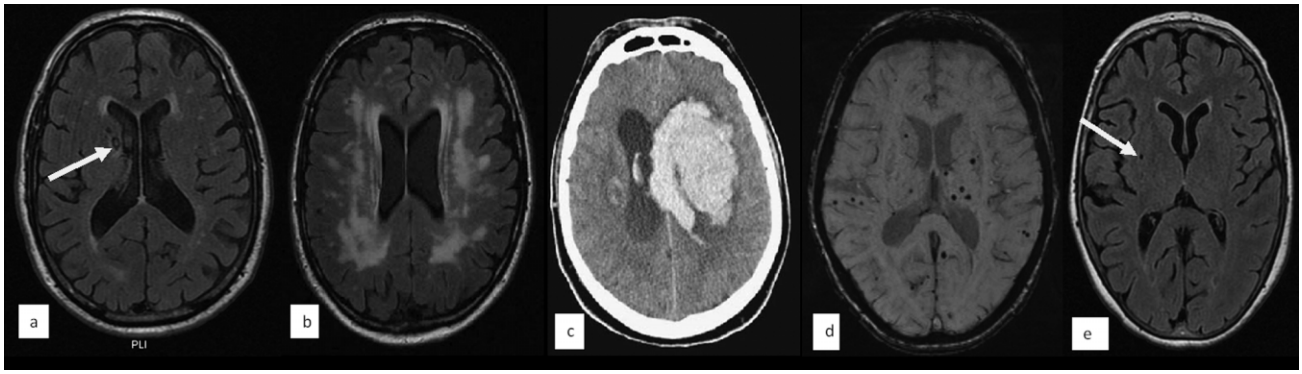

Fig. 1 Radiological phenotypes of cerebral small vessel disease (images a,b,d & e from the Tasmanian Cognition and Gait Study). (a) Deep brain infarcts (arrows) on fluid attenuated inversion recovery (FLAIR). (b) Severe white matter lesion. (c) Large intracerebral haemorrhage with smaller right-sided haemorrhage. (d) Cerebral microbleeds (arrow) on susceptibility-weighted image imaging. (e) Enlarged perivascular space (arrow) on FLAIR.

cardioembolism (7,8). The use of the term 'deep brain infarcts' avoids speculation as to what the actual underlying stroke mechanism may be.

Symptomatic deep brain infarcts account for between 20% and 30% of all stroke sub-types, with an incidence rate in developed countries of approximately 0·33/1000 per year in community-based studies of the general elderly population (9). The mortality rate for symptomatic deep brain infarcts is less than 1% at one-month and ranges between 2·4% and 10% at one-year (10). The prevalence of sub-clinical deep brain infarcts ranges from 8% to 28% depending on the type of sample studied, being less common in the general population than in clinical samples (11). Their incidence rate in population-based studies is approximately 2–3% per year in older people living in developed countries (11,12). These estimates of prevalence or incidence are subject to variability depending on whether CT or MRI is used for detection, the latter being more sensitive (1,13).

Radiology and pathology of deep brain infarcts (Fig. 1a)

Acute deep brain infarcts are best detected using diffusion-weighted imaging (DWI) MRI, and can appear hyperintense on fluid attenuated inversion recovery (FLAIR) MRI sequences. Chronic infarcts are hypointense on T1-weighted MRI and FLAIR (14), often with a hyperintense rim on FLAIR. They are commonly seen in the basal ganglia, internal capsule, and pons (15) and are considered the radiological manifestation of the 'lacune', a term first used by Déchambre in 1838 to describe small cerebral cavities following resorption of necrotic tissue related to infarction (16). The term 'lacune' then expanded to encompass small round lesions seen in white and grey matter on autopsy, as well as the scars of small residual infarcts (16). Pierre Marie and Ferrand, at the turn of the 20th century, redefined 'lacune' to its original meaning of deep cerebral infarction due to local small blood vessel occlusion (16). However, the actual pathological process underlying the vessels related to 'lacunes' remains poorly understood.

Histopathological studies of deep brain infarcts are limited by the often long time elapsed between occurrence and death (17,18). Irregular cavities are seen with surrounding gliosis, lipid-rich and haemosiderin-rich macrophages, and fragmented blood vessels. The significance of haemosiderin-rich macrophages is unclear, but may be explained by microhaemorrhage due to endothelial damage (3). In an attempt to identify the causative lesion underlying such infarcts, Fisher meticulously performed serial dissection of the vascular supply proximal to the infarction of 50 'lacunes' derived from four patients reaching autopsy (4). In all, 45/50 of these 'lacunes' had total occlusion of the perforating artery supplying the infarct (3). He observed that the majority (40/45) of these total occlusions was due to 'segmental arterial disorganization' (3), a term describing patchy, asymmetrical focal vascular changes resulting in the loss of normal arterial wall architecture and thickening of very small vessels (40–200 μm), similar to changes that he observed in larger vessels of those with poorly controlled hypertension (3). He also observed extravasation of plasma proteins into the arteriolar wall and the subsequent conversion to fibrin, which he termed 'lipohyalinosis' (5), and referred to by others as fibrinoid necrosis (Fig. 2a) (19). In a separate study, Fisher also showed that nine of 11 patients with internal capsule infarcts had obstructive vascular lesions in the proximal larger vessel supplying the region, including plaque with superimposed thrombus and plaque causing severe stenosis, coupled with mural or occlusive thrombi in the deep perforating artery (Fig. 2b) (2–4). It is uncertain whether or not such thrombi are primary events or terminal events in a prolonged process of intrinsic vasculopathy. Although such older pathological studies are the most detailed yet in the field, they were based on a small number of cases and set in a time when vascular risk factors were poorly controlled. It has been noted that deep brain infarcts still occur in those with well-controlled hypertension (13) , raising the possibility that more complex mechanisms may be at play. Advances in neuroimaging with the development of high-field MRI may provide more insight into other potential mechanisms.

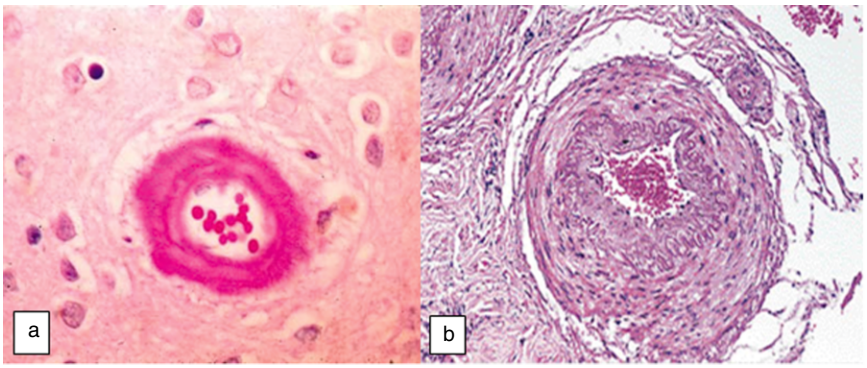

Fig. 2 Histopathology of fibrinoid necrosis and arteriosclerosis. (a) Fibrinoid necrosis: deposition of fibroid around entire circumference of vessel with loss of collagen. (b) Arteriosclerosis: lipid-laden stenosis of perforating arteriole.

Risk factors and mechanisms of deep brain infarcts

Risk factors for larger cortical strokes such as increasing age, hypertension, diabetes mellitus, smoking, prior stroke or transient ischaemic attack (TIA), excess alcohol consumption, and raised cholesterol are also associated to varying degrees with an increased risk of developing deep brain infarcts, whether symptomatic or sub-clinical (20–22). A causal role for hypertension, although likely, is by no means the only contributing factor. Early data supporting a strong association with hypertension may also have been biased by the inclusion of the risk factor in the determination of stroke sub-type (13), hence a possible circular bias. More recently, the association of hypertension and deep cerebral infarcts has been found to be less clear, with deep brain infarcts being seen in those with well-controlled or without hypertension (21). Less evidence is available to support the contribution of elevated lipid levels, smoking, and diabetes to the development of deep cerebral infarcts (20,21). The relationship between carotid stenosis and deep brain infarcts is weak, with the severity of stenosis being similar within patients suffering a recent 'lacunar' infarct in one study (7). Atrial fibrillation and ischaemic heart disease occur less frequently in those with the 'lacunar' syndrome' than those with 'non-lacunar' syndrome' (20). Genetic studies may also provide clues to understanding the pathogenesis of deep brain infarcts (20,21). In a recent genome-wide association study of sub-clinical cerebral infarcts in 9401 participants, the presence and frequency of the single nucleotide polymorphism rs2208454 (in a region possibly regulating growth factor signalling and potentially angiogenesis and neurogenesis) was associated with decreased odds of MRI infarction (23).

The mechanism of intrinsic SVD, as compared with embolic vessel occlusion, has been suggested as the principal mechanism underlying deep brain infarcts (13) with recent observational supportive evidence for this in studies of retinal vessels (24–27). Retinal vessels are like cerebral vessels in many ways including their functional similarity with the blood-brain barrier, position as end arteries, embryological origin, and calibre (26). Patients with clinical deep brain infarcts identified on DWI-MRI are more likely to have a range of retinal microvascular signs (narrowed arterioles, arteriovenous nipping, and enhanced arteriolar light reflex) suggestive of arteriosclerosis than those without, and others have postulated that endothelial dysfunction may underlie such disease (13,25,27). Ageing, and to some extent, hypertension, may also impair the integrity of the blood-brain barrier (17,28), and some have shown an increase in endothelial permeability measured with gadolinium leakage on MRI (17) in people with clinical 'lacunar' strokes. It is unclear whether this is an epiphenomenon or causal, and still leaves open the question regarding its antecedent mechanisms, with immune- or cytokine-mediated damage remaining as possible suspects (28). While intrinsic disease is a likely mechanism, a proximal embolic source cannot be completely excluded (3,4). Acute deep brain infarcts on occasion may be associated with the presence of a visible clot in a larger vessel such as the middle cerebral artery (Fig. 3), suggesting embolism from a more proximal source (29). A study of nonhuman primates showed that the direct injection of emboli into the internal carotid artery resulted in deep brain infarcts in a small proportion of cases (<6%) (30). Similarly, a proximal embolic source may be present in at least 10% of symptomatic deep brain infarcts, particularly in those with larger infarcts or those with multiple small sub-cortical lesions on DWI (7,31–33). The limited phenotyping in most prior studies of sub-cortical stroke argues for careful consideration of additional information (such as angiographic methods) before a small vessel mechanism can be assigned with reasonable confidence.

Deep brain infarcts – clinical presentations, effects on function, and treatment

Clinical syndromes (termed as 'lacunar syndromes') that are most commonly associated with acute deep brain infarcts include pure motor hemiplegia, pure sensory stroke, sensorimotor stroke, ataxic hemiparesis, and dysarthria-clumsy hand

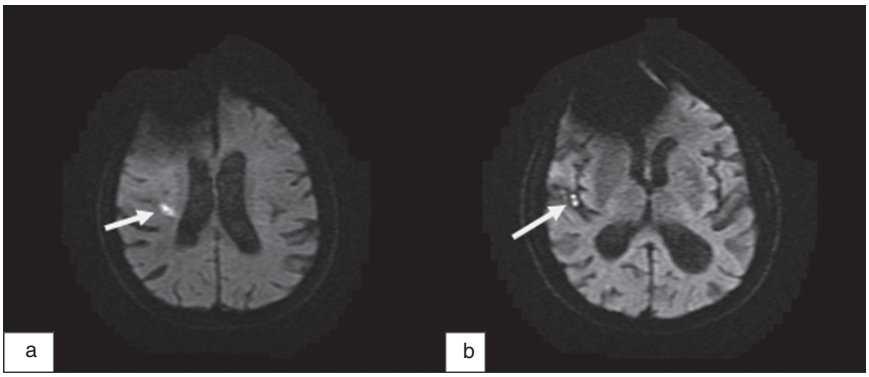

Fig. 3 Deep brain infarct with underlying thrombus present. Acute sub-cortical infarcts (a) may be associated with the presence of a visible clot in a larger vessel, such as the middle cerebral artery in the same patient (b), suggesting embolism. Right frontal lobe artefact present.

syndrome (6,34), although several others have also been described (6,34). These clinical syndromes, although closely associated with radiological evidence of deep brain infarcts, are not perfectly predictive of type of lesion or location (16,35,36) and can, therefore, limit attempts to identify underlying mechanisms and guide appropriate secondary prevention. There is evidence from prior studies that approximately 20% of patients presenting with a clinical 'lacunar syndrome' will have a nonsmall vessel mechanism underlying the stroke (35,36). Conversely, in one study, around 7% of those with radiologically confirmed deep brain infarcts were shown to have presented with atypical syndromes, such as dysarthria facial paresis, isolated dysarthria, and isolated hemiataxia (37). Moreover, between 4% and 10% of patients with clinical 'lacunar' syndromes' may actually have a deep ICH (38,39). Thus, reliance on clinical syndromes alone may introduce bias in clinical studies (13) and potentially lead to incorrect medical management.

Compared with larger and cortical strokes, acute deep brain infarcts are less severe and have better short-term physical outcomes (6,13). However, they are associated with an important risk of recurrence, and affected patients have an increased risk of developing cognitive impairment, depression, and functional impairment over time (11,16,40). A recent longitudinal study has shown that progression of deep brain infarcts has been associated with subtle decline in executive, speed, and motor control functions (41). Pharmacological management of symptomatic deep brain infarcts lies in the use of standard therapies including antiplatelet agents (22), blood pressure reduction (42), and diabetes control (43). A sub-study of Stroke Prevention by Aggressive Reduction in Cholesterol Levels (44) showed that cerebrovascular reactivity (a marker of endothelial function) in symptomatic patients with 'lacunar' stroke was unaffected by atorvastatin therapy, raising some doubt regarding the usefulness of lipid reduction. Although it is unclear whether carotid endarterectomy is effective in the secondary prevention of deep brain infarcts, previous major trials have not differentiated between types of infarcts (7,32,33,45). A large, randomized controlled trial is currently underway testing the efficacy of antiplatelet therapy and blood pressure lowering in reducing the recurrence of small sub-cortical stroke events and the incidence of cognitive dysfunction (Secondary Prevention of Small Sub-cortical Strokes) (46). Sub-clinical deep brain infarcts also have important long-term cumulative effects, contributing to a greater risk of future dementia (11), clinical stroke, falls, and mortality (47). The use of the antiplatelet agents dilazep hydrochloride and cilostazole was associated with fewer incidents of sub-clinical infarcts in two small trials of Japanese patients with type 2 diabetes mellitus (48,49), suggesting that these may need further exploration. Clearly, there is a need for larger-scale trials of vascular therapies to prevent sub-clinical deep brain infarcts.

Age-related WMLs

WMLs are distinct from deep brain infarcts, although they frequently coexist (1). In the general population, they occur in approximately 80% of Caucasians aged over 60 years (50). They are also present in 2/3 of patients with dementia and 1/3 of those diagnosed with Alzheimer's disease (51). They are seen as hyperintensities in white matter on T2 MRI sequences, and may occur around the ventricles (periventricular WML) or more peripherally (sometimes referred to as 'deep' sub-cortical WML). They occur more frequently in the frontal white matter (52), in women (50), and tend to progress among those with a large lesion load (53) which occurs in approximately a third of the general older population (53). The results of pathological and epidemiological studies suggest that an ischaemic basis due to SVD may be responsible for at least this subset of people with severe WML (53). Age and hypertension remain the only consistently identified risk factors for WML in prospective studies (54), whereas the evidence supporting the effect of other vascular risk factors is weak. There is an association between increasing frequency of migraines and the burden of WML, but only in females (55). Although there is high heritability for WML, no convincing evidence has yet been obtained to support the associations of candidate genetic

polymorphisms with these lesions (56), and future large genome-wide association studies may shed more light on this matter.

Radiology and pathology of WML (Fig. 1b)

Before the advent of MRI, WMLs were observed as diffuse areas of white matter attenuation on CT scans, and termed 'leukoaraiosis' (57). However, FLAIR is now the preferred imaging modality to detect such lesions in clinical settings. It can miss small lesions in the thalamus and posterior fossa compared with high resolution T2-weighted sequences. A number of different visual rating scales have been developed in an attempt to allow further study of clinical correlates (58,59), but there are significant limitations with such scales, particularly with regard to intra-observer variability (60). Several methods of semi-automated or automated segmentation of WMLs currently exist to compute volume of WMLs, which may be a more sensitive measure for research purposes (61). Diffusion tension imaging allows the measurement of fractional anisotropy and diffusivity of white matter tracts, to further allow measurement of the integrity of axonal membranes in WMLs and in normal-appearing white matter (62). Magnetization transfer ratio may provide another surrogate marker of axonal injury while studying WML (58,63). Although there is increasing interest in the use of high-field MRI (7 Tesla and upwards) to study SVD (64), its ability to detect WML may be approximately equivalent to the ability of 1·5 Tesla MRI (65).

Pathological examination of WML reveals a spectrum of changes ranging from myelin pallor, enlarged perivascular spaces, tissue infarction, gliosis, and axonal loss (66). As these lesions become more confluent, there is complete loss of the entire nerve fibre (66). Perivascular infiltration of foam cells, and proinflammatory mediators including Apolipoprotein E (ApoE), α2-macroglobulin, and immunoglobulin G have also been described (18,67), as have reactive astrocytosis and microglial activation (66). Other evidence also points to a loss of integrity of small vessel endothelium or the blood-brain barrier (68). Venous collagenosis has also been observed in areas of WML, although its pathogenetic significance remains uncertain (69). More recently, molecular biological studies have suggested that alteration of RNA transcription may occur in multiple genes that are involved in immune regulation, cell cycle, apoptosis, and proteolysis among others, highlighting the complexity of the phenotype (70).

Effects of WMLs on function and treatment

There is now little doubt that those with a large lesion load of WMLs clearly suffer physical, cognitive, and psychological effects. When present in large amounts, they are associated with cognitive impairment, gait disorders, falls, mood disturbance, and bladder instability (40,50). The effects on cognition manifest usually as subtle impairment of executive function, set shifting, and processing speed (41,59). Cross-sectional associations between WMLs and depression have been demonstrated (47,71). Greater WMLs, particularly in periventricular frontal regions (52), are associated with poor gait (72) and a high risk of falls (72). Importantly, in a meta-analysis of 46 longitudinal studies, WMLs were associated with a greater risk of future stroke, dementia, and death, highlighting their potential significance as surrogate markers of major disease burden in the elderly (73).

To date, there is no convincing evidence for the efficacy of pharmacological or lifestyle therapies for slowing the progression of WMLs. In small *post hoc* analyses of data from the Perindopril Protection Against Recurrent Stroke Study (PROGRESS) study (74) and the Regression of Cerebral Artery Stenosis (ROCAS) study (75), the use of an angiotensin-converting enzyme inhibitor or a statin was found to be associated with slower progression of WMLs on MRI, raising the possibility that their use may be beneficial. However, there need to be larger definitive trials in this field.

Deep ICH

Up to 50% of all ICHs are due to deep brain haemorrhage (76), the rest being lobar. They are a common but often less emphasized manifestation of SVD, but are associated with a significant mortality and morbidity rate, accounting for up to 15% of all deaths from stroke (77), with a higher fatality rate than ischaemic stroke (78). Deep ICH is strongly associated with the presence of other SVD phenotypes such as deep brain infarcts (79) and WMLs (80). Hypertension continues to be a strong risk factor in the development of deep ICH along with increasing age (76,81). Approximately 49% of ICH are deep cerebral haemorrhages (76). Putaminal and thalamic haemorrhage accounts for 19–53% and 4–26% of these, respectively (34). Caudate nucleus haemorrhage occurs in approximately 1–5% of ICH, while haemorrhages in the pons or cerebellum account for between 2% and 10% each (34).

Radiology and pathology of deep ICH (Fig. 1c)

Noncontrast CT remains the preferred mode of diagnosis due to greater availability and lesser contraindications. MRI may be helpful in the acute phase in differentiating an infarct with haemorrhagic transformation from that of a primary haemorrhage, identifying small haemorrhages, and detecting underlying vascular abnormalities or lesions (78).

On neuropathological study, there is a common finding of fibrinoid necrosis, lipohyalinosis, and medial degeneration of the vessel wall affecting deep penetrating arteries or arterioles (19).Similar findings occur in relation to small deep infarcts, except for the prominence of fibrinoid necrosis, which may be driven by a local inflammatory process among those with hypertension (82). It is as yet unclear why there would be a predisposition towards haemorrhage rather than infarction, given the similarity in vessel pathology. Previously, it has been postulated that such haemorrhages may occur due to the

rupture of microaneurysm (Charcot–Bouchard microaneurysms) in the middle or distal portions at or near bifurcations of the penetrating artery (81,82). However, there is still uncertainty and controversy whether such microaneurysms are common, and whether or not they bear any association with ICH (19). Deep brain infarcts have been found to underlie deep ICHs in approximately 10% of cases, identifying a further potential contributor to the development of ICH (83).

Clinical effects and treatment of deep ICH

The clinical effects of deep cerebral haemorrhages are dependent on the volume of haemorrhage, location, and presence of mass effect. The acute management of deep ICH as recommended by American Heart Association/American Stroke Association guidelines can be viewed elsewhere (78). Hypertension is the main target of the modifiable risk factors for secondary prevention (78).

CMBs

The widespread use of MRI has resulted in the increased recognition of CMBs (84). They are usually 2–5-mm hypointense lesions on gradient echo (GRE-T2*) or susceptibility-weighted images (SWIs) on MRI (79). In a systematic review, their prevalence was around 5% in healthy adults, 34% in people with ischaemic stroke, and 60% in people with non-traumatic ICH using GRE-T2* (85). They are more prevalent in the setting of recurrent ischaemic strokes (44%) than first-ever ischaemic strokes (23%), and in those with recurrent ICH (83%) than in first-ever ICH (52%) (85). There may be two sub-types of CMBs as suggested by findings from the Rotterdam study (86). Those in deep sub-cortical regions (deep CMBs) tend to be associated with cardiovascular risk factors and a greater load of deep brain infarcts and WMLs (86). Those in lobar regions (lobar CMBs) have been reported to be associated with the presence of ApoEϵ4 genotype (86).

Radiology and pathology of CMBs (Fig. 1d)

Lesions seen on GRE MRI correlate with haemosiderin deposition from both old and more recent haemorrhage (84). More recent advances in imaging (SWI) sequences have greater sensitivity in detecting CMBs than GRE sequences (84), with the latter only identifying 33% of SWI lesions. In a post-mortem study of Alzheimer's disease patients, SWI hypointensities corresponded to acute microhaemorrhage, haemosiderin residua of old haemorrhages, and small lacunes ringed by haemosiderin (87). In lesions where the bleeding vessel could be identified, beta-amyloid was detected in the vessel wall, suggesting that amyloid angiopathy may play a role in the pathogenesis of CMBs and partly explain the overlap between SVD and Alzheimer's disease (88). However, CMBs may occur independent of amyloid deposition in older people without a history of stroke or Alzheimer's disease (88). The strong association of CMBs with confluent WMLs also suggests that they may have shared mechanisms (84).

Clinical effects of CMBs

The clinical significance of microbleeds is a topic of current investigation, and there is reasonable strong evidence to support an association with symptomatic ICH (89). There is some evidence that the burden of CMBs is associated with cognitive impairment (90) particularly in the presence of previous stroke suggesting a cumulative effect. Studies in CADASIL have failed to demonstrate cognitive impairment associated with CMBs, but linked the number of CMBs with more functional dependence (91). At the present time, it is unclear whether CMB is a target for therapy, or is simply a surrogate marker for SVD.

Enlarged perivascular spaces

Enlarged perivascular or Virchow–Robin spaces deserve mention when discussing SVD, with increasing interest in whether they may be involved in the pathogenesis of WMLs and deep brain infarcts. They are common findings on brain MRI in older people, and may be a radiological mimic of deep brain infarcts (13). These cerebrospinal fluid-filled cavities surround small penetrating cerebral arterioles and are visible as high signal areas on T2-weighted MRI, and low signal on T1 and FLAIR sequences, usually <2 mm in diameter, although they may be larger on occasion (Fig. 1e) (92). Increasing age is associated with an increased prevalence of enlarged perivascular spaces (93). Although present in most adults, they are also more commonly seen in those diagnosed with dementia (94), and in association with greater volume of WMLs and deep brain infarcts among stroke patients (92). Their association with deep brain infarcts appears stronger than with cortical infarcts, suggesting a possible involvement in SVD (92). Mechanisms suggested in the formation of such spaces include altered permeability of the vessel wall due to inflammation or blood-brain barrier breakdown, amyloid accumulation along the vessel wall, and cerebral atrophy (92). However, their potential mechanistic involvement in SVD is still speculative.

Genetic forms of SVD

CADASIL

CADASIL is a well-described hereditary form of predominately SVD causing stroke and WMLs with minimal involvement of the large cerebral vessels (95). It is the most common of several monogenic conditions that predispose to mainly ischaemic and less commonly haemorrhagic stroke of small vessel origin (96). CADASIL is autosomal dominant with nearly 100% pen-

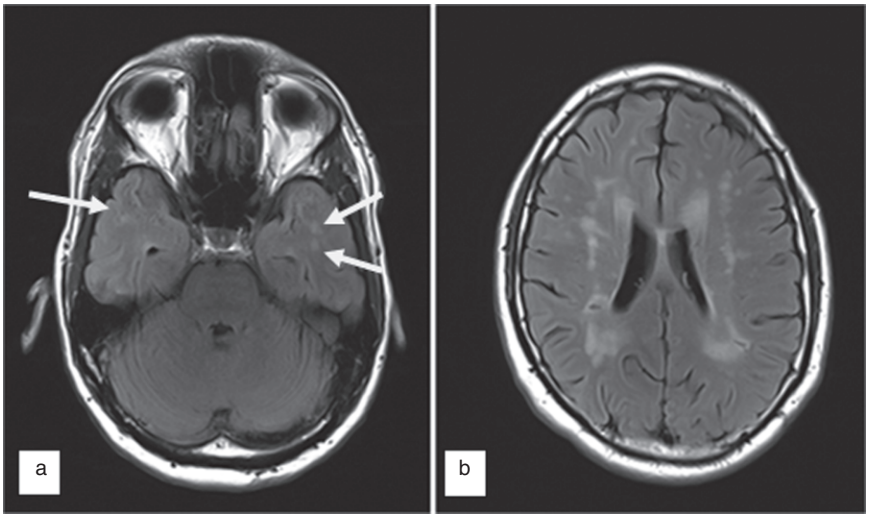

Fig. 4 T2-fluid attenuated inversion recovery magnetic resonance imaging showing hyperintensities in cerebral autosomal dominant arteriopathy with sub-cortical infarcts and leukoencephalopathy. (a) Temporal lobe hyperintensities. (b) Diffuse white matter lesions.

etrance, and associated with over 190 different mutations in the *NOTCH3* gene on chromosome 19, which codes for a cell-signalling receptor in vascular smooth muscle cells (97). It is estimated to occur in approximately 1·5–5·0 per 100 000 people and is characterized by the early onset of TIA or stroke, with patients usually developing first stroke symptoms in the fourth decade of life (97). This may be preceded by a history of migraine with aura in approximately 30–50% of patients (98). Cognitive impairment is common with 70% having a formal diagnosis of dementia by the sixth decade (98). Other clinical features are that of severe mood disturbances such as depression and apathy (30%) and seizures (10%) (99). A study of a pair of monozygotic twins with a NOTCH3 mutation demonstrated that nongenetic factors may play an important role in clinical expression, with a 14-year earlier onset of stroke seen in the twin who smoked and exercised less, and was not on a statin (100). The effect of nongenetic factors on the clinical expression of CADASIL may provide clues about their potential role in causing sporadic SVD.

Radiology, pathology, and disease mechanisms in CADASIL (Fig. 4)

MRI abnormalities that have been commonly reported include WMLs and small deep brain infarcts ('silent' or symptomatic) and are diffusely found in CADASIL patients as young as 20 years old and are present in most patients over the age of 35 years (95). WMLs in CADASIL tend to have a unique predilection for the anterior temporal pole and external capsule (101), whereas this is unusual in ageing. Confluent hyperintensities on T1-weighted imaging in both thalami have also been reported in around 12% of a case series, possibly reflecting demyelination or glial loss (102). Cerebral atrophy has been shown to progress three times more rapidly in CADASIL compared with normal ageing (103). Cerebral microhaemorrhages as detected by T2*-weighted MRI (GRE) are observed in approximately 35% of CADASIL patients (104). Larger ICHs (<3 cm) have also been reported to occur commonly in the presence of microhaemorrhages and deep brain infarcts (97).

CADASIL is a severe small vessel arteriopathy on immunohistological staining and light microscopy, characterized by the finding of granular osmiophilic material on electron microscopy affecting mainly the vascular smooth muscle cells with relative sparing of the endothelium (105–107). These pathological findings are distinct from those found in age-related WMLs or sporadic deep brain infarcts where endothelial involvement may be prominent, suggesting different underlying molecular mechanisms. The *NOTCH3* gene encodes for a single-pass transmembrane receptor protein that mediates signal transduction. A mutation in NOTCH3 results in the uneven production of cysteine residues in this protein, leading to abnormal signal transduction and impaired arterial smooth muscle differentiation and maturation (106,107). Vessels supplying deep white and grey matter are the most affected in CADASIL, but clot occlusion tends to be rare on autopsy (108). There is evidence for systemic vascular involvement with similar pathological changes being observed in the retinal arteries, spleen, liver, kidney, carotid arteries, and aortic wall, as well as the skin (105), which may be biopsied for diagnosis and can be an alternative to genetic testing. Electron microscopy of skin biospy is highly specific but has a sensitivity of only 57%. Immunohistochemistry increases the sensitivitity of the test to 90% with a specificity of approximately 98%. Genetic testing is limited by availability but remains the gold standard (109). Although most other organs do not demonstrate clinical manifestations of the disease, patchy visual field defects with narrowing and sheathing of retinal arteries were seen in one study of CADASIL (110).

Effects of CADASIL on brain function and its treatment

Cognitive impairment is the most common effect of CADASIL. It is common for a variety of different cognitive domains to be affected in the early clinical stages of the disease. Executive dysfunction is observed well before the development of dementia (98,99), which is often insidious and associated with other evidence of sub-cortical impairment in attention and retrieval memory (111). With time, other domains become affected, involving both cortical and sub-cortical functions with impairment of instrumental functions, such as language and visuospatial abilities (111). Recently, it was also found that approximately 18% of patients in a clinical series had a hippocampal pattern of verbal memory impairment, raising interesting speculation about the neural substrate involved (112). The most likely mechanism of cognitive dysfunction is the disconnection of neuronal networks with increasing burden of ischaemic lesions. It has been shown that these functional impairments are also associated with coexistent cerebral atrophy (103). However, in a seven-year follow-up study of mildly affected patients, progression in infarct load, but not WML or atrophy, was associated with global and domain-based decline (113). As the disease progresses, it is not unusual for patients to develop gait disturbances (90%), urinary incontinence (80%), and pseudobulbar palsy (50%) (96). A study following 411 confirmed cases of CADASIL found the average age at death to be 64·6 years for males and 70·7 years for females (96), with the vast majority of those who died being completely dependent on others for their personal care. Pharmacological treatments for CADASIL have been largely unsuccessful. Aspirin is often recommended for stroke prevention, but its use is not supported by evidence (98). A randomized, double-blind, placebo-controlled study of the cholinesterase inhibitor donepezil did not show a statistically significant change in the primary end-point of the vascular Alzheimer's Disease Assessment Scale cognitive sub-scale, although significant reduction in the rate of decline of a number of sub-group measures was seen (114). The clinical relevance of these findings is unclear, and the mainstay of treatment remains largely supportive in nature.

Conclusion

SVD is extremely common and has deleterious effects on brain function and overall health. A greater understanding of pathophysiology, better targeting of risk factors, and potential treatments are necessary to minimize the burden of the disease. The advent of newer neuroimaging techniques such as high-field MRI is an exciting step towards this. It is crucial to develop accurate terminology to describe disease phenotypes in a way that prevents bias and allows harmonization of research in the field. Longitudinal studies may provide a greater understanding of the mechanisms and risk factors underlying SVD. Hopefully, these steps will set the scene for randomized controlled trials in the field.

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Appendix B

Cognitive measures

The following tests were used to measure cognitive function:

Executive and attentional function

The Controlled Oral Word Association Test (COWAT – FAS and category fluency)(Lezak, 2004), the Victoria Stroop test (Spreen et al., 1998) and the digit span subtest of the Wechsler Adult Intelligence Scale – Third edition (WAIS-III)(Weschler, 1997).

The COWAT consists of two sections that test verbal fluency. In the first section, participants are asked to list as many words as possible beginning with the letters F in one minute. This task is then repeated with words beginning with the A, then S, allowing one minute for each letter. In the second section, participants are asked to list as name as many different animals as they can without repetition in one minute. Higher scores on COWAT tests indicate better function. The test-retest reliability for COWAT tests are high (intraclass correlation coefficient, ICC=0.70) and verbal fluency measures, such as COWAT are a sensitive measure of frontal lobe dysfunction (Spreen et al., 1998; Lezak, 2004).

The Victoria Stroop test is a timed task that measures the ability of participants to shift perception in response to changing demands and suppress an habitual response (in this case reading) in favour of another more unusual one (colour naming) (Spreen et al., 1998). The test involves three different white cards each containing four rows of six items that access selective attention and cognitive flexibility. The first card contains coloured dots, the second, coloured words and the third, colour names in differently coloured ink (e.g. the word blue written in red ink). Each time, the participant is asked to describe the colour of the ink. The

time for completion of the three separate tasks is recorded. Slow performance on the Stroop task is associated with frontal lobe lesions (Lezak, 2004).

The digit span task consists of two components. A list of numbers is read out loud to the participant who is then asked to repeat this list in the same order. The length of the list begins with two numbers and increase in length (and therefore difficulty) until it is nine numbers long. High scores indicate better function. Performance may be impaired by frontal lobe lesions. (Lezak, 2004) The digit span test has moderate-to-high reliability (ICC=0.66-0.89) (Spreen et al., 1998).

Processing speed

Processing speed was measured using the Symbol Search and the Digit Symbol Coding subtests of the WAIS-III (Weschler, 1997). Both of these tests were designed to assess processing speed and are relatively unaffected by intellect, memory or learning. (Spreen et al., 1998; Lezak, 2004) In the digit symbol coding test, participants are given nine symbols with a number from one to nine uniquely assigned to each symbol. Participants are then given a random list of numbers and ask to identify which symbol is correctly associated with each number. The number of correct responses in two minutes is recorded. In the symbol search, the participant is shown two symbols and asked to identify whether either of them were in a group of five symbols they are provided with. The number of correct responses in a two minute period is recorded. Both the symbol search and digit symbol coding tests have high test-retest reliability (ICC>0.88). Higher scores on these tests indicate better function.

Visuospatial function

Visuospatial function was measured using the Rey Complex figure (Lezak, 2004). The participant is first asked to copy the Rey Complex figure. This is a complex figure formed

from lines, circles and dots. The figure and the copy are removed once the participant has completed the task to the best of their ability. For scoring, the figure is broken into 18 separate units and marks awarded for accuracy of reproduction of each unit and the relative positioning within the whole design (Lezak, 2004). Higher scores reflect better visuospatial ability and incorporates planning, organizational and problem-solving strategies and therefore felt to be an indirect measure of executive function (Lezak, 2004).

Memory

Memory was assessed using the Hopkins Verbal Learning test – Revised (HVLTR) and delayed recall of the Rey Complex Figure test. The HVLTR is a brief test of immediate, delayed and recognition memory and consists of three separate tasks (Lezak, 2004). A list of 12 words is read to the participant three times. After each list reading, the participant is tested on their immediate recall of the list. Twenty minutes after the third recall attempt, a further, delayed recall is attempted. To test recognition skills, participants are asked to correctly identify the 12 previously repeated words from a list of 24 words that include 12 "distractor" words. A high score in these three components reflects better function. The HVLTR has acceptable test-retest reliability across all components (ICC=0.41-0.74) (Lezak, 2004).

Twenty-five minutes after the initial copy of the Rey Complex Figure, and without prior warning, the participant is asked to reproduce the Rey Complex figure from memory. Delayed recall of the figure is considered to be a sensitive indicator of a visuospatial memory as well as being dependent upon the same executive function skills as the immediate copy task (Spreen et al., 1998; Lezak, 2004) Test-retest reliability of both immediate and the delayed reproduction are moderate (ICC=0.47-0.59) (Spreen et al., 1998).

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