RETINAL AND CEREBRAL NEUROIMAGING BIOMARKERS FOR COGNITIVE IMPAIRMENT

SAIMA HILAL

(MBBS (Dow), MPH (NUS))

A THESIS SUBMITTED

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHARMACOLOGY

NATIONAL UNIVERSITY OF SINGAPORE

2015

Declaration

I hereby declare that this thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

_____

SAIMA HILAL

26th November 2015

ACKNOWLEGMENTS

This thesis would not have been possible without contribution and support from so many people.

My family:

None of this could have been possible without my family. From genes, environment, family teaching and everything else which have contributed together to make this a success. My wonderful parents, for their unwavering love and support, for inspiring me through their own academic career and for never giving up on me. My siblings who have stood by me and encouraged me throughout this journey so that I remain grounded. And to all the munchkins for making my weekends enjoyable even from the long distance.

Daiman boo, apa yang boleh saya katakan, semua ini tidak mungkin dapat dicapai tanpa anda di sisi saya. Anda memberikan saya sayap dan membuat segala-galanya accomplishable untuk saya. Cinta tanpa syarat anda telah membuat saya lebih kuat dan bahagia. Anda tahu saya lebih baik daripada yang saya tahu diri saya. Sayang, anda hanya luar biasa!

Alesha, my baby, you are my one and only nyawa. You have made my life colorful and gave me everlasting smile. The sun has never risen prettier than it does now ever since you are born and it has never seemed to set since then. You have been very patient throughout my career. I know you will always make me proud.

Ma dan Maklin , terima kasih kerana menjadi begitu baik hati , pemahaman dan kasih sayang dalam undang-undang. Anda telah menyokong saya secara rohani dan membimbing saya dalam perjalanan ini panjang pengajian dan kerjaya. Anda telah memberikan saya begitu banyak yang saya pernah meminta.

Supervisors:

Dr Christopher Chen, main supervisor – thank you for your guidance and giving me career planning tips throughout my MPH and PhD days. Your strong fervor for research has always pushed me beyond borders. It has been a very productive five years. Your patience, comments

and defending me on several occasions is very much appreciated. I could not have reached this far without you.

Dr M. Kamran Ikram, previous main and now a co-supervisor - I am still amazed at how we started off as colleagues in 2010 and then you became my mentor for both MPH and PhD. You have unflinchingly allowed me to work around the obstacles and helped me smooth over tons of issues be it presentations or scientific writing. I think you have seen all possible sides of me and yet you are still there to listen and give advice. You have proved yourself to be more than a mentor. You are just awesome!

Dr Wong Tien Yin, co-supervisor - thank you for agreeing to be my co-supervisor even though I was not directly involved in your projects. Your suggestions in the papers and wide expertise in research has made this entire thesis possible.

Senior colleagues:

Dr N V Ramani, a very special thanks to you for bringing me to a place where I am today. A chance email to you changed my entire life! Thank you for the generosity and warmth you have always given during brief meetings. I am truly grateful for all your help, suggestions, comments and most importantly a fatherly attitude throughout my working life. *Chuen Seng,* ah the number cruncher, how can I forget. You are a very good mentor and a friend. Thank you for all the advice and time you spend in teaching me stats over coffees. Also not to forget, for responding to my endless emails. I am sure we will continue to work together in future.

Junior colleagues:

MACC staff (ex and present) - the crazy, the sane, the wise, the dearest… it is just difficult to describe my feelings for the combination of wonderful people I worked with these five years. Administrators and managers (*Salmah, Connie, Yudi*), fellows (*Joseree, Serrie*), co-ordinators (*William, Wan Ting, Hui Shan, Lisa, Casuarine, Pei San, Napalie, Farhanah*), psychologists and raters (*Summer, Cath, Amin, Qun Lin, Eddie*), all this would not have been possible without you. Thank you very much for the multitude of wonderful memories.

SERI Colleagues:

Carol Chueng - thank you so much for being so generous and kind in making me part of your sailor team. Your support and guidance has been a great help for papers and in this thesis. *Yi Ting*, you are just fabulous. Thank you so much for being so cheerful and optimistic. We both have grown together in developing research skills over the last 5 years. It was a pleasure working with you.

External collaborators:

Utrecht: Prof Biessels – thank you very much for hosting me last year in Utrecht. It felt great to be part of your research group. It is always a pleasure to meet and discuss projects with you on microinfarcts. Thank you for your excellent supervision and I am really looking forward to our strong collaboration in the coming years. I guess I shall be seeing you next year so that we can continue to work on the interesting topic of lesion symptom mapping. *Susanne*, it was just great to work with you both in Singapore and in Utrecht. You have set a very good example for taking care of the visitor. Hope to catch up with you soon next year. *Emiel*, it was fun to have you here for 3 months. I very much enjoyed getting to know you. Thanks for being a patient and committed student and your assistance in grading microinfarcts.

Rotterdam: Henri – thanks a million for helping out with all the segmentations. You have always expedited all the processing for me without any complaints. Thank you so much for your patience, hard work and commitment for making this degree a success.

Participants of studies:

Finally my sincere thanks to all the participants and families of the Singapore Epidemiology of Eye Disease studies, Epidemiology of Dementia In Singapore study and case control study (fondly called as Harmy) for their important and valuable contribution without which this thesis would not have been possible.

LIST OF PUBLICATIONS

This thesis is based on the following original articles which constitutes individual chapters;

1. Brain markers of small vessel disease

Chapter 4:

Hilal S, Saini M, Tan CS, Catindig JA, Koay WI, Niessen WJ, Vrooman HA, Wong TY, Chen C, Ikram MK, Venketasubramanian N. Cerebral microbleeds and cognition: the epidemiology of dementia in Singapore study. Alzheimer Dis Assoc Disord. 2014; 28(2):106-12.

Chapter 5:

van Veluw SJ, **Hilal S**, Kuijf HJ, Ikram MK, Xin X, Yeow TB, Venketasubramanian N, Biessels GJ, Chen C. Cortical microinfarcts on 3T MRI: Clinical correlates in memory-clinic patients. Alzheimers Dement. 2015; 11(12):1500-9.

Chapter 6:

Hilal S, Sikking E, Chan QL, van Veluw SJ, Wong TY, Venketasubramanian N, Biessels GJ, Chen C, Ikram MK. Cortical cerebral microinfarcts on 3 Tesla Magnetic Resonance Imaging - a marker of cerebrovascular diseases. (In preparation)

2. Brain marker of large vessel disease

Chapter 7:

Hilal S, Saini M, Tan CS, Catindig JA, Dong YH, Holandez RL, Niessen WJ, Vrooman HA, Ting E, Wong TY, Chen C, Venketasubramanian N, Ikram MK. Intracranial stenosis, cerebrovascular diseases, and cognitive impairment in Chinese. Alzheimer Dis Assoc Disord. 2015; 29(1):12-7.

Chapter 8:

Hilal S, Xin X, Venketasubramanian N, Ikram MK, Chen C. Intracranial stenosis in cognitive impairment and dementia. (In preparation)

3. Brain markers of involutional changes

Chapter 9:

Hilal S, Xin X, Ang SL, Tan CS, Venketasubramanian N, Niessen WJ, Vrooman H, Wong TY, Chen C, Ikram MK. Risk Factors and Consequences of Cortical Thickness in an Asian Population. Medicine. 2015; 94(23):e852.

Chapter 10:

Hilal S, Amin SM, Venketasubramanian N, Niessen WJ, Vrooman H, Wong TY, Chen C, Ikram MK. Subcortical Atrophy in Cognitive Impairment and Dementia. J Alzheimers Dis. 2015; 48:813-23.

4. Retinal markers of cerebrovascular diseases and involutional changes

Chapter 11:

Hilal S, Ong YT, Cheung CY, Tan CS, Venketasubramanian N, Niessen WJ, Vrooman H, Anuar AR, Chew M, Chen C, Wong TY, Ikram MK. Microvascular network alterations in retina of subjects with cerebral small vessel disease. Neurosci Lett. 2014; 577:95-100.

Chapter 12:

Ong YT, **Hilal S**, Cheung CY, Xu X, Chen C, Venketasubramanian N, Wong TY, Ikram MK. Retinal vascular fractals and cognitive impairment. Dement Geriatr Cogn Dis Extra. 2014; 4(2):305-13.

Chapter 13:

Ong YT*, **Hilal S***, Cheung CY, Venketasubramanian N, Niessen WJ, Vrooman H, Anuar AR, Chew M, Chen C, Wong TY, Ikram MK. Retinal neurodegeneration on optical coherence tomography and cerebral atrophy. Neurosci Lett. 2015; 584:12-6.

* Joint first authors

TABLE OF CONTENTS

SUMMARY

Besides neurodegeneration, cerebrovascular disease (CeVD) is also considered a major cause and contributor to cognitive decline and dementia. Damage to the cerebral small and large blood vessels has been implicated in CeVD pathology. Magnetic Resonance Imaging (MRI) correlates of cerebral small vessel diseases include lacunes and white matter hyperintensities which have been widely linked to stroke, cognitive decline, dementia and mortality. However, these lesions do not fully capture the burden of CeVD in the brain. In this context, cerebral microbleeds and microinfarcts have emerged as the new imaging markers of CeVD pathology. Moreover, large vessel disease such as intracranial stenosis has increasingly gained importance in Asian population due to higher prevalence of vascular risk factors. Advancement in MRI quantitative segmentation of the brain parenchyma (cortex and subcortical regions) has also revealed subtle neuronal damage in cognitive impairment and dementia. However, the data on the determinants and the consequences of these CeVD markers and involutional changes are lacking.

Despite advances in neuroimaging techniques, damage to the cerebral small vessels is difficult to visualize in-vivo. Retina as an extension of the brain, can serve as a complimentary technique to study subtle and early microvascular and neuronal damage involved in CeVD and cognitive impairment. Retinal microvascular (vessel calibers) and neuronal changes (thinning of retinal nerve fiber layer) have been linked to cognitive impairment, dementia and poor cognitive performance on the neuropsychological testing. However, association of other retinal quantitative parameters such as fractal dimension, tortuosity and ganglion cell inner plexiform layer with CeVD and involutional changes remains to be explored.

Hence, the major objective of this proposal is to examine cerebral and retinal imaging biomarkers for cognitive impairment and dementia. Based on this objective advanced

cerebral and retinal imaging techniques are applied in two observational studies to demonstrate the following;

1) Role of cerebral small vessel diseases (cerebral microbleeds and microinfarcts) in cognitive impairment and dementia,

2) Role of large vessel disease (intracranial stenosis) in cognitive impairment and dementia,

3) Role of involutional changes (cortical thinning and subcortical structure volumes) in cognitive impairment and dementia,

4) Association of retinal microvascular and neuronal layers changes with CeVD, involutional changes and cognitive impairment.

The major findings from this thesis are:

1) Cerebral small vessel diseases (cerebral microbleeds and microinfarcts) are associated with cognitive impairment and poor performance on neuropsychological assessment.

2) Large vessel disease (intracranial stenosis) is highly prevalent in Chinese and is associated with cognitive impairment and dementia in the presence of ischemia.

3) Cortical thinning and decreasing subcortical structure volumes are associated with reduced performance in global and domain specific cognitive scores.

4) Retinal microvascular changes (fractals and tortuosity) are associated with cerebrovascular diseases on MRI scans and preclinical cognitive impairment.

5) Retinal neuronal damage reflected by thinning of ganglion cell inner plexiform layer is associated with cerebral atrophy on MRI scans.

In conclusion, both cerebral and retinal parameters may serve as:

- Surrogate markers to study the exact role of microvascular pathology and involutional changes

- To develop new treatment and prevention strategies of cognitive decline and dementia.

LIST OF TABLES

LIST OF TABLES

LIST OF TABLES

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES

CHAPTER 1:

INTRODUCTION, AIMS AND THESIS OUTLINE

1. INTRODUCTION

1.1 Aging, Dementia and Cerebrovascular Diseases

The world population is aging rapidly. The less economically developed countries gain about 10 hours per day in life expectancy[1] while the global maximum life expectancy continues to increase linearly as it has over the last 160 years due to better health care, sanitation, education and economic wellbeing.[2] People aged 60 years and over make up 12.3% of the global population which is expected to increase up to 22% by 2050. The World Health Organization (WHO) has projected that by 2025, about three quarters of world population aged 60 years and above will be living in developed countries.[3] As a result of the rapid demographic aging, the burden from common age related diseases such as dementia is therefore expected to rise dramatically.[4] This will not only affect the quality of life of patients and their care givers but will also increase the health care costs. World Alzheimer Report has pointed out that the annual societal and economic cost of dementia has increased by 35% from its previous estimate of 604 billion USD.[5] Asia and Europe are the two main regions where a number of countries will face an increase in aging population in the near future.[6] As nearly 60% of the total world population of 7 billion is living in Asia, the burden of dementia will have major implications on the Asian continent compared to Europe and United States.[7] Specifically, it is expected that not only will there be a rise in the proportion of persons aged ≥ 60 years among the total Asian population from 10% in 2010 to 24% in 2050, but also the absolute number of elderly will dramatically increase from 414 million to 1.2 billion.[7, 8]

The most common type of dementia is Alzheimer's disease (AD) followed by vascular dementia and other rare causes such as Fronto-Temporal Dementia and Lewy Body Dementia.[9] There is increasing evidence that before a clinical diagnosis of AD is made, early signs of the disease are already present. Mild cognitive impairment (MCI) or cognitive impairment no dementia (CIND) is considered as a

transitional stage between normal cognitive function and AD. Persons diagnosed with MCI or CIND are in general, at high risk of conversion to AD paralleled by increased decline in disability and mortality.[10, 11] However, the mechanism behind this increased risk remains unknown.

Besides neurodegeneration, cerebrovascular diseases (CeVD) such as strokes and white matter lesions are considered a major cause and contributor to cognitive decline and dementia in aging population.[12] Autopsy studies have shown that the vascular pathology commonly co-exist in subjects diagnosed with AD and may even trigger or potentiate the existing neurodegenerative process.[13] This is possibly because both CeVD and AD share common vascular risk factors such as hypertension, hyperlipidemia and diabetes.[14] Overt clinical symptoms of CeVD and dementia reflect irreversible brain damage thereby highlighting the importance of early markers of vascular pathology and involutional changes. These markers of microvascular damage and involutional changes may allow early identification of at-risk patients and hence could be a potential target for early intervention. Moreover, these biomarkers may also serve as surrogate markers of disease progression providing information on prognosis and treatment efficacy.

1.2 Magnetic Resonance Imaging Correlates of Cerebrovascular Diseases

Non-invasive neuroimaging techniques such as magnetic resonance imaging (MRI) play an important role in identifying both clinical and subclinical structural brain changes. Over the last two decades, MRI has been increasingly utilized in unraveling the role of cerebrovascular disease pathology involved in cognitive impairment and dementia. Manifestations of CeVD on MRI commonly include subcortical infarcts, lacunes, white matter hyperintensities (WMH), cerebral microbleeds, enlarged perivascular spaces and even brain atrophy. An extensive work on infarcts and WMH has demonstrated their link with cognitive dysfunction and mortality. Now with the

advancements in conventional MRI hard and soft wares, it is possible to visualize other important markers of CeVD which includes cerebral microbleeds and microinfarcts. Moreover, there is an increasing focus on large vessel diseases (extra and intracranial stenosis) in Asians due to higher prevalence of vascular risk factors. Besides these visible vascular diseases on MRI, it is also feasible to quantitatively assess underlying subtle changes involved in involutional changes which include cortical thickness and volumes of the brain parenchyma.

However, conventional MRI correlates of these CeVD do not fully capture the burden of vascular pathology in the brain as other small parenchymal lesions and vasculopathologic changes remain undetected in-vivo. As an expensive technique, MRI cannot feasibly be applied to large population based studies. Furthermore, there are several contraindications to MRI which includes pacemakers, metallic implants and claustrophobia which makes it less practicable in the elderly.

1.3 Retinal Imaging – A Complimentary Technique to Study Cerebral Microvascular and Involutional Changes

Retina shares several embryological, physiological and anatomical features with cerebral microcirculation while maintaining a close contact via optic nerve. In contrast to MRI, retinal imaging remains a time efficient and less expensive technique. Hence retinal imaging can be utilized as a complimentary technique to non-invasively assess subtle microvascular and involutional changes in the brain. These retinal changes may serve as biomarker of preclinical stages of the disease and might also predict the onset of the disease.

Systemic vascular diseases such as hypertension, hyperlipidemia and diabetes not only affects the cerebral microvessels but also the retinal vasculature which are visible on fundus photography as retinopathy signs- the end stage of retinal microvascular damage. These changes have been previously link to stroke, dementia

and other cardiovascular events. With the advent of the geometric computer based methods, it is now possible to quantify retinal vascular parameters which reflect early changes in the retinal vascular network. Compared to the qualitative retinopathy signs, changes in retinal microvessels can even be assessed in the absence of visible pathology and hence can provide insight into the role of microvascular pathology in the preclinical stages of the CeVD and cognitive impairment.

Apart from retinal microvessels, retinal axonal and ganglion cells are connected to the central nervous system through the optic nerve. Structural changes to the optic nerve can be non-invasively measured using high resolution techniques which can quantify retinal nerve fiber thickness. Neurodegeneration can directly affect the retinal axons and cell bodies or vice versa through transneuronal degeneration. Retinal neuronal loss can be found in dementia, stroke and other neurodegenerative diseases (Parkinson's disease and multiple sclerosis). So far, these studies have not been able to provide conclusive answers. Therefore, three dimensional architecture of the retina still remains to be analyzed.

2. OBJECTIVE AND SPECIFIC AIMS

This thesis intends to explore whether both brain and retina can serve as biomarkers for cerebrovascular and involutional changes involved in cognitive impairment and dementia.

Hence, the major objective of this thesis is to **examine the age-related structural changes in the brain and retina related to cognition using novel structural cerebral magnetic resonance (MR) and retinal imaging markers**. Based on the overall objective, the following specific aims will be addressed in this thesis;

Specific aim 1 – ***Brain markers of small vessel disease***

1a. To examine the association of cerebral microbleeds and cognitive impairment.

1b. To determine the clinical relevance of cerebral cortical microinfarcts on 3 Tesla in a memory clinic population.

1c. To study the determinants and consequences of cerebral cortical microinfarcts on 3 Tesla in a subsample of population based study.

Specific aim 2 – ***Brain marker of large vessel disease***

2a. To examine the association of Intracranial stenosis (ICS) with cognitive impairment and to show whether this association is mediated by MRI markers in a subsample of population based study.

2b. To examine the association of intracranial stenosis with cognitive impairment, dementia and their subtypes in a memory clinic population.

Specific aim 3 – ***Brain markers of involutional changes***

3a. To study the determinants and consequences of cortical thickness.

3b. To study the risk factors of subcortical structures on neuroimaging and their association with cognitive impairment and dementia.

Specific aim 4 – ***Retinal markers of cerebrovascular disease and involutional changes***

4a. To examine the link between quantitative retinal vascular parameters and MRI markers.

4b. To examine the association of quantitative retinal vascular parameters and preclinical cognitive impairment.

4c. To examine the association of retinal neuronal parameters with cerebral atrophy on MRI.

3. OUTLINE OF THE THESIS

The work described in this thesis is summarized in 14 chapters. The first 3 chapters of this thesis include scope of the study, aims and objectives, literature review and methodology. In chapters 4 to 13, the whole thesis is divided into three main parts; 1) Brain markers of cerebrovascular diseases, 2) Brain markers of involutional changes, and 3) Retinal markers of cerebrovascular diseases and involutional changes. The final chapter 14 covers the synthesis with future implications.

Chapter 1 describes the scope of the study, objective and specific aims which this thesis attempts to study.

Chapter 2 compiles the literature review related to established cerebrovascular disease markers of cognitive impairment and dementia and how the other MRI markers additionally add to the cerebrovascular disease burden and affect cognition. Moreover, I also describe how retinal imaging helps to study microvascular and involutional changes in the brain.

Chapter 3 describes the study methodology in detail which consists of study population, study design, risk factors, determinants and outcomes of interest examined in each study. Retinal imaging, neuroimaging and cognitive assessments common among different studies are described in detail. Study specific determinants and imaging (brain and retina) unique to each study are further described in each chapter.

Part I includes the next five chapters on the brain markers of cerebrovascular diseases

Chapter 4 examines the effects of cerebral microbleeds on cognition from a subsample of population based study- the Epidemiology of Dementia In Singapore study (EDIS).

Chapter 5 investigates the feasibility of detection of cerebral cortical microinfarcts (CMIs) on 3Tesla MRI and further determines the clinical relevance of this emerging new marker of CeVD in dementia. This is performed in a memory clinic setting.

Chapter 6 explores the determinants and consequences of CMIs. The association of CMIs with brief cognitive tests and cognitive domains are examined using data from the EDIS study.

Chapter 7 investigates the association of intracranial stenosis (ICS) with cognitive impairment and whether these associations are mediated by MRI markers. This is again performed in the EDIS cohort.

Chapter 8 further investigates the role of ICS in cognitive impairment and dementia. The association of ICS with vascular vs. non-vascular subtypes of cognitive impairment is also explored. This is a case control study recruiting cases from the memory clinic and controls from both community and memory clinic.

Part II includes two chapters on brain markers of involutional changes.

Chapter 9 aims to identify the risk factors of cortical thickness (a reflection of cerebral involutional changes) and its eventual effects on cognition. Correlations of cortical thickness with varying severity of cognitive impairment and cognitive performance are performed in the EDIS cohort.

Chapter 10 further explores the subcortical structure volumes in cognitive impairment and dementia. Major determinants and consequences of subcortical structures are reported from EDIS study whereas the findings on specific pattern of subcortical volume reduction in vascular vs. non vascular cognitive impairment is examined in memory clinic data.

Part III includes three chapters on retinal markers of cerebrovascular diseases and involutional changes

Chapter 11 investigates the association of the retinal vascular parameters with cerebral small vessel diseases. This is performed in the EDIS study where the subjects have both retinal and neuroimaging gradings.

Chapter 12 examines the association of retinal vascular parameters with preclinical stages of cognitive impairment. These associations are explored with both the clinical outcomes and neuropsychological assessments in participants from EDIS study.

Chapter 13 examines whether thinning of the retinal neuronal layers is associated with global and regional cerebral atrophy on MRI among participants of the EDIS study.

Chapter 14 includes a synthesis of the main findings of the chapters 4 – 13, with a discussion on the implications of these markers in cerebrovascular diseases and cognitive impairment. Finally limitations and future perspectives from this thesis are also discussed.

CHAPTER 1 – REFERENCES

1. World Health Organization. World Health Statistics 2014. WHO press. 2014; 1-180.

2. Oeppen J, Vaupel JW. Demography. Broken limits to life expectancy. Science. 2002; 296:1029-31.

3. Rizzi L, Rosset I, Roriz-Cruz M. Global epidemiology of dementia: Alzheimer's and vascular types. BioMed research international. 2014; 2014: 908915.

4. Larson EB, Langa KM. The rising tide of dementia worldwide. Lancet. 2008; 372: 430-2.

5. World Alzheimer Report. The Global Impact of Dementia - an analysis of prevalence, incidence, cost and trends. Alzheimer Disease International, London. 2015; 1-88.

6. Ferri CP, Prince M, Brayne C, et al. Global prevalence of dementia: a Delphi consensus study. Lancet. 2005;366:2112-7.

7. Catindig JA, Venketasubramanian N, Ikram MK, Chen C. Epidemiology of dementia in Asia: insights on prevalence, trends and novel risk factors. Journal of the neurological sciences. 2012;321:11-6.

8. Population Division of the Department of Economics and Social Affairs of the United Nations Secretariat. World Population Prospects: The 2008 Revision. 2008. http://esa.un.org/unpp (accessed Jan 07 2010).

9. Ritchie K, Lovestone S. The dementias. Lancet. 2002;360:1759-66.

10. Artero S, Touchon J, Ritchie K. Disability and mild cognitive impairment: a longitudinal population-based study. Int J Geriatr Psychiatry. 2001;16:1092-7.

11. Hunderfund AL, Roberts RO, Slusser TC, et al. Mortality in amnestic mild cognitive impairment: a prospective community study. Neurology. 2006;67:1764-8.

12. Pantoni L, Poggesi A, Inzitari D. Cognitive decline and dementia related to cerebrovascular diseases: some evidence and concepts. Cerebrovasc Dis. 2009;27: 191-6.

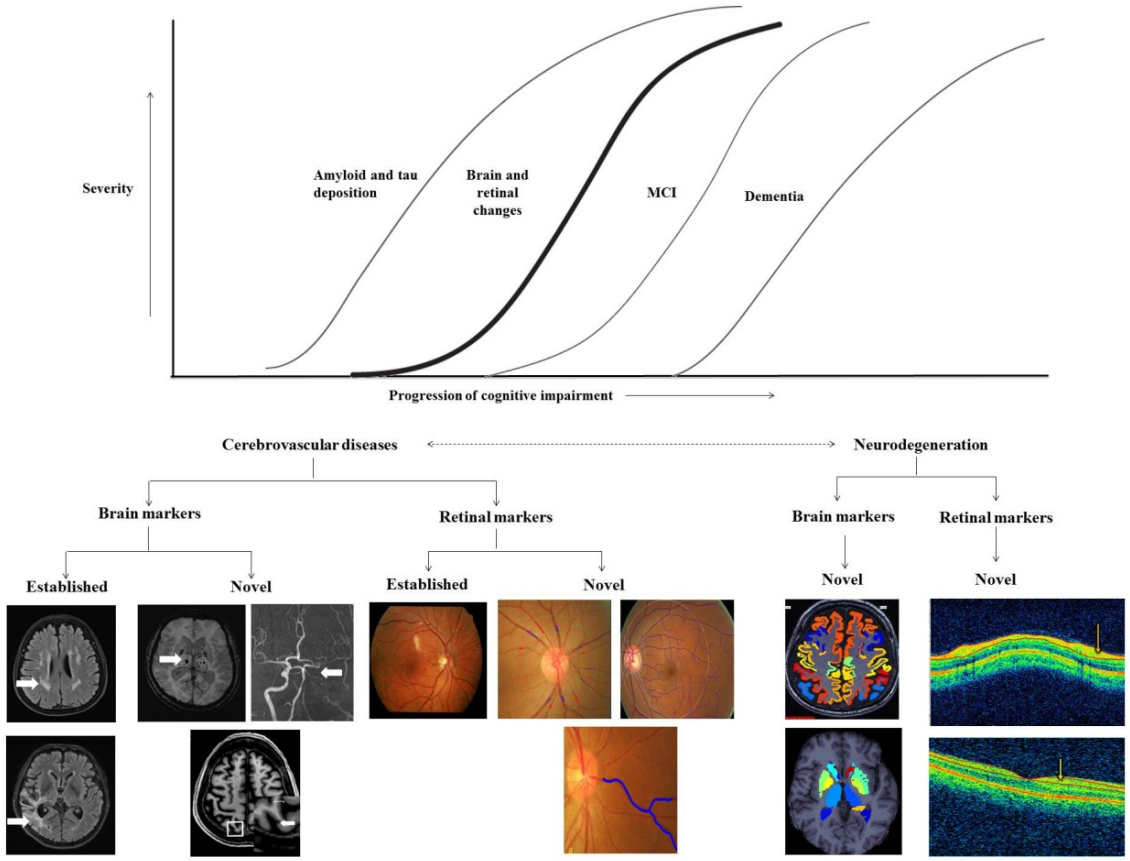
13. Attems J, Jellinger KA. The overlap between vascular disease and Alzheimer's disease--lessons from pathology. BMC medicine. 2014;12:206.

14. de Bruijn RF, Ikram MA. Cardiovascular risk factors and future risk of Alzheimer's disease. BMC medicine. 2014;12:130.

CHAPTER 1 – FIGURE

Figure 1: Markers of cerebrovascular diseases and involutional changes

In the Alzheimer's pathological cascade, amyloid and tau deposition takes place in the early stages of the disease followed by brain and retinal changes before the development of clinical symptoms. These brain and retinal changes may occur concurrently and hence certain biomarkers on cerebral and retinal imaging can reflect the microvascular and involutional changes in the brain. Manifestations of cerebrovascular diseases on MRI include infarcts, white matter hyperintensities, microbleeds, microinfarcts and intracranial stenosis. Retinal mircovascular damage is reflected by changes in calibers, fractional dimension and tortuosity. Neuronal damage in the brain can be measured by cortical thickness and subcortical structures volume whereas on the retinal images it is identified by thinning of the retinal neuronal layers.



CHAPTER 2:

LITERATURE REVIEW

1. PREVALENCE OF DEMENTIA AND COGNITIVE IMPAIRMENT- FACTS AND FIGURES

Dementia is one of the most important neurological disorders in the elderly. A recent WHO publication has reported that there are currently 35.6 million people living with dementia worldwide; this number is expected to triple by 2050.[1] The prevalence of dementia rapidly increases from 2-3% in ages 70-75 years to 20-25% in ages 80 and above. Moreover, whilst approximately 60% of patients with dementia were living in developing countries in 2001, this is expected to rise to 71% by 2040.[2] This is due to the non-uniform increase in the number of dementia patients with four fold increase in Asia compared to two fold increase in Europe and USA.[2] The prevalence of dementia in Caucasian population ranges from 5.4% in those aged >60 years to 68.3% at age 90 years and above. With respect to the pre-clinical stages of dementia [cognitive impairment no dementia (CIND)], the overall prevalence ranges from 14.9% to 22.2% in Caucasians.[2] Previous studies from Asian population have reported the corresponding figures to be 2.6-60.5% for dementia[3, 4] and 7.2-22.2% for CIND.[5, 6]

Our recent findings from Singapore population have shown an overall age standardized prevalence of cognitive impairment to be 15.2% in Chinese[7] and 25.5% in Malays.[8] These results on ethnic differences have implications beyond Singapore as they constitute the major ethnic groups in South East Asia such as Malaysia and Indonesia. This higher prevalence of cognitive impairment among Asia Pacific regions will put a significant burden on the health care systems in these regions.[8] Hence it is important to target research efforts on Asian populations so as to identify novel markers for pre-clinical cognitive impairment and dementia which are economically feasible for predicting and monitoring disease progression.

2. DEMENTIA AND COGNITIVE IMPAIRMENT

2.1 Dementia

Dementia- a clinical syndrome – is characterized by slow and progressive loss of memory and other cognitive abilities (such as language, comprehension, attention, judgment) which are sufficiently severe to interfere with a person's everyday activities. A clinical diagnosis of dementia is based on the Diagnostic and Statistical Manual of Mental Disorders – 4th edition (DSM-IV). According to this criteria, the development of multiple cognitive deficits is necessary for diagnosis of dementia which includes 1) memory impairment and 2) any one of the followings; language problems, inability to identify familiar objects/faces, impaired ability to carry out motor activities, disturbance in planning, organizing and sequencing, 3) significant impairment in social and occupational functioning, 4) sudden or gradual onset with progression in cognitive decline.[9] The history of multiple cognitive deficits is usually collected from both the patients and caregivers and is also confirmed on the objective neuropsychological testing (either brief or detailed tests).

Mostly dementia is underdiagnosed due to overlapping symptoms with other neurological and psychiatric disorders. Moreover, as memory problems are considered a normal part of aging process especially in the Asian population, dementia is often overlooked or identified at the later stage of the disease.[10]

2.2 Types of Dementia

Alzheimer's Disease

Alzheimer's disease (AD) – the most common type of dementia accounts for 60-80% of all dementia cases.[11] AD is clinically diagnosed by gradual onset and slow progression of cognitive symptoms predominantly memory and one or more other cognitive domains together with functional loss. It is distinguished from other types of dementia and neurological disorders by lack of substantial cerebrovascular diseases on MRI scans. The pathophysiology of AD has been related to the deposition of extracellular amyloid beta (Aß) plaques and intracellular neurofibrillary tangles.[12]

In normal conditions, amyloid ß peptides are formed during the metabolism of amyloid precursor protein and are rapidly removed from the brain. Due to reduced clearance of Aß peptides, they aggregate to form oligomers and eventually deposit in the brain in the form of plaques.[13] On the other hand, tau protein is a microtubule associated protein involved in stabilizing microtubules in neurons. Abnormal phosphorylation of tau proteins leads to microtubule instability and their aggregation in the form of neurofibrillary tangles. Both amyloid and tau proteins damage neuronal synapses and causes neuronal cell death.[14] These pathological changes are more common in early onset AD than in persons with late onset AD. Grossly, AD is characterized by loss of neuronal cell bodies and dendrites (gray matter) together with loss of axonal myelin sheath (white matter) of the brain.

Vascular Dementia

Vascular dementia (VaD) on the other hand, occurs after stroke and is the second most common type of dementia. Clinical history is uniquely represented by sudden onset of cognitive deficits after stroke with no recovery or continued deterioration after 3 months of clinical stroke, or abrupt deterioration of cognitive function with fluctuating or step wise progression in cognitive decline. VaD is primarily due to vascular damage and it is mostly correlated with the presence of cerebrovascular lesions on MRI scans.[15] These lesions may appear in the form of multiple infarcts, strategic single infarcts, small vessel diseases, hypoperfusion, hemorrhage or any combination thereof.

2.3 Cerebrovascular Disease and Alzheimer's Disease

Despite the clinical classification of AD and VaD, increasing evidences suggest that the cerebrovascular disease and AD pathology have significant overlap and similar brain structures might be damaged in both AD and CeVD.[16, 17] Neuropathological studies have shown that AD is accompanied by CeVD features in about a quarter to

one-third cases[18] and conversely about a half of dementia subjects with CeVD also have underlying AD pathology on autopsy.[19] This CeVD can be additive with AD pathology in impairing cognitive function and increasing the likelihood of dementia.

2.4 Cognitive Impairment

Pathological cascade of AD takes place years before the onset of clinical symptoms. By the time clinical cognitive deficits appear, irreversible neuronal damage has already taken place. Hence it is imperative to identify patients at risk of converting to dementia so that early treatment could be targeted at this stage where there is still a chance of preserving cognitive function and functional independence.

Mild Cognitive Impairment

Mild cognitive impairment (MCI) is considered a transitional stage to dementia particularly AD. MCI is characterized by subjective/informant complaints of memory problems, impairment in at least one domain in neuropsychological assessment and difficulty in performing activities without loss of functional independence.[20, 21] The annual conversion rate of MCI subjects to dementia is about 5-10% and hence is the potential target group for early intervention.[22, 23]

Cognitive Impairment No Dementia

Cognitive impairment no dementia (CIND) is a relatively recent concept and is defined based on the impairment in any objective cognitive domains in neuropsychological assessment.[24] Unlike MCI, CIND subjects do not necessarily have to have subjective complaints. CIND has been regarded as an unstable group with some persons progressing rapidly into dementia while others experience a more indolent course.[24, 25] Limited data is available on the subtypes of CIND based on the severity and their associated risk factors.

2.5 Mechanism Behind Cognitive Impairment and Dementia

It is increasingly being recognized that systemic vascular diseases such as hypertension, hypercholesterolemia and diabetes are the major risk factors for both cognitive impairment and dementia.[26, 27] It has been suggested that mid-life hypertension increases atherosclerosis and lipohyalinosis of the small vessels in the brain thus leading to cognitive dysfunction. The relationship between cholesterol and dementia is linked to the increased production of amyloid proteins in brain leading to neuronal death. Type-II diabetes mellitus on the hand is suggested to play an important role in cognitive decline by promoting ischemic cerebral changes secondary to hyperglycemia and function as a modulatory factor in association with other co-morbid conditions as hypertension and hyperlipidemia.[28] This suggests that these vascular risk factors act synergistically and promote cerebrovascular diseases through microvascular damage and increase the risk of cognitive impairment and dementia.

3. CEREBROVASCULAR DISEASES

Cerebrovascular diseases encompass both small and large vessel diseases.

Cerebral Small Vessel Diseases

Cerebral small vessel diseases are a group of pathological disorders such as arteriosclerosis, atherosclerosis, vasculitis, micro-aneurysms, fibrinoid necrosis that affects the small vessels (arteries, arterioles, veins and capillaries) in the brain.[29] These pathological changes dysregulate cerebral blood flow causing local ischemia. Disruption of the blood brain barrier results in the leakage of blood and plasma into perivascular tissue causing microhemorrhages, edema and tissue damage which is visible as ischemic infarcts. Cumulative tissue damage leads to rarefraction and demyelination as seen in white matter lesions.[30] However, unlike large vessels, cerebral small vessels cannot be visualized in vivo. Therefore, lesions in the brain parenchyma presumably caused by these small vessel changes have been adopted as

markers of small vessel diseases. These markers are identified as lacunes, white matter hyperintensities, microbleeds and enlarged perivascular spaces on MRI. In order to differentiate these markers on neuroimaging and to have unified definitions for grading these lesions, STandards for ReportIng Vascular changes on nEuroimaging (STRIVE)[31] criteria has been recommended which are as follows;

Lacunar Infarcts

Lacunar infarcts are defined as round or ovoid lesions, 3-15 mm in diameter, with low signal on T1weighted image and Fluid Attenuated Inversion Recovery (FLAIR); a high signal on T2 weighted image, and a hyperintense rim with center following the cerebrospinal fluid intensity.

White Matter Hyperintensities (WMH)

WMH are defined as signal abnormalities of variable sizes in white matter, hyperintense on T2-weighted image or FLAIR, without cavitation.

Both lacunes and WMH are endemic in the elderly population with a prevalence of up to a quarter for infarcts and 96% for any severity of WMH in persons ≥ 60 years. Several studies have also shown association of lacunes and WMH with risk of stroke[32] and development of cognitive impairment and dementia.[33-35] These lesions are also associated with cognitive decline[36, 37] and all-cause mortality[35, 38, 39] (**Table 2 – 1a**). Hence an extensive work is available on these established markers of CeVD. In the past decade, another marker that has been suggested to reflect cerebral small vessel disease is the presence of cerebral microbleeds.

Cerebral Microbleeds (CMBs)

CMB is defined as focal, rounded areas of hypointensity (T1 and T2 weighted images), 2-10 mm in diameter with blooming on T2*-weighted scans, which correspond pathologically to hemosiderin deposits surrounding small vessels. In

healthy populations the reported prevalence of CMBs ranges from 3.8% to 38.3%,[40] whereas in patients with stroke the corresponding figures may be as high as 50-70%.[41, 42] CMBs in the deep subcortical regions have been linked to hypertension whereas those in lobar are believed to be related to cerebral amyloid angiopathy (CAA). This amyloid plaque deposits in leptomeningeal and cortical arteries leading to vessel occlusion and reduction in microvessel density.[43, 44] There is a considerable debate on the exact role of CMB in the pathophysiology of cognitive impairment and dementia. The effect of CMB on cognition has been variable. Some studies have reported significant association,[45] whereas others have either failed to find an independent association of CMB and cognitive decline [46-48] or found a striking effect on a specific domain (executive function, processing speed) with no effect on other cognitive domains[49, 50] or brief test[51] (**Table 2 – 1b**). These differences might be due to different cognitive tests used (brief tests vs. detailed) and varying criteria to define CMBs.

Despite the extensive literature on cerebrovascular diseases on MRI, there is still debate that these MRI correlates of small vessel diseases do not fully capture the vascular damage in the brain parenchyma. In this context, cortical cerebral microinfarcts have gained increasing attention.

Cortical Cerebral Microinfarcts (CMIs)

Over the past decade, several neuropathological studies have suggested that CMIs are also manifestations of small vessel disease in addition to the established markers.[52, 53] They are reported to be the most wide spread form of brain infarction and are involved in the pathway between small vessel disease and cognitive impairment.[54, 55] CMIs are a common lesion in the elderly and are often seen in autopsy studies performed on both healthy elderly and patients with dementia. They are reported to be present in 24% of non-demented older adults, 43% of AD patients and 62% of

patients with vascular dementia.[55] CMIs are most commonly present in the cortex but may also appear in the subcortical regions.[56] Microscopically, these lesions appear as sharply delineated areas of tissue necrosis.[57] As CMIs can only be confirmed in pathological specimen of autopsied brain, this has its limitations as only small samples of brain tissue can be assessed and thus may not represent the true burden of such lesions.

With the advancement of high resolution MRI scanners, it is now possible to detect CMIs in-vivo using 7T MRI.[58] These lesions appear as perpendicular lesions in the cortical ribbon, <5mm in size, hyperintense on Fluid Attenuated Inversion Recovery (FLAIR) and T2 sequences and hypointense on T1 weighted images. They are distinguished from other hemorrhagic lesion, a vessel, or an artifact if they also appear as hypo or iso-intense lesions on FLAIR and T2 images. However, high resolution 7T scanning remains an expensive technique with limited accessibility in clinical settings. Furthermore there are challenges in terms of image homogeneity and strict safety regulations due to high strength magnetic field which restricts patients with metallic implants and stents to undergo 7T MRI which may still be possible with lower field strength 3T scanning. Hence it is of note that two recent reports have now shown that the CMIs are also visible on 3T mainly because of the availability of ultrahigh resolution 3T machines[58, 59] in both clinical and research settings. However limited data exist on the risk factors[60] and consequences of CMIs on 3T MRI scans [61] (**Table 2 – 2**).

Cerebral Large Vessel Diseases

Besides the small blood vessels, large vessel diseases such as atherosclerosis obstruct the lumen of both extracranial and intracranial arteries and are major risk factors of stroke and mortality. Mounting evidences suggest that the coronary artery disease,[62, 63] hypercholesterolemia[64, 65] and atherosclerosis of the internal carotid artery[66] lead to

thromboembolism which results in cardioembolic stroke.[67] Postmortem studies have shown that severe arterial atherosclerosis of the Circle of Willis is also a common finding in dementia with reported prevalence of 53% in VaD, 30% in AD patients and 20% in non-demented brains[68, 69] (**Table 2 - 3a**). It has been hypothesized that Circle of Willis occlusion can lead to hypoperfusion with selective neuronal loss and may even trigger the neurodegenerative process by promoting amyloid accumulation.[68]

Ante mortem studies have suggested that extracranial large artery disease is associated with cognitive impairment and poorer performance on several cognitive tests.[70-73] Moreover, limited data has also reported cognitive alterations in patients with stroke, transient ischemic attack and internal carotid occlusion.[74, 75] The effects of large artery stenosis on cognitive decline and risk of dementia has been contradictory with some reporting significant association whereas others do not [76] (**Table 2 - 3b**). This disparity among studies are due to different imaging modalities used (transcranial doppler, magnetic resonance angiography, duplex ultrasound) to assess carotid stenosis. Furthermore, they are largely focused on the extracranial carotid artery stenosis rather than intracranial stenosis. It has been reported that ICS in stroke patients vary among different ethnicities with a higher prevalence in Chinese (40-50%) compared to Caucasians (8-10%)[77-79] (**Table 2 - 3a**). Hence, data on ICS from asymptomatic and community-based subjects – especially of Asian populations - are largely lacking. Moreover, its association with vascular vs. non-vascular cognitive impairment and dementia subtypes has not been explored previously.

4. NEURODEGENERATION

Neurodegeneration is an umbrella term for progressive loss of structure and function of a neuron including neuronal death. Overt pathology in neurodegeneration is characterized by focal loss of neurons with reactive gliosis.[80] Neurodegeneration typically appears as cerebral atrophy and remains the key neuroimaging feature of

AD and other dementias. It involves both cortical and subcortical structures (hippocampus) and remains the strongest predictor of MCI conversion to AD[81, 82] and tracking disease progression.[83, 84] Quantitative structural imaging has provided more insights in region specific atrophy thus assisting in early diagnosis and in increasing diagnostic confidence. Recent reports have suggested that brain changes, particularly reduction in gray matter volumes are present even during normal aging [85-89] (**Table 2 - 4c**). In this regard, cortical thickness provides an advantage of capturing the physical property of the brain that can be measured in an individual in vivo[90], with thinning of the cortex corresponding to pathologic observations including cellular shrinkage, neuronal loss, and reduction of intracortical myelin.

A consistent pattern of cortical thinning in AD comprises the medial temporal lobes; inferior and anterior temporal association cortices; superior, inferior, and medial parietal association cortices; along with superior and inferior frontal association regions[91-94] (**Table 2 - 4a**). Dickerson and colleagues have demonstrated that a set of regions with cortical thinning specific to AD can reliably be used to predict progression to AD in subjects with MCI and in older individuals without cognitive impairment.[95, 96] In addition, a few studies have also suggested preclinical cognitive impairment to be associated with worse performance on cognitive testing[97, 98] (**Table 2 - 4b**). However, limited data remains on the determinants of cortical thinning[99] and its effects on cognition from elderly population in Asia.[100, 101]

Besides the cortical thickness, computational segmentations can also quantify subcortical structures volumes which include accumbens, amygdala, caudate, pallidum, putamen, thalamus, hippocampus and more recently brainstem.[102] Atrophy of these subcortical structures has been reported in a wide range of neurological and psychiatric disorders.[103-106] Some studies have now suggested smaller volumes of several subcortical structures in AD[107-111] (**Table 2 - 5**). There is only a scant literature available on the putative risk factors of subcortical atrophy in elderly with

no cognitive impairment with a primary focus on age and sex effects[88, 112-115] (**Table 2 - 5**). Moreover, the specific pattern of subcortical atrophies in vascular vs. non vascular cognitive impairment remains unclear.[116]

5. LIMITATIONS OF NEUROIMAGING

Despite the rapid advancement in neuroimaging techniques in facilitating the diagnosis of different dementia subtypes and providing invaluable tools in advancing our understanding of cerebrovascular pathophysiology, it remains an expensive technique with several contraindications for the elderly with metallic implants. Moreover, quantitative structural imaging techniques require specialized skills in standardizing and co-registering the images with constant visual inspection and manual editing. Hence, this MRI processing becomes a long and tedious procedure and is only available in selected research settings with image analysis expertise. These techniques are therefore, not suitable candidates for more widespread screening of patients at risk of cerebrovascular disease.

6. RETINA – WINDOW TO THE BRAIN

6.1 Homology in Retinal and Cerebral Microvasculature

Retina and brain are highly metabolically active tissues with high demands on metabolic substrates such as oxygen via the specialized vascular network. Since both organs share similar pattern of vascularization (macro and microvascular blood supply), there is also a similarity in their regulatory processes.[117-119] Aging affects both retinal and cerebral microvasculature by reducing blood flow and exhibiting decreased oxygen and glucose demand.[120-123] Similarly both retinal and cerebral microcirculations also exhibit morphological changes in hypertension, diabetes, stroke and other hereditary conditions such as cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and hereditary endotheliopathy with retinopathy, nephropathy and stroke (HERNS).[124]

Retinopathy is the end organ damage in retina and is reflected on the retinal photography as generalized and focal retinal arteriolar narrowing, arteriovenous nicking (from intimal thickening), media wall hyperplasia, hyaline degeneration, and vessel wall necrosis. These are typically seen in the two most important cardiovascular risk factors i.e. hypertension and diabetes.[125, 126] Both conditions cause endothelial dysfunction and blood retinal barrier break down leading to retinal haemorrhages, micoraneurysms, exudates and nerve fiber layer ischemia (cotton wool spots).[127, 128] Narrowing and occlusion of the small arterioles lead to vessel collapse and reduced network density[129] while capillary occlusion leads to neovascularization.[130] Similar microvascular changes occur in brain with luminal narrowing (replacement of tunica media and internal elastic lamina with fibrous tissue),[131] increased vessel tortuosity and vessel permeability (break down of blood brain barrier).[132] Thus, these diseases on pathology have illustrated that the events occurring in the retinal circulation are indeed, mirrored by the cerebral circulation.

6.2 Possible Early Retinal Microvascular Changes

As retinopathy reflects the severe late stages of retinal damage, it is imperative to identify the early changes taking place in retinal vessels before the development of the retinopathy signs. Over the last decade, computer-based retinal image analysis technique has enabled us to quantify possible early changes which might serve as potential biomarker for alterations in retinal microvasculature. It is based on the prevailing hypothesis that the design of the retinal vascular tree obeys simple physiological and physical principle that optimizes the operation of the system. Singapore I Vessel Assessment (SIVA) system has the ability to provide in-depth visualization of the retinal vasculature.

Retinal Vessel Calibers

Retinal arteriolar and venular calibers, which are calculated as the central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE), have emerged as a marker for preclinical stages of the diseases such as hypertension, diabetes, myocardial infarction and stroke.[133-136] However, as both of these calibers are affected by different pathological mechanisms, they are usually analyzed separately.[137, 138] Arteriolar calibers are largely affected by blood pressure, hypertension whereas venular calibers are sensitive to changes in blood glucose, diabetes, inflammation, dyslipidemia and smoking.[137] Caliber measurements are affected by pulse period and single measurement reflects significant variability across pulse cycle. Hence there is a need for other potential parameters which are less time dependent.[139]

Retinal Fractal Dimension

Fractal dimension uses a mathematical concept to characterize the complexity of natural branching vascular networks, such as those seen in the retinal, coronary and pulmonary vascular systems. It captures the optimality and efficiency of blood distribution. Fractal analysis has been used in many aspects of medicine to detect changes in the retinal vasculature during early stages of retinal diseases, such as diabetic retinopathy and glaucoma.[140] Studies show that fractal dimension increases in eyes with new vessels, and decreases with regression of these new vessels. Fractal analysis thus may offer new insights into systemic microvasculogenesis.[141, 142]

Retinal Vascular Tortuosity

Retinal vessel tortuosity reflects the curvature of the vessel path and reflects vessel integrity and barrier dysfunction. Increased retinal tortuosity has been linked to retinopathy of prematurity, hypertension and diabetes.[143]

Retinal Vascular Changes in Cerebrovascular Diseases

Several population based studies such as Atherosclerosis Risk In Communities (ARIC) study, the Cardiovascular Health Study and the Rotterdam Scan Study, which are based on healthy middle-aged and elderly population, have shown that the retinopathy signs were associated not only with incident stroke, but also with subclinical MRI-defined changes, including cerebral infarction, progression of white matter lesions, multiple cerebral microbleeds and atrophy (**Table 2 - 6a**).[144-153] These associations were independent of other cardiovascular risk factors suggesting that these retinal signs may provide additional information on cerebral small vessel diseases.

Previous studies have also shown that narrower arteriolar and wider venular calibers are associated with incident stroke.[134, 135, 151, 154] With respect to the other markers of cerebrovascular diseases, the similar caliber parameters were also linked to incident lacunar infarcts, and white matter lesions progression[151, 155, 156](**Table 2 - 6b**). However, conflicting results remain in terms of newer vascular parameters i.e. fractal dimension.[157] Furthermore, the effects of vessel tortuosity on MRI markers of cerebrovascular diseases and cognitive impairment remain to be explored. Limited studies on HERNS and CADASIL have suggested that the reduced fractal dimension and increased vessel tortuosity coexist with microvascular pathology in the brain such as microbleeds and white matter lesions.[158, 159] However no studies have yet examined the association of all retinal microvascular changes (arteriolar caliber, venular caliber, fractal dimension and tortuosity) with the cerebrovascular diseases in the preclinical stages of dementia.

Retinal Vascular Changes in Cognitive Impairment and Dementia

Several population based studies have shown that retinopathy signs are associated with AD and vascular dementia in hypertensive groups[50, 160, 161] and are even linked to cerebral atrophy on the scans.[146, 152] Besides dementia, the late changes in the retina

were also associated with cognitive impairment in some studies whereas others failed to find clear association[50, 162] (**Table 2 - 6c**). These discrepancies might be due to the differences in brief cognitive tests [Mini Mental Status Examination (MMSE) vs. Abbreviated Mental Test (AMT)][162, 163] and limitation to specific cognitive domains (psychomotor speed, executive function and visual memory).[50, 164]

With regards to the early changes in retinal vasculature, Rotterdam scan study has reported that wider venular and smaller arteriolar caliber is associated with incident dementia.[165] Another study has reported a link between smaller venular caliber and AD.[166] Reduction in fractal dimension was associated with cognitive impairment in Singapore Malay Eye Study (SiMES) and the Australian Imaging, Biomarker & Lifestyle Flagship Study of Ageing (AIBL) studies (**Table 2 – 6d**).[167, 168] However, it remains unknown if retinal microvascular changes are also associated with preclinical stages of cognitive impairment measured on a detailed neuropsychological assessment.

6.3 Homology in Retinal and Cerebral Neuronal Layers

Retina is a direct extension of the mesencephalon and is connected to the brain via the optic nerve. Retinal ganglion cells receive information from the photoreceptors in the eye and share morphological characteristics with the cerebral neurons in having the cell body, dendrites and myelinated axons. These axonal processes relay information to the visual cortex in the occipital lobe. Damage to either retinal or cerebral neurons results in degeneration in anterograde or retrograde direction due to lack of regenerative ability in axons.[169] Hence the degeneration in the brain is reflected as retinal neuronal loss or vice versa.

Optical Coherence Tomography

Optical coherence tomography (OCT) is a non-invasive, non-contact optical imaging technique for studying retinal neuronal layers in-vivo.[170] It provides high resolution,

biopsy like cross-sectional images (10-20μm) using optical backscattering of light analogous to ultrasonography. First generation OCTs [time domain (TD) OCTs] are capable of measuring the retinal nerve fiber layer thickness (RNFL) and have been successfully used in research settings to measure the optic nerve damage in glaucoma and optic neuritis.[171, 172] Second generation OCTs [spectral domain (SD) OCTs] are now able to measure thickness of retinal sublayers, the ganglion cell inner plexiform layer (GC-IPL) which is directly posterior and exterior to RNFL.[173, 174]

RNFL consists of unmyelinated axons of the retinal ganglion cells and is thickest at the peripapillary region around the optic disc. GC-IPL contains the cell bodies and dendrites of the retinal ganglion cells, and is thickest at the macular region. Damage to the retinal neuronal layers affects the dendrites prior to the ganglion cell body, hence GC-IPL is more sensitive to neuronal damage compared to RNFL.[175, 176]

Retinal Neuronal Changes in Cognitive Impairment and Dementia

Several small studies using TD- OCT have shown that retinal nerve fiber thickness is reduced in subjects with AD and MCI.[166, 177-181] Conversely, studies using the SD-OCT have shown either in-consistent or mixed results with cognitive performance.[182-184] More recent studies have shown that both RNFL and GC-IPL thickness is reduced in AD patients (**Table 2 – 7**).[185] However, these were limited by small numbers and did not take into account the other cardiovascular risk factors.

Besides the cognitive impairment and dementia, retinal neuronal changes (RNFL and GC-IPL) have also been reported in other neurodegenerative diseases such as multiple sclerosis and Parkinson's disease.[186-188] However, there is no data on the GC-IPL thinning in relation to cerebral atrophy among subjects with preclinical cognitive impairment.

Hence the main focus of this thesis is to **examine how the age-related structural changes in the brain and retina are related to cerebrovascular diseases on MRI and cognition using cerebral and retinal imaging markers.**

CHAPTER 2 – REFERENCES

1. Population Division of the Department of Economics and Social Affairs of the United Nations Secretariat. World Population Prospects: The 2008 Revision. 2008. http://esa.un.org/unpp (accessed Jan 07 2010).

2. Ferri CP, Prince M, Brayne C, et al. Global prevalence of dementia: a Delphi consensus study. Lancet. 2005;366:2112-7.

3. Larson EB, Langa KM. The rising tide of dementia worldwide. Lancet. 2008; 372:430-2.

4. Chan KY, Wang W, Wu JJ, et al. Epidemiology of Alzheimer's disease and other forms of dementia in China, 1990-2010: a systematic review and analysis. Lancet. 2013;381:2016-23.

5. Lim HJ, Lim JP, Anthony P, Yeo DH, Sahadevan S. Prevalence of cognitive impairment amongst Singapore's elderly Chinese: a community-based study using the ECAQ and the IQCODE. Int J Geriatr Psychiatry. 2003;18:142-8.

6. Wu MS, Lan TH, Chen CM, Chiu HC, Lan TY. Socio-demographic and health-related factors associated with cognitive impairment in the elderly in Taiwan. BMC public health. 2011;11:22.

7. Hilal S, Ikram MK, Saini M, et al. Prevalence of cognitive impairment in Chinese: Epidemiology of Dementia in Singapore study. J Neurol Neurosurg Psychiatry. 2013;84:686-92.

8. Hilal S, Tan CS, Xin S, et al. Prevalence of cognitive impairment and dementia in Malays - Epidemiology of Dementia in Singapore Study. Curr Alzheimer Res. 2015 [epub ahead of print].

9. Diagnostic and Statistical Manual of Mental Disorders. Fourth Edition, Text Revision ed. Washington, D.C: American Psychiatric Association; 2000.

10. Valcour VG, Masaki KH, Curb JD, Blanchette PL. The detection of dementia in the primary care setting. Arch Intern Med. 2000;160:2964-8.

11. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology. 1984;34:939-44.

12. Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. Lancet. 2006; 368:387-403.

13. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proceedings of the National Academy of Sciences of the United States of America. 1985;82:4245-9.

14. Crews L, Masliah E. Molecular mechanisms of neurodegeneration in Alzheimer's disease. Human molecular genetics. 2010;19:R12-20.

15. Roman GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology. 1993;43:250-60.

16. Attems J, Jellinger KA. The overlap between vascular disease and Alzheimer's disease--lessons from pathology. BMC medicine. 2014;12:206.

17. Jagust WJ, Zheng L, Harvey DJ, et al. Neuropathological basis of magnetic resonance images in aging and dementia. Ann Neurol. 2008;63:72-80.

18. Toledo JB, Arnold SE, Raible K, et al. Contribution of cerebrovascular disease in autopsy confirmed neurodegenerative disease cases in the National Alzheimer's Coordinating Centre. Brain. 2013;136:2697-706.

19. Knopman DS, Parisi JE, Boeve BF, et al. Vascular dementia in a population-based autopsy study. Arch Neurol. 2003;60:569-75.

20. Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7:270-9.

21. Petersen RC. Mild cognitive impairment as a diagnostic entity. Journal of internal medicine. 2004;256:183-94.

22. Mitchell AJ, Shiri-Feshki M. Rate of progression of mild cognitive impairment to dementia--meta-analysis of 41 robust inception cohort studies. Acta Psychiatr Scand. 2009;119:252-65.

23. Anderson TM, Sachdev PS, Brodaty H, Trollor JN, Andrews G. Effects of sociodemographic and health variables on Mini-Mental State Exam scores in older Australians. Am J Geriatr Psychiatry. 2007;15:467-76.

24. Graham JE, Rockwood K, Beattie BL, et al. Prevalence and severity of cognitive impairment with and without dementia in an elderly population. Lancet. 1997;349:1793-6.

25. Palmer K, Wang HX, Backman L, Winblad B, Fratiglioni L. Differential evolution of cognitive impairment in nondemented older persons: results from the Kungsholmen Project. The American journal of psychiatry. 2002;159:436-42.

26. Debette S. Vascular risk factors and cognitive disorders. Rev Neurol. 2013;169:757-64.

27. Solfrizzi V, Panza F, Colacicco AM, et al. Vascular risk factors, incidence of MCI, and rates of progression to dementia. Neurology. 2004;63:1882-91.

28. Duron E, Hanon O. Vascular risk factors, cognitive decline, and dementia. Vascular health and risk management. 2008;4:363-81.

29. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. Lancet Neurol. 2010;9:689-701.

30. Pantoni L, Garcia JH. Pathogenesis of leukoaraiosis: a review. Stroke; a journal of cerebral circulation. 1997;28:652-9.

31. Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol. 2013;12:822-38.

32. Vermeer SE, Hollander M, van Dijk EJ, Hofman A, Koudstaal PJ, Breteler MM. Silent brain infarcts and white matter lesions increase stroke risk in the general population: the Rotterdam Scan Study. Stroke; a journal of cerebral circulation. 2003; 34:1126-9.

33. Vermeer SE, Prins ND, den Heijer T, Hofman A, Koudstaal PJ, Breteler MM. Silent brain infarcts and the risk of dementia and cognitive decline. N Engl J Med. 2003;348:1215-22.

34. Benjamin P, Lawrence AJ, Lambert C, et al. Strategic lacunes and their relationship to cognitive impairment in cerebral small vessel disease. NeuroImage Clinical. 2014;4:828-37.

35. Debette S, Markus HS. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. BMJ. 2010;341:c3666.

36. Pantoni L, Poggesi A, Inzitari D. Cognitive decline and dementia related to cerebrovascular diseases: some evidence and concepts. Cerebrovasc Dis. 2009;27: 191-6.

37. Mortamais M, Artero S, Ritchie K. Cerebral white matter hyperintensities in the prediction of cognitive decline and incident dementia. International review of psychiatry. 2013;25:686-98.

38. Vermeer SE, Longstreth WT, Jr., Koudstaal PJ. Silent brain infarcts: a systematic review. Lancet Neurol. 2007;6:611-9.

39. Prins ND, Scheltens P. White matter hyperintensities, cognitive impairment and dementia: an update. Nature reviews Neurology. 2015;11:157-65.

40. Poels MM, Ikram MA, van der Lugt A, et al. Incidence of cerebral microbleeds in the general population: the Rotterdam Scan Study. Stroke. 2011; 42: 656-61.

41. Werring DJ, Coward LJ, Losseff NA, Jager HR, Brown MM. Cerebral microbleeds are common in ischemic stroke but rare in TIA. Neurology. 2005;65: 1914-8.

42. Jeerakathil T, Wolf PA, Beiser A, et al. Cerebral microbleeds: prevalence and associations with cardiovascular risk factors in the Framingham Study. Stroke. 2004; 35:1831-5.

43. Yates PA, Sirisriro R, Villemagne VL, et al. Cerebral microhemorrhage and brain beta-amyloid in aging and Alzheimer disease. Neurology. 2011;77:48-54.

44. Charidimou A, Krishnan A, Werring DJ, Rolf Jager H. Cerebral microbleeds: a guide to detection and clinical relevance in different disease settings. Neuroradiology. 2013;55:655-74.

45. Lei C, Lin S, Tao W, Hao Z, Liu M, Wu B. Association between cerebral microbleeds and cognitive function: a systematic review. J Neurol Neurosurg Psychiatry. 2013;84:693-7.

46. Martinez-Ramirez S, Greenberg SM, Viswanathan A. Microbleeds do not affect rate of cognitive decline in Alzheimer disease. Neurology. 2013;80:1266.

47. Schneider JA. Brain microbleeds and cognitive function. Stroke. 2007;38: 1730-1.

48. van der Vlies AE, Goos JD, Barkhof F, Scheltens P, van der Flier WM. Microbleeds do not affect rate of cognitive decline in Alzheimer disease. Neurology. 2012;79:763-9.

49. Werring DJ, Frazer DW, Coward LJ, et al. Cognitive dysfunction in patients with cerebral microbleeds on T2*-weighted gradient-echo MRI. Brain. 2004;127: 2265-75.

50. Qiu C, Cotch MF, Sigurdsson S, et al. Cerebral microbleeds, retinopathy, and dementia: the AGES-Reykjavik Study. Neurology. 2010;75:2221-8.

51. Cordonnier C, van der Flier WM, Sluimer JD, Leys D, Barkhof F, Scheltens P. Prevalence and severity of microbleeds in a memory clinic setting. Neurology. 2006;66:1356-60.

52. Launer LJ, Hughes TM, White LR. Microinfarcts, brain atrophy, and cognitive function: the Honolulu Asia Aging Study Autopsy Study. Ann Neurol. 2011;70:774-80.

53. Arvanitakis Z, Leurgans SE, Barnes LL, Bennett DA, Schneider JA. Microinfarct pathology, dementia, and cognitive systems. Stroke. 2011;42:722-7.

54. Smith EE, Schneider JA, Wardlaw JM, Greenberg SM. Cerebral microinfarcts: the invisible lesions. Lancet Neurol. 2012;11:272-82.

55. Brundel M, de Bresser J, van Dillen JJ, Kappelle LJ, Biessels GJ. Cerebral microinfarcts: a systematic review of neuropathological studies. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2012;32:425-36.

56. Suter OC, Sunthorn T, Kraftsik R, et al. Cerebral hypoperfusion generates cortical watershed microinfarcts in Alzheimer disease. Stroke. 2002;33:1986-92.

57. White L, Petrovitch H, Hardman J, et al. Cerebrovascular pathology and dementia in autopsied Honolulu-Asia Aging Study participants. Ann N Y Acad Sci. 2002;977: 9-23.

58. van Veluw SJ, Zwanenburg JJ, Engelen-Lee J, et al. In vivo detection of cerebral cortical microinfarcts with high-resolution 7T MRI. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2013;33:322-9.

59. Ii Y, Maeda M, Kida H, et al. In vivo detection of cortical microinfarcts on ultrahigh-field MRI. Journal of neuroimaging : official journal of the American Society of Neuroimaging. 2013;23:28-32.

60. van Dalen JW, Scuric EE, van Veluw SJ, et al. Cortical microinfarcts detected in vivo on 3 Tesla MRI: clinical and radiological correlates. Stroke. 2015; 46:255-7.

61. van Rooden S, Goos JD, van Opstal AM, et al. Increased number of microinfarcts in Alzheimer disease at 7-T MR imaging. Radiology. 2014;270:205-11.

62. Soneira CF, Scott TM. Severe cardiovascular disease and Alzheimer's disease: senile plaque formation in cortical areas. Clinical anatomy.1996;9:118-27.

63. Sparks DL, Hunsaker JC, 3rd, Scheff SW, Kryscio RJ, Henson JL, Markesbery WR. Cortical senile plaques in coronary artery disease, aging and Alzheimer's disease. Neurobiology of aging. 1990;11:601-7.

64. Kalmijn S, Launer LJ, Ott A, Witteman JC, Hofman A, Breteler MM. Dietary fat intake and the risk of incident dementia in the Rotterdam Study. Ann Neurol. 1997;42:776-82.

65. Launer LJ, White LR, Petrovitch H, Ross GW, Curb JD. Cholesterol and neuropathologic markers of AD: a population-based autopsy study. Neurology. 2001; 57:1447-52.

66. Hofman A, Ott A, Breteler MM, et al. Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam Study. Lancet. 1997;349:151-4.

67. Adams HP, Jr., Bendixen BH, Kappelle LJ, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke. 1993;24:35-41.

68. Roher AE, Esh C, Kokjohn TA, et al. Circle of willis atherosclerosis is a risk factor for sporadic Alzheimer's disease. Arteriosclerosis, thrombosis, and vascular biology. 2003;23:2055-62.

69. Beach TG, Wilson JR, Sue LI, et al. Circle of Willis atherosclerosis: association with Alzheimer's disease, neuritic plaques and neurofibrillary tangles. Acta neuropathologica 2007;113:13-21.

70. Bossema ER, Brand N, Moll FL, Ackerstaff RG, van Doornen LJ. Does carotid endarterectomy improve cognitive functioning? J Vasc Surg. 2005;41:775-81.

71. Bossema ER, Brand N, Moll FL, Ackerstaff RG, de Haan EH, van Doornen LJ. Cognitive functions in carotid artery disease before endarterectomy. Journal of clinical and experimental neuropsychology 2006;28:357-69.

72. Landgraff NC, Whitney SL, Rubinstein EN, Yonas H. Cognitive and physical performance in patients with asymptomatic carotid artery disease. J Neurol. 2010; 257:982-91.

73. Mathiesen EB, Waterloo K, Joakimsen O, Bakke SJ, Jacobsen EA, Bonaa KH. Reduced neuropsychological test performance in asymptomatic carotid stenosis: The Tromso Study. Neurology 2004;62:695-701.

74. Bakker FC, Klijn CJ, Jennekens-Schinkel A, van der Tweel I, Tulleken CA, Kappelle LJ. Cognitive impairment in patients with carotid artery occlusion and ipsilateral transient ischemic attacks. Journal of neurology. 2003;250:1340-7.

75. Silvestrini M, Paolino I, Vernieri F, et al. Cerebral hemodynamics and cognitive performance in patients with asymptomatic carotid stenosis. Neurology. 2009;72:1062-8.

76. Poels MM, van Oijen M, Mattace-Raso FU, et al. Arterial stiffness, cognitive decline, and risk of dementia: the Rotterdam study. Stroke. 2007;38:888-92.

77. Huang YN, Gao S, Li SW, et al. Vascular lesions in Chinese patients with transient ischemic attacks. Neurology. 1997;48:524-5.

78. Li H, Wong KS. Racial distribution of intracranial and extracranial atherosclerosis. Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia. 2003;10:30-4.

79. Wong KS, Huang YN, Yang HB, et al. A door-to-door survey of intracranial atherosclerosis in Liangbei County, China. Neurology. 2007;68:2031-4.

80. Przedborski S, Vila M, Jackson-Lewis V. Neurodegeneration: what is it and where are we? The Journal of clinical investigation. 2003;111:3-10.

81. Dickerson BC, Wolk DA. Biomarker-based prediction of progression in MCI: Comparison of AD signature and hippocampal volume with spinal fluid amyloid-beta and tau. Frontiers in aging neuroscience. 2013;5:55.

82. Vemuri P, Wiste HJ, Weigand SD, et al. MRI and CSF biomarkers in normal, MCI, and AD subjects: predicting future clinical change. Neurology. 2009;73:294-301.

83. Jack CR, Jr., Knopman DS, Jagust WJ, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol. 2010;9:119-28.

84. Vemuri P, Wiste HJ, Weigand SD, et al. Serial MRI and CSF biomarkers in normal aging, MCI, and AD. Neurology. 2010;75:143-51.

85. Lemaitre H, Goldman AL, Sambataro F, et al. Normal age-related brain morphometric changes: nonuniformity across cortical thickness, surface area and gray matter volume? Neurobiology of aging. 2012;33:617 e1-9.

86. Magnotta VA, Andreasen NC, Schultz SK, et al. Quantitative in vivo measurement of gyrification in the human brain: changes associated with aging. Cerebral cortex.1999;9:151-60.

87. Preul C, Hund-Georgiadis M, Forstmann BU, Lohmann G. Characterization of cortical thickness and ventricular width in normal aging: a morphometric study at 3 Tesla. Journal of magnetic resonance imaging : JMRI. 2006;24:513-9.

88. Long X, Liao W, Jiang C, Liang D, Qiu B, Zhang L. Healthy aging: an automatic analysis of global and regional morphological alterations of human brain. Academic radiology. 2012;19:785-93.

89. Salat DH, Buckner RL, Snyder AZ, et al. Thinning of the cerebral cortex in aging. Cerebral cortex. 2004;14:721-30.

90. Fischl B, Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. Proceedings of the National Academy of Sciences of the United States of America. 2000;97:11050-5.

91. Lerch JP, Pruessner JC, Zijdenbos A, Hampel H, Teipel SJ, Evans AC. Focal decline of cortical thickness in Alzheimer's disease identified by computational neuroanatomy. Cerebral cortex. 2005;15:995-1001.

92. Sabuncu MR, Desikan RS, Sepulcre J, et al. The dynamics of cortical and hippocampal atrophy in Alzheimer disease. Arch Neurol. 2011;68:1040-8.

93. Frisoni GB, Testa C, Zorzan A, et al. Detection of grey matter loss in mild Alzheimer's disease with voxel based morphometry. J Neurol Neurosurg Psychiatry. 2002;73:657-64.

94. Singh V, Chertkow H, Lerch JP, Evans AC, Dorr AE, Kabani NJ. Spatial patterns of cortical thinning in mild cognitive impairment and Alzheimer's disease. Brain. 2006;129:2885-93.

95. Dickerson BC, Fenstermacher E, Salat DH, et al. Detection of cortical thickness correlates of cognitive performance: Reliability across MRI scan sessions, scanners, and field strengths. Neuroimage. 2008;39:10-8.

96. Karas G, Sluimer J, Goekoop R, et al. Amnestic mild cognitive impairment: structural MR imaging findings predictive of conversion to Alzheimer disease. AJNR Am J Neuroradiol. 2008;29:944-9.

97. Chang YL, Jacobson MW, Fennema-Notestine C, et al. Level of executive function influences verbal memory in amnestic mild cognitive impairment and predicts prefrontal and posterior cingulate thickness. Cerebral cortex. 2010;20:1305-13.

98. Paajanen T, Hanninen T, Aitken A, et al. CERAD Neuropsychological Total Scores Reflect Cortical Thinning in Prodromal Alzheimer's Disease. Dementia and geriatric cognitive disorders extra. 2013;3:446-58.

99. van Velsen EF, Vernooij MW, Vrooman HA, et al. Brain cortical thickness in the general elderly population: the Rotterdam Scan Study. Neuroscience letters. 2013; 550:189-94.

100. Seo SW, Im K, Lee JM, et al. Cortical thickness in single- versus multiple-domain amnestic mild cognitive impairment. Neuroimage. 2007;36:289-97.

101. Seo SW, Im K, Lee JM, et al. Effects of demographic factors on cortical thickness in Alzheimer's disease. Neurobiology of aging. 2011;32:200-9.

102. Fischl B, Salat DH, Busa E, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron. 2002;33:341-55.

103. van den Bogaard SJ, Dumas EM, Acharya TP, et al. Early atrophy of pallidum and accumbens nucleus in Huntington's disease. J Neurol. 2011;258:412-20.

104. Mak E, Bergsland N, Dwyer MG, Zivadinov R, Kandiah N. Subcortical atrophy is associated with cognitive impairment in mild Parkinson disease: a combined investigation of volumetric changes, cortical thickness, and vertex-based shape analysis. AJNR Am J Neuroradiol. 2014;35:2257-64.

105. Hartberg CB, Sundet K, Rimol LM, et al. Subcortical brain volumes relate to neurocognition in schizophrenia and bipolar disorder and healthy controls. Progress in neuro-psychopharmacology & biological psychiatry. 2011;35:1122-30.

106. Schoonheim MM, Popescu V, Rueda Lopes FC, et al. Subcortical atrophy and cognition: sex effects in multiple sclerosis. Neurology. 2012;79:1754-61.

107. Stepan-Buksakowska I, Szabo N, Horinek D, et al. Cortical and subcortical atrophy in Alzheimer disease: parallel atrophy of thalamus and hippocampus. Alzheimer Dis Assoc Disord. 2014;28:65-72.

108. de Jong LW, van der Hiele K, Veer IM, et al. Strongly reduced volumes of putamen and thalamus in Alzheimer's disease: an MRI study. Brain. 2008;131:3277-85.

109. Roh JH, Qiu A, Seo SW, et al. Volume reduction in subcortical regions according to severity of Alzheimer's disease. J Neurol. 2011;258:1013-20.

110. Cho H, Seo SW, Kim JH, et al. Changes in subcortical structures in early- versus late-onset Alzheimer's disease. Neurobiology of aging. 2013;34:1740-7.

111. Mrzilkova J, Zach P, Bartos A, Tintera J, Ripova D. Volumetric analysis of the pons, cerebellum and hippocampi in patients with Alzheimer's disease. Dement Geriatr Cogn Disord. 2012;34:224-34.

112. Li W, van Tol MJ, Li M, et al. Regional specificity of sex effects on subcortical volumes across the lifespan in healthy aging. Human brain mapping. 2014;35:238-47.

113. Fjell AM, Westlye LT, Amlien I, et al. High consistency of regional cortical thinning in aging across multiple samples. Cerebral cortex. 2009;19:2001-12.

114. Walhovd KB, Fjell AM, Reinvang I, et al. Effects of age on volumes of cortex, white matter and subcortical structures. Neurobiology of aging. 2005;26: 1261-70.

115. Goodro M, Sameti M, Patenaude B, Fein G. Age effect on subcortical structures in healthy adults. Psychiatry research. 2012;203:38-45.

116. Thong JY, Du J, Ratnarajah N, et al. Abnormalities of cortical thickness, subcortical shapes, and white matter integrity in subcortical vascular cognitive impairment. Human brain mapping. 2014;35:2320-32.

117. Lassen NA. Autoregulation of cerebral blood flow. Circulation research 1964;15:suppl:201-4.

118. Hardy P, Varma DR, Chemtob S. Control of cerebral and ocular blood flow autoregulation in neonates. Pediatric clinics of North America. 1997;44:137-52.

119. Delaey C, Van De Voorde J. Regulatory mechanisms in the retinal and choroidal circulation. Ophthalmic research. 2000;32:249-56.

120. Schultz SK, O'Leary DS, Boles Ponto LL, Watkins GL, Hichwa RD, Andreasen NC. Age-related changes in regional cerebral blood flow among young to mid-life adults. Neuroreport. 1999;10:2493-6.

121. Embleton SJ, Hosking SL, Roff Hilton EJ, Cunliffe IA. Effect of senescence on ocular blood flow in the retina, neuroretinal rim and lamina cribrosa, using scanning laser Doppler flowmetry. Eye. 2002;16:156-62.

122. Abernethy WB, Bell MA, Morris M, Moody DM. Microvascular density of the human paraventricular nucleus decreases with aging but not hypertension. Experimental neurology. 1993;121:270-4.

123. Azemin MZ, Kumar DK, Wong TY, et al. Age-related rarefaction in the fractal dimension of retinal vessel. Neurobiology of aging. 2012;33:194 e1-4.

124. Patton N, Aslam T, Macgillivray T, Pattie A, Deary IJ, Dhillon B. Retinal vascular image analysis as a potential screening tool for cerebrovascular disease: a rationale based on homology between cerebral and retinal microvasculatures. J Anat. 2005;206:319-48.

125. Tso MO, Jampol LM. Pathophysiology of hypertensive retinopathy. Ophthalmology. 1982;89:1132-45.

126. Grosso A, Cheung N, Veglio F, Wong TY. Similarities and differences in early retinal phenotypes in hypertension and diabetes. J Hypertens. 2011;29:1667-75.

127. Bhargava M WT. Current Concepts In Hypertensive Retinopathy. Retinal Physician. 2013;10:43-54.

128. Leishman R. The retinal vessels in hypertensive disease. Proceedings of the Royal Society of Medicine. 1952;45:419-26.

129. Hammes HP, Feng Y, Pfister F, Brownlee M. Diabetic retinopathy: targeting vasoregression. Diabetes. 2011;60:9-16.

130. Cai J, Boulton M. The pathogenesis of diabetic retinopathy: old concepts and new questions. Eye. 2002;16:242-60.

131. Furuta A, Ishii N, Nishihara Y, Horie A. Medullary arteries in aging and dementia. Stroke. 1991;22:442-6.

132. Fredriksson K, Nordborg C, Kalimo H, Olsson Y, Johansson BB. Cerebral microangiopathy in stroke-prone spontaneously hypertensive rats. An immunohistochemical and ultrastructural study. Acta neuropathologica. 1988;75: 241-52.

133. Hubbard LD, Brothers RJ, King WN, et al. Methods for evaluation of retinal microvascular abnormalities associated with hypertension/sclerosis in the Atherosclerosis Risk in Communities Study. Ophthalmology. 1999;106:2269-80.

134. Wong TY, Kamineni A, Klein R, et al. Quantitative retinal venular caliber and risk of cardiovascular disease in older persons: the cardiovascular health study. Arch Intern Med. 2006;166:2388-94.

135. Ikram MK, de Jong FJ, Bos MJ, et al. Retinal vessel diameters and risk of stroke: the Rotterdam Study. Neurology. 2006;66:1339-43.

136. Ikram MK, Witteman JC, Vingerling JR, Breteler MM, Hofman A, de Jong PT. Retinal vessel diameters and risk of hypertension: the Rotterdam Study. Hypertension. 2006;47:189-94.

137. Sun C, Wang JJ, Mackey DA, Wong TY. Retinal vascular caliber: systemic, environmental, and genetic associations. Survey of ophthalmology. 2009;54:74-95.

138. Liew G, Sharrett AR, Wang JJ, et al. Relative importance of systemic determinants of retinal arteriolar and venular caliber: the atherosclerosis risk in communities study. Arch Ophthalmol. 2008;126:1404-10.

139. Cheung CY, Hsu W, Lee ML, et al. A new method to measure peripheral retinal vascular caliber over an extended area. Microcirculation. 2010;17:495-503.

140. Cheung CY, Thomas GN, Tay W, et al. Retinal vascular fractal dimension and its relationship with cardiovascular and ocular risk factors. American journal of ophthalmology. 2012;154:663-74 e1.

141. Mainster MA. The fractal properties of retinal vessels: embryological and clinical implications. Eye.1990;4:235-41.

142. Avakian A, Kalina RE, Sage EH, et al. Fractal analysis of region-based vascular change in the normal and non-proliferative diabetic retina. Curr Eye Res. 2002;24:274-80.

143. Cheung CY, Zheng Y, Hsu W, et al. Retinal vascular tortuosity, blood pressure, and cardiovascular risk factors. Ophthalmology. 2011;118:812-8.

144. Wong TY, Klein R, Couper DJ, et al. Retinal microvascular abnormalities and incident stroke: the Atherosclerosis Risk in Communities Study. Lancet. 2001; 358:1134-40.

145. Wong TY, Klein R, Sharrett AR, et al. Cerebral white matter lesions, retinopathy, and incident clinical stroke. JAMA : the journal of the American Medical Association. 2002;288:67-74.

146. Wong TY, Mosley TH, Jr., Klein R, et al. Retinal microvascular changes and MRI signs of cerebral atrophy in healthy, middle-aged people. Neurology. 2003;61: 806-11.

147. Mitchell P, Wang JJ, Wong TY, Smith W, Klein R, Leeder SR. Retinal microvascular signs and risk of stroke and stroke mortality. Neurology. 2005;65: 1005-9.

148. Cooper LS, Wong TY, Klein R, et al. Retinal microvascular abnormalities and MRI-defined subclinical cerebral infarction: the Atherosclerosis Risk in Communities Study. Stroke. 2006;37:82-6.

149. Longstreth W, Jr., Larsen EK, Klein R, et al. Associations between findings on cranial magnetic resonance imaging and retinal photography in the elderly: the Cardiovascular Health Study. Am J Epidemiol. 2007;165:78-84.

150. Qiu C, Cotch MF, Sigurdsson S, et al. Microvascular lesions in the brain and retina: The age, gene/environment susceptibility-Reykjavik study. Ann Neurol. 2009; 65:569-76.

151. Yatsuya H, Folsom AR, Wong TY, Klein R, Klein BE, Sharrett AR. Retinal microvascular abnormalities and risk of lacunar stroke: Atherosclerosis Risk in Communities Study. Stroke. 2010;41:1349-55.

152. Kawasaki R, Cheung N, Mosley T, et al. Retinal microvascular signs and 10-year risk of cerebral atrophy: the Atherosclerosis Risk in Communities (ARIC) study. Stroke. 2010;41:1826-8.

153. Qiu C, Cotch MF, Sigurdsson S, et al. Retinal and cerebral microvascular signs and diabetes: the age, gene/environment susceptibility-Reykjavik study. Diabetes. 2008;57:1645-50.

154. Wieberdink RG, Ikram MK, Koudstaal PJ, Hofman A, Vingerling JR, Breteler MM. Retinal vascular calibers and the risk of intracerebral hemorrhage and cerebral infarction: the Rotterdam Study. Stroke. 2010;41:2757-61.

155. Ikram MK, De Jong FJ, Van Dijk EJ, et al. Retinal vessel diameters and cerebral small vessel disease: the Rotterdam Scan Study. Brain : a journal of neurology. 2006;129:182-8.

156. Bettermann K, Slocomb JE, Shivkumar V, Lott ME. Retinal vasoreactivity as a marker for chronic ischemic white matter disease? Journal of the neurological sciences. 2012;322:206-10.

157. Kawasaki R, Che Azemin MZ, Kumar DK, et al. Fractal dimension of the retinal vasculature and risk of stroke: a nested case-control study. Neurology. 2011; 76:1766-7.

158. Cavallari M, Falco T, Frontali M, Romano S, Bagnato F, Orzi F. Fractal analysis reveals reduced complexity of retinal vessels in CADASIL. PloS one. 2011; 6:e19150.

159. Vahedi K, Massin P, Guichard JP, et al. Hereditary infantile hemiparesis, retinal arteriolar tortuosity, and leukoencephalopathy. Neurology. 2003;60:57-63.

160. Baker ML, Marino Larsen EK, Kuller LH, et al. Retinal microvascular signs, cognitive function, and dementia in older persons: the Cardiovascular Health Study. Stroke. 2007;38:2041-7.

161. Schrijvers EM, Buitendijk GH, Ikram MK, et al. Retinopathy and risk of dementia: the Rotterdam Study. Neurology. 2012;79:365-70.

162. Gatto NM, Varma R, Torres M, et al. Retinal microvascular abnormalities and cognitive function in Latino adults in Los Angeles. Ophthalmic Epidemiol. 2012; 19:127-36.

163. Liew G, Mitchell P, Wong TY, et al. Retinal microvascular signs and cognitive impairment. J Am Geriatr Soc. 2009;57:1892-6.

164. Lesage SR, Mosley TH, Wong TY, et al. Retinal microvascular abnormalities and cognitive decline: the ARIC 14-year follow-up study. Neurology. 2009;73:862-8.

165. de Jong FJ, Schrijvers EM, Ikram MK, et al. Retinal vascular caliber and risk of dementia: the Rotterdam study. Neurology. 2011;76:816-21.

166. Berisha F, Feke GT, Trempe CL, McMeel JW, Schepens CL. Retinal abnormalities in early Alzheimer's disease. Invest Ophthalmol Vis Sci. 2007;48: 2285-9.

167. Cheung CY, Ong S, Ikram MK, et al. Retinal Vascular Fractal Dimension is Associated with Cognitive Dysfunction. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association. 2014;23:43-50.

168. Frost S, Kanagasingam Y, Sohrabi H, et al. Retinal vascular biomarkers for early detection and monitoring of Alzheimer's disease. Translational psychiatry. 2013;3:e233.

169. London A, Benhar I, Schwartz M. The retina as a window to the brain-from eye research to CNS disorders. Nature reviews Neurology. 2013;9:44-53.

170. Gabriele ML, Wollstein G, Ishikawa H, et al. Three dimensional optical coherence tomography imaging: advantages and advances. Progress in retinal and eye research. 2010;29:556-79.

171. Petzold A, de Boer JF, Schippling S, et al. Optical coherence tomography in multiple sclerosis: a systematic review and meta-analysis. Lancet Neurol. 2010;9: 921-32.

172. Sakata LM, Deleon-Ortega J, Sakata V, Girkin CA. Optical coherence tomography of the retina and optic nerve - a review. Clinical & experimental ophthalmology. 2009;37:90-9.

173. Koh VT, Tham YC, Cheung CY, et al. Determinants of ganglion cell-inner plexiform layer thickness measured by high-definition optical coherence tomography. Invest Ophthalmol Vis Sci. 2012;53:5853-9.

174. Kergoat H, Kergoat MJ, Justino L, Robillard A, Bergman H, Chertkow H. Normal optic nerve head topography in the early stages of dementia of the Alzheimer type. Dement Geriatr Cogn Disord. 2001;12:359-63.

175. Liu M, Duggan J, Salt TE, Cordeiro MF. Dendritic changes in visual pathways in glaucoma and other neurodegenerative conditions. Experimental eye research. 2011;92:244-50.

176. Buckingham BP, Inman DM, Lambert W, et al. Progressive ganglion cell degeneration precedes neuronal loss in a mouse model of glaucoma. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2008;28:2735-44.

177. Moschos MM, Markopoulos I, Chatziralli I, et al. Structural and functional impairment of the retina and optic nerve in Alzheimer's disease. Curr Alzheimer Res. 2012;9:782-8.

178. Kirbas S, Turkyilmaz K, Anlar O, Tufekci A, Durmus M. Retinal nerve fiber layer thickness in patients with Alzheimer disease. Journal of neuro-ophthalmology : the official journal of the North American Neuro-Ophthalmology Society. 2013;33: 58-61.

179. Larrosa JM, Garcia-Martin E, Bambo MP, et al. Potential new diagnostic tool for Alzheimer's disease using a linear discriminant function for Fourier domain optical coherence tomography. Invest Ophthalmol Vis Sci. 2014;55:3043-51.

180. Parisi V, Restuccia R, Fattapposta F, Mina C, Bucci MG, Pierelli F. Morphological and functional retinal impairment in Alzheimer's disease patients. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology. 2001;112:1860-7.

181. Iseri PK, Altinas O, Tokay T, Yuksel N. Relationship between cognitive impairment and retinal morphological and visual functional abnormalities in

Alzheimer disease. Journal of neuro-ophthalmology : the official journal of the North American Neuro-Ophthalmology Society. 2006;26:18-24.

182. Garcia-Martin ES, Rojas B, Ramirez AI, et al. Macular thickness as a potential biomarker of mild Alzheimer's disease. Ophthalmology. 2014;121:1149-51 e3.

183. Laude A, Lascaratos G, Henderson RD, Starr JM, Deary IJ, Dhillon B. Retinal nerve fiber layer thickness and cognitive ability in older people: the Lothian Birth Cohort 1936 study. BMC ophthalmology. 2013;13:28.

184. van Koolwijk LM, Despriet DD, Van Duijn CM, et al. Association of cognitive functioning with retinal nerve fiber layer thickness. Invest Ophthalmol Vis Sci. 2009;50:4576-80.

185. Marziani E, Pomati S, Ramolfo P, et al. Evaluation of retinal nerve fiber layer and ganglion cell layer thickness in Alzheimer's disease using spectral-domain optical coherence tomography. Invest Ophthalmol Vis Sci. 2013;54:5953-8.

186. Garcia-Martin E, Larrosa JM, Polo V, et al. Distribution of retinal layer atrophy in patients with Parkinson disease and association with disease severity and duration. American journal of ophthalmology. 2014;157:470-8.

187. Adam CR, Shrier E, Ding Y, Glazman S, Bodis-Wollner I. Correlation of inner retinal thickness evaluated by spectral-domain optical coherence tomography and contrast sensitivity in Parkinson disease. Journal of neuro-ophthalmology : the official journal of the North American Neuro-Ophthalmology Society. 2013;33:137-42.

188. Ratchford JN, Saidha S, Sotirchos ES, et al. Active MS is associated with accelerated retinal ganglion cell/inner plexiform layer thinning. Neurology. 2013;80: 47-54.

189. van Es AC, van der Grond J, de Craen AJ, et al. Cerebral microbleeds and cognitive functioning in the PROSPER study. Neurology. 2011;77:1446-52.

190. Poels MM, Ikram MA, van der Lugt A, et al. Cerebral microbleeds are associated with worse cognitive function: the Rotterdam Scan Study. Neurology. 2012;78:326-33.

191. Wong KS, Ng PW, Tang A, Liu R, Yeung V, Tomlinson B. Prevalence of asymptomatic intracranial atherosclerosis in high-risk patients. Neurology. 2007;68: 2035-8.

192. De Silva DA, Ancalan M, Doshi K, Chang HM, Wong MC, Chen C. Intracranial large artery disease in Alzheimer's disease and vascular dementia among ethnic Asians. Eur J Neurol. 2009;16:643-5.

193. Karas GB, Scheltens P, Rombouts SA, et al. Global and local gray matter loss in mild cognitive impairment and Alzheimer's disease. Neuroimage. 2004;23:708-16.

194. Trivedi MA, Wichmann AK, Torgerson BM, et al. Structural MRI discriminates individuals with Mild Cognitive Impairment from age-matched controls: a combined neuropsychological and voxel based morphometry study. Alzheimers Dement. 2006;2:296-302.

195. Ryan NS, Keihaninejad S, Shakespeare TJ, et al. Magnetic resonance imaging evidence for presymptomatic change in thalamus and caudate in familial Alzheimer's disease. Brain. 2013;136:1399-414.

196. Cheung N, Mosley T, Islam A, et al. Retinal microvascular abnormalities and subclinical magnetic resonance imaging brain infarct: a prospective study. Brain. 2010;133:1987-93.

197. De Silva DA, Manzano JJ, Liu EY, et al. Retinal microvascular changes and subsequent vascular events after ischemic stroke. Neurology. 2011;77:896-903.

198. Wong TY, Klein R, Sharrett AR, et al. Retinal arteriolar narrowing and risk of diabetes mellitus in middle-aged persons. JAMA : the journal of the American Medical Association. 2002;287:2528-33.

199. Ding J, Strachan MW, Reynolds RM, et al. Diabetic retinopathy and cognitive decline in older people with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. Diabetes. 2010;59:2883-9.

200. Haan M, Espeland MA, Klein BE, et al. Cognitive function and retinal and ischemic brain changes: the Women's Health Initiative. Neurology. 2012;78:942-9.

201. Ong SY, Cheung CY, Li X, et al. Visual Impairment, Age-Related Eye Diseases, and Cognitive Function: The Singapore Malay Eye Study. Arch Ophthalmol. 2012;12:12.

202. Ding J, Strachan MW, Fowkes FG, et al. Association of retinal arteriolar dilatation with lower verbal memory: the Edinburgh Type 2 Diabetes Study. Diabetologia. 2011;54:1653-62.

203. Kim DH, Newman AB, Hajjar I, et al. Retinal microvascular signs and functional loss in older persons: the cardiovascular health study. Stroke. 2011;42: 1589-95.

204. Paquet C, Boissonnot M, Roger F, Dighiero P, Gil R, Hugon J. Abnormal retinal thickness in patients with mild cognitive impairment and Alzheimer's disease. Neuroscience letters. 2007;420:97-9.

205. Kesler A, Vakhapova V, Korczyn AD, Naftaliev E, Neudorfer M. Retinal thickness in patients with mild cognitive impairment and Alzheimer's disease. Clinical neurology and neurosurgery. 2011;113:523-6.

CHAPTER 2 – TABLES

Table 2 – 1 (a): Association of established cerebral small vessel disease markers (infarcts and white matter hyperintensities) with stroke and cognition

Authors and Study	Study type and population	Cerebrovascular disease markers	Outcome/Evaluation	Findings with effect measure and 95% confidence interval or p value
Vermeer S, et al. 2003 [32] (Rotterdam Scan Study)	Prospective population based study; n= 1015; ages 60-90 years	Silent brain infarcts and white matter hyperintensities	Risk of stroke	- Silent brain infarcts, HR: 3.5 (2.0 , 6.0) - White matter hyperintensities, HR: 3.5 (1.4 , 8.6)
Vermeer S, et al. 2003 [33] (Rotterdam Scan Study)	Prospective longitudinal population based study; n= 1015; ages 60-90 years	Silent brain infarcts	Risk of dementia and cognitive decline	HR: 2.26 (1.09 , 4.70)
Vermeer S, et al. 2007 [38] (Review)	Population and hospital based, case series; n= 105; ages 34-97 years	Silent brain infarcts	Physical, cognitive disabilities, risk of stroke	- Associated with physical, depressive symptoms, worse cognitive ability, subsequent stroke, risk of dementia
Benjamin P, et al. 2014 [34] (St George's Cognition and Neuroimaging in Stroke (SCANS) study)	Cross sectional study; n= 120; ages ≥ 60 years	Lacunes count	Cognitive tests: Trail making test, Modified Wis- consin Card Sorting Test, Phonemic Fluency, Wechsler Adult Intelligence Scale-III, Digit symbol substitution, Speed of Information Processing Task, Grooved Pegboard Task, Digit Span Task, Wechsler Memory Scale-III, Logical Memory and Visual Reproduction	- Executive function, ß: −0.377 (p= 0.001) - Processing speed, ß: −0.430 (p= 0.001) - Working memory, ß: −0.207 (p= 0.028) - Episodic memory, ß: −0.189 (p= 0.045)
Debette S, et al. 2010 [35] (Systemic review)	Longitudinal studies; n= 53 articles	White matter hyperintensities	Dementia, mortality	- Incident dementia, HR: 1.9 (1.3 , 2.8) - Mortality, HR: 2.0 (1.6 , 2.7)
Mortamais M, et al. 2013 [37] (Review)	Longitudinal studies in general population	White matter hyperintensities	Risk of dementia, risk of conversion to dementia and cognitive decline	- Risk of dementia, HR: 2.9 (1.3 , 6.3) - Conversion to AD, HR: 1.2 (0.7 , 2.2) - Conversion to other dementias, HR 5.8 (1.2 – 26.6) - Cognitive decline, HR: 3.30 (1.33 , 8.22)
Prins ND, et al. 2015 [39] (Review)	Population and hospital based studies	White matter hyperintensities	Risk of dementia, cognitive decline, disability	- WMH severity and progression linked to cognitive decline and dementia risk - Large confluent WMH with normal cognition can lead to disability within one year of assessment, HR: 2.36 (1.65, 3.81)

HR= Hazard ratio; ß= mean difference

Table 2 – 1 (b): Association of cerebral small vessel disease marker (cerebral microbleeds) with cognition

Authors and Study	Study type and population	Cerebrovascular disease markers	Outcome/Evaluation	Findings with effect measure and 95% confidence interval or p value
Werring DJ, et al. 2004 [49]	Case-control hospital based study; n= 55; age ≥ 60 years	Cerebral microbleeds	Cognitive dysfunction assessed by detailed neuropsychological tests	- Microbleed an independent predictor of executive function impairment OR: 1.32 (1.01 , 1.70) - Modest correlation with other domains Spearman's r = 0.44, (p = 0.03)
Qui C, et al. 2010 [50] (AGES-Reykjavik Study)	Cross-sectional study; n= 3906; age= 76 years	Cerebral microbleeds	California Verbal Learning Test, Digit Symbol Substitution Test, Salt house Figure Comparison Test, Stroop Test, Digit backwards	People with multiple (≥2) CMBs had lower *Z* scores on tests of processing speed (ß: -0.25 (-0.37, -0.12) and executive function (ß: -0.19 (-0.31, -0.07)
van Es AC, et al. 2011[189] (PROSPER study)	Hospital based study; n= 439; age 70-82 years	Cerebral microbleeds	MMSE, Picture Word Learning Test (immediate and delayed), Letter Digit Coding Test, Stroop Color Word Test	Only infratentorial MBs associated with lower score on the Immediate Picture-Word Learning test (p=<0.01) and delayed Picture-Word Learning (p=0.01)
Lei C, et al. 2013 [45] (Systemic review)	Cross sectional studies; n= 7 articles	Cerebral microbleeds	Cognitive function using brief and detailed tests	- Cerebral microbleeds (presence vs. absence), OR: 3.06 (1.59 , 5.89) - Cerebral microbleeds (count), ß: -1.06 (-2.10 , -0.02)
Poels MMF, et al. 2012[190] (Rotterdam Scan Study)	Prospective population based study; n= 3979; age 60-90years	Cerebral microbleeds	MMSE, Word Verbal Learning Test, Stroop test, Letter-Digit Substitution Task, Purdue Pegboard test, Word Fluency Test	Higher number of microbleeds was associated with lower MMSE score (ß: -0.54) and worse performance on tests of information processing speed (ß: -0.46) and motor speed (ß: -0.42)
van der Vlies A, et al. 2012 [48]	Prospective memory clinic study; n= 221; age ≥ 60 years	Cerebral microbleeds	Cognitive decline using mini mental status examination (MMSE)	Microbleeds not associated with cognition - Baseline MMSE score, ß: 0.34 (p= 0.65) - Rate of decline, ß: 0.09 (p= 0.79)

PROSPER=Prospective Study of Pravastatin in the Elderly at Risk; MMSE= mini mental status examination; OR= odds ratios; CMB= cerebral microbleeds; ß= mean difference

Table 2 – 2: Studies with prevalence and association of cerebral small vessel disease (microinfarcts) with other neuroimaging correlates and cognition

Authors and Study	Study type and population	Cerebrovascular disease markers	Methodology	Diagnosis/Evaluation	Findings with effect measure and 95% confidence interval or p value
Postmortem					
Launer LJ, et al. 2011[52] (Honolulu Asia Aging Study)	Hospital based neuropathological study; n=436; age > 73 years	Cerebral microinfarcts	Autopsy	Cognitive Abilities Screening Instrument/ Dementia and non-dementia	- Microinfarcts associated with poorer performance in cognitive testing in non-demented group, β: −0.103 (p=0.003) - No association in demented group, β: −0.56, (p=0.104)
Brundel M, et al. 2012 [55] (Systemic review)	Neuropathological studies; n= 32 articles; age 44-101 years	Cerebral microinfarcts	Autopsy	Dementia and non-dementia	- Prevalence of microinfarcts in non-demented 24%, AD 43% and VaD 62% - Microinfarcts associated with clinical diagnosis of dementia, neuropathological confirmed AD, clinical diagnosis of VaD and cognitive dysfunction - Microinfarcts associated with other cerebrovascular diseases (infarcts, leukoencephalopathy and CAA)
Smith EE, et al 2012 [54] (Review)	Neuropathological-imaging studies	Cerebral microinfarcts	Autopsy	Dementia and non-dementia	Microinfarcts disrupt important cognitive networks and account for some of the neurological dysfunction associated with other MRI lesions such as lacunar infarcts and white matter hyperintensities
Antemortem					
van Veluw SJ, et al 2013 [58]	Hospital based study; n= 24; age 65-80 years	Cerebral cortical microinfarcts	7T and 3T imaging	Dementia and non-dementia	CMIs can be detected noninvasively using 7T and on 3T MRI. Histopathologic validation of these lesions with similar characteristics on ex vivo MRI confirmed these lesions as CMIs
Ii Y, et al. 2013 [59]	Hospital based study; n= 70; age 41-86 years	Cerebral cortical microinfarcts	3T imaging	Cognitively impaired	Multiple small CMIs visible in intracortical regions on 3T
van Rooden S, et al 2014 [61]	Hospital based study; n= 32; age > 50 years	Cerebral cortical microinfarcts	7T imaging	AD and controls/ MMSE	Patients with AD have more microinfarcts than controls on 7T and is associated with worse global performance on MMSE (p= 0.009)
van Dalen JW, et al 2015 [60]	Population based study; n= 194; age 72-80 years	Cerebral cortical microinfarcts	3T imaging	Non demented hypertensive	Prevalence of CMIs on 3T is 6%. Age and history of stroke are the major risk factors.

T= Tesla; AD= Alzheimer's disease; MMSE= Mini Mental Status Examination; β = mean difference; VaD=Vascular dementia; CAA= cerebral amyloid angiopathy; MRI= Magentic resonance imaging; CMIs= cortical cerebral microinfarcts

Table 2 – 3 (a): Studies with prevalence of cerebral large vessel disease -extra and intracranial arterial stenosis in asymptomatic, stroke & demented subject

Authors and Study	Study type and population	Cerebrovascular disease markers	Methodology	Diagnosis/Evaluation	Findings with effect measure and 95% confidence interval or p value
Postmortem					
Roher, et al. 2003 [68]	Neuropathological study; n= 54; age > 80 years	Large artery stenosis (intracranial)	Autopsy	AD and non-dementia	- Average stenosis in AD is 30% and in non-demented brains is 20%. - Atherosclerosis-induced brain hypoperfusion contributes to the clinical and pathological manifestations of AD
Beach, et al. 2007 [69]	Case-control neuropathological study; controls n= 92, cases n= 305; age ≥ 70 years	Large artery stenosis (intracranial)	Autopsy	Dementia and non-dementia	- AD vs. controls, OR: 1.31 (1.04 ,1.69) - VaD vs. controls, OR: 2.50 (1.52, 4.10)
Antemortem					
Huang YN, et al. 1997 [77]	Hospital based study; n= 96; age 12-81 years	Large artery stenosis (extracranial and intracranial)	Transcranial doppler and duplex ultrasound	Transient ischemic attack	- Extracranial stenosis was 19% and intracranial stenosis was 51%. - Stenosis of internal carotid and middle cerebral arteries is common in Chinese
Li H, et al. 2003 [78] (Review)	Hospital based studies	Large artery stenosis (extracranial and intracranial)	Angiography, ultrasound, MR angiography	Stroke	- Intracranial stenosis in Asians is 30-83% and in whites it is 8-10%. - Extracranial stenosis in Asians is 6-33% whereas in whites it is 85%
Wong KS, et al. 2007 [191]	Population based study; n= 642; age ≥ 40 years	Large artery stenosis (intracranial)	Transcranial doppler ultrasound	Asymptomatic Chinese subjects	- Prevalence of intracranial stenosis was 6.9%. - Significant risk factors were hypertension, glycosuria, heart disease and history of stroke
De Silva DA et al. 2009 [192]	Hospital based study; AD n= 56, VaD n= 47; age 69-89 years	Large artery stenosis (intracranial stenosis)	Magnetic Resonance Angiography	AD and VaD	Prevalence of intracranial stenosis among VaD patients was 53%, significantly higher than AD patients (18%; P = < 0.001)

AD= Alzheimer's disease; VaD=Vascular dementia; OR= odds ratios

Table 2 – 3 (b): Studies showing association of cerebral large vessel disease- <u>extra cranial arterial stenosis with cognition</u> in stroke, asymptomatic and symptomatic subjects

Authors and Study	Study type and population	Cerebrovascular disease markers	Methodology	Diagnosis/Evaluation	Findings with effect measure and 95% confidence interval or p value

Bakker FC, et al. 2003 [74]	Hospital based study;
	cases n= 39;
	controls n= 46;
	age 40-78 years
		Large artery stenosis (extracranial)	Digital subtraction angiograms	Transient ischemic attack
				
				Cognitive tests include: Standard progressive matrices, Wechsler memory scale, Verbal learning and memory test; Visual retention test, Modified card sorting test, Trail making test, Word production test, Reaction time
					Patients with carotid artery occlusion and ipsilateral TIA performed worse on cognitive testing compared to controls

Silvestrini M, et al. 2009 [75]	Hospital based study; n= 102 age ≥ 60 years	Large artery stenosis (extracranial)	Duplex ultrasound	Asymptomatic subjects Cognitive tests include: Phonemic Verbal Fluency, Category Verbal Fluency, Coloured Progressive Matrices, Complex Figure Copy Test	Subjects with stenosis had significantly worse performance on phonemic verbal fluency compared to controls (p= <0.05)
Bossema ER, et al. 2006 [71]	Case control hospital based study; n= 64; age ≥ 60 years	Large artery stenosis (extracranial)	Duplex ultrasound	Symptomatic and asymptomatic	Patients with severe stenosis of one or both carotid arteries were impaired in cognitive functioning (p=0.042)
Bossema ER, et al. 2005 [70]	Case control hospital based study; cases n= 60, controls n= 23 age ≥ 60 years	Large artery stenosis (extracranial)	Duplex ultrasound	Symptomatic and asymptomatic subjects Cognitive tests include: Digit Span, Word Learning Test, Doors Test, Verbal Fluency, Trail Making Test, Motor Planning Test, Finger Tapping Test Subjects underwent carotid endarterectomy	Significant improvements up to 1 year were demonstrated for the - Retrieval of verbal material (p=0.008), - Planning speed of movement (p=0.02), - Finger tapping (p=0.04)

TIA= transient ischemic attack

Table 2 – 3 (b) continued: Studies showing association of cerebral large vessel disease (extra cranial arterial stenosis) with cognition in stroke, asymptomatic and symptomatic subjects

Authors and Study	Study type and population	Cerebrovascular disease markers	Methodology	Diagnosis/Evaluation	Findings with effect measure and 95% confidence interval or p value
Mathiesen EB, et al. 2004 [73] (Tromso study)	Case control hospital based study; cases n= 189, controls n= 201 age 55-74 years	Large vessel stenosis (extracranial)	Doppler ultrasound	Asymptomatic subjects Digit Span Forward and Backward Test, Seashore Rhythm Test, Trail Making Test (Parts A and B), Grooved Pegboard, Verbal and Visual Paired Associates immediate and 30-minute delayed recall, Controlled Oral Word Association Test, Wechsler Adult Intelligence Scale	Patients with stenosis perform worse in the following tests; - Seashore Rhythm, OR: 2.28 (1.67, 4.47) - Trail Making Test A, OR:3.88 (1.85, 8.14) - Trail Making Test B, OR:4.21 (1.94, 9.14) - Verbal Pair Association, immediate recall, OR: 2.83 (1.43, 5.62) - Visual Pair Association, immediate recall, OR: 3.21 (1.58, 6.49) - Grooved Pegboard Test, OR: 4.38 (1.93, 9.91)
Landgraff NC, et al. 2010 [72]	Hospital based study; n= 79; age 48-88 years	Large vessel stenosis (extracranial)	Computed Tomographic Arteriography/ MRI	Asymptomatic subjects Cognitive tests include RBANS testing for immediate memory, visuospatial/ constructional, language, attention and delayed memory	- In complete occluded group significant cognitive deficits were found in all domains except immediate memory - In the moderately stenotic group, there was significant cognitive decline in all domains - In the severely stenotic group, there was significant cognitive deficit in all domains with the exception of language
Poels MMF, et al. 2007 [76] (Rotterdam scan study)	Prospective population based study; n= 2767; age ≥ 55 years	Large vessel stenosis (extracranial)	Arterial stiffness measured by Pulse wave velocity and Carotid distensibility	Normal subjects Cognitive tests include MMSE, Letter-Digit Substitution Task, Stroop Test and Word Fluency Test	Arterial stiffness is not associated with cognitive decline and risk of dementia, HR: 0.91 (0.75 , 1.10)

MRI= magnetic resonance imaging; RBANS= Repeatable Battery for the Assessment of Neuropsychological Status; MMSE= mini mental status examination; OR= odds ratios; HR= hazards ratios

Table 2 – 4 (a): Studies showing cerebral thickness/volume in cognitive impairment and Alzheimer's dementia

Authors and Study	Study type, population and ethnicity	Markers of involutional changes	Methodology	Diagnosis/ Evaluation	Findings with effect measure and 95% confidence interval or p value
Karas GB, et al. 2004 [193]	Hospital based study; AD = 33, controls = 14, MCI = 22 age ≥ 70 years; Caucasians	Gray matter volume (GM)	Voxel based morphometry (VBM)	Dementia, MCI and controls	- AD subjects had lower mean global GM volume compared to controls (p= < 0.001) - Global GM volume in the MCI group was intermediate between AD and controls (p=<0.001) - VBM showed MCI had local reductions in gray matter in the medial temporal lobe, the insula, and thalamus compared to controls - MCI subjects had more GM in the parietal association areas, anterior and the posterior cingulate compared to AD
Trivedi MA, et al 2006 [194]	Hospital based study; MCI = 15, controls = 15 age ≥ 70 years; Caucasians	Gray matter, white matter volumes	Voxel based morphometry	MCI and controls	- MCI patients display significantly less GM volume in medial temporal lobe and posterior cingulate gyrus compared to controls (p=<0.01) - Discriminative accuracy for distinguishing MCI from controls was 87%
Karas G et al. 2008 [96]	Hospital based study; MCI = 24; age ≥ 70 years; Caucasians	Gray matter volume	Voxel based morphometry	MCI	- Converters had more left parietal atrophy and left lateral temporal lobe atrophy than stable MCI patients (p=<0.001)
Frisoni et al. 2002 [93]	Hospital based study; AD = 29 controls = 26; age ≥ 65 years; Caucasians	Gray matter density	Voxel based morphometry	AD and controls	AD subjects had more localised atrophic regions in the temporal and cingulate gyri, precuneus, insular cortex, caudate nucleus, and frontal cortex compared to controls (p=<0.0001)
Singh V et al. 2006 [94]	Hospital based study; controls= 34, MCI = 62 and AD = 42; age ≥ 70 years; Caucasians	Cortical thickness	Automatic segmentation MNI	AD, MCI and controls	- Cortical thickness decreased significantly when controls were compared to MCI, mainly in the medial temporal lobe region and in some regions of the frontal and the parietal cortices (p<0.05). - With the progression of disease from MCI to AD, a general thinning of the entire cortex was observed
Lerch JP et al. 2005 [91]	Hospital based study; AD = 19, controls=17; age ≥ 60 years; Caucasians	Cortical thickness	Automatic segmentation	AD and controls	- Cortical thickness decline in AD in temporal, orbitofrontal and parietal regions, with the most in medial temporal lobes with a loss of >1.25 millimeters of cortical thickness. - Focal cortical areas decline with progression of the disease as measured by time from baseline scan (p= <0.006) as well as the Mini-Mental State Exam (p=0.06)

AD= Alzeimer's disease; MCI= mild cognitive impairment

Table 2 – 4 (b): Studies showing association between cortical thickness and cognition in cognitive impairment and Alzheimer's dementia

Authors and Study	Study type and population	Markers of involutional changes	Methodology	Diagnosis/Evaluation	Findings with effect measure and 95% confidence interval or p value
Chang YL, et al. 2010 [97] (ADNI study)	Hospital and community based; MCI= 358 controls = 222 age 55-90 years; Caucasians	Cortical thickness	FREE SURFER	MCI and controls Cognitive tests include; MMSE, Auditory Verbal Learning Test, Long delay free recall and a recognition trial, logical memory subtest, Trail making test (A & B), Digit span (backward), Animal fluency and ADAS- Cog	- Compared to MCI high executive function (EF) group, MCI low EF demonstrated cortical thinning in frontal lobe (p=<0.0025) - Compared to MCI high EF, MCI low EF performed worse in verbal memory (p=<0.005)
Paajanen T, et al. 2013 [98]	Hospital and population based study; AD = 27, MCI = 30, controls = 16 age ≥ 70 years; Caucasians	Cortical thickness	FREE SURFER	AD, MCI and controls Cognitive tests include; CERAD battery (Verbal Fluency, 15-item Boston Naming Test, MMSE, 10-item Word List Learning, Recall and Recognition Test, Constructional Praxis, Constructional Praxis Recall)	- CERAD total scores correlated with mean cortical thickness, (r: 0.34–0.38, p= < 0.001) and MMSE (r: 0.19, p = 0.01). - Of the vertex clusters that showed thinning in progressive MCI, 60–75% related to the CERAD total scores and 3% to the MMSE
Seo SW, et al. 2007 [100]	Hospital based study; MCI = 31, controls = 61 age ≥ 65 years; Asian (Korean)	Cortical thickness	Automatic segmentation using CLASP algorithm	Single and multiple domain a MCI and controls Cognitive tests include; Digit Span, Boston Naming Test, Rey–Osterrieth Complex Figure Test, Seoul Verbal Learning Test, Controlled Oral Word Association Test, Stroop Test	- Relative to controls, Single domain –aMCI patients showed cortical thinning in the left medial temporal lobe (p=<0.05) - Multi domain - aMCI patients showed cortical thinning in the left medial temporal lobe, precuneus, and anterior and inferior basal temporal, insular, and temporal association cortices (p=<0.01)
Seo SW, et al. 2011 [101]	Hospital based study; AD = 196, controls = 142, age ≥ 60 years; Asian (Korean)	Cortical thickness	Automatic segmentation using CLASP algorithm	AD and controls Cognitive tests include; Digit Span, Boston Naming Test, Rey–Osterrieth Complex Figure Test, Seoul Verbal Learning Test, Controlled Oral Word Association Test, Stroop Test	High levels of education in the AD group correlated with cortical thinning in the frontal and temporo-parietal association cortices (p=<0.001)

ADNI= Alzheimer Disease Neuroimaging Initiative; AD= Alzheimer's disease; MCI= mild cognitive impairment; MMSE= mini mental status examination; CERAD= Consortium to Establish a Registry for Alzheimer's Disease; CLASP= Consortium Of Local Authorities Special Programme; r= spearman's correlation coefficient

Table 2 – 4 (c): Studies showing effects of demographics and other risk factors on cortical thickness/volumes in healthy subjects

Authors and Study	Study type, population and ethnicity	Markers of involutional changes	Methodology	Diagnosis/ Evaluation	Findings with effect measure and 95% confidence interval or p value
Magnotta VA, et al. 1999 [86]	Community based study; n=148; age 18-82 years; Caucasians	Gyral curvature, sulcul curvature, and cortical depth (cortical thickness)	BRAIN SURF Gyrification measurement	Healthy volunteers	- Sulcal (more flattened and less curve) and gyral (sharp and steeply curved) changes over time (p=<0.001). - Cortical thickness decreases over time (p=<0.001)
Preul C, et al 2006 [87]	Community based study; n= 525; age 17-68 years; Caucasians	Cortical thickness and ventricular enlargement	Automatic segmentation using voxels	Healthy volunteers	- Cortical thickness decreases with age (r: –0.49, p =0.01; r: –0.502, p= <0.01 in males and r: –0.461, p= <0.01 in females). - Ventricles enlarge with age (r: 0.67, p= 0.01; r: 0.71, p= 0.01 for males, and r: 0.63, p= 0.01 for female subjects
Lemaitre H, et al. 2012 [85]	Community based study; n= 216; age 18-87 years; Caucasians	Cortical thickness, volume and surface area	FREE SURFER	Healthy volunteers	- Age related volume reductions in middle frontal gyrus, the superior frontal gyrus and the frontal pole (p=<0.001) - Age-related changes in cortical thickness reductions in superior frontal gyrus, the paracentral gyrus, pars opercularis and triangularis of the inferior frontal gyrus (p= <0.001) - Age-related reduction in surface area in middle frontal gyrus and the superior frontal gyrus
Long X, et al. 2012 [88]	Community subjects; n= 314; age 18-94 years; Caucasians	Cortical surface area, cortical thickness, curvature index, white matter volume	FREE SURFER	Healthy subjects	- Significant cortical thinning observed in parietal (r: 0.553, p= < 0.001) and insula regions (r: 0.405, p= < 0.001) with aging - Surface area and mean curvature less affected by aging relative to cortical thickness and white matter volume
Salat DH, et al. 2004 [89]	Community based study; n= 106; age 18-93 years; Caucasians	Cortical thickness	FREE SURFER	Healthy subjects	- Aging associated with prefrontal lobe atrophy (r^2: 0.25) - Additional atrophy in in frontal cortex near motor cortex (r^2: 0.34) and calcarine cortex (r^2: 0.38)
van Velsen EF, et al. 2013[99] (Rotterdam scan study)	Prospective population based study; n= 1092 age ≥ 55 years; Caucasians	Cortical thickness	FREE SURFER	Healthy subjects	- Women had thicker cortex than men (p=< 0.01) - With increasing age, cortical thickness decreased (approximately 0.2% per year), with the largest age effects for the occipital and temporal lobes - Higher education, higher diastolic blood pressure and larger intra-cranial volume were related to a larger cortical thickness - Diabetes mellitus and higher HDL cholesterol levels were related to a thinner cortex

r= spearman's correlation coefficient; r^2= Pearson's correlation coefficient

Table 2 – 5: Studies showing association of subcortical volumes/density in Alzheimer's dementia, cognitive impairment and cognitively normal subjects

Authors and Study	Study type and population	Markers of involutional changes	Methodology	Diagnosis/ Evaluation	Findings with effect measure and 95% confidence interval or p value
Frisoni, et al. 2002 [93]	Hospital based study; AD = 29 controls = 26; age ≥ 65 years	Gray matter density	Voxel based morphometry	AD and controls	- AD patients had more atrophy in right and left hippocampal/amygdalar complex (p=<0.0001). All parts of the hippocampus (head, body, and tail) were affected. - More localised atrophic regions observed in the temporal and cingulate gyri, precuneus, insular cortex, caudate nucleus, and frontal cortex p=<0.0001)
de Jong LW, et al. 2008 [108]	Hospital based study; AD = 69, memory complainers= 70; age ≥ 60 years	Subcortical structure volume	FMRIB's Integrated Registration and Segmentation Tool (FIRST)	AD and subjective memory complaints Cognitive tests used were; Cambridge Cognitive Examination-Revised and MMSE	- Significant reduction in hippocampus (p=<0.05), thalamus (p=<0.01) and putamen (p=<0.01) in AD patients compared to memory complainers - Decreased volumes of left putamen and thalamus correlate independently to poorer cognitive test results (p=<0.001)
Mrzilková J, et al. 2012 [111]	Hospital based study; AD = 26 controls = 29; age ≥ 65 years	Volumes	Manual volumetric MR analysis	AD with MMSE ≥ 18 and < 18 scores Cognitive tests include: MMSE, Mattis Dementia Rating Scale, Trail Making Test version A and B, Disability Assessment in Dementia, 7-Minute Screen, verbal fluency tests and Edinburgh Handedness Inventory	- Hippocampus volume reduction in both AD groups ≥18 MMSE score (p=0.006) and <18 MMSE score (p=0.02) compared to controls - No reduction in pons and cerebellar volumes
Roh JH, et al. 2011 [109]	Hospital based study; AD= 179, controls = 57; age ≥ 60 years	Subcortical structure volume	Automatic segmentation using Markov random field model	AD and controls. Severity of disease defined by clinical dementia rating scale (CDR) Cognitive tests include; Digit Span, Boston Naming Test, Rey–Osterrieth Complex Figure Test, Seoul Verbal Learning Test, Controlled Oral Word Association Test, Stroop Test	- Volume loss in amygdala and hippocampus in very mild stage of AD (CDR=0.5) - Volume reduction in thalamus and putamen in mild to moderate stages of AD (CDR= 1and 2) - Globus pallidus and caudate reduction in moderate stages (CDR= 2, p = <0.01) - All these structures correlated with cognitive performance (p=<0.01)
Ryan N, et al. 2013 [195]	Hospital based; AD = 20, controls =20; age 30-50 years	Gray matter density	Voxel based morphometry	Presymptomatic, symptomatic AD and controls	Atrophy in caudate (p=<0.001) and thalamus (p=<0.0025) in presymptomatic mutation carriers compared to controls

AD= Alzheimer's disease; MCI= mild cognitive impairment; MMSE= mini mental status examination

Table 2 – 5 (continued): Studies showing association of subcortical volumes in Alzheimer's dementia, cognitive impairment & cognitively normal subjects

Authors and Study	Study type and population	Markers of involutional changes	Methodology	Diagnosis/ Evaluation	Findings with effect measure and 95% confidence interval or p value
Fjell AM, et al. 2009 [113]	Case control study; AD = 96, controls = 1143; age 18-87 years	Subcortical structure volume	FREE SURFER	AD and controls	- Pallidum corrected for intracranial volume showed slightly higher age correlations for men (p=<0.05) - No age effects on men and women cortex - Analysis in AD subjects showed no age and sex interactions
Cho H, et al. 2013 [110]	Case control study; AD = 36, controls = 14 age 50-80 years	Subcortical structure volumes	FREE SURFER	Early onset (EO) and late onset (LO) ADs and controls	- No differences in the volumes of subcortical structures between patients with EOAD and LOAD. - Patients with EOAD showed more rapid volumetric decline in the caudate (p=<0.001), putamen (p=0.003), and thalamus (p=0.001) than patients with LOAD on 3 years of longitudinal follow-up,
Thong JY, et al. 2014 [116]	Case control study; VCI = 55, controls = 25 age ≥ 55 years	Cortical thickness, subcortical shapes	Automatic segmentation	VCI (moderate/ severe and mild) and controls	- Cortex in moderate/severe VCI was thinner in the parietal and lateral temporal cortices than that in VCI mild. - Compared to controls, mild VCI and moderate/severe VCI showed smaller shapes in the thalamus, putamen and globus pallidus
Walhovd KB, et al. 2005 [114]	Community study; n= 73; age 20-88 years	Cortical thickness, subcortical structures	FREE SURFER	Healthy volunteers	- Age effects all cortical and subcortical structures (p= <0.0001) except pallidum and the 4th ventricle - Age relationships for cortex, amygdala, thalamus, accumbens and caudate were linear (p=<0.003) - Age relationship for cerebral white matter, hippocampus, brainstem, cerebellar white, and gray matter, lateral, inferior lateral and 3rd ventricles volume were curvilinear (p=<0.05)
Long X, et al. 2012 [88]	Community subjects; n= 314; age 18-94 years	Cortical surface area, cortical thickness, curvature index, white	FREE SURFER	Healthy subjects	Moderate atrophy observed in subcortical gray matter structures, including the thalamus (r^2: 0.476, p= < 0.001), nucleus accumbens (r^2: 0.525, p= < 0.001), pallidum (r^2: 0.461, p= < 0.001) and putamen (r^2: 0.533, p= < 0.001) with age
Goodro M, et al. 2012 [115]	Community subjects; n= 226; age 19-86 years	Subcortical structures volumes	FMRIB's Integrated Registration and Segmentation Tool	Healthy volunteers	Older subjects (60–85 years of age) showed a stronger correlation with structural volume for the ventricles (p=<0.001), hippocampus (p=<0.07), amygdala (p=<0.01) than middle aged (35–60 years of age) subjects
Li W, et al. 2014 [112]	Community study; n= 76; age 19-69 ye ars	Subcortical structure volumes	FREE SURFER	Healthy subjects	- Age-related absolute atrophy found in the basal ganglia and thalamus in males, females showed disproportionate degeneration - Hippocampus decline only observed in males (p=0.004) - Subcortical structures showed significantly smaller absolute volumes in females than in males (p= < 0.05)

AD= Alzheimer's disease; VCI = Vascular cognitive impairment; MMSE= Mini Mental Status Examination; r^2= Pearson's correlation coefficient;

Table 2 - 6 (a): Studies showing association of retinopathy signs with MRI markers of cerebrovascular diseases

Authors and Study	Study type and population	Retinopathy	Outcome	Findings with effect measure and 95% confidence interval or pvalue
Wong TY, et al. 2001[144] (ARIC)	Prospective population based study; n= 10358; age 51-72 years	Microaneurysms, soft exudates, blot and flame-hemorrhages, arteriovenous nicking	Incident stroke	Any retinopathy associated with incident stroke, - HR: 2.58 (1.59, 4.20) Arteriovenous nicking associated with incident stroke, - HR: 1.60 (1.03, 2.47)
Wong TY, et al. 2002[145] (ARIC)	Prospective population based study; n=1684; age 51-72 years	Microaneurysms, soft exudates, blot and flame-hemorrhages, arteriovenous nicking	Incident clinical stroke	- Persons with WMH had higher incidence of stroke, HR: 3.4 (1.5, 7.7) - Persons with both WMH and retinopathy had higher incidence of stroke, HR: 18.1 (5.9, 55.4)
Wong TY, et al. 2003 [146] (ARIC)	Prospective population based study; n=1684; age 51-72 years	Microaneurysms, soft exudates, blot flame-hemorrhages, AV nicking	Sulcal widening and ventricular enlargement	- Sulcal widening, OR: 1.9 (1.2, 3.0) - Ventricular enlargement, OR: 1.5 (1.0, 2.3)
Mitchell P, et al. 2005 [147] (BMES)	Prospective population based study; n=3583; age>49 years	Microaneurysms, retinal hemorrhages	Incident stroke/TIA/ death	Combined (stroke, TIA and death), RR: 1.7 (1.0, 2.8) Incident stroke, RR: 3.5 (1.5, 8.2)
Cooper LS, et al. 2006 [148] (ARIC)	Cross-sectional population-based study; n=1684; age 55-74 years	Arteriovenous nicking, focal arteriolar narrowing, retinal hemorrhages, soft exudates and microaneurysms, arterio-venous ratio	MRI defined infarcts	Cerebral infarcts associated with retinal microvascular abnormalities, - Arteriovenous nicking, OR: 1.90 (1.25, 2.88) - Focal arteriolar narrowing, OR: 1.89 (1.22, 2.92) - Blot hemorrhages, OR: 2.95 (1.30 , 6.71) - Soft exudates, OR: 2.08 (0.69, 6.31) - Microaneurysms, OR: 3.17 (1.05, 9.64) - Arteriovenous ratio, OR: 1.74 (0.95, 3.21)
Longstreth W, et al. 2007 [149] (CHS)	Prospective population based study; n=1285; age>65 years	Retinopathy, focal arteriolar narrowing, arteriovenous nicking, and arteriovenous ratio	Prevalent infarcts and WMH, incident infarcts and worsening of WMH	Arteriovenous ratio associated with, - Prevalent infarcts, OR: 1.18 (1.05, 1.34), - White matter grade, ß: 0.093; (p =0.011), - Incident infarct, OR: 1.26 (1.09, 1.46) - Worsening white matter grade, OR: 1.12 (0.98, 1.29) Arteriovenous nicking associated with, - Prevalent infarcts, OR: 1.84 (1.23, 2.76) - Incident infarcts, OR: 1.84 (1.15, 2.94)

ARIC= Atheroscelorsis Risk in Communities Study; BMES= Blue Mountain Eye Study; CHS= Cardiovascular Health Study; TIA= Transient Ischemic Attack; Magnetic Resonance Imaging; WMH= white matter hyperintensities; HR= hazard ratios; OR= odds ratios; RR= relative risk; β = mean difference

Table 2 - 6 (a) continued: Studies showing association of retinopathy signs with MRI markers of cerebrovascular diseases

Authors and Study	Study type and population	Retinopathy	Outcome	Findings with effect measure and 95% confidence interval or p value
Qiu C, et al. 2009[150] (AGES- Reykjavik study)	Cross-sectional study; n=4176; mean age=76 years	Retinal focal arteriolar signs (arteriolar narrowing arterio-venous nicking), Retinopathy lesions (retinal blot hemorrhages, microaneurysms)	Cerebral infarcts and WMHs	- Retinal focal arteriolar signs associated with increasing load of subcortical, OR: 1.40 (1.09, 1.79) and periventricular WMHs, OR: 1.64 (1.36, 1.97) - Arteriovenous nicking was significantly associated with subcortical infarcts, OR: 1.33 (1.09, 1.62)
Yatsuya H, et al. 2010 [151] (ARIC)	Prospective population based study; n=10496; age 45-64 years	Microaneurysms, retinal hemorrhages, arteriovenous nicking, arteriolar narrowing	Incident non-lacunar thrombotic and cardioembolic	Retinopathy signs (aneurysm, hemorrhage), - Non-lacunar thrombotic, HR: 2.41 (1.47, 3.95) - Cardioembolic, HR: 2.25 (1.09, 4.65) - Retinal narrowing associated with incident infarct, HR: 2.22 (1.11, 4.48) - Arteriovenous nicking associated with incident infarct, HR: 2.38 (1.20, 4.71)
Cheung N, et al. 2010 [196] (ARIC)	Prospective population based study; n=810; age≥ 55 years	Microaneurysms, soft exudates, blot and flame-hemorrhages, arteriovenous nicking	Incident infarct, incident WMH, WMH progression	Retinopathy (microaneurysms, retinal hemorrhages) with; - Incident cerebral infarct, OR: 2.82 (1.42, 5.60) - Incident lacunar infarct, OR: 3.19 (1.56, 6.50) Retinal arteriovenous nicking with; - Incident cerebral infarct, OR: 2.82 (1.66, 4.76) - Lacunar infarct, OR: 2.48 (1.39, 4.40) - WMH incidence, OR: 2.12 (1.18, 3.81) - Progression of WMH, OR: 2.22 (1.00, 5.88)
Kawasaki, et al. 2010 [152] (ARIC)	Prospective population based study; n=810; age≥ 55 years	Microaneurysms, soft exudates, blot and flame-hemorrhages, arteriovenous nicking	10-year sulcal widening and ventricular enlargement	- Retinopathy, OR: 2.03 (1.20, 4.42) - Arteriovenous nicking, OR: 2.19 (1.23, 3.90)
de Silva DA, et al. 2011[197] (MCRS)	Prospective population based study; n=652,	Arteriovenous nicking, arteriolar narrowing	Recurrent vascular events (cerebrovascular, coronary, vascular death, and composite vascular events)	- Arteriovenous, HR: 2.28 (1.20, 4.33) - Focal arteriolar narrowing, HR: 2.75 (1.14, 6.63)
Qiu C, et al. 2008[153] (AGES- Reykjavik study)	Cross-sectional study; n=4218, mean age=76 years	AV nicking, focal arteriolar narrowing, microaneurysms/ hemorrhages	Multiple cerebral microbleeds	- AV nicking, OR: 1.44 (1.06, 1.95) - Focal arteriolar narrowing, OR: 1.45 (1.01, 2.09) - Microaneurysms/ hemorrhages, OR: 1.75 (1.25, 2.45)

ARIC= Atheroscelorsis Risk in Communities Study; MCRS= Multi-center Retinal Stroke Study; WMH= white matter hyperintensities; OR= odds ratios; HR= hazard ratios

Table 2 - 6 (b): Studies showing association of retinal vascular parameters with MRI markers of cerebrovascular diseases

Authors and Study	Study type and population	Retinal vascular parameters	Outcome	Findings with effect measure and 95% confidence interval or p value
Wong TY, et al. 2006 [134] (CHS)	Prospective cohort study; n=1992; age 69-97 years	Retinal vascular calibers	Incident stroke	Venular widening, HR: 2.2 (1.1, 4.3)
Ikram MK, et al. 2006 [135] (RSS)	Prospective cohort study; n=5540; age ≥ 55 years	Retinal vascular calibers	Incident stroke	Large venular diameters associated with increased risk for - Stroke, HR: 1.12 (1.02, 1.24) - cerebral infarction, HR: 1.15 (1.02, 1.29)
Ikram MK, et al. 2006 [155] (RSS)	Prospective cohort study; n=490; age 60-90 years	Retinal vascular calibers	Changes in WMH and incident lacunar infarcts	Large venular diameters associated with increased risk for, - Periventricular WMH progression, HR: 1.71 (1.11, 2.61) - Subcortical WMH progression, HR: 1.72 (1.09, 2.71) - Incident lacunar infarcts, HR: 1.59 (1.06, 2.39)
Yatsuya H, et al. 2010 [151] (ARIC)	Prospective population based study; n=10496; age 45-64 years	Retinal vascular calibers	Incident lacunar stroke	- Arteriolar narrowing, OR: 1.67 (1.23, 2.26) - Venular widening, OR: 1.44 (1.09, 1.91)
Wieberdink RG, et al. 2010 [154] (RSS)	Prospective cohort study; n=5518; age≥ 55 years	Retinal vascular calibers	Incident stroke	Large venular caliber associated with an increased risk for - Stroke, HR: 1.20 (1.09, 1.33), - Cerebral infarction, HR: 1.28 (1.13, 1.46) - Intracerebral hemorrhage, HR: 1.53 (1.09, 2.15)
Kawasaki R, et al. 2011[157] (BMES)	Nested case-control study; stroke =10, controls=184, age ≥ 70 years	Fractal dimension	Incident stroke or mortality	OR: 1.39 (1.06, 1.83)
Bettermann K et al. 2012 [156]	Case-control study; chronic ischemic white matter disease =12, controls = 14; age 43-85 years	Retinal vasoreactivity	Chronic WMH	Increased WMH (p=0.006)

ARIC= Atheroscelorsis Risk in Communities Study; BMES= Blue Mountain Eye Study; CHS= Cardiovascular Health Study; RSS= Rotterdam Scan Study; WMH= white matter hyperintensities; HR= hazard ratios; OR= odds ratios

Table 2 - 6 (c): Studies showing association of retinopathy signs with cognition

Authors and Study	Study type and population	Retinopathy signs	Outcome/ Evaluation	Findings with effect measure and 95% confidence interval or p value
Baker et al. 2007 [160] (CHS)	Cross-sectional population based study, n=2211, age 69-97 years	Microaneurysms, soft exudates, blot and flame-hemorrhages, arteriolar narrowing	Dementia Cognitive test: Digit-Symbol Substitution Test, MMSE	Retinopathy associated with; - Dementia, OR: 2.10 (1.04, 4.24) - Lower mean Digit-Symbol Substitution Test scores (p=0.002) Focal arteriolar narrowing associated with - Dementia, OR: 3.02 (1.51, 6.02)
Qiu C, et al. 2010 [50] (AGES-Reykjavik Study)	Cross-sectional study; n=3906, age 66-96 years	Microaneurysms, soft exudates, blot and flame-hemorrhages	Vascular dementia Cognitive test: California Verbal Learning Test, Digit Symbol Substitution Test, Salt house Figure Comparison Test, Stroop Test, Digit backwards	OR: 1.95 (1.04 to 3.62) Persons with multiple microbleeds and retinopathy had lower Z scores on tests of - Processing speed, ß: -0.25 (-0.37, -0.12) - Executive function, ß: -0.19 (-0.31, -0.07)
Schrijvers EM, et al. 2012 [161] (RSS)	Cross-sectional population based study, n=6273, age≥55 years	Microaneurysms, soft exudates, blot and flame-hemorrhages,	Dementia, AD and Vascular dementia	- Dementia, OR: 1.92 (1.24, 2.98) - AD, OR: 1.89 (1.15, 3.10) - Vascular dementia, OR: 2.00 (0.71, 5.63)
Wong TY, et al. 2002 [198] (ARIC)	Cross-sectional population based study; n=8734, age 51-70 years	Microaneurysms, soft exudates, blot and flame-hemorrhages	Cognitive function tested on; Delayed word recall test, Digit Symbol subtest, Word Fluency test	- Delayed Word Recall Test, OR: 2.60 (1.70, 3.99) - Digit Symbol subtest, OR: 1.91 (1.04, 3.49) - Word Fluency Test, OR: 2.03 (1.07, 3.86)
Liew G, et al. 2009 [163]	Cross-sectional population based study; n=1988, age 49-97 years	Microaneurysms, hemo-rrhages, hard/soft exudates	Cognitive impairment defined on MMSE (≤23)	OR: 1.7 (1.0, 3.2) in hypertensives
Lesage SR, et al. 2009 [164] (ARIC)	Prospective population based study; n=803, age 55-72 years	Microaneurysms, soft exudates, blot and flame-hemorrhages	Cognitive function tested on; Delayed word recall test, Digit Symbol subtest, Word Fluency test	- Decline in Word Fluency, score difference: -1.70 (-3.3, -0.02) - Decline in Digit Symbol, OR: 2.18 (1.02, 4.64)
Ding J, et al. 2010 [199] (The Edinburgh Type 2 Diabetes Study)	Cross-sectional population based study; n=1044; age 60-75 years	Retinopathy	Faces and Family Pictures Sub-test, Matrix Reasoning, Letter-Number Sequencing, Digit Symbol Test, Borkowski Verbal Fluency Test, Trail Making Test	- General cognitive ability (Π^2= 0.020, p=<0.001) - Verbal Fluency Test, (Π^2= 0.020, p=0.001) - Trail Making Test, (Π^2=0.012, p=0.009) - Digit Span Test, (Π^2=0.032, p=0.001)
Haan M, et al. 2012 [200] (WHIMS & WHISE)	Prospective population based study; n=505 women; age 64-79 years	Retinopathy	Cognitive dysfunction defined on 10 year follow up change in Modified MMSEscores	Lower MMSE score, mean difference: 1.01, (p = 0.019)
Ong SY, et al. 2012 [201] (SIMES)	Cross-sectional population based study, n=1179, age 60-80 years	Retinopathy	Cognitive dysfunction defined on Abbreviated Mental Test	OR: 5.57 (1.56, 19.91) in diabetics

ARIC= Atheroscelorsis Risk in Communities Study; CHS= Cardiovascular Health Study; RSS=Rotterdam Scan Study; WHIMS= Women's Health Initiative Memory Study; WHISE=Women's Health Initiative Sight; Examination Study; SIMES= Singapore Malay Eye Study; MMSE= mini mental status examination; AD= Alzheimer's disease

Table 2 - 6 (d): Studies showing association of retinal vascular parameters with cognition

Authors and Study	Study type and population	Retinal structural changes	Outcome/ Evaluation	Findings with effect measure and 95% confidence interval or p value
Berisha F, et al. 2007 [166]	Case-control study; AD =9, controls = 8 mean age= 74.3 years (AD), mean age= 74.3 years (controls)	Retinal vascular calibers	AD	- Significant narrowing of the retinal venous blood column diameter in AD (131.7 +/- 10.8 μm) compared with control (148.3 +/- 12.7 μm), (p= 0.01)
de Jong FJ, et al. 2011[165] (RSS)	Prospective cohort study; n=5553, age≥50 years,	Retinal vascular calibers	Incident dementia, Incident vascular dementia	Large venular caliber associated with; - Incident dementia, HR: 1.11 (1.00, 1.22) - Incident Vascular dementia, HR: 1.44 (1.10, 1.89) Smaller arteriolar caliber associated with; - Incident dementia, HR: 1.05 (0.96, 1.16) - Incident Vascular dementia, HR: 1.33 (0.99, 1.78)
Liew G, et al. 2009 [163]	Cross-sectional population based study; n=1988, age 49-97 years	Retinal vascular calibers	Cognitive impairment defined on MMSE (≤ 23)	Retinal venular dilation, OR: 1.8 (1.0, 3.2) Retinal venular dilation, OR: 2.7 (1.2, 6.1) in hypertensives
Ding J, et al. 2011 [202] (The Edinburgh Type 2 Diabetes Study)	Cross-sectional population based study; n=954; age 60-75 years	Retinal vascular calibers	Faces and Family Pictures Sub-test, Matrix Reasoning, Letter-Number Sequencing, Digit Symbol Test, Borkowski Verbal Fluency Test, Trail Making Test	- Increasing venular caliber associated with lower logical memory score, β: -0.069, (p=<0.05) - Increasing arteriolar caliber associated with lower Logical memory scores, β: -0.080, (p=<0.01)
Kim DH, et al. 2011[203] (CHS)	Cross-sectional population based study; n=1744, age≥ 65 years	Retinal vascular calibers	Digit Symbol Substitution Test score	- Large venular caliber, mean difference: −4.81 (−8.81,−0.81), p = 0.018 - Smaller arteriolar caliber, mean difference: −4.51 (−6.38, −2.64), p = <0.001
Gatto NM, et al. 2012 [162] (Los Angeles Latino Eye Study)	Cross-sectional population based study; n= 809; mean age=70.3 years	Retinal vascular calibers	Low Cognitive Abilities Screening Instrument-Short (CASI-S) score	OR: 2.04 (1.14, 3.66)
Cheung CY, et al. 2010 [167] (SIMES)	Cross-sectional population based study, n=1202, age≥ 60 years	Fractal dimension	Abbreviated Mental Test	OR: 1.71 (1.03, 2.82)

RSS= Rotterdam Scan Study; CHS= Cardiovascular Health Study; SIMES= Singapore Malay Eye Study; AD= Alzheimer's disease; MMSE= mini mental status examination; OR= odds ratios; β= mean difference

Table 2 – 7: Studies showing retinal neuronal changes with cognitive impairment and Alzheimer's dementia

Authors and Study	Study type and population	Retinal structural changes	Methodology	Outcome	Findings with effect measure and 95% confidence interval or p value
Kergoat H, et al. 2001 [203]	Case-control study; AD = 30, controls = 30, mean age= 72.0 years (AD), mean age= 72.1 years (controls)	RNFL thickness	Scanning laser polarimetry	AD	RNFL thickness was not significantly different in AD compared to controls (p=>0.05)
Kergoat H, et al. 2001 [174]	Case-control study; AD =27, controls = 27, mean age=70.1 years (AD), mean age=71.7 years (controls)	RNFL thickness	Scanning laser polarimetry	Early AD	RNFL thickness was not significantly different in AD compared to controls (p=>0.05)
Parisi V, et al. 2001 [180]	Case-control study, AD =17, controls= 14 mean age=70.4 years (AD), mean age=71 years (controls)	RNFL thickness	Time-domain OCT	AD	- Significant reduction in RNFL thickness in AD (99.9±8.95µm) compared to controls (59.5±16.7µm) (p=<0.01), - Significant reduction in RNFL thickness in all quadrants (p=<0.01)
Iseri P, et al. 2001 [181]	Case-control study, AD =14, controls= 15, mean age= 70.1 years (AD), mean age= 65.1 years (controls)	RNFL thickness	Time-domain OCT	AD	- Significant reduction in mean RNFL thickness in AD (59.5±16.70µm) compared to controls (99.9±8.95µm) (p=<0.01) – Significant reduction in RNFL thickness in all quadrants (p=<0.05)
Berisha F, et al. 2007 [166]	Case-control study; AD =9, controls = 8 mean age= 74.3 years (AD), mean age= 74.3 years (controls)	RNFL thickness	Time-domain OCT	AD	- Significant reduction in RNFL thickness in superior quadrant of AD patients (92.2 ± 21.6 µm) compared to controls (113.6 ± 10.7 µm) (p=0.02)
Paquet C, et al. 2007 [204]	Case-control study; AD= 26, MCI= 23, controls= 15 mean age=78.3 years (AD), mean age=78.7 years (MCI), mean age=75.5 years (controls)	RNFL thickness	Time-domain OCT	mild, moderate-severe AD, MCI	- Significant reduction in mean RNFL thickness in MCI (89.3 ± 2.7µm, p<0.001), mild AD (89.2 ± 2.9µm, p=<0.01) and moderate-severe AD (76.6 ± 3.8µm, p=<0.001) compared to controls (102.2 ± 1.8µm) - Significant reduction in RNFL thickness in moderate-severe AD compared to MCI patients (p=<0.01)

AD= Alzheimer's disease; MCI= mild cognitive impairment; RNFL= retinal nerve fiber layer; OCT= Optical Coherence Tomography

Table 2 – 7 (continued): Studies showing retinal neuronal changes with cognitive impairment and Alzheimer's dementia

Authors and Study	Study type and population	Retinal structural changes	Methodology	Outcome	Findings with effect measure and 95% confidence interval or p value
Kesler A, et al. 2011 [205]	Case-control study; AD= 30, MCI= 24, controls= 24, mean age=72.1 years	RNFL thickness	Time-domain OCT	AD and MCI	- Reduced average RNFL thickness in AD and MCI ($p<0.05$) compared to controls - Reduced RNFL thickness in superior and inferior quadrants in AD ($p<0.05$) compared to controls - Reduced RNFL thickness in inferior quadrant in MCI compared to controls ($p<0.05$) - No significant difference between AD and MCI
Moschos MM, et al 2012 [177]	Case-control study; AD= 30, controls = 30; age 42-84 years	RNFL thickness	Time-domain OCT	AD	Reduced RNFL thickness in inferior ($p=<0.0001$), superior ($p<0.0001$) and temporal quadrants ($p=0.024$) in AD compared to controls
Kirbas S, et al. 2013 [178]	Case-control study; AD= 40, controls= 40; mean age=69.3 years (AD), mean age=68.9 years (controls)	RNFL thickness	Spectral domain OCT	AD	- Reduced average RNFL thickness in AD compared to controls ($p=0.001$) - Reduced RNFL thickness in superior quadrant in AD compared to controls ($p=0.001$)
Larossa JM, et al. 2014 [179]	Case-control study; AD=151, controls= 61 age 55-90 years	RNFL thickness	Spectral domain OCT and Spectralis OCT	AD	- Reduced superior ($p=0.010$), inferior ($p<0.001$), temporal ($p=0.023$) RNFL thickness in AD by Spectral domain OCT - Reduced average ($p=0.049$), nasal ($p=0.005$), nasal inferior ($p=0.020$), temporal inferior ($p<0.001$), temporal superior ($p<0.001$) RNFL thickness by Spectralis OCT
Garcia-Martin ES, et al. 2014 [182]	Case-control study; AD=20, controls = 28, mean age=79.3 years (AD), mean age=72.1 years (controls)	Macular RNFL thickness	Spectral domain OCT and 3D OCT	AD	- Reduced RNFL thickness in macular region ($p=<0.01$)
Marziani E, et al. 2013[185]	Case-control study; AD=21, controls=21; mean age=79.3 years (AD), mean age=77.0 years (controls)	Macular RNFL and GCIPL thickness	Spectral domain OCT and Spectralis OCT	AD	- Reduced macular RNFL thickness in all sectors, mean diff -8.5 to-4.2μm ($p<0.02$) in AD compared to controls - Reduced macular RNFL+GCL thickness in all sectors, mean diff -15.7 to 7.3μm ($p<0.005$) in AD compared to controls

AD= Alzheimer's disease; MCI= mild cognitive impairment; RNFL= retinal nerve fiber layer; GCIPL= ganglion cell inner plexiform layer; OCT= Optical Coherence Tomography

CHAPTER 3:

MATERIALS AND METHODS

1. STUDY POPULATION

To achieve the specific aims mentioned in **chapter 1**, the following studies were used;

a. Epidemiology of Dementia In Singapore study (EDIS)

b. Case Control study from memory clinic

a. Epidemiology of Dementia In Singapore study (EDIS)

The EDIS study drew subjects from the on-going population-based community-dwelling study of Chinese, Malays and Indians cohorts aged 40-80 years who participated in the Singapore Epidemiology of Eye Disease (SEED; n=7,454), which comprises the Singapore Chinese Eye Study (SCES; n=3,353), Singapore Malay Eye Study -2 (SiMES-2; n=1,901) and Singapore Indian Eye Study -2 (SINDI-2; n=2,200).

As part of the SEED study, participants were randomly selected from the community, and were invited to Singapore Eye Research Institute (SERI) for interview and clinical assessments.[1, 2] Briefly, SiMES, SINDI and SCES were designed to study the prevalence and risk factors for major eye diseases including age-related macular degeneration,[3] diabetic retinopathy,[4] glaucoma,[5] cataract [6] and myopia.[7] Information on participants was collected by means of a questionnaire, physical examination and laboratory based tests. The questionnaire included data on demographics, lifestyle factors, personal and family health history and medication use. Physical examination included anthropometry, blood pressure, pulse rate measurement and extensive eye examination including digital fundal photography. Laboratory examinations included serum creatinine, serum lipids, plasma glucose, glycosylated hemoglobin (HbA1c) and urine for albumin and creatinine. Blood samples were stored for future biomarkers and genetic analysis.

As part of the first phase of the EDIS study, SEED participants who were 60 years and above (n=3,800) (44% of the total population) also underwent cognitive screening using the Abbreviated Mental Test (AMT) and a self-report of progressive

forgetfulness (PF), both of which have been previously validated in Singapore.[8-10] Screen positives were defined as AMT $\leq$ 6, among those with up to 6 years of formal education, or $\leq$ 8 among those with more than 6 years of formal education; or if the caregiver confirmed progressive forgetfulness. Subsequently, these screen-positive subjects (n=1,598) were invited to participate in the second phase of the EDIS study, which was conducted at the Centre for Life Sciences, National University of Singapore (NUS). Participants who declined the initial invitation were contacted again at a later time to increase the participation rate. Those who declined at the first attempt were mailed study brochures, and offered free transportation and pick up services. A person was termed 'uncontactable' if he/she failed to respond after 6 attempts.[11] The total number of subjects who agreed to participate in phase II were 957. Brief study assesment flow chart is provided in (**Figure 3 – 1**).

b. Case Control Study

For the case control study, the cases (CIND and dementia) with subjective complaints of memory loss and cognitive impairment on neuropsychological assessment were recruited from two study sites in Singapore (i.e. memory clinics from National University Hospital and Saint Luke's Hospital). Controls were recruited from both memory clinics and the community (Epidemiology of Dementia In Singapore study, with a similar catchment area as cases). Controls (from memory clinic and community) were defined as those with subjective cognitive complaints but were cognitively normal on objective neuropsychological assessment (**Figure 3 – 2**). Patients with other diagnoses, or significant neurological comorbidities (e.g. Parkinson's disease), or loss of functional independence (modified Rankin Scale 4), were excluded from the study.

2. EXAMINATION PROCEDURES

Participants in the EDIS study (during the second phase) and of case control study, underwent standardized extensive clinical, neuropsychological evaluation, laboratory tests, neuroimaging, and retinal photography.

Questionnaire

A detailed questionnaire was administered by the interviewer to collect relevant demographic and medical information. Data collected included age, gender, education, marital status, occupation, ability to live independently, handedness, previous head trauma, smoking, alcohol consumption and family history of dementia. Previous medical history including stroke, cardiovascular diseases, hypertension, hyperlipidemia, diabetes mellitus, vitamin B 12 deficiency, thyroid disease, urinary and bowel incontinence, Parkinson's disease and psychiatric illnesses were noted, and subsequently verified by medical records. The Instrumental activities of daily living and Barthel activities of daily living indices were assessed for functional status.[12, 13]

Physical Examination and Clinical Assessment

Clinical assessment included height, weight, blood pressure, pulse rate, ankle and brachial blood pressures and indices (e.g. ankle brachial index), modified versions of National Institutes of Health Stroke Scale, Hachinski Ischemic Scale and frontal release signs. Clinical history and Clinical Dementia Rating Scale (CDR) evaluations were performed by clinicians, in accordance with established clinical guidelines for the evaluation of cognitive impairment and dementia.

Vascular risk factors

Systolic and diastolic blood pressures were measured using a digital automatic blood pressure monitor (OMRON-HEM 7203, Japan) after the subject rested for five minutes. Blood pressure was measured twice, five minutes apart. The mean of the

two readings was considered as the relevant blood pressure. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, or use of antihypertensive medication. Mean arterial blood pressure was calculated as two-thirds of the diastolic blood pressure plus one-third of the systolic blood pressure. Diabetes mellitus was defined as glycated hemoglobin ≥ 6·5 %, or use of anti-diabetic medication. Hyperlipidemia was defined as total cholesterol levels ≥ 4·14 mmol/l, or use of lipid lowering medication.

Blood Tests

A total of 20 cc of blood was drawn in the fasting state. All blood samples were sent to National University Hospital Laboratory for measurements on the same day. Blood tests included the following: full blood count, glucose, lipids, creatinine, alanine transaminase, aspartate transaminase, calcium, albumin, thyroid function, vitamin B12, folate, syphilis screen, homocysteine, high sensitivity C-reactive protein.

Additionally the blood samples from case control study were collected and stored at the Neuroscience Research Laboratory for future genetic and biomarker analysis.

Neuroimaging

Sequences

MRI scans were performed on a 3T Siemens Magnetom Trio Tim scanner, using a 32-channel head coil, at the Clinical Imaging Research Centre of the NUS. A number of standardized and advanced MRI brain sequences were performed to allow morphologic, microstructure and functional assessments. These included;

- High-resolution T1-weighted Magnetization Prepared Rapid Gradient Recalled Echo (MPRAGE) sequence (repetition time, TR = 7.2 ms, time to echo, TE =3.3 ms, matrix = 256×256×180 mm^3) was used to obtain high resolution anatomical information and to detect microinfarcts.

- Fluid attenuated inversion recovery (FLAIR) (TR = 9.3 ms, TE = 140 ms, matrix = 256×192 mm^3) and T2 sequences (TR = 3,000 ms, time to echo, TE = 10.1 ms, matrix = 256×247 mm^3) for assessment of signal alterations in brain tissues and to detect infarcts, white matter hyperintensities and confirm microinfarcts (previously detected on T1).

- Susceptibility Weighted Imaging (SWI) sequence (TR = 27 ms, TE = 20 ms, matrix = 240× 240 mm^3) was used to detect microbleeds.

- Finally, a three dimensional Time of Flight (ToF) Magnetic Resonance Angiography (MRA) (TR = 24 ms, TE = 4.1 ms, spatial resolution = 0.6×0.6×0.6 mm^3, flip angle of 20°, 192mm field of view, 218 × 256 acquisition matrix, slice thickness of 0·80mm, distance factor -22.73% and an acquisition time of 6 minutes and 28 seconds) was conducted to assess the intracranial vessels.

Scanning time was approximately 60 minutes. Subjects with claustrophobia, contraindications for MRI, or those who were unable to tolerate the procedure, underwent a non-contrast enhanced Computed Tomography (CT) scan, which was performed in axial slices at 5mm intervals rostrally from the orbitomeatal line. Scanning time was approximately 3 minutes.

Visual grading of MRI scans

All MRI scans were visually graded for infarcts, white matter hyperintensities, cerebral microbleeds, atrophy, intracranial stenosis and cerebral cortical microinfarcts (**Figure 3 – 3**). The details of each marker is described below;

Infarcts:

- Lacunar infarcts were defined on focal lesions measuring ≥ 3mm to < 15mm, hyperintense rim on T2 FLAIR with center following CSF intensity and hyperintensity on T2 weighted images. Differentiation of lacunes from perivascular spaces was based on morphology of typical vascular shape and following the orientation of perforating vessels and absence of FLAIR rim.[14]

- Cortical infarcts were defined as focal lesions involving cortical gray matter, signal following cerebrospinal fluid intensity, hyperintense rim on FLAIR images, and tissue loss of variable magnitude, with prominent adjacent sulci and ipsilateral ventricular enlargement.[14]

  The anotomical location together with arterial territory of these infarcts were noted and collected. The inter- and intrarater reliability as expressed by kappa statistic ranged from 0.59 to 0.80.

Cerebral microbleeds (CMBs):

CMBs were defined as focal, rounded areas of hypointensity (T1 and T2 weighted images), 2-10 mm in diameter with blooming on Susceptibility Weighted Imaging (SWI) sequences using Brain Observer Micro Bleed Scale (BOMBS).[15] Symmetrical hypointensities in the basal ganglia, choroid plexus and pineal gland caused by calcification, hypointense lesions within the subarachnoid space, or those possibly associated with traumatic brain injury, hemorrhagic infarcts or vascular malformation were carefully excluded. CMBs were categorized according to their location into cortical [cortical gray matter and gray-white matter junction], subcortical white matter [subcortical or periventricular white matter], subcortical gray matter [basal ganglia and the thalamus] and infratentorial [brain stem and cerebellum] (**Figure 3 - 4**). Furthermore, lobar location was defined as cortical, subcortical or periventricular white matter, whereas deep as subcortical gray matter, and the white matter of the corpus callosum, internal and external capsule.

Cortical cerebral microinfarcts (CMIs):

CMIs were defined as hypointense on T1, <5 mm in diameter, restricted to the cortex, perpendicular to the cortical surface, and distinct from perivascular spaces. The location of a hypointense cortical lesion found on T1 was explored on FLAIR and T2-weighted images. The lesion was rated as a definite cortical CMI if the location was hyperintense or isointense on FLAIR and T2. The lesion was discarded as a CMI if at the same location a hypointense signal was found on FLAIR or T2, indicating the

T1 hypointense lesion was either due to a hemorrhagic lesion, a vessel, or an artifact **(Figure 3 – 5)**. Possible cortical CMIs in tissue affected by larger cortical infarcts were discarded.

Intracranial stenosis (ICS):

ICS was defined as narrowing exceeding 50% of the luminal diameter in any of the intracranial vessels assessed on 3D TOF MRA. The images were first visually assessed on the coronal sequences and then on reconstruction. The final decision on stenosis (>50%) was based on the reconstruction sections. The arteries that were assessed were vertebral, basilar, internal carotids, posterior cerebral, middle cerebral and anterior cerebral arteries. Radiologists and clinicians, who were blinded to clinical data, graded each participant's MRA independently. The inter-rater reliability expressed as kappa statistic ranged from 0.51 to 0.79.

Quantitative MRI grading

Intracranial volume and white matter hyperintensities volume

Total intracranial volume and white matter hyperintensities were quantified by automatic segmentation using the proton density-weighted T1 sequence, T2 weighted images and FLAIR sequences. Briefly, cerebrospinal fluid, gray matter and white matter were segmented by an atlas-based k-nearest neighbour classifier on multi-modal MRI data. This classifier was trained by registering brain atlases to the subject. The resulting gray matter segmentation was used to automatically find a white matter hyperintensities threshold in a FLAIR image. False positive lesions were removed by ensuring that the lesions are within the white matter. This method has been previously validated on the manual segmentations.[16, 17] Total brain volume and white matter hyperintensities were calculated for the five regions (frontal, parietal, occipital, temporal and central regions) (**Figure 3 – 6**).

Cortical thickness

Cortical thickness was calculated using a model-based automated procedure (FreeSurfer, v.5.1.0) on T1-weighted images (TR = 7.2 ms, TE = 3.3 ms, matrix = 256 × 256 × 180 mm^3). Cortical thickness was measured at each vertex by taking the shortest distance between white matter/gray matter boundary and pial surface.[18] Whole brain (global) and regional (lobar) averages of cortical thickness were expressed in micrometers (μm). Lobar average was calculated from right and left thicknesses using the parcellation guide on gyral and sulcal structures of cerebral cortex.[18] Lobar averages were calculated for the frontal, parietal, occipital, temporal, insular and limbic regions.

Subcortical structure volume

Volumes of subcortical structures (accumbens, amygdala, caudate, pallidum, putamen, thalamus, hippocampus and brainstem) were segmented using a model based automated procedure (FreeSurfer, v.5.1.0) on T1 weighted images (TR= 7.2 ms, TE= 3.3 ms, matrix = 256×256×180 mm^3). Segmentation was performed by rigid-body registration and nonlinear normalization of images to a probabilistic brain atlas. In the segmentation process, each voxel of the MRI volumes was labeled automatically as a corresponding brain region based on a parcellation guide. Finally the volumes of accumbens, amygdala, caudate, pallidum, putamen thalamus and hippocampus were calculated separately for left and right hemispheres.[19]

The segmentation technique of both cortical thicknesses and subcortical structures is shown in **Figure 3 – 7**.

Retinal assessment

Assessment of Retinal Vasculature

Retinal microvascular changes were assessed using non-mydriatic retinal fundus photography. Retinal photographs centered on the optic disc and the macula was taken from both eyes after pupil dilation using 1% tropicamide. The Singapore I

Vessel Assessment (SIVA) system is a semi-automated computer-based program designed to assess quantitative structural retinal vascular parameters from optic-disc. Retinal fundus images were centered at the optic disc and were taken 0.5 to 2.0 disc diameter away from the optic disc margin. The major parameters extracted from this system include retinal vascular diameter, fractal dimension, and tortuosity (**Figure 3 – 5**).

Retinal Vascular Caliber

The program calculates retinal arteriolar and venular calibers as central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE), based on the revised Knudtson-Parr-Hubbard formula.[20, 21]

Retinal Vascular Fractal Dimension

Retinal vascular dimension was evaluated from the skeletonized vascular network using the box-counting method, and represents a "global" measure that summarizes the whole branching pattern of the retinal vascular tree.[20, 21] Larger values indicate a more complex branching pattern.

Retinal Vascular Tortuosity

Retinal vascular tortuosity was computed as the integral of the curvature square along the path of the vessel, normalized by the total path length; this measure is dimensionless as it represents a ratio measure.[20, 21] The estimates were summarized as retinal arteriolar and venular tortuosity separately, representing the average tortuosity of arterioles and venules, respectively. Retinal vascular tortuosity reflects the extent of curvature in the vessels; a smaller tortuosity value indicates a straighter retinal vessel.

Assessment of Retinal Neuronal Layers

Besides the retinal microvascular changes, spectral domain optical coherence tomography (SD-OCT) was used to assess the retinal neuronal changes. SD-OCT can quantify the thicknesses of both retinal nerve fiber layer and ganglion cell inner

plexiform layer. After pupil dilatation, SD-OCT (Cirrus HD-OCT; Carl Zeiss Meditec) was used to acquire macular and optic disc scans using the macular cube 200x200 and optic nerve head cube 200x200 scan protocols respectively in each eye. Scans were repeated if motion artifacts (as indicated by blood vessels discontinuity) or saccades were detected. Details of the Cirrus HD-OCT macular and optic disc scan protocols have been described in detail elsewhere.[22, 23] Peripapillary RNFL thickness parameters (average, superior quadrant, nasal quadrant, inferior quadrant and temporal quadrant) were derived automatically from optic nerve head cube scan. The built-in algorithm automatically detects the optic disc center and positions a calculation circle of diameter 3.46mm around the optic disc on the RNFL thickness map.

Using the same software, a series of GC-IPL parameters (average, superior, superonasal, inferonasal, inferior, inferotemporal, superotemporal sectors) from macular cube scan were derived automatically. The software detects and measures the GC-IPL thicknesses automatically within a 14.13mm^2 elliptical annulus area centered on the fovea from 3-dimensions. The ganglion cell analysis algorithm detects and yields the combined thickness of the GCL and the IPL (**Figure 3 – 8**). Additional details are available in **chapter 13**.

Neuropsychological Test Battery

Trained research psychologists administered brief cognitive screening tests, the Mini-Mental State Examination (MMSE), the Montreal Cognitive Assessment (MoCA), the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) and a formal neuropsychological battery locally validated for Singaporean elderly.[11] This battery assessed seven domains, five of which were non-memory domains (executive function, attention, language, visuoconstruction and visuomotor speed) and two

memory domains (visual and verbal memory). The list of the neuropsychological tests used for respective domains are described below;

- Executive Function (Frontal Assessment Battery,[24] Maze Task[25]),
- Attention (Digit Span, Visual Memory Span[26] and Auditory Detection[27]),
- Language (Boston Naming Test[28] and Verbal Fluency[29]),
- Visuomotor speed (Symbol Digit Modality Test[30], Digit Cancellation[31]),
- Visuoconstruction (Weschler Memory Scale – Revised (WMS-R) Visual Reproduction Copy Task,[24] Clock Drawing,[32]AIS-R subtest of Block Design),[33]
- Verbal Memory (Word List Recall[34] and Story Recall),
- Visual Memory (Picture Recall, WMS-R Visual Reproduction). [26]

For each participant, raw scores from each individual test within a domain were first transformed to standardized Z-scores using the mean and standard deviation [SD] of that test in this cohort. A higher Z-score reflected a better performance on that test. Subsequently, for each participant a mean Z-score for each domain was calculated by averaging the Z-scores of all the individual tests within that domain. These mean Z-scores of each domain were then standardized using the mean and SD of that domain-specific mean Z-score. Finally, composite Z-score reflecting global cognitive functioning was calculated by averaging the seven domain-specific mean Z-scores, which were also standardized using the corresponding mean and SD. The modified 15-item Geriatric Depression Scale (GDS) was also administered to all subjects.[35]

Diagnosis of Cognitive Impairment and Dementia

Diagnoses of cognitive impairment and dementia were made at weekly consensus meetings attended by study clinicians, neuropsychologists, clinical research fellows, research coordinators and research assistants. The clinical features, blood investigations, psychometrics and neuroimages were reviewed. Subjects with no objective evidence of impairment in cognitive domains were classified as no cognitive impairment (NCI). Cognitive impairment without dementia (CIND) was

defined as impairment in at least one domain of the neuropsychological test battery without functional impairment.

- CIND-mild was diagnosed when less than or two domains were impaired,
- CIND-moderate was diagnosed when more than two domains were impaired.

Dementia was diagnosed according to the DSM-IV criteria. Details on the CIND severity, vascular CIND and etiological diagnosis of dementia are described in details in **chapters 5** and **10**.

CHAPTER 3 – REFERENCES

1. Rosman M, Zheng Y, Wong W, et al. Singapore Malay Eye Study: rationale and methodology of 6-year follow-up study (SiMES-2). Clinical & experimental ophthalmology. 2012;40:557-68.

2. Lavanya R, Jeganathan VS, Zheng Y, et al. Methodology of the Singapore Indian Chinese Cohort (SICC) eye study: quantifying ethnic variations in the epidemiology of eye diseases in Asians. Ophthalmic Epidemiol. 2009;16:325-36.

3. Kawasaki R, Yasuda M, Song SJ, et al. The prevalence of age-related macular degeneration in Asians: a systematic review and meta-analysis. Ophthalmology. 2010;117:921-7.

4. Lim LS, Lamoureux E, Saw SM, Tay WT, Mitchell P, Wong TY. Are myopic eyes less likely to have diabetic retinopathy? Ophthalmology. 2010;117:524-30.

5. Shen SY, Wong TY, Foster PJ, et al. The prevalence and types of glaucoma in malay people: the Singapore Malay eye study. Invest Ophthalmol Vis Sci. 2008; 49:3846-51.

6. Tan AC, Wang JJ, Lamoureux EL, et al. Cataract prevalence varies substantially with assessment systems: comparison of clinical and photographic grading in a population-based study. Ophthalmic Epidemiol. 2011;18:164-70.

7. Saw SM, Chan YH, Wong WL, et al. Prevalence and risk factors for refractive errors in the Singapore Malay Eye Survey. Ophthalmology. 2008;115: 1713-9.

8. Sahadevan S, Lim PP, Tan NJ, Chan SP. Diagnostic performance of two mental status tests in the older chinese: influence of education and age on cut-off values. Int J Geriatr Psychiatry. 2000;15:234-41.

9. Sahadevan S, Tan NJ, Tan T, Tan S. Cognitive testing of elderly Chinese people in Singapore: influence of education and age on normative scores. Age Ageing. 1997;26:481-6.

10. Chong MS, Chin JJ, Saw SM, et al. Screening for dementia in the older Chinese with a single question test on progressive forgetfulness. Int J Geriatr Psychiatry. 2006;21:442-8.

11. Hilal S, Ikram MK, Saini M, et al. Prevalence of cognitive impairment in Chinese: Epidemiology of Dementia in Singapore study. J Neurol Neurosurg Psychiatry. 2013;84:686-92.

12. Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. Gerontologist. 1969;9:179-86.

13. Mahoney FI, Barthel DW. Functional Evaluation: The Barthel index. Md State Med J. 1965;14:61-5.

14. Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol. 2013;12:822-38.

15. Cordonnier C, Potter GM, Jackson CA, et al. improving interrater agreement about brain microbleeds: development of the Brain Observer MicroBleed Scale (BOMBS). Stroke. 2009;40:94-9.

16. de Boer R, Vrooman HA, Ikram MA, et al. Accuracy and reproducibility study of automatic MRI brain tissue segmentation methods. Neuroimage. 2010;51: 1047-56.

17. Vrooman HA, Cocosco CA, van der Lijn F, et al. Multi-spectral brain tissue segmentation using automatically trained k-Nearest-Neighbor classification. Neuroimage. 2007;37:71-81.

18. van Velsen EF, Vernooij MW, Vrooman HA, et al. Brain cortical thickness in the general elderly population: the Rotterdam Scan Study. Neuroscience letters. 2013;550:189-94.

19. Fischl B, Salat DH, Busa E, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron, 2002;33,341-55.

20. Ong YT, De Silva DA, Cheung CY, et al. Microvascular structure and network in the retina of patients with ischemic stroke. Stroke. 2013;44,2121–27.

21. Cheung CY, Ong YT, Ikram MK, et al. Microvascular network alterations in the retina of patients with Alzheimer's disease. Alzheimers Dement. 2014;10,135–42.

22. Koh VT, Tham YC, Cheung CY, et al. Determinants of ganglion cell-inner plexiform layer thickness measured by high-definition optical coherence tomography. Invest Ophthalmol Vis Sci. 2012;53:5853-9.

23. Cheung CY, Chen D, Wong TY, et al. Determinants of quantitative optic nerve measurements using spectral domain optical coherence tomography in a population-based sample of non-glaucomatous subjects. Invest Ophthalmol Vis Sci. 2011;52:9629-35.

24. Dubois B, Slachevsky A, Litvan I, Pillon B. The FAB: a Frontal Assessment. Battery at bedside. Neurology. 2000;55:1621-26.

25. Porteus SD. The Maze Test and clinical psychology. Palo Alto, CA: Pacific Books; 1959.

26. Wechsler D. Wechsler Memory Scale – Revised. 3rd ed. San Antonio, TX: Jovanovich; 1997.

27. Lewis RF, Rennick PM. Manual for the Repeatable Cognitive Perceptual-Motor Battery. Clinton Township, MI: Axon; 1979.

28. Mack WJ, Freed DM, Williams BW, Henderson VW. Boston Naming Test: shortened versions for use in Alzheimer's disease. J Gerontol. 1992;47:154-58.

29. Isaacs B, Kennie AT. The Set test as an aid to the detection of dementia in old people. Br J Psychiatry. 1973;123:467-70.

30. Smith A. Symbol Digit Modalities Test. Los Angeles, CA1973.

31. Diller L, Ben-Yishay Y, Gerstman LJ. Studies in cognition and rehabilitation in hemiplegia. New York: New York University Medical Center Institute of Rehabilitation Medicine; 1974.

32. Sunderland T, Hill JL, Mellow AM, et al. Clock drawing in Alzheimer's disease. A novel measure of dementia severity. J Am Geriatr Soc. 1989;37:725-29.

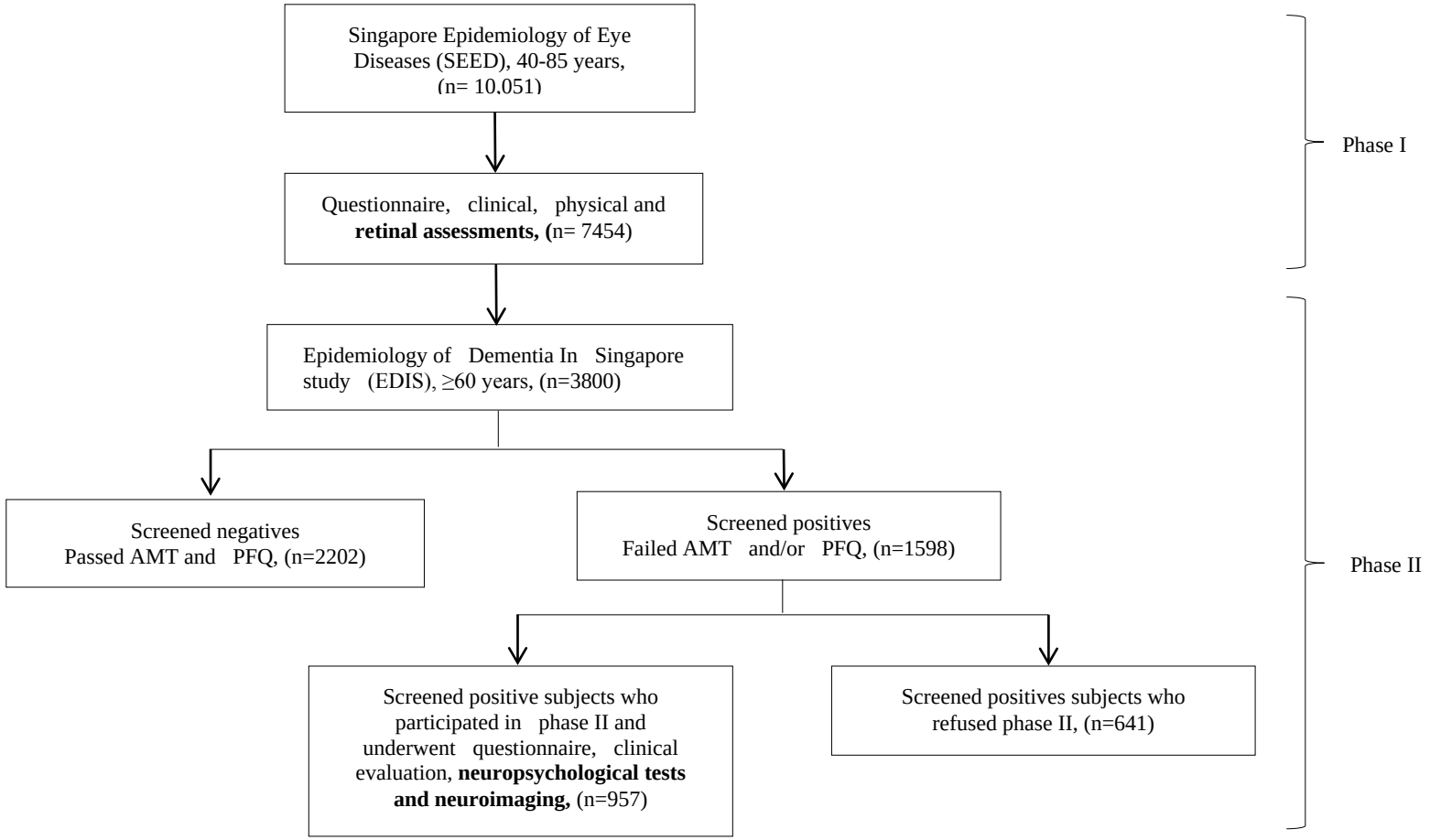
33. Wechsler D. Wechsler Adult Intelligence Scale - Revised. San Antonio, TX: Harcourt Brace Jovanovich; 1981.

34. Sahadevan S, Tan NJ, Tan T, Tan S. Cognitive testing of elderly Chinese people in Singapore: influence of education and age on normative scores. Age Ageing. 1997;26:481-86.

35. Yesavage JA, Brink TL, Rose TL, et al. Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatr Res. 1982;17:37-49.

CHAPTER 3 – FIGURES

Figure 3 – 1: Flow chart of study assessments performed in Epidemiology of Dementia In Singapore study



AMT= Abbreviated Mental Test; PFQ=Progressive forgetfulness Questionnaire

Figure 3 – 2: Flow chart of study assessments performed in case control study

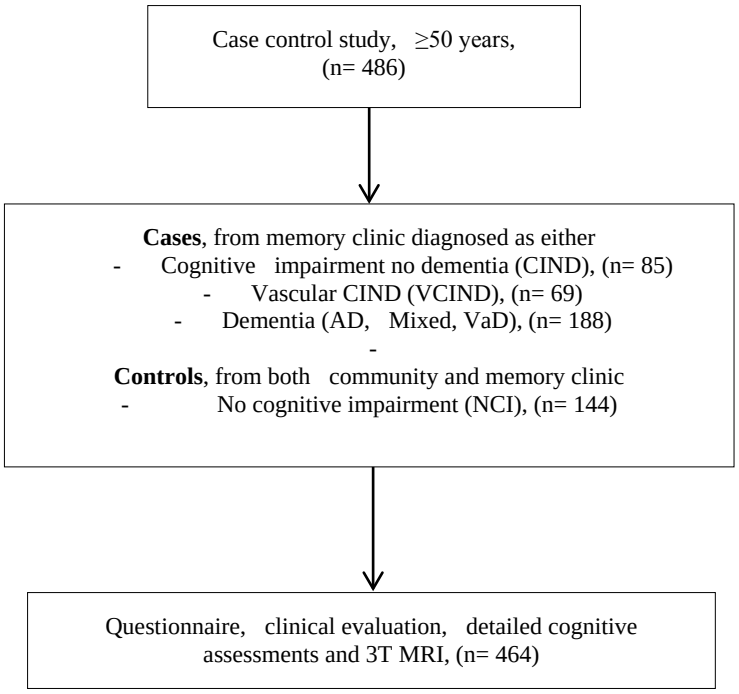


Figure 3 – 3: Markers of cerebrovascular diseases visually graded on MRI scans.

(A) Lacunes, visible on Fluid Attenuated Inversion Recovery (FLAIR) as round or ovoid hypointense lesions with hyperintense rim. (B) White matter hyperintensities, identified as signal abnormality of variable size in white matter without cavitation on FLAIR. (C) Cerebral microbleeds, visible as focal, rounded areas of hypointensity on Susceptibility Weighted Images (SWI). (D) Cortical cerebral microinfarcts, appear as hypointense, perpendicular lesions in cortical ribbon on T1. (E) Intracranial stenosis, identified as flow void in internal carotid artery on Magnetic Resonance Angiography (MRA). (F) Atrophy, visible as widening of sulcus, enlargement of ventricles and shrinkage of medial temporal lobe (enlargement of choroid fissure, widening of temporal horns and reduced height of hippocampus.

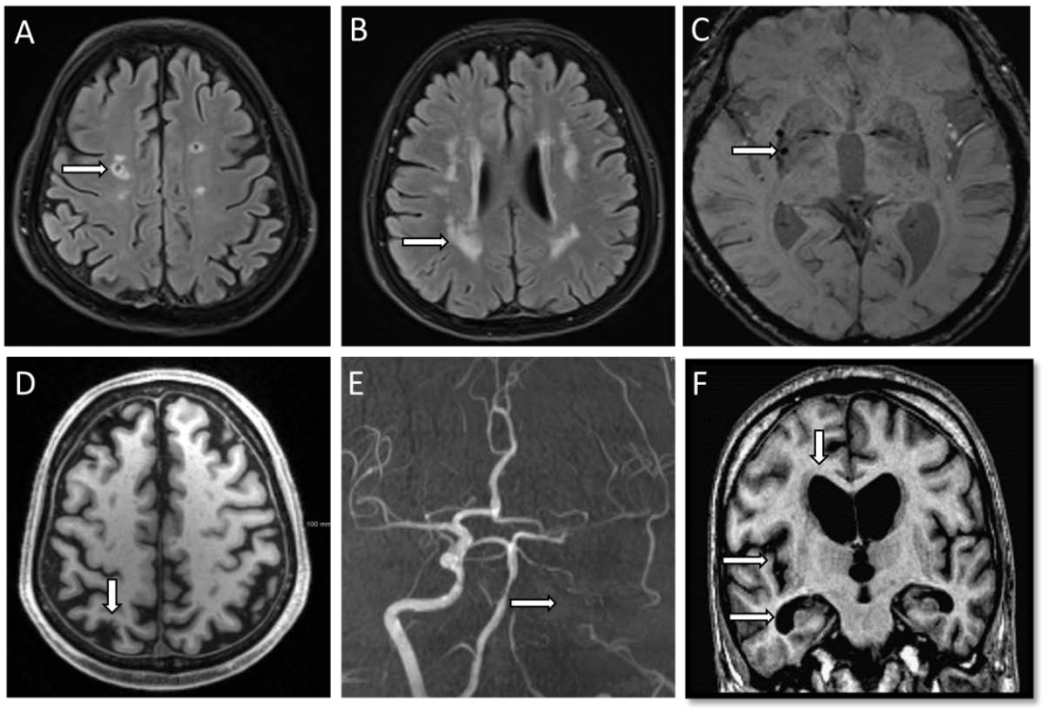


Figure 3 – 4: Cerebral microbleeds on Susceptibility Weighted Images (SWI)

Microbleeds visible as focal, rounded areas of hypointensity, 2-10 mm in diameter with blooming on SWI. Mircobleeds were categorized according to their location into infratentorial (A), deep (B) and lobar (C).

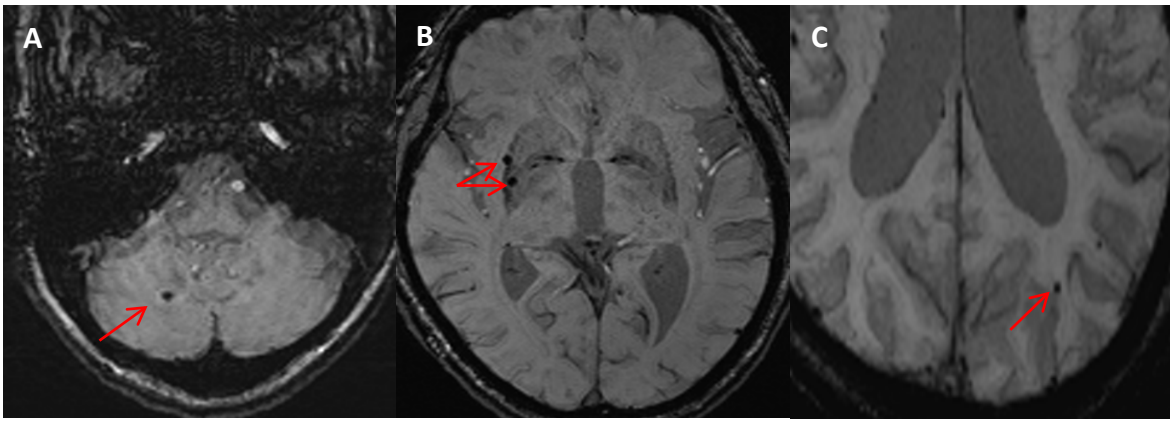


Figure 3 – 5: Cortical cerebral microinfarct (CMI) on 3T MRI

CMI in an 87-year old woman, visible as a hypointense lesion on T1 in sagittal (A), coronal (B), and axial sections (C). This CMI was confirmed as a hyperintense lesion on fluid-attenuated inversion recovery (D) and T2 weighted images (E) and not hypointense on susceptibility weighted sequence (F). Scale bar is set at 5mm.

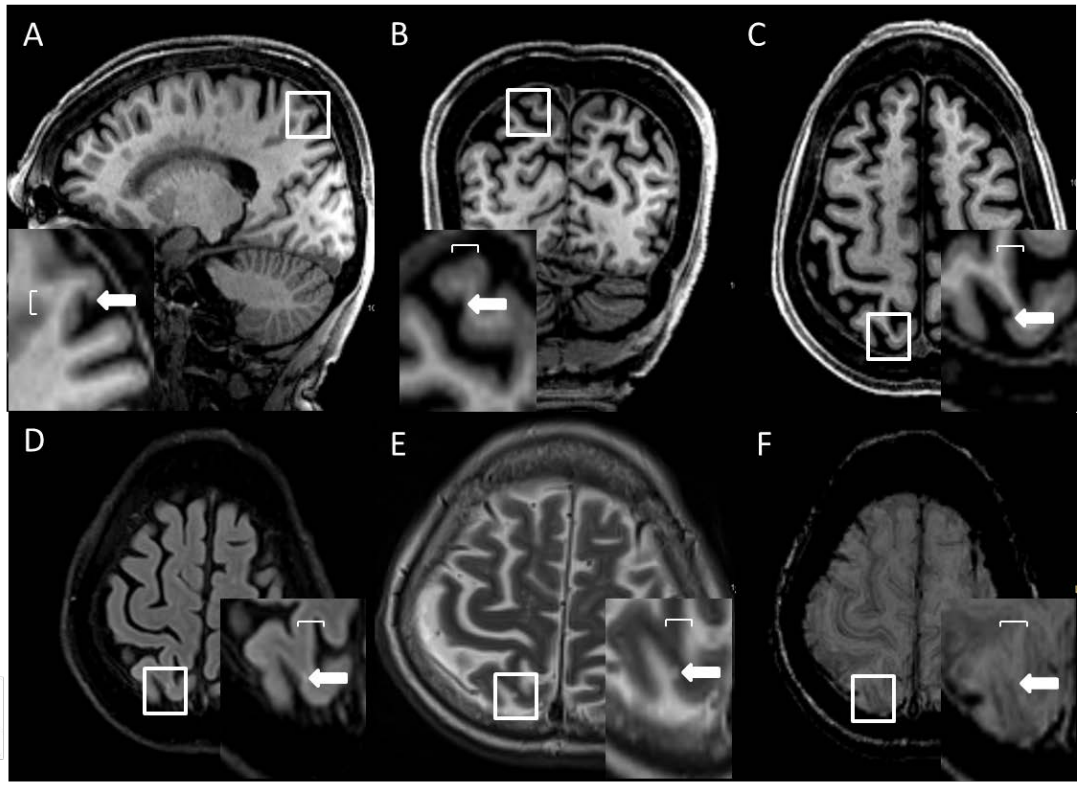


Figure 3 – 6: Quantitative segmentations of the brain parenchyma.

(A) Segmentations of the white and gray matter and cerebrospinal fluid (in colours). (B) Segmentations of the white matter hyperintensities together with the brain parenchyma. (C) Cerebral cortex parcellated into sulci and gyri cortices (displayed by colours) based on anatomical regions. (D) Subcortical gray matter structures identified as colours

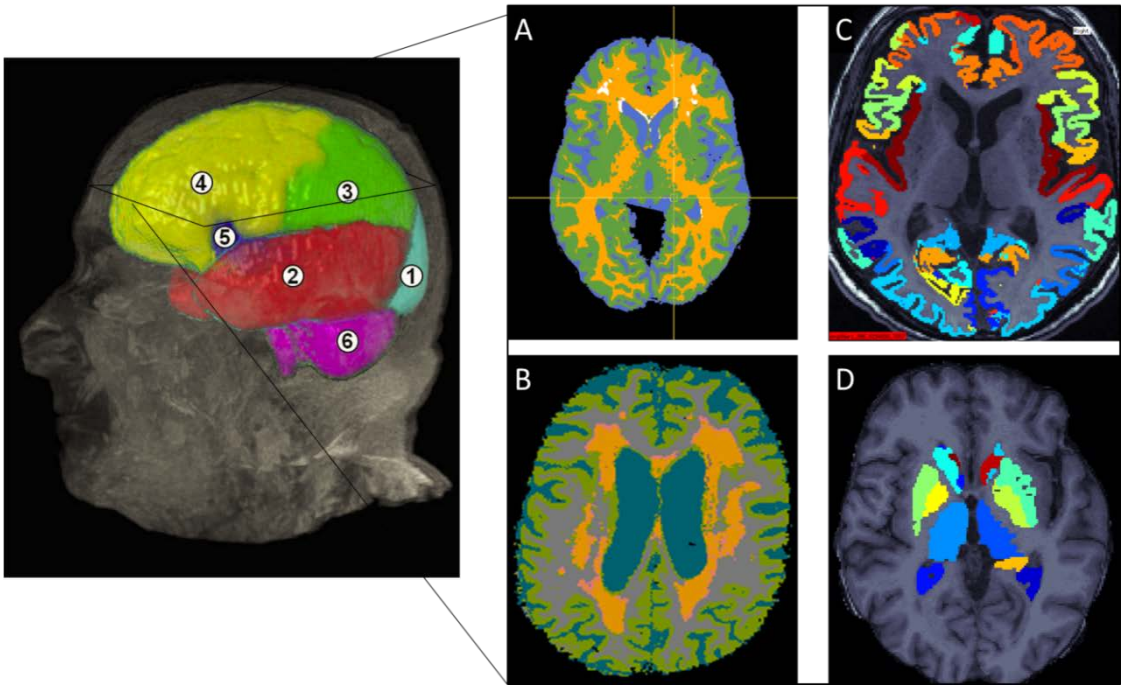


Figure 3 – 7: Measurement of retinal microvascular parameters using Singapore I Vessel Assessment (SIVA)

All retinal microvascular parameters are measured within the grid drawn over a region 0.5 to 2 disc diameter away from the optic disc. SIVA programme automatically traces the vessels (A) Calibers are calculated separately for arterioles (red) and venules (blue). (B) Fractal dimension calculated from the skeletonized line using box counting method. (C) Tortuosity is derived from the integral of the curvature square along the path of the vessel, normalized by the total path length.

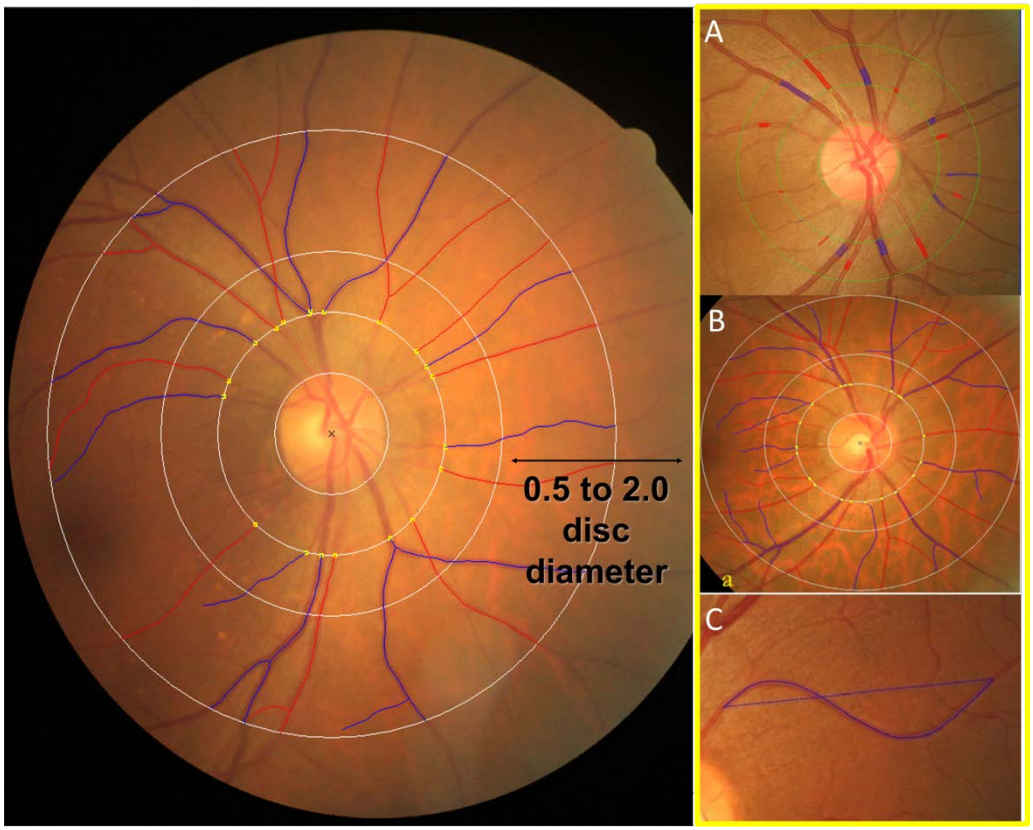
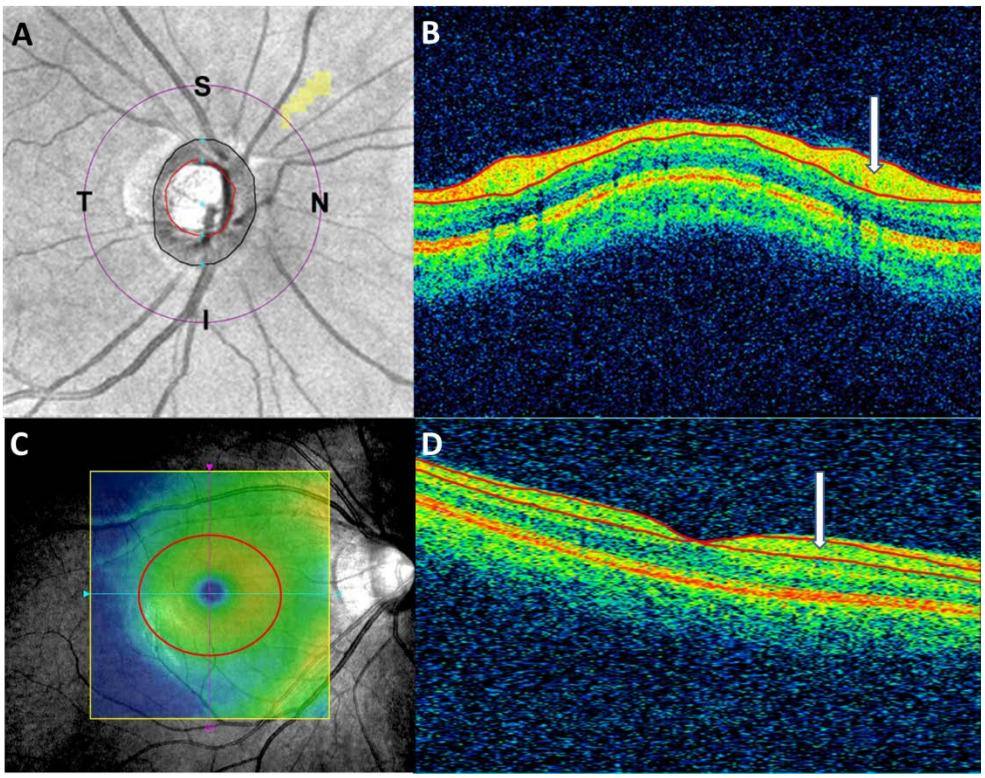


Figure 3 – 8: Measurement of retinal neuronal layers using spectral domain optical coherence tomography (SD-OCT)

Retinal nerve fiber layer (RNFL) is measured at the purple circle (A) around the optic disc using optic nerve head cube scans where RNFL is automatically delineated as seen in a cross section (between two red lines) (B). Ganglion cell inner plexiform layer (GC-IPL) is measured at red circle on the fovea (C) using macular cube scans where GC-IPL is automatically delineated as shown on the cross section (between the two red lines) (D).



PART I:

BRAIN MARKERS OF CEREBROVASCULAR DISEASES

CHAPTER 4:

Cerebral Microbleeds and Cognition- The Epidemiology of Dementia In Singapore study (EDIS)

1. INTRODUCTION

Cerebral microbleeds (CMB) are radiologically defined lesions on magnetic resonance imaging (MRI) sequences, most commonly on gradient-echo (GE) T2* or susceptibility weighted images (SWI), which correspond pathologically to hemosiderin deposits surrounding small vessels.[1, 2] In healthy populations the reported prevalence of CMBs ranges from 3.8% to 38.3%, whereas in patients with stroke the corresponding figures may be as high as 50-70%.[3-6]

Histo-pathological studies have shown that CMBs are associated with surrounding tissue damage.[7, 8] Although a direct impact of CMBs on cognitive function has been hypothesized, results from studies have varied.[9-12] CMBs are associated with both a higher amyloid burden and are also known to occur in patients with Alzheimer's disease. Furthermore, previous studies showed that CMBs occur concomitantly with white matter hyperintensities (WMH) and lacunar stroke.[12-14] Thus, an independent effect of CMBs on cognition may be implicated only if other associated pathologies are accounted for.[15]

With respect to Asian populations, studies from Japan reported that presence of CMBs is related to a poorer cognitive function.[16, 17] However, in these studies cognitive function was assessed solely by the mini mental status examination (MMSE), and 1.5T MRI was utilized for assessment of CMBs. As yet, there are no data from Chinese populations on the association with cognitive impairment as assessed by an extensive neuropsychological test battery. Therefore, in the present study, we investigated the association of CMBs with cognition, as assessed by a comprehensive neuropsychological evaluation among Chinese subjects from the population-based Singapore Chinese Eye Study (SCES), who failed an initial cognitive screening and were recruited into the on-going Epidemiology of Dementia in Singapore (EDIS) Study. Furthermore, when examining this association we took into account the presence of other MRI features, as reflected by markers of cerebral small vessel disease and involutional changes on MRI.

2. METHODS

2.1 Study Population

The ongoing Epidemiology of Dementia in Singapore (EDIS) study drew subjects from the population-based study among Chinese aged 40-85 years, who participated in the Singapore Chinese Eye Study (SCES). In order to use the limited resources in an efficient way, it was decided to focus on those subjects who were most likely to have some cognitive problems. Hence, in the first phase of the EDIS Study, Chinese participants from SCES aged ≥ 60 years (n=1,538) were screened using the Abbreviated Mental Test (AMT) and a self-report of progressive forgetfulness. Screen-positives were defined as AMT score ≤ 6, among those with ≤ 6 years of formal education, or ≤ 8 among those with > 6 years of formal education; or if the subject or caregiver reported progressive forgetfulness. Screen-positive subjects (n=612) were invited to take part in the second phase of this study, which included an extensive neuropsychological test battery and brain magnetic resonance imaging (MRI). Of these 612 participants, 300 agreed to participate in phase II and hence were included in the present study. Ethics approval for EDIS was obtained from the Singapore Eye Research Institute (SERI) and National Healthcare group (NHG) Institutional Review Boards. Informed consent was obtained for all participants prior to recruitment. The details of the study methodology have been described elsewhere.[18]

2.2 Neuroimaging

MRI Acquisition

MRI scans were performed on a 3T Siemens Magnetom Trio Tim scanner using a 32-channel head coil at the Clinical Imaging Research Centre, National University of Singapore, Singapore. A number of standardized and advanced MRI Brain sequences were performed including Susceptibility Weighted Imaging (SWI) sequences to detect CMBs as described in **Chapter 3**. Subjects with claustrophobia,

contraindications for MRI, or those who were unable to tolerate the procedure were excluded.

Grading of Cerebral Microbleeds (CMB)

The presence, location and number of CMBs were graded on SWI images according to the Brain Observer Micro Bleed Scale (BOMBS)[19] (**Chapter 3**).

Other markers on MRI

Other MRI markers of cerebrovascular diseases (lacunes and WMH volume) and involutional changes (total brain volume) have been described in detail in **Chapter 3**.

2.3 Cognitive Assessment

A formal neuropsychological battery, previously validated for the Singaporean elderly, was administered to all participants.[20] The details of cognitive domains, utilizing respective neuropsychological tests have been described in **Chapter 3**.

2.4 Assessment of Other Risk Factors

Demographic and vascular risk factors including age, sex, education, smoking, hypertension, diabetes, hyperlipidemia, height, weight and history of stroke were collected and verified by medical records.[18] Data on medication use included use of antiplatelets or anticoagulants. Education was categorized into < Primary 6 and ≥ Primary 6. Smoking was categorized into ever smokers (past and current smokers) vs. never smokers. Body mass index (BMI) was calculated as the weight in kg divided by the square of height in meters.

2.5 Statistical Analysis

Baseline characteristics are presented as means ± standard deviation [SD] or number (percentage), and were compared between subjects with and without CMB. Chi-square test was used for categorical variables, student's t-test for normally distributed continuous variables and Mann-Whitney U test for skewed distributed continuous variable (WMH).

Regarding quantitative MRI markers, WMH volume was logarithmically transformed, to ensure a normal distribution. With respect to the associations between

CMBs and other MRI markers: logistic regression models were constructed for lacunes, and linear regression models for WMH and total brain volume. These models were adjusted initially for age and sex; subsequently for smoking, mean arterial blood pressure, cholesterol, random blood glucose and, finally, for the other MRI markers.

For the associations of CMBs with cognition, linear regression models were constructed for composite and domain-specific Z-scores. These regression models with cognition were adjusted initially for age, sex and education; subsequently for mean arterial blood pressure, cholesterol, random blood glucose, smoking, BMI, antiplatelet/ anticoagulant, GDS and, finally, for the other MRI markers.

For all the models, measures of association were expressed with the corresponding 95% confidence intervals (CI). In order to examine the robustness of the associations, CMBs were included in these models as (1) per CMB increase and (2) multiple (≥2) versus none/single (<2) CMB. P-values < 0.05 were considered statistically significant. In view of the multiple tests performed on the specific cognitive domains (7 domains), we also used the Bonferroni correction to obtain an adjusted significance level for each domain-specific test: 0.05/7=0.007. These analyses were performed using standard statistical software (Statistical Package for Social Science, SPSS V20, SPSS Inc., USA).

3. RESULTS

A total of 1,538 Chinese subjects participated in phase I of the EDIS Study, of whom 612 were screen positive and thus were invited for the second phase. Out of 612 screened positive participants, 300 subjects agreed to participate in phase II. Compared to those who did not participate in phase II (n=312), those who participated were younger (mean age 69.9), more often women, had a higher education and higher socio-economic status, less often hypertensive, whereas the proportion of hyperlipidemia was higher (**Table 4-1**). Of these 300 participants, 18

subjects had MRI scans that could not be graded. Of the remaining 282 subjects, 91(32.3%) subjects had any CMBs. Among subjects with CMBs, 55 (60.4%) had a single CMB, 19 (20.8%) had 2 CMB and 17 (18.7%) > 2 CMBs. Lobar CMBs were present in 75 (82.4%), of which 36 (39.6%) were cortical CMBs. The range of CMB counts was 0 to 43. Baseline characteristics of the participants with and without CMB are shown in **Table 4–2**. Subjects with CMB more often used antiplatelets and anticoagulants and had more lacunes and a higher WMH volume compared to those without CMBs.

Increasing age was associated with higher prevalence of CMBs: among persons aged 60-64 years the prevalence was 28.6% increasing to 35.7% in those older than 75 years. With respect to other MRI markers, the most consistent associations were found between CMBs and lacunes, which were independent even after adjusting for WMH and total brain volumes. For WMH, only the model, which included CMBs as "a one lesion increase", suggested an association with WMH volume: 0.05 (95% CI 0.01; 0.09) (**Table 4-3**). However, this was not supported by the categorized analysis. Finally, CMBs were not associated with total brain volume.

With respect to cognition, **Table 4-4** shows that there was an association between CMB and global composite Z–score (difference in mean Z-score per CMB increase: -0.06 [-0.11; -0.01]). Furthermore, these associations were independent of other cardiovascular risk factors and other markers of cerebral small vessel disease. The findings were further supported when CMBs were categorized as multiple versus none/single.

As we found a significant association with the global composite Z-score, further analyses were conducted with domain-specific Z-scores (**Table 4-5**). In the fully adjusted models CMBs were associated with executive function, attention and visuoconstruction. Similar associations were observed when CMBs were categorized as ≥ 2 versus < 2. Finally, when applying Bonferroni-corrected significance level of

0.007 to the domain specific analyses, only the association with visuoconstruction reached this revised level of significance.

4. DISCUSSION

In this study of an elderly Chinese population, we showed that the presence and number of CMBs were - independent of other markers of cerebral small vessel disease – associated with poorer cognitive function.

There is considerable debate about the exact role of CMBs in the pathophysiology of cognitive impairment and dementia. A recent systemic review and meta-analysis reported an association between CMBs and cognitive impairment.[21] Furthermore, the RUN DMC study examining non-demented subjects (50-85 years) with cerebral small vessel disease also reported significant associations of presence and number of CMBs with global cognitive function, as measured by the Cognitive Index, psychomotor speed and attention, though no association was found with the MMSE.[9] On the other hand, data from the Rotterdam Study (n=3,979) has suggested that the number of CMBs is associated with MMSE scores after additional adjustment for brain atrophy, WMH volume and lacunar infarcts, suggesting that concomitant occurrence of traditional markers of cerebral small vessel disease explained their findings.[10] In contrast, several other studies in Caucasians have failed to find an independent association between CMB and cognitive decline.[22,23] Finally, a clinic-based study among subjects with Alzheimer's disease (mean age 68 ± 9 years) showed that the presence and number of CMB was neither associated with baseline MMSE, nor with change in MMSE over a period of 3 years.[24]

With respect to Asian populations, thus far two Asian studies have shown an association of CMB with lower scores on MMSE.[16, 17] However, these studies lacked detailed neuropsychological assessment, utilized low resolution MRI scans and other cerebrovascular diseases (strokes and WMH) were not taken into consideration. With respect to the Chinese population, ours is the first study to suggest that CMBs are

associated with cognitive function independent of other markers of cerebral small vessel disease. In order to examine the robustness of our findings, we also examined these associations using a different categorization (≥2 versus <2), apart from the "per CMB increase" analyses. This categorization further supported our original findings.

In relation to MRI markers of cerebrovascular disease, previous studies have reported an association of CMBs with silent brain infarcts, lacunar infarcts and WMH.[14,25,26] Our observations confirm the association of cerebral CMBs with other markers of cerebral small vessel disease, including lacunes and – less clearly – WMH.

Some methodological issues need to be discussed. First, only half of the screen positive subjects took part in phase II of the study, as described previously.[18] This might have led to an underestimation of the prevalence of CMBs on MRI and subjects with poorer cognitive function and subsequently attenuation of the effect sizes. Despite this underestimation, we still found an association with the composite and several domain-specific Z-scores. Second, we did not have sufficient number of CMBs to specifically examine the association between location of CMBs (such as lobar region) and cognition. Third, due to the cross-sectional design of our study the temporal relationship between the presence of CMBs and the development of cognitive decline cannot be assessed. Fourth, due to the small number of dementia cases (n=5) in this sample, we were unable to determine the effect of CMBs on clinically defined dementia. Fifth, even after adjusting for MRI markers such as WMH, lacunes and total brain volume, we cannot exclude the possibility of residual confounding by other effects of small vessel disease not fully captured by the current MRI markers. Finally, for the domain specific analyses, although we found several significant associations at a nominal significance level of 0.05, after applying Bonferroni correction only the association with visuoconstruction reached the revised significance level of 0.007. Probably due to low power of our study we were not able to examine the large number of specific cognitive domains separately.

The strengths of this study include: utilization of a validated comprehensive neuropsychological test battery for the evaluation of cognitive function and a multi-modal MRI (3T) to visualize CMBs and other markers of cerebral small vessel disease. Furthermore, quantitative MRI markers, such as WMH and total brain volume were utilized and adjusted for in our analysis.

5. CONCLUSION

In this study among Chinese subjects, CMBs were, independent of other concomitant markers of cerebral small vessel disease, associated with poorer cognitive function. Future studies with a prospective design are required to further elucidate the exact role of this novel marker of cerebral small vessel disease in the pathophysiology of cognitive impairment and dementia.

CHAPTER 4 – REFERENCES

1. Nandigam RN, Viswanathan A, Delgado P, et al. MR imaging detection of cerebral microbleeds: effect of susceptibility-weighted imaging, section thickness, and field strength. AJNR Am J Neuroradiol. 2009;30:338-43.

2. Koennecke HC. Cerebral microbleeds on MRI: prevalence, associations, and potential clinical implications. Neurology. 2006;66:165-71.

3. Poels MM, Ikram MA, van der Lugt A, et al. Incidence of cerebral microbleeds in the general population: the Rotterdam Scan Study. Stroke. 2011;42:656-61.

4. Werring DJ, Coward LJ, Losseff NA, et al. Cerebral microbleeds are common in ischemic stroke but rare in TIA. Neurology. 2005;65:1914-18.

5. Lee SH, Bae HJ, Kwon SJ, et al. Cerebral microbleeds are regionally associated with intracerebral hemorrhage. Neurology. 2004;62:72-6.

6. Jeerakathil T, Wolf PA, Beiser A, et al. Cerebral microbleeds: prevalence and associations with cardiovascular risk factors in the Framingham Study. Stroke. 2004;35:1831-35.

7. Gouw AA, Seewann A, van der Flier WM, et al. Heterogeneity of small vessel disease: a systematic review of MRI and histopathology correlations. J Neurol Neurosurg Psychiatry. 2011;82:126-35.

8. Tatsumi S, Shinohara M, Yamamoto T. Direct comparison of histology of microbleeds with postmortem MR images: a case report. Cerebrovasc Dis. 2008;26:142-46.

9. van Norden AG, van den Berg HA, de Laat KF, et al. Frontal and temporal microbleeds are related to cognitive function: the Radboud University Nijmegen Diffusion Tensor and Magnetic Resonance Cohort (RUN DMC) Study. Stroke. 2011;42:3382-86.

10. Poels MM, Ikram MA, van der Lugt A, et al. Cerebral microbleeds are associated with worse cognitive function: the Rotterdam Scan Study. Neurology 2012;78:326-33.

11. Cordonnier C, van der Flier WM, Sluimer JD, et al. Prevalence and severity of microbleeds in a memory clinic setting. Neurology. 2006;66:1356-60.

12. Patel B, Lawrence AJ, Chung AW, et al. Cerebral microbleeds and cognition in patients with symptomatic small vessel disease. Stroke. 2013;44:356-61.

13. Yates PA, Sirisriro R, Villemagne VL, et al. Cerebral microhemorrhage and brain beta-amyloid in aging and Alzheimer disease. Neurology. 2011;77:48-54.

14. Wardlaw JM, Lewis SC, Keir SL, et al. Cerebral microbleeds are associated with lacunar stroke defined clinically and radiologically, independently of white matter lesions. Stroke. 2006;37:2633-36.

15. Charidimou A, Krishnan A, Werring DJ, et al. Cerebral microbleeds: a guide to detection and clinical relevance in different disease settings. Neuroradiology. 2013;55:655-74.

16. Takashima Y, Mori T, Hashimoto M, et al. Clinical correlating factors and cognitive function in community-dwelling healthy subjects with cerebral microbleeds. Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association. 2011;20:105-10.

17. Yakushiji Y, Nishiyama M, Yakushiji S, et al. Brain microbleeds and global cognitive function in adults without neurological disorder. Stroke. 2008;39:3323-28.

18. Hilal S, Ikram MK, Saini M, et al. Prevalence of cognitive impairment in Chinese: Epidemiology of Dementia in Singapore study. J Neurol Neurosurg Psychiatry. 2013;84:686-92.

19. Cordonnier C, Potter GM, Jackson CA, et al. improving interrater agreement about brain microbleeds: development of the Brain Observer MicroBleed Scale (BOMBS). Stroke. 2009;40:94-9.

20. Yeo D, Gabriel C, Chen C, et al. Pilot Validation of a customized neuropsychological battery in elderly Singaporeans. Neurological Journal of South East Asia. 1997:123.

21. Lei C, Lin S, Tao W, Hao Z, Liu M, Wu B. Association between cerebral microbleeds and cognitive function: a systematic review. J Neurol Neurosurg Psychiatry. 2013;84:693-97.

22. Martinez-Ramirez S, Greenberg SM, Viswanathan A. Microbleeds do not affect rate of cognitive decline in Alzheimer disease. Neurology. 2013;80:1266.

23. Schneider JA. Brain microbleeds and cognitive function. Stroke. 2007;38:1730-31.

24. van der Vlies AE, Goos JD, Barkhof F, Scheltens P, van der Flier WM. Microbleeds do not affect rate of cognitive decline in Alzheimer disease. Neurology. 2012;79:763-69.

25. Lovelock CE, Cordonnier C, Naka H, et al. Antithrombotic drug use, cerebral microbleeds, and intracerebral hemorrhage: a systematic review of published and unpublished studies. Stroke. 2010;41:1222-28.

26. Vernooij MW, van der Lugt A, Ikram MA, et al. Prevalence and risk factors of cerebral microbleeds: the Rotterdam Scan Study. Neurology. 2008;70:1208-14

CHAPTER 4 – TABLES

Table 4 – 1: Baseline characteristics of screen positive participants in phase II compared to non-participants

Risk Factors	Participated in phase II		P-value*
	Yes (n = 282)	No (n = 312)	
Age (years)	69.8 (6.3)	71.4 (6.5)	**0.003**
Women, no. (%)	146 (51.8)	172 (55.1)	0.408
No formal education, no. (%)	124 (43.9)	171 (55)	**0.007**
Low Socioeconomic status, no. (%)	184 (65.2)	242 (77.5)	**<0.001**
Hypertension, no. (%)	208 (73.5)	258 (83)	**0.005**
Diabetes mellitus, no. (%)	78 (27.6)	77 (24.8)	0.698
Hyperlipidemia, no. (%)	245 (74.8)	147 (47.3)	**<0.001**
Mean arterial blood pressure, mmHg, (SD)	97.1 (9.7)	99.1 (11.9)	**0.026**
Random blood glucose, mmol/l (SD)	6.6 (2.7)	6.7 (2.9)	0.603
Total cholesterol, mmol/l (SD)	5.8 (1.7)	5.2 (1.1)	**0.010**
Ever smokers, no. (%)	86 (30.5)	93 (29.9)	0.271
Alcohol drinking, no. (%)	18 (6.4)	19 (6.1)	0.883
Body mass index, kg/m^2, (SD)	23.9 (3.4)	23.5(3.4)	0.166

Abbreviation: SD, standard deviation; kg/m^2, kilogram per meter square; mmHg, millimeters of mercury; mmol/l, millimoles per liter

* $p < 0.05$; significant

Table 4 – 2: Baseline characteristics of the participants with and without cerebral microbleeds on MRI (n= 282)

Baseline characteristics	CMB absence (n=191)	CMB present (n=91)	P value
Age, years (SD)	70.1 (6.4)	71.2 (5.9)	0.19
Women, n (%)	105 (55)	45 (49.5)	0.44
Education, (Primary ≤ 6 years)	124 (64.9)	60 (65.9)	0.89
Body mass index, kg/m^2 (SD)	19.1 (2.8)	18.9 (2.8)	0.62
Hypertension, n (%)	146 (76.4)	69 (75.8)	1.00
Diabetes, n (%)	50 (26.2)	25 (27.5)	0.89
Hyperlipidemia, n (%)	112 (58.6)	55 (60.4)	0.80
Mean arterial blood pressure, mmHg (SD)	100.2 (10.1)	100.0 (13.3)	0.88
Random blood glucose, mmol/l (SD)	6.7 (2.9)	6.6 (2.5)	0.65
Total cholesterol, mmol/l (SD)	5.0 (0.9)	4.8 (0.8)	0.12
Ever smokers, n (%)	54 (28.3)	32 (35.2)	0.27
Antiplatelets/ Anticoagulants, n (%)	23 (12)	26 (28.6)	**0.001**
Presence of lacunes, n (%)	25 (13.1)	27 (29.7)	**0.002**
Total brain volume, ml (SD)	889.0 (87.8)	902.6 (90.9)	0.23
Total WMH volume, ml, median (IQR)[#]	1.7 (0.5-4.4)	2.9 (0.5-6.9)	**0.02**

Abbreviations: CMB, cerebral microbleed; MRI, magnetic resonance imaging; WMH, white matter hyperintensities; SD, standard deviation; kg/m^2, kilogram per meter square; mmHg, millimeters of mercury; mmol/l, millimoles per liter; ml, milliliters; IQR, interquartile range

* $p < 0.05$; significant

The median, IQR and Wilcoxon Rank-Sum test was used as the variable had a skewed distribution

Table 4 – 3: Association between the presence of cerebral microbleeds and MRI markers of cerebrovascular disease and involutional changes (n = 282)

	Presence of lacunes	WMH volume	Total brain volume
	OR (95%CI)	**Mean difference (95%CI)**	**Mean difference (95%CI)**
Per CMB increase			
Model I*	**1.34 (1.09; 1.63)**	**0.04 (0.01; 0.07)**	0.63 (-2.12; 3.37)
Model II†	**1.33 (1.05; 1.69)**	**0.07 (0.03; 0.12)**	-1.31 (-6.27; 3.66)
Model III‡	1.25 (0.95; 1.63)§	**0.05 (0.01; 0.09)**‖	-2.47 (-7.53; 2.59)¶
CMB, ≥ 2 versus <2			
Model I*	**3.42 (1.55; 7.54)**	0.25 (-0.01; 0.49)	13.94 (-11.92; 39.82)
Model II†	**3.23 (1.38; 7.56)**	0.17 (-0.08; 0.42)	4.05 (-22.52; 30.61)
Model III‡	**2.94 (1.16; 7.45)§**	0.07 (-0.17; 0.31)‖	2.51 (-24.15; 29.17)¶
CMB 0, 1, or ≥ 2			
Model I*			
0	Reference	Reference	Reference
1	1.83 (0.82; 4.06)	0.11 (-0.10; 0.32)	5.71 (-16.18; 27.60)
≥ 2	**4.00 (1.74; 9.23)**	**0.26 (0.02; 0.51)**	16.06 (-10.47; 42.58)
Model II†			
0	Reference	Reference	Reference
1	1.69 (0.71; 4.08)	0.14 (-0.07; 0.35)	0.48 (-22.25; 23.22)
≥ 2	**3.77 (1.53; 9.33)**	0.20 (-0.05; 0.46)	4.87 (-22.21; 31.95)
Model II†			
0	Reference	Reference	Reference
1	1.63 (0.61; 4.38)§	0.09 (-0.10; 0.28)‖	-1.31 (-23.83; 21.22)¶
≥ 2	**3.24 (1.30; 9.06)§**	0.11 (-0.15; 0.36)‖	3.03 (-24.50; 30.55)¶

Abbreviations: OR, odds ratios; CI, confidence interval; CMB, cerebral microbleed; MRI, magnetic resonance imaging; WMH, white matter hyperintensities.

*Adjusted for age and sex.

†Adjusted for age, sex, smoking, mean arterial blood pressure, cholesterol, and random blood glucose.

‡Adjusted for age, sex, smoking, mean arterial blood pressure, cholesterol, random blood glucose, and other MRI markers.

§Other MRI markers include WMH and total brain volume.

‖Other MRI markers include lacunes and total brain volume.

¶Other MRI markers include lacunes and WMH volume.

Bold values represent statistically significant associations at P<0.05.

Table 4 – 4: Association between cerebral microbleeds and cognitive-impairment expressed as odd ratios and mean difference with 95% confidence intervals

	Composite Z-score **Mean difference (95% CI)**
Per CMB increase	
Model I*	**-0.04 (-0.07;-0.01)**
Model II†	**-0.09 (-0.14; -0.04)**
Model III‡	**-0.06 (-0.11; -0.01)**
CMB, ≥ 2 versus <2	
Model I*	**-0.34 (-0.59; -0.08)**
Model II†	**-0.31 (-0.57; -0.04)**
Model III‡	-0.18 (-0.43; 0.08)
CMB 0, 1, or ≥2	
Model I	
0	Reference
1	0.08 (-0.13; 0.29)
≥2	-0.32 (-0.58; -0.06)
Model II	
0	Reference
1	0.06 (-0.16; 0.28)
≥2	-0.32 (-0.59; -0.05)
Model III	
0	Reference
1	0.08 (-0.13; 0.29)
≥2	-0.18 (-0.45; 0.09)

Abbreviation: CI, confidence interval; CMB, cerebral microbleed; MRI, magnetic resonance imaging; WMH, white matter hyperintensities

* Adjusted for age, sex and education

† Adjusted for age, sex, education, mean arterial blood pressure, cholesterol, random blood glucose, smoking, body mass index, antiplatelets/anticoagulants and Geriatric Depression Scale

‡ Adjusted for age, sex, education, mean arterial blood pressure, cholesterol, random blood glucose, smoking, body mass index, antiplatelets/anticoagulants, Geriatric Depression Scale and other MRI markers (lacunes, WMH and total brain volumes)

Bold values represent statistically significant associations at P<0.05

Table 4 – 5: Association between the number of cerebral microbleeds (per lesion increase) and specific cognitive domains expressed as mean differences with 95% confidence intervals

	Executive function	Attention	Language	Visuomotor speed	Visuoconstruction	Verbal memory	Visual memory
	B (95%CI)	B (95%CI)	B (95%CI)	B (95%CI)	B (95%CI)	B (95%CI)	B (95%CI)
Model I*	**-0.05 (-0.08; -0.02)**	-0.02 (-0.05; 0.01)	-0.03 (-0.06; 0.00)	-0.02 (-0.05; 0.00)	**-0.05 (-0.07; -0.02)#**	-0.02 (-0.05; 0.01)	**-0.04 (-0.06; -0.01)**
Model II†	**-0.10 (-0.16; -0.04)**	**-0.09 (-0.15; -0.04)**	**-0.08 (-0.14; -0.02)**	**-0.05 (-0.09; -0.00)**	**-0.09 (-0.15; -0.04)#**	**-0.06 (-0.11; -0.00)**	**-0.06 (-0.12; -0.01)**
Model III‡	**-0.07 (-0.13; -0.02)**	**-0.06 (-0.12; -0.01)**	-0.06 (-0.12; 0.01)	-0.02 (-0.07; 0.03)	**-0.08 (-0.13; -0.02)#**	-0.03 (-0.08; 0.03)	-0.03 (-0.08; 0.02)

Abbreviations: B= mean difference; CI= confidence interval

* Adjusted for age, sex and education

† Adjusted for age, sex, education, mean arterial blood pressure, cholesterol, random blood glucose, smoking, body mass index, antiplatelets/anticoagulants and Geriatric Depression Scale

‡ Adjusted for age, sex, education, mean arterial blood pressure, cholesterol, random blood glucose, smoking, body mass index, antiplatelets/anticoagulants, Geriatric Depression Scale and other MRI markers (lacunes, WMH and total brain volumes)

Significant after accounting for multiple testing with Bonferroni correction

Bold values represent statistically significant associations at P<0.05

CHAPTER 5:

Cortical Microinfarcts on 3T MRI: Clinical Correlates in Memory-Clinic Patients

1. INTRODUCTION

Cerebrovascular disease is an important contributor to cognitive decline and dementia in the aging population.[1] On autopsy, vascular pathology is found in the majority of patients with clinically diagnosed dementia.[2] This vascular pathology frequently involves the cerebral small vessels. In vivo, signs of cerebral small vessel disease (SVD) on conventional magnetic resonance imaging (MRI) include white matter hyperintensities (WMHs), lacunes, and microbleeds.[3,4] However, these conventional MRI markers do not fully capture the burden of SVD in cognitive decline and dementia. In this context, cerebral microinfarcts (CMIs) have attracted increasing attention.[5] CMIs are regarded as the most widespread form of brain infarction and hence could play an important role in cognitive decline and dementia.[5,6] A systematic review with a pooled analysis of autopsy studies showed that CMIs are observed in 24% of non-demented older subjects, in 43% of patients with Alzheimer's disease (AD), and in 62% of patients with vascular dementia (VaD).[6] Moreover, autopsy studies link CMIs to ante-mortem cognitive decline, also independent of Alzheimer pathology.[7,8] Recently, it has been shown that cortical CMIs can be visualized in vivo using high-field 7 tesla (7T) MRI,[9] and that these CMIs can also be detected on 3T MRI scans.[9,10]

In this study, we examined the frequency of cortical CMIs on 3T MRI in a multi-ethnic Asian memory clinic population with a high vascular burden from Singapore. Furthermore, we investigated their association with vascular risk factors, cognition, and conventional SVD markers.

2. METHODS

2.1 Study Population

This study involves patients from the National University Health System Memory Ageing and Cognition Centre Cohort recruited from the memory clinics of the National

University Hospital and St. Luke's Hospital in Singapore. Patients received a referral diagnosis prior to enrolment into the study. Five diagnostic categories were eligible for inclusion in this study, which were based on the referral diagnosis. 1) 'No cognitive impairment' (NCI): this diagnosis was given to patients visiting the memory clinic who had no objective cognitive impairment on formal neuropsychological tests, or functional loss. 2) 'Cognitive impairment no dementia' (CIND), with (2a) or without (2b) a history of stroke was diagnosed in patients who were impaired in at least one cognitive domain of a formal neuropsychological test battery, but did not meet DSM-IV (Diagnostic and Statistical Manual of Mental Disorders - Fourth Edition) criteria for dementia. Subjects were considered to have failed a test it they scored lower than age and education-adjusted 1.5 SDs below established normal means on individual tests. Failure in at least half of the tests in a domain was considered as impairment in that domain. Ischemic stroke was assessed based on medical history, and confirmed by neuroimaging. Patients with a history of hemorrhagic stroke were excluded. 3) AD was diagnosed in accordance with the NINCDS—ADRDA criteria.[11] 4) VaD was diagnosed in accordance with the NINDS-AIREN criteria.[12] Patients with other diagnoses, or significant neurological co-morbidities (e.g. Parkinson's disease), or loss of functional independence (modified Rankin Scale >4), were not included in the cohort.

As part of the cohort study these diagnoses were confirmed in a multidisciplinary consensus meeting, attended by neurologists, psychologists, and a neuroradiologist. All study patients underwent a standardized extensive physical, clinical, and neuropsychological assessment as well as 3T MRI, all on the same day, at the National University of Singapore. For the present study, we selected all consecutive patients (N=251), meeting the abovementioned criteria, included between December 2010 and September 2013. Of these 251 subjects, 13 were excluded due to missing T1-, FLAIR, or T2-weighted images, resulting in a total of 238 patients included in the current analyses.

Ethical approval for this study was obtained from the National Healthcare Group Domain-Specific Review Board (DSRB). The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained, in the preferred language of the patients, by bilingual study coordinators prior to recruitment into the study. Consent for patients lacking capacity was provided by their legal representative, as allowed by the DSRB.

The vascular risk profile was recorded for each patient, which included: a) Diabetes mellitus: defined as a history or previous diagnosis of diabetes mellitus, or use of glucose-lowering medication; b) Hypertension: defined as a history or previous diagnosis of hypertension, or use of antihypertensive medication; c) Hyperlipidemia: defined as a history or previous diagnosis of hyperlipidemia, or use of lipid-lowering medication; d) Cardiovascular disease: defined as a previous diagnosis of myocardial infarction, congestive heart failure, atrial fibrillation, or intervention procedures such as angioplasty, or stenting.

2.2 Cognitive Assessment

The Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) as well as a formal neuropsychological battery, previously validated for elderly Singaporeans,[13] were administered. The subtests to assess the seven cognitive domains have been described previously in **Chapter 3**.

The assessment was administered according to the patient's preferred language (i.e. English, Mandarin, or Malay). All individual raw test scores were transformed to standardized z-scores using the means and SDs of the whole group (N=238). Further information on calculation of Z scores have been described in **Chapter 3**.

2.3 MRI Protocol

All scans were acquired on a 3T Siemens Magnetom Trio Tim system, with a 32-channel receiver head-coil, at the Clinical Imaging Research Centre of the National University of

Singapore. The standardized protocol for 3D T1-weighted, a 2D multislice T2-weighted, fluid-attenuated inversion recovery (FLAIR) and susceptibility weighted imaging (SWI), for the assessment of markers of SVD and intracranial stenosis has been described previously in **Chapter 3**.

2.4 MRI Rating

Rating criteria for cortical CMIs were based on a previous study that included histological validation (**Figure 5-1)**.[9] In that study 15 CMIs were found on 7T in 6/22 subjects. A proportion (4/15 = 27%) of those CMIs in 2 subjects could also be visualized on 3T MRI, especially on the 3D T1-weighted image (**Figure 5-2**).[9] Based on those results, the rating criteria for cortical CMIs on 3T for the present study were defined in the similar fashion as decribed in **Chapter 3** (**Figure 5-3**).

The reliability of these 3T rating criteria were tested, using scans from the database of a previous study.[14] From this dataset, 3T scans were selected based on the earlier 7T evaluation by a single rater [SvV]. The validation set included 12 subjects with CMIs on the 7T MR images with appropriate 3T MR images, and 11 subjects without CMIs on 7T. Cortical CMIs were identified by one visual rater [SvV] on the 3T FLAIR, T1, and T2 images of these 23 subjects, using the 3T rating criteria as described above, blinded to the 7T results and clinical information. Identified cortical CMI locations on 3T were then compared to the 7T FLAIR, T1, and T2 of the same subject to verify the presence of a CMI. It was found that 7/8 (88%) of the identified cortical CMI locations on 3T in these subjects, matched with CMIs on the 7T MRI. One of the 8 CMI locations proved to be a sulcus on the higher resolution 7T images. The 7 CMIs identified on 3T represented 27% of the total number of CMIs (N=26) identified by the same rater on the 7T MRI scans in this dataset.

Cortical CMIs were assessed by one experienced rater [SvV]. The intra-rater agreement for cortical CMIs, as assessed with the intraclass correlation coefficient (ICC) on a

representative subset of 3T MRI scans, was excellent (ICC=0.97) and good, as assessed with Dice's similarity coefficient (DSC) (DSC=0.65).[15] CMI size was estimated on T1 along the longest axis of the lesion.

Microbleeds were assessed by one rater [SH] on T2*-weighted images using the Brain Observer MicroBleeds Scale (BOMBS) criteria. The intra-rater agreement was excellent (ICC=0.89).

The presence of any large cortical infarct (>5 mm), any cerebellar infarct, or any subcortical infarct (i.e. a large subcortical infarct and/or a lacunar infarct) was assessed on FLAIR and T1, by two independent raters [SH, NW]. Subsequently, both infarct and WMH volume were segmented manually [NW] on FLAIR and T1-weighted images using an in-house developed tool based on MeVisLab (MeVis Medical Solutions AG, Bremen, Germany).[16,17]

Total brain volume and intracranial volume were quantified using a unified segmentation approach as implemented in Statistical Parametric Mapping 12b.[18] WMH and infarct volumes were censored in the segmentation using a mask based on the manual delineation of WMHs and infarcts, but were considered as part of total brain volume. Intracranial volume was calculated using total brain volume, intraventricular, and extracortical cerebrospinal fluid volume.

The presence of intracranial stenosis, assessed by one rater [SH] on time of flight, was defined as a narrowing exceeding 50% of the luminal diameter of either the vertebral, basilar, internal carotid, posterior cerebral, middle cerebral, or anterior cerebral artery.

All MRI ratings were performed blinded to clinical information and without knowledge of cortical CMI ratings.

2.5 Statistical Analyses

Differences between patients without and with ≥1 or ≥3 (upper tertile) cortical CMIs on MRI were assessed using independent t-tests for continuous variables, chi-square tests for

dichotomous variables, and Mann-Whitney U tests for non-parametric data. Linear regression was used for the association of cortical CMIs (determinant) with MMSE, MoCA, cognitive domains, the composite z-score, and diagnosis (outcomes), adjusted for age, gender, and level of education. Linear regression was used for the association of cortical CMIs (determinant) with total brain volume and WMH volume (log transformed) (outcomes), adjusted for age, gender, and intracranial volume. The B from the linear regression models reflects a 'mean difference' between patients with cortical CMIs and those without cortical CMIs on MRI. Binary logistic regression was used for the association of cortical CMIs with vascular risk factors (except BMI, which was assessed using linear regression), the presence of intracranial stenosis, (subcortical and cortical) infarcts, (deep and lobar) microbleeds, and the presence of confluent WMHs, adjusted for age and gender. Dummy variables were constructed for the analysis, using chi-square tests, of diagnosis in relation to cortical CMIs. P-values <0.05 were regarded as statistically significant. All analyses were performed using IBM SPSS Statistics, version 20.0.

3. RESULTS

3.1 Demographics and Vascular Risk Factor Profile

The mean age of the 238 patients in this study was 72.5 ± 9.1 years (range 50 - 95), including 117 (49%) men. Demographic and vascular risk factor profile characteristics of patients with and without cortical CMIs on MRI are presented in **Table 5-1**. Seventy-five patients (32%) had cortical CMIs (Figure 3), ranging between 1 - 43 CMIs, with a median of 1. Of the patients with cortical CMIs, 39 (52%) had one, 11 (15%) had two, and 25 (33%) had three or more cortical CMIs (median 1in those with ≥3 CMIs: median 5, range 31 - 43). Median size of CMIs was 3 mm, only 8% was larger than 3 mm. Cortical CMIs were found throughout the brain, with a slight predilection for parietal

cortical areas (**Supplementary Figure 5-1**). Presence of cortical CMIs was not related to age, gender, race, or level of education. Presence of CMIs was associated with hyperlipidemia, a history of stroke, and cardiovascular disease, but not with other vascular risk factors.

3.2 Cognitive Profile

The association of cortical CMIs with cognition is presented in **Table 5-2**. The presence of cortical CMIs was associated with lower MMSE score and a lower overall composite z-score, and worse performance on the specific domains, including language and visuoconstruction. The presence of ≥3 cortical CMIs was also associated with impaired executive function.

3.3 MRI Findings

The association of cortical CMIs with other MRI findings is presented in **Table 5-3**.

The presence of cortical CMIs was associated with the presence of both large cortical and subcortical infarcts, and both deep and lobar microbleeds. In patients with a large cortical infarct, cortical CMIs were often not always restricted to the same hemisphere as the infarct. The relation between cortical CMIs with hyperlipidemia and cardiovascular disease did not alter when we adjusted for presence of large cortical infarcts.

The presence of cortical CMIs was associated with smaller brain volume, and larger WMH volume. The association of the presence of cortical CMIs with brain volume did not alter when we subsequently adjusted for WMH volume, presence of infarcts, or presence of microbleeds.

The presence of cortical CMIs was associated with the presence of any intracranial stenosis. Of note, location of the CMI and the stenosis appeared to be interrelated. Cortical CMIs in the right middle cerebral artery (MCA) territory were more common in patients with a right MCA stenosis (patients with a right MCA stenosis N=16; 50% CMIs in right MCA territory; patients without a right MCA stenosis N=213; 17% CMIs in right

MCA territory; p=0.001). The same was true for left MCA stenosis (patients with a left MCA stenosis N=14; 36% CMIs in left MCA territory; patients without a left MCA stenosis N=215; 12% CMIs in left MCA territory; p=0.010).

The association of the presence of cortical CMIs with MMSE was independent of the presence of infarcts, attenuated after adjusting for WMH volume, the presence of microbleeds, and after adjustment for brain volume (as percentage of intracranial volume), a marker for brain atrophy. The association of the presence of cortical CMIs with the domain language was independent of the presence of infarcts and microbleeds, and attenuated after adjusting for WMH volume, and after adjustment for brain volume. The association of the presence of cortical CMIs with the domain visuoconstruction was independent of the presence of infarcts, microbleeds, and WMH, and attenuated after adjustment for brain volume (**Table 5-4**).

3.4 Clinical Diagnosis

The presence of cortical CMIs was linked to the assigned referral diagnoses (Table 4). Patients with cortical CMIs on MRI were less often diagnosed with NCI (0.27 [0.09 ; 0.85] p=0.025) or CIND without stroke (0.34 [0.12 ; 0.92] p=0.033), whereas patients with cortical CMIs were more often diagnosed with VaD (2.86 [1.17 ; 6.99] p=0.021), compared to patients without cortical CMIs (**Supplementary Table 5-1**).

4. DISCUSSION

This study showed that cortical CMIs are a common finding on 3T MRI in a memory clinic population. Presence of CMIs was associated with several distinct clinical features, including reduced performance in the domains of language and visuoconstruction, domains that are not typically related to other MRI markers of vascular disease. On MRI, presence of cortical CMIs was related to markers of SVD as well as large vessel disease.

Until recently, cortical CMIs could not be visualized on MRI, giving rise to the term 'the invisible lesion'.[5] Recently, it was shown that cortical CMIs can be visualized with 7T MRI, but also with 3T MRI.[9] In the present study we show that CMIs are a common finding on 3T MRI scans from a memory clinic population. This is important because 3T is more widely available than 7T, allowing a more widespread evaluation of the clinical relevance of CMIs in the context of aging, cerebrovascular disease, and dementia in future clinical studies. In the present cohort, cortical CMIs were more common in patients with dementia (36%) compared to patients without dementia (27%), which is in line with neuropathological findings[6] and a previous 7T MRI study.[19] It should be acknowledged, however, that 3T MRI only detects the larger CMIs. Neuropathological studies report that sizes of CMIs vary between 50 μm and 5 mm.[6] The vast majority of CMIs that are captured on 3T MRI are 2-3 mm. Hence, these CMIs on MRI are likely to represent only a small fraction of the largest lesions from a much larger underlying total CMI burden. The same applies, albeit to a lesser extent, for 7T, as again only the larger CMIs are detected. Indeed, estimates from neuropathological studies indicate that CMI counts are much higher than observed in the present study.[20] Nevertheless, as shown here, the CMIs that are detected by MRI do have important clinical correlates.

Of the demographic and vascular risk factors examined in this study, only hyperlipidemia, a history of stroke, and a history of cardiovascular disease were associated with the presence of cortical CMIs. Relatively few autopsy studies have systematically examined the relation between CMIs and demographic or vascular risk factors. Some autopsy studies found an association of CMIs with advanced age at death,[21,22] but other studies did not.[8] No relation with gender has been found,[8, 21,23] which is in line with our findings. Severe hypertension was identified as a risk factor for microscopic infarcts upon autopsy in one population-based study.[23] Another population-based autopsy study found only an association between higher systolic blood pressure

and CMIs in individuals younger than 80 years of age at entry.[24]As far as we know, the relation between CMIs and dyslipidemia has not been explored in post-mortem studies. The strong association of hyperlipidemia, rather than hypertension, with cortical CMIs in our study is remarkable, as hypertension is the most important risk factor for the conventional markers of SVD (i.e. lacunar infarcts, microbleeds, WMHs). This implies that CMIs may also occur in the context of other etiological processes.

We found that cortical CMIs were associated with worse cognitive performance, in particular tasks assessing cortical function (i.e. language and visuoconstruction). Interestingly, tasks that are known to be related to vascular damage in subcortical regions (e.g. attention, visuomotor speed) were relatively less affected. The significant association with MMSE score in contrast to MoCA score further underlines this finding, as the MoCA incorporates more tests of executive function. Few autopsy studies have looked into the relation of CMIs and specific cognitive domains. A relation between cortical CMIs and worse performance on semantic memory, perceptual speed, and visuospatial abilities was found in one study, whereas subcortical CMIs were not associated with any of the cognitive domains.[8]

Cortical CMIs were strongly associated with larger cortical infarcts. They were not solely found in cortical areas surrounding the infarct, but also in other cortical areas and the other hemisphere. This suggests global underlying vessel pathology instead of a more local manifestation of cerebrovascular disease. Cortical CMIs are generally considered as manifestations of cerebral SVD.[5] Our results suggest that CMIs are likely to be attributable to different aetiologies. The strong associations of cortical CMIs with larger cortical infarcts and the spatial relation with presence of intracranial stenosis suggest that they are also related to large vessel disease. Possibly CMIs downstream from a large vessel stenosis are due to hypoperfusion, but microemboli might also be a causative factor. Neuropathological studies indeed confirm that CMIs are linked to SVD, in

particular cerebral amyloid angiopathy,[22,23, 25-28] but can also be attributed to large vessel disease.[29] The present study cohort was enriched for patients with ischemic stroke, but patients with hemorrhagic stroke were excluded. In future studies it would be interesting to assess CMI burden also in patients with hemorrhagic stroke, for example in the context of cerebral amyloid angiopathy, because CMIs appear to be common in such patients.[26-28, 30]

This study further showed that cortical CMIs are related to brain atrophy. Neuropathological studies have suggested that a single CMI on routine pathological examination indicates the presence of hundreds up to a thousand CMIs in a single brain.[20] It could be argued that this lesion burden by itself contributes to volume loss. With our current scan protocol, only the largest of the whole CMI spectrum can be captured. Extending the neuropathological findings, several CMIs on MRI could therefore indicate the presence of many more smaller CMIs. Nevertheless, whether many CMIs in a single brain by themselves explain global brain atrophy remains to be determined. Even hundreds of CMIs still only account for a total lesion volume of less than one ml, which is still only a fraction of total cortical volume. The relation of cortical CMIs with worse cognitive performance in this study lost statistical significance when we adjusted for atrophy measured by brain volume as part of intracranial volume. Apparently, cortical atrophy and CMIs may be linked through shared aetiologies or risk factors. The interrelation between atrophy, CMIs, and cognition should be a topic of future studies.

There are some limitations to this study that need to be considered. Cortical CMI rating criteria have been developed and validated with histology on 7T MRI. The current 3T rating criteria proved to be very consistent with 7T MRI, in the sense that 88% of 3T MRI CMIs proved to be CMIs on 7T in our validation study. However, it needs to be acknowledged that the sensitivity of 3T to detect cortical CMIs is much lower than 7T (27% of CMIs on 7T are detected by 3T). The current translation of cortical CMI rating

that was developed on 7T MRI to conventional MRI is of importance, as it allows the assessment of this novel marker of cerebrovascular disease in much larger groups of patients and in the general population. Longitudinal studies are needed to further unravel the clinical importance of cortical CMIs. To further improve cortical CMI rating, we suggest scan protocol improvements, including the use of 3T 3D FLAIR images. Finally, it remains to be investigated if the current findings are generalizable to non-Asian populations and patients from other memory clinics, or comparable in those with a different vascular risk factor profile.

5. CONCLUSION

Our 3T MRI study showed that cortical CMIs are a common finding in an Asian memory clinic population. Cortical CMIs are associated with cerebral SVD, but most strongly with cortical infarcts. In contrast with subcortical SVD, cortical CMIs are particularly related with worse language and visuoconstructive abilities, domains considered cortical in nature. Hence, CMIs may be regarded as a distinct marker of cerebrovascular disease in dementia.

CHAPTER 5 – REFERENCES

1. Pendlebury ST, Rothwell PM. Prevalence, incidence, and factors associated with pre-stroke and post-stroke dementia: a systematic review and meta-analysis. Lancet Neurol. 2009;8:1006-18.

2. Kalaria RN, Kenny RA, Ballard CG, et al. Towards defining the neuropathological substrates of vascular dementia. J Neurol Sci. 2004;226:75-80.

3. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. Lancet Neurol. 2010;9:689-701.

4. Wardlaw JM, Smith EE, Biessels GJ, et al. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol. 2013;12:822-38.

5. Smith EE, Schneider JA, Wardlaw JM, Greenberg SM. Cerebral microinfarcts: the invisible lesion. Lancet Neurol. 2012;11:272-82.

6. Brundel M, de Bresser J, van Dillen JJ, et al. Cerebral microinfarcts: a systematic review of neuropathological studies. J Cereb Blood Flow Metab. 2012;32:425-36.

7. Launer LJ, Hughes TM, White LR. Microinfarcts, brain atrophy, and cognitive function: the Honolulu Asia Aging Study Autopsy Study. Ann Neurol. 2011;70:774-80.

8. Arvanitakis Z, Leurgans SE, Barnes LL, et al. Microinfarct pathology, dementia, and cognitive systems. Stroke. 2011;42:722-27.

9. Van Veluw SJ, Zwanenburg JJ, Engelen-Lee J, et al. In vivo detection of cerebral cortical microinfarcts with high-resolution 7T MRI. J Cereb Blood Flow Metab. 2013;33:322-29.

10. Ii Y, Maeda M, Kida H, et al. In vivo detection of cortical microinfarcts on ultrahigh-field MRI. J Neuroimaging. 2013;23:28-32.

11. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of department of health and human services task force on Alzheimer's disease. Neurology. 1984;34:939-44.

12. Wiederkehr S, Simard M, Fortin C, van Reekum R. Validity of the clinical diagnostic criteria for vascular dementia: a critical review. J Neuropsychiatry Clin Neurosci. 2008;20:150-77.

13. Yeo D, Gabriel C, Chen C, et al. Pilot validation of a customized neuropsychological battery in elderly Singaporeans. Neurol J South East Asia. 1997;123:1-8.

14. Van Veluw SJ, Heringa SM, Kuijf HJ, et al. Cerebral cortical microinfarcts at 7Tesla MRI in patients with early Alzheimer's disease. J Alzheimers Dis. 2014;39:163-67.

15. Kuijf HJ, van Veluw SJ, Viergever MA, et al. How to assess the reliability of cerebral microbleed rating? Front Aging Neurosci. 2013;26:57.

16. Ritter F, Boskamp T, Homeyer A, et al. Medical image analysis: A visual approach. IEEE Pulse. 2011;2:60-70.

17. Kuijf HJ. Image processing techniques for quantification and assessment of brain MRI, Utrecht University, 2013; ISBN: 978-90-393-6037-8.

18. Ashburner J, Friston KJ. Unified segmentation. Neuroimage. 2005;26:839-851.

19. Van Rooden S, Goos JD, van Opstal AM et al. Increased number of microinfarcts in Alzheimer disease at 7-T MR imaging. Radiology. 2013;270:205-211.

20. Westover MB, Bianchi MT, Yang C, et al. Estimating cerebral microinfarct burden from autopsy samples. Neurology. 2013;80:1365-1369.

21. Yu L, Boyle PA, Leurgans S, et al. Disentangling the effects of age and APOE on neuropathology and late life cognitive decline. Neurobiol Aging. 2014;35:819-826.

22. Longstreth WT Jr, Sonnen JA, Koepsell TD, et al. Associations between microinfarcts and other macroscopic vascular findings on neuropathologic examination in 2 databases. Alzheimer Dis Assoc Disord. 2009;23:291-94.

23. Troncoso JC, Zonderman AB, Resnick SM, et al. Effect of infarcts on dementia in the Baltimore Longitudinal Study of Aging. Ann Neurol. 2008;64:168-76.

24. Wang LY, Larson EB, Sonnen JA, et al. Blood pressure and brain injury in older adults: findings from a community-based autopsy study. J Am Geriatr Soc. 2009;57:1675-1981.

25. Schneider JA, Boyle PA, Arvanitakis Z, et al. Subcortical infarcts, Alzheimer's disease pathology, and memory function in older persons. Ann Neurol. 2007;62:59-66.

26. Kövari E, Herrmann FR, Hof PR, et al. The relationship between cerebral amyloid angiopathy and cortical microinfarcts in brain ageing and Alzheimer's disease. Neuropathol Appl Neurobiol. 2013;39:498-509.

27. Soontornniyomkij V, Lynch MD, Mermash S, et al. Cerebral microinfarcts associated with severe cerebral beta-amyloid angiopathy. Brain Pathol. 2010;20:459-67.

28. Haglund M, Passant U, Sjobeck M, et al. Cerebral amyloid angiopathy and cortical microinfarcts as putative substrates of vascular dementia. Int J Geriatr Psychiatry. 2006;21:681-87.

29. Zheng L, Vinters HV, Mack WJ, et al. Cerebral atherosclerosis is associated with cystic infarcts and microinfarcts but not Alzheimer pathologic changes. Stroke. 2013;44:2835-41.

30. Van Veluw SJ, Jolink WM, Hendrikse J, et al. Cortical microinfarcts on 7T MRI in patients with spontaneous intracerebral hemorrhage. J Cereb Blood Flow Metab. 2014;34:1104-6.

CHAPTER 5 – TABLES

Table 5 – 1: Patient characteristics

Characteristics	Without CMIs (N=163)	With CMIs (N=75)	OR (95%CI)	P-value	Multiple (≥3) CMIs (N=25)	OR (95%CI)	P-value
Demographics							
Age (years), mean (SD)	72.5 ± 9.2	72.4 ± 9.0	-	0.976	73.4 ± 9.5	-	0.643
Male, no. (%)	76 (47)	41 (55)	-	0.249	17 (68)	-	**0.047**
Race (Chinese)*, no (%)	130 (78)	55 (73)	-	0.298	21 (84)	-	0.639
Education†, median (min-max)	1 [0-3]	1 [0-3]	-	0.960	1 [0-3]	-	0.792
Vascular risk factor profile							
Current smoking, no (%)	14 (9)	10 (13)	1.58 [0.65 ; 3.86]	0.312	3 (12)	1.11 [0.28 ; 4.41]	0.884
Alcohol use, no (%)	5 (3)	2 (3)	0.81 [0.15 ; 4.36]	0.804	0 (0)	n/a	n/a
Body-mass index, mean (SD)	23.7 ± 3.7	24.6 ± 4.6	0.88 [-0.22 ; 1.98]§	0.118	24.4 ± 4.5	0.69 [-0.95 ; 2.33]§	0.407
Hypertension, no (%)	120 (74)	60 (80)	1.42 [0.73 ; 2.76]	0.304	19 (76)	1.10 [0.41 ; 2.98]	0.852
Diabetes mellitus, no (%)	56 (34)	34 (45)	1.59 [0.91 ; 2.78]	0.104	10 (40)	1.26 [0.52 ; 3.03]	0.609
Hyperlipidemia, no (%)	103 (63)	65 (87)	**3.79 [1.81 ; 7.95]**	**0.000**	24 (96)	**14.06 [1.84 ; 107.35]**	**0.011**
History of stroke‡, no (%)	53 (33)	39 (52)	**2.40 [1.30 ; 4.41]**	**0.005**	15 (60)	**3.35 [1.29 ; 8.68]**	**0.013**
History of CVD, no (%)	25 (15)	28 (37)	**3.28 [1.72 ; 6.24]**	**0.000**	13 (52)	**5.76 [2.29 ; 14.51]**	**0.000**

Abbreviations: CMI, cerebral microinfarcts; OR, odds ratio; CI, confidence interval; n/a, not available; SD, standard deviation; CVD, cardiovascular diseases

Bold text indicates P<0.05. *Chinese (N = 185), Malay (N = 33), Indian (N = 15), mixed (N = 2), others (N = 3)

†Nil (N = 52), primary (N = 88), secondary (N = 69), tertiary (N = 28). ‡Based on self-reported stroke. Odds ratio in binary logistic regression, adjusted for age and gender, compared with patients without CMIs. §Mean difference in BMI between groups, adjusted for age and gender

Table 5 – 2: Cognitive profile

Characteristics	without CMIs (N=163)	with CMIs (N=75)	B [95% CI]	P-value	Multiple (≥3) CMIs (N=25)	B [95% CI]	P-value
Cognitive profile							
Mini-mental state examination	21.0 ± 6.2	19.5 ± 5.9	-1.49 [-2.89; -0.08]	**0.038**	18.8 ± 6.2	-2.17 [-4.37; 0.04]	0.054
Montreal Cognitive Assessment	16.5 ± 7.2	15.2 ± 6.9	-1.38 [-2.96; 0.20]	0.086	14.2 ± 6.2	-2.19 [-4.63; 0.25]	0.078
Composite z-score	0.08 ± 1.05	-0.17 ± 0.10	-0.20 [-0.42; 0.01]	0.067	-0.37 ± 0.79	-0.38 [-0.74; -0.03]	**0.036**
Executive function	0.06 ± 1.00	-0.13 ± 1.00	-0.18 [-0.41; 0.05]	0.133	-0.36 ± 1.05	-0.39 [-0.76; -0.01]	**0.042**
Attention	0.03 ± 1.00	-0.07 ± 1.02	-0.11 [-0.34; 0.13]	0.375	-0.19 ± 1.06	-0.21 [-0.59; 0.17]	0.282
Language	0.09 ± 1.04	-0.21 ± 0.89	-0.28 [-0.53; -0.04]	**0.023**	-0.44 ± 0.62	-0.48 [-0.87; -0.09]	**0.017**
Verbal memory	0.05 ± 1.05	-0.11 ± 0.88	-0.13 [-0.37; 0.10]	0.268	-0.24 ± 0.78	-0.21 [-0.59; 0.17]	0.273
Visual memory	0.07 ± 1.06	-0.15 ± 0.84	-0.21 [-0.45; 0.03]	0.086	-0.34 ± 0.55	-0.37 [-0.77; 0.02]	0.063
Visuoconstruction	0.10 ± 1.03	-0.21 ± 0.89	-0.30 [-0.52; -0.08]	**0.008**	-0.44 ± 0.77	-0.51 [-0.87; -0.15]	**0.005**
Visuomotor speed	0.07 ± 1.06	-0.15 ± 0.83	-0.19 [-0.40; 0.01]	0.067	-0.27 ± 0.87	-0.25 [-0.60; 0.09]	0.153

Abbreviations: CMI, cerebral microinfarcts; CI, confidence interval

NOTE. Data are presented as mean ± standard deviation (SD). B: Mean difference between patients with cortical CMIs and patients without, from linear regression analyses, adjusted for age, gender, and level of education. Z-scores were based on the mean and SDs of the whole patient group (N = 238)

Table 5 – 3: MRI findings

Characteristics	Without CMIs (N=163)	With CMIs (N=75)	B [95% CI]	P-value	Multiple (≥3) CMIs (N=25)	B [95% CI]	P-value
Intracranial volume (ml)	1434 ± 137	1459 ± 152	10 [-19 ; 39]	0.506	1497 ± 166	24 [-21 ; 68]	0.298
Brain volume (ml)	918 ± 114	897 ± 109	-32 [-50 ; -13]	**0.001**	905 ± 117	-35 [-64 ; -7]	**0.017**
WMH volume (ml), log	15.4 ± 16.7	21.6 ± 21.1	0.4 [0.1 ; 0.7]	**0.008**	27.8 ± 26.9	0.5 [0.0 ; 1.0]	**0.035**
			OR [95% CI]			**OR [95% CI]**	
Intracranial stenosis	31 (20)	24 (34)	2.04 [1.08 ; 3.84]	**0.027**	11 (46)	3.39 [1.37 ; 8.43]	**0.009**
Presence of infarcts	56 (34)	48 (65)	3.35 [1.87 ; 6.02]	**0.000**	21 (84)	8.90 [2.86 ; 27.65]	**0.000**
- cortical infarcts	12 (7)	27 (36)	6.91 [3.24 ; 14.77]	**0.000**	18 (72)	29.64 [10.26 ; 85.64]	**0.000**
- subcortical infarcts	42 (26)	33 (44)	2.19 [1.22 ; 3.94]	**0.009**	11 (44)	1.88 [0.77 ; 4.58]	0.163
Presence of microbleeds	83 (51)	52 (72)	2.42 [1.33 ; 4.43]	**0.004**	21 (84)	5.04 [1.64 ; 15.48]	**0.005**
- deep microbleeds	28 (17)	25 (35)	2.46 [1.30 ; 4.65]	**0.006**	10 (40)	3.04 [1.22 ; 7.57]	**0.017**
- lobar microbleeds	70 (43)	45 (63)	2.15 [1.21 ; 3.80]	**0.009**	19 (76)	4.36 [1.63 ; 11.65]	**0.003**
Presence of WMH*	101 (62)	56 (75)	1.93 [1.02 ; 3.67]	**0.044**	21 (84)	3.53 [1.08 ; 11.55]	**0.037**

CMI, cerebral microinfarcts; WMH, white matter hyperintensities; OR, odds ratio; MRI, magnetic resonance imaging; CI, confidence interval.

NOTE. Data are presented as mean ± standard deviation, or number (percentage). B: mean difference between patients with cortical CMIs and patients without, from linear regression analyses, adjusted for age, gender, and intracranial volume. Odds ratio in binary logistic regression, adjusted for age and gender, compared with patients without CMIs.

*Presence of WMHs is defined as beginning confluence or large confluent area, on Fazekas scale

Table 5 – 4: Association of cortical CMIs with cognition, adjusted for other MRI markers

MRI markers included in the model	B [95% CI]	P-value
MMSE (in points)		
CMIs alone	-1.49 [-2.89 ; -0.08]	**0.038**
CMIs + presence of infarcts	-1.61 [-3.07 ; -0.15]	**0.031**
CMIs + WMH volume	-1.22 [-2.63 ; 0.18]	0.087
CMIs + presence of microbleeds	-1.34 [-2.79 ; 0.11]	0.070
CMIs + brain volume (% ICV)	-0.68 [-2.00 ; 0.65]	0.315
Language (z-score)		
CMIs alone	-0.28 [-0.53 ; -0.04]	**0.023**
CMIs + presence of infarcts	-0.28 [-0.53 ; -0.02]	**0.034**
CMIs + WMH volume	-0.24 [-0.49 ; 0.01]	0.057
CMIs + presence of microbleeds	-0.27 [-0.52 ; -0.02]	**0.038**
CMIs + brain volume (% ICV)	-0.15 [-0.38 ; 0.08]	0.196
Visuoconstruction (z-score)		
CMIs alone	-0.30 [-0.52 ; -0.08]	**0.008**
CMIs + presence of infarcts	-0.26 [-0.49 ; -0.03]	**0.027**
CMIs + WMH volume	-0.26 [-0.48 ; -0.04]	**0.019**
CMIs + presence of microbleeds	-0.28 [-0.51 ; -0.05]	**0.015**
CMIs + brain volume (% ICV)	-0.18 [-0.38 ; 0.03]	0.086

Abbreviations: CMI, cerebral microinfarcts; MRI, magnetic resonance imaging; CI, confidence interval; MMSE, Mini-Mental State Examination; WMH, white matter hyperintensities; ICV, intracranial volume.

NOTE. B: mean difference between patients with cortical CMIs and patients without, from linear regression analyses, adjusted for age, gender, level of education, and for each of the individual MRI marker indicated in the corresponding row.

Supplementary Table 5 – 1: Clinical diagnosis

Characteristics	Without CMIs (N=163)	With CMIs (N=75)	OR [95%CI]	P-value	Multiple (≥3) CMIs (N=25)	OR [95% CI]	P-value
Referral diagnosis							
No cognitive impairment	26 (16)	4 (5)	0.27 [0.09 ; 0.85]	**0.025**	1 (4)	0.20 [0.02 ; 1.79]	0.151
CIND, without stroke	29 (18)	5 (7)	0.34 [0.12 ; 0.92]	**0.033**	1 (4)	0.20 [0.03 ; 1.55]	0.124
CIND with stroke	32 (20)	23 (31)	1.80 [0.94 ; 3.47]	0.078	7 (28)	1.39 [0.51 ; 3.82]	0.523
Alzheimer's disease	66 (40)	31 (41)	1.13 [0.60 ; 2.12]	0.708	8 (32)	0.61 [0.22 ; 1.67]	0.333
Vascular dementia	10 (6)	12 (16)	2.86 [1.17 ; 6.99]	**0.021**	8 (32)	7.10 [2.37 ; 21.21]	**0.000**

Abbreviations: CMI, cerebral microinfarcts; CIND, cognitive impairment no dementia;

NOTE: Data are presented as number (percentage). OR: odds ratio in binary logistic regression, adjusted for age, gender, and level of education, compared to patients without CMIs.

CHAPTER 5 – FIGURES

Figure 5 – 1: Cortical microinfarct on 7T postmortem MRI and histology

A cortical microinfarct on 7 tesla post-mortem MRI and histology, in the brain of an 83 year-old male with pathologically confirmed vascular dementia. A presumed cortical microinfarct (arrow) was identified on post-mortem 7 tesla 3D T1 (A; 0.4 mm isotropic voxels), which was less conspicuous on post-mortem 7 tesla 3D FLAIR (B; 0.4 mm isotropic voxels). After sampling and histological verification of the area indicated with the white square, this cortical lesion was verified as a microinfarct (C; Hematoxylin & Eosin stain). The adjacent section, immunostained against Glial Fibrillary Acidic Protein, confirmed the presence of gliosis (D).

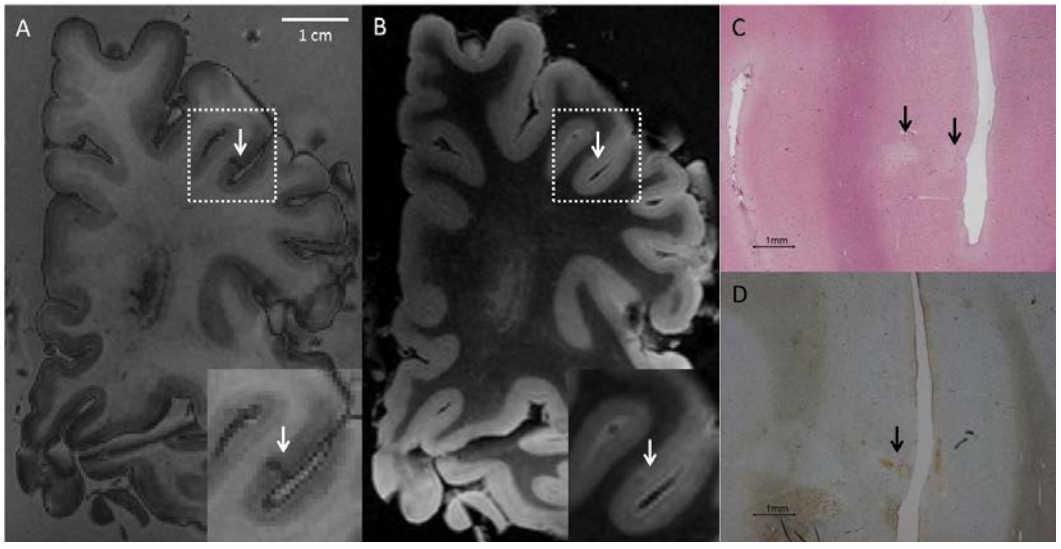


Figure 5 - 2: Same cortical microinfarct on 7T visible on 3T MRI

The same cortical microinfarct on 7 tesla MRI (top row) and 3 tesla MRI (bottom row) in a 66 year-old non-demented Dutch female. A cortical microinfarct (arrow) was found on 7 tesla 3D FLAIR (A; 0.8 mm isotropic voxels), and 7 tesla 3D T1 (B; 1.0 mm isotropic voxels). The same cortical microinfarct could not be retrieved on the 3 tesla FLAIR (C; 1.0x1.3x3.0 mm^3 voxels), but could be identified on the 3 tesla 3D T1 (D; 1.0 mm isotropic voxels), made on the same day as the 7 tesla scans. Adapted from Van Veluw et al. 2013 *JCBFM*.

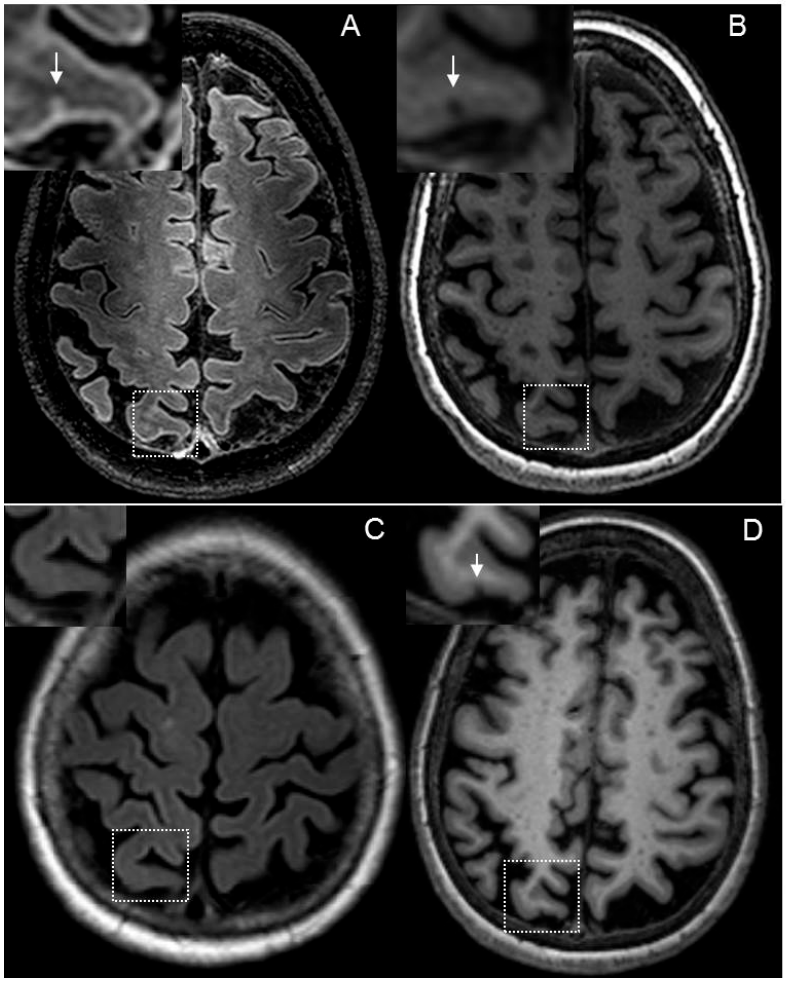
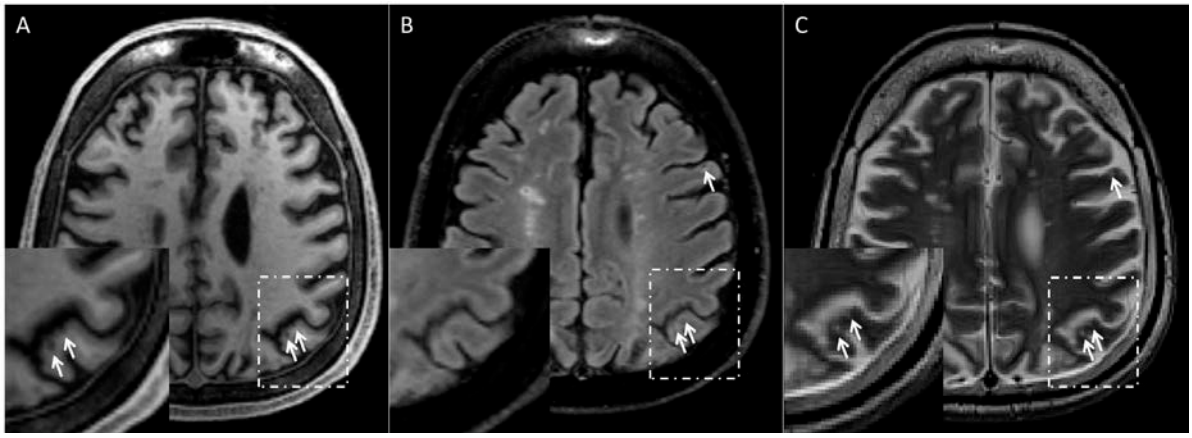


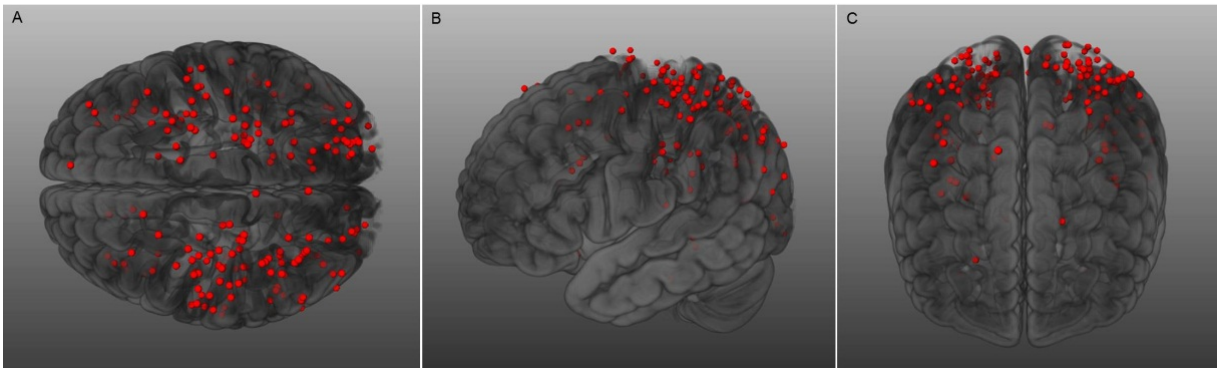
Figure 5 – 3: Cortical microinfarcts on 3T visible on different sequences

Three cortical microinfarcts on the 3 tesla MR images of a 63-year old Singaporean male with vascular cognitive impairment no dementia. Depicted are a 3D T1 (A), FLAIR (B), and T2 (C) image. This patient had 32 cortical microinfarcts, of which 3 are captured in these images (arrows).



Supplementary figure 5 – 1: 3D representation of the cortical microinfarcts

A 3D representation of total cortical microinfarct distribution. Cortical microinfarcts are represented by red dots in a transversal (A), sagittal (B), and coronal (C) view of the brain. There is a strong predilection of microinfarcts to be present in the parietal region



CHAPTER 6:

Cortical Cerebral Microinfarcts on 3 Tesla Magnetic Resonance Imaging - A Marker of Cerebrovascular Diseases

1. INTRODUCTION

Cerebrovascular disease (CeVD) is a common pathological finding in older individuals and a major cause and contributor to cognitive decline and dementia.[1, 2] Brain parenchymal damage in cognitive decline secondary to CeVD or vascular cognitive impairment, is conventionally visualised on magnetic resonance imaging (MRI) as infarcts, white matter hyperintensities (WMH), cerebral microbleeds and atrophy. However, autopsy studies have shown that cerebral microinfarcts (CMIs) are highly prevalent in dementia (43% in Alzheimer's Disease and 62% in Vascular dementia), as well as in non-demented elderly subjects (up to 33%) and are strongly associated with cognitive impairment and dementia.[3, 4] CMIs have reported sizes ranging from 50μm to ~5mm,[4] although small and previously held to be "invisible" lesions during life, they may be present in sufficient numbers to impair cognition and predict poor outcome in elderly with CeVD.[5, 6]

Recently, it has been shown that CMIs can be detected *in-vivo* using 7 Tesla (T) Magnetic Resonance Imaging (MRI).[7] However, due to the limited accessibility of 7T scanners in the clinical setting, there has been a successful effort to extend the detection of CMIs using 3T MRI in order to understand their clinical relevance in larger populations.[8, 9] A recent study from a memory clinic population in Singapore, has shown the feasibility of detection of cortical CMIs on 3T MRI scans and an association with cognitive dysfunction and dementia.[10] However, limited data are available on the risk factors of CMIs and no data exist on the effects of CMI on cognition in the general elderly population. We, therefore, examined the risk factors of CMIs and their association with cognition in a subsample from a population-based study in Singapore.

2. METHODS

2.1 Study Population

The Epidemiology of Dementia In Singapore (EDIS) study drew participants from ongoing Singapore Epidemiology of Eye Disease (SEED) study, a population-based study of three ethnic cohorts : Chinese (Singapore Chinese Eye Study [SCES]),[11] Malay (Singapore Malay Eye Study [SiMES-2]),[12] and Indians (Singapore Indian Eye Study [SINDI-2]). The details of the study methodology have been described in **Chapter 3**.[11] Ethics approval for the EDIS study was obtained from the Singapore Eye Research Institute, and National Healthcare Group Domain-Specific Review Board. The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained prior to their recruitment into the study.

2.2 Demographic and Cardiovascular Risk Factor Assessment

The demographic and cardiovascular risk factors were collected for all the subjects in a similar fashion[11] as described in **Chapter 3**. Hypertension, diabetes and hyperlipidemia were taken as presence and absence. Education was categorized into ≤ 6 years or > 6 years. Smoking was categorized into ever (past and current smokers) and never smokers. Body mass index (BMI) was calculated as the weight (kg) divided by the square of the height (meters). Stroke was defined as the presence of focal neurological deficits whereas cardiovascular diseases were defined based on the presence of ischemic heart disease, congestive heart failure, atrial fibrillation and cardiac bypass.

2.3 Neuroimaging

MRI was performed on a 3T Siemens Magnetom Trio Tim scanner, using a 32-channel head coil, at the Clinical Imaging Research Centre of the National University of Singapore. Subjects with claustrophobia, contraindications for MRI, or those who were

unable to tolerate the procedure were excluded. For each participant, the following MRI markers were determined:

Cortical CMIs

Cortical CMIs were graded on T1, T2 weighted and Fluid-Attenuated Inversion Recovery (FLAIR) sequences, according to a validated protocol [10] described in **Chapter 3**.

MRI rating for CMIs in EDIS study was independently performed by two trained graders (SH, ES). A set of 20 alternate scans were graded for CMIs by the two graders, blinded to subject's characteristics. All the identified cortical CMIs were then discussed in the weekly consensus meetings. Any disagreement was further discussed with a third experienced grader (SvV) to make a final decision. A subset of 60 scans was randomly selected to assess inter-rater reliability, which showed good to excellent agreement (kappa=0.83).

Other MRI Markers

Quantitative MRI analyses (WMH volume) and visual gradings (cortical infarcts, lacunar infarcts, cerebral microbleeds and intracranial stenosis) were collected using the protocol and definitions as mentioned in **Chapter 3**.

2.4 Cognitive Assessment

The Mini-Mental Status Examination (MMSE), Montreal Cognitive Assessment (MoCA), and an extensive neuropsychological battery, which has been previously validated in Singaporean elderly, was administered to assess cognitive function. The criteria for defining CIND and calculation of z scores have been described in detail in **Chapter 3**.

2.5. Statistical Analysis

In order to examine the differences in demographic, vascular risk factors and MRI markers, the chi square test was used for categorical variables, student t test for continuous variables and Mann Whitney U test for skewed distributed continuous variables (WML). WMH volume was logarithmically transformed, to ensure a normal distribution for regression analysis. With respect to the associations between risk factors and CMIs, Poisson regression models were used to compute relative risk (RR) and 95% confidence intervals (CI), initially adjusting for age, gender and each risk factor separately. The fully adjusted model consisted of risk factors significant from first model with each MRI marker added one at a time.

For clinical outcomes (CIND-mild, CIND-moderate and dementia), multiple logistic regression analysis was used to compute odds ratios (OR) and 95%confidence interval (CI). These regression models with cognition were initially adjusted for age, gender, education and subsequently for ethnicity and vascular risk factors. In order to examine whether the association between CMI and cognition was independent of other MRI markers of CeVD, we additionally adjusted the models for each MRI marker separately. With regards to the association between CMIs and cognitive profile, linear regression models were constructed for MMSE, MoCA, composite, and domain-specific Z-scores. The models were adjusted in the similar fashion as described above. P-value < 0·05 was considered statistically significant. In view of the multiple tests performed on the specific cognitive domains (7 domains), we also used the Bonferroni correction to obtain an adjusted significance level for each domain specific test: 0.05/7~0.007. Statistical analysis was performed using standard statistical software (Statistical Package for Social Science, SPSS V23, SPSS Inc., USA).

3. RESULTS

Assessments of subjects were performed from August 12, 2010 to July 24, 2015. Out of 957 subjects who participated in EDIS study in phase II, 88 had no MRI scans and 8 had ungradable scans. **Supplementary table 6-1** presents baseline data of included and excluded subjects (screened positive- non-participants and without/ungradable MRI scans). Those who were excluded were likely to be older, more often Chinese, had lower education and had higher frequency of hypertension, and lower frequency of hyperlipidemia. Of the 861 subjects with gradable MRIs, 54 (6·3%) subjects had ≥1 cortical CMIs. Among subjects with cortical CMIs, 33 (61·1%) had a single cortical CMI, 15 (27·8%) with 2-4 and six (11.1%) had ≥ 5, with a range of 0 to 13. Cortical CMIs were present throughout the brain with a strong predilection for parietal lobes (41·9%) followed by frontal (20·9%), occipital (11·6%) and temporal (4·7%) lobes. Out of 861 subjects, 275 (31.9%) subjects were diagnosed with CIND-mild, 290 (33.6%) with CIND-moderate and 40 (4.6%) with dementia. Baseline characteristics of subjects with and without cortical CMIs are shown in **table 6-1**. Subjects with cortical CMIs were likely to be older, of Malay ethnicity. had higher frequency of hypertension, smoking and history of stroke. Moreover, compared to subjects without cortical CMIs, the prevalence of cerebral small (lacunar infarcts, WMH, and microbleeds) and large vessel (cortical infarcts and intracranial stenosis) diseases on MRI was higher in persons with cortical CMIs.

Table 6-2 shows the association of risk factors with cortical CMIs. In fully adjusted models, the most important demographic and cardiovascular risk factors were increasing age (per year increase, OR: 1·09; 1·06-1·12), Malay ethnicity (Malay vs. Chinese, OR: 2·29; 95%CI: 1·42-3·69 and Malay vs. Indians, OR: 1·79; 95%CI: 1·14-2·81), hypertension (yes vs. no, OR: 4·33; 95%CI: 1·58-11·83), diabetes (yes vs. no, OR: 1·59;

95%CI: 1·10-2·31) and history of stroke (yes vs. no, OR: 4·85; 95%CI: 3·17-7·43). MRI markers of both large (cortical infarcts and intracranial stenosis) and small (lacunar infarcts, WMH and microbleeds) vessel diseases were associated with increasing number of cortical CMIs. When the analysis was restricted to strictly lobar microbleeds, the association remains unaltered.

The presence of cortical CMIs was significantly associated with both CIND moderate (OR: 3·28; 95%CI: 1·26-8·58) and dementia (OR: 11.88; 95%CI: 2.18-64.78) after adjusting for age, gender and education. On further adjustment with cardiovascular risk factors and MRI markers, the association remained unaltered. However, after including lacunar infarcts in the model, the association became attenuated but still showed a trend towards significance (**Table 6-3**). When the analysis was performed with cortical CMI numbers as risk factor, an independent association was observed between increasing numbers of cortical CMIs and CIND moderate and dementia.

Cortical CMIs was significantly associated with worse performance on MMSE (mean difference in MMSE scores: -1·85; 95%CI: -2·84; 0·86), MOCA (mean difference in MOCA scores: -2·55; 95% CI: -3·79; -1·29), and composite Z-scores (mean difference in composite Z-score: -0·42; 95% CI: -0·62; -0·21) in age, gender and education adjusted models. Following further adjustments for ethnicity, cardiovascular risk factors, and MRI markers, the associations of CMIs with cognitive profiles remained statistically significant (**Table 6-4**). Finally, in the domain-specific analyses, CMIs were independently associated with executive function, visual memory, and verbal memory after adjustments for age, gender, education, ethnicity, cardiovascular risk factors, and MRI markers. After applying Bonferroni correction, most of the associations remained statistically significant (**Table 6-5**). Lastly, all the associations with cognitive profiles remain unaltered after excluding subjects with dementia.

4. DISCUSSION

In this study, we found that cortical CMIs are a novel MRI marker of cerebrovascular disease in a general elderly population and are associated with worse cognitive functioning, in particular executive function, verbal, and visual memory. In terms of the clinical outcomes, persons with cortical CMIs were more likely to have significant cognitive impairment. These associations remained independent of cardiovascular risk factors and other MRI markers suggesting that these lesions do play an important role in cognitive impairment and dementia.

To our knowledge, this is the first study to report the detection of cortical CMIs on 3T MRI from a population based study. We have found a cortical CMI prevalence of 6·3%- comparable with the previous prevalence of 6% in hypertensive subjects[9] but less than the 32% from a memory clinic based study[10]. The observed lower prevalence of cortical CMIs in the present study might be due to fact that our subjects were drawn from a population-based study. Moreover, it has been shown that only about 25% of cortical CMIs identified on 7T are detectable on 3T.[7] However, it should be acknowledged that cortical CMIs identified on either 3T or 7T are usually larger and hence only reflects a small fraction of the total CMI burden. Indeed, autopsy studies have shown that the actual CMI prevalence is much higher (24%-62%) then what is observed in the present study,[4] thus indicating that the CMIs observed on MRI are an underestimate.

So far, relatively few autopsy and MRI studies have examined the association between demographic and cardiovascular risk factors with CMIs. The reported effects of age on CMIs have been variable with some studies reporting an association with advanced age[13, 14] whereas others report no association.[7, 15] The association of cortical CMIs with increasing age in our study is further supported by a recent study where persons with cortical CMIs were older compared to those without CMI.[9] Moreover, we also reported

that the men were more likely to have CMIs compared to women which differ from the findings reported previously where no relation with gender was found. [9, 10, 16] This gender difference might due to the increased vulnerability of males to cardiovascular risk factors and stroke. In terms of ethnic differences, Malays have a higher prevalence of ApoE4 carriers[17] compared to Chinese and Indians which increases their susceptibility to develop cerebral amyloid angiopathy (CAA) – a possible mechanism behind CMIs.[18] Besides genetic factors, environmental factors such as lifestyle, cardiovascular risk factors and their complex interactions may also explain the underlying differences in CMI prevalence among the three ethnicities.[17]

Among the cardiovascular risk factors, hypertension, systolic blood pressure and diabetes were identified as the major risk factors for CMIs in a few neuropathological studies.[14, 19, 20] Similar to these findings, our study has also shown an independent association of hypertension and diabetes with increasing numbers of cortical CMIs. The possible mechanism behind high systolic blood pressure and diabetes leading to microinfarcts has been attributed to decreased luminal diameter (atherosclerosis and lipohyalinosis) in the small penetrating arteries in the cerebrum.[20, 21] Besides, hypertension and diabetes, history of stroke was also recognized as an important risk factor for CMIs in our study. This highlights the importance of CMIs occurring in parallel with strokes and the vascular pathology underlying development of CMIs.

With respect to MRI markers, we found - in accordance with a previous study[10] - that both cerebral large (cortical infarcts and intracranial stenosis) and small vessel (lacunar infarcts, white matter lesions, and microbleeds) disease markers are associated with the presence of cortical CMIs, thus suggesting that these lesions have a heterogeneous etiology. It has previously been suggested that CMIs may represent proxies for both small and large infarcts or even diffuse injury.[16] On the basis of these findings, we could

hypothesize that mechanisms such as arteriosclerosis, microembolisms and hypoperfusion might contribute to the development of CMIs.[18, 22] Moreover, we have also reported an association with lobar cerebral microbleeds which indicate that CMIs might also be CAA related- attributed to reduced blood flow in small cortical arteries due to the deposition of amyloid in the vessel wall.[23]

With regards to cognition, the present study shows that the persons with cortical CMIs are more likely to have worse cognitive function in terms of clinical outcomes (CIND / dementia), brief tests (MMSE and MoCA) and global cognitive performance. Moreover, persons with cortical CMIs performed worse in tasks of executive function, verbal and visual memory independent of cardiovascular risk factors and other MRI markers of cerebrovascular disease pathology. However, the association of cortical CMIs with language and visuomotor speed were attenuated only in the presence of infarcts leading to the possibility this association is partly mediated by cerebral ischemic damage. So far, only one autopsy study has shown the association of CMIs with specific cognitive domains such as semantic memory, perceptual speed and visuospatial function.[16] The role of cortical CMIs in causing cognitive dysfunction has also been shown in a study on memory clinic patients where those with cortical CMI had worse language and visuoconstructive abilities.[10] Possibly this association with executive function and memory domains can be attributed by the abundance of cortical CMIs in the frontal and parietal cortex. Moreover, executive function, visuomotor speed and language are also commonly affected in cognitive impairment secondary to both small vessel and macroscopic ischemic damage.[24] Impairment of these cognitive domains in persons with cortical CMIs might be meditated or modified by processes such as hypoperfusion with hypoxia, oxidative stress, and inflammation.[16] Finally, patients with single or multiple CMIs may also have an unrecognized burden of hundreds of thousands more CMIs in the

rest of the brain.[16, 25] This may explain why neuropathological studies have shown that CMIs disrupt important cognitive networks underlying the cognitive dysfunction observed in these subjects.[5]

Limitations of this study include: first, 46·1% of the screened positive subjects were excluded from these analyses. Compared to the included participants, these excluded subjects were relatively older, less educated, and more likely to have hypertension. However, despite this non-participation of subjects more likely to have cortical CMIs, we still found significant associations with CMIs and in turn with cognition. Second, due to the cross-sectional design of our study the temporal relationship between risk factors and cortical CMIs and its effect on cognitive impairment could not be assessed. Third, due to the lower resolution of 3T MRI compared to 7T, smaller cortical CMIs <2mm might have gone undetected. Despite this under detection on 3T, we nevertheless found significant associations of risk factors with CMIs and its link with cognitive functioning. Furthermore, as the grading of the 3T MRI scans was independent of clinical characteristics of the subjects, it is likely that the true effects with risk factors and cognition may have been even larger. Strengths of the study include: subjects were selected from a population-based study, extensive neuropsychological tests were used to diagnose cognitive impairment and dementia. The final models with cognition were adjusted for all possible risk factors of cortical CMIs to show an independent effect of cortical CMIs with cognition.

5. CONCLUSION

In conclusion, in this study in the general elderly population, we found that cortical CMIs are indeed a distinct MRI marker of cerebrovascular disease and are associated with worse cognitive functioning, in particular executive function, verbal, and visual memory. Future longitudinal studies focusing on the clinical relevance of cortical CMIs may

provide novel insights into the pathophysiological link between cortical CMIs and cognition. It would be interesting to investigate the effects of this novel, emerging MRI marker on vascular cognitive impairment in addition to the traditional small vessel disease markers and its additional value in predicting cognitive decline. Finally, it would be of importance to determine if interventions can effectively reduce the incidence of CMIs and hence cognitive decline in clinical trials or observational studies.

CHAPTER 6 – REFERENCES

1. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. Lancet Neurol. 2010;9:689-701.

2. Pantoni L, Poggesi A, Inzitari D. Cognitive decline and dementia related to cerebrovascular diseases: some evidence and concepts. Cerebrovasc Dis. 2009;27:191-6.

3. Sonnen JA, Santa Cruz K, Hemmy LS, et al. Ecology of the aging human brain. Arch Neurol. 2011;68:1049-56.

4. Brundel M, de Bresser J, van Dillen JJ, Kappelle LJ, Biessels GJ. Cerebral microinfarcts: a systematic review of neuropathological studies. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism . 2012;32:425-36.

5. Smith EE, Schneider JA, Wardlaw JM, Greenberg SM. Cerebral microinfarcts: the invisible lesions. Lancet Neurol. 2012;11:272-82.

6. Kalaria RN. Cerebrovascular disease and mechanisms of cognitive impairment: evidence from clinicopathological studies in humans. Stroke. 2012;43:2526-34.

7. van Veluw SJ, Zwanenburg JJ, Engelen-Lee J, et al. In vivo detection of cerebral cortical microinfarcts with high-resolution 7T MRI. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2013; 33:322-9.

8. Ii Y, Maeda M, Kida H, et al. In vivo detection of cortical microinfarcts on ultrahigh-field MRI. Journal of neuroimaging : official journal of the American Society of Neuroimaging. 2013;23:28-32.

9. van Dalen JW, Scuric EE, van Veluw SJ, et al. Cortical microinfarcts detected in vivo on 3 Tesla MRI: clinical and radiological correlates. Stroke. 2015;46:255-7.

10. van Veluw SJ, Hilal S, Kuijf HJ, et al. Cortical microinfarcts on 3T MRI: Clinical correlates in memory-clinic patients. Alzheimers Dement 2015. S1552-5260(15)00123-5.

11. Hilal S, Ikram MK, Saini M, et al. Prevalence of cognitive impairment in Chinese: Epidemiology of Dementia in Singapore study. J Neurol Neurosurg Psychiatry 2013;84:686-92.

12. Rosman M, Zheng Y, Wong W, et al. Singapore Malay Eye Study: rationale and methodology of 6-year follow-up study (SiMES-2). Clinical & experimental ophthalmology 2012;40:557-68.

17. Yu L, Boyle PA, Leurgans S, Schneider JA, Bennett DA. Disentangling the effects of age and APOE on neuropathology and late life cognitive decline. Neurobiology of aging. 2014;35:819-26.

18. Longstreth WT, Jr., Sonnen JA, Koepsell TD, Kukull WA, Larson EB, Montine TJ. Associations between microinfarcts and other macroscopic vascular findings on neuropathologic examination in 2 databases. Alzheimer Dis Assoc Disord.2009;23:291-4.

19. Brundel M, Reijmer YD, van Veluw SJ, et al. Cerebral microvascular lesions on high-resolution 7-Tesla MRI in patients with type 2 diabetes. Diabetes. 2014;63:3523-9.

20. Arvanitakis Z, Leurgans SE, Barnes LL, Bennett DA, Schneider JA. Microinfarct pathology, dementia, and cognitive systems. Stroke. 2011;42:722-7.

21. Venketasubramanian N, Sahadevan S, Kua EH, Chen CP, Ng TP. Interethnic differences in dementia epidemiology: global and Asia-Pacific perspectives. Dement Geriatr Cogn Disord. 2010;30:492-8.

22. Zheng L, Vinters HV, Mack WJ, Zarow C, Ellis WG, Chui HC. Cerebral atherosclerosis is associated with cystic infarcts and microinfarcts but not Alzheimer pathologic changes. Stroke. 2013;44:2835-41.

23. Troncoso JC, Zonderman AB, Resnick SM, Crain B, Pletnikova O, O'Brien RJ. Effect of infarcts on dementia in the Baltimore longitudinal study of aging. Ann Neurol. 2008;64:168-76.

24. Wang LY, Larson EB, Sonnen JA, et al. Blood pressure and brain injury in older adults: findings from a community-based autopsy study. J Am Geriatr Soc. 2009;57: 1975-81.

25. Sonnen JA, Larson EB, Brickell K, et al. Different patterns of cerebral injury in dementia with or without diabetes. Arch Neurol. 2009; 66:315-22.

26. van Rooden S, Goos JD, van Opstal AM, et al. Increased number of microinfarcts in Alzheimer disease at 7-T MR imaging. Radiology. 2014;270:205-11.

27. Charidimou A, Gang Q, Werring DJ. Sporadic cerebral amyloid angiopathy revisited: recent insights into pathophysiology and clinical spectrum. J Neurol Neurosurg Psychiatry. 2012;83:124-37.

28. Aggarwal NT, Schneider JA, Wilson RS, Beck TL, Evans DA, Carli CD. Characteristics of MR infarcts associated with dementia and cognitive function in the elderly. Neuroepidemiology. 2012;38:41-7.

29. Auriel E, Westover MB, Bianchi MT, et al. Estimating Total Cerebral Microinfarct Burden From Diffusion-Weighted Imaging. Stroke. 2015;46:2129-35.

CHAPTER 6 – TABLES

Table 6 – 1: Baseline demographic and clinical characteristics of subjects (n=861)

Characteristics	With CMIs (n= 54)	Without CMIs (n= 807)	P value[a]
Demographics,			
Age, years, mean (SD)	74.3 (6.6)	70.0 (6.6)	**<0.001**
Men, no. (%)	30 (55.6)	373 (46.2)	0.183
Ethnicity,			
Chinese, no. (%)	13 (24.1)	268 (33.2)	**0.004**
Malays, no. (%)	30 (55.6)	270 (33.5)	
Indians, no. (%)	11 (20.4)	269 (33.3)	
Cardiovascular risk factors,			
Hypertension, no. (%)	51 (94.4)	643 (79.7)	**0.008**
Hyperlipidemia, no. (%)	40 (74.1)	609 (75.5)	0.818
Diabetes, no. (%)	26 (48.1)	297 (36.8)	0.096
Mean arterial blood pressure, mmHg, mean (SD)	100.7 (12.5)	98.1 (10)	0.071
Total Cholesterol, mmol/l, mean (SD)	4.7 (1.2)	5.0 (1.1)	0.054
Random blood glucose, mean (SD)	7.3 (3.0)	7.1 (3.1)	0.593
Smoking, no. (%)	21 (38.9)	207 (25.7)	**0.033**
Body mass index, kg/m^2, mean (SD)	19.6 (2.8)	20.1 (3.6)	0.329
History of stroke, no. (%)	12 (22.2)	35 (4.3)	**<0.001**
History of cardiovascular diseases, no. (%)	6 (11.1)	59 (7.3)	0.306
MRI markers,			
Presence of any infarcts, no. (%)	35 (64.8)	140 (17.3)	**<0.001**
Lacunes, no. (%)	29 (53.7)	127 (15.7)	**<0.001**
Cortical infarcts, no. (%)	13 (24.1)	15 (1.9)	**<0.001**
White matter hyperintensity volume, median (IQR)	5.79 (18.7)	1.47 (3.9)	**<0.001**
Presence of cerebral microbleeds, no. (%)	27 (50.9)	267 (33.9)	**0.012**
Presence of intracranial stenosis, no. (%)	17 (32.1)	98 (12.5)	**<0.001**

Abbreviation: SD, Standard deviation; no, number; mmHg, millimeter of mercury; mmol/l, millimoles per liters; kg/m^2, kilogram per meter square; MRI, magnetic resonance imaging; IQR, interquartile range; ml, milliliters; CMI, cerebral microinfarct

[a] p-value < 0.05 was considered statistically significant

Table 6 – 2: Association of risk factors (as determinants) with cortical CMIs (as outcome)

Risk factors	Per CMI increase	
	Model I	**Model II**
	RR (95% CI)	**RR (95%CI)**
Demographics,		
Age, (per year increase)	**1.10 (1.07-1.13)**	**1.09 (1.06-1.12)**
Gender (men vs. women)	**1.82 (1.26-2.63)**	**1.77 (1.22-2.56)**
Ethnicity,		
Malay vs. Chinese	**2.44 (1.51-3.93)**	**2.29 (1.42-3.69)**
Malay vs. Indian	**1.66 (1.07-2.56)**	**1.79 (1.14-2.81)**
Chinese vs. Indian	0.71 (0.41-1.23)	0.78 (0.44-1.38)
Cardiovascular risk factors,		
Hypertension, (yes vs. no)	**5.69 (2.09-15.48)**	**4.33 (1.58-11.83)**
Hyperlipidemia, (yes vs. no)	1.42 (0.89-2.24)	0.91 (0.56-1.45)
Diabetes, (yes vs. no)	**1.82 (1.27-2.62)**	**1.59 (1.10-2.31)**
Smoking (ever vs. never)	1.38 (0.90-2.15)	1.36 (0.85-2.17)
BMI (kg/m^2)	1.01 (0.96-1.07)	0.98 (0.93-1.04)
History of stroke, (yes vs. no)	**4.95 (3.27-7.49)**	**4.85 (3.17-7.43)**
History of cardiovascular disease, (yes vs. no)	1.19 (0.69-2.07)	0.90 (0.52-1.58)
MRI markers,		
Cortical infarct, (yes vs. no)	**16.74 (11.56-24.23)**	**13.64 (9.40-19.78)**
Lacunar infarct, (yes vs. no)	**7.03 (4.73-10.44)**	**4.94 (3.25-7.50)**
WMH volume, ml, log transformed	**3.22 (2.24-4.61)**	**2.28 (1.55-3.33)**
Presence of cerebral microbleed (yes vs. no)	**2.16 (1.46-3.20)**	**1.72 (1.15-2.57)**
Presence of intracranial stenosis (yes vs. no)	**5.19 (3.54-7.62)**	**3.57 (2.40-5.31)**

Abbreviation: CMI, cerebral microinfarct; RR, rate ratios; CI, confidence interval; BMI, body mass index; MRI, magnetic resonance imaging; ml, milliliters; WMH, white matter hyperintensity

Model I included age, gender, and each associated factor separately

Model II included age, gender, ethnicity, hypertension, diabetes and smoking (significant from model I)

Table 6 – 3: Association between cortical cerebral microinfarcts (presence vs. absence) and cognition (clinical outcomes)

	CIND mild (n=275) OR (95%CI)	CIND moderate (n=290) OR (95%CI)	Dementia (n=40) OR (95%CI)	CIND moderate/dementia (n=330) OR (95%CI)
Model I	1.26 (0.46-3.40)	**3.28 (1.26-8.58)**	**11.88 (2.18-64.78)**	**3.66 (1.43-9.39)**
Model II	1.23 (0.44-3.44)	**3.57 (1.31-9.68)**	**13.95 (2.45-79.30)**	**3.91 (1.47-10.39)**
Model III				
Intracranial stenosis	1.25 (0.45-3.49)	**3.49 (1.26-9.68)**	**13.51 (2.27-80.48)**	**3.82 (1.41-10.36)**
Cerebral microbleeds	1.24 (0.44-3.44)	**3.63 (1.33-9.93)**	**12.61 (2.14-74.21)**	**3.96 (1.48-10.62)**
White matter hyperintensity volume	1.19 (0.43-3.35)	**2.94 (1.05-8.22)**	**11.52 (1.73-76.98)**	**3.23 (1.18-8.84)**
Lacunar infarcts	1.04 (0.37-2.93)	2.38 (0.86-6.64)	4.47 (0.58-34.46)	2.44 (0.89-6.73)
Cortical infarcts	1.17 (0.41-3.31)	**2.85 (1.01-8.05)**	**10.39 (1.65-65.25)**	**3.02 (1.08-8.38)**

Abbreviations: CIND, cognitive impairment no dementia; OR, odds ratios; CI, confidence interval

Model I included age, gender, and education

Model II included age, gender, race, education, hypertension, diabetes

Model I + II + each individual MRI marker added separately

Table 6 – 4: Association between cortical cerebral microinfarcts (presence vs. absence) and cognition

CMI, presence vs. absence	MMSE Mean difference (95%CI)	MOCA Mean difference (95%CI)	Composite Z scores Mean difference (95%CI)
Model I	**-1.85 (-2.84; -0.86) p=<0.001**	**-2.55 (-3.79; -1.29) p=<0.001**	**-0.42 (-0.62; -0.21) p=<0.001**
Model II	**-1.83 (-2.81; -0.84) p=<0.001**	**-2.47 (-3.69; -1.24) p=<0.001**	**-0.39 (-0.59; -0.19) p=<0.001**
Model III			
Intracranial stenosis	**-1.93 (-2.90; -0.96) p=<0.001**	**-2.34 (-3.57; -1.12) p=<0.001**	**-0.38 (-0.58; -0.18) p=<0.001**
Cerebral microbleeds	**-1.95 (-2.92; -0.99) p=<0.001**	**-2.51 (-3.74; -1.28) p=<0.001**	**-0.41 (-0.61; -0.21) p=<0.001**
WMH volume	**-1.49 (-2.48; -0.52) p=0.003**	**-2.04 (-3.26; -0.81) p=0.001**	**-0.31 (-0.50; -0.11) p=0.002**
Lacunar infarcts	**-1.53 (-2.53; -0.53) p=0.003**	**-2.01 (-3.25; -0.76) p=0.002**	**-0.29 (-0.49; -0.09) p=0.004**
Cortical infarcts	**-1.11 (-2.13; -0.09) p=0.032**	**-1.95 (-3.23; -0.67) p=0.003**	**-0.31 (-0.52; -0.10) p=0.003**

Abbreviations: CMI, cerebral microinfarct; MMSE, Mini Mental Status Examination; MOCA, Montreal Cognitive Assessment; CI, confidence interval; WMH, white matter hyperintensity

Model I included age, gender, and education

Model II included age, gender, education, ethnicity, hypertension, diabetes, smoking

Model I + II + each individual MRI marker added separately

Table 6 – 5: Association between cortical CMIs and specific cognitive domains expressed as mean differences with 95% confidence intervals

	Executive function Mean difference (95%CI)	Attention Mean difference (95%CI)	Language Mean difference (95%CI)	Visuomotor speed Mean difference (95%CI)	Visuoconstruction Mean difference (95%CI)	Visual memory Mean difference (95%CI)	Verbal memory Mean difference (95%CI)
Model I	**-0.42 (-0.65; -0.19) p=<0.001**	**-0.30 (-0.53; -0.07) p=0.010**	**-0.37 (-0.61; -0.13) p=0.002**	**-0.29 (-0.49; -0.09) p=0.004**	**-0.22 (-0.44; -0.00) p=0.045**	**-0.46 (-0.67; -0.24) p=<0.001**	**-0.41 (-0.64; -0.19) p=<0.001**
Model II	**-0.40 (-0.63; -0.17) p=0.001**	**-0.28 (-0.50; -0.06) p=0.011**	**-0.36 (-0.60; -0.12) p=0.003**	**-0.26 (-0.46; -0.07) p=0.008**	-0.19 (-0.41; 0.02) p=0.070	**-0.44 (-0.66; -0.22) p=<0.001**	**-0.39 (-0.62; -0.17) p=0.001**
Model III							
ICS	**-0.41 (-0.64; -0.17) p=0.001***	**-0.27 (-0.49; -0.05) p=0.015**	**-0.36 (-0.61; -0.12) p=0.003***	**-0.26 (-0.45; -0.06) p=0.009**	-0.19 (-0.41; 0.02) p=0.069	**-0.44 (-0.66; -0.22) p=<0.001***	**-0.36 (-0.58; -0.14) p=0.002***
CMB	**-0.43 (-0.66; -0.19) p=<0.001***	**-0.29 (-0.51; -0.07) p=0.009**	**-0.36 (-0.61; -0.12) p=0.003***	**-0.29 (-0.49; -0.10) p=0.003***	-0.21 (-0.42; 0.00) p=0.054	**-0.46 (-0.68; -0.24) p=<0.001***	**-0.39 (-0.62; -0.17) p=0.001***
WMH volume	**-0.33 (-0.56; -0.09) p=0.005***	**-0.22 (-0.44; -0.00) p=0.047**	**-0.27 (-0.51; -0.03) p=0.028**	**-0.21 (-0.40; -0.01) p=0.039**	-0.11 (-0.33; 0.09) p=0.295	**-0.35 (-0.57; -0.13) p=0.002***	**-0.33 (-0.56; -0.11) p=0.003***
Lacunar infarcts	**-0.30 (-0.54; -0.07) p=0.011**	-0.21 (-0.43; 0.01) p=0.065	**-0.29 (-0.53; -0.04) p=0.021**	-0.18 (-0.38; 0.01) p=0.069	-0.12 (-0.34; 0.09) p=0.275	**-0.34 (-0.57; -0.12) p=0.002***	**-0.32 (-0.54; -0.09) p=0.005***
Cortical infarcts	**-0.34 (-0.58; -0.09) p=0.006***	-0.21 (-0.44; 0.01) p=0.066	-0.25 (-0.49; 0.00) p=0.054	**-0.22 (-0.43; -0.02) p=0.033**	-0.16 (-0.38; 0.06) p=0.164	**-0.33 (-0.56; -0.11) p=0.004***	**-0.34 (-0.57; -0.11) p=0.004***

Abbreviations: CMI, cerebral microinfarct; CI, confidence interval; ICS, intracranial stenosis; CMB, cerebral microbleed; WMH, white matter hyperintensity

Model I included age, gender, and education

Model II included age, gender, education, ethnicity, hypertension, diabetes, smoking

Model I + II + each individual MRI marker added separately

*Statistically significant after Bonferroni correction (0.05/7 ~ 0.007)

Supplementary table 6 – 1: Comparison of baseline characteristics of included and excluded subjects

Characteristics	**Included (n=861)**	**Excluded (n=737)**	**P value[a]**
Age (years)	70.2 (6.7)	71.9 (6.9)	**<0.001**
Women, no. (%)	457 (53.1)	419 (56.9)	0.131
Race, no. (%)			
Chinese	281 (32.6)	332 (45)	**<0.001[b]**
Malays	300 (34.8)	184 (25)	
Indians	280 (32.5)	221 (30)	
Primary education > 6 years, no. (%)	316 (36.7)	200 (27.1)	**<0.001**
Hypertension, no. (%)	679 (78.9)	618 (83.9)	**0.011**
Diabetes, no. (%)	322 (37.4)	250 (33.9)	0.148
Hyperlipidemia, no. (%)	633 (73.5)	486 (65.9)	**0.001**
Mean arterial blood pressure, mmHg, (SD)	97.4 (10.5)	97.8 (11.3)	0.486
Random blood glucose, mmol/l, (SD)	7.09 (3.12)	7.07 (3.08)	0.890
Total cholesterol, mmol/l, (SD)	5.11 (1.2)	5.14 (1.16)	0.562
Smoking, no. (%)	209 (24.3)	180 (24.4)	0.945
Body mass index, kg/m^2, (SD)	23.5 (4.6)	23.6 (4.6)	0.648

Abbreviation: SD, Standard deviation; no, number; mmHg, millimeter of mercury; mmol/l, millimoles per liters; kg/m^2, kilogram per meter square

[a] p-value < 0.05 was considered statistically significant

[b] p-value for overall ethnic comparison

CHAPTER 7:

Intracranial Stenosis, Cerebrovascular Diseases and Cognitive Impairment in Chinese

1. INTRODUCTION

Intracranial stenosis (ICS) in stroke patients has been suggested to vary among different ethnicities with higher prevalence (40-50%) reported in Chinese, Africans, and Hispanics as compared to Caucasians (8-10%).[1-3] This difference in the prevalence figures may, next to differences in study populations, be influenced by the imaging modalities [transcranial Doppler ultrasound (TCD) vs. Magnetic Resonance Angiography (MRA)] and criteria used to define ICS. Furthermore, data on ICS from asymptomatic and community-based subjects – especially Asian populations - are largely lacking. One study using TCD among asymptomatic subjects from rural China (mean age: 53.5 years) reported a prevalence of ICS of 6.9%. Another study in asymptomatic predominantly white US subjects, ICS was identified in 12.9% using TCD. However, this was a relatively older population with a mean age of 71.4 years.[4] The use of TCD may limit the ability to diagnose ICS, as this is not feasible in patients with poor bone windows and is also rater dependent with high inter- and intra-observer variability.[5] More recently, with the application of higher resolution imaging with flow enhancement, investigators have started to employ MRA in population-based research settings, thereby creating opportunities to examine the determinants and consequences of ICS.[6]

With respect to cognitive impairment, studies have suggested that extra carotid artery disease is associated with impaired neuropsychological test performance, probably as a consequence of cerebral ischemic damage.[7] However, the majority of these studies have focused on extracranial carotid artery stenosis, rather than ICS.[8-12] Specifically, the association between ICS and cognitive impairment has not been investigated previously. We, therefore, examined the association of ICS with cognitive impairment in a Chinese population from Singapore, and whether this association is mediated by the presence of other markers of involutional changes or cerebrovascular diseases on magnetic resonance imaging (MRI).

2. METHODS

2.1 Study population

The ongoing Epidemiology of Dementia in Singapore (EDIS) study drew subjects from the population-based study among Chinese aged 40-85 years, who participated in the Singapore Chinese Eye Study (SCES).[13] The details of study population in Chinese subjects have been described in **Chapter 4**.

2.2 Neuroimaging

MRI Acquisition

Magnetic Resonance Imaging (MRI) and intracranial MRA were performed on a 3T Siemens Magnetom Trio Tim scanner, using a 32-channel head coil, at the Clinical Imaging Research Centre of the National University of Singapore. The study details on MRA were provided previously in **Chapter 3**.

Intracranial Stenosis on MRA

ICS was defined as narrowing exceeding 50% of the luminal diameter in any of the intracranial vessels assessed on 3D TOF MRA as mentioned in **Chapter 3**.The images were first visually assessed on the coronal sequences and then on reconstruction. The final decision on stenosis (>50%) was based on the reconstruction sections (**Figure 7-1**).

Other Markers on MRI

Other markers of cerebrovascular diseases (infarcts, white matter hyperintensities and cerebral microbleeds) and involutional changes (total brain volume) were also graded on MRI using the same methods described in **Chapter 3**.

2.3 Cognitive Assessment

An extensive neuropsychological battery, which has been previously validated in Singaporean elderly, was administered to assess cognitive function.[14] Details on subtests for assessing cognitive domains and calculation of Z-scores have been described in detail

in **Chapter 3**. The modified 15-item Geriatric Depression Scale (GDS) was also administered to all subjects.[15]

Cognitive impairment without dementia (CIND) was defined as impairment in at least one domain of the neuropsychological test battery using education-adjusted cutoffs of 1.5 standard deviations below established normal means on individual tests. CIND was classified into mild (when ≤ 2 domains were impaired) and moderate (when > 2 domains were impaired).[16] The diagnosis of dementia was made according to DSM-IV criteria.

2.4 Assessment of Other Risk Factors

Demographic and vascular risk factors including age, gender, education, smoking, hypertension, diabetes, hyperlipidemia, height, weight and history of stroke were collected and verified by medical records (details in **Chapter 3**). Education was categorized into < Primary 6 and ≥ Primary 6. Smoking was categorized into non-smokers and smokers (past and current smokers). Body mass index (BMI) was calculated as the weight in kg divided by the square of height in meters.

2.5 Statistical Analysis

To assess the differences between included and excluded subjects, Chi-square tests were utilized for categorical variables and Student t-tests were used for continuous variables. We analyzed the associations between presence of ICS and Z-scores for cognition using multiple linear regression models expressing the effect sizes as mean differences in Z-score between those with ICS and those without ICS together with 95% confidence intervals (CI). Models were initially adjusted for age, gender and education and additionally for mean arterial blood pressure, cholesterol, random blood glucose, smoking, BMI and GDS. For clinical outcomes (CIND-mild, CIND-moderate, dementia), multiple logistic regression models were used to compute odds ratios (OR) and 95% CI. To examine whether the association between ICS and cognitive impairment are mediated by the presence of specific MRI lesions, we adjusted both the linear and logistic models

additionally for each MRI marker. Standardized total brain volume and WMH volume, presence of microbleeds and lacunar infarcts were entered into the model one at a time. In order to calculate standardized volumes, the mean total brain volume of the study population was subtracted from individual volumes; the results were then divided by the standard deviation (of our population) to obtain standardized brain volume. The total brain volume was then standardized with total intracranial volume in order to have a true measure of atrophy. The same procedure was also applied to standardize WMH volumes first with the study population and then with total white matter volume. P-value < 0.05 was considered statistically significant. In view of the multiple tests performed on the specific cognitive domains (7 domains), we also used the Bonferroni correction to obtain an adjusted significance level for each domain specific test: 0.05/7=0.007. Statistical analysis was performed using standard statistical software (Statistical Package for Social Science, SPSS V20, SPSS Inc., USA).

3. RESULTS

Table 7-1 shows the comparison between included (n=278) and excluded (n=1260) subjects. Compared to excluded subjects, those that were included were more likely to be older and diabetic and less likely to be hypertensive with low mean arterial blood pressure. The baseline characteristics of the participants with and without ICS are shown in **Table 7-2**. Those who had ICS were more likely to have hypertension, diabetes and presence of infarcts on their MRI scans. Out of 278 included subjects, 77 (27.7%) were diagnosed with CIND-mild, 74 (26.6%) with CIND-moderate and 4 (1.4%) with dementia. Due to the small numbers of dementia cases, these subjects were combined together with CIND-moderate as “significant cognitive impairment” for further analysis.

Twenty-nine subjects (10.4%) were diagnosed with ICS of which 10 were symptomatic (history of stroke) and 19 asymptomatic. Among persons aged 60-64 years, the prevalence was 5% increasing to 16.7% in those older than 75 years. ICS was diagnosed

in 5 (4.1%) subjects with normal cognition, 9 (11.7%) with CIND-mild and 15 (19.2%) with CIND-moderate/dementia. The presence of ICS was significantly associated with both CIND-moderate/dementia as well as composite Z-scores (**Table 7-3**). Following further adjustments with MRI markers including standardized total brain volume, WMH volume and presence of cerebral microbleeds, the associations of ICS with both the clinical outcomes and the composite Z-scores remained statistically significant. After adjustment for the presence of lacunar infarcts, however, these associations attenuated and partly became non-significant, suggesting that these associations are partially mediated through infarcts.

With respect to specific domains, ICS was related to executive function, language, visuomotor speed, verbal and visual memory after adjusting for age, gender, education, and vascular risk factors (**Table 7-4**). In terms of additional adjustment for MRI markers, similar trends were also seen for the domain specific analyses: additional adjustment for standardized total brain volume, WMH volume and presence of cerebral microbleeds did not alter these associations. However, these associations did become non-significant after including presence of infarcts in the model, except for executive function. Finally, when applying Bonferroni corrected significance level of 0.007 (~0.05/7 domains) to the domain specific analyses, none of these associations in these models reached this revised level of significance.

4. DISCUSSION

In this study we found that persons with ICS were more likely to have poorer overall cognitive performance. In particular, these persons performed worse on tasks of executive function, language, visuomotor speed, verbal and visual memory. In terms of clinical outcomes, persons with ICS were more likely to have significant cognitive

impairment. Additional adjustment for MRI markers revealed that these associations may be partially mediated by the presence of lacunar infarcts on MRI.

Several studies have shown that cognitive deficits were present in both symptomatic (with history of stroke) and asymptomatic subjects with extracranial carotid artery occlusion.[17,18,11,12] Additionally, a few studies on extracranial carotid stenosis and cognition have shown that stroke-free subjects with moderate carotid stenosis (stenosis of 50-69%) have poorer performance on several cognitive tests than persons without carotid stenosis.[9,19] In the Tromso study, tests of attention, particularly sustained attention, and psychomotor speed were strongly associated with carotid stenosis, while a weaker association was observed between tests of memory and carotid stenosis independent of clinical ischemic episodes or structural vascular MRI changes.[10] Several studies have also shown that patients with asymptomatic carotid stenosis improved significantly with respect to their neuropsychological assessments after prophylactic carotid surgery, including improvement in attention, visual memory and psychomotor speed.[8,20] However, it was unclear whether these associations were mediated through the presence of infarcts (or other lesions on MRI), as these studies lacked such data.[21-23]

The present study extends these previous findings by showing that persons with ICS were also more likely to have poorer cognitive function.[8] However, the association between ICS and cognitive impairment attenuated when adjusting for the presence of lacunar infarcts on MRI leading to the possibility that this association is partially mediated by cerebral ischemia. It has been hypothesized that cognitive deficits in patients with carotid stenosis may occur as a result of diffuse ischemic damage.[24,25] It has also been suggested that patients with carotid stenosis have cerebral hypoperfusion which is associated with white matter lesions and cerebral atrophy. However, hypoperfusion was not directly measured in this or earlier studies.[10] The specific role of carotid disease in producing cognitive alterations was also reported by a study in which cognitive dysfunction was

observed in patients with TIA and internal carotid occlusion irrespective of the presence of cerebrovascular disease (WMH) or the localization (retinal versus cerebral) of symptoms.[26] However, in this study the presence of WMH was graded visually and other markers such as brain volume and microbleeds were not taken into consideration.

Some methodological issues need to be discussed. First, almost half of the screened positive subjects refused to participate in phase II. These subjects were relatively older, less educated, and had higher mean arterial blood pressure compared to those who participated, which may have led to the underestimation of prevalence of ICS in our sample. Furthermore, those excluded subjects might also be more cognitively impaired suggesting that the effect sizes described in this study may be underestimated. Second, due to the cross-sectional design of our study the temporal relationship between the presence of ICS and the development of cognitive decline cannot be assessed. Third, due to the small number of dementia cases, we were unable to determine the effect of ICS on clinically defined dementia. However, the dose-response relationship with significant cognitive impairment, suggests that these findings may be extendable to dementia. Also with relatively small numbers of ICS, we were not able to examine asymptomatic ICS separately. Finally, for the domain specific analyses, although we found several significant associations at a nominal significance level of 0.05, after applying Bonferroni correction none of these associations reached the revised significance level of 0.007, probably due to low power of our study to examine a large number of specific cognitive domains separately. Finally, conventional cerebral angiograms are considered to be the gold standard to diagnose occlusive disease of the intracerebral arteries; however, it would not have been feasible or ethical to conduct such tests in these subjects, who were drawn from a community-dwelling population.

The strengths of the study are: an extensive neuropsychological battery was used to determine the cognitive dysfunction and the use of cerebral MRI to grade the presence of

cerebrovascular diseases. Furthermore, quantitative MRI measures such as the total brain volume and WMH volume were adjusted for in this study, which are more reliable compared to visual scales.

5. CONCLUSION

In conclusion, in this study we showed that ICS was - independent of vascular risk factor - related to overall poorer cognitive performance. Although the effect of ICS on cognitive impairment may be partly mediated through infarcts, other mechanisms may also be involved. Future studies focusing on perfusion and cerebrovascular reserve may provide novel insights into the pathophysiological link between ICS and cognition.

CHAPTER 7 – REFERENCES

1. Huang YN, Gao S, Li SW, et al. Vascular lesions in Chinese patients with transient ischemic attacks. Neurology. 1997;48:524-25.

2. Li H, Wong KS. Racial distribution of intracranial and extracranial atherosclerosis. Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia. 2003;10:30-4.

3. Wong KS, Huang YN, Yang HB, et al. A door-to-door survey of intracranial atherosclerosis in Liangbei County, China. Neurology. 2007;68:2031-34.

4. Elmore EM, Mosquera A, Weinberger J. The prevalence of asymptomatic intracranial large-vessel occlusive disease: the role of diabetes. Journal of neuroimaging : official journal of the American Society of Neuroimaging. 2003;13:224-7.

5. Ceravolo MG, Minciotti P, Orlandini M, Provinciali L. Intra- and inter-observer variability of basal flow velocity and vascular reactivity measurements using transcranial Doppler sonography. Neurol Res. 1992;14:122-4.

6. Schellinger PD, Richter G, Kohrmann M, Dorfler A. Noninvasive angiography (magnetic resonance and computed tomography) in the diagnosis of ischemic cerebrovascular disease. Techniques and clinical applications. Cerebrovasc Dis. 2007;24:16-23.

7. Bossema ER, Brand N, Moll FL, Ackerstaff RG, van Doornen LJ. Does carotid endarterectomy improve cognitive functioning? J Vasc Surg. 2005;41:775-81.

8. Bossema ER, Brand N, Moll FL, Ackerstaff RG, de Haan EH, van Doornen LJ. Cognitive functions in carotid artery disease before endarterectomy. Journal of clinical and experimental neuropsychology. 2006;28:357-69.

9. Landgraff NC, Whitney SL, Rubinstein EN, Yonas H. Cognitive and physical performance in patients with asymptomatic carotid artery disease. J Neurol. 2010;257:982-91.

10. Mathiesen EB, Waterloo K, Joakimsen O et al. Reduced neuropsychological test performance in asymptomatic carotid stenosis: The Tromso Study. Neurology. 2004;62:695-701.

11. Breteler MM, Claus JJ, Grobbee DE, Hofman A. Cardiovascular disease and distribution of cognitive function in elderly people: the Rotterdam Study. BMJ. 1994;308:1604-8.

12. Poels MM, van Oijen M, Mattace-Raso FU, Hofman A, Koudstaal PJ, Witteman JC, Breteler MM. Arterial stiffness, cognitive decline, and risk of dementia: the Rotterdam study. Stroke. 2007;38:888-92.

14. Hilal S, Ikram MK, Saini M et al. Prevalence of cognitive impairment in Chinese: Epidemiology of Dementia in Singapore study. J Neurol Neurosurg Psychiatry. 2013;84:686-92.

15. Yesavage JA, Brink TL, Rose TL, et al. Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatr Res.1982;17:37-49.

16. Narasimhalu K, Ang S, De Silva DA, et al. Severity of CIND and MCI predict incidence of dementia in an ischemic stroke cohort. Neurology. 2009;73:1866-72.

17. Benke T, Neussl D, Aichner F. Neuropsychological deficits in asymptomatic carotid artery stenosis. Acta neurologica Scandinavica. 1991;83:378-81.

18. Hamster W, Diener HC. Neuropsychological changes associated with stenoses or occlusions of the carotid arteries. A comparative psychometric study. Eur Arch Psychiatry Neurol Sci. 1984;234:69-73.

19. Silvestrini M, Paolino I, Vernieri F, et al. Cerebral hemodynamics and cognitive performance in patients with asymptomatic carotid stenosis. Neurology. 2009;72:1062-68.

20. Borroni B, Tiberio G, Bonardelli S, et al. Is mild vascular cognitive impairment reversible? Evidence from a study on the effect of carotid endarterectomy. Neurol Res. 2004;26:594-97.

21. De Groot JC, De Leeuw FE, Oudkerk M et al. Periventricular cerebral white matter lesions predict rate of cognitive decline. Ann Neurol. 2002;52:335-41.

22. Mungas D, Reed BR, Jagust WJ, et al. Volumetric MRI predicts rate of cognitive decline related to AD and cerebrovascular disease. Neurology. 2002;59:867-73.

23. O'Brien JT, Erkinjuntti T, Reisberg B, et al. Vascular cognitive impairment. Lancet Neurology. 2003;2:89-98.

24. Brand N, Bossema ER, Ommen MvM, Moll FL, Ackerstaff RG. Left or right carotid endarterectomy in patients with atherosclerotic disease: ipsilateral effects on cognition? Brain Cogn. 2004;54:117-23.

25. Rao R. The role of carotid stenosis in vascular cognitive impairment. Eur Neurol. 2001;46:63-9.

26. Bakker FC, Klijn CJ, Jennekens-Schinkel A, van der Tweel I, Tulleken CA, Kappelle LJ, et al. Cognitive impairment in patients with carotid artery occlusion and ipsilateral transient ischemic attacks. Journal of neurology. 2003;250:1340-47.

CHAPTER 7 – TABLES

Table 7 - 1: Baseline characteristics of subjects who were screen positive at phase I and participated in phase II (n = 278) compared with those who were excluded from this analyses (n = 1260)

Risk Factors	Excluded* Yes (n = 1260)	Included† No (n = 278)	P-value
Age (years)	68.7 (6.3)	69.7 (6.2)	**0.02**
Women, no. (%)	582 (46.2)	143 (51.4)	0.11
No formal education, no. (%)	480 (38.1)	120 (43.2)	0.11
Low Socioeconomic status, no. (%)	812 (66)	180 (66.4)	0.89
Hypertension, no. (%)	991 (78.7)	204 (73.4)	**0.05**
Diabetes mellitus, no. (%)	261 (20.7)	72 (25.9)	**0.05**
Hyperlipidemia, no. (%)	661 (52.5)	146 (52.5)	0.98
Mean arterial blood pressure, mmHg, (SD)	99.9 (11.2)	97.1 (9.7)	**<0.001**
Random blood glucose, mmol/l (SD)	6.7 (2.8)	6.6 (2.7)	0.61
Total cholesterol, mmol/l (SD)	5.3 (1.1)	5.2 (1.0)	0.13
Ever smokers, no. (%)	377 (29.9)	71 (25.5)	0.15
Alcohol drinking, no. (%)	122 (9.7)	18 (6.5)	0.10
Body mass index, kg/m^2, mean (SD)	23.5 (3.7)	23.9 (3.4)	0.08

Abbreviations: SD, standard deviation; kg/m^2, kilogram per meter square; mmol/l, millimoles per liter; mmHg, millimeters of mercury

*Excluded were those subjects who were either screen negative (n=926) at phase I and hence were not invited for phase II of the EDIS study or those subjects who were screen positive at phase I, but refused participation at phase II or had missing data (n=334)

† Participants at phase II

Values in bold indicates P<0.05.

Table 7 - 2: Baseline demographic and clinical characteristics of the subjects with and without ICS (n= 278)

Baseline characteristics	ICS absence (n=249)	ICS present (n=29)	P value
Age, years (SD)	70.2 (6.3)	72.2 (6.1)	0.10
Women, n (%)	134 (53.8)	12 (41.4)	0.24
Education, (Primary ≤ 6 years)	158 (63.5)	22 (75.9)	0.22
Body mass index, kg/m^2, mean (SD)	18.9 (2.8)	19.6 (2.9)	0.24
Hypertension, n (%)	184 (73.9)	27 (93.1)	**0.02**
Diabetes mellitus, n (%)	59 (23.7)	14 (48.3)	**0.007**
Hyperlipidemia, n (%)	143 (57.4)	22 (75.9)	0.07
MABP, mmHg, mean (SD)	100.1 (11.3)	100.1 (10.5)	0.98
Random blood glucose, mmol/l (SD)	6.4 (2.3)	8.6 (4.6)	**<0.001**
Fasting total cholesterol, mmol/l (SD)	4.9 (0.87)	4.6 (0.95)	0.06
Ever smokers, n (%)	73 (29.3)	13 (44.8)	0.09
Presence of lacunar infarcts, n (%)	35 (14.1)	16 (55.2)	**<0.001**
Presence of cerebral microbleeds, n (%)	78 (31.5)	12 (41.4)	0.29
Total brain volume, ml, mean (SD)	893.5 (89.6)	904.2 (85.5)	0.54
Total WMH volume, ml, median (IQR)	1.90 (4.44)	2.77 (11.46)	0.20

Abbreviations: ICS, intracranial stenosis; SD, standard deviation; kg/m^2, kilogram per meter square; MABP, mean arterial blood pressure; mmol/l, millimoles per liter; IQR, interquartile range; ml, milliliters; WMH, white matter hyperintensities volume

Values in bold indicates P<0.05.

Table 7 – 3: Association between intracranial stenosis (presence vs. absence) and cognitive function

	CIND-mild (n=77) OR (95% CI)	CIND-moderate/dementia (n=78) OR (95% CI)	Composite Z scores (n=278) Mean differences (95%CI)
Model I*	**3.47 (1.00–12.03)**	**6.42 (1.61–25.65)**	**-0.54 (-0.80; -0.27)**
Model II†	2.89 (0.75–11.17)	**5.54 (1.23–24.98)**	**-0.48 (-0.77; -0.18)**
Model III‡			
Total brain volume	2.89 (0.75–11.16)	**5.12 (1.12–23.40)**	**-0.47 (-0.77; -0.17)**
WMH volume	3.01 (0.78–11.60)	**5.99 (1.31–27.33)**	**-0.48 (-0.78; -0.19)**
Presence of microbleeds	2.88 (0.75–11.08)	**5.35 (1.19–24.04)**	**-0.47 (-0.76; -0.17)**
Presence of infarcts	2.54 (0.64–10.14)	3.14 (0.50–19.82)	**-0.32 (-0.62; -0.03)**
All MRI markers	2.69 (0.68-10.75)	2.99 (0.47-19.15)	**-0.35 (-0.64, -0.05)**

Abbreviations: CIND, cognitive impairment no dementia; WMH, white matter hyperintensities; MRI, magnetic resonance imaging; OR, odds ratios

*Model I: adjusted for age, sex, and education

†Model II: model I+ mean arterial blood pressure, cholesterol, random blood glucose, smoking, body mass index, and Geriatric Depression Scale

‡Model III: model II+ individual MRI markers

Values in bold indicates P<0.05

Table 7 - 4: Association between intracranial stenosis (presence versus absence) and specific cognitive domains expressed as mean differences with 95% confidence intervals (n=278)

	Executive function Mean difference (95%CI)	Attention Mean difference (95%CI)	Language Mean difference (95%CI)	Visuomotor speed Mean difference (95%CI)	Visuoconstruction Mean difference (95%CI)	Verbal memory Mean difference (95%CI)	Visual memory Mean difference (95%CI)
Model I*	**-0.60 (-0.93; -0.28)**	**-0.29 (-0.60; 0.01)**	**-0.44 (-0.78; -0.11)**	**-0.42 (-0.67; -0.16)**	**-0.41 (-0.71; -0.12)**	**-0.51 (-0.81; -0.20)**	**-0.51 (-0.79; -0.23)**
Model II†	**-0.56 (-0.93; -0.20)**	-0.31 (-0.64; 0.02)	**-0.39 (-0.76; -0.02)**	**-0.39 (-0.67; -0.10)**	-0.31 (-0.63; 0.02)	**-0.45 (-0.78; -0.07)**	**-0.43 (-0.75; -0.12)**
Model III‡							
TBV	**-0.56 (-0.93; -0.20)**	-0.31 (-0.64; 0.02)	**-0.38 (-0.76; -0.01)**	**-0.38 (-0.67; -0.09)**	-0.31 (-0.64; 0.02)	**-0.44 (-0.77; -0.11)**	**-0.42 (-0.73; -0.10)**
WMH volume	**-0.58 (-0.93; -0.22)**	-0.31 (-0.64; 0.02)	**-0.39 (-0.77; -0.03)**	**-0.39 (-0.68; -0.11)**	-0.31 (-0.64; 0.01)	**-0.46 (-0.79; -0.13)**	**-0.44 (-0.75; -0.12)**
Microbleeds	**-0.56 (-0.92; -0.20)**	-0.30 (-0.64; 0.03)	**-0.38 (-0.75; -0.01)**	**-0.39 (-0.67; -0.10)**	-0.30 (-0.63; 0.03)	**-0.46 (-0.72; -0.06)**	**-0.43 (-0.75; -0.12)**
Infarcts	**-0.42 (-0.78; -0.06)**	-0.20 (-0.53; 0.14)	-0.27 (-0.64; 0.11)	-0.26 (-0.55; 0.02)	-0.23 (-0.56; 0.11)	-0.29 (-0.61; 0.05)	-0.27 (-0.59; 0.04)
All MRI markers	**-0.46 (-0.82; -0.09)**	-0.22 (-0.55; 0.12)	-0.29 (-0.67; 0.08)	-0.27 (-0.55; 0.02)	-0.26 (-0.59; 0.08)	-0.30 (-0.64; 0.03)	-0.27 (-0.59; 0.04)

Abbreviations: TBV, total brain volume; WMH, white matter hyperintensities; MRI, magnetic resonance imaging, CI, confidence interval

* Model I: Adjusted for age, gender and education

† Model II: Model I + mean arterial blood pressure, cholesterol, random blood glucose, smoking, body mass index and Geriatric Depression Scale

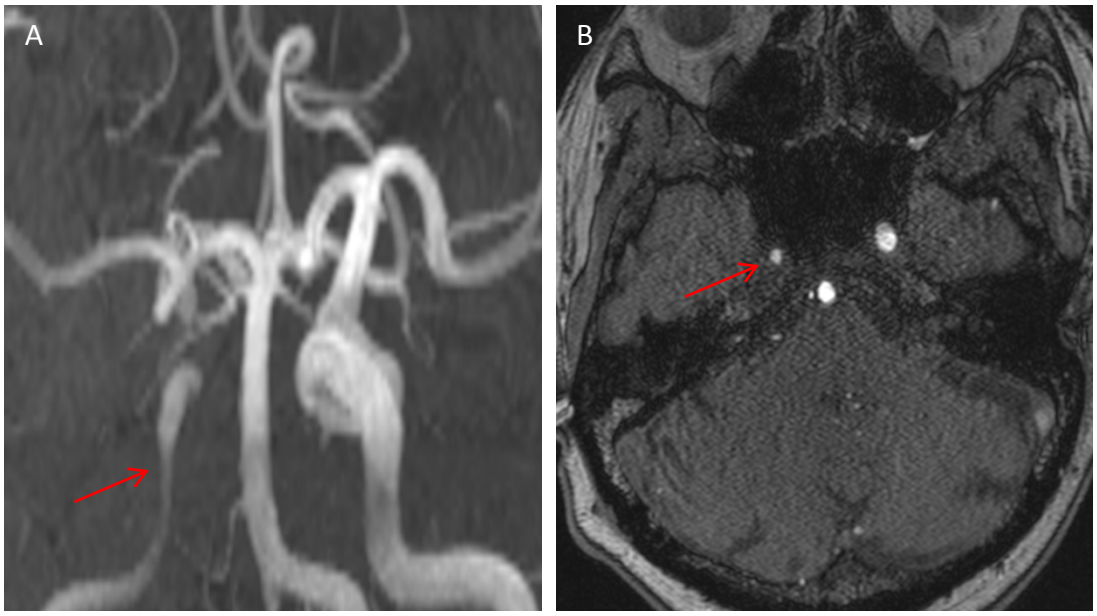
‡ Model III: Model II + individual MRI markers

Values in bold indicates P<0.05

CHAPTER 7 – FIGURE

Figure 7 – 1: Intracranial stenosis on Magnetic Resonance Angiography (MRA)

Intracranial stenosis is defined as the narrowing exceeding 50% of the luminal diameter in any of the intracranial vessels assessed on 3D Time of Flight MRA. The images are first visually accessed on the coronal view (A) and confirmed on the reconstruction (B).



CHAPTER 8:

Intracranial Stenosis in Cognitive Impairment and Dementia

1. INTRODUCTION

Cerebrovascular diseases have been increasing implicated as a cause and contributor to cognitive impairment and dementia.[1] There is a strong association between ischemic strokes and white matter hyperintensities with cognitive decline. In this context, intracranial stenosis (ICS) has gained increasing attention due to its role in causing ischemic damage and hence cognitive dysfunction.[2] The occurrence of ICS has been attributed largely to systemic vascular risk factors such as hypertension and diabetes[3, 4] – the risk factors also associated with Alzheimer's disease (AD) and Vascular Dementia (VaD). Previous post mortem studies have shown that severe arterial atherosclerosis of the Circle of Willis is a common finding in dementia found in 53% of VaD and 34% of AD patients.[5]

Reports from ante mortem studies have suggested that the ICS in stroke subjects varies across ethnicities with a higher prevalence (30-83%) reported in Asians compared to Caucasians (8-10%).[6, 7] This difference in prevalence might arise from different imaging modalities [Transcranial Doppler ultrasound (TCD) vs. Magnetic Resonance Angiography (MRA)] and criteria used to define ICS. Furthermore, a high prevalence of ICS on MRA is also reported in VaD compared to AD patients (53% vs. 18%) in a hospital based study consistent with the previous neuropathological findings.[8]

With respect to cognition, a few studies[9-12] have shown that both extra and intracranial arterial stenosis affects neuropsychological test performance possibly due to hypoperfusion and structural brain damage.[13] However, the exact role of ICS in causing vascular pathology in preclinical cognitive impairment and different types of dementia has been less studied. Moreover, these studies lacked a disease free comparison group[8] and have not taken into account the other markers of cerebrovascular diseases. In the

present study, we examined the association of ICS in relation to cognitive impairment and dementia in the presence of other markers of cerebrovascular diseases. Moreover, we investigated its association in vascular vs. non vascular subtypes of cognitive impairment from a memory clinic setting in Singapore.

2. METHODS

For the present memory clinic-based study, we employed a case-control design. Cases (CIND and dementia) were recruited from two study sites in Singapore (i.e. memory clinics from National University Hospital and Saint Luke's Hospital). Controls were recruited from both memory clinics and the community (with a similar catchment area as cases). Controls (from memory clinic and community) were defined as those who may have subjective complaints of memory impairment, but were cognitively normal on objective neuropsychological assessment. Details of this study have been described previously.[14] All subjects underwent physical, clinical and neuropsychological assessments and neuroimaging at the National University of Singapore.

Ethics approval was obtained from the Singapore Eye Research Institute, and National-Healthcare Group Domain-Specific Review Board. The study is conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained in the preferred language of the participants by bilingual study coordinators prior to their recruitment into the study.

2.1 Demographic and cardiovascular risk factor assessment

Detailed questionnaire collecting information on age, gender, race, education and smoking history was described in **Chapter 3**. Previous medical history of hypertension, hyperlipidemia, diabetes mellitus, was also noted and subsequently verified by medical records.

2.2 Neuroimaging

MRI and three dimensional time-of-flight MRA images (3D TOF MRA) was performed on a 3Tesla Siemens Magnetom Trio Tim scanner, using a 32-channel head coil, at the Clinical Imaging Research Centre of the National University of Singapore. The MRA acquisition details have been described in **Chapter 3**.

Intracranial stenosis on MRA

ICS was defined based on the criteria published previously.[9] Briefly, arterial narrowing exceeding 50% of the luminal diameter in any of the intracranial vessels were assessed on 3D TOF MRA images and were recorded as mentioned in **Chapter 3**.

Other MRI markers

Other markers of cerebrovascular diseases (infarcts, cerebral microbleeds and white matter hyperintensities) and involutional changes (total intracranial volume) were also graded on MRI, details of which have been provided in **Chapter 3**.

2.3 Diagnosis of cognitive impairment and dementia

An extensive neuropsychological battery, which has been previously validated in Singaporean elderly,[15] was administered to assess cognitive function. Besides the objective tests, the various diagnostic groups of the participants were also made at a weekly consensus meeting:

- Subjects with no objective evidence of neuropsychological deficits were classified as having no cognitive impairment (NCI).
- CIND was determined by clinical judgment and was defined as no significant loss of independence in daily activities, and impairment in at least one domain of

the neuropsychological test battery. Participants were considered to have failed a test if they scored 1.5 SD below education-adjusted cut-off values on each individual test. Failure in at least half of the tests in each domain was considered as impairment in that domain.

- The diagnosis of dementia was made according to DSM-IV criteria. The etiological diagnoses of dementia were based on the internationally accepted criteria;
    - AD was diagnosed using the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA).
    - VaD was defined using the National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherché et l' Enseignement en Neurosciences (NINDS-AIREN) criteria.
- The subtypes of vascular and non-vascular cognitive impairment were defined as;
    - Vascular cognitive impairment included (VCI) (a) CIND with a history of ischemic stroke within the past 6–24 months or neuroimaging evidence of cerebral infarction, or (b) VaD,
    - Non-vascular cognitive impairment (non-VCI) included (a) CIND without a history of ischemic stroke or infarcts on neuroimaging or (b) AD.

2.4 Statistical Analysis

In order to compare the baseline characteristics between cases (CIND and dementia) and controls (NCI), analysis of covariance or chi square tests were used. In case of non-uniform data (WMH), difference between the groups was determined using Kruskal-Wallis test. WMH volume was logarithmically transformed due to the skewed

distribution for further analysis. Association between ICS and CIND/dementia were determined initially using logistic regression models with odds ratios (OR) and 95%Confidence interval (CI). Further regression analyses were then constructed separately for VCI and non-VCI and then stratified by its subtypes. All models were initially adjusted for age and gender and additionally for hypertension, hyperlipidemia, and diabetes. Finally in order to examine whether the association between ICS and CIND/dementia remains independent of other cerebrovascular diseases, we adjusted logistic models additionally for each MRI marker. P-value < 0.05 was considered statistically significant. Statistical analysis was performed using standard statistical software (Statistical Package for Social Science, SPSS V23, SPSS Inc., USA).

3. RESULTS

Assessments of subjects were performed from August 12, 2010 to July 28, 2015. Out of the 462 subjects, 13 had no MRI scans and 25 had ungradable scans. Of the remaining 424 subjects, there were 96 controls and 328 cases [177 (53.9%) CIND and 151 (46%) dementia]. **Table 8-1** shows the baseline characteristics of the cases and controls. An increasing frequency of risk factors was observed from NCI to CIND and dementia. Compared to controls, subjects with CIND or dementia were older, had more women and attained lower education. A higher prevalence of hypertension and diabetes and lower BMI was present in the cognitively impaired subjects. Moreover, an increasing trend was observed for all the MRI markers whereas a decreasing trend for brain atrophy was observed from NCI to dementia. Among different diagnostic groups, ICS was identified in 8 (8.3%) NCI, 6 (5.6%) CIND, 24 (19.8%) AD, 20 (28.6%) VCIND and 17 (56.7%) VaD subjects.

Table 8-2 shows the association of ICS with CIND and dementia. ICS was only related to dementia (age/gender adjusted OR: 4.56; 95%CI: 1.88-11.11) but not with CIND

(age/gender adjusted OR: 2.07; 95%CI: 0.87-4.94). After adjustment for cardiovascular risk factors, the presence of ICS remain significantly associated with dementia (OR: 3.69; 95%CI: 1.46-9.31). Following further adjustments with MRI markers including total intracranial volume, presence of cerebral microbleeds, and infarcts, the associations of ICS with dementia remained statistically significant. On adding WMH volume into the model, these associations attenuated and partly became non-significant.

On further analysis comparing VCI vs. non-VCI groups, ICS was only associated with VCI in age/gender adjusted model (OR: 5.85; 95%CI: 2.52-13.56). This association remained independent of cardiovascular risk factors and other MRI markers. No association was observed with non-VCI (**Table 8-3**). With respect to subtypes analysis, ICS was related to both AD (OR: 3.51; 95%CI: 1.28-9.62) in non-VCI group and VCIND (OR: 3.97; 95%CI: 1.59-9.87) and VaD (OR: 11.36; 95%CI: 3.84-33.64) in VCI group in age and gender adjusted models (**Table 8-4**). These associations remain unaltered after including cardiovascular risk factors in the model. In terms of additional adjustment for total brain volume and cerebral microbleeds, similar trends were seen for both VCI and non-VCI subtypes. However, these associations become non-significant after including WMH volume in the model in case of AD and presence of infarcts and WMH for VaD. An independent association was only observed for VCIND group (OR: 4.23; 95%CI: 1.43-12.51).

4. DISCUSSION

This study showed that the persons with ICS are more likely to have vascular cognitive impairment and dementia compared to the controls independent of cardiovascular risk factors. Additional adjustments with MRI markers revealed that the ICS induce cognitive dysfunction possibly through decreased perfusion and cerebral ischemic damage.

Several studies have previously reported the prevalence of ICS in stroke and cognitively impaired individuals including dementia.[16-18] The range of reported prevalence varies from 11-85% in stroke subjects[17] to 18-53% in dementia.[8] This wide difference in prevalence might be due to the diversities in, 1) imaging modalities, 2) measures of neuropsychological function and 3) limited information on degree of stenosis. The ICS prevalence of 19.8% in AD, 28.6% in VCIND and 56.7% in VaD in the present study – though towards the higher side – is in concordant with the previous findings using MRA.[8, 19]

With respect to cognition, it has been reported that the cognitive deficits are commonly present in symptomatic (history of stroke) subjects with extra and intracranial arterial stenosis possibly through cerebral hypoxia.[12,20-22] However, majority of these studies did not control for other relevant neurovascular variables such as cerebral infarction and white matter hyperintensities thus introducing heterogeneity in neurological condition of the patients. Furthermore, no data exist on the association of ICS with vascular cognitive impairment and its subtypes. The present study extends these previous findings by showing that the persons with ICS are more likely to have vascular cognitive impairment independent of other structural MRI markers. This could be linked to the multiple cortical and subcortical ischemic damage (increased resistance and reduced vascular reactivity of the small vessels) or reduction in anatomic connectivity and perfusion deficits secondary to ICS. Moreover, due to the lack of underlying AD pathology, the cognitive reserve capacity of the brain is preserved and hence promotes recovery of cognitive function. By contrast, in dementia, vascular ischemic processes decrease the cognitive reserve of the brain to compensate for ongoing involutional changes thus preventing recovery of cognitive function. The similar mediating factors for both AD and VaD in this study might be due to the several overlapping neuropathological features between the two

disease types.[23] Moreover, the pathogenesis behind AD and VaD might also involve two separate interaction processes. In case of AD, hypoxic injury not only accelerates amyloid beta deposition (especially in the hemisphere with the ICS) but also triggers secondary degeneration induced by inflammatory processes whereas in VaD, hypoperfusion interacts with the existing cerebrovascular diseases and initiates secondary neurodegenerative process.[24] This multifactorial nature of mechanisms underlying cognitive impairment in patients with large-artery atheroma, may explain the link between ICS and dementia (AD and VaD) observed in this study.

Our study has some limitations. First, as this data was examined cross-sectionally it is not possible to establish the temporal association between these ICS and the development of cognitive impairment. Second, cases and half of the controls were derived from two locations, memory clinic and community, although representative of the elderly population in Singapore. The control group was relatively younger and had less burden of vascular risk factors compared to cognitively impaired individuals which could have resulted in selection bias and residual confounding. Also there is a higher burden of vascular risk factors (hypertension, hyperlipidemia and diabetes) in our sample which limits generalizability of the results to the general population. Third, due to some overlapping symptoms and similar MRI features between AD and VaD, there is a chance of misclassification bias. Strengths of the study include; extensive neuropsychological assessment to diagnose cognitive impairment and dementia, availability of 3T MRA neuroimaging to grade and classify individuals with ICS which is relatively feasible to conduct in a large population based study.

5. CONCLUSION

In conclusion, ICS is associated with vascular cognitive impairment and dementia in this study in the presence of cerebral ischemic damage. This further suggests that ICS is a

marker of cerebral or generalized atherosclerosis. Further studies focusing on cerebral perfusion and cognitive reserve are required to determine the pathophysiological link between ICS and cognition.

CHAPTER 8 – REFERENCES

1. Kling MA, Trojanowski JQ, Wolk DA, Lee VM, Arnold SE. Vascular disease and dementias: Paradigm shifts to drive research in new directions. Alzheimers Dement. 2013;9:76-92.

2. Rao R. The role of carotid stenosis in vascular cognitive impairment. Eur Neurol. 2001;46:63-9.

3. Bae HJ, Lee J, Park JM, Kwon O, Koo JS, Kim BK, et al. Risk factors of intracranial cerebral atherosclerosis among asymptomatics. Cerebrovasc Dis. 2007;24:355-60.

4. Ingall TJ, Homer D, Baker HL, Jr., Kottke BA, O'Fallon WM, Whisnant JP. Predictors of intracranial carotid artery atherosclerosis. Duration of cigarette smoking and hypertension are more powerful than serum lipid levels. Arch Neurol. 1991;48:687-91.

5. Beach TG, Wilson JR, Sue LI, Newell A, Poston M, Cisneros R, et al. Circle of willis atherosclerosis: Association with alzheimer's disease, neuritic plaques and neurofibrillary tangles. Acta neuropathologica. 2007;113:13-21.

6. Huang YN, Gao S, Li SW, Huang Y, Li JF, Wong KS, et al. Vascular lesions in chinese patients with transient ischemic attacks. Neurology. 1997;48:524-5.

7. Li H, Wong KS. Racial distribution of intracranial and extracranial atherosclerosis. Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia. 2003;10:30-4.

8. De Silva DA, Ancalan M, Doshi K, Chang HM, Wong MC, Chen C. Intracranial large artery disease in alzheimer's disease and vascular dementia among ethnic asians. Eur J Neurol. 2009;16:643-5.

9. Hilal S, Saini M, Tan CS, Catindig JA, Dong YH, Holandez RL, et al. Intracranial stenosis, cerebrovascular diseases, and cognitive impairment in chinese. Alzheimer Dis Assoc Disord. 2015;29:12-7.

10. Mathiesen EB, Waterloo K, Joakimsen O, Bakke SJ, Jacobsen EA, Bonaa KH. Reduced neuropsychological test performance in asymptomatic carotid stenosis: The tromso study. Neurology. 2004;62:695-701.

11. Bakker FC, Klijn CJ, Jennekens-Schinkel A, Kappelle LJ. Cognitive disorders in patients with occlusive disease of the carotid artery: A systematic review of the literature. Journal of neurology. 2000;247:669-76.

12. Benke T, Neussl D, Aichner F. Neuropsychological deficits in asymptomatic carotid artery stenosis. Acta neurologica Scandinavica. 1991;83:378-81.

13. Klijn CJ, Kappelle LJ, Tulleken CA, van Gijn J. Symptomatic carotid artery occlusion. A reappraisal of hemodynamic factors. Stroke. 1997;28:2084-93.

14. Hilal S, Chai YL, Ikram MK, Elangovan S, Yeow TB, Xin X, et al. Markers of cardiac dysfunction in cognitive impairment and dementia. Medicine. 2015;94:e297.

15. Hilal S, Ikram MK, Saini M, Tan CS, Catindig JA, Dong YH, et al. Prevalence of cognitive impairment in chinese: Epidemiology of dementia in singapore study. J Neurol Neurosurg Psychiatry. 2013;84:686-92.

16. Wong KS, Ng PW, Tang A, Liu R, Yeung V, Tomlinson B. Prevalence of asymptomatic intracranial atherosclerosis in high-risk patients. Neurology. 2007;68:2035-38.

17. Wong KS, Huang YN, Yang HB, Gao S, Li H, Liu JY, et al. A door-to-door survey of intracranial atherosclerosis in liangbei county, china. Neurology. 2007;68:2031-34.

18. Wong LK. Global burden of intracranial atherosclerosis. Int J Stroke. 2006;1:158-9.

19. Man BL, Fu YP, Chan YY, Lam W, Hui CF, Leung WH, et al. Use of magnetic resonance angiography to predict long-term outcomes of ischemic stroke patients with concurrent stenoses in hong kong. Cerebrovasc Dis. 2009;28:112-8.

20. Hamster W, Diener HC. Neuropsychological changes associated with stenoses or occlusions of the carotid arteries. A comparative psychometric study. Eur Arch Psychiatry Neurol Sci. 1984;234:69-73.

21. Poels MM, van Oijen M, Mattace-Raso FU, Hofman A, Koudstaal PJ, Witteman JC, et al. Arterial stiffness, cognitive decline, and risk of dementia: The rotterdam study. Stroke. 2007;38:888-92.

22. Breteler MM, Claus JJ, Grobbee DE, Hofman A. Cardiovascular disease and distribution of cognitive function in elderly people: The rotterdam study. BMJ. 1994;308:1604-8.

23. Iadecola C, Gorelick PB. Converging pathogenic mechanisms in vascular and neurodegenerative dementia. Stroke. 2003;34:335-7.

24. Thiel A, Cechetto DF, Heiss WD, Hachinski V, Whitehead SN. Amyloid burden, neuroinflammation, and links to cognitive decline after ischemic stroke. Stroke. 2014;45:2825-9.

CHAPTER 8 – TABLES

Table 8 – 1: Baseline demographic and clinical characteristics of the subjects (n=424)

Characteristics	NCI (n=96)	CIND (n=177)	Dementia (n=151)	P value
Age, years, mean (SD)	68.4 (6.1)	71.5 (8.3)	76.1 (7.9)	**<0.001**
Males, no. (%)	44 (45.8)	91 (51.4)	57 (37.7)	**0.046**
Education, (Total number of years), mean (SD)	9.86 (5.04)	7.20 (4.81)	4.87 (4.51)	**<0.001**
Hypertension, no. (%)	58 (60.4)	121 (68.4)	126 (83.4)	**<0.001**
Hyperlipidemia, no. (%)	70 (72.9)	136 (76.8)	110 (72.8)	0.653
Diabetes, no. (%)	20 (20.8)	63 (35.6)	64 (42.4)	**0.002**
Smoking, no. (%)	20 (20.8)	49 (27.7)	38 (25.2)	0.461
Body mass index, mean (SD)	19.5 (3.1)	19.1 (3.2)	18.3 (3.3)	**0.017**
Cardiovascular disease, no (%)	8 (8.3)	31 (17.5)	28 (18.5)	0.072
Presence of infarcts, no (%)	21 (21.9)	76 (42.9)	70 (46.4)	**<0.001**
White matter hyperintensities volume, ml, median (IQR)	0.69 (1.06)	4.65 (18.87)	11.77 (16.36)	**<0.001**
Total brain volume, ml, mean (SD)	920.3 (83.9)	888.3 (144.8)	843.5 (199.6)	**0.007**
Presence of microbleeds, no. (%)	49 (51)	80 (45.5)	93 (63.7)	**0.004**
Intracranial stenosis, no. (%)	8 (8.3)	26 (14.7)	41 (27.2)	**<0.001**

Abbrevations: NCI, no cognitive impairment; CIND, cognitive impairment no dementia; SD, standard deviation; No., number; IQR, interquartile range; ml milliliters

Table 8 – 2: Association between intracranial stenosis (presence versus absence) and cognition

	CIND (n=177) OR (95% CI)	Dementia (n=151) OR (95% CI)
Model I*	2.07 (0.87-4.94)	**4.56 (1.88-11.11)**
Model II†	2.04 (0.83-5.01)	**3.69 (1.46-9.31)**
Model III‡		
Total intracranial volume	2.35 (0.89-6.15)	**3.89 (1.37-11.10)**
Presence of microbleeds	2.18 (0.88-5.38)	**3.59 (1.42-9.12)**
Presence of infarcts	1.78 (0.71-4.46)	**3.50 (1.38-8.92)**
WMH volume	2.26 (0.85-6.04)	2.86 (0.91-8.98)

Abbreviation: CIND, cognitive impairment; OR, odds ratios; WMH, white matter hyperintensity

* Model I: Adjusted for age and gender

† Model II: Model I +hypertension, hyperlipidemia, diabetes

‡ Model III: model II + individual MRI markers

Table 8 – 3: Association of intracranial stenosis with vascular vs. non vascular cognitive impairment

	Non VCI (n=228) OR (95% CI)	VCI (n=100) OR (95% CI)
Model I*	1.67 (0.68-4.13)	**5.85 (2.52-13.56)**
Model II†	1.64 (0.64-4.19)	**4.49 (1.88-10.75)**
Model III‡		
Total intracranial volume	1.67 (0.59-4.75)	**4.11 (1.59-10.65)**
Presence of microbleeds	1.64 (0.64-4.21)	**4.39 (1.83-10.54)**
Presence of infarcts	1.63 (0.64-4.17)	**4.11 (1.51-11.16)**
WMH volume	1.41 (0.49-4.07)	**4.27 (1.54-11.86)**

Abbreviation: VCI, vascular cognitive impairment; OR, odds ratios; WMH, white matter hyperintensity

* Model I: Adjusted for age and gender

† Model II: Model I +hypertension, hyperlipidemia, diabetes

‡ Model III: model II + individual MRI markers

Table 8 – 4: Association of intracranial stenosis with subtypes of vascular vs. non vascular cognitive impairment

	Non VCI (n=228)		VCI (n=100)	
	CIND (n=107)	**AD (n=121)**	**VCIND (n=70)**	**VaD (n=30)**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Model I*	0.55 (0.17-1.82)	**3.51 (1.28-9.62)**	**3.97 (1.59-9.87)**	**11.36 (3.84-33.64)**
Model II†	0.53 (0.15-1.92)	**3.36 (1.17-9.65)**	**3.50 (1.38-8.90)**	**7.44 (2.33-23.77)**
Model III‡				
Total brain volume	0.55 (0.13-2.35)	**3.95 (1.19-13.03)**	**4.31 (1.55-11.96)**	**9.57 (2.26-40.59)**
Presence of microbleeds	0.55 (0.15-2.01)	**3.15 (1.09-9.09)**	**3.36 (1.31-8.63)**	**7.46 (2.32-24.05)**
Presence of infarcts	0.54 (0.15-1.97)	**3.52 (1.23-10.08)**	**3.67 (1.27-10.61)**	3.78 (0.92-15.63)
WMH volume	0.56 (0.14-2.28)	3.26 (0.91-11.63)	**4.23 (1.43-12.51)**	4.01 (0.72-22.43)

Abbreviation: VCI, vascular cognitive impairment; CIND, cognitive impairment no dementia; AD, Alzheimers disease; VCIND, Vascular cognitive impairment no dementia; VaD, Vascular dementia; OR, odds ratios; WMH, white matter hyperintensity

* Model I: Adjusted for age and gender

† Model II: Model I +hypertension, hyperlipidemia, diabetes

‡ Model III: model II + individual MRI markers

PART II:

BRAIN MARKERS OF INVOLUTIONAL CHANGES

CHAPTER 9:

Risk Factors and Consequences of Cortical Thickness in an Asian Population

1. INTRODUCTION

Neurodegeneration - a hallmark of dementia - is characterized by loss of neuronal tissue in both gray and white matter. This brain atrophy is not only seen in clinically manifest Alzheimer's disease (AD), but may already be present in the preclinical stages [for which the terms cognitive impairment no dementia (CIND) or mild cognitive impairment (MCI) have been coined].[1-4] Furthermore, it has been suggested that these brain changes may even be present during normal aging.[5-8]

Recent advances in neuroimaging enable us to assess early age-related brain changes. Of particular interest is cortical thickness, which reflects the width of the cortical gray matter,[9] and has been proposed to be a reliable marker of brain atrophy.[10] Previous studies have shown that patients with AD have cortical thinning in frontal, temporal and parietal regions compared to controls, consistent with pathological patterns of atrophy described in AD.[1,11,12] In addition, a few studies has suggested that even during the preclinical stages of dementia cortical thinning is associated with worse performance on cognitive tests.[13,14] Overall, these studies were mainly limited to Caucasian populations, had small sample sizes, [15-17] and lacked detailed neuropsychological tests.[13,14]

With respect to Asian populations, it has been proposed that – besides neurodegeneration - cerebrovascular disease may play a prominent role in the development of dementia, due to the higher prevalence of vascular risk factors among Asians compared to Caucasians.[18-21] Nevertheless, it remains important to determine the exact role of involutional changes in Asian populations, particularly in the preclinical stages of dementia. Thus far, several studies from Korea have shown regional differences in temporo-parietal and prefrontal regions in both AD patients and subjects with MCI compared to controls.[22-24] However, the association between cortical thickness and cognitive impairment in elderly Asian populations has not been explored extensively.

We, therefore, examined whether demographic and cardiovascular risk factors were related to cortical thickness. Furthermore, we examined in an elderly Asian population from Singapore the association of global and lobe-specific cortical thicknesses with cognitive impairment, including preclinical stages of dementia.

2. METHODS

2.1. Study Population

The on-going Epidemiology of Dementia in Singapore (EDIS) study draws participants from the Singapore Epidemiology of Eye Disease (SEED) study, a multi-ethnic population-based study among persons aged 40 to 85 years among Chinese (Singapore Chinese Eye Study [SCES]), Malay (Singapore Malay Eye Study [SiMES-2]), and Indians (Singapore Indian Eye Study [SINDI-2]). For this study, we focused on Chinese [25] and Malay components [26] of the EDIS Study, as the recruitment of the Indians is still on-going. In the first phase of the EDIS study, participants aged ≥ 60 years (n=2,666) were screened using the Abbreviated Mental Test and a self-report of progressive forgetfulness. Screen-positive subjects (n=1,097) were invited to take part in the second phase of this study, which included an extensive neuropsychological test battery and brain MRI. Of these 1,097 participants, 623 agreed to participate in phase II and hence were included in the present study. The details of the study methodology have been described elsewhere.[25] Ethics approval for EDIS study was obtained from the Singapore Eye Research Institute, and National Healthcare Group Domain-Specific Review Board. The study is conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained in the preferred language of the participants by bilingual study coordinators prior to their recruitment into the study.

2.2. Demographic and Cardiovascular Factor Assessment

During a personal interview a detailed questionnaire was administered to collect relevant demographic and medical information. Details of all the study assessments have been described previously in **Chapter 3**.

2.3. Neuroimaging

Cortical thickness was segmented throught an automated model based approach (**Figure 9 – 1**). Details of cortical thickness and other quantitative MRI data (total intracranial volume and white matter hyperintensities volume) have been described in **Chapter 3**.

2.4. Cognitive Assessment

An extensive neuropsychological battery, which has been previously validated in Singaporean elderly, was administered to assess cognitive function[25] (**Chapter 3).** The diagnosis of CIND and dementia utilized in the study has been described previously in **Chapter 3**.

2.5. Statistical Analysis

In order to examine differences in baseline characteristics between included and excluded subjects, chi-square test were used for categorical variables and student t-test for continuous variables. Trends in baseline characteristics across different diagnostic groups were examined using Analysis of Variance (ANOVA) and a p-value for the trend test was computed.

Associations of potential demographic and cardiovascular risk factors with global and lobar cortical thicknesses were explored using multiple linear regression models. All continuous variables (age, mean arterial blood pressure, non-fasting blood glucose, total cholesterol, BMI, total intracranial volume) were standardized (by dividing each variable by its SD). For each continuous variable, mean differences in cortical thicknesses were expressed per SD increase/decrease in that variable. Model I was adjusted for age, gender and education. Subsequently, in the fully adjusted model (Model II), all potential risk

factors were included in the same model to determine the independent effect of each potential factor with cortical thickness.

Next, we examined the associations of global and lobar cortical thicknesses with clinical outcomes (CIND and dementia) using logistic regression models [odds ratios (OR) with 95% confidence interval (CI)] and with composite Z score using linear regression models [mean difference with 95% CI]. The effect sizes of these associations with cognition were expressed per SD decrease in cortical thickness.

P-values < 0.05 were considered statistically significant. In view of the multiple tests performed in the lobe-specific analyses, we used Bonferroni correction to obtain a revised statistical significance level of 0.05/6 ~ 0.008. Furthermore, we used revised levels of statistical significance for the cognitive domain-specific analyses: 0.05/7 ~ 0.007 when analyzing the associations with global cortical thickness, and 0.05/7*6 ~ 0.001 when analyzing the associations with lobar cortical thicknesses. Statistical analysis was performed using standard statistical software (Statistical Package for Social Sciences, SPSS V22, SPSS Inc., USA).

3. RESULTS

Assessments of study participants were performed from August 12, 2010 to December 21, 2013. Out of 623 subjects who participated in phase II, 36 had no MRI scans and 3 had ungradable scans. Furthermore, 12 subjects who had a cortical infarct were excluded, as these infarcts may influence the cortical thickness measurements. **Supplementary table 9-1** presents baseline data of both the included and excluded subjects. In brief, excluded subjects were likely to be older, were more often Chinese, had lower education and had higher frequency of hypertension and lower frequency of hyperlipidemia. Out of 572 included subjects, 171 (29.9%) were diagnosed with CIND-mild, 197 (34.4%) with CIND-moderate and 28 (4.9%) with dementia. **Table 9-1** provides baseline

characteristics of the included participants according to the different diagnostic groups. In brief, increasing age, female gender, Malay ethnicity, higher proportion of hypertension, diabetes, and hyperlipidemia were related to severity of cognitive impairment. Also, an increasing frequency was observed for several MRI markers. Conversely, a decreasing trend was observed for education, BMI, total intracranial volume and IADL.

Table 9-2 shows the association of potential risk factors with mean global cortical thickness. In fully adjusted models (Model II), the most important risk factors of cortical thickness were: increasing age [mean difference in cortical thickness per SD increase in age: -30.9μm; 95% CI: -40.2; -21.7; p<0.001], gender [women versus men: 25.4μm; 95% CI: 2.1; 48.7; p=0.029], Malay ethnicity [Malay versus Chinese: -57.4μm; 95% CI: -74.5; -40.3; p<0.001], BMI [mean difference per SD increase in BMI: -9.5; 95% CI: -18.1; -0.8; p=0.022] and presence of lacunar infarct [presence versus absence: -25.8; 95% CI: -48.6; -3.1; p=0.034]. A borderline significant association was observed for non-fasting glucose levels [mean difference per SD increase in glucose levels: -8.6μm; 95% CI: -16.4.; 0.3; p=0.059].

The association between potential risk factors and lobe-specific cortical thickness are presented in **Supplementary table 9-2.** After Bonferroni correction, the most consistent associations with smaller cortical thicknesses across the different lobes were found for increasing age and Malay ethnicity. Women had thicker cortical thicknesses in particular in the parietal and temporal lobes. The association between higher BMI and smaller cortical thickness was most prominent in the frontal region [mean difference per SD increase in BMI: -14.7; 95% CI: -23.9; -5.45; p=0.002]. In terms of MRI markers of cerebral small vessel disease, WMH were associated with temporal thinning, whereas increasing number of microbleeds were related to insular thinning.

With respect to clinical outcomes (**Table 9-3**), smaller global cortical thickness was significantly associated with CIND moderate/dementia [OR: 1.70; 95% CI: 1.19-2.44;

p=0.004]. This association persisted even after excluding 28 dementia cases [OR: 1.69; 95% CI: 1.18-2.43; p=0.004]. Smaller cortical thickness was also related to poorer global cognitive functioning as reflected by the composite Z-scores [mean difference composite Z-score per SD decrease in cortical thickness: -0.094; 95%CI: -0.159; -0.030, p=0.004]. Lobe-specific analyses showed that these associations were mainly driven by the parietal, occipital, temporal and limbic lobes. Specifically, the associations with the temporal and occipital lobes remained statistically significant after Bonferroni correction.

Finally, in the domain-specific analyses (**Table 9-4**), global cortical thickness was related to executive function [mean difference per SD decrease in cortical thickness: -0.129; 95% CI: -0.207; -0.051; p=0.001], visuoconstruction [mean difference per SD decrease in cortical thickness: -0.099; 95% CI: -0.172; -0.027; p=0.007] and visual memory [mean difference per SD decrease in cortical thickness: -0.111; 95% CI: -0.183; -0.039; p=0.003]. In the lobe-specific analyses, the most consistent associations at the nominal significance level of 0.05 were found between the occipital and temporal lobes with the various cognitive domains. However, after applying Bonferroni correction, most of these associations did not remain statistically significant.

4. DISCUSSION

In this study, we found that persons with smaller cortical thickness – in particular in the temporal and occipital lobes - were more likely to have cognitive impairment, including the preclinical stages of dementia. More specifically, these persons performed worse on tasks in executive function, visuoconstruction and visual memory. Finally, the most important risk factors were increasing age, male gender, Malay ethnicity, increased blood glucose, high BMI and presence of lacunar infarction on MRI.

Several studies reported a smaller global cortical thickness with increasing age.[7, 27, 28] However, across these studies this effect of age was variable with some reporting the

largest decrease in frontal and temporal lobes,[27] whereas others found the strongest effects in the occipital and parietal regions.[6, 29] The wide age distribution of these studies (ranging from 18 to 82 years) may underlie these differences. Despite these variations, the overall trend – that increasing age was related to smaller cortical thickness – is similar across all these studies, which is further supported by our current findings.

In our study women had relatively thicker cortex compared to men. This gender difference may be related to the protective effect of estrogen on involutional changes.[30] This is in line with other studies reporting similar gender differences in cortical thickness. [31] In terms of ethnic differences, Malays had a thinner global and lobe-specific cortical thicknesses compared to Chinese. A higher prevalence of vascular risk factors (hypertension, diabetes and hyperlipidemia) and a higher frequency of Apoε4 carriers have been reported among Malays. These factors may lead to an increased susceptibility to involutional changes in Malays and hence may underlie this difference.[32]

With respect to cardiovascular risk factors, we found – in accordance with other studies – that increased blood glucose levels were associated (borderline significantly) with global cortical thinning.[15,31,33] The mechanisms leading to involutional changes are linked to episodes of hypo- and hyperglycemia, alterations to the blood-brain barrier and increased production of glycated endproducts.[34, 35] Besides glucose levels, an independent association was found for BMI, especially in the frontal lobe. A previous study suggested that adiposity was associated with frontal gray matter atrophy in middle and old aged persons, possibly through increased vascular pathology and reduced blood supply eventually leading to brain atrophy.[36] Further studies are needed to elucidate the exact mechanisms through which BMI and adiposity are related to cortical thinning. Finally, several MRI markers of cerebral small vessel disease showed some associations with

smaller global and lobe-specific cortical thicknesses, indicating an interaction between cerebrovascular and involutional changes.[37-39]

With respect to cognition, we found that a smaller global cortical thickness is linked to cognitive impairment suggesting that diffuse involutional change beyond medial temporal lobe and hippocampus atrophy is already present in the preclinical stages of dementia.[24] More specifically, thinner cortex in temporal and occipital lobes showed consistent patterns with worse performance in all cognitive domains. Patho-physiologically, the temporal and occipital lobes may show thinning in the early stages of dementia, as these regions are especially susceptible to the toxic effects of neurofibrillary tangles and amyloid plaques,[40,41] and hence are early sites for these depositions. It has been reported that the burden of these depositions was correlated with the extent of atrophy and reduced metabolism in these regions,[42] and functionally with cognitive dysfunction. Our current findings suggest that in Asian populations, besides the contribution of cerebrovascular disease, involutional changes as reflected by cortical thickness plays an important role in cognitive impairment, including the preclinical stages of dementia.

Limitations of the study include: first, 47.9% of the screened positive subjects were excluded from these analyses. Compared to the included participants, these excluded subjects were relatively older, less educated and more likely to have hypertension and hyperlipidemia. However, despite this non-participation we still found significant associations with cortical thickness. Furthermore, these excluded subjects might be more cognitively impaired, suggesting that the reported effect sizes in this study might be an underestimation. Second, due to the cross-sectional design of our study the temporal relationship between the presence of cortical thickness and cognitive impairment could not be assessed. Third, due to the small number of cases with dementia, we were not able to examine these cases separately in multi-variable models as this resulted in unstable

effect sizes and wide confidence intervals. However, the dose-response relationship with the preclinical stages of cognitive impairment suggests that these findings may also be extendable to dementia. Strengths of the study include: subjects were selected from a population-based study, extensive neuropsychological tests were used to diagnose cognitive impairment and dementia, and automated and standardized image processing was used to quantify cortical thickness.

5. CONCLUSION

In conclusion, persons with smaller cortical thickness – in particular in the temporal and occipital lobes - were more likely to have cognitive impairment, suggesting a contribution of diffuse cortical thinning beyond the medial-temporal lobe to cognitive function. These findings support the notion that cortical thinning is a biomarker of involutional changes in the brain not only in dementia, but also in its preclinical stages.

CHAPTER 9 – REFERENCES

1. Frisoni GB, Testa C, Zorzan A, et al. Detection of grey matter loss in mild Alzheimer's disease with voxel based morphometry. J Neurol Neurosurg Psychiatry. 2002;73:657-64.
2. Karas G, Sluimer J, Goekoop R, et al. Amnestic mild cognitive impairment: structural MR imaging findings predictive of conversion to Alzheimer disease. AJNR Am J Neuroradiol. 2008;29:944-9.
3. Karas GB, Scheltens P, Rombouts SA, et al. Global and local gray matter loss in mild cognitive impairment and Alzheimer's disease. Neuroimage. 2004;23:708-16.
4. Trivedi MA, Wichmann AK, Torgerson BM, et al. Structural MRI discriminates individuals with Mild Cognitive Impairment from age-matched controls: a combined neuropsychological and voxel based morphometry study. Alzheimers Dement. 2006;2:296-302.
5. Lemaitre H, Goldman AL, Sambataro F, et al. Normal age-related brain morphometric changes: nonuniformity across cortical thickness, surface area and gray matter volume? Neurobiology of aging. 2012;33:617 e611-619.
6. Long X, Liao W, Jiang C, Liang D, Qiu B, Zhang L. Healthy aging: an automatic analysis of global and regional morphological alterations of human brain. Academic radiology. 2012;19:785-93.
7. Magnotta VA, Andreasen NC, Schultz SK, et al. Quantitative in vivo measurement of gyrification in the human brain: changes associated with aging. Cerebral cortex. 1999;9:151-60.

8. Preul C, Hund-Georgiadis M, Forstmann BU, Lohmann G. Characterization of cortical thickness and ventricular width in normal aging: a morphometric study at 3 Tesla. Journal of magnetic resonance imaging : JMRI. 2006;24:513-9.

9. Fischl B, Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. Proceedings of the National Academy of Sciences of the United States of America. 2000;97:11050-5.

10. Singh V, Chertkow H, Lerch JP, Evans AC, Dorr AE, Kabani NJ. Spatial patterns of cortical thinning in mild cognitive impairment and Alzheimer's disease. Brain. 2006;129:2885-93.

11. Lerch JP, Pruessner JC, Zijdenbos A, Hampel H, Teipel SJ, Evans AC. Focal decline of cortical thickness in Alzheimer's disease identified by computational neuroanatomy. Cerebral cortex. 2005;15:995-1001.

12. Sabuncu MR, Desikan RS, Sepulcre J, et al. The dynamics of cortical and hippocampal atrophy in Alzheimer disease. Arch Neurol. 2011;68:1040-8.

13. Chang YL, Jacobson MW, Fennema-Notestine C, et al. Level of executive function influences verbal memory in amnestic mild cognitive impairment and predicts prefrontal and posterior cingulate thickness. Cerebral cortex. 2010;20:1305-13.

14. Paajanen T, Hanninen T, Aitken A, et al. CERAD Neuropsychological Total Scores Reflect Cortical Thinning in Prodromal Alzheimer's Disease. Dementia and geriatric cognitive disorders extra. 2013;3:446-58.

15. Brundel M, van den Heuvel M, de Bresser J, Kappelle LJ, Biessels GJ. Cerebral cortical thickness in patients with type 2 diabetes. Journal of the neurological sciences. 2010;299:126-30.

16. Leritz EC, Salat DH, Williams VJ, et al. Thickness of the human cerebral cortex is associated with metrics of cerebrovascular health in a normative sample of community dwelling older adults. Neuroimage. 2011;54:2659-71.

17. Luders E, Narr KL, Thompson PM, et al. Gender effects on cortical thickness and the influence of scaling. Human brain mapping. 2006;27:314-24.

18. Mok V, Srikanth V, Xiong Y, et al. Race-ethnicity and cerebral small vessel disease - Comparison between Chinese and White populations. Int J Stroke. 2014;100:36-42.

19. Khattar RS, Swales JD, Senior R, Lahiri A. Racial variation in cardiovascular morbidity and mortality in essential hypertension. Heart (British Cardiac Society). 2000;83:267-71.

20. Xu G, Meyer JS, Huang Y, Chen G, Chowdhury M, Quach M. Cross-cultural comparison of mild cognitive impairment between China and USA. Curr Alzheimer Res. 2004;1:55-61.

21. Catindig JA, Venketasubramanian N, Ikram MK, Chen C. Epidemiology of dementia in Asia: insights on prevalence, trends and novel risk factors. Journal of the neurological sciences. 2012;321:11-6.

22. Seo SW, Im K, Lee JM, et al. Effects of demographic factors on cortical thickness in Alzheimer's disease. Neurobiology of aging. 2011;32:200-9.

23. Ye BS, Seo SW, Yang JJ, et al. Comparison of cortical thickness in patients with early-stage versus late-stage amnestic mild cognitive impairment. Eur J Neurol. 2014;21:86-92.

24. Seo SW, Im K, Lee JM, et al. Cortical thickness in single- versus multiple-domain amnestic mild cognitive impairment. Neuroimage. 2007;36:289-97.

25. Hilal S, Ikram MK, Saini M, et al. Prevalence of cognitive impairment in Chinese: Epidemiology of Dementia in Singapore study. J Neurol Neurosurg Psychiatry. 2013;84:686-92.

26. Rosman M, Zheng Y, Wong W, et al. Singapore Malay Eye Study: rationale and methodology of 6-year follow-up study (SiMES-2). Clinical & experimental ophthalmology. 2012;40:557-68.

27. Fjell AM, Westlye LT, Amlien I, et al. High consistency of regional cortical thinning in aging across multiple samples. Cerebral cortex. 2009;19:2001-12.

28. Salat DH, Buckner RL, Snyder AZ, et al. Thinning of the cerebral cortex in aging. Cerebral cortex. 2004;14:721-30.

29. Ziegler DA, Piguet O, Salat DH, Prince K, Connally E, Corkin S. Cognition in healthy aging is related to regional white matter integrity, but not cortical thickness. Neurobiology of aging. 2010;31:1912-26.

30. Cowell PE, Sluming VA, Wilkinson ID, et al. Effects of sex and age on regional prefrontal brain volume in two human cohorts. The European journal of neuroscience. 2007;25:307-18.

31. van Velsen EF, Vernooij MW, Vrooman HA, et al. Brain cortical thickness in the general elderly population: the Rotterdam Scan Study. Neuroscience letters. 2013;550:189-94.

32. Venketasubramanian N, Sahadevan S, Kua EH, Chen CP, Ng TP. Interethnic differences in dementia epidemiology: global and Asia-Pacific perspectives. Dement Geriatr Cogn Disord. 2010;30:492-98.

33. Wisse LE, de Bresser J, Geerlings MI, et al. Global brain atrophy but not hippocampal atrophy is related to type 2 diabetes. Journal of the neurological sciences. 2014;344:32-6.

34. Schmidt R, Launer LJ, Nilsson LG, et al. Magnetic resonance imaging of the brain in diabetes: the Cardiovascular Determinants of Dementia (CASCADE) Study. Diabetes. 2004;53:687-92.

35. Lunetta M, Damanti AR, Fabbri G, Lombardo M, Di Mauro M, Mughini L. Evidence by magnetic resonance imaging of cerebral alterations of atrophy type in young insulin-dependent diabetic patients. Journal of endocrinological investigation. 1994;17:241-5.

36. Willette AA, Kapogiannis D. Does the brain shrink as the waist expands? Ageing research reviews. 2015;20C:86-97.

37. Iadecola C. The overlap between neurodegenerative and vascular factors in the pathogenesis of dementia. Acta neuropathologica. 2010;120:287-96.

38. Thong JY, Hilal S, Wang Y, et al. Association of silent lacunar infarct with brain atrophy and cognitive impairment. J Neurol Neurosurg Psychiatry. 2013;84:1219-25.

39. Jouvent E, Viswanathan A, Chabriat H. Cerebral atrophy in cerebrovascular disorders. Journal of neuroimaging : official journal of the American Society of Neuroimaging. 2010;20:213-8.

40. Lewis DA, Campbell MJ, Terry RD, Morrison JH. Laminar and regional distributions of neurofibrillary tangles and neuritic plaques in Alzheimer's disease: a quantitative study of visual and auditory cortices. The Journal of neuroscience : the official journal of the Society for Neuroscience. 1987;7:1799-1808.

41. Hof PR, Morrison JH. Quantitative analysis of a vulnerable subset of pyramidal neurons in Alzheimer's disease: II. Primary and secondary visual cortex. The Journal of comparative neurology. 1990;301:55-64.

42. Buckner RL, Snyder AZ, Shannon BJ, et al. Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2005;25:7709-17.

CHAPTER 9 – TABLES

Table 9 – 1: Baseline characteristics of study participants

	All subjects (n=572)	NCI (n=176)	CIND mild (n=171)	CIND moderate (n=197)	Dementia (n=28)	P for trend
Age, years, (SD)	70.5 (6.77)	67.2 (5.06)	70.1 (6.06)[a]	73.6 (6.58)[ab]	78.9 (5.11)[abc]	**<0.001**
Women, n (%)	313 (54.7)	74 (42)	83 (48.5)	132 (67)[ab]	24 (85.7)[ab]	**<0.001**
Race, n (%)						
Chinese	275 (48.1)	123 (69.9)	75 (43.9)	73 (37.1)	4 (14.3)	**<0.001**
Malays	297 (51.9)	53 (30)	96 (56.1)[a]	124 (62.9)[a]	24 (85.7)[ab]	
Primary education >6years, n (%)	157 (27.4)	83 (47.2)	42 (24.6)[a]	28 (14.2)[a]	0	**<0.001**
Instrumental activities of daily living, (SD)	8.1 (2.8)	7.2 (0.6)	7.5 (1.2)	8.6 (2.5)[ab]	16.5 (6.4)[abc]	**<0.001**
Hypertension, n (%)	459 (80.2)	135 (76.7)	140 (81.9)	175 (88.8)[a]	25 (89.3)	**0.001**
Diabetes, n (%)	172 (30.1)	43 (24.4)	49 (28.7)	69 (35)	12 (42.9)	**0.008**
Hyperlipidemia, n (%)	388 (67.8)	108 (61.4)	118 (69)	155 (78.7)[a]	20 (71.4)	**0.001**
Mean arterial blood pressure, mmHg, (SD)	97.89 (10.7)	100.3 (10.4)	101.1 (10.1)	99.6 (10.9)	104.9 (13.3)	0.750
Non-fasting blood glucose, mmol/l, (SD)	6.88 (2.92)	6.5 (2.6)	7.0 (3.2)	6.9 (2.7)	7.9 (4.2)	0.054
Total cholesterol, mmol/l, (SD)	5.22 (1.18)	5.0 (0.9)	5.0 (1.0)	5.1 (1.3)	5.1 (1.1)	0.777
Smoking, n (%)	76 (25.6)	54 (30.7)	57 (33.3)	52 (26.4)	2 (7.1)	0.078
Body mass index, kg/m^2, (SD)	22.2 (4.11)	19.9 (3.2)	19.9 (3.7)	19.6 (3.6)	17.7 (4.3)[ab]	**0.019**
Total intracranial volume, ml, (SD)	1074.8 (120.9)	1108.8 (106.5)	1072.6 (131.6)[a]	1054.8 (102.6)[a]	1001.3 (195.3)[ab]	**<0.001**
Presence of lacunar infarcts, n (%)	112 (19.6)	13 (7.4)	30 (17.5)	56 (28.4)[ab]	13 (46.4)[ab]	**<0.001**
WMH, ml, median (IQR)	1.91 (5.27)	1.3 (3.1)	1.6 (4.2)	2.9 (7.4)	8.5 (15.6)	**<0.001**
Cerebral microbleeds, no. (%)	201 (35.1)	49 (27.8)	62 (36.3)	78 (39.6)	12 (42.9)	**0.002**
Global cortical thickness, μm, mean (SD)	2362.0 (108.9)	2404.1 (94.3)	2366.6 (100.0)[a]	2334.1 (112.3)[a]	2266.6 (108.1)[abc]	**<0.001**

Abbreviations: NCI, no cognitive impairment; CIND, cognitive impairment no dementia; SD, Standard deviation; n, number; mmHg, millimeter of mercury; mmol/l, millimoles per liters; kg/m^2, kilogram per meter square; IQR, interquartile range; μm, micrometers; WMH, white matter hyperintensities

Superscript letters indicate representing group is significantly different from NCI (a), CIND mild (b) or CIND moderate (c) based on ANOVA ($p<0.05$)

Table 9 – 2: Multi-variable adjusted associations between potential risk factors and global cortical thickness (n=572)

	Global cortical thickness (µm)			
	Model I* Mean difference (95% CI)	**P value**	**Model II† Mean difference (95% CI)**	**P value**
Age (years), per SD decrease	**-36.7 (-45.6; -27.8)‡**	**<0.001**	**-30.9 (-40.2; -21.7)**	**<0.001**
Gender (women vs men)	**34.7 (17.4; 52.0)‡**	**<0.001**	**25.4 (2.1;48.7)**	**0.029**
Primary education > 6 years	16.0 (-4.8; 36.8)‡	0.131	5.3 (-14.9; 25.5)	0.606
Ethnicity (Malay vs Chinese)	**-63.1 (-79.0; -47.3)**	**<0.001**	**-57.4 (-74.5; -40.3)**	**<0.001**
Instrumental activities of daily living, per score increase	**-6.14 (-9.3; -2.9)**	**<0.001**	-2.43 (-5.8; 0.9)	0.161
Mean arterial blood pressure (mmHg), per SD increase	-1.3 (-9.8; 7.2)	0.758	4.8 (-3.6; 13.2)	0.242
Non-fasting blood glucose (mmol/l), per SD increase	**-14.3 (-22.8; -5.8)**	**0.001**	**-8.1 (-16.4; 0.3)**	**0.059**
Total Cholesterol (mmol/l), per SD increase	3.6 (-5.1; 12.2)	0.418	4.4 (-4.1; 12.9)	0.322
Smoking (Yes vs No)	-1.6 (-24.3; 21.0)	0.888	-1.8 (-23.7; 20.1)	0.860
Body mass index (kg/m^2), per SD increase	**-15.9 (-24.4; -7.6)**	**<0.001**	**-9.5 (-18.1; -0.8)**	**0.022**
Intracranial volume (ml), per SD increase	-2.5 (-12.3; 7.3)	0.619	-2.1 (-12.0; 7.8)	0.612
Presence of lacunar infarcts	**-41.6 (-63.1; -20.1)**	**<0.001**	**-25.8 (-48.6; -3.1)**	**0.034**
WMH (ml, Log-transformed) per SD increase	**-32.3 (-52.7; -11.9)**	**0.002**	-16.5 (-38.7; 5.8)	0.169
Per cerebral microbleed increase	**-0.8 (-1.6; 0.0)**	**0.051**	-0.5 (-1.2; 0.3)	0.300

Abbreviations: µm, micrometer; CI, confidence interval; SD, standard deviation; Log, log transformed; mmHg, millimeter of mercury; mmol/l, millimoles per liters; kg/m^2, kilogram per meter square; ml, milliliters; WMH, white matter hyperintensities

* Model I adjusted for age, gender and education

‡ In Model I, the effect sizes for these three variables were from a basic model containing only age, gender and education.

† Model II fully adjusted for age, gender, education, race, non-fasting blood glucose, blood cholesterol, mean arterial blood pressure, BMI, smoking, presence of lacunes, white matter hyperintensities volume, number of cerebral microbleeds, intracranial volume and independent activities of daily living

Table 9 – 3: Multivariable-adjusted odds ratios for clinical outcomes and mean differences in global cognitive functioning per standard deviationdecrease in global and lobe-specific cortical thickness

	CIND mild (n=171)	CIND moderate (n=197)	CIND moderate/dementia (n=225)	Composite Z scores
Per standard deviation decrease	OR (95%CI)*	OR (95%CI)*	OR (95%CI)*	Mean difference (95%CI)*
Mean global thickness	1.19 (0.88-1.61)	**1.69 (1.18-2.43)**	**1.70 (1.19-2.44)**	**-0.094 (-0.159; -0.030)**
	p=0.252	**p=0.004**	**p=0.004**	**p=0.004**
Lobe-specific cortical thickness:				
Frontal lobe	1.14 (0.87-1.50)	1.26 (0.92-1.73)	1.26 (0.92-1.72)	-0.027 (-0.086; 0.032)
	p=0.332	p=0.153	p=0.157	p=0.364
Parietal lobe	1.12 (0.84-1.51)	**1.51 (1.07-2.13)**	**1.51 (1.07-2.13)**	**-0.083 (-0.146; -0.020)**
	p=0.444	**p=0.020**	**p=0.020**	**p=0.010**
Occipital lobe	1.27 (0.94-1.72)	**1.68 (1.21-2.33)†**	**1.68 (1.21-2.33)†**	**-0.118 (-0.181; -0.054)†**
	p=0.122	**p=0.002**	**p=0.002**	**p=<0.001**
Temporal lobe	1.08 (0.79-1.47)	**1.68 (1.14-2.47)**	**1.70 (1.16-2.50)†**	**-0.135 (-0.203; -0.067)†**
	p=0.640	**p=0.009**	**p=0.007**	**p=<0.001**
Insula	0.89 (0.68-1.17)	1.31 (0.95-1.79)	1.31 (0.96-1.79)	-0.031 (-0.092; 0.029)
	p=0.425	p=0.096	p=0.092	p=0.313
Limbic lobe	1.16 (0.86-1.55)	**1.49 (1.06-2.11)**	**1.51 (1.07-2.13)**	**-0.080 (-0.143; -0.017)**
	p=0.326	**p=0.021**	**p=0.018**	**p=0.013**

Abbreviations: CIND, cognitive impairment no dementia; OR, odds ratios; CI, confidence interval

*Fully adjusted models (age, gender, education, race, non-fasting blood glucose, blood cholesterol, mean arterial blood pressure, BMI, smoking, presence of lacunes, white matter hyperintensities volume, number of cerebral microbleeds and intracranial volume)

† Statistically significant after Bonferroni correction (0.05/6~0.008)

Table 9 – 4: Multivariable-adjusted mean differences in composite and domain-specific cognitive function per standard deviation decrease in global and lobe-specific cortical thicknesses

SD decrease in cortical thickness	**Executive function Mean difference (95%CI)***	**Attention Mean difference (95%CI)***	**Language Mean difference (95%CI)***	**Visuomotor speed Mean difference (95%CI)***	**Visuoconstruction Mean difference (95%CI)***	**Visual memory Mean difference (95%CI)***	**Verbal memory Mean difference (95%CI)***
Global	**-0.129 (-0.207; -0.051)† p=0.001**	-0.065 (-0.134; 0.004) p=0.065	-0.059 (-0.137; 0.019) p=0.138	-0.060 (-0.126; 0.006) p=0.076	**-0.099 (-0.172; -0.027)† p=0.007**	**-0.111 (-0.183; -0.039)† p=0.003**	-0.058 (-0.135; 0.019) p=0.141
Lobes							
Frontal	-0.057 (-0.128; 0.015) p=0.120	-0.001 (-0.064; 0.062) p=0.979	-0.016 (-0.087; 0.055) p=0.657	-0.013 (-0.074; 0.047) p=0.662	-0.053 (-0.119; 0.013) p=0.114	-0.038 (-0.104; 0.028) p=0.264	0.008 (-0.062; 0.078) p=0.821
Parietal	**-0.142 (-0.218; -0.066)‡ p=<0.001**	-0.057 (-0.125; 0.011) p=0.098	-0.022 (-0.098; 0.054) p=0.573	-0.042 (-0.107; 0.023) p=0.202	**-0.078 (-0.149; -0.007) p=0.032**	**-0.098 (-0.168; -0.027) p=0.007**	-0.068 (-0.143; 0.007) p=0.077
Occipital	**-0.138 (-0.215; -0.061)‡ p=<0.001**	**-0.102 (-0.170; -0.034) p=0.003**	**-0.085 (-0.161; -0.008) p=0.030**	**-0.077 (-0.142; -0.011) p=0.022**	**-0.110 (-0.182; -0.039) p=0.003**	**-0.115 (-0.186; -0.043) p=0.002**	**-0.099 (-0.175; -0.023) p=0.011**
Temporal	**-0.129 (-0.211; -0.046) p=0.002**	**-0.107 (-0.181; -0.034) p=0.004**	**-0.128 (-0.210; -0.046) p=0.002**	**-0.095 (-0.165; -0.025) p=0.009**	**-0.134 (-0.210; -0.057)‡ p=0.001**	**-0.155 (-0.231; -0.078)‡ p=<0.001**	**-0.081 (-0.163; -0.000) p=0.051**
Insula	-0.008 (-0.082; 0.066) p=0.829	-0.041 (-0.106; 0.024) p=0.213	-0.018 (-0.091; 0.055) p=0.632	-0.050 (-0.112; 0.013) p=0.117	-0.045 (-0.114; 0.023) p=0.192	-0.047 (-0.115; 0.021) p=0.178	0.016 (-0.057; 0.088) p=0.668
Limbic	**-0.106 (-0.183; -0.029) p=0.007**	-0.054 (-0.123; 0.014) p=0.117	-0.061 (-0.137; 0.016) p=0.121	-0.057 (-0.122; 0.008) p=0.085	**-0.070 (-0.141; 0.001) p=0.055**	**-0.108 (-0.179; -0.037) p=0.003**	-0.037 (-0.112; 0.039) p=0.344

Abbreviations: CI, confidence interval ; SD, standard deviation

* Fully adjusted models (age, gender, education, race, non-fasting blood glucose, blood cholesterol, mean arterial blood pressure, BMI, smoking, presence of lacunar infarcts, white matter hyperintensities volume, number of cerebral microbleeds and intracranial volume)

† Statistically significant after Bonferroni correction (0.05/7 ~ 0.007)

‡ Statistically significant after Bonferroni correction (0.05/(7*6) ~ 0.001)

Supplementary table 9 – 1: Comparison of baseline characteristics of included and excluded subjects

	Included (n=572)	**Excluded (n=525)**	**P value***
Age (years)	70.5 (6.77)	71.9 (6.81)	**0.001**
Women, n (%)	313 (54.7)	303 (57.7)	0.318
Race, n (%)			
Chinese	275 (48.1)	337 (64.2)	**<0.001**
Malays	297 (51.9)	188 (35.8)	
Primary education > 6 years, n (%)	157 (27.4)	110 (21)	**0.012**
Hypertension, n (%)	459 (80.2)	450 (85.7)	**0.016**
Diabetes, n (%)	172 (30.1)	156 (29.7)	0.898
Hyperlipidemia, n (%)	388 (67.8)	311 (59.2)	**0.003**
Mean arterial blood pressure, mmHg, (SD)	97.89 (10.7)	98.88 (11.7)	0.144
Random blood glucose, mmol/l, (SD)	6.88 (2.92)	6.93 (3.05)	0.805
Total cholesterol, mmol/l, (SD)	5.22 (1.18)	5.20 (1.16)	0.832
Smoking, n (%)	76 (25.3)	40 (26.9)	0.570
Body mass index, kg/m^2, (SD)	22.2 (4.11)	22.5 (4.21)	0.342

Abbreviation: SD, Standard deviation; n, number; mmHg, millimeter of mercury; mmol/l, millimoles per liters; kg/m^2, kilogram per meter square

* p-value < 0.05 was considered statistically significant

Supplementary table 9 – 2: Multi-variable adjusted associations between potential risk factors and lobe-specific cortical thicknesses

	Frontal lobe (µm) Beta (95%CI)*	Parietal lobe (µm) Beta (95%CI)*	Occipital lobe (µm) Beta (95%CI)*	Temporal lobe (µm) Beta (95%CI)*	Insula (µm) Beta (95%CI)*	Limbic lobe (µm) Beta (95%CI)*
Age (years)	-0.39 (-14.2; 6.36) p=0.453	**-36.7 (-49.2; -24.3)† p=<0.001**	**-48.4 (-60.6; -36.3)† p=<0.001**	**-41.9 (-53.7; -30.3)† p=<0.001**	-12.4 (-26.6; 1.68) p=0.084	**-14.6 (-24.5; -4.67)† p=0.004**
Gender (Women vs men)	9.82 (-15.1; 34.7) p=0.438	**51.6 (21.6; 81.7)† p=0.001**	14.2 (-15.1; 43.5) p=0.341	**39.1 (10.9; 67.3)† p=0.007**	14.3 (-19.8; 48.4) p=0.411	**29.0 (5.01; 53.1) p=0.018**
Race (Malay vs Chinese)	**-45.0 (-63.6; -26.4)† p=<0.001**	**-59.2 (-81.6; -36.7)† p=<0.001**	**-39.3 (-61.2; -17.4)† p=<0.001**	**-65.7 (-86.7; -44.7)† p=<0.001**	**-75.6 (-101.2; -50.1)† p=<0.001**	**-57.8 (-75.8; -39.9)† p=<0.001**
Primary education ≥ 6 years	5.42 (-16.1; 26.9) p=0.621	3.29 (-22.7; 29.3) p=0.803	4.86 (-20.5; 30.2) p=0.706	9.08 (-15.3; 33.4) p=0.464	20.2 (-9.30; 49.7) p=0.179	3.72 (-17.0; 24.5) p=0.725
IADL, per score increase	-1.75 (-5.38; 1.88) p=0.343	-3.44 (-7.82; 0.93) p=0.123	-1.42 (-5.69; 2.85) p=0.513	**-5.98 (-10.1; -1.89)† p=0.004**	-1.89 (-6.87; 3.08) p=0.455	-2.90 (-6.40; 0.60) p=0.104
MABP, per SD increase	0.87 (-8.11; 9.85) p=0.849	6.79 (-4.05; 17.6) p=0.219	7.12 (-3.46; 17.7) p=0.187	**11.6 (1.39; 21.7) p=0.026**	7.19 (-5.14; 19.5) p=0.253	1.09 (-7.59; 9.76) p=0.806
NFBG, per SD increase	**-8.89 (-17.8; 0.05) p=0.051**	-6.22 (-17.0; 4.57) p=0.258	**-12.8 (-23.3; -2.26) p=0.017**	-9.05 (-19.1; 1.04) p=0.079	**-12.5 (-24.8; -0.28) p=0.046**	**-9.34 (-17.9; -0.71) p=0.034**
Cholesterol, per SD increase	-1.23 (-10.3; 7.84) p=0.789	4.95 (-6.01; 15.9) p=0.376	**12.4 (1.73; 23.1) p=0.023**	-0.41 (-10.7; 9.85) p=0.937	0.30 (-12.2; 12.8) p=0.962	3.19 (-5.57; 11.9) p=0.474
Smoking (Yes vs No)	-4.61 (-27.9; 18.7) p=0.698	5.39 (-22.7; 33.5) p=0.707	-12.7 (-40.2; 14.8) p=0.364	-2.59 (-28.9; 23.8) p=0.847	-4.92 (-36.9; 27.1) p=0.763	-2.26 (-24.8; 20.3) p=0.844
BMI, per SD increase	**-14.7 (-23.9; -5.45)† p=0.002**	-8.26 (-19.4; 2.88) p=0.146	**-14.1 (-24.9; -3.17) p=0.011**	-8.79 (-19.2; 1.64) p=0.098	-11.4 (-24.1; 1.26) p=0.078	**-8.91 (-17.8; 0.01) p=0.050**
TIV, (ml), per SD increase	-7.34 (-17.9; 3.27) p=0.174	-8.95 (-21.7; 3.85) p=0.170	2.89 (-9.60; 15.4) p=0.650	4.76 (-7.22; 16.7) p=0.780	-10.7 (-25.3; 3.83) p=0.148	**-11.7 (-21.9; -1.45) p=0.025**
Presence of lacunar infarcts	-11.7 (-36.0; 12.4) p=0.340	**-34.7 (-64.1; -5.54) p=0.020**	**-31.7 (-60.2; -3.13) p=0.030**	**-31.3 (-58.6; -3.89) p=0.025**	-23.7 (-56.9; 9.56) p=0.162	-18.1 (-41.5; 5.29) p=0.129
WMH (ml, Log) per SD increase	-18.4 (-42.1; 5.33) p=0.128	-1.88 (-30.5; 26.7) p=0.897	-7.44 (-35.4; 20.5) p=0.601	**-38.9 (-65.7; -12.0)† p=0.005**	**-42.7 (-75.3; -10.1) p=0.010**	**-24.9 (-47.8; -1.95) p=0.033**
Per CMB increase	-0.36 (-1.20; 0.48) p=0.399	-0.09 (-1.11; 0.92) p=0.854	-0.21 (-1.19; 0.78) p=0.679	-0.46 (-1.41; 0.49) p=0.340	**-1.67 (-2.83; -0.52)† p=0.005**	-0.46 (-1.27; 0.35) p=0.267

Abbreviations: µm, micrometer; beta, mean difference; CI, confidence interval; mmHg, millimeter of mercury; SD, standard deviation; mmol/l, millimoles per liters; kg/m^2, kilogram per meter square; ml, milliliters; Log, log transformed; no., number; BMI, body mass index; IADL, independent activities of daily living; MABP, mean arterial blood pressure; TIV, total intracranial volume

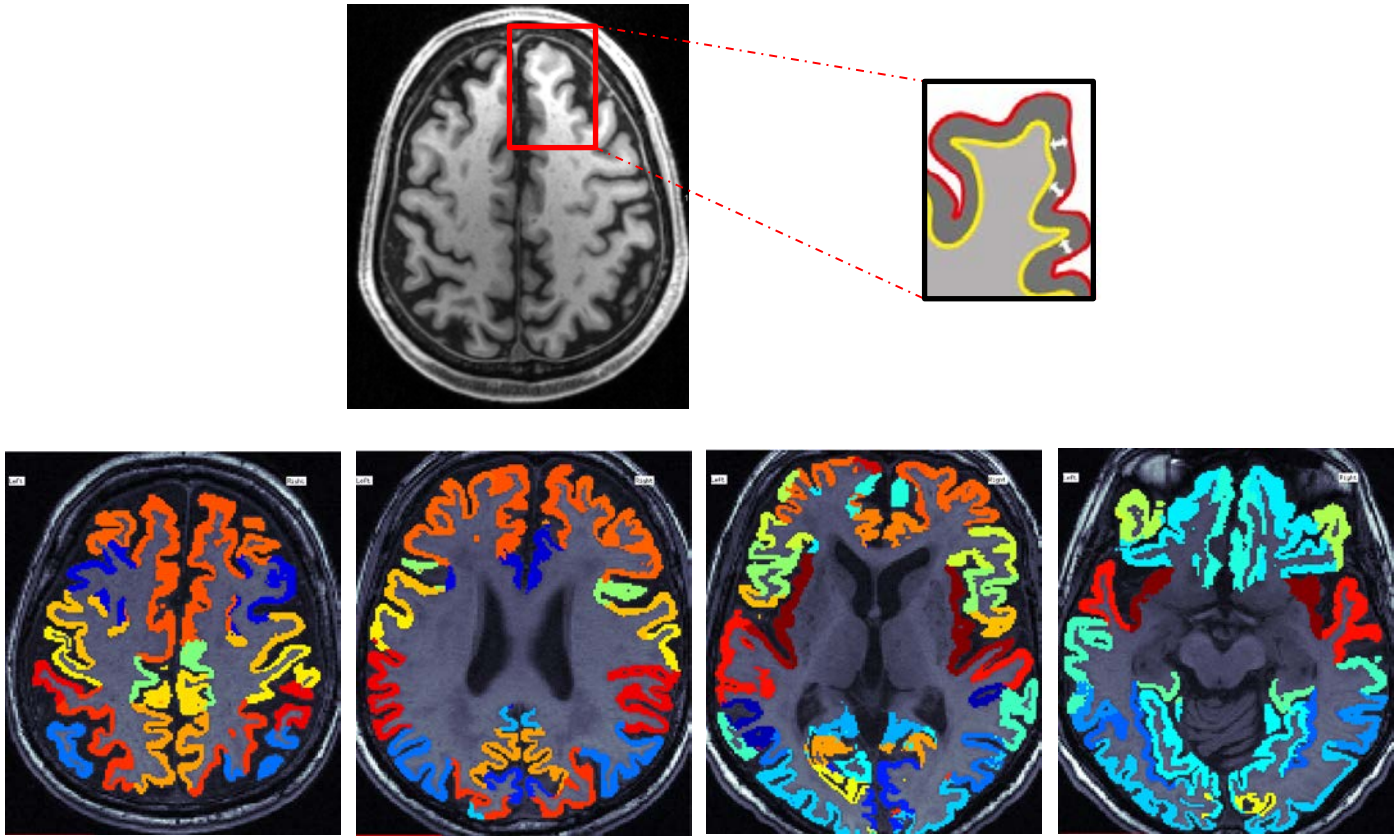
* Fully adjusted models (age, gender, education, race, non-fasting blood glucose, blood cholesterol, mean arterial blood pressure, BMI, smoking, presence of lacunar infarcts, white matter hyperintensities volume, number of cerebral microbleeds, intracranial volume and IADL)

† Statistically significant after Bonferroni correction (0.05/6~0.008)

CHAPTER 9 – FIGURES

Figure 9 – 1: Segmentation of cortical thickness through model based automated software

Cortical thickness is calculated on T1-weighted images at each vertex by taking the shortest distance between white matter/gray matter boundary and pial surface. Whole brain (global) and regional (lobar) cortical thickness are calculated using the parcellation guide on gyral and sulcal structures of cerebral cortex.



CHAPTER 10:

Subcortical Structure Volume in Cognitive Impairment and Dementia

1. INTRODUCTION

Loss of neuronal cell bodies and their connections - referred to as atrophy - is a hallmark of dementia, in particular Alzheimer's disease (AD). AD is characterized by neurodegeneration of the cortex and subcortical structures including hippocampus. These changes are not only present in clinically manifest stages of dementia, but are already present in preclinical-stages [mild cognitive impairment (MCI)/cognitive impairment no dementia (CIND)].[1,2] Several postmortem and in-vivo studies have shown that such atrophy may even occur during normal aging.[3,4] It has been reported that pathological changes (amyloid, tau or iron deposition) in these structures are related to cognitive dysfunction in a wide range of neurological and psychiatric disorders.[5] Furthermore, several histopathological studies have shown smaller volumes of thalamus, putamen, pallidum and caudate nuclei in patients with AD.[6,7] Magnetic Resonance Imaging (MRI) acquisition and analyses tools enable accurate measurement of subcortical structural volumes in-vivo, including the accumbens, amygdala, caudate, pallidum, putamen, thalamus, hippocampus and brainstem.[8] Previous studies using these techniques have mainly focused on AD patients,[6,7,9,10] whereas the preclinical-stages of dementia have been less well studied.[11] Furthermore, reduction of subcortical structural volumes in vascular compared to non-vascular subtypes of cognitive impairment remains unclear.

In view of the paucity of epidemiological data on subcortical volume in cognitive impairment, we first examined the risk factors of subcortical structure volumes in a non-demented population from Epidemiology of Dementia in Singapore study, and secondly their association with cognitive impairment and dementia using data from a case-control study in a memory clinic setting.

2. MATERIALS AND METHODS

2.1 Study Population

For the current analyses, subjects were drawn from two ongoing studies in Singapore. The first is the Epidemiology of Dementia in Singapore (EDIS) study which draws participants from the Singapore Epidemiology of Eye Disease (SEED) study.[12,13] In the first phase of the EDIS study, participants aged ≥60 years (n=2,666) were screened using the Abbreviated Mental Test and a self-report of progressive forgetfulness. Screen-positive subjects (n=1,097) were invited to take part in the second phase of this study, which included an extensive neuropsychological test battery and brain MRI. Of these 1,097 participants, 623 agreed to participate in phase II and hence were included in the present study. The details of the study methodology has been further described in **Chapter 3**.[12]

The second is a memory clinic-based study, which employs a case-control design. Cases (CIND and dementia) were recruited from two study sites in Singapore (i.e. memory clinics from National University Hospital and Saint Luke's Hospital). Controls were recruited from both memory clinics and the community (with a similar catchment area as cases). Details of this study have been described previously in **Chapter 3**.[14]All subjects underwent physical, clinical and neuropsychological assessments and neuroimaging at the National University of Singapore.

Ethics approval for both studies was obtained from the Singapore Eye Research Institute, and National-Healthcare Group Domain-Specific Review Board. The study is conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained in the preferred language of the participants by bilingual study coordinators prior to their recruitment into the study.

2.2 Demographic and Cardiovascular Risk Factor Assessment

Detailed assessments for demographics and cardiovascular risk factors have been described in **Chapter 3**.

2.3 Neuroimaging

Subcortical structure was segmented through an automated based model approach (**Figure 10-1**). Other quantitative MRI data (intracranial volume and white matter hyperintencities) together with visual gradings (lacunar infarcts and cerebral microbleeds) was obtained by automatic segmentation as mentioned in **Chapter 3**.

2.4 Cognitive Assessment

An extensive neuropsychological battery, which has been previously validated in Singaporean elderly, was administered to assess cognitive function.[14] Details on subtests for testing cognitive function and calculation of z scores in both EDIS and case control study have been described in **Chapter 3**.

Additionally, in the case-control study, various diagnostic groups were defined using the same criteria as described extensively in **Chapter 8**.

2.5 Statistical Analysis

In order to examine the differences between cases and controls, chi square test were used for categorical variables and t test for continuous variables. For skewed distributed variable (WMH), Mann-Whitney U test was utilized. All continuous variables (age, total intracranial volume and subcortical structures volumes) were standardized (by dividing each variable by its SD). For each continuous variable, mean differences in subcortical structures volumes were expressed as per SD increase/decrease in that variable. In the EDIS Study, association between risk factors and volumes of subcortical structures was explored using multiple linear regression models adjusting initially for age and gender. Subsequently, in the fully adjusted model, all potential risk factors were included in the same model to determine the independent effect of each factor with volumes of subcortical structures. Next, we examined the association of subcortical structures volume with composite and domain specific Z-scores [mean difference with 95% confidence interval (CI)]. The effect sizes of these associations with cognition were expressed as per SD decrease in subcortical structures volumes. In view of multiple

testing performed between specific domains and subcortical structures, we used revised statistical significance level of 0.05/8*7~0.0009.

With respect to the clinical outcomes, data from the case-control study was utilized where the association of subcortical structures with CIND and dementia were constructed using logistic regression models with odds ratios (OR) and 95%CI. Lastly, in order to investigate whether reduction in subcortical structure volumes differ between vascular and non-vascular cognitive impairment, logistic regression models were used adjusting for all the possible confounders. Statistical analysis was performed using standard statistical software (Statistical Package for Social Science, SPSS V22, SPSS Inc., USA).

3. RESULTS

Assessments of subjects were performed from August 12, 2010 to August 21, 2014. Out of 623 subjects who participated in EDIS study in phase II, 36 had no MRI scans and 7 had ungradable scans. Out of the remaining 580 subjects from EDIS study, 30 were diagnosed with dementia and hence were excluded, leaving 550 subjects for analysis. In the case-control study, there were initially 410 subjects of whom 4 had no MRI scans and 28 had ungradable scans. Of the remaining 378 subjects, there were 90 controls and 288 cases [154 (40.7%) CIND and 134 (35.4%) dementia]. When subjects were classified based on VCI criteria, there were 86 (22.8%) VCI and 202 (53.4%) non-VCI cases.

Table 10-1 presents baseline characteristics of the subjects in the two studies. **Table 10-2** shows the association of determinants with volumes of subcortical structures among EDIS participants. In multivariate adjusted models, increasing age was associated with smaller volumes of all subcortical structures. Women had significantly smaller amygdala, pallidum, putamen and brainstem volumes compared to men, whereas Malays had smaller amygdala, thalamus and hippocampus volumes compared to Chinese participants. Among cardiovascular risk factors, diabetes, presence of lacunar infarcts and WMH were

significantly associated with several subcortical structures. Lastly, increasing total intracranial volume was significantly associated with larger volumes of all the subcortical structures.

With respect to cognitive function, analysis of the EDIS sample showed that smaller accumbens [mean change in Z-score per SD decrease in volume: -0.08 (95%CI: -0.16; -0.01)], amygdala [mean change in Z-score per SD decrease: -0.13 (95%CI: -0.21; -0.05)], caudate [mean change in Z-score per SD decrease: -0.07 (95%CI: -0.15;-0.00)], thalamus [mean change in Z-score per SD decrease: -0.08 (95%CI: -0.16; -0.01)], and brainstem [mean change in Z-score per SD decrease: -0.09 (95%CI: -0.17; -0.03)] were significantly associated with lower composite Z-scores (**Table 10-3**). After Bonferroni correction in domain-specific analysis, only smaller amygdala volume remains statistically significantly associated with the language domain.

In the case-control study, a trend for increasing subcortical atrophy was observed from CIND to dementia (**Table 10-4**). In multivariate adjusted models, smaller accumbens, caudate, putamen and hippocampus volumes were associated with CIND whereas smaller volumes of all subcortical structures except for pallidum were significantly associated with dementia. On further analysis comparing VCI and non-VCI groups, a specific pattern was observed in the subcortical structures with smaller volumes of caudate and pallidum associated with VCI whereas smaller amygdala volume was only associated with the non-VCI group (**Table 10-4**). Smaller accumbens, putamen and hippocampus volumes were equally related to both VCI and non-VCI groups.

4. DISCUSSION

Findings from this study suggest that important risk factors for smaller subcortical structures were age, female sex, ethnicity, diabetes, presence of lacunar infarcts on MRI, and WMH volume. Moreover, reduction of subcortical grey matter volume is not only

observed in dementia, but also in the preclinical stages of cognitive impairment. Furthermore, besides VCI, subcortical structures were also related to non-VCI.

Several studies have shown age-related volume changes in subcortical structures.[15] This age effect has been variable among studies with some reporting the greatest decrease in putamen, amygdala and accumbens in elderly subjects,[16] whereas other studies report or small reductions in caudate, putamen, thalamus and brainstem volumes.[16,17] Overall our findings are consistent with the majority of these studies, namely subcortical volumes of deep gray matter nuclei decreases with increasing age.

Our study also shows that women had smaller volumes of amygdala, pallidum, putamen and brainstem compared to men which are consistent with previous studies.[5] This effect remains even after adjustments for age and total intracranial volume. Smaller subcortical structure volume in women has been attributed to the loss of protective effects of estrogen after menopause. In terms of ethnic differences, Malays had smaller amygdala, thalamus and hippocampus volumes compared to Chinese. It has been reported previously that Malays have a higher prevalence of ApoE4 carriers.[18] Moreover we also postulate that Malays may have a lower cognitive reserve based on lower occupational attainment and lower education which may explain the underlying differences.

With respect to cardiovascular risk factors, we found that the presence of diabetes was associated with volume reduction in putamen,[19] thalamus[20] and hippocampus[21,22] which is in line with the previous literature. Moreover, we also report an independent association of diabetes with smaller pallidum, and brainstem volumes. The underlying mechanism may be the link between diabetic vascular disease, impaired circulation and silent ischemic damage.[23] Moreover, it has also been suggested that hyperinsulinemia may affect brain amyloid clearance, leading to its deposition and hence its neurotoxicity.[24] Finally, vascular pathology as reflected by cerebral small vessel diseases (lacunar infarcts and WMH) was independently associated with volume reduction in several subcortical

structures. Subcortical lesions such as the lacunes and WMH may induce focal atrophy in the surrounding gray matter nuclei and interact with neurodegenerative process through disruption of white matter tracts.[25, 26] However, the positive association of lacunes and WMH with caudate and separately of WMH with pallidum volumes in our study was unexpected. There could be two possible explanation to these findings; firstly, it has been reported that an increased neuronal hypertrophy and/or inflammation precedes clinically manifest AD[27] giving rise to increase volumes of caudate and pallidum. Secondly, periventricular WMH are difficult to distinguish from gray matter especially the caudate nucleus on T1 sequences and hence may lead to an artificially increased volume of basal ganglia nuclei.[16]

With respect to cognition, we found that the reduced volumes of all subcortical structures except pallidum and putamen were significantly associated with cognitive impairment reflecting that the involutional process in deep gray matter structures takes place early in the process of dementia. This may be explained by the fact that the amygdala, accumbens and thalamus are directly connected to hippocampus and reductions in these structures have been reported in early stages of AD.[9] Pathophysiologically, amygdala, accumbens and hippocampus are particularly vulnerable to amyloid and tau deposition in the early stages of AD which then extends further to involve the thalamic nuclei and caudate. [28, 29] Moreover, it has been reported that the anterior thalamic nuclei are directly connected to the medial limbic portion of the temporal lobe and cingulate gyrus,[6] which controls language, learning and memory and hence this was reflected by the impairment in language and visuomotor speed domains. The association of smaller brainstem volume with cognitive dysfunction is inconsistent with previous findings where brainstem volume did not significantly change during the aging process.[17, 30] This may be due to the low resolution MRI (1.5T) scan used in these studies which makes the accurate

segmentation of the whole brainstem difficult to achieve. However, deposition of neurofibrillary tangles and neurotransmitter alterations in brainstem nuclei has been shown previously in AD,[31, 32] and thus supports our findings on the positive association between brainstem volume reduction and cognition.

The association of basal ganglia nuclei (caudate and pallidum) volume with VCI further supports our previous findings that these nuclei are the common site for subcortical lesions (lacunes and WMH), which in turn induces volumes reduction secondary to ischemic changes.[26] This is also in agreement with a prior study where it was shown that subjects with both mild and severe vascular cognitive impairment have similar pattern of shape abnormalities in lentiform and hippocampus.[33] In contrast, a smaller amygdala was only associated with non-VCI consistent with previous literature where amygdalar atrophy is considered an early marker for AD type neuropathology.[21] The preservation or lack of association of the similar nuclei (caudate and pallidum) in non-VCI cases is congruent with previous report where it has been suggested that such structures are only affected in the late stages of involutional changes.[9] The association of accumbens, putamen and hippocampal atrophy with both VCI and non-VCI group suggests that these structures are equally affected in both vascular and neurodegenerative type cognitive impairment.

Both samples included in this study had their limitations: first, in the EDIS study 43.2% of the screened positive subjects did not participate in the second phase of the study. These subjects were relatively older, less educated and more likely to have hypertension and hyperlipidemia (data not shown). Furthermore, those excluded subjects might also be more cognitively impaired. This exclusion may have led to an underestimation of the effect sizes. However, despite this non-participation we still found significant associations. Due to the relatively small number of cases with dementia (n=30) in the EDIS study, we could only focus on preclinical stages of dementia in this sample.

Second, with respect to the case-control study, controls were relatively younger compared to the cases, and had a lower burden of vascular risk factors, which could have resulted in sampling bias and residual confounding. Third, another limitation applicable to both samples was the cross-sectional design, which did not allow us to assess the temporal relationship between the subcortical volume reduction and cognitive impairment. Nevertheless, despite the various limitations of these two complimentary studies, both provided consistent associations in the same direction on the association between subcortical structures and cognition. Strengths of the study include: subjects for EDIS were selected from a population-based study, extensive neuropsychological tests were used to diagnose cognitive impairment and dementia, and automated and standardized image processing was used to quantify subcortical structure volumes.

5. CONCLUSION

Smaller subcortical grey matter volume is not only observed in dementia, but also in the preclinical stages of cognitive impairment. Furthermore, besides VCI, subcortical structures were also related to non-VCI. Further prospective studies are needed to unravel the role of subcortical volume reduction as a biomarker in predicting cognitive decline.

CHAPTER 10 – REFERENCES

1. Frisoni GB, Testa C, Zorzan A, Sabattoli F, Beltramello A, Soininen H, Laakso MP. Detection of grey matter loss in mild Alzheimer's disease with voxel based morphometry. J Neurol Neurosurg Psychiatry. 2002;73,657-64.
2. Karas G, Sluimer J, Goekoop R et al. Amnestic mild cognitive impairment: structural MR imaging findings predictive of conversion to Alzheimer disease. AJNR Am J Neuroradiol. 2008;29,944-9.
3. Lemaitre H, Goldman AL, Sambataro F, Verchinski BA, Meyer-Lindenberg A, Weinberger DR, Mattay VS. Normal age-related brain morphometric changes: nonuniformity across cortical thickness, surface area and gray matter volume? Neurobiol Aging. 2012;33,611-9.
4. Long X, Liao W, Jiang C, Liang D, Qiu B, Zhang L. Healthy aging: an automatic analysis of global and regional morphological alterations of human brain. Acad Radiol. 2012;19,785-93.
5. Li W, van Tol MJ, Li M, et al. Regional specificity of sex effects on subcortical volumes across the lifespan in healthy aging. Hum Brain Mapp. 2014;35,238-47.
6. Stepan-Buksakowska I, Szabo N, Horinek D, et al. Cortical and subcortical atrophy in Alzheimer disease: parallel atrophy of thalamus and hippocampus. Alzheimer Dis Assoc Disord. 2014;28,65-72.
7. de Jong LW, van der Hiele K, Veer IM, et al. Strongly reduced volumes of putamen and thalamus in Alzheimer's disease: an MRI study. Brain, 2008; 131,3277-85.
8. Fischl B, Salat DH, Busa E, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. Neuron, 2002;33, 341-55.

9. Roh JH, Qiu A, Seo SW, et al. Volume reduction in subcortical regions according to severity of Alzheimer's disease. J Neurol. 2011;258,1013-20.

10. Cho H, Seo SW, Kim JH, et al. Changes in subcortical structures in early- versus late-onset Alzheimer's disease. Neurobiol Aging. 2013;34,1740-47.

11. Roberts RO, Knopman DS, Przybelski SA, et al. Association of type 2 diabetes with brain atrophy and cognitive impairment. Neurology. 2014;82,1132-41.

12. Hilal S, Ikram MK, Saini M, et al. Prevalence of cognitive impairment in Chinese: Epidemiology of Dementia in Singapore study. J Neurol Neurosurg Psychiatry. 2013;84,686-92.

13. Rosman M, Zheng Y, Wong W, et al. Singapore Malay Eye Study: rationale and methodology of 6-year follow-up study (SiMES-2). Clin Experiment Ophthalmol. 2012;40,557-68.

14. Hilal S, Chai YL, Ikram MK, et al. Markers of cardiac dysfunction in cognitive impairment and dementia. Medicine. 2015;94,e297.

15. Walhovd KB, Fjell AM, Reinvang I, et al. Effects of age on volumes of cortex, white matter and subcortical structures. Neurobiol Aging. 2005;26,1261-70.

16. Goodro M, Sameti M, Patenaude B, Fein G. Age effect on subcortical structures in healthy adults. Psychiatry Res. 2012;203,38-45.

17. Luft AR, Skalej M, Schulz JB, et al. Patterns of age-related shrinkage in cerebellum and brainstem observed in vivo using three-dimensional MRI volumetry. Cereb Cortex. 1999;9,712-21.

18. Venketasubramanian N, Sahadevan S, Kua EH, Chen CP, Ng TP. Interethnic differences in dementia epidemiology: global and Asia-Pacific perspectives. Dement Geriatr Cogn Disord. 2010;30,492-8.

19. Falvey CM, Rosano C, Simonsick EM, Harris T, Strotmeyer ES, Satterfield S, Yaffe K. Macro- and microstructural magnetic resonance imaging indices

associated with diabetes among community-dwelling older adults. Diabetes Care. 2013;36,677-82.

20. Moulton CD, Costafreda SG, Horton P, Ismail K, Fu CH. Meta-analyses of structural regional cerebral effects in type 1 and type 2 diabetes. Brain Imaging Behav. 2015 [epub ahead of print].

21. den Heijer T, Vermeer SE, van Dijk EJ, Prins ND, Koudstaal PJ, Hofman A, Breteler MM. Type 2 diabetes and atrophy of medial temporal lobe structures on brain MRI. Diabetologia. 2003;46,1604-10.

22. Cherbuin N, Sachdev P, Anstey KJ. Higher normal fasting plasma glucose is associated with hippocampal atrophy: The PATH Study. Neurology. 2012;79, 1019-26.

23. Barbagallo M, Dominguez LJ. Type 2 diabetes mellitus and Alzheimer's disease. World J Diabetes. 2014;5,889-93.

24. Cukierman-Yaffe T, Gerstein HC, Williamson JD, et al. Relationship between baseline glycemic control and cognitive function in individuals with type 2 diabetes and other cardiovascular risk factors: the action to control cardiovascular risk in diabetes-memory in diabetes (ACCORD-MIND) trial. Diabetes Care. 2009;32,221-6.

25. Iadecola C. The overlap between neurodegenerative and vascular factors in the pathogenesis of dementia. Acta Neuropathol. 2010;120,287-96.

26. Thong JY, Hilal S, Wang Y, et al. Association of silent lacunar infarct with brain atrophy and cognitive impairment. J Neurol Neurosurg Psychiatry. 2013;84,1219-25.

27. Fortea J, Sala-Llonch R, Bartres-Faz D, et al. Increased cortical thickness and caudate volume precede atrophy in PSEN1 mutation carriers. J Alzheimers Dis. 2010;22,909-922.

28. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. 1991;82,239-259.

29. Kawakami I, Hasegawa M, Arai T, et al. Tau accumulation in the nucleus accumbens in tangle-predominant dementia. Acta Neuropathol Commun. 2014;2, 40.

30. Mrzilkova J, Zach P, Bartos A, Tintera J, Ripova D. Volumetric analysis of the pons, cerebellum and hippocampi in patients with Alzheimer's disease. Dement Geriatr Cogn Disord. 2012;34,224-234.

31. Matthews KL, Chen CP, Esiri MM, Keene J, Minger SL, Francis PT . Noradrenergic changes, aggressive behavior, and cognition in patients with dementia. Biol Psychiatry. 2002;51,407-416.

32. Chen CP, Eastwood SL, Hope T, McDonald B, Francis PT, Esiri MM. Immunocytochemical study of the dorsal and median raphe nuclei in patients with Alzheimer's disease prospectively assessed for behavioural changes. Neuropathol Appl Neurobiol. 2000;26,347-355.

33. Thong JY, Du J, Ratnarajah N, et al. Abnormalities of cortical thickness, subcortical shapes, and white matter integrity in subcortical vascular cognitive impairment. Hum Brain Mapp. 2014;35,2320-2332.

CHAPTER 10 – TABLES

Table 10 – 1: Baseline characteristics of the subjects

	Epidemiology of dementia in Singapore (EDIS)	Hospital based study		P value*
	(n= 580)	Controls (n=90)	Cases (n=288)	
Age (years)	70.5 (6.7)	67.6 (5.6)	73.6 (8.6)	**<0.001**
Women, n (%)	317 (54.7)	50 (55.6)	155 (53.8)	0.773
Race, n (%)				
Chinese	280 (48.3)	81 (90)	227 (78.8)	0.059
Malays	300 (51.7)	2 (2.2)	35 (12.2)	
Indians	-	6 (6.7)	21 (7.3)	
Others	-	1 (1.1)	5 (0.02)	
Total years of education, mean (SD)	6.6 (36.9)	10 (4.9)	6.6 (4.9)	**<0.001**
Hypertension, n (%)	466 (80.3)	51 (56.7)	215 (74.7)	**0.001**
Diabetes, n (%)	175 (30.2)	19 (21.1)	116 (40.3)	**0.001**
Hyperlipidemia, n (%)	394 (67.9)	64 (71.1)	212 (73.6)	0.641
MRI markers				
Presence of lacunar infarcts, n (%)	112 (19.3)	17 (19.3)	88 (30.9)	**0.035**
WMH volume, median (IQR)	2.03 (5.93)	0.99 (2.71)	4.58 (13)	**<0.001**
Cerebral microbleeds, n (%)	205 (35.3)	43 (47.8)	150 (52.1)	0.386
TIV volume, mean (SD)	1075.5 (120.5)	1123.2 (115.9)	1077.7 (147.7)	0.066
Subcortical structures				
Accumbens, mm^3, mean (SD)	878.3 (183.6)	936.3 (172.1)	746.4 (198.1)	**<0.001**
Amygdala, mm^3, mean (SD)	2652.5 (460.7)	2803.3 (483.7)	2363.5 (599.1)	**<0.001**
Caudate, mm^3, mean (SD)	6591.1 (1050.6)	6761.9 (923.2)	6648.4 (1465.3)	0.489
Pallidum, mm^3, mean (SD)	3165.7 (460.7)	3228.9 (452.8)	3067.9 (575.3)	**0.016**
Putamen, mm^3, mean (SD)	9442.8 (1305.1)	9693.6 (1350.3)	8648.6 (1568.6)	**<0.001**
Thalamus, mm^3, mean (SD)	11032.6 (1130.7)	11481.2 (1293.6)	10681.7 (1378.1)	**<0.001**
Hippocampus, mm^3, mean (SD)	7031.0 (920.7)	7423.2 (877.4)	6173.6 (1278.6)	**<0.001**
Brainstem, mm^3, mean (SD)	19404.8 (2422)	19982.1 (2548.3)	18620 (2422.6)	**<0.001**

Abbreviations: SD= standard deviation, IQR= interquartile range; WMH= white matter hyperintensities; TIV= total intracranial volume, mm= millimeters

*****p value denotes differences between cases and controls; significant < 0.05.

Chi square test was used for categorical variables, T-test for continuous variables, and Mann-Whitney U test for skewed distributed variable (white matter lesions).

Table 10 – 2: Association of demographics and cardiovascular risk factors with volumes of subcortical structures in the EDIS study (n=550)

	Accumbens volume Mean difference (95%CI)*	Amygdala volume Mean difference (95%CI)*	Caudate volume Mean difference (95%CI)*	Pallidum volume Mean difference (95%CI)*	Putamen volume Mean difference (95%CI)*	Thalamus volume Mean difference (95%CI)*	Hippocampal volum Mean difference (95%CI)*	Brainstem Mean difference (95%CI)*
Age, years, per SD increase	**-0.39 (-0.47; -0.31)**	**-0.39 (-0.46; -0.32)**	**-0.11 (-0.19; -0.04)**	**-0.19 (-0.27; -0.12)**	**-0.32 (-0.40; -0.24)**	**-0.24 (-0.32; -0.17)**	**-0.44 (-0.51; -0.37)**	**-0.12 (-0.19; -0.04)**
	p=<0.001	**p=<0.001**	**p=0.004**	**p=<0.001**	**p=<0.001**	**p=<0.001**	**p=<0.001**	**p=0.004**
Gender (women vs. men)	0.03 (-0.14; 0.20)	**-0.46 (-0.62; -0.30)**	-0.13 (-0.30; 0.04)	**-0.44 (-0.60; -0.27)**	**-0.28 (-0.46; -0.10)**	-0.04 (-0.20; 0.12)	0.06 (-0.09; 0.22)	**-0.32 (-0.48; -0.15)**
	p=0.713	**p=<0.001**	p=0.124	**p=<0.001**	**p=0.002**	p=0.610	p=0.434	**p=<0.001**
Race (Malays vs. Chinese)	-0.06 (-0.21; 0.09)	**-0.18 (-0.32; -0.05)**	0.03 (-0.12; 0.18)	0.08 (-0.06; 0.22)	-0.04 (-0.19; 0.11)	**-0.24 (-0.38; -0.10)**	**-0.24 (-0.38; -0.11)**	-0.13 (-0.27; 0.02)
	p=0.415	**p=0.009**	p=0.679	p=0.252	p=0.581	**p=0.001**	**p=<0.001**	p=0.084
Hypertension	0.10 (-0.09; 0.29)	0.00 (-0.18; 0.18)	-0.13 (-0.32; 0.07)	-0.17 (-0.36; 0.02)	0.02 (-0.18; 0.22)	-0.13 (-0.32; 0.05)	-0.14 (-0.31; 0.04)	**-0.23 (-0.43; -0.04)**
	p=0.304	p=0.983	p=0.206	p=0.070	p=0.864	p=0.162	p=0.138	**p=0.018**
Diabetes	-0.15 (-0.31; 0.01)	-0.02 (-0.17; 0.13)	-0.13 (-0.29; 0.03)	**-0.16 (-0.31; -0.01)**	**-0.29 (-0.44; -0.11)**	**-0.26 (-0.41; -0.11)**	**-0.21 (-0.35; -0.06)**	**-0.36 (-0.51; -0.19)**
	p=0.059	0.768	p=0.103	**p=0.039**	**p=0.001**	**p=0.001**	**p=0.006**	**p=<0.001**
Hyperlipidemia	0.08 (-0.08; 0.25)	-0.01 (-0.16; 0.15)	-0.00 (-0.16; 0.16)	-0.05 (-0.21; 0.11)	0.03 (-0.14; 0.20)	-0.00 (-0.16; 0.15)	0.01 (-0.14; 0.16)	**-0.19 (-0.36; -0.03)**
	p=0.319	p=0.923	p=0.999	p0.544	p=0.721	p=0.960	p=0.913	**p=0.019**
Presence of lacunar infarcts	**-0.26 (-0.47; -0.06)**	-0.11 (-0.29; 0.08)	**0.23 (0.03; 0.43)**	**-0.23 (-0.42; -0.03)**	**-0.22 (-0.43; -0.01)**	**-0.23 (-0.42; -0.04)**	-0.02 (-0.21; 0.16)	**-0.22 (-0.42; -0.02)**
	p=0.011	p=0.261	**p=0.026**	**p=0.022**	**p=0.038**	**p=0.018**	p=0.817	**p=0.030**
WMH volume (ml, log), per SD increase	**-0.48 (-0.68; -0.29)**	**-0.29 (-0.47; -0.11)**	**0.56 (0.37; 0.76)**	**0.25 (0.06; 0.43)**	0.09 (-0.10; 0.29)	-0.17 (-0.35; 0.01)	**-0.19 (-0.37; -0.01)**	**-0.23 (-0.42; -0.04)**
	p=<0.001	**p=0.001**	**p=<0.001**	**p=0.009**	p=0.350	p=0.067	**p=0.034**	**p=0.017**
Cerebral microbleeds,	-0.01 (-0.02; 0.01)	0.00 (-0.01; 0.01)	0.00 (-0.01; 0.02)	-0.01 (-0.02; 0.00)	0.00 (-0.01; 0.01)	-0.00 (-0.02; 0.01)	0.00 (-0.01; 0.01)	-0.00 (-0.01; 0.01)
	p=0.374	p=0.958	P=0.643	p=0.159	p=0.942	p=0.499	p=0.832	p=0.791
TIV, ml, per SD increase	**0.16 (0.07; 0.25)**	**0.29 (0.21; 0.38)**	**0.35 (0.26; 0.44)**	**0.42 (0.34; 0.51)**	**0.25 (0.16; 0.35)**	**0.48 (0.39; 0.56)**	**0.32 (0.23; 0.39)**	**0.38 (0.29; 0.47)**
	p=0.001	**p=<0.001**	**P=<0.001**	**p=<0.001**	**p=<0.001**	**p=<0.001**	**p=<0.001**	**p=<0.001**

Abbreviations: SD= standard deviation; CI= confidence interval; WMH= white matter hyperintensities; ml= milliliters; TIV= total intracranial volume

*Multivariate adjusted linear regression model with 95% confidence interval using risk factors as determinant and each subcortical structures as outcome. The values provided in each column refer to the mean difference in volume of subcortical structure with respect to the risk factors. All models are adjusted for age, gender, race, hypertension, diabetes, hyperlipidemia, presence of lacunar infarcts, white matter hyperintensities volume, cerebral microbleeds, total intracranial volume.

Table 10 – 3: Multivariate adjusted estimated change in cognitive performance (mean difference) per standard deviation change in volumes of subcortical structures (EDIS)

Per SD decrease in volume	**Composite Z-score Mean difference (95% CI)***	**Executive function Mean difference (95%CI)***	**Attention Mean difference (95%CI)***	**Language Mean difference (95%CI)***	**Visuomotor speed Mean difference (95%CI)***	**Visuoconstruction Mean difference (95%CI)***	**Visual memory Mean difference (95%CI)***	**Verbal memory Mean difference (95%CI)***
Accumbens	**-0.08 (-0.16; -0.01)**	**-0.09 (-0.18; -0.00)**	-0.07 (-0.14; 0.01)	**-0.10 (-0.19; -0.01)**	**-0.08 (-0.16; -0.01)**	-0.05 (-0.13; 0.02)	-0.08 (-0.16; 0.01)	-0.01 (-0.09; 0.07)
	p=0.019	**p=0.047**	p=0.072	**p=0.025**	**p=0.020**	p=0.177	p=0.066	p=0.740
Amygdala	**-0.13 (-0.21; -0.05)**	-0.08 (-0.18; 0.01)	**-0.09 (-0.18; -0.01)**	**-0.19 (-0.28; -0.09)†**	-0.05 (-0.13; 0.03)	**-0.11 (-0.19; -0.02)**	**-0.13 (-0.22; -0.04)**	-0.08 (-0.17; 0.01)
	p=0.001	p=0.085	**p=0.024**	**p=<0.001**	p=0.199	**p=0.014**	**p=0.003**	p=0.072
Caudate	**-0.07 (-0.15; -0.00)**	-0.05 (-0.14; 0.04)	-0.05 (-0.13; 0.03)	-0.04 (-0.13; 0.05)	**-0.08 (-0.15; -0.01)**	-0.08 (-0.15; 0.00)	-0.08 (-0.16; 0.01)	-0.06 (-0.14; 0.03)
	p=0.047	p=0.297	p=0.200	p=0.422	**p=0.034**	p=0.062	p=0.071	p=0.187
Pallidum	-0.04 (-0.11; 0.04)	-0.04 (-0.13; 0.06)	-0.04 (-0.12; 0.04)	-0.01 (-0.10; 0.09)	-0.06 (-0.14; 0.01)	-0.05 (-0.13; 0.03)	-0.02 (-0.10; 0.07)	-0.02 (-0.10; 0.07)
	p=0.354	p=0.436	p=0.353	p=0.884	p=0.110	p=0.215	p=0.713	p=0.696
Putamen	-0.06 (-0.13; 0.01)	-0.07 (-0.16; 0.01)	-0.03 (-0.11; 0.04)	-0.05 (-0.14; 0.04)	-0.04 (-0.11; 0.03)	-0.03 (-0.11; 0.04)	-0.06 (-0.14; 0.02)	-0.05 (-0.13; 0.03)
	p=0.115	p=0.095	p=0.390	p=0.242	p=0.308	p=0.385	p=0.134	p=0.249
Thalamus	**-0.08 (-0.16; -0.01)**	-0.00 (-0.09; 0.09)	-0.07 (-0.15; 0.02)	**-0.11 (-0.21; -0.02)**	**-0.11 (-0.19; -0.04)**	-0.07 (-0.15; 0.02)	-0.06 (-0.15; 0.03)	-0.07 (-0.16; 0.02)
	p=0.037	p=0.973	p=0.107	**p=0.024**	**p=0.004**	p=0.112	p=0.192	p=0.108
Hippocampus	-0.06 (-0.14; 0.02)	-0.06 (-0.16; 0.04)	-0.03 (-0.11; 0.05)	**-0.14 (-0.23; -0.04)**	-0.03 (-0.10; 0.05)	-0.05 (-0.13; 0.04)	-0.06 (-0.15; 0.03)	-0.02 (-0.11; 0.07)
	p=0.120	p=0.226	p=0.474	**p=0.007**	p=0.510	p=0.280	p=0.215	p=0.692
Brainstem	**-0.09 (-0.17; -0.03)**	-0.04 (-0.13; 0.05)	-0.06 (-0.14; 0.02)	**-0.11 (-0.19; -0.01)**	**-0.12 (-0.19; -0.05)**	-0.07 (-0.15; 0.02)	**-0.09 (-0.17; -0.01)**	**-0.12 (-0.20; -0.04)**
	p=0.008	p=0.346	p=0.125	**p=0.024**	**p=0.001**	p=0.110	**p=0.036**	**p=0.004**

Abbreviations: SD= standard deviation; CI= confidence interval

* Multivariate adjusted linear regression model with 95% confidence interval using each subcortical structure volume as determinant and composite/domain specific Z-scores as outcome. The values provided in each column refer to the mean difference in Z-score per SD decrease in volume of subcortical structure. All models are adjusted for age, gender, race, hypertension, diabetes, hyperlipidemia, presence of lacunar infarcts, white matter hyperintensities volume, cerebral microbleeds, total intracranial volume.

†Bonferroni corrected= P<0.0009

Table 10 – 4: Multivariate adjusted estimated change in cognitive performance (ratios) per standard deviation change in volumes of subcortical structures (case control study)

Per SD decrease	**CIND (n=154) OR (95%CI)***	**Dementia (n=134) OR (95%CI)***	**VCI (n=86) OR(95% CI)***	**Non VCI (n=202) OR (95% CI)***
Accumbens volume	**1.64 (1.12-2.41) p=0.011**	**3.57 (1.81-7.03) p=<0.001**	**2.00 (1.15 – 3.49) p=0.014**	**1.83 (1.23 – 2.72) p=0.003**
Amygdala volume	1.40 (0.96-2.06) p=0.085	**4.73 (2.13-10.48) p=<0.001**	1.44 (0.78 – 2.65) p=0.241	**1.87 (1.23 – 2.86) p=0.004**
Caudate volume	**1.74 (1.09-2.78) p=0.020**	**1.94 (1.04-3.59) p=0.036**	**1.94 (1.07 – 3.52) p=0.030**	1.55 (0.98 – 2.45) p=0.063
Pallidum volume	0.97 (0.66-1.43) p=0.870	1.22 (0.72-2.06) p=0.467	**1.84 (1.04 – 3.24) p=0.036**	0.79 (0.52 – 1.21) p=0.796
Putamen volume	**1.66 (1.13-2.46) p=0.010**	**1.92 (1.10-3.34) p=0.021**	**1.61 (1.00 – 2.60) p=0.053**	**1.79 (1.19 – 2.69) p=0.005**
Thalamus volume	1.16 (0.77-1.75) p=0.483	**2.22 (1.18-4.19) p=0.014**	1.64 (0.96 – 2.81) p=0.070	1.11 (0.71 – 1.75) p=0.640
Hippocampus volume	**2.06 (1.29-3.29) p=0.003**	**10.05 (4.02-25.15) p=<0.001**	**2.76 (1.39 – 5.48) p=0.004**	**2.86 (1.75 – 4.69) p=<0.001**
Brainstem volume	1.06 (0.72-1.55) p=0.784	**2.07 (1.14-3.76) p=0.017**	1.46 (0.88 - 2.43) p=0.147	1.16 (0.78 - 1.72) p=0.472

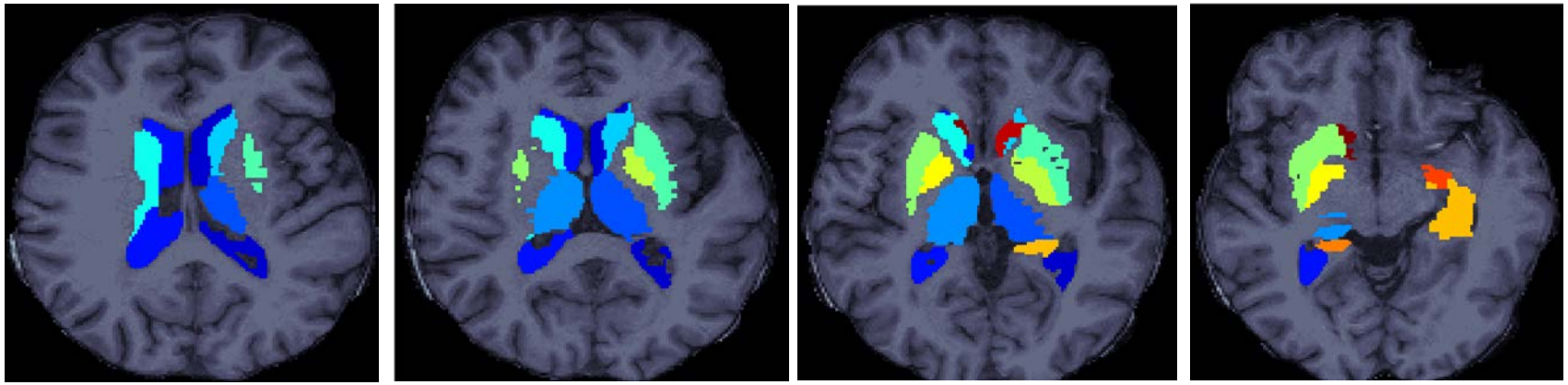
Abbreviations: CIND= cognitive impairment no dementia; VCI= vascular cognitive impairment; OR= odds ratios; CI= confidence interval; SD= standard deviation

* Multivariate adjusted logistic regression model with 95% confidence interval using each subcortical structure volume as determinant and clinical categories of cognitive impairment as outcome. All models are adjusted for age, gender, race, hypertension, diabetes, hyperlipidemia, presence of lacunar infarcts, white matter hyperintensities volume, cerebral microbleeds, total intracranial volume.

CHAPTER 10 – FIGURE

Figure 10– 1: Volumes of subcortical structures segmented through model based automated procedure

Each voxel of the MRI volumes on T1 sequence was labeled automatically as a corresponding brain region based on a parcellation guide. The volumes of accumbens, amygdala, caudate, pallidum, putamen thalamus and hippocampus were calculated separately for left and right hemispheres



PART III:

RETINAL MARKERS OF CEREBROVASCULAR DISEASES AND INVOLUTIONAL CHANGES

CHAPTER 11:

Microvascular Network Alterations in Retina of Subjects with Cerebral Small Vessel Disease

1. INTRODUCTION

In addition to Alzheimer's disease (AD), cerebrovascular disease is an important cause and contributor to cognitive decline and dementia.[1] Magnetic resonance imaging (MRI) has become increasingly important in unraveling the role of vascular pathology involved in cognitive impairment and dementia. Improvements in MR scanner hard- and software have made it possible to visualize brain pathology more accurately. Recently, cerebral microbleeds (CMB) detected on MRI have been suggested as another manifestation or marker of cerebral small vessel disease, in addition to traditional markers such as white matter lesions (WML) and lacunar infarcts.[2]

However, direct *in vivo* visualization of the involvement of cerebral small vessels is difficult to achieve. As the retinal and cerebral microvasculature share several anatomical and physiological features,[3] the retina may provide a non-invasive "window" into the status of these small cerebral vessels. Previous studies have shown that the clinically visible retinopathy signs are associated with an increased risk of clinical cerebrovascular disease, including stroke and dementia, and subclinical markers of cerebral small vessel disease.[4] Furthermore, quantitative changes in retinal vessel width, such as narrower arteriolar caliber and wider venular caliber, have been shown to be associated with these subclinical and clinical age-related brain pathologies.[5]

More recently, a series of novel quantitative retinal vascular parameters such as fractal dimension and tortuosity have been proposed to provide information on the cerebral microvasculature even before the appearance of retinopathy signs. It has been shown that both patients with ischemic stroke and those with AD have a sparser and more tortuous microvascular network in the retina,[6,7] suggesting that AD and stroke may share similar underlying microvascular pathology. However, data on the association between these novel retinal vascular parameters and cerebral small vessel disease, in particular CMB,

are largely lacking. Therefore, we investigated whether there was a link between these novel quantitative retinal parameters and cerebral small vessel disease on MRI.

2. MATERIALS AND METHODS

2.1 Study Population

The Epidemiology of Dementia in Singapore (EDIS) study draws participants from the Singapore Epidemiology of Eye Disease (SEED) Study, which is a population-based study among Chinese, Malays and Indians.[2] In the present study we restricted analysis to the Chinese component of EDIS, the description of which has been described in **Chapter 3**.

2.2 Retinal Photography

Retinal vascular parameters were extracted through fundus photographs and included retinal vascular caliber, fractal dimension, and tortuosity.[6,7] Details of these parameters and reliability assessment have been described in **Chapter 3**.

2.3 Neuroimaging

Markers of cerebrovascular diseases (lacunes, CMB and WML) and involutional changes (total brain and intracranial volume) were collated for each subject using the same protocol as described previously in **Chapter 3**.

2.4 Assessment of Other Vascular Risk Factors

The details of vascular risk factors assessment have been described in **Chapter 3**.

2.5 Statistical Analyses

Quantitative retinal vascular measures (retinal vascular caliber, fractal dimension, and tortuosity) from arterioles and venules were used as determinants and expressed as per standard deviation (SD) increase or decrease, whereas markers of cerebral small vessel disease were taken as outcomes. To examine the associations with lacunar infarcts and CMBs, logistic regression was used. As WML volumes were not normally distributed,

values were log-transformed. Linear regression was used to model log-transformed WML volumes. These models were initially adjusted for age and sex, additionally for smoking, body mass index, mean arterial blood pressure, fasting blood glucose and total cholesterol; and finally for other MRI markers. In order to examine the robustness of any association with CMB, we decided to use different categorizations for CMB, as described previously[2]: (a) multiple (≥2) versus none/single (<2) CMB, (b) multiple categories of CMBs (0, 1 or ≥ 2) and (c) CMB counts using Poisson regression. For the Poisson regression models, outliers were excluded to satisfy the goodness-of-fit criterion assessed with the Pearson's Chi-square statistic. For all the models, association measures were expressed with the corresponding 95% confidence intervals (CI). All statistical analyses were performed on standard statistical software (SPSS Version 17, SPSS Inc., USA).

3. RESULTS

Out of the 300 subjects who participated in the second phase of the EDIS Study, 39 subjects were excluded from the analysis: 18 had no MRI scans (due to claustrophobia, or contraindications), and 21 subjects did not have gradable retinal photographs. Baseline characteristics of the remaining 261 subjects are shown in **Table 11-1**. With respect to qualitative MRI markers, 46 subjects (17.6%) had evidence of ischemic stroke on their MRI scan, of whom 36 (13.8%) had lacunes; while 83 subjects (31.8%) had CMB present, of whom 33 (12.6%) had multiple CMB, including 2 subjects who had more than 20 CMB (23 and 43 CMBs). Among these 33 subjects, 22 had isolated multiple lobar CMBs, 9 isolated multiple deep or posterior fossa CMBs and 2 combination of lobar, deep or posterior fossa CMB. With respect to quantitative MRI markers, mean total brain volume was 895.6 ml (standard error of the mean [SEM]: 5.4) and mean total intracranial volume 1096 ml (SEM: 6.1). As WML volume showed a skewed distribution, the median WML volume was 1.90 ml (interquartile range: 4.48). **Figure 11-1** shows the prevalence

of these markers of cerebral small vessel disease stratified according to 5-year age categories. All MRI lesions showed a higher prevalence with increasing age. CMBs were more prevalent in the younger age categories compared to lacunes, whereas in those aged ≥80 years, the prevalence of CMB and lacunes were comparable. Although WML volume is not directly comparable across age categories with the other markers, the median WML volume in the youngest category was relatively small and showed a steep increase at older age.

Table 11-2 describes age and sex-adjusted associations of retinal vascular parameters with log-transformed WML volumes, and the presence of lacunes and multiple CMB. The presence of lacunes was not associated with any retinal vascular parameters. WML volume was initially associated with increasing venular caliber in age and sex-adjusted models (**Table 11-2**), while multiple CMB was associated with narrower arteriolar caliber, wider venular caliber and reduced arteriolar fractal dimensions. However, associations with WML were attenuated when adjusted for cardiovascular risk factors. Associations with multiple CMB remained consistent, after adjustment for vascular risk factors and MRI markers (**Table 11-3**). Similar associations were observed when different categorizations of CMBs were used. In addition to narrower arteriolar caliber, wider venular caliber, and smaller arteriolar fractal dimension, analyses using Poisson regression models showed that higher arteriolar tortuosity was also associated with an increasing number of CMBs (**Table 11-3**). **Figure 11-2** shows examples of subjects with CMB who had relatively smaller fractal dimension and higher tortuosity compared to subjects without CMB.

4. DISCUSSION

In this study, persons with a sparser and more tortuous retinal vascular network were more likely to have CMBs, independent of vascular risk factors and other cerebral

markers such as WML and lacunes. These data suggest that early retinal changes may provide insight into specific early markers of cerebral small vessel disease, such as CMBs.

Thus far there have been no data from population-based studies examining the link between these novel retinal changes and CMBs. Though, using the same latest computer-assisted method to measure the retinal microvasculature, we did recently observe similar retinal microvascular network changes (a sparser and more tortuous retinal network) in patients with stroke and Alzheimer's disease.[6,7] Furthermore, there are several reports describing rare hereditary conditions involving both retinal and cerebral microvessels including cerebroretinal vasculopathy, hereditary endotheliopathy with retinopathy, nephropathy and stroke (HERNS), cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) and hereditary retinal arteriolar tortuosity. For example, persons with CADASIL have a decreased retinal vascular fractal dimension compared to healthy controls.[8] In patients with hereditary retinal arteriolar tortuosity, besides the presence of retinal arteriolar tortuosity and retinal hemorrhages, CMBs have been observed on MRI.[9] Although these studies in rare hereditary conditions affecting the retinal and brain microvessels consists of small numbers or case series, these data do provide initial evidence that these novel retinal parameters such as fractal dimension or tortuosity may be early markers of microvascular pathology in the brain as reflected on MRI, such as CMBs. Taken together, findings from our current study further support data from histopathological studies that CMBs are a marker of microvascular pathology.[10]

Most studies that have examined the link between retinal changes and cerebral small vessel disease have focused on clinically visible retinopathy signs and on WMH and lacunar infarcts. Data from both the Atherosclerosis Risks in Communities Study and the Cardiovascular Health Study reported that persons with retinopathy signs were more

likely to have both WML and subclinical infarcts.[4,11] More recently, the AGES-Reykjavik Study reported that retinopathy signs were also associated with the presence of multiple CMB.[12] Taken together, these studies provided evidence that microvascular lesions in both the retina and the brain may occur concomitantly as part of generalized microvascular disease resulting from common pathophysiological mechanisms. Retinopathy signs are, however, relatively late indicators of target organ damage in the eye and probably reflect advanced stages of structural microvascular damage including breakdown of the blood-retina barrier (**Figure 11-3**; blue line).

Initial studies using the computer analysis techniques focused on earlier markers such as generalized arteriolar narrowing and venular dilatation. Longitudinal data from the Rotterdam Study showed that specifically wider venular caliber was associated with progression of both periventricular and subcortical white matter lesions, and incident lacunar infarcts on MRI.[5] However, in the cross-sectional analyses from this study there was no statistically significant association between retinal calibers and these markers of cerebral small vessel disease. The lack of an association in the cross-sectional analyses may suggest that early changes in the retinal microvasculature (e.g. venular dilatation) do not reflect the actual severity of cerebral small vessel disease, but may precede the development of WMH and lacunes. Furthermore, in our cohort we found that in contrast to WMH and lacunes, prevalence of CMB was already high at a younger age, suggesting that CMBs may manifest before the appearance of the other cerebral small vessels disease markers. These data are in accordance with observations made in the spontaneously hypertensive stroke-prone (SHRSP) rat model showing that the development of CMBs as an important early milestone in the pathogenesis of cerebral small vessel disease.[13] Overall, our current analyses showed that these early novel retinal parameters such as fractal dimension and tortuosity showed a significant cross-sectional association with CMBs, but not with more traditional makers of cerebral small vessel

disease (**Figure 11-3**; green line). Finally, our findings suggest that although these MRI lesions can occur concomitantly and increase with advancing age, there may be differences in pathophysiology underlying these lesions.

Several methodological issues need to be discussed. Firstly, nearly 50% of screen-positive subjects did not participate in the second phase of the EDIS study.[2] Those (n=312) who did not participate were relatively older and had higher mean arterial blood pressure. This might have led to an underestimation of the prevalence of MRI markers of cerebral small vessel disease. Secondly, also due to small numbers, we were not able to study in detail the associations with the specific location of CMB. Strengths of our study include quantitative assessment of retinal photographs using standardized protocols, and quantitative measurement of MRI markers such as white matter lesion volume.

5. CONCLUSION

In conclusion, we report that elderly persons with early retinal changes such as a sparser and more tortuous retinal microvascular network are more likely to have CMBs on MRI independent of cardiovascular risk factors and other markers of small vessel disease on MRI. This provides further evidence that CMB may be an early manifestation of cerebral small vessel disease.

As reduced fractal dimension were significantly associated with

CHAPTER 11 – REFERENCES

1. Pantoni L, Poggesi A, Inzitari D. Cognitive decline and dementia related to cerebrovascular diseases: some evidence and concepts. Cerebrovasc. Dis. 2009;27,191–6.

2. Hilal S, Saini M, Tan CS, et al. Cerebral microbleeds and cognition in a Chinese population. Alzheimer Dis. Assoc. Disord. 2014;28,106–22.

3. Patton N, Aslam T, Macgillivray T, Pattie A, Deary IJ, Dhillon B. Retinal vascular image analysis as a potential screening tool for cerebrovascular disease: a rationale based on homology between cerebral and retinal microvasculatures. J. Anat. 2005; 206,319–48.

4. Ikram MK, Cheung CYL, Wong TY, Chen CPLH. Retinal pathology as biomarker for cognitive impairment and Alzheimer's disease. J. Neurol. Neurosurg. Psychiatry. 2012;83,917–22.

5. Ikram MK, Ong YT, Cheung CY, Wong TY. Retinal vascular caliber measurements: clinical significance, current knowledge and future perspectives. Ophthalmologica. 2013;229,125–36.

6. Ong YT, De Silva DA, Cheung CY, et al. Microvascular structure and network in the retina of patients with ischemic stroke. Stroke. 2013;44,2121–27.

7. Cheung CY, Ong YT, Ikram MK, et al. Microvascular network alterations in the retina of patients with Alzheimer's disease. Alzheimers Dement. 2014;10,135–42.

8. Cavallari M, Falco T, Frontali M, Romano S, Bagnato F, Orzi F. Fractal analysisreveals reduced complexity of retinal vessels in CADASIL. PLoS One. 2011;6 e19150.

9. Vahedi K, Massin P, Guichard JP, et al. Hereditary infantile hemiparesis, retinal arteriolar tortuosity, and leukoencephalopathy. Neurology. 2003;60,57–63.

10. Charidimou A, Werring DJ. Cerebral microbleeds and cognition in cerebrovascular disease: an update. J. Neurol. Sci. 2012;322,50–5.

11. Longstreth W, Larsen EK, Klein R, Wong TY, Sharrett AR, Lefkowitz D, Manolio TA. Associations between findings on cranial magnetic resonance imaging and retinal photography in the elderly: the Cardiovascular Health Study. Am.J. Epidemiol. 2007;165,78–84.

12. Qiu C, Cotch MF, Sigurdsson S, et al. Cerebral microbleeds, retinopathy, and dementia: the AGES-Reykjavik Study. Neurology. 2010;75,2221–8.

13. Schreiber S, Bueche CZ, Garz C, et al. The pathologic cascade of cerebrovascular lesions in SHRSP: is erythrocyte accumulation a nearly phase? J. Cereb. Blood Flow Metab. 2012;32,278–90.

CHAPTER 11 – TABLES

Table 11 – 1: Baseline characteristics of study participants (n=261)

Baseline characteristics	Mean / N
Age, years	70 (0.4)
Males,	122 (46.7)
BMI, kg/m^2	24.1 (0.2)
Hypertension,	196 (75.1)
Diabetes,	69 (26.4)
Hyperlipidemia,	154 (59.0)
Systolic blood pressure, mmHg	147.0 (1.2)
Diastolic blood pressure, mmHg	76.7 (0.7)
Mean arterial blood pressure, mmHg	100.2 (0.7)
Fasting blood glucose, mmol/l	5.1 (0.09)
Fasting total cholesterol, mmol/l	4.9 (0.05)
Ever smokers,	76 (29.1)

Abbreviations: BMI= body mass index; kg/m^2= kilogram per meter square; mmHg= millimeters of mercury; mmol/l= millimoles per liter

Numbers between brackets are percentage or standard error of the mean

Table 11 – 2: Age-sex adjusted regression models of retina vascular parameters for white matter hyperintensities (WMH) volume, and multiple cerebral microbleeds (CMB≥2)

Retinal Parameter	**WMH volume∗ Mean difference (95%CI) (n=261)**	**Lacunes† OR (95% CI) (n=36/261)**	**Multiple CMBs† OR (95% CI) (n=33/261)**
Caliber			
Arteriolar, per SD decrease‡	0.32 (-0.05; 0.69)	1.26 (0.65; 2.45)	**2.10 (1.06; 4.15)**
Venular, per SD increase‡	**0.38 (0.02; 0.75)**	1.57 (0.83; 2.98)	**2.29 (1.19; 4.40)**
Fractal dimension, per SD decrease			
Arteriolar	0.06 (-0.16; 0.27)	1.31 (0.90; 1.91)	**1.89 (1.27; 2.82)**
Venular	-0.10 (-0.32; 0.12)	1.11 (0.95; 1.64)	1.30 (0.88; 1.92)
Tortuosity, per SD increase			
Arteriolar	-0.05 (-0.27; 0.16)	0.78 (0.52; 1.17)	1.07 (0.73; 1.56)
Venular	0.12 (-0.09; 0.33)	0.99 (0.67; 1.46)	0.94 (0.63; 1.40)

Abbreviations: WMH= white matter hyperintensities; CMB= cerebral microbleeds; OR= odds ratios; CI= confidence interval; SD= standard deviation

∗Linear regression models with log-transformed WMH volumes as the dependent variable.

†Logistic regression models with the presence of lacunes or multiple CMB as the dependent variable.

‡Adjusted for fellow vessel caliber

Table 11 – 3: Multivariable-adjusted odds ratios (ORs) for the presence of multiple cerebral microbleeds (CMBs) and rate ratios (RRs) for the CMB counts (with 95% confidence intervals) presented as per standard deviation difference in retinal parameters

	ORs for presence of multiple CMB*		**RRs for CMB counts†**	
Retinal Parameter	**Model I‡ OR (95% CI) (n=32/258)**	**Model II§ OR (95% CI) (n=32/258)**	**Model I‡ RR (95% CI) (n=256)**	**Model II§ RR (95% CI) (n=256)**
Caliber				
Arteriolar, per SD decrease#	**2.17 (1.04; 4.51)**	2.07 (0.98; 4.38)	**1.48 (1.01; 2.17)**	1.42 (0.98; 2.05)
Venular, per SD increase#	**2.40 (1.20; 4.77)**	**2.23 (1.09; 4.56)**	**1.39 (1.00; 1.93)**	1.31 (0.93; 1.84)
Fractal dimension, per SD decrease				
Arteriolar	**1.84 (1.22; 2.78)**	**1.79 (1.17; 2.73)**	**1.39 (1.08; 1.80)**	**1.37 (1.06; 1.78)**
Venular	1.22 (0.82; 1.83)	1.24 (0.82; 1.88)	1.15 (0.91; 1.45)	1.15 (0.91; 1.46)
Tortuosity, per SD increase				
Arteriolar	1.17 (0.80; 1.38)	1.29 (0.87; 1.93)	**1.25 (1.01; 1.55)**	**1.29 (1.03; 1.61)**
Venular	0.96 (0.64; 1.45)	0.96 (0.64; 1.44)	0.99 (0.76; 1.29)	0.98 (0.76; 1.27)

Abbreviations: OR=odds ratios; RR= rate ratios; CMB= cerebral microbleeds; SD= standard deviation

*Multivariable-adjusted odds ratios for the presence of multiple cerebral microbleeds (with 95% confidence intervals)

†Multivariable-adjusted rate ratios for the cerebral microbleed counts (with 95% confidence intervals). Additionally, 2 outliers (subjects with 23 and 43 CMBs) were excluded to satisfy the goodness-of-fit criterion for Poisson regression models

‡Model I: Adjusted for age, sex, smoking, body mass index, mean arterial blood pressure, fasting blood glucose and cholesterol

§Model II: Adjusted for confounders from model I, and total white matter volume, total brain volume/total intracranial volume, and presence of stroke

#Adjusted for fellow vessel caliber

CHAPTER 11 – FIGURES

Figure 11 – 1: Distribution of cerebral small vessel disease markers by 5-year age categories

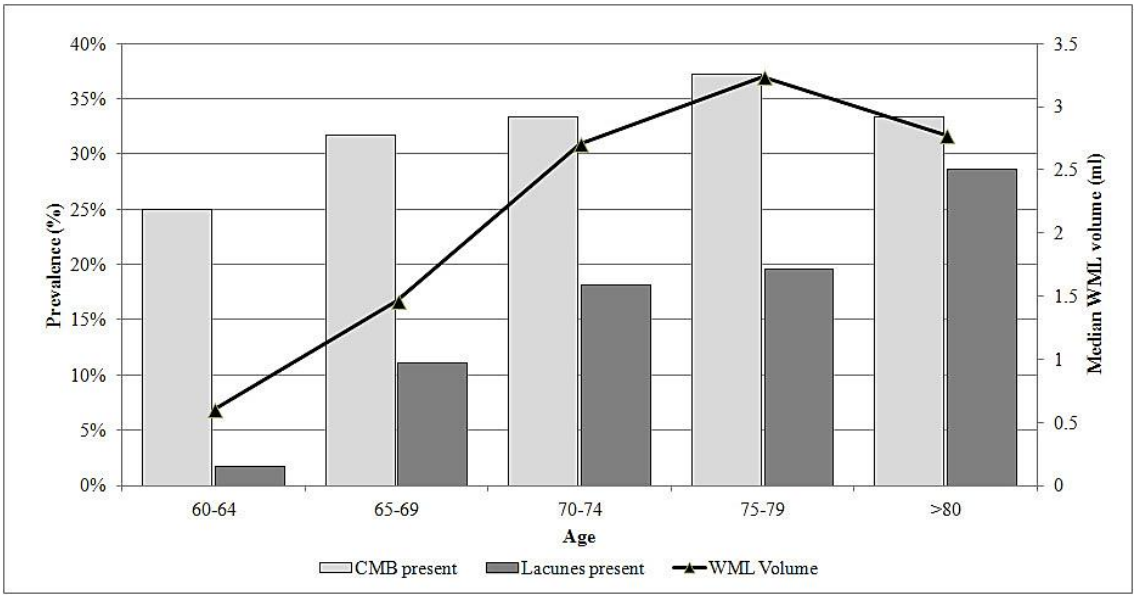


Figure 11 – 2: MRI scans and corresponding retinal photographs of study subjects

MRI scans (A-D) showing absence (left column) or presence (right column) of cerebral microbleeds (CMB; white arrows) and retinal fundus photos graded by computer software (A'-D') showing vessel path tracing of images from participants, with red for arterioles and blue for venules. In a subject with CMB retinal arteriolar fractal dimension (C,C') is smaller compared to a subject without CMB (A,A'). In another subject with CMB, retinal arteriolar tortuosity (D,D') is higher compared to a subject without CMB (B,B').

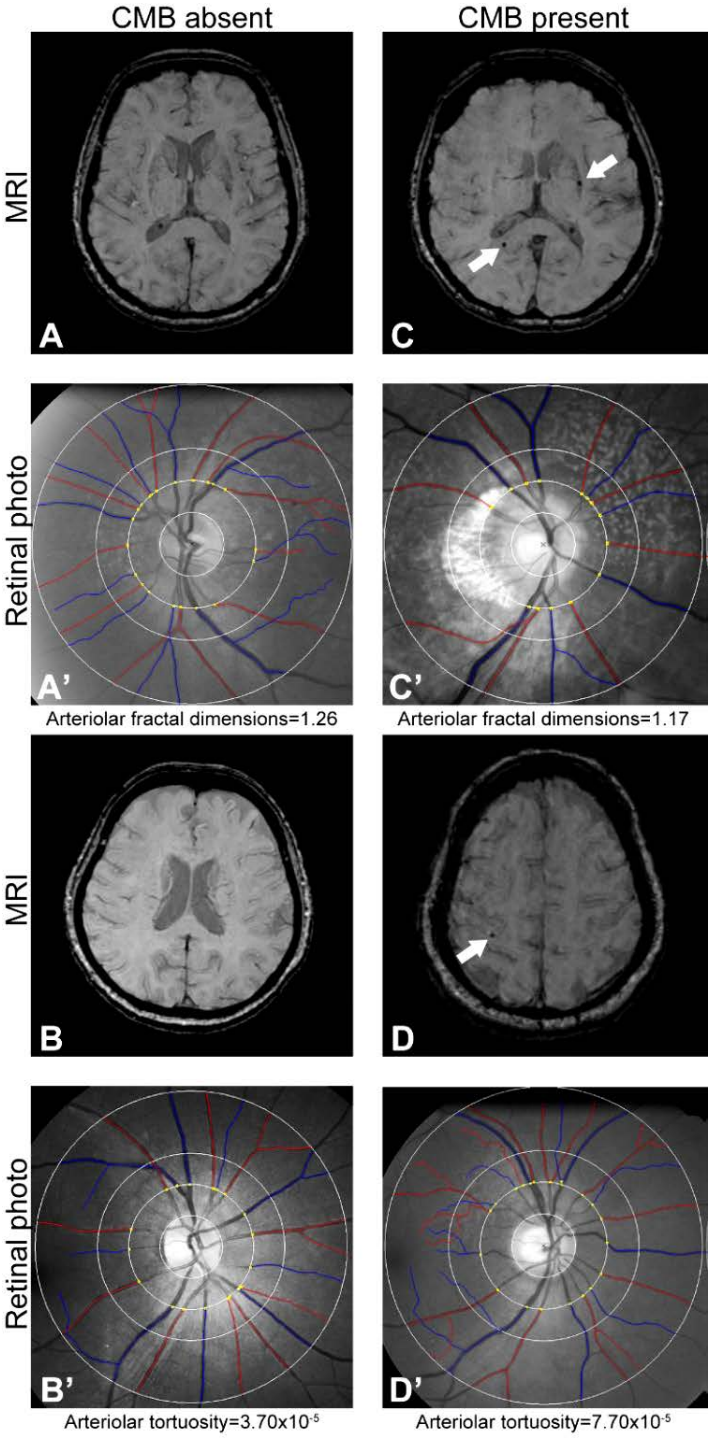
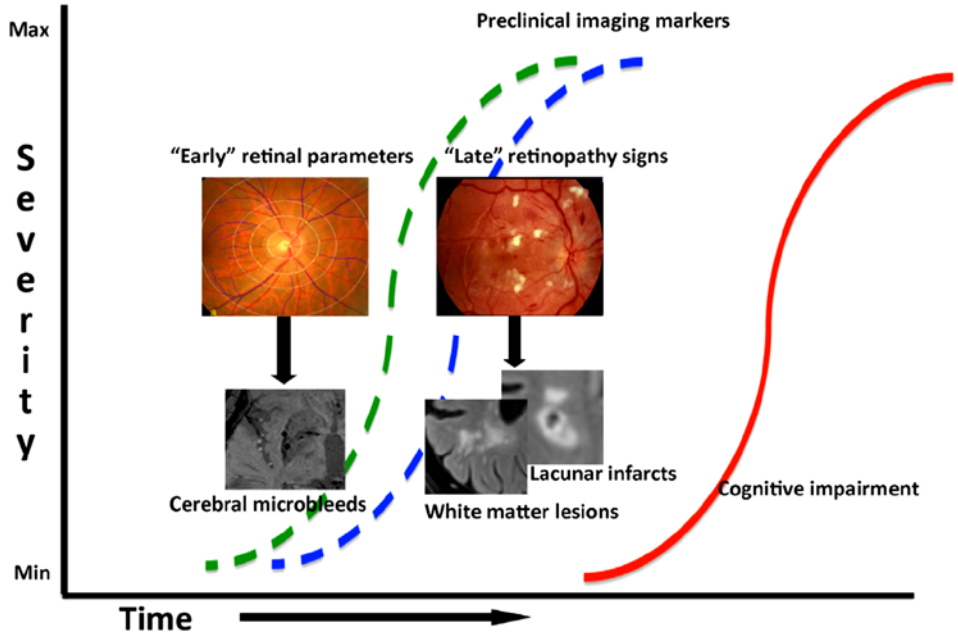


Figure 11 – 3: Representation of postulated timeline of preclinical imaging markers in cognitive impairment

Among preclinical imaging markers for cognitive impairment, white matter lesions and lacunar infarcts may be relatively "late" markers as they are related to retinopathy signs (blue line), whereas cerebral microbleeds are related to "early" retinal parameters such as fractal dimension and tortuosity (green line) and hence may be early manifestation of cerebral small vessel disease.



CHAPTER 12:

Retinal Microvascular Network Changes in Mild Cognitive Impairment

1. INTRODUCTION

An increasing amount of evidence suggests that vascular pathology is an independent and important contributor to the development of dementia, including Alzheimer's disease and its preclinical stages.[1] In particular, cerebral small vessel disease has been associated with increased risk of cognitive decline and dementia.[2,3] Although modern neuroimaging modalities have contributed immensely to our understanding of microvascular pathology in dementia and cognitive impairment, it remains difficult to directly observe the cerebral microvasculature in vivo. As the retinal and cerebral microvasculature share many anatomical and physiological aspects, the retina provides a viable window to directly observe changes to the cerebral microvasculature.[4]

Thus far, studies have shown that traditional signs of retinal microvascular damage, such as retinopathy signs (e.g. retinal hemorrhage) are associated with both dementia and its earlier preclinical stages.[5-7] However, retinopathy signs are relatively late indicators of damage in the eye, and indicate advanced stages of structural microvascular damage, such as breakdown of the blood-retina barrier, and are not commonly seen. With recent advances in digital retinal imaging and analysis techniques, we are now able to quantify objectively the structure and pattern of the retinal microvascular network, which may reflect earlier and more subtle changes before the appearance of overt signs. Novel retinal vascular parameters such as fractal dimensions, which reflect the optimality of the vascular network, are of particular interest, as they have recently been found to be associated with both stroke and dementia.[8,9] In view of these associations with clinical disease, we hypothesized that these early retinal vascular network changes may also be present even in the preclinical stages of dementia. In this study, we examined the association between retinal vascular network parameters, particularly vascular fractal dimensions, and preclinical cognitive impairment in a Chinese population from Singapore.

2. METHODS

2.1 Study Population

The Epidemiology of Dementia in Singapore (EDIS) study draws participants from the Singapore Epidemiology of Eye Disease (SEED) Study, which is a population-based study among Chinese, Malays and Indians.[2] In the present study we restricted analysis to the Chinese component of EDIS, the description of which has been described in **Chapter 3**.

2.2 Assessment of Retinal Vascular Parameters

Retinal fundus photographs were taken from each eye after pupil dilation and graded according to a standardized protocol as described in **Chapter 3**. The following retinal vascular parameters were extracted and used for analysis: retinal vascular fractal dimension, tortuosity, and caliber. Details of these parameters and reliability assessment have been described in the previous **Chapter 3**.

2.3 Neuropsychological Assessment

Detailed neuropsychological assessments with subtests and Z-score calculation has been described in detail in **Chapter 3.**

2.4 Diagnosis of Cognitive Impairment and Dementia

Weekly consensus meetings were held with study clinicians, neuropsychologists, clinical research fellows, research coordinators, and research assistants. Details from the clinical assessment, blood investigations, neuropsychological testing and MRI scans were reviewed. Diagnostic criteria for CIND and dementia have been described in detail in **Chapter 3**. Seven participants who were diagnosed with dementia were excluded from final analysis.

2.5 Assessment of Other Risk Factors

Details on the demographic and vascular risk factors assessment have been described in detail in **Chapter 3**. Scans were graded by one radiologist and two clinicians blinded to

the neuropsychological and clinical data for the presence of stroke and cerebral microbleeds (Brain Observer Microbleed Scale). White matter lesions (WML) volume, total brain volume, and total intracranial volume were quantified by automatic segmentation as described in **Chapter 3** at the Erasmus University Medical Center Rotterdam, The Netherlands.[16-18]

2.6 Statistical Analysis

For the comparison of baseline demographic and risk factors between participants with gradable and ungradable retinal fundus images, and between the different diagnostic groups, Pearson's chi-square test was used for categorical variables with independent t-tests and analysis of variance (ANOVA) for continuous variables. Kruskal-Wallis one-way analysis of variance was used to compare WML volumes, MMSE scores, and MoCA scores as they were not normally distributed. Multinomial logistic regression models were constructed to calculate odds ratios (OR) and their 95% confidence intervals (CI) for CIND-mild and CIND moderate by per standard deviation (SD) increase or decrease in retinal vascular parameters. Models were firstly adjusted for age and sex, then additionally for risk factors of education level, socioeconomic status, mean arterial blood pressure, fasting blood glucose, serum cholesterol, smoking status, and MRI brain imaging markers. Similarly adjusted linear regression models were also constructed for z-scores from individual domains and the composite Z-score for all domains to test for linear relationships between retinal vascular parameters and cognitive performance. All statistical analysis was performed using SPSS Version 17.0 (SPSS Inc., USA).

3. RESULTS

Of the 300 Chinese participants recruited into the EDIS study, 7 participants diagnosed with clinical dementia were excluded from this study. From the remaining 293 participants, the additional 25 excluded participants (due to poor retinal image quality)

from the analysis were similar in baseline characteristics compared to the included participants, except for education level (less with Primary education and above, p=0.045), socioeconomic status (lower, p=0.006), and presence of any previous stroke on neuroimaging (higher, p=0.043). In the 268 eligible participants, 121 participants were NCI, 78 CIND-mild, and 69 CIND-moderate. In general, participants who were CIND-mild or CIND-moderate were more likely to be women, older, had lower education and socioeconomic status, higher diastolic blood pressures, prevalent stroke, higher WML volume, and lower total brain volume/intracranial volume ratio (**Table 12-1**).

In multinomial age-sex adjusted logistic regression models, reduced retinal arteriolar fractal dimension was associated with higher risk of being CIND-moderate, while reduced venular fractal dimension was associated with higher risk of being CIND-mild and CIND-moderate (**Table 12-2**). After further adjustment for other risk factors such as socioeconomic status, blood pressure, glucose, cholesterol levels, and MRI markers, reduced fractal dimensions remained associated with both clinical outcomes of CIND-mild and CIND-moderate (**Table 12-3**).

In age-sex adjusted linear regression models for global cognitive function as expressed as composite VDB Z-scores in the entire cohort showed that both reduced arteriolar and venular fractal dimensions and reduced arteriolar vessel tortuosity was associated with poorer cognitive performance (**Table 12-2**). However, the association of cognitive impairment with arteriolar tortuosity was attenuated after additional adjustment for other risk factors and MRI markers (**Table 12-3**).

As there were significant associations between fractal dimensions and global cognitive function, associations with specific cognitive domains were also investigated in this cohort. Reduced fractal dimensions were associated with lower scores in verbal memory, visuoconstruction, and visuomotor speed (**Table 12-4**).

4. DISCUSSION

In this Chinese population, persons with a sparser vascular network in the retina were more likely to have poorer global cognitive performance, and have significant cognitive impairment, independent of traditional risk factors and MRI markers. In particular, they performed worse in specific cognitive domains of verbal memory, visuoconstruction and visuomotor speed.

Thus far, previous studies examining the relationship between retinal microvascular changes and cognitive dysfunction have mainly focused on clinically visible retinopathy signs. In the Atherosclerosis Risk in Communities (ARIC) study,[10] classic retinopathy lesions were clearly associated with cognitive impairment. However, findings from other studies, including the Los Angeles Latino Eye Study (LALES), the Cardiovascular Health Study (CHS), the Blue Mountain Eye Study (BMES), and the AGES-Reykjavik Study, have been less clear.[11-13] For example, in the BMES,[13] these associations were only present among subjects with hypertension, whereas in the AGES-Reykjavik Study,[6] retinopathy combined with the presence of cerebral microbleeds was associated with cognition. These discrepancies could partly be due to not only differences in the cognitive tests used, such as the Mini Mental State Examination, or the Abbreviated Mental Test,[11,13,14] but also differences in the specific cognitive domains tested such as psychomotor speed, executive function, and verbal memory.[10,11] Finally, retinopathy signs are considered relatively late indicators of vascular damage in the eye, and indicate advanced stages of structural microvascular damage. Recent advances in digital retinal imaging have enabled us to quantify early changes in the retinal microvasculature.

In this present study, we focused on retinal vascular fractal dimensions. In addition to its potential to reflect earlier and more subtle changes before the appearance of overt signs, fractal dimensions in particular have the advantage of being parameters that do not vary with pulse cycles such as vessel diameters. One previous study has shown that decreased

fractal dimensions was related to cognitive dysfunction.[14] However, in the study only a brief 10-point screening test (Abbreviated Mental Test) was employed. In the current study, an extensive neuropsychological test battery was employed to assess a range of cognitive domains, allowing us to not only study individual domains, but also comprehensively stage our subjects into categories with increasing severity of impairment. Our data provide additional support that these changes in the retinal vascular network are associated with cognitive impairment. Furthermore, our findings that reduced arteriolar and venular fractal dimensions are associated with preclinical stages of dementia are in line with previous studies showing that these retinal parameters are linked to not only clinical outcomes such as acute ischemic stroke and dementia, but also markers of cerebral small vessel disease such as lacunar infarcts and cerebral microbleeds.[8,9,15-17] Pathophysiologically, a sparser network as reflected by a reduced fractal dimension is a consequence of retinal vessel rarefaction and collapse, which may lead to hypoxia in the retina.[18] Similarly in the brain, destruction and occlusion of the small perforating vessels have been observed,[19] suggesting that there may be parallel pathological mechanisms at work in the brain and retina leading to microvascular changes. Taken together, these morphological changes in the retinal microvasculature suggest that subtle microvascular changes may already be present in the preclinical stages of dementia, further providing evidence for vascular disease as an important contributor to the development of cognitive impairment and dementia.

Some methodological issues need to be discussed. Since approximately half of the screen positive subjects declined to take part in phase II of the study (cognitive assessment and neuroimaging phase), eligible subjects who refused to participate may have had poorer cognitive function leading to an underestimation of the effect sizes.[20] Nevertheless, we still found a consistent association between retinal vascular network complexity and cognitive impairment, suggesting that the true association may be stronger. Strengths of

our study include comprehensive and standardized assessment of cognitive ability over a range of domains, and quantitative assessment of retinal photographs using standardized semi-automated protocols.

5. CONCLUSION

In conclusion, our study found that a sparser retinal microvascular network is associated with cognitive impairment and poorer performance on cognitive scores, independent of cardiovascular risk factors and MRI markers of cerebral small vessel disease. This provides additional evidence for the importance of microvascular pathology in the development of cognitive impairment.

CHAPTER 12 – REFERENCES

1. Gorelick PB, Scuteri A, Black SE, et al. Vascular contributions to cognitive impairment and dementia. Stroke. 2011;42:2672-713.

2. Wardlaw JM, Sandercock PA, Dennis MS, Starr J. Is breakdown of the blood-brain barrier responsible for lacunar stroke, leukoaraiosis, and dementia? Stroke. 2003;34:806-12.

3. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. Lancet Neurol. 2010;9:689-701.

4. Patton N, Aslam T, Macgillivray T, Pattie A, Deary IJ, Dhillon B. Retinal vascular image analysis as a potential screening tool for cerebrovascular disease: a rationale based on homology between cerebral and retinal microvasculatures. J Anat. 2005;206:319-48.

5. Ong SY, Cheung CY, Li X, et al. Visual impairment, age-related eye diseases, and cognitive function: the Singapore Malay Eye study. Arch Ophthalmol. 2012;130:895-900.

6. Qiu C, Cotch MF, Sigurdsson S, et al. Cerebral microbleeds, retinopathy, and dementia: the AGES-Reykjavik Study. Neurology. 2010;75:2221-8.

7. Wong TY, Klein R, Sharrett AR, et al. Retinal microvascular abnormalities and cognitive impairment in middle-aged persons: the Atherosclerosis Risk in Communities Study. Stroke. 2002;33:1487-92.

8. Cheung CY, Ong YT, Ikram MK, et al. Microvascular network alterations in the retina of patients with Alzheimer's disease. Alzheimers Dement. 2014;10:135-42.

9. Ong YT, De Silva DA, Cheung CY, et al. Microvascular structure and network in the retina of patients with ischemic stroke. Stroke. 2013;44:2121-27.

10. Lesage SR, Mosley TH, Wong TY, et al. Retinal microvascular abnormalities and cognitive decline: the ARIC 14-year follow-up study. Neurology. 2009;73:862-8.

11. Baker ML, Marino Larsen EK, Kuller LH, et al. Retinal microvascular signs, cognitive function, and dementia in older persons: the Cardiovascular Health Study. Stroke. 2007;38:2041-7.

12. Gatto NM, Varma R, Torres M, et al. Retinal microvascular abnormalities and cognitive function in Latino adults in Los Angeles. Ophthalmic Epidemiol. 2012;19:127-136.

13. Liew G, Mitchell P, Wong TY, et al. Retinal microvascular signs and cognitive impairment. J Am Geriatr Soc. 2009;57:1892-6.

14. Cheung CY, Ong S, Ikram MK, et al. Retinal Vascular Fractal Dimension is Associated with Cognitive Dysfunction. J Stroke Cerebrovasc Dis. 2014;23:43-50.

15. Kawasaki R, Che Azemin MZ, Kumar DK, et al. Fractal dimension of the retinal vasculature and risk of stroke: a nested case-control study. Neurology. 2011;76:1766-7.

16. Doubal FN, MacGillivray TJ, Patton N, Dhillon B, Dennis MS, Wardlaw JM. Fractal analysis of retinal vessels suggests that a distinct vasculopathy causes lacunar stroke. Neurology. 2010;74:1102-7.

17. Yatsuya H, Folsom AR, Wong TY, et al. Retinal microvascular abnormalities and risk of lacunar stroke: Atherosclerosis Risk in Communities Study. Stroke. 2010;41:1349-55.

18. Hammes HP, Feng Y, Pfister F, Brownlee M. Diabetic retinopathy: targeting vasoregression. Diabetes. 2011;60:9-16.

19. Tomita Y, Kubis N, Calando Y, et al. Long-term in vivo investigation of mouse cerebral microcirculation by fluorescence confocal microscopy in the area of focal ischemia. J Cereb Blood Flow Metab. 2005;25:858-67.

20. Hilal S, Ikram MK, Saini M, et al. Prevalence of cognitive impairment in Chinese: Epidemiology of Dementia in Singapore study. J Neurol Neurosurg Psychiatry. 2013;84:686-92.

CHAPTER 12 – TABLES

Table 12 – 1: Baseline characteristics of participants by diagnosis of cognitive impairment

Characteristic	NCI (n=121)	CIND-mild (n=78)	CIND-moderate (n=69)	P*
Female, n (%)	53 (43.8)	42 (53.8)	47 (68.1)	**0.005**
Age, mean (SD)	67.3 (4.8)	71.1 (6.3)	74.1 (5.4)	**<0.001**
Above primary education, n (%)	110 (90.9)	61 (76.9)	41 (59.4)	**<0.001**
Low socioeconomic status, n (%)	58 (48.7)	52 (71.2)	57 (83.8)	**<0.001**
BMI, mean (SD)	23.9 (3.2)	23.8 (3.3)	24.5 (4.1)	0.456
Systolic blood pressure, mean (SD)	146.3 (19.1)	145.4 (17.9)	149.1 (21.6)	0.477
Diastolic blood pressure, mean (SD)	78.9 (10.1)	75.2 (10.1)	74.8 (10.9)	**0.010**
Hypertension, n (%)	88 (72.7)	59 (75.6)	61 (88.4)	**0.040**
Random blood glucose, mmol/L (SD)	6.41 (2.50)	6.61 (3.09)	6.65 (2.44)	0.810
Diabetes, n (%)	26 (21.5)	19 (24.4)	22 (31.9)	0.278
Serum total cholesterol, mmol/L (SD)	5.32 (1.01)	5.06 (0.97)	5.33 (1.05)	0.493
Hyperlipidemia, n (%)	67 (55.4)	48 (61.5)	48 (69.6)	0.154
Ever smokers, n (%)	35 (28.9)	24 (30.8)	22 (31.9)	0.906
MMSE score, median (IQR)	27 (2)	25 (4)	21 (5)	**<0.001†**
MoCA score, mean (IQR)	24 (4)	20 (5)	16 (6)	**<0.001†**
MRI markers				
Presence of stroke, n (%)	5 (4.1)	14 (17.9)	21 (30.4)	**<0.001**
Presence of CMB, n (%)	33 (27.3)	24 (30.8)	23 (33.3)	0.510
WML volume, median ml (IQR)	1.37 (3.19)	1.82 (4.26)	3.28 (10.39)	**0.002†**
TBV/ICV, ratio (SD)	0.820 (0.018)	0.817 (0.018)	0.812 (0.018)	**0.013**

Abbreviations: BMI= body mass index; MMSE= mini mental status examination; MoCA= montreal cognitive assessment; CMB= cerebral microbleeds; TBV= total brain volume; ICV= intracranial volume, NCI= no cognitive impairment; CIND= cognitive impairment no dementia

*Chi-square test was used for categorical variables and Student's t-test for continuous variables unless stated otherwise

†Kruskal-Wallis test was used for white matter lesion volume, MMSE score and MoCA score

Table 12 – 2: Age-sex adjusted associations of retinal vascular parameters with global cognitive performance, expressed as mean differences (95%CI) in normalized test scores, and with diagnosis of cognitive impairment status expressed as odds ratios (95%CI)

Retinal vascular parameters	Age-sex adjusted (268)		
	B (95%CI)*	OR (95%CI)	
	Composite VDB score (n=267)	CIND-mild (n=78)	CIND-moderate (n=69)
Caliber			
Arteriolar, per SD decrease†	0.072 (-0.091, 0.234)	0.70 (0.39-1.24)	0.81 (0.43-1.53)
Venular, per SD increase†	-0.080 (-0.240, 0.081)	0.99 (0.57-1.72)	1.17 (0.63-2.16)
Fractal Dimension			
Arteriolar, per SD decrease	**-0.165 (-0.247, -0.073)**	1.37 (0.99-1.89)	**1.73 (1.19-2.53)**
Venular, per SD decrease	**-0.151 (-0.242, -0.061)**	**1.38 (1.00-1.90)**	**1.79 (1.24-2.60)**
Tortuosity			
Arteriolar, per SD increase	**0.143 (0.053, 0.234)**	0.86 (0.63-1.17)	0.73 (0.51-1.05)
Venular, per SD increase	0.073 (-0.017, 0.164)	0.90 (0.67-1.23)	0.85 (0.60-1.22)

Abbreviations: B= mean difference; OR= odds ratios; CI= confidence interval; CIND= cognitive impairment no dementia; VDB= vascular dementia battery; SD= standard deviation

*Expressed as mean differences with 95% confidence intervals

† Adjusted additionally for other vessel caliber

Table 12 – 3: Multivariable adjusted associations of retinal vascular parameters with global cognitive performance, expressed as mean differences (95%CI) in normalized test scores, and with diagnosis of cognitive impairment status expressed as odds ratios (95% CI)

Retinal vascular parameter	Model I* (244)			Model II† (243)		
	B (95%CI)	OR (95% CI)		B (95%CI)	OR (95% CI)	
	Composite VDB score‡ (n=244)	CIND-mild (n=66)	CIND-moderate (n=62)	Composite VDB score‡ (n=243)	CIND-mild (n=66)	CIND-moderate (n=61)
Fractal Dimension						
Arteriolar, per SD decrease	**-0.119 (-0.200, -0.037)**	1.40 (0.98-2.01)	**1.86 (1.20-2.88)**	**-0.103 (-0.187, -0.020)**	**1.46 (1.01-2.12)**	**1.86 (1.17-2.93)**
Venular, per SD decrease	**-0.108 (-0.190, -0.026)**	**1.52 (1.05-2.21)**	**2.09 (1.35-3.22)**	**-0.109 (-0.191, -0.028)**	**1.54 (1.06-2.25)**	**2.15 (1.38-3.34)**
Tortuosity						
Arteriolar, per SD increase	0.072 (-0.010, 0.315)	0.95 (0.66-1.36)	0.82 (0.53-1.25)	0.063 (-0.019, 0.144)	0.93 (0.65-1.34)	0.82 (0.53-1.27)

Abbreviations: B= mean difference; OR= odds ratios; CI= confidence interval; VDB= vascular dementia battery; CIND= cognitive impairment no dementia; SD= standard deviation

* Adjusted for age, gender, race, education level, low socioeconomic status, mean arteriolar blood pressure, random blood glucose, total cholesterol and presence of stroke

† Adjusted for age, gender, race, education level, low socioeconomic status, mean arteriolar blood pressure, random blood glucose and total cholesterol, presence of stroke and cerebral microbleeds, total white matter lesion volume, and total brain volume/intracranial volume

‡ Expressed as mean differences with 95% confidence intervals

Table 12 – 4: Associations between retinal vascular fractal dimensions with specific cognitive domain scores expressed as mean differences (95%CI)

	Executive Function	Attention	Language	Visual Memory	Verbal Memory	Visuoconstruction	Visuomotor speed
	B (95%CI)	**B (95%CI)**	**B (95%CI)**	**B (95%CI)**	**B (95%CI)**	**B (95%CI)**	**B (95%CI)**
Arteriolar fractal dimension per SD decrease							
Model I*	-0.082	-0.057	-0.087 (-0.183,	-0.083	**-0.145**	**-0.146**	**-0.116**
	(-0.181, 0.018)	(-0.144, 0.030)	0.008)	(-0.172, 0.005)	**(-0.248, -0.042)**	**(-0.246, -0.047)**	**(-0.199, -0.033)**
Model II†	-0.053	-0.041	-0.069	-0.078	**-0.135**	**-0.126**	**-0.122**
	(-0.154, 0.048)	(-0.131, 0.049)	(-0.167, 0.029)	(-0.168, 0.013)	**(-0.242, -0.029)**	**(-0.228, -0.025)**	**(-0.206, -0.037)**
Venular fractal dimension per SD decrease							
Model I*	-0.022	**-0.127**	-0.044	**-0.093**	**-0.113**	**-0.146**	**-0.109**
	(-0.122, 0.079)	**(-0.214, -0.041)**	(-0.141, 0.053)	**(-0.182, -0.004)**	**(-0.217, -0.008)**	**(-0.246, -0.046)**	**(-0.192, -0.025)**
Model II†	-0.022	**-0.126**	-0.047	**-0.096**	**-0.113**	**-0.144**	**-0.110**
	(-0.121, 0.078)	**(-0.213, -0.040)**	(-0.143, 0.049)	**(-0.184, -0.008)**	**(-0.218 -0.008)**	**(-0.243, -0.045)**	**(-0.193, -0.026)**

Abbreviations: B= mean difference; CI= confidence interval; SD= standard deviation

∗ Adjusted for age, gender, race, education level, mean arteriolar blood pressure, fasting blood glucose, total cholesterol, and presence of stroke

†Adjusted for age, gender, race, education level, mean arteriolar blood pressure, fasting blood glucose, total cholesterol, presence of stroke, cerebral microbleeds, total white matter lesion volume, and total brain volume/intracranial volume

CHAPTER 13:

Retinal Neurodegeneration on Optical Coherence Tomography and Cerebral Atrophy

1. INTRODUCTION

Alzheimer's disease is characterized by brain atrophy in the cortical and subcortical grey and white matter especially the hippocampus and entorhinal cortex. Structural neuroimaging has shown that diffuse atrophy is present even in the early stages of dementia.[1-3] Advanced automated segmentation techniques, such as voxel based morphometry, allow quantification of grey and white matter volumes using magnetic resonance imaging (MRI).[4] Grey matter loss is related to progressive mild cognitive impairment (MCI) and conversion to dementia in the MCI group.[5-7] Global and regional grey matter volume measurements are now crucial biomarkers in detecting neuronal loss and progression of cognitive decline. However, MRI remains a time-consuming and expensive technique. Moreover, some patients have contraindications for undergoing MRI such as claustrophobia, cardiac pacemakers, and inability to tolerate the procedure.

As the retina shares developmental, physiological and anatomical features with the brain,[8,9] retinal imaging is now increasingly used in studying neurodegenerative disease. Both histopathological and clinical studies have shown that patients with Alzheimer's disease have functional visual deficits and anatomical changes in retinal structures.[10,11]

Structural changes to the optic nerve can be non-invasively measured *in vivo* using techniques such as spectral domain-optical coherence tomography (SD-OCT). Recent advances in SD-OCT have made it possible to automatically measure the retinal nerve fiber layer (RNFL) and the ganglion cell-inner plexiform layer (GC-IPL). Unmyelinated axons of the retinal ganglion cells form the RNFL, while the GC-IPL contains the cell bodies and dendrites of these cells. Previous studies have linked RNFL thinning to a number of brain diseases, such as Alzheimer's disease,[12-14] Parkinson's disease, and multiple sclerosis.[15-17] However, studies have not examined if neuronal changes in the retina are associated with global or regional cerebral atrophy assessed from MRI.

Therefore, we examined the relationship of RNFL and GC-IPL thickness, with cerebral white and grey matter volumes on MRI in an elderly population from Singapore.

2. METHODS

2.1 Study Population

The on-going Epidemiology of Dementia in Singapore (EDIS) study draws participants from the Singapore Epidemiology of Eye Disease (SEED) study, a multi-ethnic population-based study among persons aged 40 to 85 years. For this study, we focused on participants drawn from the first follow-up examination of the Singapore Malay Eye Study (SiMES) component of the SEED study who had OCT data available.[18] In order to use the limited MRI imaging resources efficiently, it was decided to focus on those subjects who were most likely to have cognitive problems. Hence, in the first phase of the EDIS-SiMES Study, participants from SiMES aged ≥ 60 years (n=1014) were screened using the Abbreviated Mental Test (AMT) and a self-report of progressive forgetfulness. Screen-positive subjects (n=448) were invited to take part in the second phase of this study, which included an extensive neuropsychological test battery and brain MRI. Of these 448 participants, 307 agreed to participate in phase II and hence were included in the present study. Cognitively impaired no dementia (CIND) was defined as impairment in one or more domains in the neuropsychological test battery, as described previously.[19] Dementia was diagnosed in accordance to the Diagnostic and Statistical Manual of Mental Disorders-IV criteria. The details of the study methodology have been described elsewhere.[19] Ethics approval for the EDIS study was obtained from the SingHealth Institutional Review Board and the National Healthcare Group Domain-Specific Review Board. The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained prior to recruitment.

2.2 Neuroimaging

Intracranial volume (ICV), grey matter and white matter volumes were quantified by automatic segmentation at the Erasmus University Medical Center Rotterdam, The Netherlands as mentioned in **Chapter 3**.

2.3 Assessment of Retinal Neuronal Layers

SD-OCT (Cirrus HD-OCT; Carl Zeiss Meditec) macular and optic disc cube scans were obtained from study participants and assessed according to standardized protocol described in **Chapter 3**. Participants were also excluded if they had a diagnosis of glaucoma from study ophthalmologists. Macular GC-IPL thickness and peripapillary RNFL thickness measurements were taken from a randomly selected eye from each participant for analysis. **Figure 13-1** shows detailed cross-sectional image of the retinal layers centered at the macula using a SD-OCT.

2.4 Assessment of Other Vascular Risk Factors

Other vascular risk factors assessment has been described in detail in **Chapter 3**. Axial length for study eyes was measured using IOL Master V3.01 (Carl Zeiss; Meditec AG Jene, Germany).

2.5 Statistical Analysis

Grey and white matter volumes were used as determinants and expressed as per standard deviation (SD) decrease, whereas GC-IPL and RNFL thickness were taken as outcomes. Linear regression models were used to estimate mean change [95% confidence intervals (CI)] in GC-IPL and RNFL thickness per SD decrease in total brain volume, grey matter, and white matter volumes. Models were initially adjusted for age and sex, additionally MABP, blood glucose, serum cholesterol, with axial length and OCT scan signal strength from the eye chosen, and total intracranial volume to correct for head size. RNFL models were additionally adjusted for optic disc area. Separate linear regression models were constructed for occipital, temporal, frontal, parietal lobes and the central regions. All

statistical analyses were performed on standard statistical software (SPSS Version 17, SPSS Inc., USA).

3. RESULTS

Screening and assessments of the Malay cohort was performed from February 2011 to July 2013. Of the 307 subjects who participated in the second phase of the EDIS Study, 143 were excluded from the analysis: 21 had no MRI scans (due to claustrophobia, or contraindications), 14 were diagnosed with glaucoma, 87 did not complete OCT scanning, while 34 had ungradable OCT scans. Comparison of the 164 participants included and 143 participants excluded are shown in **Table 13-1**. In summary, excluded participants were more likely to be older, have diabetes, lower BMI, and more likely to be CIND or dementia. Mean total brain volume was 863.5ml (SD, 89.1ml), mean grey matter volume 507.2ml (SD, 53.2ml), mean white matter volume 357.9ml (SD, 42.1ml) and mean total intracranial volume 1056.0ml (SD, 100.1ml). Mean RNFL thickness was 90.6 μm (SD, 10.3 μm), while mean GC-IPL thickness was 78.8 μm (SD, 7.2 μm).

Decreasing total brain volume was weakly associated with GC-IPL thinning (mean change in GC-IPL per SD decrease in brain volume: -1.26 μm, 95%CI -2.53 to 0.01μm), while decreasing total grey matter volume was more strongly associated with GC-IPL thinning (mean change in GC-IPL per SD decrease in grey matter volume: -1.40μm, 95%CI -2.66 to -0.14μm) in age-sex adjusted models. In contrast, reduction in white matter volume was not significantly associated with GC-IPL thinning (mean change in GC-IPL per SD decrease in white matter volume: -0.80μm, 95%CI -2.02 to 0.41μm). However, these associations became non-significant after additional adjustment for MABP, blood glucose, plasma cholesterol levels, smoking, axial length, OCT scan signal strength and total intracranial volume. Both age-sex and multivariable-adjusted models

for RNFL thickness were not statistically associated with total brain, grey or white matter volume.

Region-specific analyses (**Table 13-2** and **Table 13-3**) showed that decreasing grey matter volume in both occipital and temporal lobes was associated with GC-IPL thinning in both age-sex and multivariable-adjusted models, whereas only decreasing temporal lobe grey matter volume was associated with RNFL thinning independent of risk factors, axial length and intracranial volume. Reduction in grey and white matter volumes in the frontal, parietal lobes and central regions were not significantly associated with GC-IPL or RNFL thickness changes.

When participants with dementia (n=3; all Alzheimer's Disease) were further excluded from analysis, these associations remained unaltered.

4. DISCUSSION

In this study, we found that reduction in grey matter volume in the occipital and temporal lobes was associated with GC-IPL thinning in the retina, independent of systemic vascular risk factors, in elderly persons without glaucoma or clinical retinal diseases. However, there was no evidence of an association between global grey and white matter volumes or regional white matter volumes with GC-IPL and RNFL thickness. This suggests that thinning in the retinal neuronal layers may provide insight into region-specific grey matter atrophy in elderly persons at risk of cognitive decline.

Thickness measurements of the GC-IPL and RNFL are both markers of retinal ganglion cell structural integrity, with the RNFL being mainly composed of retinal ganglion cell axons, whereas the GC-IPL is composed of both the cell bodies and dendrites of the retinal ganglion cells. As reduction in dendritic complexity and area occurs prior to retinal ganglion cell death and loss,[20] this suggests that the GC-IPL may be more informative and sensitive to neurodegenerative damage compared to the RNFL.

Our findings that reduction in grey matter volumes in the occipital and temporal lobes is linked to RNFL and GC-IPL thinning suggest the presence of a neurodegenerative pathway linking these regions of the brain directly to the eye. The visual association cortex in the inferior temporal lobe and the occipital lobes are particularly susceptible to neurofibrillary tangles and amyloid plaque deposition,[21,22] and this correlates with atrophy and reduced metabolism especially in the occipital and temporoparietal regions.[23] As a result, these pathological depositions disrupt connections within the visual tract, potentially causing retrograde neuronal degeneration down the optic nerve,[21] resulting in region-specific relationships between the temporal and occipital lobe atrophy with retinal neuronal damage. Retrograde damage to the optic nerve, and hence retinal ganglion cells, would then be reflected as RNFL and GC-IPL thinning, as shown in a number of clinical studies in mild cognitive impairment and Alzheimer's disease.[12, 14, 24]

The association of occipital and temporal lobe grey matter atrophy with GC-IPL thinning was present in persons with no dementia. In conjunction with how the occipital and adjacent temporal lobes appear to be an early site of amyloid and neurofibril accumulation,[21, 22, 25] this suggests that GC-IPL thinning may reflect involutional changes in these regions of interest even before the onset of dementia. Hence, the GC-IPL shows promise as a novel early biomarker of involutional changes in the brain. Future studies are warranted to further assess if the GC-IPL is useful in tracking grey matter volume changes longitudinally, and if this association is related to cognitive performance and decline.

There are a few limitations to our study. Firstly, about 33.6% of screen-positive subjects did not participate in the second phase of the EDIS study, and of those who participated, nearly 40% of them either did not successfully complete OCT scanning or had scans unsuitable for analysis. Therefore, the generalizability of our study results may be limited. Secondly, the cross-sectional design of the study limits the interpretation of the

results with respect to the cause and effect. Third, visual fields were not tested in our subjects. Hence, we were unable to examine whether retinal thinning was correlated to visual field defects. Fourth, as is the case with any technology (including OCT and MRI), each has its own limitations and contraindications. For OCT, ocular co-morbidities and any inability to undergo OCT assessment form the most important limitations. In terms of prognostic utility, we do not think that OCT on its own will be sufficient to assess involutional changes in the brain. Different and complementary imaging modalities such as the OCT and MRI should be combined to obtain a thorough assessment of involutional changes in the brain. In terms of limitations, this means that subjects with prominent ocular co-morbidities may benefit more from a brain MRI, while subjects with pacemakers, claustrophobia, who cannot undergo MRI, may benefit more from OCT to get an impression of the extent of neurodegneration. The main strengths of our study include direct *in vivo* quantitative assessment of neuronal damage in the retina and the brain using OCT and MRI, and a cohort spanning varying degrees of cognitive impairment status.

5. CONCLUSION

In conclusion, retinal neuronal damage, as reflected by GC-IPL thinning, is independently associated with grey matter loss in the occipital and temporal lobes. In contrast, there was no evidence of an association between global grey and white matter volumes or regional white matter volumes with GC-IPL and RNFL thickness. These findings suggest that changes in retinal ganglion cells as assessed by high-resolution OCT technology may provide novel markers for specific involutional changes in the brain.

CHAPTER 13 – REFERENCES

1. Karas GB, Scheltens P, Rombouts SA, et al. Global and local gray matter loss in mild cognitive impairment and Alzheimer's disease. Neuroimage. 2004;23:708-16.

2. Trivedi MA, Wichmann AK, Torgerson BM, et al. Structural MRI discriminates individuals with Mild Cognitive Impairment from age-matched controls: a combined neuropsychological and voxel based morphometry study. Alzheimers Dement. 2006;2:296-302.

3. Frisoni GB, Testa C, Zorzan A, et al. Detection of grey matter loss in mild Alzheimer's disease with voxel based morphometry. J Neurol Neurosurg Psychiatry. 2002;73:657-64.

4. Whitwell JL. Voxel-based morphometry: an automated technique for assessing structural changes in the brain. J Neurosci. 2009;29:9661-4.

5. Hämäläinen A, Tervo S, Grau-Olivares M, et al. Voxel-based morphometry to detect brain atrophy in progressive mild cognitive impairment. Neuroimage. 2007;37:1122-31.

6. Whitwell JL, Shiung MM, Przybelski SA, et al. MRI patterns of atrophy associated with progression to AD in amnestic mild cognitive impairment. Neurology. 2008;70:512-20.

7. Leung KK, Bartlett JW, Barnes J, et al. Cerebral atrophy in mild cognitive impairment and Alzheimer disease: rates and acceleration. Neurology. 2013;80:648-54.

8. Ikram MK, Cheung CY, Wong TY, Chen CP. Retinal pathology as biomarker for cognitive impairment and Alzheimer's disease. J Neurol Neurosurg Psychiatry. 2012;83:917-22.

9. London A, Benhar I, Schwartz M. The retina as a window to the brain-from eye research to CNS disorders. Nat Rev Neurol. 2013;9:44-53.

10. Iseri PK, Altinaş O, Tokay T, Yüksel N. Relationship between cognitive impairment and retinal morphological and visual functional abnormalities in Alzheimer disease. J Neuroophthalmol. 2006;26:18-24.

11. Chang LY, Lowe J, Ardiles A, et al. Alzheimer's disease in the human eye. Clinical tests that identify ocular and visual information processing deficit as biomarkers. Alzheimers Dement. 2014;10:251-61.

12. Berisha F, Feke GT, Trempe CL, McMeel JW, Schepens CL. Retinal abnormalities in early Alzheimer's disease. Invest Ophthalmol Vis Sci. 2007;48:2285-89.

13. Paquet C, Boissonnot M, Roger F, Dighiero P, Gil R, Hugon J. Abnormal retinal thickness in patients with mild cognitive impairment and Alzheimer's disease. Neurosci Lett. 2007;420:97-9.

14. Kesler A, Vakhapova V, Korczyn AD, Naftaliev E, Neudorfer M. Retinal thickness in patients with mild cognitive impairment and Alzheimer's disease. Clin Neurol Neurosurg. 2011;113:523-6.

15. Garcia-Martin E, Larrosa JM, Polo V, et al. Distribution of retinal layer atrophy in patients with Parkinson disease and association with disease severity and duration. Am J Ophthalmol. 2014;157:470-8.

16. Khanifar AA, Parlitsis GJ, Ehrlich JR, et al. Retinal nerve fiber layer evaluation in multiple sclerosis with spectral domain optical coherence tomography. Clin Ophthalmol. 2010;4:1007-13.

17. Gordon-Lipkin E, Chodkowski B, Reich DS, et al. Retinal nerve fiber layer is associated with brain atrophy in multiple sclerosis. Neurology. 2007;69:1603-9.

18. Rosman M, Zheng Y, Wong W, et al. Singapore Malay Eye Study: rationale and methodology of 6-year follow-up study (SiMES-2). Clin Experiment Ophthalmol. 2012;40:557-68.

19. Hilal S, Saini M, Tan CS, et al. Ankle-brachial index, cognitive impairment and cerebrovascular disease in a chinese population. Neuroepidemiology. 2014;42:131-8.

20. Williams PA, Thirgood RA, Oliphant H, et al. Retinal ganglion cell dendritic degeneration in a mouse model of Alzheimer's disease. Neurobiol Aging. 2013;34:1799-1806.

21. Lewis DA, Campbell MJ, Terry RD, Morrison JH. Laminar and regional distributions of neurofibrillary tangles and neuritic plaques in Alzheimer's disease: a quantitative study of visual and auditory cortices. J Neurosci. 1987;7:1799-1808.

22. Hof PR, Morrison JH. Quantitative analysis of a vulnerable subset of pyramidal neurons in Alzheimer's disease: II. Primary and secondary visual cortex. J Comp Neurol. 1990;301:55-64.

23. Buckner RL, Snyder AZ, Shannon BJ, et al. Molecular, structural, and functional characterization of Alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. J Neurosci. 2005;25:7709-17.

24. Marziani E, Pomati S, Ramolfo P, et al. Evaluation of retinal nerve fiber layer and ganglion cell layer thickness in Alzheimer's disease using spectral-domain optical coherence tomography. Invest Ophthalmol Vis Sci. 2013;54:5953-8.

25. McKee AC, Au R, Cabral HJ, et al. Visual association pathology in preclinical Alzheimer disease. J Neuropathol Exp Neurol. 2006;65:621-30.

CHAPTER 13 – TABLES

Table 13 – 1: Comparison of baseline characteristics of included and excluded participants of this study

Baseline characteristics	Included (n=164)	Excluded (n=143)	p-value*
Age, years (SD)	69.0 (6.5)	73.3 (6.8)	**<0.001**
Males, n (%)	70 (42.7)	69 (48.3)	0.359
BMI, kg/m^2 (SD)	21.0 (4.1)	20.0 (4.0)	**0.045**
Hypertension, n (%)	138 (84.1)	131 (91.6)	0.056
Diabetes, n (%)	44 (26.8)	59 (41.3)	**0.008**
Hyperlipidemia, n (%)	133 (81.1)	112 (78.3)	0.571
Systolic blood pressure, mmHg (SD)	150.1 (19.5)	154.1 (21.6)	0.091
Diastolic blood pressure, mmHg (SD)	79.7 (11.6)	78.4 (12.0)	0.352
Mean arterial blood pressure, mmHg (SD)	101.7 (11.3)	102.8 (11.5)	0.435
Random blood glucose, mmol/l (SD)	6.86 (3.02)	7.51 (3.04)	0.073
Total cholesterol, mmol/l (SD)	5.27 (1.31)	5.17 (1.35)	0.531
Ever Smokers, n (%)	46 (28.0)	44 (30.8)	0.617
Cognitive status			
Not cognitively impaired, n (%)	36 (22.0)	22 (15.4)	**<0.001**
CIND, n (%)	125 (76.2)	101 (70.6)	
Dementia, n (%)	3 (1.8)	20 (14.0)	

Abbreviations: SD= standard deviation; BMI= body mass index; kg/m^2= kilogram per meter square; mmHg= millimeters of mercury; mmol/l= millimoles per liter; CIND= cognitive impairment no dementia

*Bold values signifies p <0.05

Table 13 – 2: Age-sex-adjusted estimated mean change in GC-IPL and RNFL thicknesses (95%CI) per standard deviation change in MRI markers

	GC-IPL thickness B (95%CI), μm	RNFL thickness B (95%CI), μm
per SD decrease in Occipital lobe		
Grey+White matter volume	**-1.83 (-3.05, -0.61)**	**-2.33 (-4.16, -0.51)**
Grey matter volume	**-2.04 (-3.20, -0.88)**	**-2.46 (-4.20, -0.71)**
White matter volume	-0.80 (-2.04, 0.45)	**-1.23 (-3.08, 0.62)**
per SD decrease in Temporal lobe		
Grey+White matter volume	**-2.32 (-3.52, -1.11)**	**-2.77 (-4.58, -0.96)**
Grey matter volume	**-2.55 (-3.71, -1.40)**	**-3.03 (-4.78, -1.28)**
White matter volume	-1.14 (-2.35, 0.06)	-1.40 (-3.20, 0.39)
per SD decrease in Frontal lobe		
Grey+White matter volume	-0.97 (-2.22, 0.28)	-1.35 (-3.20, 0.50)
Grey matter volume	-0.95 (-2.19, 0.30)	-0.99 (-2.84, 0.87)
White matter volume	-0.71 (-1.90, 0.48)	-1.41 (-3.17, 0.35)
per SD decrease in Parietal lobe		
Grey+White matter volume	-1.12 (-2.30, 0.06)	-1.18 (-2.94, 0.58)
Grey matter volume	-1.23 (-2.39, 0.08)	-1.19 (-2.91, 0.53)
White matter volume	-0.73 (-1.91, 0.44)	-0.90 (-2.65, 0.85)
per SD decrease in Central lobe		
Grey+White matter volume	-1.17 (-2.43, 0.09)	-1.76 (-3.63, 0.11)
Grey matter volume	**-1.50 (-2.74, -0.27)**	**-1.94 (-3.78, -0.10)**
White matter volume	-0.51 (-1.70, 0.67)	-1.03 (-2.79, 0.73)

Abbreviations: GC-IPL= ganglion cell inner plexiform layer; RNFL= retinal nerve fiber layer; B= mean difference, CI= confidence interval; μm= micrometer; SD= standard deviation

Table 13 – 3: Multivariable-adjusted estimated mean change in GC-IPL and RNFL thicknesses (95%CI) per standard deviation change in MRI markers

	GC-IPL thickness (95%CI), μm*	RNFL thickness (95%CI), μm*†
per SD decrease in Occipital lobe		
Grey+White matter volume	-1.77 (-6.55, 0.01)	-1.87 (-4.44. 0.69)
Grey matter volume	**-1.78 (-3.20, -0.36)**	-1.72 (-3.79, 0.34)
White matter volume	0.07 (-1.68, 1.81)	-0.27 (-2.79, 2.25)
per SD decrease in Temporal lobe		
Grey+White matter volume	**-3.45 (-5.40, -1.49)**	-2.70 (-2.61, 0.21)
Grey matter volume	**-2.94 (-4.46, -1.41)**	**-2.56 (-4.85, -0.27)**
White matter volume	-0.55 (-2.57, 1.46)	0.14 (-2.76, 3.05)
per SD decrease in Frontal lobe		
Grey+White matter volume	0.05 (-2.67, 2.76)	1.16 (-2.72, 5.03)
Grey matter volume	-0.34 (-3.20, 1.53)	0.59 (-2.09, 3.26)
White matter volume	0.81 (-1.51, 3.14)	0.56 (-2.69, 3.82)
per SD decrease in Parietal lobe		
Grey+White matter volume	-0.24 (-2.72, 2.23)	2.83 (-0.75, 6.43)
Grey matter volume	-0.88 (-2.79, 1.03)	0.93 (-1.85, 3.71)
White matter volume	0.67 (-1.27, 2.61)	2.18 (-0.56, 4.93)
per SD decrease in Central lobe		
Grey+White matter volume	-0.06 (-2.56, 2.44)	-0.57 (-4.16, 3.03)
Grey matter volume	-1.10 (-2.80, 0.60)	-1.28 (-3.73, 1.16)
White matter volume	1.02 (-0.80, 2.83)	0.77 (-1.85, 3.39)

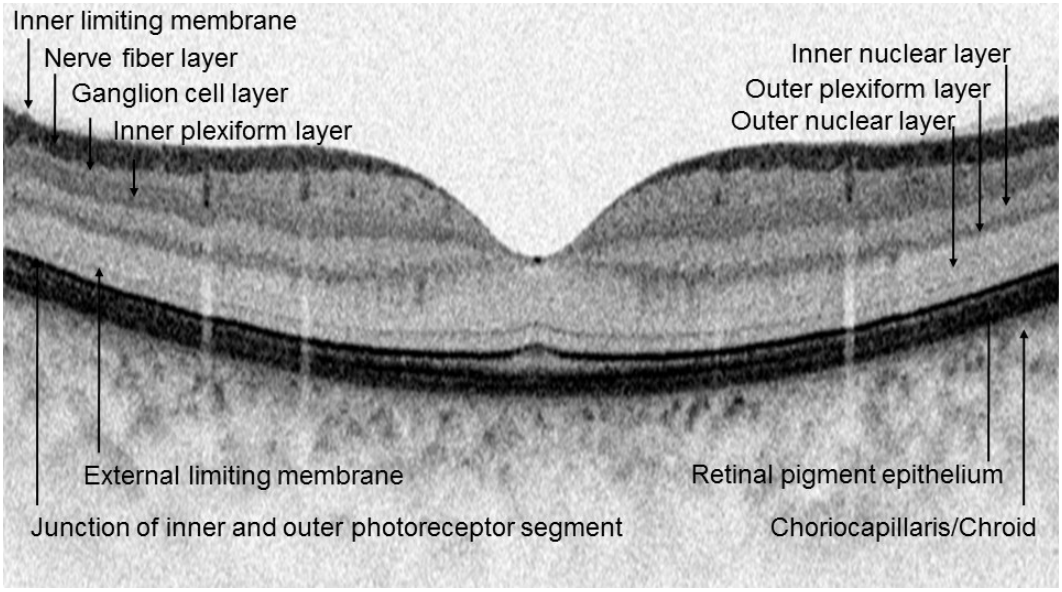
Abbreviations: GC-IPL= ganglion cell inner plexiform layer; RNFL= retinal nerve fiber layer; B= mean difference, CI= confidence interval; μm= micrometer; SD= standard deviation

*Adjusted for age, sex, age, gender, mean arterial blood pressure, plasma blood glucose, serum cholesterol, smoking, axial length, OCT signal strength and total intracranial volume.

† Additionally adjusted for optic disc area.

CHAPTER 13 – FIGURE

Figure 13 – 1: Detailed retinal layers of a cross-sectional SD-OCT image centered at the macula



CHAPTER 14:

SYNTHESIS, IMPLICATIONS AND FUTURE PERSPECTIVES

SYNTHESIS

One of the main goals of this thesis was to explore markers of cerebrovascular diseases and involutional changes on MR and retinal imaging and to subsequently link them with cognitive dysfunction. A range of cerebral markers and retinal parameters were identified and evaluated using visual and automated approaches in a large population based cohort and in a diseased population. A schematic representation of these markers identified through visual and quantitative techniques are summarized in **Figure 14 – 1** and **2**. Subsequently, these markers/parameters were applied to study several other manifestations of brain changes in the context of aging, cerebrovascular diseases and cognitive impairment. A summary of these findings and their link with cognition is shown in figure **14 – 3**. In the following sections, I will first synthesize my main findings from **chapters 4 – 13** and will show their joint effects using Principle Component Analysis (PCA). I will also suggest a cerebrovascular disease burden score and will assess its clinical correlates with respect to cognitive impairment and dementia. Finally, I will expand on the implications of these markers which are then followed by future perspectives and recommendations.

1. SYNTHESIS OF MAIN FINDINGS

The main findings described in the chapters above are discussed in the same order in which they appear in this thesis; first the MRI markers of cerebral small and large vessel diseases, followed by markers of involutional changes and their effects on cognition and finally retinal microvascular and neuronal markers of cerebrovascular diseases and involutional changes. A summary of these imaging markers has been presented in **Figure 14 – 4**.

1.1 Cerebral Small Vessel Disease Markers

Findings from both EDIS and memory clinic studies have shown that the cerebral small vessel disease as reflected by cerebral microbleeds and cortical microinfarcts (CMIs) were not only related to clinical outcomes (cognitive impairment and dementia) but were also associated with the worse performance on cognitive testing. This high lights the importance of microvascular pathology in cerebrovascular disease related cognitive decline. This is in line with other findings on established markers of cerebrovascular diseases such as lacunes and white matter hyperintensities which often co-exist in Alzheimer's pathology and in vascular dementia.

Age, gender, cardiovascular risk factors (hypertension, hyperlipidemia and diabetes), stroke and atrial fibrillation were the major risk factors of CMIs. The most striking findings were that CMIs which were previously reported to be a manifestation of small vessel disease were also related to large vessel disease. This shows that CMIs represent proxies for either small or large vessel infarcts or even diffuse cerebral injury through arteriosclerosis, microembolism and hypoperfusion. Using a detailed neuropsychological assessment, we further showed that the reduced performance in global cognitive scores together with domains of executive function, language, visuomotor speed, verbal and visual memory were associated with cerebral small vessel disease markers. However, in the memory clinic setting, this association was attenuated in the presence of atrophy suggesting common pathways involved in CMIs and atrophy. Our study findings from both EDIS and case control studies, therefore supports a major step in developing the non-invasive means of detecting CMIs on MRI scans during life and further unravel their role in aging and dementia.

1.2 Cerebral Large Vessel Disease Marker

In the population based study (EDIS), we reported that the intracranial stenosis influence cognition through ischemic changes in the brain. On the formal neuropsychological tests, we found that the persons with ICS were more likely to have significant cognitive impairment and performed worse in the domains of executive function, language, visuomotor speed, verbal and visual memory albeit in the presence of infarcts. From the memory clinic study, we also showed that intracranial stenosis was associated with vascular cognitive impairment, and with dementia subtypes i.e. AD and Vascular dementia (VaD). The association with dementia became attenuated in the presence of white matter hyperintensities and infarcts. This suggests that the cerebral ischemia (through possible perfusion deficits) remains the common mechanism behind ICS and cognition. Moreover, despite having different etiologies, the association of ICS with different subtypes of dementia suggests that similar underlying micro- and macrovascular pathologies exist in both AD and cerebrovascular disease related cognitive decline. Hence, in summary both studies have shown that ICS effects cognition through cerebral ischemic damage.

1.3 Markers of involutional changes

We have used quantitative MRI techniques to segment cortical thickness and subcortical structure volumes and have reported their risk factors and relation to cognitive impairment and dementia. The major common risk factors were found to be age, gender, Malay ethnicity, diabetes and lacunar infarcts thus, high lighting the fact that these cortical and subcortical structures are sensitive to increasing age, hormonal effects, ethnic differences, systemic diseases and small vessel diseases. Moreover, we have also reported independent association of global and lobar specific region thinnings and subcortical structural volumes with severe cognitive impairment (CIND moderate and dementia). Analysis with neuropsychological testing has further confirmed our hypothesis that

involutional changes in the gray matter (cortex and deep gray matter nuclei) takes place early in the process of dementia due to the possible progression of tau and amyloid pathology from the hippocampus to the rest of the brain.

Our most interesting finding was a specific pattern of subcortical volume reduction in vascular vs. non vascular cognitive impairment. Smaller caudate and pallidum were specific to vascular cognitive impairment as they are the common sites of subcortical lacunes whereas the amygdala was particularly affected in non-vascular cognitive impairment due to increased sensitivity to AD type neuropathology. However, the association of other subcortical structures with cognitive impairment has suggested that subcortical structures are equally affected in both vascular and non-vascular cognitive impairment.

1.3 Retinal markers

Microvascular Markers

Besides the visual assessment of retinopathy signs which have been extensively studied previously, we have utilized the automated image analysis technique which can quantify subtle changes in the retinal microvasculature by identifying changes in retinal vessel calibers, fractal dimension and tortuosity. We have found that the retinal venular widening, smaller arteriolar fractals and increased arteriolar tortuosity were associated with cerebral microbleeds on the MRI scans. Compared with previous studies where lacunes and white matter hyperintensities were linked to retinopathy, we also found the association of cerebral microbleeds with retinal microvascular changes suggesting that microbleeds may be a possible early marker of cerebrovascular disease.

In terms of the clinical outcome, we have also reported an association of reduced arteriolar and venular fractals with mild and moderate cognitive impairment. On detailed

neuropsychological assessment, we also showed that reduced retinal arteriolar and venular fractals were related to worse performance on verbal memory, visuoconstruction and visuomotor speed domains, thus further confirming that the retinal microvascular changes occur in preclinical cognitive impairment.

Neuronal Markers

Spectral domain optical coherence tomography was used to quantify retinal nerve fiber layer (RNFL) and ganglion cell inner plexiform layer (GC-IPL) and hence these were examined in relation to cognitive impairment. We found that the GC-IPL thinning was independently associated with cerebral atrophy in the occipital and temporal region whereas RNFL was related only to temporal atrophy. These findings suggest that the GC-IPL show promise as an early and sensitive marker of involutional changes in the brain.

2. LINKING MARKERS OF CEREBROVASCULAR DISEASES

As mentioned previously, the aim of the work described in this thesis was to focus on markers of CeVD and involutional changes using both cerebral and retinal imaging and assess the clinical correlates of these lesions with cognitive impairment and dementia. Interestingly, all the markers showed a dose response relationship with increasing severity of cognitive impairment. The consistent pattern observed in the association of CeVD markers with cognitive function specifically in the domains of executive function, visuoconstruction, language and memory reflects underlying vascular mechanisms in cognitive dysfunction.

Although my previous chapters (**4-13**) were concentrated on individual marker of CeVD, there is a need to explore the joint effect of all markers of CeVD on cognitive dysfunction. Previous data has suggested that small vessel diseases such as WMH, lacunes, and microbleeds often occur together and are inversely associated with cognitive

impairment. As these CeVD markers produce similar features of brain damage on imaging, it is appropriate to explore the inter-correlations between these variables. It is likely that markers with similar underlying pathology might co-exist and affect cognitive ability in the elderly.

Principle component analysis

Validated visual scores of MRI markers for CeVD (lacunes, WMH, microbleeds, intracranial stenosis, cortical infarcts and CMIs) were used to identify the clusters of MRI markers in the Epidemiology of Dementia In Singapore study (EDIS). We first performed principal component analysis (PCA) to reduce the selected six MRI markers into principal components (PCs). PCA revealed two PCs: PC1 was mainly driven by CMIs, cortical infarcts, and intracranial stenosis whilst PC2 by WMH, lacunes and microbleeds. The largest variance in PC1 was contributed by CMIs whereas in PC2, it was mainly driven by WMH. After identifying these components, we further performed regression analysis with cognition. In multivariate adjusted models, both PC1 and PC2 were independently linked with cognition (**Table 14 – 1** and **14 – 2**). On adding the two components into the same model, an independent association was observed with CIND-moderate/dementia for both PC1 [Odds ratios (OR): 2.39; 95%CI: 1.26-4.56] and PC2 [OR: 1.77; 95%CI: 1.11-2.81]. A similar association was also observed with composite Z scores (**Table 14 – 3**).

Based on the above findings, a total CeVD measure may therefore better account for the global effect of CeVD on brain than the individual MRI features. Hence, it would be ideal to provide not only an efficient measure to assess the global CeVD burden but also to account for the individual contribution of small and large vessel diseases on cognition

using a weighted assessment which can be feasibly administered in a clinical and research setting.

CeVD burden score

In order to explore whether a weighted assessment of total CeVD burden can improve understanding of the cognitive consequences of CeVD, we first performed regression analysis between different markers of CeVD. The results showed that the presence of WMH was significantly correlated with multiple lacunes (φ=0.28; P<0.001) and multiple microbleeds (φ=0.27; P<0.001). In addition, multiple lacunes were associated with multiple microbleeds (φ=0.27; P<0.001) and cortical infarcts (φ=0.28; P<0.001). Intracranial stenosis was associated solely with cortical infarcts (φ=0.30; P<0.001). Moderate-to-severe WMH was independently associated with lower global cognition (β [SE]=−0.21 [0.16]; P<0.001). Other CeVD markers did not show an independent association with global cognition. Moreover, the presence of any ≥2 CeVD indicators was found to be significantly associated with worse global cognitive performance (β [SE]=−0.09 [0.18]; P<0.01), independent of WMH.

On the basis of linear regression coefficient established between CeVD indicators and cognitive performance as above, we devised a weighted CeVD burden score with 2 points awarded when moderate or severe WMH was present and one point awarded when at least 2 CeVD markers were present.

Hence, a 4-category CeVD burden score was generated as suggested below:

None/very mild CeVD burden score, 0= none/mild WMH and <2 other CeVD indicators.
Mild CeVD burden score, 1= none/mild WMH and ≥2 other CeVD indicators.
Moderate CeVD burden score, 2= moderate/severe WMH and <2 other CeVD indicators.
Severe CeVD burden score, 3= moderate/severe WMH and ≥2 other CeVD indicators.

An inverse association of CeVD burden score with global cognitive score and domain-specific cognitive performance is shown in **figure 14 – 5**. Poor cognitive score was associated with moderate CeVD, whilst severe CeVD showed further compromised cognitive performance in language (p=0.003) and visuomotor speed (p=0.007) domains. Moreover, a more impaired visual memory score was found in cases with severe CeVD compared to moderate CeVD (p=0.006). The association of the total CeVD burden score with cognition suggests that there is a threshold effect: when moderate CeVD burden is reached, global cognitive dysfunction begins to occur.

In summary, we provided two measures of MRI burden. First from the PC analysis which showed two components comprising MRI markers of small and large vessel diseases are equally important in cognitive dysfunction and secondly from the simple and pragmatic CeVD burden score, defined by a combination of WMH and other small and large vessel disease markers, which is inversely related to global cognitive status. This further confirms that the individual MRI markers are jointly indicative of an underlying CeVD and hence might be useful in tracking their impact on cognition and disease over time. Our results imply a cumulative effect of different CeVD markers on general cognitive ability, possibly reflecting more extensive vascular pathology in the brain that can be estimated using a CeVD burden score.

These results are promising and have already provided valuable clues on the role of microvascular changes in CeVD and cognitive dysfunction.

3. IMPLICATIONS OF STUDY FINDINGS

This thesis has highlighted the brain imaging biomarkers of cerebrovascular disease and involutional changes involved in cognitive impairment and dementia. Moreover, I have also shown that the retinal imaging can be employed as a complimentary tool in studying

microvascular and involutional changes in the cerebral vascular and degenerative diseases.

3.1 Cerebrovascular Disease Markers

As mentioned in **chapters 1** and **2**, cerebrovascular diseases are a major cause and contributors to cognitive impairment and dementia. However, the exact mechanism underlying cerebrovascular disease pathology and its functional consequences (cognitive impairment) remains unknown. Currently, there are no adequate treatment options available. Furthermore, it is also unclear why some patients with cerebrovascular diseases develop cognitive dysfunction while others do not. Moreover, as Asian populations are reported to have a higher burden of vascular risk factors and cerebrovascular diseases compared to Caucasians, it is imperative to identify reliable markers of cerebrovascular diseases before an individual develop dementia in order to facilitate development of new treatments and prevention strategies.

Brain Markers of Vascular Changes

Cerebral microbleeds and CMIs have emerged as new imaging markers of cerebral small vessel disease besides the other conventional MRI visible lesions (lacunes and white matter hyperintensities). The development of higher resolution and sensitive sequences has sparked scientific and clinical interests in these lesions due to their possible link with cognitive dysfunction. It has previously been argued that established cerebrovascular MR lesions did not fully capture the burden of the total cerebrovascular disease pathology as the observed associations with clinical outcomes are often inconsistent and weak.[1] Hence, microbleeds and CMIs could potentially account for the unexplained variances observed in the previous studies. Furthermore, as both these lesions can be feasibly detected on 3Tesla as shown by our studies, there has been an effort to understand the clinical

relevance of these lesions in research settings. As described in this thesis, we were able to describe their prevalence and demonstrated their link with cognitive dysfunction independent of other cerebrovascular disease markers. Hence, microbleeds and CMIs are potentially early markers of cerebrovascular diseases and are related to cognitive decline and dementia.

Besides the cerebral small vessel diseases, intracranial stenosis (ICS) secondary to large artery atherosclerosis has been reported to be highly prevalent in Asians due to higher prevalence of vascular risk factors. The prevalence of ICS in Chinese is much higher (40-50%) compared to Caucasians (8-10%).[2] In this context, both EDIS and case control studies provide an ideal platform to study the prevalence of ICS not only in asymptomatic Chinese subjects but also in vascular cognitive impairment, AD, Vascular dementia. We have already shown that ICS leads to cognitive dysfunction in the presence of cerebral ischemic changes (infarcts and white matter hyperintensities). The cognitive deficits develop as a result of microcirculation defects, increased resistance in the small blood vessels and reduced vascular reactivity leading to cerebral hypoperfusion. This hypoperfusion not only triggers but may also accelerate neurodegenerative process by facilitating amyloid beta deposition.[3] This further suggests that the ICS does not directly reduce cognitive functioning but in fact is a marker of cerebral or generalized atherosclerosis.[4]

Retinal Markers of Vascular Changes

We have demonstrated retinal imaging to be a simple and feasible tool for studying microvascular changes in cerebrovascular diseases and cognitive impairment. Semi-automated techniques for analyzing retinal photography are able to quantify subtle changes in the retinal vessels. Quantiative parameters such as fractal dimension and

tortuosity are of particular interest as they are not affected by pulse wave velocity unlike vessel calibers. The association of reduced fractal dimension with cerebral microbleeds in our study suggests that this might be an early and sensitive marker of cerebrovascular damage in the brain. Retinal vascular changes mirror changes in the cerebral microvasculature through impaired vascular perfusion, vessel wall dysfunction and blood retinal-brain damage. Moreover, the association of smaller fractal density with preclinical cognitive impairment suggests that cerebral hypoperfusion occurs relatively early in the course of cognitive dysfunction. This further confirms that microvascular damage is common in both cerebrovascular diseases and cognitive impairment.

Hence, based on our results on brain and retinal vascular markers, we have consistently shown that vascular pathology is indeed an important contributor to cognitive impairment and dementia. This further suggests that cerebrovascular diseases should be accounted for in all the studies focusing on cognitive impairment, AD and vascular dementias.

3.2 Markers of involutional changes

Neurodegeneration remains the pathological hall mark of AD and is even observed in preclinical cognitive impairment and normal aging. This process encompasses neuronal loss in both cortical and subcortical structures. Although atrophy is generally considered a late biomarker for AD, it is the most versatile and prominent marker of neuronal changes in the brain. Hence it is viable to detect subtle changes in the brain which could potentially serve as useful markers for tracking diseases progression.

Brain Markers of Neuronal Loss

Robust, automated procedures have made it possible to segment gray matter in brain parenchyma which is present in both cerebral cortex and deep subcortical structures. Age,

sex, race, cardiovascular risk factors and other cerebrovascular disease markers, all triggers and affects the involutional changes as shown by our findings from the population based study. Moreover, pathological changes possibly induced by risk factors in both cortical and subcortical structures gives rise to wide range of cognitive deficits as reflected by reduced cognitive performance. The improved techniques of detecting subtle cortical thinning and subcortical structure volumes offer insight into early degenerative damage and further confirm that these changes are indeed taking place much earlier in the dementia cascade. Moreover, the association of subcortical atrophy in both vascular and non-vascular cognitive impairments suggests vascular insufficiency promoting neurodegeneration or vice versa, thus shedding light onto an additive or synergistic effects between AD pathology and cerebrovascular diseases.

Retinal Marker of Neuronal Loss

Development in the Optical Coherence Tomography (OCT), has resulted in the segmentation of two key neuronal layers; Retinal Nerve Fiber Layer (RNFL) and Ganglion Cell Inner Plexiform Layer (GC-IPL). Thinning of the GC-IPL was related to both the occipital and temporal atrophy in the brain, suggesting that the GC-IPL is a sensitive marker for involutional changes. By using OCT, we have demonstrated that cerebral atrophy, measurement of which requires expertise and depends on expensive technique, is now easily accessible. Hence, changes in the retinal ganglion cell can serve as biomarkers for region specific involutional changes in the brain.

Thus, these sensitive cerebral and retinal neuronal markers can reflect underlying cerebral neuronal damage resulting from AD or cerebrovascular disease related pathology and for tracking disease progression.

4. STRENGTHS AND LIMITATIONS

Strengths and limitations pertaining to EDIS and case-control studies have been described in detail in each chapter. In the following sections, I highlight the common strengths and limitations relevant to the two studies.

4.1 Strengths

The major strengths of the studies include use of the large population based sample (EDIS), detailed neuropsychological assessment to diagnose cognitive impairment and dementia and availability of 3T MRI scans to grade and classify individuals based on the cerebrovascular diseases. Both studies had standardized protocols to assess MRI and retinal quantitative changes using semi-automated and automated techniques which ensured direct comparison between the two studies. All the final analyses were adjusted for possible confounders to show independent effects of imaging markers with cognition.

4.2 Limitations

Although efforts were made to minimize the potential source of bias in the two studies, there still remain some common limitations which can contribute to following errors:

Temporal Relationship

In both the EDIS (cross-sectional) and case control studies, it was difficult to determine the temporal relationship between MRI and retinal imaging markers with cognitive dysfunction which limits the interpretation of the study findings.

Selection Bias

In the EDIS study, 47.2% of the screened positive subjects did not participate in the second phase of the study. Compared to the participants, the non-participants were

relatively older, less educated, and more likely to have hypertension. Hence it is likely that they might have had poor cognitive function and/or had higher prevalence of cerebrovascular diseases which could lead to underestimation of effect sizes. Moreover, of those who participated, nearly 40% did not complete OCT scanning or had ungradable scans for analysis. Therefore, the generalizability of our results may be limited. Moreover it would have been ideal to perform the MR imaging in individuals who were cognitively normal (passed AMT and PFQ). This would have allowed us to report on the prevalence of incidental findings of cerebrovascular diseases in cognitively asymptomatic individuals.

In relation to case control study, cases and half of the controls were recruited from two locations i.e. memory clinic and the community. The control group consisted of relatively younger subjects who had less burden of cerebrovascular diseases on their MRI compared to cognitively impaired individuals. Also there was a higher burden of vascular risk factors (hypertension, hyperlipidemia and diabetes) in this sample which limited the generalizability of our results to the general population.

Information Bias

The classification of cognitive outcomes (CIND and dementia) together with functional loss was corroborated using clinical history which was subjected to underreporting from both the patients and caregivers, leading to information bias. In terms of retinal imaging, as only one eye was randomly selected without taking into account the severity of the disease in either of the eye, this may have led to misclassification bias.

Confounding

Even though standardized procedures were used for assessments (collection of risk factors, MRI markers and cognitive assessments) in both the studies, there were still

differences in the definition of cardiovascular risk factors (medical history vs. single measurement of blood pressure, cholesterol and blood sugar) which could have resulted in residual confounding. Other possible causes of residual confounding include adjustment for categorical variables instead of the continuous ones and the presence of unknown confounders which might not have accounted for in both the studies.

5. FUTURE PERSPECTIVES AND RECOMMENDATIONS

Throughout the thesis, I have consistently demonstrated that both the brain and retinal imaging markers can provide novel insights into the pathophysiology of cerebrovascular disease and cognitive impairment. However, there are still other areas that need to be explored and requires attention.

5.1 Structural to Functional Parameters

Our present findings were based on the structural changes on both MR and retinal imaging which usually indicate irreversible damage. Future studies focusing on the functional or dynamic biomarkers would be able to help uncover early pathological mechanisms behind cerebrovascular diseases and involutional changes. The future directions and recommendations of each biomarker are given below;

Magnetic Resonance Imaging (MRI)

Cerebral Microbleeds and Susceptibility Mapping Images

Susceptibility weighted imaging which is extensively used to grade microbleeds is limited by undesirable artifacts created by veins and calcium depositions. Addition of MR phase data or quantitative susceptibility mapping images which identify true iron containing lesions, can provide better tool for diagnosing microbleeds.[5, 6] They could also be used in conjunction with Pittsburgh compound B Positron Emission Tomography

(PiB-PET)[6] which better identifies amyloid deposition and hence might be able to provide the clinical relevance of these lesions in relation to CAA.

Cerebral Microinfarcts, Perfusion and White Matter Integrity

Our findings provide clues that CMIs are possible expressions of both small vessel and large vessel diseases which can be the result of a) small vessel changes, b) microemboli secondary to atrial fibrillation or stenosis in an upstream vessel, or c) hypoperfusion. Future studies can benefit from the use of transcranial doppler (TCD) or arterial spin labeling (ASL) technique with brain density maps to help unravel the perfusion deficits in the whole brain and in area surrounding the lesion. Another interesting aspect to explore the link between CMIs and heart would be to determine its link with cardiac biomarkers (brain natriuretic peptide, troponin T and growth differentiation factor 15) of ischemic heart disease, atrial fibrillation and congestive heart failure in conjunction with TCD and echo cardiogram.

Moreover, the eventual outcome of CMIs on cognition remains mixed as evident from our samples and previous study.[7] Do CMIs directly disrupt neuronal connections or co-occur in parallel with other cerebrovascular diseases to produce cognitive dysfunction remains unknown? A combination of structural sequences together with its effect on white matter integrity using diffusion tensor imaging (DTI) or functional connectomes can help unravel the true meaning of these lesions. Moving on from cortical to subcortical microinfarcts might be another useful strategy to understand complete burden of cerebrovascular diseases in cognitive impairment and dementia.

Intracranial Stenosis and Cerebral Hemodynamics

We have demonstrated the effects of intracranial stenosis on cognitive performance through cerebral ischemic damage. Future studies on intracranial stenosis can benefit

from functional imaging such as perfusion techniques (ASL) which is able to detect alterations in the cerebrovascular reactivity in both the symptomatic and asymptomatic individuals. Detection of cerebral hemodynamics through perfusion may serve as a useful strategy in the management of subjects with carotid stenosis even in the absence of signs or symptoms of cerebrovascular disease.[8] However, the contemporary medical therapy (antiplatelet and statin therapy) vs. surgical endarterectomy as means of treatment for asymptomatic carotid stenosis remains inconclusive. Other valuable methods to identify high-risk asymptomatic carotid stenosis besides MRA is the transcranial Doppler embolus detection, advanced carotid imaging and plaque characterization.[9] These various imaging parameters can be combined to estimate the individual risk of ischemia.[10, 11] However, their added value needs to be confirmed in large, multicenter studies.

Cortical Thickness, Subcortical Structural Volumes and White Matter Integrity

The automated segmentation tool has the advantage of being stable due to the cytoarchitectural features of the gray matter and thus is a desirable measure of disease related subtle alterations.[12] Mesaures of cortical thinning and decreasing subcortical structure volume can serve as reliable tools to identify MCI from AD and it can even detect, at risk patients, of converting into dementia. Future studies can reliably use these techniques in conjunction with tractography and voxel based methods utilizing multiple novel analysis approaches. These will include remote effects of cortical thinning in relation to subcortical infarcts,[13] localization of white matter hyperintensities on white matter tracts and their effects on cognitive domains using lesion symptom mapping approach[14] and relation of cortical thickness and subcortical structure volume with white matter integrity using Diffusion Tensor Imaging (DTI) and functional MRI.[15]

Retinal Imaging

Retinal imaging has the potential to study cerebrovascular diseases and involutional changes in the brain. We have demonstrated the association of structural retinal imaging in relation to cerebral small vessel and degenerative changes and preclinical cognitive impairment. The potential of retinal imaging biomarkers for screening, prognostic and assessment purposes need to be further evaluated in future prospective studies.

Retinal Microvascular Changes and Cerebral Hemodynamics

Using combined retinal vascular parameters (calibers, fractal dimension and tortuosity) instead of individual marker, might be better in providing specificity and etiologies for different subtypes of dementia and other diseases (cardiovascular and kidney diseases). Moreover, utilizing functional biomarkers from retinal vascular imaging which includes dynamic vessel analyzer,[16] doppler flowmetry[17] and retinal oximeter[17] may be able to provide insights into cerebral hemodynamics in cerebrovascular diseases and dementia.

Retinal Neuronal Changes and Cerebral Involutional Changes

According to our findings, GC-IPL thinning was more related to cerebral gray matter loss compared to RNFL. The latter comprises axonal processes of the ganglion cell and hence may be more related to white matter changes. This might be better assessed at the microstructural level i.e. white matter integrity using DTI and functional connectome. Adaptive optics in combination with OCT may be able to provide detailed retinal structural images (nerve bundle layers, capillaries and photoreceptors) and hence can be used to study amyloid plaque deposition and dynamics.[18]

5.2 Longitudinal Design

As mentioned in the paragraph of limitations, our findings were based on the cross sectional and case-control data which limits the cause-effect relationship between the

imaging markers and cognitive impairment. Future population based studies using a prospective longitudinal design can provide more insights on the temporal relationship of these MRI (microbleeds, microinfarcts and ICS) and retinal defined structural changes (microstructural and neuronal layer chnages) with the risk of other cerebrovascular diseases, cognitive decline and conversion of MCI to dementia.

5.3 Composite CeVD markers

Integrating results from PCA and joint CeVD burden score can direct studies towards a better understanding of CeVD and should be validated across cohorts with wide age ranges, longitudinal design and in animal models. Specifically, studies across a wider age range may provide insights into the temporal sequence of CeVD markers, whilst longitudinal studies are necessary to demonstrate causality. Animal models are required to investigate mechanisms and targets for therapies which will eventually justify interventional clinical trials.

5.4 Furture extension of current work

Apart from above recommendations, I also suggest the following as future extension of the current work:

1. Determine interaction between markers of cerebral small vessel diseases and explore their synergistic effect on cognitive dysfunction,
2. A meta-analysis on the previous literature based on these markers and how they differ from current findings,
3. Evaluation of the causal link between markers of cerebrovascular diseases/neurodegeneration and cognition using Structural Equation Modelling and path analysis,

4. Longitudinal extension of the current work by repeating followup of the study participants
5. Adding markers of involutional changes and retinal imaging in both PCA and CeVD burden score whihc might be useful to capture complete CeVD burden.

5.5 Translating Imaging Biomarkers into Clinical Practice

As cerebrovascular diseases remains the most common cause of cognitive impairment and dementia, it seems prudent that, its detection during life requires attention. Due to availability of 3T MRI scans in clinical settings, I recommend that the detection of micobleeds, microinfarcts and intracranial stenosis should routinely be conducted in patients presenting in the clinics with symptoms of cognitive impairment, besides the infarcts and white matter hyperintensities. Moreover, they should also be screened in subjects who are at risk of cognitive decline and dementia. In addition to MR scanning, retinal imaging could potentially be used as a research tool to provide a better picture of the underlying cerebrovascular diseases and involutional changes. Lastly, both cerebral and retinal imaging markers can provide new clues for the implementation of therapeutic and preventive interventions in clinical trials. In particular, these imaging biomarkers can be used as a “surrogate” for preclinical brain diseases to show efficacy of a drug in a trial setting and to identify and select individuals who will gain most from the use of particular drugs or interventions.

5. CONCLUSION

Cerebrovascular diseases are a collective term including both small and large vessel alterations and cerebral atrophy. In this thesis, I have investigated the MRI manifestation of cerebrovascular diseases (microbleeds and intracranial stenosis) and uncovered clinical implications of a new imaging marker (CMIs). Specifically, I was able to show the

impact of the cerebrovascular disease markers on cognition and also identify their major risk factors. Furthermore, I have also shown that the retinal imaging is an additional imaging tool in aiding our understanding of the mechanism behind cerebrovascular and involutional changes. Future studies should be directed towards understanding the longitudinal implications of these cerebrovascular disease markers, to the pathophysiological mechanism that drives the formation of these markers, and their impact on structure and function of aging brain. This will direct the studies towards the development of new treatment and prevention strategies of cognitive decline and dementia.

CHAPTER 14 – REFERENCES

1. Smith EE, Schneider JA, Wardlaw JM, Greenberg SM. Cerebral microinfarcts: the invisible lesions. Lancet Neurol. 2012;11:272-82.

2. Li H, Wong KS. Racial distribution of intracranial and extracranial atherosclerosis. Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia. 2003;10:30-4

3. Thiel A, Cechetto DF, Heiss WD, Hachinski V, Whitehead SN. Amyloid burden, neuroinflammation, and links to cognitive decline after ischemic stroke. Stroke. 2014;45: 2825-9.

4. Mathiesen EB, Waterloo K, Joakimsen O, Bakke SJ, Jacobsen EA, Bonaa KH. Reduced neuropsychological test performance in asymptomatic carotid stenosis: The Tromso Study. Neurology. 2004;62:695-701.

5. Klohs J, Deistung A, Schweser F, et al. Detection of cerebral microbleeds with quantitative susceptibility mapping in the ArcAbeta mouse model of cerebral amyloidosis. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2011;31:2282-92.

6. McAuley G, Schrag M, Barnes S, et al. Iron quantification of microbleeds in postmortem brain. Magnetic resonance in medicine. 2011;65:1592-1601.

7. van Veluw SJ, Heringa SM, Kuijf HJ, Koek HL, Luijten PR, Biessels GJ. Cerebral cortical microinfarcts at 7Tesla MRI in patients with early Alzheimer's disease. Journal of Alzheimer's disease : JAD. 2014;39:163-7.

8. Fearn SJ, Hutchinson S, Riding G, Hill-Wilson G, Wesnes K, McCollum CN. Carotid endarterectomy improves cognitive function in patients with exhausted cerebrovascular reserve. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2003;26:529-36.

9. Singh TD, Kramer CL, Mandrekar J, Lanzino G, Rabinstein AA. Asymptomatic Carotid Stenosis: Risk of Progression and Development of Symptoms. Cerebrovasc Dis 2015;40:236-43.

10. Naylor AR. Time to rethink management strategies in asymptomatic carotid artery disease. Nature reviews Cardiology. 2012;9:116-24.

11. Naylor AR, Schroeder TV, Sillesen H. Clinical and imaging features associated with an increased risk of late stroke in patients with asymptomatic carotid disease. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2014;48:633-40.

12. Singh V, Chertkow H, Lerch JP, Evans AC, Dorr AE, Kabani NJ. Spatial patterns of cortical thinning in mild cognitive impairment and Alzheimer's disease. Brain. 2006;129:2885-93.

13. Duering M, Righart R, Wollenweber FA, Zietemann V, Gesierich B, Dichgans M. Acute infarcts cause focal thinning in remote cortex via degeneration of connecting fiber tracts. Neurology. 2015;84:1685-92.

14. Biesbroek JM, Kuijf HJ, van der Graaf Y, et al. Association between subcortical vascular lesion location and cognition: a voxel-based and tract-based lesion-symptom mapping study. The SMART-MR study. PloS one. 2013;8,e60541.

15. Bennett IJ, Madden DJ. Disconnected aging: cerebral white matter integrity and age-related differences in cognition. Neuroscience. 2014;276:187-205.

16. Frost S, Kanagasingam Y, Sohrabi H, et al. Retinal vascular biomarkers for early detection and monitoring of Alzheimer's disease. Translational psychiatry. 2013;3,e233.

17. Harazny JM, Raff U, Welzenbach J, et al. New software analyses increase the reliability of measurements of retinal arterioles morphology by scanning laser Doppler flowmetry in humans. J Hypertens. 2011;29:777-82.

18. Lombardo M, Serrao S, Devaney N, Parravano M, Lombardo G. Adaptive optics technology for high-resolution retinal imaging. *Sensors.* 2013;13:334-66.

Table 14 - 1: Association of component 1 with cognition

	CIND mild (n=173) OR (95%CI)	CIND moderate (n=202) OR (95%CI)	CIND moderate/Dementia (n=230) OR (95%CI)	Composite Z scores (n=579) Mean difference (95%CI)
Model I	1.53 (0.92 - 2.53)	**3.06 (1.64 – 5.69)**	**3.17 (1.71 – 5.87)**	**-0.16 (-0.23; -0.10)**
Model II	1.24 (0.75 - 2.04)	**2.50 (1.31 – 4.79)**	**2.55 (1.33 – 4.88)**	**-0.14 (-0.19; -0.07)**
Model III	1.36 (0.81 – 2.28)	**2.46 (1.29 – 4.71)**	**2.51 (1.32 – 4.80)**	**-0.14 (-0.21; -0.07)**

Model I: included age, gender and education

Model II: included age, gender, education, race, mean arterial blood pressure, total cholesterol, random blood glucose, smoking, BMI, peripheral arterial disease

Model III: included age, gender, race, mean arterial blood pressure, total cholesterol, random blood glucose, smoking, BMI, peripheral arterial disease, total intracranial volume, gray matter volume, white matter volume, hippocampus volume

Table 14 - 2: Association of component 2 with cognition

	CIND mild (n=173) OR (95%CI)	CIND moderate (n=202) OR (95%CI)	CIND moderate/Dementia (n=230) OR (95%CI)	Composite Z scores (n=579) Mean difference (95%CI)
Model I	**1.52 (1.09 – 2.10)**	**1.74 (1.22 – 2.47)**	**1.78 (1.26 – 2.51)**	**-0.18 (-0.25; -0.11)**
Model II	**1.44 (1.00 - 2.07)**	**1.50 (1.00 – 2.25)**	**1.54 (1.03 – 2.31)**	**-0.09 (-0.17; -0.03)**
Model III	1.41 (0.96 – 2.06)	**1.62 (1.05 – 2.51)**	**1.67 (1.08 – 2.58)**	**-0.13 (-0.21; -0.05)**

Model I: included age, gender and education

Model II: included age, gender, education, race, mean arterial blood pressure, total cholesterol, random blood glucose, smoking, BMI, peripheral arterial disease

Model III: included age, gender, race, mean arterial blood pressure, total cholesterol, random blood glucose, smoking, BMI, peripheral arterial disease, total intracranial volume, gray matter volume, white matter volume, hippocampus volume

Table 3: Association of components 1 and 2 with cognition

	CIND mild (n=173) OR (95%CI)	CIND moderate (n=202) OR (95%CI)	CIND moderate/Dementia (n=230) OR (95%CI)	Composite Z scores (n=579) Mean difference (95%CI)
Model I				
Component 1	1.41 (0.87-2.28)	**2.76 (1.46-5.23)**	**2.81 (1.48-5.31)**	**-0.16 (-0.22; -0.10)**
Component 2	**1.48 (1.07-2.05)**	**1.76 (1.21-2.56)**	**1.77 (1.22-2.57)**	**-0.18 (-0.24; -0.11)**
Model II				
Component 1	1.18 (0.73-1.91)	**2.43 (1.27-4.65)**	**2.46 (1.28-4.69)**	**-0.14 (-0.20; -0.08)**
Component 2	1.43 (0.99-2.06)	**1.59 (1.04-2.44)**	**1.63 (1.06-2.49)**	**-0.11 (-0.18; -0.03)**
Model III				
Component 1	1.30 (0.79-2.14)	**2.37 (1.24-4.52)**	**2.39 (1.26-4.56)**	**-0.14 (-0.21; -0.08)**
Component 2	1.39 (0.95-2.04)	**1.73 (1.09-2.74)**	**1.77 (1.11-2.81)**	**-0.14 (-0.21; -0.06)**

Model I: included age, gender and education

Model II: included age, gender, education, race, mean arterial blood pressure, total cholesterol, random blood glucose, smoking, BMI, peripheral arterial disease

Model III: included age, gender, race, mean arterial blood pressure, total cholesterol, random blood glucose, smoking, BMI, peripheral arterial disease, total intracranial volume, gray matter volume, white matter volume, hippocampus volume

CHAPTER 14 – FIGURES

Figure 14 - 1: MR qualitative and quantitative markers of cerebrovascular diseases and involutional changes

Schematic representation of MRI qualitative (visible) and quantitative (invisible) markers of cerebrovascular disease and involutional changes. It is now possible to detect the subtle changes in the brain parenchyma through the automated software. This has led to the better understanding of the association between qualitative and quantitative markers of cerebrovascular diseases/involutional changes and cognitive dysfunction.

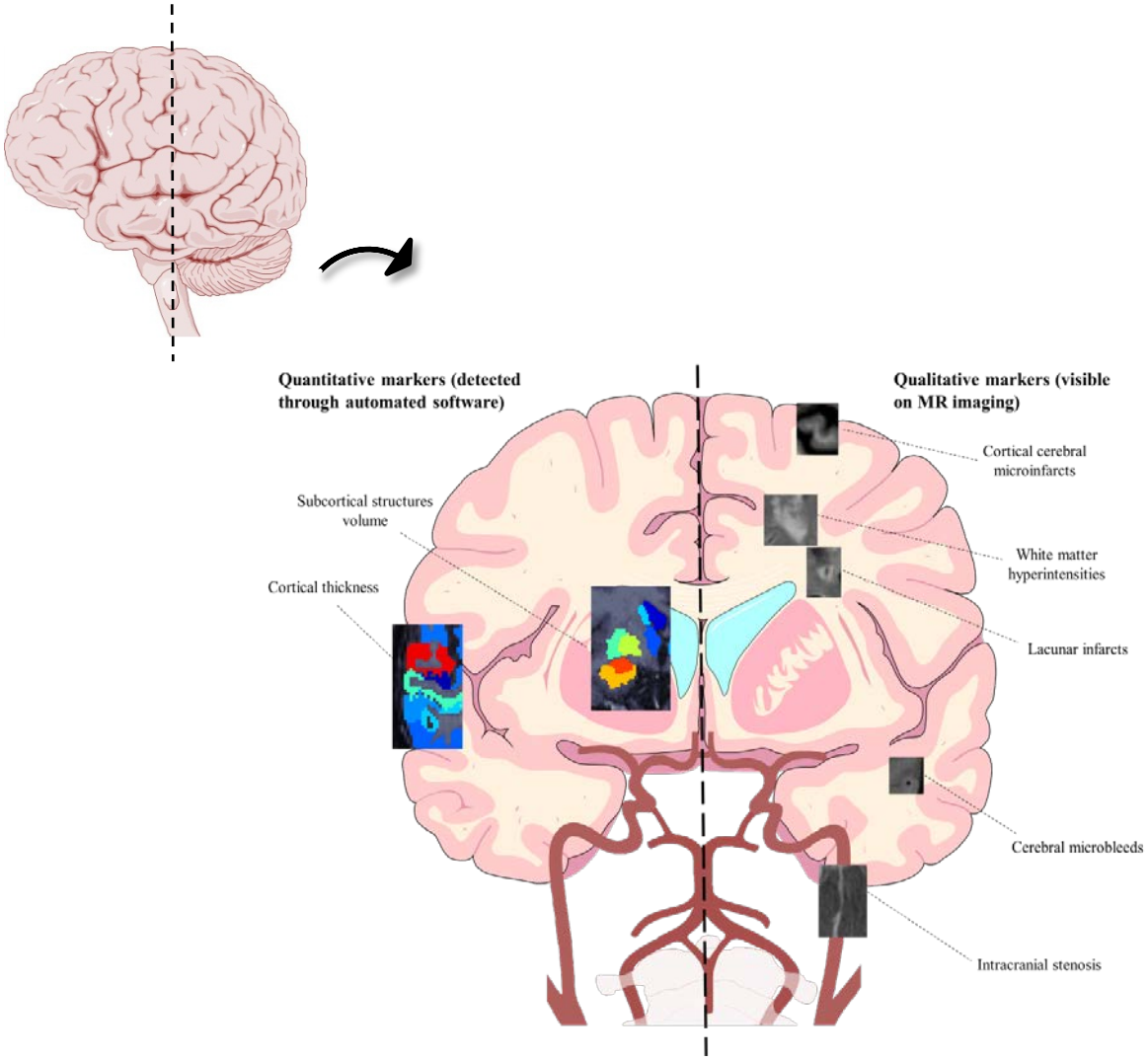


Figure 14 - 2: Retinal qualitative and quantitative markers of cerebrovascular diseases and involutional changes

Schematic representation of retinal qualitative and quantitative cerebrovascular disease and involutional changes markers. It is now possible to detect the subtle changes in the retinal microvascular and neuronal layers through the automated software. This has led to the better understanding of the association between qualitative and quantitative markers with cerebrovascular diseases and cognitive dysfunction.

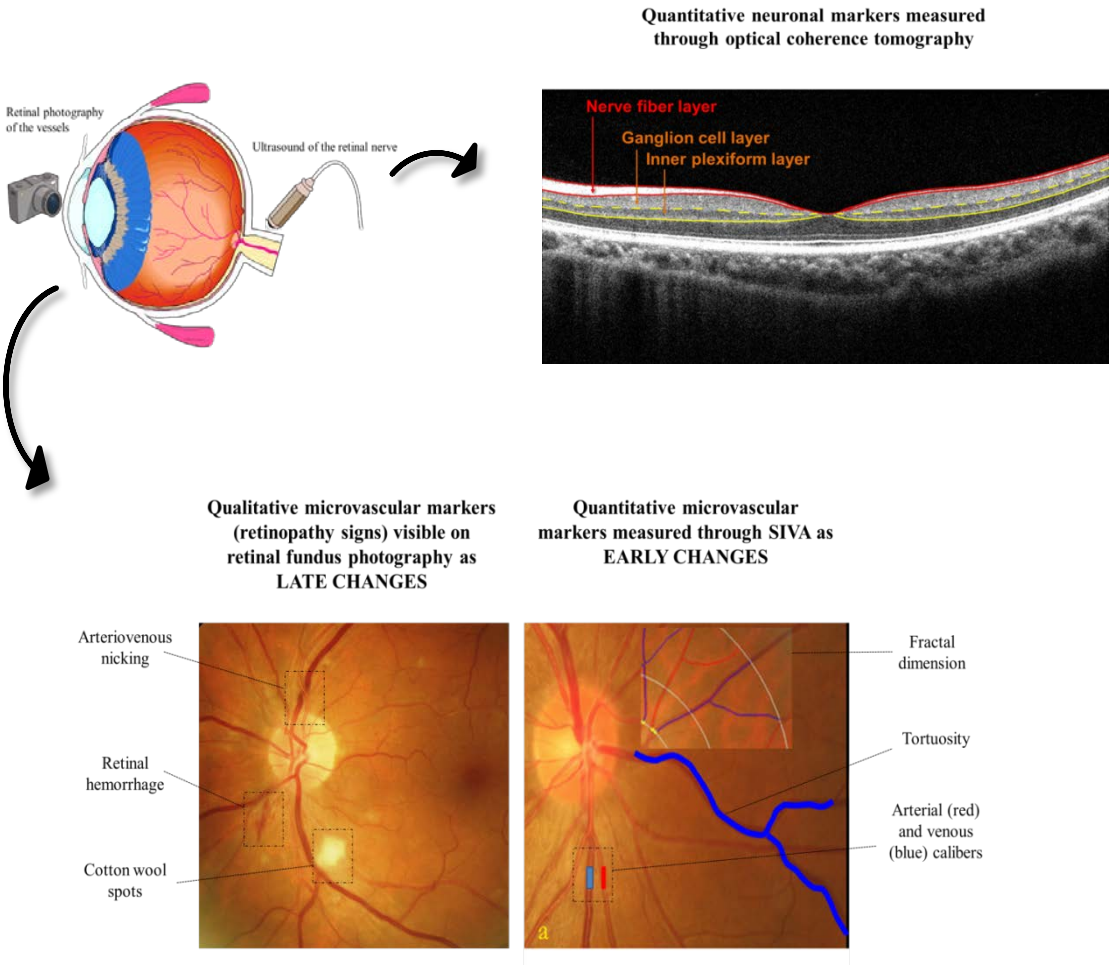


Figure 14 - 3: Summary of the cerebral and retinal markers of cerebrovascular diseases and neurodegeneration and their link with cognition

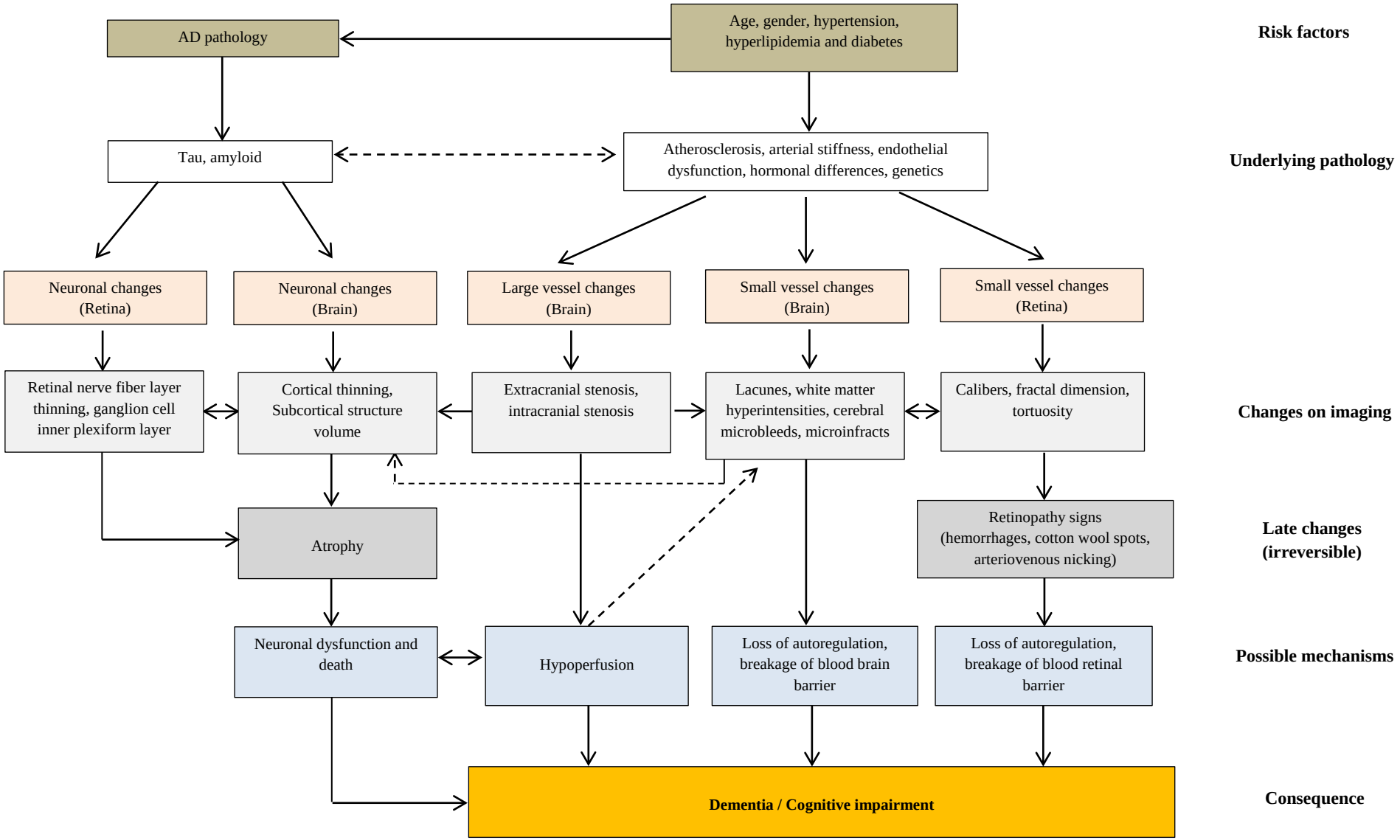


Figure 14 – 4: Summary of the cerebral and retinal markers of cerebrovascular diseases and involutional changes

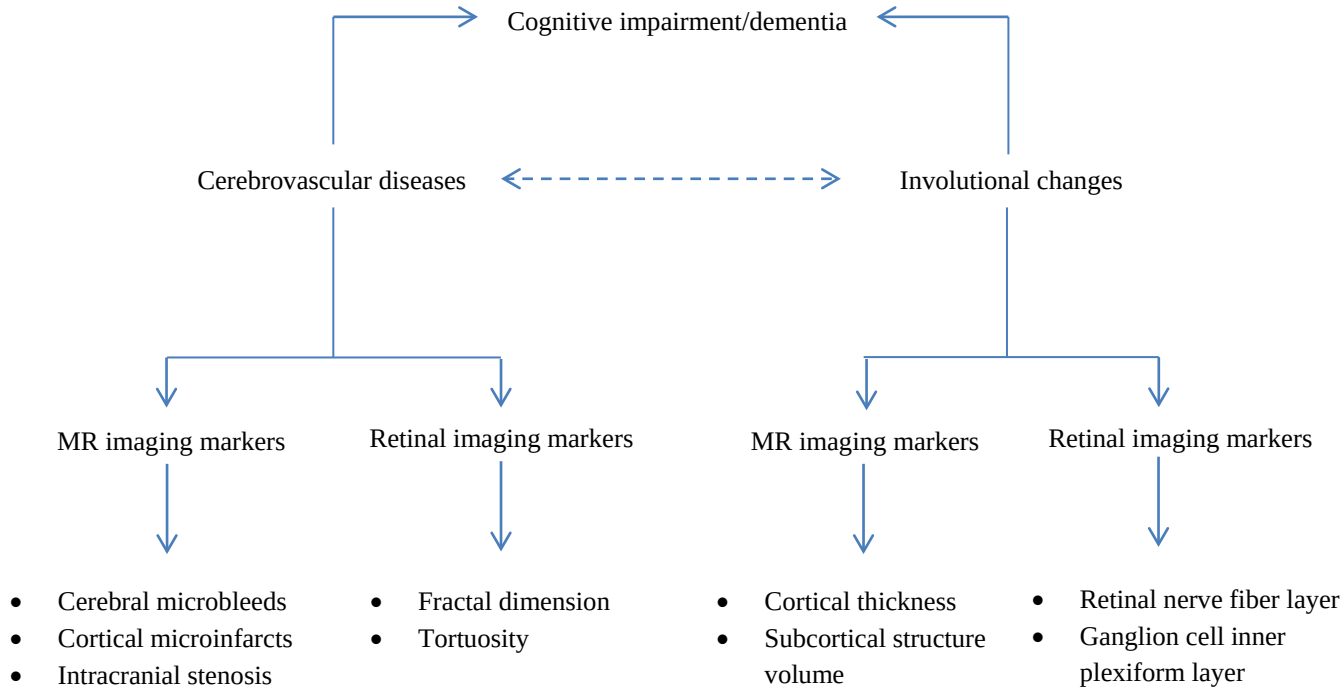
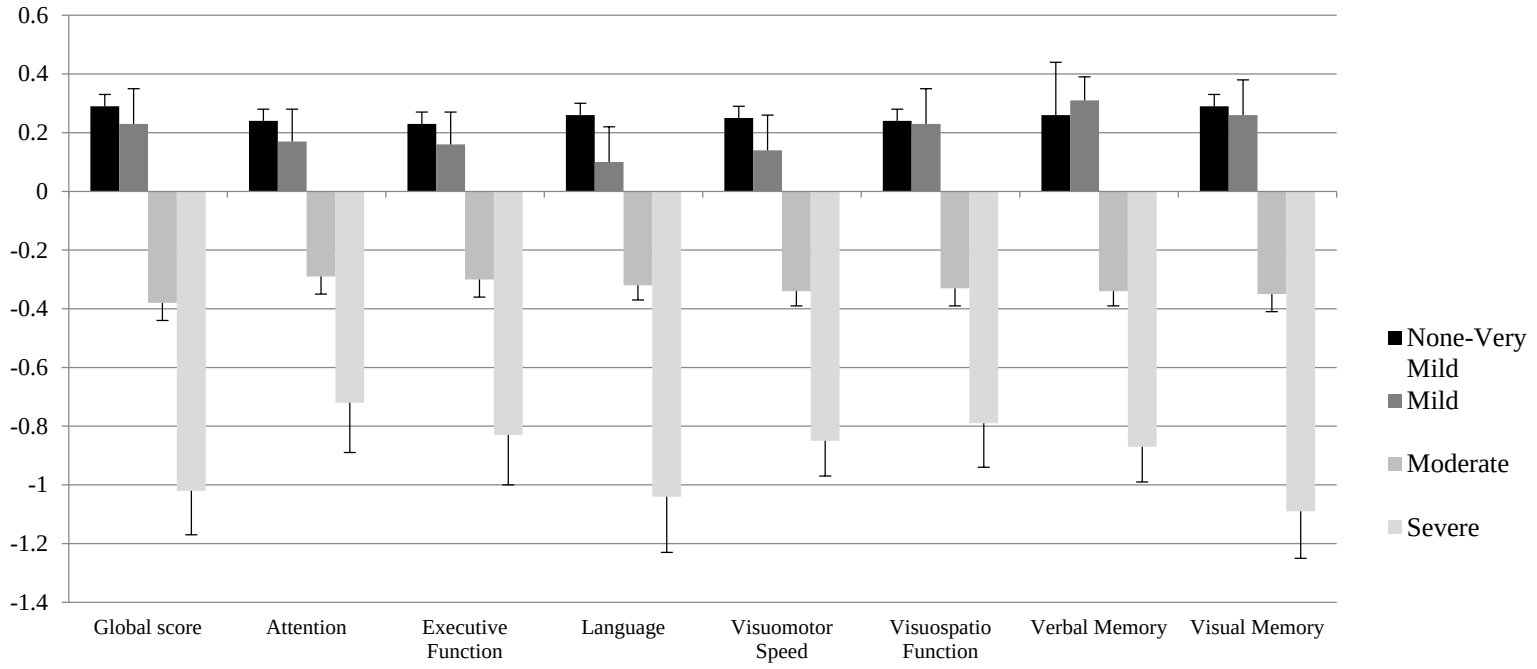


Figure 14 - 5: Global and domain-based neurocognitive performance in different CeVD severity groups with error bars



SUMMARY OF PUBLICATIONS

Aims	Studies	Main findings/ High lights	Status	Authorship
Aim 1: Brain markers of small vessel disease				
a. Cerebral microbleeds (CMB)	EDIS	CMB is associated with worse performance on cognitive testing (executive function, attention and visuoconstruction)	Alzheimer Dis Assoc Disord. 2014; 28(2):106-12	1st
b. Cerebral cortical microinfarcts (CMIs)	Case control	CMIs are novel marker of cerebrovascular disease and associated with worse performance in language and visuoconstruction.	Alzheimers Dement. 2015; S1552-5260(15):00123-5	2nd
c. Cerebral cortical microinfarcts (CMIs)	EDIS	CMIs are associated with severe cognitive impairment and worse performance in the domains of executive function, verbal and visual memory	Revision for Neurology	1st
Aim 2: Brain marker of large vessel disease				
a. Intracranial stenosis (ICS)	EDIS	ICS is associated with severe cognitive impairment and in domains of executive function, language, visuomotor speed, verbal and visual memory.	Alzheimer Dis Assoc Disord. 2015;29(1):12-7	1st
b. Intracranial stenosis (ICS)	Case control	ICS is associated with vascular cognitive impairment and dementias (AD and VaD).	In preparation. Submit to Journal of cerebral blood flow and metabolism	1st
Aim 3: Brain markers of involutional changes				
a. Cortical thickness	EDIS	Decreasing global and regional cortical thickness is significantly associated with cognitive impairment and dementia	Medicine 2015; 94(23):e852	1st
b. Subcortical structure volumes	EDIS and case control	Subcortical volume reduction is related to cognitive impairment and dementia. Subcortical structures are equally affected in both vascular and non vascular cognitive impairment	J Alzheimers Dis. 2015; 48(3): 813-23	1st
Aim 4: Retinal markers of cerebrovascular diseases and involutional changes				
a. Retinal microvascular changes	EDIS	Smaller arteriolar and wider venular calibers and smaller fractal dimension associated with multiple CMB	NeurosciLett. 2014; 577:95–100	1st
b. Retinal microvascular changes	EDIS	Reduced fractal dimension associated with worse performance on verbal memory, visuoconstruction and visuomotor speed	Dement geriatr cogn disord extra. 2014; 4:305-13	2nd
c. Retinal neuronal changes	EDIS	Smaller occipital and temporal gray matter volumes are associated with GC-IPL thinning whereas smaller temporal gray matter volume associated with RNFL thinning	Neurosci Lett. 2014; 584C;12-16	1st

BIBLOGRAPHY

SAIMA HILAL

PhD candidate

Department of Pharmacology, National University of Singapore

Memory Aging and Cognition Center, National University Health System

EDUCATION

National University of Singapore, Singapore
Masters in Public Health (MPH), *specialization Clinical Epidemiology* 2011-2013

Dow Medical College, Karachi, Pakistan
B. Med, B. Surg. (MBBS) 2001-2006

SCHOLARSHIPS

Pakistan Federal Board Overseas Scholarship
Scholarship for medicine programme at Dow Medical College 2001 - 2005

AWARDS

- Travel fellowship 2016
Alzheimer's Association International Conference

- International Training Grant (Internationale Stichting Alzheimer Onderzoek) 2015

- Young Investigator Award (Bursary) 2015
The International Society of Vascular Behavioral and Cognitive Disorders 2015

- Best Oral Presenter
Asian Society Against Dementia 2015

- Travel Fellowship
Alzheimer's Association International Conference 2014

- Best Oral Presenter
Asian Society Against Dementia 2013

- Travel Fellowship
Alzheimer's Association International Conference 2012

- Higher Secondary School Silver Medal and Cash Prize 2000

- Secondary School Cash Prize 1998

MEMBERSHIPS

- Alzheimer's Association International Society to Advance Alzheimer's Research and Treatment (ISTAART)
- Asian Society Against Dementia (ASAD)
- Student Committee, The International Society of Vascular Behavioral and Cognitive Disorders (VasCog)

PRESENTATIONS

Prevalence and risk factors of white matter hyperintensities: comparison between population based studies in Asia 2015
Oral presentation, The International Society of Vascular Behavioral and Cognitive Disorders, Tokyo, Japan

Growth differentiation factor 15: a novel marker of cerebrovascular disease in cognitive impairment and dementia 2015
Poster presentation, The International Society of Vascular Behavioral and Cognitive Disorders, Tokyo, Japan

Subcortical atrophy in cognitive impairment and dementia 2015
Oral presentation, Asian Society Against Dementia, Kumamoto, Japan

Cerebral cortical microinfarcts- findings from 3Tesla Magnetic Resonance Imaging 2015
Oral presentation, Asian Society Against Dementia, Kumamoto, Japan

Retinal Microvascular Alterations in Alzheimer's Disease and Vascular Dementia 2015
Oral presentation, Alzheimer's Association International Conference, Washington DC, USA

Specific patterns of subcortical volume reduction in vascular cognitive impairment no dementia and vascular dementia 2015
Poster presentation, European Stroke Organization Committee, Glasgow, UK

High-definition optical coherence tomography and MRI-defined neuroimaging markers in an Asian population 2014
Oral presentation, Alzheimer's Association International Conference, Copenhagen, Denmark

Are there ethnic differences in the prevalence of cerebral small vessel disease? 2014
Poster presentation, Annual Graduate Scientific Congress, NUS, Singapore

Determinants and consequences of cortical thickness in an Asian population- The Epidemiology of Dementia in Singapore study 2014
Oral presentation, Asian Society Against Dementia, Colombo, Sri Lanka

TRAININGS

- Infarcts and cortical thinning segmentation techniques at Klinikum der Universität München, Ludwig-Maximilians-University, Munich, Germany 2015
- Cerebral cortical microinfarcts gradings at Utrecht Medical Center, Netherlands 2014
- Diffusion Tensor Imaging segmentation at DUKE-NUS, Singapore ongoing

COURSES

- MR Imaging, National University Hospital, Singapore 2014
- Grant writing workshop, National University Hospital, Singapore 2015

COLLABORATIONS

- **Erasmus Medical Center, Rotterdam, Netherlands** - Quantitative neuroimaging segmentation
- **Utrecht Medical Center, Utrecht, Netherlands** – Cerebral cortical microinfarcts
- **Singapore Eye Research Institute, Singapore** – Retinal imaging segmentation

ACADEMIC EDITOR AND REVIEWING EXPERIENCE

- Medicine
- International Ophthalmology
- Journal of Neurology, Neurosurgery and Psychiatry
- PLoS one

APPENDICES

APPENDIX 1 – LIST OF ABBREVIATIONS

Abbreviation	Term
AD	Alzheimer's Disease
AGES-Reykjavik	Age, Gene/Environment Susceptibility-Reykjavik
AIBL	Australian Imaging, Biomarker & Lifestyle
aMCI	Amnestic Mild Cognitive Impairment
AMT	Abbreviated Mental Test
ARIC	Atherosclerosis Risk in Communities
AV nicking	Arteriovenous nicking
BMES	Blue Mountains Eye Study
BMI	Body Mass Index
BOMBS	Brain Observer Microbleed Scale
CAA	Cerebral Amyloid Angiopathy
CADASIL	Cerebral Autosomal-Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy
CASI-S	Cognitive Abilities Screening Instrument-Short
CDR	Clinical Dementia Rating scale
CERAD	Consortium to Establish a Registry for Alzheimer’s Disease
CeVD	Cerebrovascular diseases
CHS	Cardiovascular Health Study
CI	Confidence interval
CIND	Cognitive Impairment No Dementia
CMB	Cerebral microbleeds
CMIs	Cerebral microinfarcts
CRAE	Central Retinal Arteriolar Equivalent
CRVE	Central Retinal Venular Equivalent
CT	Computed Tomography
DSM	Diagnostic and Statistical Manual of Mental Disorders
DSRB	Domain-Specific Review Board
DSC	Dice Similarity Coefficient
EDIS	Epidemiology of Dementia In Singapore study
EOAD	Early Onset Alzheimer's Disease
FLAIR	Fluid Attenuated Inversion Recovery
FIRST	FMRIB’s Integrated Registration and Segmentation Tool

Abbreviation	Term
GC-IPL	Ganglion cell-Inner Plexiform Layer
GDS	Geriatric Depression Scale
GE	Gradient Echo
HD-OCT	High Definition-Optical Coherence Tomography
HERNS	Hereditary Endotheliopathy with Retinopathy, Nephropathy and Stroke
HR	Hazard Ratio
IADL	Instrumental Activities of Daily Living
ICC	Intraclass Correlation Coefficient
ICS	Intracranial stenosis
IQCODE	Informant Questionnaire on Cognitive Decline in the Elderly
LALES	Los Angeles Latino Eye Study
LOAD	Late Onset Alzheimer's Disease
MABP	Mean Arterial Blood Pressure
MCA	Middle Cerebral Artery
MCI	Mild cognitive impairment
MMSE	Mini Mental Status Examination
MoCA	Montreal Cognitive Assessment
MPRAGE	Magnetization Prepared Rapid Gradient Recalled Echo
MRA	Magnetic Resonance Angiography
MRI	Magnetic Resonance Imaging
NCI	No Cognitive Impairment
NINDS-ADRDA	National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
NINDS-AIREN	National Institute for Neurological Disorders and Stroke-Association Internationale pour la Recherche et l'Enseignement en Neurosciences
OCT	Optical Coherence Tomography
OR	Odds Ratios
PF	Progressive Forgetfulness
RNFL	Retinal Nerve Fiber Layer
RR	Relative Risk
RSS	Rotterdam Scan Study
RUN-DMC	Radboud University Nijmegen Diffusion tensor and MRI Cohort
SCES	Singapore Chinese Eye Study
SD	Standard Deviation
SD-OCT	Spectral Domain-Optical Coherence Tomography

Abbreviation	Term
SEED	Singapore Epidemiology of Eye Disease
SiMES	Singapore Malay Eye Study
SINDI	Singapore Indian Eye Study
SIVA	Singapore I Vessel Analysis
STRIVE	STandards for ReportIng Vascular changes on nEuroimaging
SVD	Small Vessel Disease
SWI	Susceptibility-weighted imaging
T	Tesla
TBV	Total Brain Volume
TCD	Transcranial Doppler
TD-OCT	Time Domain-Optical Coherence Tomography
TE	Time to Echo
TIV	Total Intracranial volume
ToF	Time of Flight
TR	Repetition Time
VaD	Vascular dementia
VCI	Vascular Cognitive Impairment
WAIS	Wechsler Adult Intelligence Scale
WHO	World Health Organization
WMH	White Matter Hyperintensities
WMS	Weschler Memory Scale

APPENDIX 2 – SUMMARY OF WORK DONE

Epidemiology of Dementia In Singapore study (EDIS)

- Subject assessments (questionnaire, physical examination, clinical history)
- Neuroimaging gradings
- Participation in consensus for clinical diagnosis of subjects
- Study coordination (retinal imaging, neuroimaging segmentations) with multi-disciplinary teams which includes;
    - Singapore Eye Research Institute
    - Erasmus Univerity Medical Center
    - Utrecht Medical Center
- Data cleaning and data management
- Data analysis
- Manuscript writing

Case control study

- Subject assessments (questionnaire, physical examination, clinical history)
- Neuroimaging gradings
- Participation in consensus for clinical diagnosis of subjects
- Study coordination (neuroimaging segmentations) with multi-disciplinary teams which includes;
    - Erasmus Univerity Medical Center
    - Utrecht Medical Center
- Data cleaning and data management
- Data analysis
- Manuscript writing

APPENDIX 3 – LIST OF OTHER PUBLICATIONS

1. **Hilal S**, Tan CS, Xin S, Amin SM, Wong TY, Chen C, Venketasubramanian N, Ikram MK. Prevalence of cognitive impairment and dementia in Malays – Epidemiology of Dementia in Singapore Study. Curr Alzheimer Res. 2015 [epub ahead of print].

2. **Hilal S**, Chai YL, Ikram MK, Elangovan S, Yeow TB, Xin X, Chong JY, Venketasubramanian N, Richards AM, Chong JP, Lai MK, Chen C. Markers of cardiac dysfunction in cognitive impairment and dementia. Medicine 2015; 94:e297.

3. Xu X, **Hilal S**, Collinson SL, Chong EJ, Ikram MK, Venketasubramanian N, Chen CL. Association of Magnetic Resonance Imaging Markers of Cerebrovascular Disease Burden and Cognition. Stroke 2015; 46:2808-14.

4. Saini M, Suministrado MS, **Hilal S**, Dong YH, Venketasubramanian N, Ikram MK, Chen C. Prevalence and risk factors of Acute Incidental Infarcts. Stroke 2015; 46:2722-7.

5. Xu X, Ang SL, **Hilal S**, Chan QL, Wong TY, Venketasubramanian N, Ikram MK, Chen CL. Association of neuropsychiatric symptoms and sub-syndromes with cognitive impairment in community-dwelling Asian elderly. Int Psychogeriatr. 2015; 1-9.

6. Cheung CY, Ong YT, **Hilal S**, Ikram MK, Low S, Ong YL, Venketasubramanian N, Yap P, Seow D, Chen CL, Wong TY. Retinal Ganglion Cell Analysis Using High-Definition Optical Coherence Tomography in Patients with Mild Cognitive Impairment and Alzheimer's Disease. J Alzheimers Dis. 2015; 45:45-56.

7. Wong MY, Chen CL, Ong YT, **Hilal S**, Ikram MK, Kumari N, Yip CC, Venketasubramanian N, Yap P, Seow D, Wong TY, Cheung CY. High prevalence of undiagnosed eye diseases in individuals with dementia. J Am Geriatr Soc 2015, 63:192-4.

8. Verhaaren BF, Debette S, Bis JC, Smith JA, Ikram MK, Adams HH, Beecham AH, Rajan KB, Lopez LM, Barral S, van Buchem MA, van der Grond J, Smith AV, Hegenscheid K, Aggarwal NT, de Andrade M, Atkinson EJ, Beekman M, Beiser AS, Blanton SH, Boerwinkle E, Brickman AM, Bryan RN, Chauhan G, Chen CP, Chouraki V, de Craen AJ, Crivello F, Deary IJ, Deelen J, De Jager PL, Dufouil C, Elkind MS, Evans DA, Freudenberger P, Gottesman RF, Guðnason V, Habes M, Heckbert SR, Heiss G, **Hilal S**, Hofer E, Hofman A, Ibrahim-Verbaas CA, Knopman DS, Lewis CE, Liao J, Liewald DC, Luciano M, van der Lugt A, Martinez OO, Mayeux R, Mazoyer B, Nalls M, Nauck M, Niessen WJ, Oostra BA, Psaty BM, Rice

KM, Rotter JI, von Sarnowski B, Schmidt H, Schreiner PJ, Schuur M, Sidney SS, Sigurdsson S, Slagboom PE, Stott DJ, van Swieten JC, Teumer A, Töglhofer AM, Traylor M, Trompet S, Turner ST, Tzourio C, Uh HW, Uitterlinden AG, Vernooij MW, Wang JJ, Wong TY, Wardlaw JM, Windham BG, Wittfeld K, Wolf C, Wright CB, Yang Q, Zhao W, Zijdenbos A, Jukema JW, Sacco RL, Kardia SL, Amouyel P, Mosley TH, Longstreth WT Jr, DeCarli CC, van Duijn CM, Schmidt R, Launer LJ, Grabe HJ, Seshadri SS, Ikram MA, Fornage M. Multiethnic genome-wide association study of cerebral white matter hyperintensities on MRI. Circ Cardiovasc Genet. 2015; 8:398-409.

9. **Hilal S**, Saini M, Tan CS, Catindig JA, Dong YH, Leon LB, Niessen WJ, Vrooman H, Wong TY, Chen C, Venketasubramanian N, Ikram MK. Ankle-brachial index, cerebrovascular disease and cognitive impairment in a Chinese population. Neuroepidemiology 2014; 42:131-8.

10. Saini M, Tan CS, **Hilal S**, Dong Y, Ting E, Ikram MK, Sharma VK, Venketasubramanian N, Chen C. Computer tomography for prediction of cognitive outcomes after ischemic cerebrovascular events. J Stroke Cerebrovasc Dis. 2014;23:1921-7.

11. Cheung C, Ong YT, Ikram MK, Ong SY, Li X, **Hilal S**, Catindig JA, Venketasubramanian N, Yap P, Seow D, Chen CP, Wong TY. Microvascular network alterations in the retina of patients with Alzheimer's disease. Alzheimers Dement. 2014; 5260: 2871-9.

12. **Hilal S**, Ikram MK, Saini M, Tan CS, Catindig JA, Dong YH, Lim LB, Ting EY, Koo EH, Cheung CY, Qiu A, Wong TY, Chen CL, Venketasubramanian N. Prevalence of cognitive impairment in Chinese: The Epidemiology of Dementia in Singapore Study. J Neurol Neurosurg Psychiatry. 2013; 84:686–92.

13. Thong YJ, **Hilal S**, Wang Y, Soon HW, Dong Y, Collinson SL, Anh TT, Ikram MK, Wong TY, Venketasubramanian N, Chen C, Qiu A. Association of silent lacunar infarct with brain atrophy and cognitive impairment. J Neurol Neurosurg Psychiatry. 2013; 84:1219-25.

14. Dong YH, Lee WY, **Hilal S**, Saini M, Wong TY, Chen CL, Venketasubramanian N, Ikram MK. Comparison of Montreal Cognitive Assessment and the Mini-Mental State Examination in detecting multiple domain-Mild Cognitive Impairment in community-dwelling Chinese elderly. Int Psychogeriatr. 2013;25:1831-8.

15. Dong Y, Gan DZ, Tay SZ, Koay WI, Collinson SL, **Hilal S**, Venketasubramanian N, Chen C. Patterns of neuropsychological impairment in Alzheimer's disease and mixed dementia. J Neurol Sci. 2013; 333:5-8.

16. Saini M, Ikram K, **Hilal S**, Qiu A, Venketasubramanian N, Chen C. Silent Stroke: Not Listened to Rather Than Silent. Stroke 2012; 43:3102-4.

17. Wang Y, Catindig JA, **Hilal S**, Soon HW, Ting E, Wong TY, Venketasubramanian N, Chen C, Qiu A. Multi-stage segmentation of white matter hyperintensity, cortical and lacunar infarcts. Neuroimage 2012; 60:2379-88.

18. Dong YH, Pang WS, Lim LBS, Yang YH, Morris JC, **Hilal S**, Venketasubramanian N, Chen C. The informant AD8 is superior to participant AD8 in detecting cognitive impairment in a memory clinic setting. J Alzheimers Dis. 2012; 35:159-68.