# Using neuroimaging to understand cognitive impairment in patients with symptomatic carotid artery disease

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## Declaration

Except where noted in the acknowledgement or text, I declare that this dissertation is my own work and is based on research that was undertaken by me in the Radiological Sciences research group, Sir Peter Mansfield Imaging Centre, School of Medicine, University of Nottingham.

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# Abstract

## Introduction

Patients with symptomatic carotid artery disease have increased risk of cognitive impairment. Multiple mechanisms of cognitive impairment have been proposed. However, the contribution of each mechanism to cognitive impairment and the interrelation between these mechanisms remain unclear. Lesional and non-lesional damage of brain tissues may lead to cognitive impairment in patients with symptomatic carotid artery disease. The research presented aims to use whole brain statistical brain mapping and quantitative neuroimaging techniques to understand vascular cognitive impairment (VCI) in a group of patients with symptomatic carotid artery disease.

## Methods

Studies presented in this thesis were based on available multimodal MRI and cognitive performance data from a study in patients with symptomatic carotid artery disease. Totally 108 subjects had FLAIR and diffusion MRI scans and 62 of them had resting-state fMRI scans in a 3T MRI scanner. All participants (n=108) underwent cognitive assessments using Addrenbrooke's cognitive examination-revised (ACE-R). Voxel-based lesion symptom mapping (VLSM) technique was used to identify the contribution of ischaemic lesion presence to cognitive performance. Tract-based spatial statistics (TBSS) approach was used to investigate the association between microscopic damage of white matter and cognitive performance. Independent component analysis (ICA) and seed-based analysis approaches were used to investigate functional disconnection of large-scale cognitive networks and its contribution on cognitive impairment. The

association between cognitive performance and ischaemic lesions volumes and microscopic damage of brain tissues were also assessed.

**Results**

The presence of chronic subcortical ischaemic lesions located in strategic white matter tracts including anterior thalamic radiation, forceps minor and forceps major contributed to global cognitive impairment in patients with symptomatic carotid artery disease. Ischaemic lesion volumes and microscopic damage of brain tissues including strategic brain areas, strategic white matter tracts, normal appearing white matter and white matter tract skeleton were related to cognitive impairment. Functional disconnection of large-scale cognitive networks including default mode network (DMN), dorsal attention network (DAN), left fronto-parietal network and salience network was found in patients with probable vascular mild cognitive impairment (VaMCI). Functional disconnection beyond the large-scale cognitive networks, showing as decreased functional connectivity between bilateral hippocampi, bilateral dorsal lateral prefrontal cortex (DLPFC), anterior cingulate cortex (ACC) and posterior cingulate cortex (PCC) with the rest of the brain, was also found in patients with probable VaMCI. Also, the thesis presented preliminary results of the role of lesional and non-lesion brain damage in fluency performance in patients with symptomatic carotid artery disease.

**Conclusions**

This thesis provides further evidence for the role and to the nature of disconnection in VCI and proposes several disconnection related imaging biomarkers.

## Abbreviations

ACE-R: Addenbrooke's Cognitive Examination-Revised

AD: Alzheimer's disease

ADC: Apparent diffusion coefficient

AUC: Area under the curve

BOLD: Blood-oxygenation-level dependent

CAA: Cerebral amyloid angiopathy

CADASIL: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy

DAN: Dorsal attention network

DMN: Default mode network

DTI: Diffusion tensor imaging

DWI: Diffusion-weighted imaging

FA: Fractional anisotropy

FLAIR: Fluid-attenuated inversion recovery

FLIRT: FMRIB's Linear Image Registration Tool

FMRIB: Functional MRI of the Brain

FWHM: Full-width-at-half-maximum

FPN: Fronto-parietal network

HIS: Hachinski Ischaemia Scale

ICA: Independent component analysis

MD: Mean diffusivity

MELODIC: Multivariate exploratory linear optimized decomposition into independent components

MMSE: Mini-Mental Stating Examination

MRI: Magnetic resonance imaging

MTA: Medial temporal lobe atrophy

NIHR: National Institute for Health Research

NIHSS: National Institutes of Health Stroke Scale

NINDS-AIREN: Neurological Disorders and Stroke-Association Internationale pour la Recherché et l'Enseignement en Neurosciences

MoCA: Montreal Cognitive Assessment

NAWM: Normal appearing white matter

PD: Parkinson Disease

ROC: Receiver operating characteristic

ROI: Region-of-interest

SAL: Salience network

SPM: Statistic Parametric Mapping

SVD: small vessel disease

TBSS: Tract-based spatial statistics

TIA: transient ischaemic attack

TILL: Total ischaemic lesion volumes

TOAST: Trial of Org 10172 in Acute Stroke Treatment

VaD: Vascular dementia

VBM: Voxel-based morphometry

VCI: Vascular cognitive impairment

VASCOG: Congress of the Internal Society for Vascular Behavioural and Cognitive disorders

VLSM: Voxel-based lesion-symptom mapping

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# Chapter 1 Introduction

## 1.1 Vascular cognitive impairment

### 1.1.1 Background

Dementia describes a set of clinical symptoms that encompass memory loss and difficulties with problem-solving, thinking or language, leading to impairments in activities of daily living. Alzheimer's disease (AD), vascular dementia (VaD), dementia with Lewy bodies and fronto-temporal dementia are common types of dementia (Robinson, Tang et al. 2015). Dementia has become a global concern in a rapidly ageing population (Prince 2014). According to a report of dementia in the UK (Prince, Knapp et al. 2015), the total age-standardised 65+ population prevalence of dementia is 7.1% (based on 2013 population data). The total number of people with dementia in the UK is expected to increase to over 1 million by 2025 and over 2 million by 2051 if age-specific prevalence remains stable and increases are only driven by demographic ageing. The economic burden of dementia in the UK is £26.3 billion with an average cost of £32,250 per person.

Vascular cognitive impairment (VCI), an umbrella term to replace VaD, encompasses a range of cognitive deficits that show a presumed vascular cause. This term includes all forms of cognitive deficits from mild cognitive impairment to dementia of vascular origin. The classification of VaD should be based on cognitive decline from a prior baseline and deficits in performance in

more than 2 cognitive domains which are of sufficient severity to affect the subject's activities of daily living. In contrast, the classification of vascular mild cognitive impairment (VaMCI) should be based on cognitive decline from a prior baseline and deficits of at least one cognitive domain (Gorelick, Scuteri et al. 2011). The details of the classifications of VaD and VaMCI will be introduced in Part 1.1.5.

VaD is the second most common type of dementia. The prevalence of VaD in subjects between age 65-69 to 80+ years ranged from 2.2% to 16.3% in clinical studies performed in the European countries (Jellinger 2013). The incidence of VaD was about 3.8 (cases per 1000 persons per year) and its incidence increased with age from 0.7 or 3.9 (less than 70 years old) to 6-8 up to 50 (more than 80 years old) (Bowler 2007) (Fratiglioni, Launer et al. 2000) (Kuller, Lopez et al. 2005). According to a review of autopsy studies, the frequency of VaD ranged from 0.03% to 85.2% with the average prevalence around 11% (real mean 8%-15% in Western studies, 22%-35% in Japan) (Jellinger 2008). High prevalence of mixed pathologies including AD and vascular pathology was also identified in previous autopsy studies with the prevalence of AD plus vascular pathology ranging from 4.1% to 93% as reviewed in autopsy studies of elderly subjects (Jellinger 2013).

Pendlebury and Rothwell investigated the prevalence of post-stroke dementia by conducting a quantitative meta-analysis (Pendlebury and Rothwell 2009). All studies they included to estimate post-stroke dementia used standard diagnostic criteria (Table 1. 1) (Spitzer 1989) (World Health Organization. 1992) as an outcome measurement. According to their findings, in hospital-based studies,

the prevalence of post-stroke dementia ranged from 12% of first-ever strokes in which pre-stroke dementia was excluded to 41.3% of recurrent strokes in which pre-stroke dementia was included. The prevalence of post-stroke dementia was estimated to be 7.4% in population-based studies.

**Table 1. 1 Summary of current diagnostic criteria sets for vascular dementia**

- National Institute of Neurological Disorders and Stroke-Association Internationale pour la Recherché et l'Enseignement en Neurosciences (NINDS-AIREN) criteria (Roman, Tatemichi et al. 1993)
- State of California Alzheimer's Disease Diagnostic and Treatment Centers (ADDTC) criteria (Chui, Victoroff et al. 1992)
- DSM-IV criteria (First, Spitzer et al. 1997)
- ICD-10 criteria (World Health 1992)

No meta-analysis was conducted to systematically review the prevalence of post-stroke cognitive impairment. Studies mentioned below investigated the prevalence in patients with cognitive impairment which is a milder end of the cognitive decline spectrum. Generally, the prevalence of post-stroke cognitive impairment ranged from 20.7% to 48.91%. In most of hospitalised-based studies, the prevalence of post-stroke cognitive impairment was more than 40% (Sachdev, Brodaty et al. 2006) (Yu, Cho et al. 2013) (Jacquin, Binquet et al. 2014) with an expectation of one study showing the prevalence around 20% (Narasimhalu, Ang et al. 2011). The prevalence of post-stroke cognitive impairment in community-based studies was lower than that in hospitalised-based studies. For example, using the Abbreviated Mental Test or MMSE to assess cognitive impairment after stroke in a community-based population, the

prevalence of cognitive impairment 3 months, 5 years and 14 years after stroke was 22%, 22% and 21%, respectively (Douiri, Rudd et al. 2013). The relatively low prevalence detected in community-based studies might be explained by diagnostic uncertainty in community sample and the use of less sensitive cognitive assessment.

### 1.1.2 Risk factors

#### Demographic factors

Age is well established to be related to VCI (Gorelick 2004) (Gorelick, Scuteri et al. 2011). In general, the prevalence of VCI increases after age 65 years as age increases (Solfrizzi, Panza et al. 2004) (Busse, Bischkopf et al. 2003) while the trend of VCI prevalence after 90 years old has not been fully established (Fitzpatrick, Kuller et al. 2004) (Fratiglioni, Launer et al. 2000) (Corrada, Brookmeyer et al. 2010). The age-related increases in VCI seem to follow the pattern of stroke (Pendlebury and Rothwell 2009) however post-stroke dementia may be more frequent in subjects less than 80 years old (Ivan, Seshadri et al. 2004).

There is no consensus on the association between sex and VCI. According to the findings of two studies, the incidence of VCI was higher in men than in women (Fitzpatrick, Kuller et al. 2004) (Ruitenberg, Ott et al. 2001). However, a contradictory result was concluded by a pooled analysis of incidence studies showing that there was no difference in the incidence of VCI between men and women (Andersen, Launer et al. 1999).

**Education**

VCI was found to be related to low education level based on the evidence from a population-based study (Ott, Breteler et al. 1995). However, this association needs to be interpreted with caution because cognitive tests usually have an education component, therefore confounding the relation between education level and VCI.

**Smoking**

Several attempts have been made to investigate whether smoking is an independent risk factor for cognitive impairment and dementia. Elderly smokers had increased risks of dementia and cognitive decline as demonstrated in a meta-analysis (Anstey, von Sanden et al. 2007). Also, according to their findings, smoking was found to be associated with poor cognitive performance in middle age subjects. However, there is no consensus on the association between smoking and certain cognitive domains. For instance, current smokers were found to have impairment of psychomotor speed and cognitive flexibility (Kalmijn, van Boxtel et al. 2002). Additionally, smoking was found to be related to increased risk of memory impairment in middle age subjects (Sabia, Marmot et al. 2008).

**Hypertension**

It is well established that hypertension in midlife is a risk factor for VCI (Launer, Ross et al. 2000) (Yamada, Mimori et al. 2009). However, the association between hypertension and cognitive decline in late-life shown in previous

studies was less consistent. For example, cognitive decline in late-life was found to be related to higher systolic blood pressure while the association between cognitive performance and diastolic blood pressure remained inconsistent (Birns and Kalra 2009). Based on the evidence, the statement from the American Heart Association/American Stroke Association has suggested that treatment of hypertension is recommended for patients at risk of VCI (Gorelick, Scuteri et al. 2011).

According to the statement from the American Heart Association/American stroke Association, alcohol intake control, weight control, physical activity, treatment of hyperglycemia and hypercholesterolemia may be reasonable in subjects at risk for VCI (Gorelick, Scuteri et al. 2011). Further studies are needed to investigate whether these factors are independent risk factors for VCI. Additionally, since AD and VCI share similar risk factors, risk factors for AD such as Apolipoprotein E (APOE) ε4 allele, may be also a risk factor for VCI (Neergaard, Dragsbaek et al. 2016).

### 1.1.3 Pathologic changes that relate to VCI

Pathologic changes in the brain that relate to VCI are manifold. The major cerebrovascular lesions that associate with VCI include gross large infarcts in supply territories of large cerebral arteries, lacunar infarctions, multiple microinfarcts in cortical border zones, pseudolaminar cortical necrosis, hippocampal sclerosis and white matter hyperintensities (Jellinger 2013). There are mainly two classifications to distinguish cerebrovascular lesions.

The first classification distinguishes cerebrovascular lesions based on their patterns.

***I. Multifocal lesions***

There are mainly six kinds of cerebrovascular lesions included in the form of multifocal lesions: (1) large territorial infarcts caused by large vessel disease; (2) watershed or borderzone infarcts due to haemodynamic impairment and carotid artery disease; (3) micro-infarcts spread in the brain that mainly relate to embolism; (4) subcortical small and medium-sized lesions in functionally important brain areas; (5) lacunar infarctions, and (6) white matter hyperintensities (Jellinger 2008).

***II. Focal disease***

Unilateral or symmetric cerebrovascular lesions that locate in strategic brain areas alone including thalamus, hippocampus, frontal and basal forebrain could lead to VCI. This could be mainly explained by ischaemia due to small and large vessel disease, haemodynamic impairment, cardiac embolism and cerebral ischaemic events due to various aetiologies (Jellinger 2008).

The second classification distinguishes cerebrovascular lesions that relate to large and small vessel disease (SVD).

### *I. Large vessel disease*

Atherosclerosis of intra- and extracranial vessels leading to hypoperfusion or local thromboembolism, and embolism due to cardiac sources could cause multiple small and large infarcts involving major cerebral arteries areas.

Atherosclerosis is a degenerative disorder which leads to intima proliferation and cholesterol accumulation within the vessel wall of medium and large size arteries. There are three main ways that carotid atherosclerosis leads to cerebrovascular ischaemic events including amaurosis fugax, TIA and ischaemic stroke. The first way is by thrombosis, which is when plaque ruptures, blood clots are formed in injured blood vessel, therefore, causing a blockage and reducing blood flow to the brain. The second way is by embolisation, which happens when the surface of an unstable plaque ruptures, then bits of plaque break off and travel into arteries, therefore leading to disruption of blood supply to brain tissues. The third way is when plaque narrows and blocks of carotid arteries. The prevalence of carotid atherosclerosis more than 50% on duplex ultrasound in a general population is from 2% to 8% (Alexandrov 2011), being 5% in women and 7% in men (Oleary, Polak et al. 1992). A higher prevalence of 5% to 9% is anticipated in patients over 65 years old (Goessens, Visseren et al. 2007). The association between atherosclerosis and cognitive decline identified from studies is not consistent. On one hand, carotid atherosclerosis has been found to be a major risk factor for ischaemic stroke and related cognitive impairment (Arntzen, Schirmer et al. 2012). It is estimated that atherosclerosis in extracranial or large intracranial arteries accounted for about 50% of all ischaemic strokes and TIAs (Warlow, Sudlow et al. 2003). Additionally, several studies have proposed that

atherosclerosis was related to the presence of AD pathology (Roher, Esh et al. 2004) (Honig, Kukull et al. 2005). On the other hand, Dolan and colleagues suggested that atherosclerosis independently caused dementia through vascular pathways that were not related to AD pathology and ischaemic stroke (Dolan, Crain et al. 2010).

***II. SVD***

Signs of SVD on neuroimaging include lacunar infarctions and white matter hyperintensities of presumed vascular origin, recent small subcortical infarcts, perivascular space and cerebral microbleeds (Wardlaw, Smith et al. 2013).

SVD causes about a fifth of all strokes worldwide and it is the most common vascular cause of dementia (Pantoni 2010) (Wardlaw, Smith et al. 2013). According to the evidence provided by a meta-analysis, the prevalence of dementia after lacunar stroke was around 20% (Makin, Turpin et al. 2013). The role of each kind of neuroimaging feature of SVD in VCI will be introduced in 1.3.

### 1.1.4 Types of cerebrovascular lesions that relate to VCI

There are several types of cerebrovascular lesions that relate to VCI (Jellinger 2007) (Jellinger 2008) (Jellinger 2013). An example of infarcts, lacunar infarcts and white matter hyperintensities was shown in Figure 1. 1.

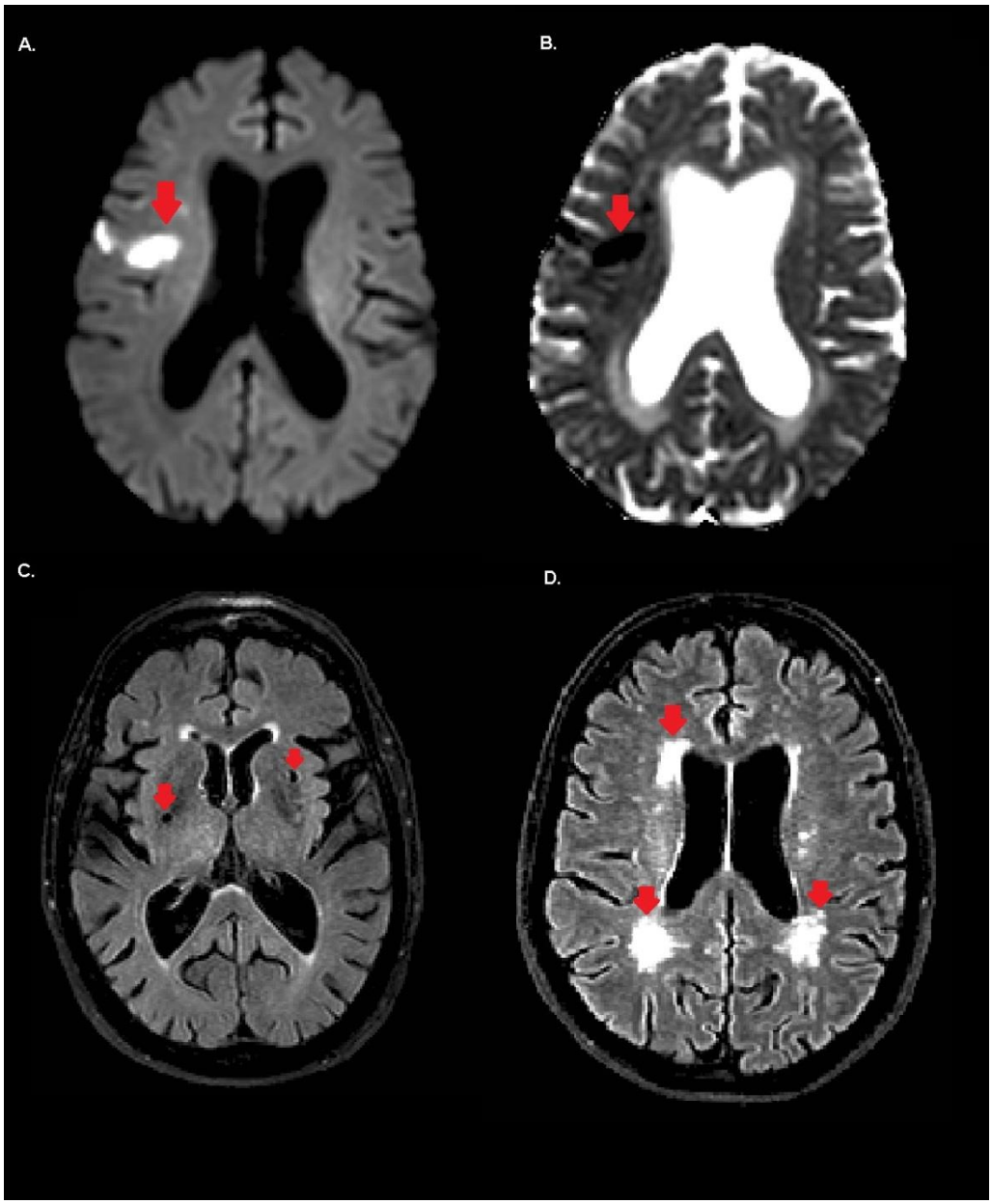



**Figure 1. 1 Types of cerebrovascular lesions. An acute ischaemic infarct showing as hyperintensity on diffusion weighted images (A) and showing as hypointensity on ADC maps (B). (C) Lacunar infarcts showing as hypointensity on FLAIR images. (D) White matter lesions showing as hyperintensity on FLAIR images. All images were obtained from the scans of ICAD patients.**

### 1.1.5 Diagnostic criteria of VCI

In this thesis, a recently proposed set of diagnostic criteria (Gorelick, Scuteri et al. 2011) was used to define cognitive deficits that associate with cerebrovascular disease. As stated in part 1.1.1, the diagnosis of VaD should be based on cognitive decline from a prior baseline and deficits in more than 2 cognitive domains which are of sufficient severity to affect the subject's activities of daily living. In contrast, the classification of vascular mild cognitive impairment (VaMCI) should be based on cognitive decline from a prior baseline and deficits of performance in at least one cognitive domain. Also, the term *probable* is used to characterise the most 'pure' forms of VaMCI or VaD, showing no history of gradually progressive cognitive disorder before or after the cerebrovascular events that suggests the presence of neurodegenerative disorders. The term *possible* to characterise VaMCI or VaD when there is an evidence of other neurodegenerative disorders that may affect cognitive performance.

The establishment of the presence of cognitive impairment not only requires a subjective report, but also requires an objective evidence of cognitive disorder provided by a formal neuropsychological test battery or a shorter bedside screening test. Using a bedside screening test seems to be more practical because a formal neuropsychological test battery is more time consuming and sometimes it may be unavailable in clinical settings. However, it is noteworthy that the bedside screening test ideally should cover a range of cognitive domains. It has been widely accepted that VCI patients have predominantly impaired executive function and slowed information processing (O'Brien, Erkinjuntti et al. 2003),

thus meaning that screening tests should at least cover these two domains. Additionally, bedside screening tests ideally cover other cognitive domains including learning and memory, language, visuo-constructional-perceptual ability, praxis-gnosis-body schema and social cognition as recommended by VASCOG statement (Sachdev, Kalaria et al. 2014). The details of cognitive assessment tools will be described in Part 1.2.

It is often difficult to determine whether the cognitive impairment is only caused by vascular pathology or caused by the underlying neurodegenerative pathology as the prevalence of mixed pathology is high, ranging from 14.2% to 44% in community-based autopsy studies (Snowdon, Greiner et al. 1997) (Schneider, Aggarwal et al. 2009) (Bennett, Wilson et al. 2013) (Troncoso, Zonderman et al. 2008) (Dolan, Crain et al. 2010) (Savva, Wharton et al. 2009) (Brayne, Richardson et al. 2009, Richardson, Stephan et al. 2012) (Matsui, Tanizaki et al. 2009) (White 2009) (Launer, Hughes et al. 2011). Therefore, it is essential to establish a predominantly vascular aetiology for diagnosing VCI. The overlap between vascular and neurodegenerative pathology, especially AD pathology, makes the diagnosis more challenging. Also, the co-existing of AD and vascular pathology could influence the progression and severity of cognitive impairment, therefore, lowering the diagnostic threshold of cognitive impairment (Attems and Jellinger 2014).

Significant evidence of cerebrovascular diseases demonstrated on neuroimaging is critical for establishing a predominant vascular aetiology and a link with the observed cognitive impairment. The role of cerebrovascular lesions in VCI will be introduced later in part 1.3.

### 1.1.6 Cognitive profile of VCI

Executive dysfunction, attention impairment and slowed information processing speed are prominent in VCI (O'Brien, Erkinjuntti et al. 2003) (Gorelick, Scuteri et al. 2011) (van Rooij, Kessels et al. 2016). As shown in a study investigating the domain-specific trends in cognitive impairment after stroke (Hurford, Charidimou et al. 2013), the prevalence of impairment in speed and attention and frontal executive function within 1 month after stroke were 72.4% and 34.4%, respectively. Three months after stroke, the prevalence of speed and attention impairment decreased to 37.9% while the prevalence of frontal executive dysfunction decreased to 16.2%.

Edwards et al. (2013) investigated the domain-specific cognitive impairment in lacunar stroke by conducting a systematic review, showing that global cognition and information processing speed were more severely impaired while executive function and attention/working memory show mild to moderate dysfunction. At the same time, other cognitive domains including memory, language and visuospatial abilities were also impaired as identified in their review. The more severely impaired global cognitive performance could be explained by the cumulative effects of the impairment of several cognitive domains. They concluded that patients with stroke showed a broader cognitive impairment profile because all major cognitive domains were involved (Edwards, Jacova et al. 2013).

## 1.2 Neuropsychometric screening tools

As proposed by the VOSCOG statement, a cognitive screening test covering a range of cognitive domains is ideal to assess cognitive performance in patients with VCI (Sachdev, Kalaria et al. 2014). This section will introduce several neuropsychometric screening tools that are commonly used to measure cognitive impairment.

### 1.2.1 Mini-Mental State Examination (MMSE)

The MMSE was originally developed to screen for dementia in a psychiatric setting and has been extended to screen global cognitive impairment in clinical services (Folstein 1990). However, several studies showed that MMSE was invalid to screen cognitive impairment after stroke due to several reasons including ceiling effects caused by the low level of task difficulty, a lack of measurements of executive function, and not completely ruling out the effects of age and educational level (Nys, van Zandvoort et al. 2005) (Pendlebury, Mariz et al. 2012) (Bour, Rasquin et al. 2010). A recent systematic review summarised that MMSE was only useful to screen post-stroke dementia but not mild to moderate cognitive impairment (Burton and Tyson 2015). Also, using MMSE could not predict any further impairment or improvement of cognitive performance over time (Bour, Rasquin et al. 2010). Thus, it seems that MMSE is not an appropriate neuropsychometric screening tool to measure VCI. Other cognitive screening tools with better sensitivity and specificity need to be considered.

### 1.2.2 Montreal Cognitive Assessment (MoCA)

MoCA (http://www.mocatest.org/) is a 30-point and simple cognitive screening tool which assesses the performance of attention and concentration, memory, visuoconstructional skills, language, conceptual thinking, calculations and orientation. It usually takes approximately ten minutes to complete this assessment (Nasreddine, Phillips et al. 2005). It has been shown that MoCA is a valid neuropsychometric screening tool to detect cognitive impairment after stroke. Several studies validated MoCA against a neuropsychological battery and demonstrated that MoCA had good sensitivity and specificity to detect cognitive impairment after stroke (Godefroy, Fickl et al. 2011) (Pendlebury, Mariz et al. 2012) (Cumming, Churilov et al. 2013). Two other studies compared MoCA with MMSE and showed that MoCA was superior to MMSE for the detection of cognitive impairment after stroke (Dong, Sharma et al. 2010) (Wong, Lam et al. 2013). However, these two studies did not validate MoCA against a neuropsychological battery. A recently published systematic review concluded that MoCA was the most valid cognitive screening tool to detect cognitive impairment in patients with stroke (Burton and Tyson 2015).

### 1.2.3 Addenbrooke's Cognitive Examination-Revised (ACE-R)

The ACE-R (100-point test) includes assessment of memory (26/100), language (26/100), visuospatial function (16/100), fluency (14/100) and attention/orientation (18/100) performance. It incorporates the questions on the MMSE and expands on the assessment of memory, language, visuospatial and fluency. It has been shown to have excellent sensitivity and specificity for the diagnosis of dementia at the cut-off score of 88/100 and 82/100 (Mioshi, Dawson

et al. 2006) (Larner and Mitchell 2014). In all research mentioned in this PhD thesis, the cut-off score of 82/100 is selected as suggested by the Australian Administration and Scoring Guide of ACE-R (http://www.ftdrg.org/wp-content/uploads/ACE_R_Aus_guide-2011.pdf). According to this guideline, the cut-off score of 88/100 is recommended for screening while 82/100 is recommended for research purpose.

It is still not clear whether ACE-R has good sensitivity and specificity for cognitive impairment after stroke/TIA as the results from the literature are inconsistent. Morris and colleagues have shown that ACE-R was not suitable for screening cognitive impairment after acute stroke even though it was more accurate than MMSE (Morris, Hacker et al. 2012). In contrast, it has been suggested that both ACE-R and MoCA had good sensitivity and specificity for diagnosing MCI one year after stroke and TIA against a neurological battery assessment (Pendlebury, Mariz et al. 2012). Also, ACE-R and other screening tools had similar accuracy to detect dementia and impairment of multiple domains after stroke (Lees, Selvarajah et al. 2014). Additionally, as suggested by a systematic review, ACE-R can be used to detect any cognitive impairment globally and in multiple domains after stroke even though they proposed MoCA as the most valid cognitive screening tool for measuring cognitive impairment after stroke (Burton and Tyson 2015). However, the decision of using ACE-R in the cognitive sub-studies included in this PhD thesis was made in 2010, which was prior to Burton's published paper.

There is no consensus on the cut-off scores for each cognitive domain in ACE-R even though some attempts have been made to define the optimal cut-off score

of each cognitive domain according to age (Mioshi, Dawson et al. 2006) (Kwak, Yang et al. 2010) (Morris, Hacker et al. 2012) (Yoshida, Terada et al. 2012) (Table 1. 2). The adapted cut-off scores of each cognitive domain in this PhD thesis will be described in Chapter 3.

**Table 1. 2 Lower limits of normal for cognitive domains in ACE-R according to age range, summarised from (Mioshi, Dawson et al. 2006) (Kwak, Yang et al. 2010) (Yoshida, Terada et al. 2012)**

| Age range | Attention& Orientation | Memory | Fluency | Language | Visuospatial |
|---|---|---|---|---|---|
| Yoshida, Terada et al. 2012 | | | | | |
| 50-59 | 18 | 22 | 8 | 25 | 14 |
| 60-69 | 18 | 21 | 8 | 25 | 15 |
| 70-79 | 17 | 20 | 7 | 23 | 15 |
| 80-86 | 17 | 18 | 5 | 24 | 16 |
| Kwak, Yang et al. 2010 | | | | | |
| 50-59 | 17 | 21 | 10 | 20 | 13 |
| 60-69 | 16 | 19 | 9 | 20 | 13 |
| 70-80 | 16 | 17 | 8 | 18 | 12 |
| Morris, Hacker et al. 2012 | | | | | |
| 50-59 | 17 | 18 | 9 | - | 15 |
| 60-69 | 17 | 19 | 8 | - | 14 |
| 70+ | 16 | 17 | 9 | - | 14 |
| Mioshi, Dawson et al. 2006 | | | | | |
| 50-59 | 17 | 18 | 9 | 24 | 15 |
| 60-69 | 17 | 19 | 8 | 21 | 14 |
| 70-75 | 16 | 17 | 9 | 22 | 14 |

## 1.3 Neuroimaging correlates of VCI

### 1.3.1 Cerebral infarcts

The association between brain infarcts and VCI remains controversial. It is generally accepted that VCI could exist without the presence of cerebral infarcts (Sachdev, Kalaria et al. 2014). In contrast, several studies stated the contributions of brain infarcts volumes on VCI. For example, Sachdev and colleagues demonstrated that infarct volume was a significant determinant of dementia in patients with ischaemic stroke (Sachdev, Brodaty et al. 2006) (Table 1. 3). Also, a recent study showed that infarct volumes significantly predicted cognitive outcome assessed by MoCA three months after stroke (Munsch, Sagnier et al. 2016) (Table 1. 3). Additionally, infarct volume was found to be related to cognitive performance assessed by the National Institutes of Health Stroke Scale (NIHSS) (Agis, Goggins et al. 2016) (Table 1. 3). The significant correlation between brain infarct volumes and cognitive impairment after stroke might be explained by the fact that large ischaemic infarcts affected a large brain area including cortical and subcortical areas, therefore leading to worse cognitive performance (Kalaria and Ballard 2001). Tomlinson and colleagues described that 100mL of brain tissue loss was sufficient to cause dementia (Tomlinson, Blessed et al. 1970).

The association between infarct location and VCI seems to be more consistent as stated in previous studies. It has been found that cerebral infarcts located in more than one location were related to impaired memory, processing speed and executive function (Saczynski, Sigurdsson et al. 2009) (Table 1. 3). Also, in

Munsch et al study, they showed infarct location was an independent predictor of post-stroke cognitive impairment in multivariate analyses even though infarct volume was found to be related to cognitive impairment in univariate analyses (Munsch, Sagnier et al. 2016). According to Munsch et al study, infarct location seemed to contribute more to cognitive impairment than infarct volume. However, since VCI could occur without the presence of infarcts, the association between infarct volumes and location and VCI should be carefully considered.

**Table 1. 3 Studies investigating the role of cerebral infarcts on cognitive impairment**

| Study | Study cohort description | Cognitive assessment | Results |
|---|---|---|---|
| Sachdev, Brodaty et al, 2006 | N=169, aged: 50-85 years, 3-6 months after stroke | Neuropsychological battery | • Stroke volume is a significant determinant of VaD.<br>• Premorbid functioning is a determinant of vascular mild cognitive impairment. |
| Munsch, Sagnier et al , 2016 | N=428, ischaemic stroke | MoCA | • Stroke location and volume are predictive of poor MoCA.<br>• In multivariable analyses, stroke location remains the strongest independent predictor of MoCA. |
| Agis, Goggins et al, 2016 | N=67, acute ischaemic stroke | NIHSS | • Infarct volume is related with NIHSS. |
| Saczynski, Sigurdsson et al, 2009 | N=4030 | Neuropsychological battery | • Patients with infarcts in multiple location has slower processing speed and poorer memory and executive function compared with patients with no infarcts.<br>• The presence of either subcortical infarcts only or cortical infarcts only is related with poorer memory performance.<br>• A combination of cerebellar and subcortical infarcts is related with slower processing speed.<br>• A combination of cortical, subcortical and cerebellar infarcts is related with slower processing speed. |

### 1.3.2 Lacunar infarcts

The role of volumes and location of lacunar infarctions on VCI is validated in previous studies. For instance, Liem and colleagues stated that volumes of lacunar infarctions were independently associated with cognitive impairment in patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) which is considered as a 'pure' model to study VCI (Liem, van der Grond et al. 2007) (Table 1. 4). The same conclusion was also shown in other two research groups' work (Viswanathan, Gschwendtner et al. 2007) (Dichgans, Filippi et al. 1999) (Table 1. 4). It has been also demonstrated that the number and volume of lacunar infarctions were related to executive dysfunction and slowed processing speed in SVD patients (Benjamin, Lawrence et al. 2014). Additionally, Koga and colleagues showed that the number of lacunar infarctions was related to frontal lobe dysfunction measured by modified Stroop test (Koga, Takashima et al. 2009) (Table 1. 4).

The location of lacunar infarctions also contributes to cognitive impairment. An autopsy study showed lacunar infarctions in thalamus and basal ganglia were associated with cognitive deficits in elderly subjects (Gold, Kovari et al. 2005) (Table 1. 4). MRI studies also verified the role of the location of lacunar infarctions in cognitive impairment. For example, Benjamin and colleagues showed that lacunar infarctions located in thalamus was related to impairment of processing speed in patients with SVD (Benjamin, Lawrence et al. 2014) (Table 1. 4). Also, Benisty and colleagues demonstrated that lacunar infarctions located in thalamus were related to global cognitive impairment and executive

dysfunction and those located in putamen/pallidum were related to memory impairment (Benisty, Gouw et al. 2009) (Table 1. 4).

Even though previous studies mentioned above have shown the impact of lacunar infarcts on VCI, its contribution on global cognition is weakened when combining with other MRI measures. For instance, Viswanathan et al (2010) have shown that among brain atrophy indexed as a decreased brain parenchymal fraction, volumes of lacunar infarcts and ultra-structural damage of white matter showing as increased mean values of apparent diffusion coefficient (ADC), brain atrophy rather than volume of lacunar infarcts showed the strongest correlation with global cognitive impairment in patients with CADASIL. They explained the inconsistency may be caused by the different phases of disease in different study cohorts. They suggested that lacunar infarct volumes had the strongest influence on VCI in the early phase of CADASIL while the impact of brain atrophy became prominent in the late phase of the disease (Viswanathan, Godin et al. 2010).

**Table 1. 4 Studies investigating the role of lacunar infarctions on cognitive impairment**

| Study | Study cohort description | Cognitive assessment | Results |
|---|---|---|---|
| Liem, van der Grond et al. 2007 | N=62, CADASIL | Neuropsychological battery | ● Lacunar infarction load is the most important MRI parameter associated with cognitive impairment in CADASIL |
| Viswanathan, Gschwendtner et al. 2007 | N=142, CADASIL | Mattis Dementia Rating Scale, MMSE | ● Overall lacunar infarction burden seems to have the most important on cognitive function in CADASIL |
| Benjamin, Lawrence et al. 2014 | N=121, lacunar stroke | Neuropsychological battery | ● Lacunar infarction number and volume are related with worse executive function and processing speed<br>● Lacunar infarctions located in thalamus are related with impaired processing speed |
| Benisty, Gouw et al. 2009 | n=633, non-disabled subjects beyond 65 years old | MMSE, a modified AD assessment scale and compound Z scores for memory , executive function, speed and motor control | ● The presence of lacunar infarctions in the thalamus is associated with global cognitive impairment and executive dysfunctions<br>● There is a significant negative association between the presence of lacunar infarctions putamen/pallidum and memory |
| Gold, Kovari et al. 2005 | N=72, elderly individuals, autopsy studies | Clinical Dementia Rating (CDR) scale | ● Lacunar infarctions located in thalamus and basal ganglia are associated with CDR scores |
| Koga, Takashima et al. 2009 | N=350, non-demented elderly individuals | MMSE and modified Stroop test (MST) | ● Impaired frontal lobe function is related to number of lacunar infarctions |

### 1.3.3 White matter hyperintensities

White matter hyperintensities are another type of cerebrovascular lesions that relate to VCI (Jellinger 2013). White matter hypernintensities are prevalent in the general population, ranging from 11-21% in subjects at age from 55 to 85

years old to 94% in subjects at age from 80 to 82 (Ylikoski, Erkinjuntti et al. 1995) (Garde, Mortensen et al. 2000). Empirical evidence has been provided to validate the association between white matter lesions and cognitive impairment (Table 1. 5). According to a recently published meta-analysis, the presence of white matter lesions was related to global cognitive impairment (Fisher Z: -0.10), memory (Fisher Z: -0.08), processing speed (Fisher Z: -0.11), attention and executive functions (Fisher Z: -0.11) and perception/construction (Fisher Z: -0.15) (Kloppenborg, Nederkoorn et al. 2014). Several studies showed the contribution of white matter lesions on MCI and AD. For example, Smith and colleagues demonstrated that white matter hyperintensities predicted the progression from normal cognitive status to MCI (adjusted hazard ratio: 3.3) (Smith, Egorova et al. 2008). In a community-based study of middle-aged subjects, white matter lesion volumes were related to an increased risk of dementia (hazard ratio: 2.22) and an increased risk of amnestic MCI in subjects beyond 60 years (hazard ratio: 2.47) (Debette, Beiser et al. 2010). In another population-based study including non-demented elderly people, volumes of periventricular white matter lesions at baseline and the progression of periventricular white matter lesion volumes were related to slowed processing speed (van den Heuvel, ten Dam et al. 2006). Silbert and colleagues demonstrated that progression of total and periventricular white matter lesion volumes, rather than baseline white matter lesion volumes, predicted more of cognitive impairment (Silbert, Howieson et al. 2009).

There are several studies demonstrating the contribution of white matter lesions on cognitive impairment in patients with vascular pathology. For instance, Prins and colleagues showed that severity of periventricular white matter lesions was

related to executive dysfunction and slowed processing speed in SVD patients (Prins, van Dijk et al. 2005). In patients with history of cerebrovascular diseases, baseline white matter lesion volumes were associated with cognitive impairment (hazard ratio: 7.7) (Dufouil, Godin et al. 2009). In contrast, non-significant correlation between white matter lesion volumes and cognitive impairment in stroke patients was also identified (Firbank, Burton et al. 2007).

The mechanisms of white matter lesions causing cognitive impairment could be explained from several perspectives (Debette and Markus 2010). Firstly, white matter lesions could directly lead to the damage of white matter tracts that connecting subcortical neural networks, therefore leading to cognitive impairment. This explanation is further strengthened when considering the association between white matter lesions and executive dysfunction which is assumed to relate to the damage of cortical-subcortical circuits (Nordahl, Ranganath et al. 2006). Moreover, the pathology of white matter lesions could interact with other pathologies such as AD pathology and vascular risk factors, therefore lowering the threshold of dementia diagnosis (Zlokovic 2005) (Prins, van Dijk et al. 2004).

**Table 1. 5 Studies investigating the role of white matter lesions on cognitive impairment**

| Study | Study cohort description | White matter lesions measure | Cognitive assessment | Results |
|---|---|---|---|---|
| Smith, Egorova et al. 2008 | normal control: n=67; MCI: n=156; | quantitative, dichotomised | neuropsychological battery | • White matter lesion is a predictor of progression from normal to MCI (adjusted hazard ratio: 3.30) |
| Debette, Beiser et al. 2010 | n=1344, mean age: 62 | quantitative, continuous and dichotomised | neuropsychological battery | • White matter lesion volumes are associated with an increased risk of dementia (HR=2.22)<br>• White matter lesion volumes are associated with amnestic mild cognitive impairment in participants aged > or =60 years (HR=2.47) |
| Prins, van Dijk et al. 2005 | n=832, mean age :71; SVD | SQ (0-9) for PVH, quantitative for DWMH | MMSE and cognitive index) and domain-specific (Stroop, Letter Digit Substitution Task, Verbal Fluency test, 15-word verbal learning test | • Increasing severity of periventricular white matter lesions is related with impaired executive function and slowed processing speed. |
| Silbert, Howieson et al. 2009 | n=98, mean age: 62 | quantitative, continuous | MMSE | • Progression of total and periventricular white matte lesion volumes are related with cognitive impairment |
| Dufouil, Godin et al. 2009 | n=226, patients with cerebrovascular history | SQ, categorised in four grades | MMSE; dementia: DSM-IV | • Higher white matter lesion volume is a strong predictor of dementia and cognitive impairment in patients with cerebrovascular disease history |
| Firbank, Burton et al. 2007 | n=79, stroke survivors, mean age: 80 | quantitative, continuous | CAMCOG | • No association between global cognitive impairment and white matter lesion volumes |
| van den Heuvel, ten Dam et al. 2006 | n=554, non-demented elderly population | quantitative | domain-specific (picture word learning test; letter digit coding test; abbreviated Stroop test) | • Volume of periventricular white matter lesions and the progression of periventricular white matter lesion volumes are related with slowed processing speed.<br>• No association between deep white matter lesion volumes and declines in cognitive domains |

### 1.3.4 Brain atrophy

Atrophy of medial temporal lobe and hippocampi are thought to strongly support the diagnosis of AD (Scheltens and van de Pol 2012) and it has been demonstrated that medial temporal lobe atrophy could differentiate AD from other forms of dementia including Lewy bodies dementia and VCI (Burton, Barber et al. 2009). However, the low specificity of using atrophy of medial temporal lobe and hippocampi to diagnose AD are increasingly being recognised because atrophy of medial temporal lobe and hippocampi are also found in VCI (Fein, Di Sclafani et al. 2000) (Bastos-Leite, van der Flier et al. 2007) (Wong, Wang et al. 2015) (Selnes, Grambaite et al. 2015). Additionally, the presence of atrophy of medial temporal lobe and hippocampi increased the risk of post-stroke dementia (Leys, Henon et al. 2005). In subcortical ischaemic vascular disease, it has been found that hippocampal atrophy, which may be caused by a mixture of vascular and neurodegenerative pathology, was strongly correlated with cognitive impairment (Fein, Di Sclafani et al. 2000). A recent study investigated the association between volume measurements on MRI and VCI assessed by the established diagnostic criteria (NINDS-CSN) (Wong, Wang et al. 2015). They found that left hippocampal atrophy was related to the impairment of executive function (β: 0.255), language (β: 0.277) and memory (β: 0.279) while right hippocampal atrophy was associated with visuospatial (β: 0.204) and memory dysfunction (β: 0.229). Additionally, global cognitive impairment was found to be related to atrophy of bilateral hippocampi (left: β: 0.297; right: β: 0.225). Even though there has been increasing evidence to demonstrate an association between atrophy of medial temporal lobe and hippocampi with cognitive

impairment in patients with vascular pathology, the exactly underlying mechanism and its role as a contributor to VCI is still unclear.

Apart from the findings of a correlation between atrophy of specific brain regions and cognitive impairment in patients with vascular pathology, the role of diffuse cortical atrophy in cognitive impairment in subcortical ischaemic vascular disease (Fein, Di Sclafani et al. 2000) has been demonstrated. Fein et al (2000) have found that cortical atrophy was associated with the severity of cognitive impairment in patients with subcortical ischaemic vascular disease. They concluded that the cortical atrophy may partially be caused by the magnitude of ischaemic process indexed by the extent of white matter lesions (Fein, Di Sclafani et al. 2000). Similar findings but different underlying mechanisms of cortical atrophy in subcortical VaD have been suggested by a study using 3D volumetric analysis approach (Seo, Ahn et al. 2010). They found out that the frontal cortical thinning was mainly found in subcortical VaD patients while temporoparietal cortical atrophy was predominant in AD patients. According to their findings, patients with subcortical VaD had a distinct pattern of cortical atrophy such that the cortical thinning in subcortical VaD could not be completely explained by co-existing AD pathology. A recent study has shown that ischaemic vascular disease and AD had different effects on cortical atrophy and cognition due to the diverse pathological changes in the brain (Zheng, Vinters et al. 2016). Based on their findings, the effect of ischaemic vascular disease on cognition appeared to occur through cerebral infarctions and cortical grey matter atrophy while the effect of AD on cognition appeared to occur through a direct pathway. All these findings highlight cortical atrophy may have an independent effect on cognition in patients with vascular pathology. However,

more research is needed to investigate whether patients with VCI have a distinct pattern of cortical atrophy which could be differentiated from that in AD patients. In the meantime, the exact underlying mechanism of cortical atrophy leading to VCI is still not clear.

### 1.4 Neuroimaging techniques for VCI

Magnetic resonance imaging (MRI), as a non-invasive technique, plays a key role in the diagnosis of cerebrovascular diseases, specifically by identification of cerebrovascular lesions using conventional MRI techniques. Advanced MRI techniques could provide information on the mechanisms of VCI. This section will introduce several conventional and advanced MRI techniques that have been widely used for investigating brain structural and functional alterations in VCI patients.

#### 1.4.1 Conventional MRI

Conventional MRI techniques including T1-weighted images, T2-weighted images and fluid-attenuated inversion recovery (FLAIR) images are included in a typical ischaemic stroke MRI protocol. T1-weighted images differentiate white matter from grey matter and measure atrophy of cortex and subcortical grey matter. T2-weighted images and FLAIR images provide information on the delineation of white matter hypernintensities. However, the conventional MRI techniques have low sensitivity to evaluate the evolution of brain ischaemia.

### 1.4.2 Diffusion-weighted imaging (DWI)

Diffusion-weighted imaging (DWI) is a technique that quantifies the Brownian motion of water molecules within tissue (Johansen-Berg and Behrens 2009). The motion of water molecules is restricted in biological tissues because of cell membranes, vascular structures and axon cylinders. In biological tissues, DWI signal is derived from water molecules motion in the intracellular space, extracellular space and intravascular space. Biological tissue cellularity and the integrity of cell membranes are inversely associated with the degree of restriction of water molecules diffusion. Water molecules diffusion is more restricted in biological tissue with a high cellular density while it is less restricted in biological tissue with a low cellular density.

Stejskal and Tanner adapted a standard T2-weighted spin-echo sequence to detect and quantify water molecules motion in vivo in 1965 (Stejskal and Tanner 1965) and this approach has been the basis of DWI sequences until now. The sensitivity of DWI sequence could be varied by changing "b value" which is the strength of a diffusion gradient. A small b value is usually required for water molecules with a large degree of motion or a great diffusion distance. By contrast, a large b value is required for water molecules with a small degree of motion or a short diffusion distance. DWI sequence is usually performed using at least two b values (b=0 s/mm$^2$ and another b value more than 0 s/mm$^2$).

The diffusion properties of water molecules movements can be quantified by calculating the apparent diffusion coefficient (ADC). The ADC is calculated for each pixel in an image and displayed as a parametric map. Low ADC values

reflect areas of restricted diffusion in highly cellular areas while high ADC values reflect less cellular areas.

The evolution of ischaemic brain lesions from ischaemia to structural infarcts could be shown using DWI techniques. During the hyperacute phase (<3h) after ischaemic cerebrovascular events, the structural brain change caused by cytotoxic cell swelling could be identified on diffusion-weighted images (Carey, Seitz et al. 2013), showing as hyperintensity on diffusion-weighted images and hypointensity on ADC maps. The ischaemic lesions could be still shown as normal appearance on conventional T2-weighted images. After that, ischaemic lesions undergo dynamic changes including tissue degradation, brain oedema formation, subsequent lesion shrinkage and atrophy of brain parenchyma and ventricles (Carey, Seitz et al. 2013). During the acute (3-24 h) and subacute (1-14 days) phase, ischaemic lesions could be clearly showed as hyperintensity on diffusion weighted images but hypointensity on ADC maps. After about 30 days (chronic phase), the infarction lesions are clearly visible on conventional MRI images (T1- and T2-weighted images) but not on diffusion-weighted images (Lutsep, Albers et al. 1997).

### 1.4.3 Diffusion tensor imaging (DTI)

Diffusion tensor imaging (DTI) is an extension of DWI. The formalism of diffusion tensor was introduced into MRI by Basser and colleagues in 1994 (Basser, Mattiello et al. 1994). It quantifies the magnitude and directionality of water molecules in a voxel into directional components. With DWI, diffusion is

fully described using a single parameter. However, diffusion can no longer be characterised by a single parameter in the presence of anisotropy but requires a tensor, $\underline{D}$, which describes the direction and degree of anisotropy of diffusion (Le Bihan, Mangin et al. 2001). The diffusion tensor could be illustrated using a diffusion ellipsoid with the eigenvectors defining the directions of principal axis of diffusion and ellipsoidal radii defined by the eigenvectors (Alexander, Lee et al. 2007).

Mean diffusivity (MD) and fractional anisotropy (FA) are two main parameters derived from diffusion tensor images (Basser, Mattiello et al. 1994). MD reflects the average magnitude of molecular displacement by diffusion, which could be calculated by the following equation:

$$MD = (\lambda_1+\lambda_2+\lambda_3)/3$$

where λ is the eigenvalue of the axis length of the ellipsoid which is equivalent to the ADC value derived from diffusion-weighted images.

FA is a measure of the directionality of water molecular displacement by diffusion, which ranges from 0 (purely isotropic diffusion) and 1 (highly anisotropic diffusion). It is derived from the following equation:

$$FA = \sqrt{\frac{(\lambda_1 - MD)^2 + (\lambda_2 - MD)^2 + (\lambda_3 - MD)^2}{2({\lambda_1}^2 + {\lambda_2}^2 + {\lambda_3}^2)}}$$

Brain chronic ischaemia could lead to increased MD and decreased FA of ischaemic infarcts (Sotak 2002). Within 3-5 days after ischaemic event, an increased FA, which is thought to be caused by cell swelling during cytotoxic oedema therefore leading to reduced space between the myelin fiber bundles, has been observed. During the subacute and chronic phase of ischaemic stroke, decreased FA is expected due to the loss of anisotropic character of brain tissue therefore leading to ultra-structural damage (Armitage, Bastin et al. 1998). The details of ischaemic infarcts evolution and its relation with diffusivity have been described in the previous section (1.4.2).The details of FA and MD values reflecting disrupted white matter integrity will be introduced in Chapter 6.

### 1.4.4 BOLD functional MRI (fMRI)

Functional MRI (fMRI) detects the blood-oxygenation-level-dependent (BOLD) changes in the MRI signal. Oxygenated haemoglobin is diamagnetic which does not affect the surrounding magnetic field while deoxygenated haemoglobin is paramagnetic therefore causing magnetic field distortion and resulting in reduced $T_2^*$ values. T2* relaxation refers to decay of transverse magnetisation caused by a combination of spin-spin relaxation and magnetic field inhomogeneity (Chavhan, Babyn et al. 2009). Under normal conditions, the conversion of oxygenated haemoglobin into deoxygenated haemoglobin remains constant within the capillary bed. However, when neuronal activity increases, an overcompensating increase of oxygenated haemoglobin occurs in blood flow because the vascular system supplies more oxygenated haemoglobin than is

needed. Correspondingly, this leads to a decrease in the amount of deoxygenated haemoglobin and therefore a decrease in the signal loss due to $T_2^*$ effects. BOLD fMRI is based on the physiological response related to brain activations and shows the difference in signal on $T_2^*$-weighted images as a function of the amount of deoxygenated haemoglobin (Huettel, Song et al. 2009).

Functional connectivity, which is defined as the synchrony of neuronal activity among direct or indirect linked brain regions, can be studied without performing any tasks or stimuli. Resting-state fMRI technique, which investigates the temporal correlation of BOLD signal between brain regions in the absence of a task or stimulus, has been increasingly used to map the functional organisation of the brain (Friston, Frith et al. 1993) (Aertsen, Gerstein et al. 1989) (van den Heuvel and Pol 2010). The observed spontaneous BOLD signals within resting-state networks are predominantly dominated by low frequency (<0.1Hz) while respiratory (0.1-0.5 Hz) and cardiovascular (0.6-1.2 Hz) oscillations are dominated by relatively higher frequency (Cordes, Haughton et al. 2001). Those low frequency spontaneous fluctuations of BOLD signals are thought to mainly arise from a combination of cognitive processes and internal neural dynamics (Buckner 2010) (Rosazza and Minati 2011). This makes the spontaneous fluctuations of BOLD signals a robust measure to investigate functional connectivity between brain regions on a whole-brain scale (Gusnard and Raichle 2001) (Greicius, Krasnow et al. 2003) (van den Heuvel and Pol 2010).

Several imaging analysis approaches have been developed in order to map the functional connectivity of brain networks. Seed-based functional connectivity analysis (Biswal, Yetkin et al. 1995) (Cordes, Haughton et al. 2000) (Fransson

2005) (Andrews-Hanna, Snyder et al. 2007) (Song, Zhou et al. 2008) (van den Heuvel and Pol 2010) (Margulies, Bottger et al. 2010) and independent component analysis (ICA) (van de Ven, Formisano et al. 2004) (Beckmann, DeLuca et al. 2005) (van den Heuvel and Pol 2010) (Margulies, Bottger et al. 2010) are two major post-processing methodologies applied to resting-state fMRI data. Additionally, clustering (van den Heuvel, Mandl et al. 2008) (Salvador, Suckling et al. 2005) (Cohen, Fair et al. 2008) and graph theory analysis (Astolfi, Fallani et al. 2007, Bullmore and Sporns 2009) (Margulies, Bottger et al. 2010) have been increasingly used in analysing resting-state fMRI to examine brain functional networks.

#### 1.4.4.1 Seed-based functional connectivity analysis

Seed-based functional connectivity analysis, a hypothesis-driven approach, is the most straightforward method to investigate the correlation between time-series of a *priori* region-of-interest (ROI) and that of the rest of the brain, resulting in a functional connectivity map which provides a clear view of functional connections of the seed region (Biswal, Yetkin et al. 1995). The primary advantage of seed-based analysis approach is that it shows brain regions that most strongly functionally connected with the seed region. Given that it only shows functional connectivity information of the *priori* defined regions, it is difficult to investigate functional connectivity on a whole-brain scale. Also, the influence of noise due to residual head motion and induced by scanners could bias the results by analysing the fMRI data using seed-based approach (Cole, Smith et al. 2010). In addition, the selection, size and shape of regions could be potential drawbacks as they may bias the results (Margulies, Bottger et al. 2010).

#### 1.4.4.2 Independent component analysis (ICA)

The ICA-based analysis is one of the model-free methods and the most popular approach to examine functional connectivity patterns at a network level rather than at a regional level. It assumes that resting-state data set comprises a mix of independent signals from a number of spatially distributed sources. Using ICA-based analysis, the dataset is decomposed into several independent spatial or temporal components. Each component can be considered as a brain functional network including brain regions showing similar BOLD activities or a noise component induced by scanners or physiological activities. A high level of consistency in terms of the networks reported by ICA studies has been shown, meaning that ICA is a robust analysis approach to examine multiple connectivity patterns (Calhoun, Adali et al. 2002) (Esposito, Aragri et al. 2008) (van den Heuvel and Pol 2010). The main advantage of ICA approach over seed-based analysis method is the avoiding of prior spatial assumptions. Also, resting-state networks identified by ICA approach can be less prone to noise than those from seed-based analysis approach. Thus, ICA could be used as a method for identifying noise components in the BOLD signal. Several resting-state networks, which consist of structurally distinct but functionally strongly linked brain regions, have been identified by a group of neuroimaging studies. The details of these resting-state networks will be introduced in Chapter 7.

# Chapter 2 Aims and hypotheses of the thesis

## 2.1 Knowledge gap

The mechanisms underpinning cognitive impairment in patients with symptomatic carotid artery disease seem to be multifactorial. Those studies mentioned in Chapter 1 show the relationships between cognitive impairment and structural brain damage as shown using neuroimaging techniques. However, they fail to provide evidence for the mechanisms underlying VCI.

Also, there is increasing recognition that the prevalence of mixed pathology including neurodegenerative and vascular pathology is high (Sachdev, Kalaria et al. 2014) and the co-existing of these two pathologies have an additive effect on cognition (Vemuri, Lesnick et al. 2015). Therefore, it is still unclear whether the association between vascular pathology, observed on neuroimaging, and cognitive impairment is confounded by the probably co-existing neurodegenerative pathology. Additionally, the extent to which cognitive impairment is contributed by disconnection related neuroimaging markers or hippocampal pathology markers is also unclear. Another question needed to be answered is how best to determine a certain degree of vascular pathology is sufficient to account for the observed cognitive impairment in patients with symptomatic carotid artery disease.

Several studies have shown that disconnection could be one of the mechanisms underlying VCI. However, they demonstrated their hypothesis in patients with CADASIL which is considered as a ‘pure’ model to study VCI. Therefore, it remains unclear whether disconnection could still explain probable VaMCI in a group of elderly patients with symptomatic carotid artery disease.

## 2.2 Thesis aims and hypotheses

The main purpose of this thesis is to investigate neuroimaging correlates of VCI in a group of patients with symptomatic carotid artery disease. Different subsets of neuroimaging changes including lesional and non-lesional changes which can be mechanistically interpreted as reflecting disconnection will be investigated.

The studies presented here included three themes.

> **Theme I:** Relationship between VCI and cerebrovascular lesional damage including cerebrovascular topography and severity of cerebrovascular lesions damage.

The first theme will be examined in Chapter 5. The main hypothesis of this chapter is that cognitive impairment is related to the strategic topography of cerebrovascular lesions and the identification of this relationship could implicitly refer the role of damage of certain brain circuits in VCI. A hypothesis-driven voxel-based lesion symptom mapping approach is utilised to investigate the relationship between the frequency of cerebrovascular lesions and cognitive impairment. The second hypothesis is that severity of cerebrovascular lesions damage is related to cognitive impairment. Diffusional changes within cerebrovascular lesion areas will be extracted to investigate the contribution of the severity of cerebrovascular lesions damage on cognitive impairment.

**Theme II:** Relationship between VCI and microscopic damage of non-lesional brain tissues

The second theme will be examined in Chapter 6. The hypothesis is that microscopic damage of non-lesional brain tissues contributes to cognitive impairment in patients with symptomatic carotid artery disease. To test this hypothesis, DTI technique is employed to investigate the role of microscopic damage of strategic brain areas, normal appearing white matter and widespread white matter tract skeleton on VCI.

**Theme III:** Relationship between VCI and functional disconnection of large-scale cognitive networks

The third theme will be examined in Chapter 7. The main hypothesis of this chapter is that functional disconnection of large-scale cognitive networks contributes to cognitive impairment in patients with symptomatic carotid artery disease. To test this hypothesis, resting-state fMRI techniques will be utilised to obtain the large-scale cognitive networks. A secondary hypothesis is that functional disconnection of large-scale cognitive networks mediates the association between structural disconnection and cognitive impairment.

To sum up, this thesis aims to use neuroimaging techniques to investigate VCI in patients with symptomatic carotid artery disease. It is hoped that this research will contribute to the continuing search for potential biomarkers to assess the effects of interventions tailored to improve cognitive performance. Additionally, this research attempts to unbundle the contributions of vascular pathology to cognitive impairment in patients with symptomatic carotid artery disease.

# Chapter 3 General methods

Studies in Chapter 4, 5, 6, 7 and 8 were co-recruited from an independent research funded by the National Institute for Health Research (NIHR), entitled "Neurovascular Imaging in patients with symptomatic carotid artery disease to define high risk of stroke (ICAD)". The ethics application was submitted by Professor Dorothee Auer. The patient recruitment, clinical examination, cognitive assessment, organisation and supervision of MRI scans were conducted by Dr. Akram Hosseini (PhD thesis title: Neurovascular imaging to define the risk of cerebrovascular ischaemic events and cognitive impairment in patients with carotid artery disease) and Richard Simpson who are the other two research investigators of the ICAD study. The author of this thesis participated in the process of cognitive assessments and MRI scans of several participants as an observer when Dr. Akram Hosseini recruited participants. Even though the author of this thesis did not participate in the whole recruitment process of the ICAD study, the author of this thesis had three years of experience as a MRI scanning assistant during the PhD to engage with NHS patients and MRI scans. The details will be provided below. All of the other work including data analyses and interpretation of results mentioned in this PhD thesis were completed by the author of this thesis unless mentioned specifically.

## 3.1 Subject recruitment

The aims of the main ICAD study (Hosseini et al., 2016, under review) were to determine whether: (1) intraplaque haemorrhage detected by MRI can predict recurrent cerebral infarction in patients with symptomatic carotid stenosis and (2) the addition of biomarkers to intraplaque haemorrhage MRI data can further

improve the prediction of recurrent cerebral infarction. As stated in the ethical application, the ICAD study was aimed to recruit 200 patients over 3 years.

Participants were recruited from NHS clinics at Nottingham University Hospitals including the City Hospital, the Queen's Medical Centre, King's Mill Hospital and the Derby Royal Infirmary Hospital from 2010 to 2015.

The inclusion criteria for the main ICAD study are as follows:

- Recent (6 months) non-disabling cerebrovascular event with carotid artery stenosis of >30% confirmed by carotid duplex scans as part of clinical care. The CAVATAS criteria, based in the NASCET method of angiographic measurement, was used for measuring artery stenosis (McCabe, Pereira et al. 2005).
- Life expectancy of > 3 years
- Able to consent
- Age range ≥ 19 years. No discrimination of sex, race or other comorbidities

Exclusion criteria are:

- MRI contraindication (pacemaker, metal implant, claustrophobia)
- Any medical conditions that could compromise patient safety, i.e. pregnant women, severe ischaemic heart disease
- Planned ipsilateral carotid endarterectomy
- Other documented neurodegenerative conditions or diseases which may contribute to cognitive impairment. The clinical history of each participants was carefully reviewed by Dr Akram Hosseini or Dr Richard Simpson.

- Aphasic and dysphasic patients were excluded if they were found during the interview conducted by the research investigators (Dr Akram Hosseini and Dr Richard Simpson)

### 3.2 Clinical assessment

On attending, all subjects gave written informed consent prior to the participation. Consenting participants were interviewed by Dr. Akram Hosseini or Richard Simpson for study eligibility and clinical information including cardiovascular risk factors and demographic information.

### 3.3 Protocol for cognitive assessment

All participants underwent a cognitive assessment using ACE-R (http://www.stvincents.ie/dynamic/File/Addenbrookes_A_SVUH_MedEl_tool.pdf) if they agreed. The cognitive assessments were conducted by Dr. Akram Hosseini or Richard Simpson. The cut-off score used in this PhD thesis to differentiate cognitively impaired patients from cognitively intact patients is 82/100. The reason to choose this cut-off score has been mentioned in Chapter 1. Given that only Morris and colleague have determined the cut-off scores of cognitive domains in ACE-R in a group of subjects with cerebrovascular disease which is similar to that in the ICAD study, the cut-off scores of cognitive domains in ACE-R published in Morris and colleagues was selected to categorise cognitive status (normal vs abnormal) of attention &orientation, memory, fluency and visuospatial ability (Morris, Hacker et al. 2012) (Table 1. 2). Since the cut-off score of language component was not provided in Morris's paper, it was selected from previous research which demonstrated a cut-off score

of 20/26 of ACE-R language component showed 90% sensitivity and 95% specificity in stroke patients (Gaber, Parsons et al. 2011).

### 3. 4 Protocol for MRI assessment

All participants underwent an MRI scan on the day of clinical and cognitive assessment. MRI scanning was performed using a 3 Tesla Achieva MRI scanner (Philips, Eindhoven, Netherlands) at the Queen's Medical Centre, Nottingham. This scanner is mainly used for clinical routine scans. All participants were scanned using the same MRI protocol. The scan usually lasted up to 40 minutes. All participants completed the MRI safety questionnaire prior to the scan. The scanning sequences included in this PhD thesis were as follows:

- DTI: Spin echo single-shot diffusion-weighted EPI, b = 1000 s/mm$^2$, 1 b0 volume, 15 directions, TR/TE 4.2s/57ms, FOV 224 × 224, matrix 112 × 112, 45 slices of 3mm thickness
- FLAIR: TR/TE/TI 10.5/0.14/2.75 s, FOV 224 × 192, matrix 224 × 181, 45 slice of 3mm thickness
- VENDI: a plaque haemorrhage sensitive blood nulled, water selective T1 weighted 3-dimensional gradient echo sequence; TR/TE/TI 8.8/4.1/570ms, FOV 92 × 162, matrix 384 × 384, 102 slices of 0.9mm thickness; covering from the top of brain to the heart on coronal images
- Resting-state functional MRI (eye-closed): TR/TE 2200ms/35ms, FOV: 208×105×208 mm, 54 slices of 3mm thickness

Raw images were exported from the MRI scanner on DVD. Then the data were imported into the Academic Radiology **pnjpuck** server by Dr. Akram Hosseini or Richard Simpson. The process of importing raw data used the **dtoa** programme, an in-house software which converted the raw data into Analyze format written by Dr. Paul Morgan in the University of Nottingham (http://www.nottingham.ac.uk/~msapm1/software/dtoa.html).

The diffusion toolbox of FSL (Behrens, Johansen-Berg et al. 2003) was used to pre-process DTI images and compute FA and MD maps.

### 3. 5 Quality assurance of images

#### 3. 5.1 FLAIR images

Each subject's FLAIR images were visually inspected to exclude patients with motion artefacts. An example of a subject's FLAIR images with motion noise is provided in Figure 3. 1.

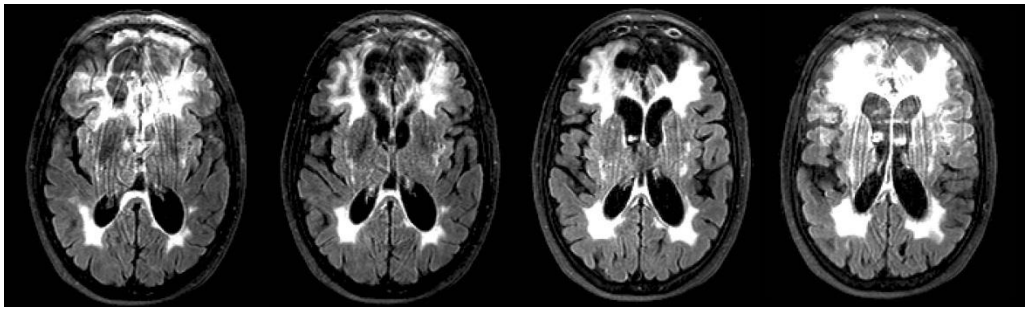


**Figure 3. 1 An example of FLAIR images with motion noise from an ICAD subject**

#### 3. 5.2 DTI images

FA and MD images were visually examined on each subject in order to exclude subjects with noise due to slice dropouts, eddy-current induced geometric

distortions, systematic vibration and ghosting (insufficient or incorrect fat suppression). An example of an ICAD subject's DTI images with noise is provided in Figure 3. 2.

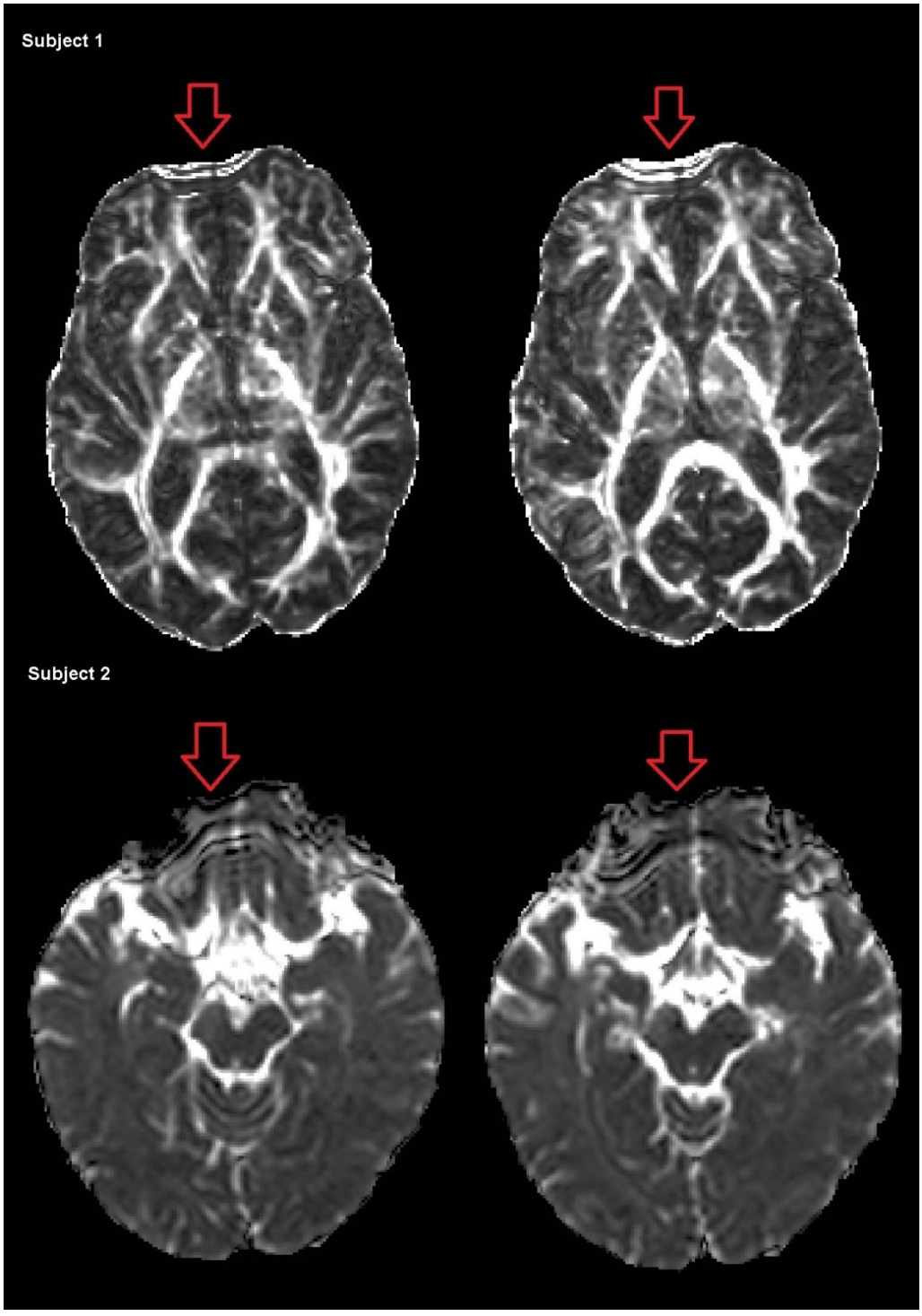



**Figure 3. 2 Examples of DTI images with noise (2 subjects of ICAD study)**

The quality assurance of resting-state fMRI images will be introduced in Chapter 7.

### 3.6 Medial temporal lobe atrophy (MTA) assessment

As introduced in Chapter 1, atrophy of whole brain or specific brain regions is related to VCI. In order to assess MTA and investigate how much it contributes to cognitive decline in elderly patients with symptomatic carotid artery disease, the MTA scale, a visual score of assessing MTA, was applied on each hemisphere of every subject (Scheltens, Leys et al. 1992). The MTA scale is usually rated on coronal T1 weighted images and based on a visual score of the width of the choroid fissure, the width of the temporal horn and the height of the hippocampal formation (Table 3. 1 and Figure 3. 3). Patients with MTA are defined as the averaged MTA score (averaged score of both hemispheres) higher than 1.5 in patients < 75 years old, or that higher than 2 in patients ≥ 75 years old, or that higher than 2.5 in patients ≥ 85 years old (Ferreira, Cavallin et al. 2015). Given that no high resolution T1 weighted images were obtained for the main ICAD study, the reconstructed coronal FLAIR images were used to apply the MTA scale. The reconstruction of FLAIR images was performed using MRIcron (http://www.mccauslandcenter.sc.edu/mricro/). MTA scale was also applied on all subjects who had 3D coronal T1-weighted images covering the temporal lobe (VENDI images) to validate the reliability of using re-constructed coronal FLAIR images to assess MTA (Figure 3. 3). Visual rating on all subjects was performed twice for testing inter- and intra-rater reliability. The first time was done by the author of this thesis and the second time was done by Quratulain

Shaikh who is a neurology trainee and completed a taught MSc in Translational neuroimaging supervised by Professor Dorothee Auer. Cohen's Kappa coefficient was used to assess inter- , intra- rater reliability and the agreement of applying MTA scale on FLAIR with that on coronal T1-weighted gradient echo images. The strength of agreement was decided according to the value of Cohen's Kappa coefficient (Table 3. 2) (Landis and Koch 1977).

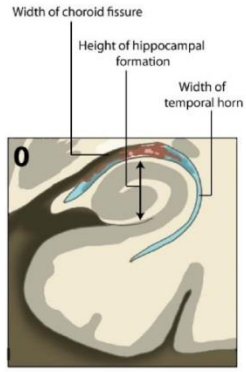
**Table 3. 1 MTA scale for medial temporal lobe atrophy**

| Score | Width of choroid fissure | Width of temporal horn | Height of hippocampal formation |
|---|---|---|---|
| 0 | N | N | N |
| 1 | ↑ | N | N |
| 2 | ↑↑ | ↑↑ | ↓ |
| 3 | ↑↑↑ | ↑↑↑ | ↓↓ |
| 4 | ↑↑↑ | ↑↑↑ | ↓↓↓ |

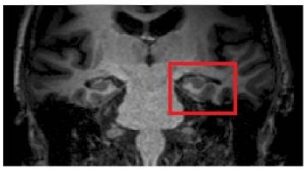
**Score 0:** no atrophy
**Score 1:** only widening of choroid fissure
**Score 2:** also widening of temporal horn of lateral ventricle
**Score 3:** moderate loss of hippocampal volume (decrease in height)
**Score 4:** severe volume loss of hippocampus

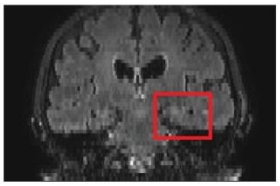
**Coronal T1 weighted images**

**Re-constructed coronal FLAIR images**

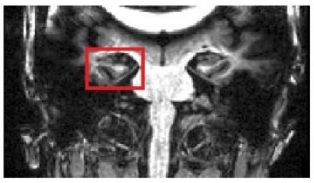


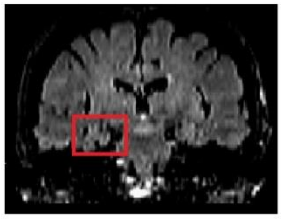

**MTA = 0**

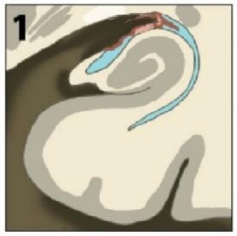




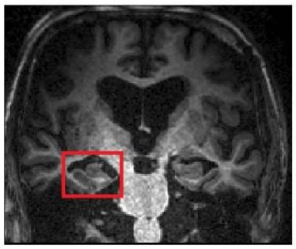
**MTA = 1**

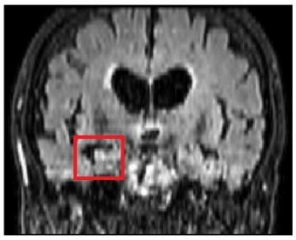


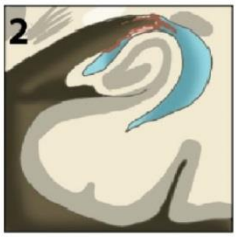





**MTA = 2**

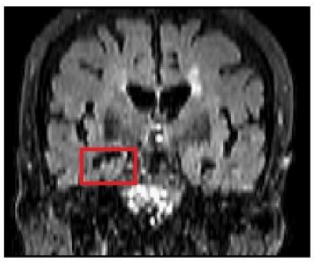


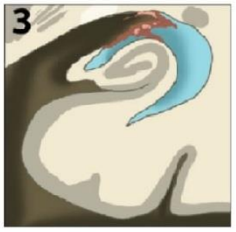





**MTA = 3**








**MTA = 4**



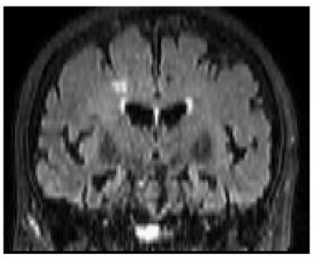




**Figure 3. 3 Examples of applying Scheltens scale on coronal T1 weighted images and re-constructed coronal FLAIR images. The figures on the left side and in the middle were obtained from ICAD subjects. The figures on the right side were obtained online (https://www.imaios.com/en/e-Cases/Channels/Radiology/Radiological-classifications-commonly-used-in-medical-imaging/MTA-scale-for-Medial-Temporal-lobe-Atrophy-Scheltens#).**

**Table 3. 2 Strength of agreement based on the value of Cohen's Kappa coefficient**

| Value of K | Strength of agreement |
|---|---|
| <0.01 | poor |
| 0.01-0.20 | Slight |
| 0.21 - 0.40 | Fair |
| 0.41 - 0.60 | Moderate |
| 0.61 - 0.80 | Substantial |
| 0.81 - 1.00 | Almost perfect |

### 3. 7 Cerebrovascular lesion volumes measurements

#### 3.7.1 Cerebrovascular lesions outlining

Lacunar infarctions were defined as small (<20mm) hypointense subcortical areas on FLAIR images with or without FLAIR hyperintense rim. Hyperintense lesions on b1000 images that were hypointense on MD maps were defined as acute ischaemic infarctions. Subcortical FLAIR hyperintense lesions were outlined to determine chronic subcortical ischaemic lesions, and cortical FLAIR hypointensities were outlined to reflect chronic cortical infarctions (Figure 3. 4).The cerebrovascular lesions outlining process was discussed with and supervised by an experienced neuroradiologist (Professor Dorothee Auer, the

main supervisor). To guarantee the quality of cerebrovascular lesion outlining, the whole process was conducted twice using the same software (NeuROI: http://www.nottingham.ac.uk/research/groups/clinicalneurology/neuroi.aspx). The cerebrovascular lesion mask of each participant created in the first time was overlaid onto that created in the second time. The cerebrovascular lesion outlining was re-done for the third time if the masks created in the first and second time were mismatched. All the steps were done by the author of this thesis.

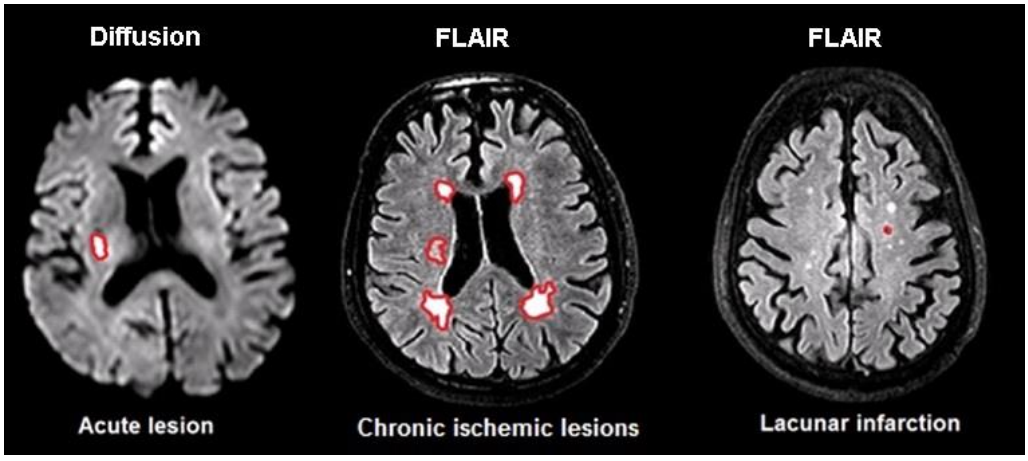



**Figure 3. 4 Examples of outlining cerebrovascular lesions on diffusion and FLAIR images. Left: an acute lesion on diffusion b1000 image. Middle: chronic subcortical ischaemic lesions on FLAIR image. Right: a lacunar infarction on FLAIR image.**

### 3.7.2 Cerebrovascular lesion volume measurements

Volumes of each type of cerebrovascular lesions were automatically calculated using the FSLstats command line:

*fslstats (acute lesions/chronic subcortical ischaemic lesions/lacunar infarctions/cortical infarctions filename).hdr -V*

Then, volumes of each type of cerebrovascular lesions were normalised to the total brain volumes by using the following equation:

$$Normalised\ cerebrovascular\ lesion\ volume = \frac{Volumes\ of\ cerebrovascular\ lesions}{Total\ brain\ volumes}$$

The calculation of total brain volumes will be introduced in 3.8.

The total ischaemic lesion volume (TILL) was calculated by adding the volumes of non-overlapping lacunar infarctions, acute lesions, chronic subcortical ischaemic lesions and cortical infarctions and then normalised to total brain volumes. Given that the histogram of TILL showed a right-skewed distribution, logarithmic transformation was then applied on TILL (logTILL) (Figure 3. 5).

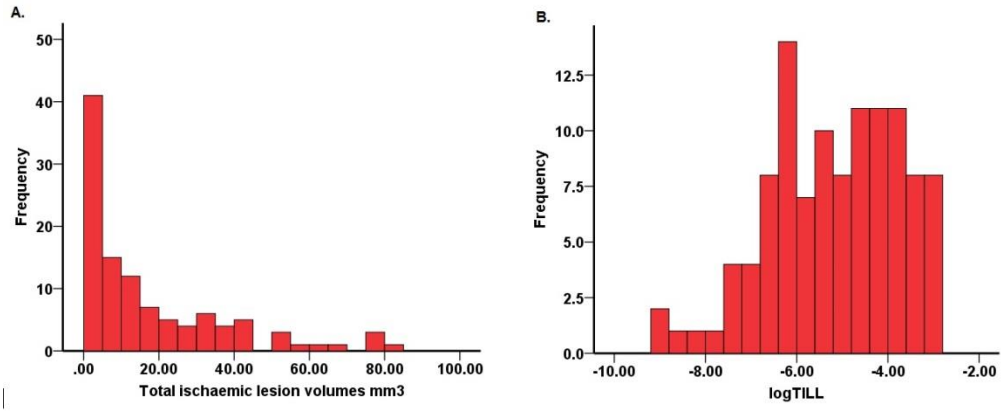



**Figure 3. 5 Histograms of TILL and logTILL. (A) illustrates the right-skewed distribution of TILL. (B) illustrates the distribution of TILL after data transformation.**

### 3. 8 Brain volume measurements

SIENAX, part of FSL, was used to automatically calculate the total volume of brain tissues (Smith, De Stefano et al. 2001) (Smith, Zhang et al. 2002). Given that no high resolution T1 weighted images were obtained in this study, FLAIR

images were used instead to calculate brain volume. All the steps mentioned below were part of SIENAX and the results of brain volumes were automatically reported. Firstly, brain and skull were separately extracted from the whole brain FLAIR images using BET (Smith 2002). Then the brain images were registered to MNI152 space in order to obtain the volumetric scaling factor and to be used as a normalisation for head size. After that, tissue-specific segmentation with partial volume estimation was conducted to calculate the total volume of brain tissues. A report including the estimated volumes of the grey matter, white matter and the whole brain tissue was provided in SIENAX. 50% of participants were randomly selected to conduct the brain volume measurements for the second time using the same method in order to make sure the results were consistent.

# Chapter 4 General results

The results of clinical characteristics, cognitive profile, MTA and cerebrovascular lesion volume measurements will be provided in this chapter.

## 4.1 Study cohort

Totally 180 subjects were recruited in the main ICAD study. The inclusion of subjects in this cognitive sub-study was illustrated in Figure 4. 1. Specifically, 147 [mean ±SD: 74.7 ± 10.1; female: 61(41.5%)] out of 180 subjects (81.6%) agreed to undergo cognitive assessment using ACE-R. One subject had Parkinson Disease (PD) therefore was excluded from the analysis. Totally 2 subjects had neither FLAIR nor DTI images with additionally 11 subjects did not have any DTI images. After quality assurance, 25 subjects were excluded due to noise on FLAIR (8 subjects) or DTI images (17 subjects). Totally 108 subjects were included in the analyses of Chapter 5 and Chapter 6.

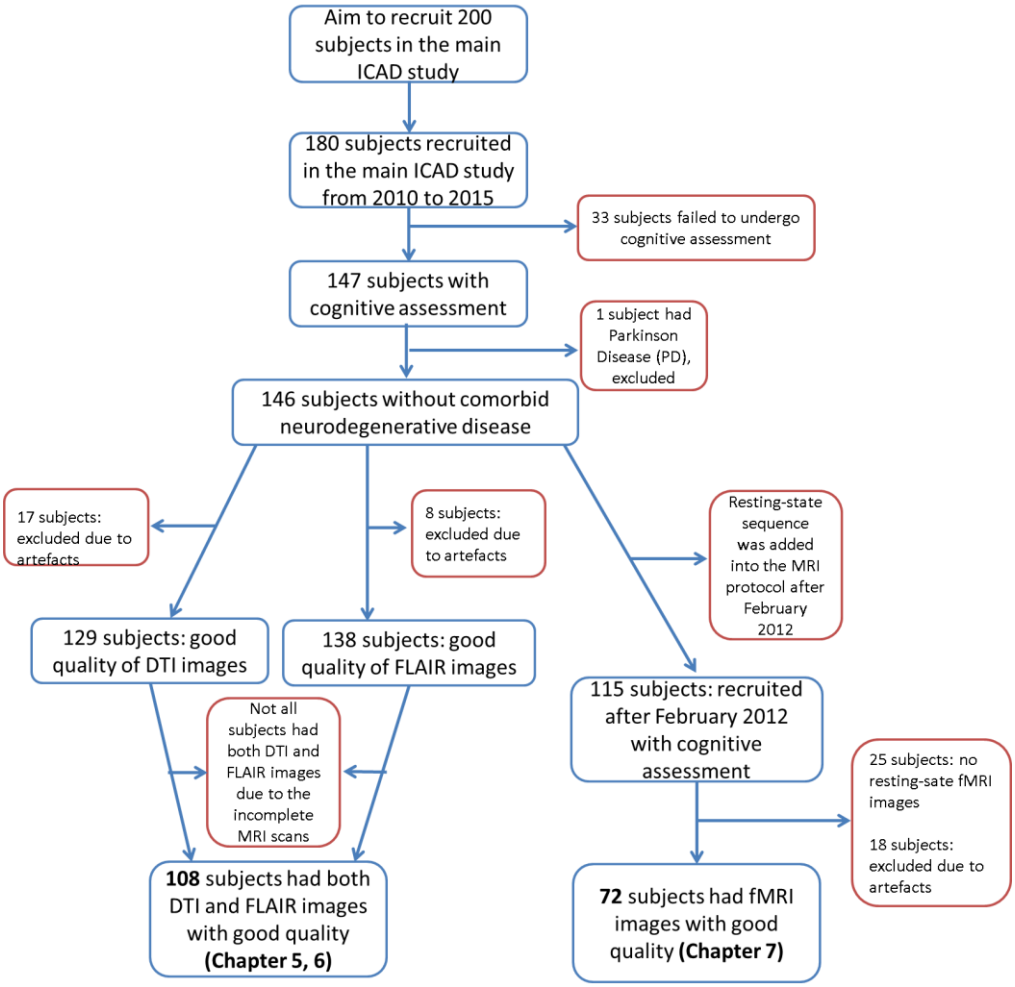



**Figure 4. 1 Flow chart of patient inclusion in the study of each chapter**

## 4.2 Results of cognitive assessment

In the cohort of 108 subjects (Chapter 5 and Chapter 6), 53 subjects (49.1%) had global cognitive impairment, 47 subjects (43.5%) had memory dysfunction, 40 subjects (37%) had impaired performance of fluency, 21 subjects (19.4%) had dysfunction of attention & orientation, 14 subjects (13%) had impaired language performance, and 26 subjects (24.1%) had visuospatial dysfunction (Figure 4. 2). The cognitive profile of the cohort of 108 subjects is provided in Table 4. 1.

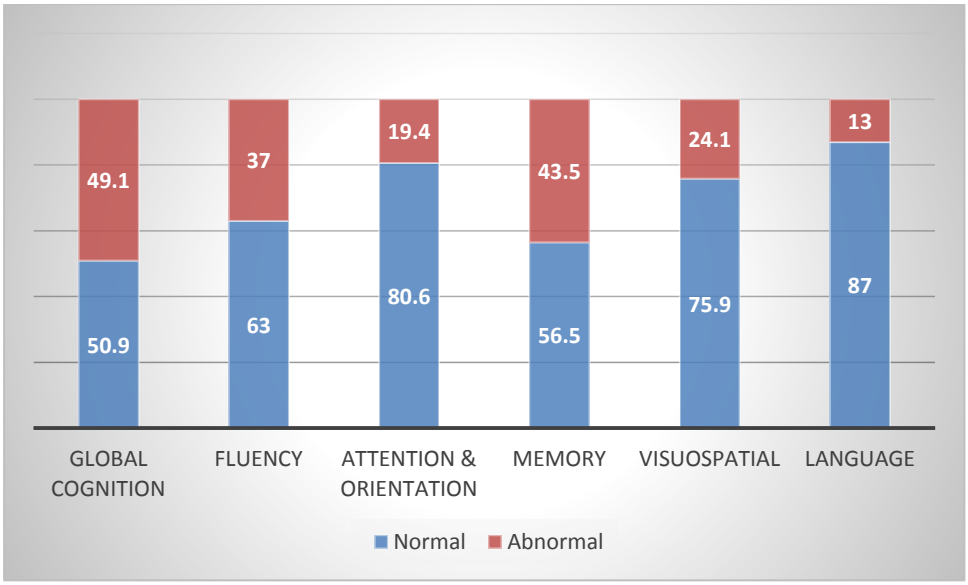



**Figure 4. 2 The percentage of subjects with normal and abnormal performance in global cognition and each cognitive domain in the cohort of 108 subjects (Chapter 5 and Chapter 6).**

**Table 4. 1 Cognitive profile of the cohort including 108 subjects (Chapter 5 and Chapter 6)**

| | Minimum | Maximum | Mean ± SD |
|---|---|---|---|
| ACE-R | 50 | 99 | 81.5 ± 10.5 |
| Memory | 4 | 26 | 17.8 ± 5.3 |
| Attention & orientation | 8 | 18 | 16.8 ± 1.8 |
| Fluency | 2 | 14 | 9.2 ± 2.7 |
| Visuospatial | 9 | 16 | 14.4 ± 1.6 |
| Language | 13 | 26 | 23.3 ± 2.6 |

The diagnostic criteria based on the level of certainty were applied in this study cohort (Gorelick, Scuteri et al. 2011). Dr. Akram Hosseini with 9 years of clinical neurology experience went through the clinical information of all cognitively impaired subjects and concluded that all cognitively impaired subjects in this study cohort had probable VaMCI.

### 4.3 Results of demographic and clinical characteristics

Independent-samples T test, Mann-Whitney U test and Chi square test in SPSS 21 were used to compare the clinical characteristics.

In the study cohort including 108 subjects (Chapter 5 and Chapter 6), patients with abnormal cognition were older (P=0.027) and had more subjects with peripheral vascular disease (P=0.017) and more subjects who ever smoked before (P=0.015) compared with patients with normal cognition (Table 4. 2).

Age was significantly different between patients with normal fluency (mean ± SD: 72.7±9.1) and patients with abnormal fluency (77.7±8.6) (P=0.006). Other demographics and clinical characteristics including sex, symptom type (stroke/TIA), AF, PVD, IHD, hypertension, smoking status, symptomatic side and ipsilateral and contralateral degree of stenosis were not significantly different between patients with normal and abnormal fluency.

**Table 4. 2 Comparison of demographic characteristics of between patients with normal and abnormal cognition in the study cohort including 108 subjects (Chapter 5 and Chapter 6)**

| Characteristics | The whole study cohort<br>n=108 | Normal cognition<br>n=55 | Abnormal cognition<br>n=53 | P value [a] |
|---|---|---|---|---|
| **Age, mean (SD) (years)** | 74.6(9.2) | 72.7(9.5) | 76.6(8.6) | **0.027*** |
| **Female, n (%)** | 42(38.9) | 22(40.0) | 20(37.7) | 0.845 |
| **Atrial fibrillation, n (%)** | 24(22.2) | 10(18.2) | 14(26.4) | 0.359 |
| **Peripheral vascular disease, n (%)** | 17(15.7) | 4(7.3) | 13(24.5) | **0.017*** |
| **Ischemic heart disease, n (%)** | 34(31.5) | 16(29.1) | 18(34.0) | 0.680 |
| **Hypertension, n (%)** | 88(81.5) | 44(80.0) | 44(83.0) | 0.806 |
| **Smoking, n (%)** | 71(65.7) | 30(54.5) | 41(77.4) | **0.015*** |
| **Diabetes mellitus, n (%)** | 24(22.2) | 12(21.8) | 12(22.6) | 0.918 |
| **Symptomatic type (Stroke), n (%)** | 53(49.1) | 23(41.8) | 30(56.6) | 0.178 |
| **Symptomatic side (Left), n (%)** | 52(48.1) | 24(43.6) | 28(52.8) | 0.441 |

| | | | | |
|---|---|---|---|---|
| **Ipsilateral degree of carotid stenosis[b], n (%)** | | | | 0.154 |
| **30-49%** | 50(46.3) | 29(52.7) | 21(39.6) | |
| **50-69%** | 45(41.7) | 18(32.7) | 27(50.9) | |
| **>70%** | 13(12.0) | 8(14.5) | 5(9.4) | |
| **Contralateral degree of carotid stenosis[c], n (%)** | | | | 0.179 |
| **0-29%** | 71(65.7) | 38(69.1) | 33(62.3) | |
| **30-49%** | 13(12.0) | 9(16.4) | 4(7.5) | |
| **50-69%** | 15(13.9) | 5(9.1) | 10(18.9) | |
| **>70%** | 9(8.3) | 3(5.5) | 6(11.3) | |
| **Time from symptom to MRI, mean (SD) (days)** | 35.1(31.0) | 36.9(27.0) | 33.3(33.9) | 0.519 |

a: Comparison between patients with normal and abnormal cognition

b,c: The measurement of the degree of stenosis was followed the CAVATAS criteria, based in the NASCET method of angiographic measurement

*: Statistical significant at the level of $P<0.05$

## 4.4 Results of MTA measurements

### 4.4.1 Inter- and intra- rater reliability

The Cohen's Kappa coefficient of intra-rater reliability of applying Scheltens score on reconstructed FLAIR images was 0.821 (95%CI: 0.693-0.948; P<0.001; Table 4. 3), indicating an 'almost perfect' agreement. The inter-rater reliability of applying Scheltens scale on reconstructed FLAIR images was 0.770 (95%CI: 0.637-0.904; P<0.001; Table 4. 4), indicating a 'substantial' agreement (Landis and Koch 1977).

Totally 50 subjects had coronal T1-weighted gradient echo images which can be used to assess MTA. The agreement of applying Scheltens scale on FLAIR with that on coronal T1-weighted gradient echo images was considered as 'substantial' (Cohen's Kappa coefficient: 0.803; 95%CI: 0.624-0.985; P<0.001, Table 4. 5).

**Table 4. 3 Cross tabs of intra-rater reliability**

| | | First time of MTA assessment by Rater 1 | | |
|---|---|---|---|---|
| | | MTA negative | MTA positive | Total |
| Second time of MTA assessment by Rater 1 | MTA negative | 79 | 5 | 84 |
| | MTA positive | 2 | 22 | 24 |
| Total | | 81 | 27 | 108 |

**Table 4. 4 Cross tabs of inter-rater reliability**

| | | MTA assessment by Rater 1 | | |
|---|---|---|---|---|
| | | MTA negative | MTA positive | Total |
| MTA assessment by Rater 2 | MTA negative | 73 | 2 | 75 |
| | MTA positive | 8 | 25 | 33 |
| Total | | 81 | 27 | 108 |

**Table 4. 5 Cross tabs of agreement of MTA assessment on T1-weighted gradient echo images and on FLAIR images**

| | | MTA assessed on VENDI | | |
|---|---|---|---|---|
| | | MTA negative | MTA positive | Total |
| MTA assessed on FLAIR | MTA negative | 34 | 0 | 34 |
| | MTA positive | 4 | 12 | 16 |
| Total | | 38 | 12 | 50 |

### 4.4.2 Results of MTA measurement and its role in global cognition

The number of patients with MTA and that of patients without MTA was significantly different between patients with normal and abnormal cognition in the study cohort including 108 subjects (P<0.001) and in the study sub-cohort including 72 subjects (P=0.001) (Table 4. 6).

**Table 4. 6 the comparison of MTA measurements between patients with normal and abnormal cognition**

| Main study cohort (n=108) (Chapter 5 and Chapter 6) | | |
|---|---|---|
| | Normal cognition (n=55) | Abnormal cognition (n=53) |
| MTA negative | 52 | 33 |
| MTA positive | 3 | 20 |

### 4.5 Results of brain volume measurements

The mean (SD) of whole brain tissue volume in the cohort including 108 subjects was 1439.174 (124.922) $cm^3$. There was no significant difference of whole brain tissue volumes between patients with normal (1448.244 ± 112.99) $cm^3$ and abnormal cognition (1429.761 ± 136.66) $cm^3$ (P=0.445).

### 4.6 Results of cerebrovascular lesion volume measurements

The effect of cerebrovascular lesion volumes on cognition was investigated in the cohort including 108 subjects.

Patients with abnormal cognition had larger normalised volumes of chronic subcortical ischaemic lesions (effect size: 0.075, P=0.004), lacunar infarctions (effect size: 0.039, P=0.041), and logTILL (effect size: 0.099, P=0.001). Additionally, they had more cortical infarctions (effect size: 0.040, P=0.038) than patients with normal cognition (Table 4. 7).

Global cognitive performance was significantly correlated with logTILL ($r$=0.240, P=0.013) after controlling for age. This correlation still reached significance when MTA was additionally controlled ($r$=0.192, P=0.046). Additionally, normalised lacunar infarction volumes were associated with global cognitive performance when age and MTA were both controlled ($r$=0.200, P=0.038) (Table 4. 7).

**Table 4. 7 Cerebrovascular lesion characteristics in the study cohort including 108 subjects (Chapter 5 and Chapter 6)**

| | Study cohort (n=108) | Normal cognition (n=55) | Abnormal cognition (n=53) | P[f] | r | P[g] | r | P[h] |
|---|---|---|---|---|---|---|---|---|
| **Presence of acute lesions[a], n (%)** | 46(42.6%) | 19(34.5) | 27(50.9) | 0.085 | - | - | - | - |
| **Normalised acute lesion volumes, mean(SD, median, IQR)×10$^4$** | 4.3(8.8, 0, 5.0) | 2.6(5.1, 0 ,4.0) | 5.9(11.3, 0.2, 8.0) | 0.059 | 0.163 | 0.088 | 0.130 | 0.179 |
| **Presence of lacunar infarctions[b], n (%)** | 24(22.2%) | 9(16.4) | 15(28.3) | 0.136 | - | - | - | - |
| **Normalised lacunar infarction volumes, mean(SD)× 10$^5$** | 1.8(5.2) | 0.8(2.6) | 2.9(6.7) | **0.045*** | 0.181 | 0.060 | 0.200 | **0.038*** |
| **Normalised chronic subcortical ischaemic lesion[c] volumes, mean(SD)× 10$^3$** | 11.7(13.6) | 8.0(10.5) | 15.5(15.5) | **0.004*** | 0.028 | 0.085 | 0.012 | 0.253 |
| **Presence of cortical infarctions[d], n (%)** | 4(3.7%) | 0(0) | 4(7.5) | **0.038*** | - | - | - | - |
| **logTILL[e], mean(SD)** | -5.12(1.42) | -5.6(1.5) | -4.7(1.3) | **0.001*** | 0.240 | **0.013*** | 0.192 | **0.046*** |

**a:** acute lesions are defined as hyperintensities on b1000 images but hypointensities on MD maps.
**b:** lacunar infarctions are defined as small (<2cm) hypointense subcortical areas on FLAIR images.
**c:** chronic subcortical ischaemic lesions are defined as subcortical FLAIR hyperintensities.
**d:** cortical infarctions are defined as cortical FLAIR hypointensities.
**e:** logTILL=log (total ischaemic lesion volumes/total brain volumes).
**f:** P value of comparison between patients with normal and abnormal cognition.
**g**: Correlation between global cognitive performance and normalised lesion volumes, controlled for age
**h**: Correlation between global cognitive performance and normalised lesion volumes, controlled for age and MTA

# Chapter 5 Topography, volume and severity of cerebrovascular ischaemic lesions damage in patients with symptomatic carotid artery disease

## Abstract

The aim of this chapter was to investigate the relationship between VCI and cerebrovascular lesional damage including cerebrovascular topography and severity of cerebrovascular lesions damage in patients with symptomatic carotid artery disease. The main hypothesis of this chapter is that global cognitive impairment is related to the strategic topography of cerebrovascular lesions. The association between the presence of cerebrovascular lesions and global cognitive performance in 108 participants was investigated by using voxel-based lesion symptom mapping (VLSM) method. The correlation between severity of cerebrovascular lesions damage, indexed by mean diffusivity (MD) increases, and global cognitive impairment was investigated using regression analysis. All analyses were conducted in both the main cohort (n=108) and in the MTA negative (MTA-ve) sub-cohort (n=85) in order to examine whether the main findings are confounded by the presence of MTA. Global cognitive impairment in patients with symptomatic carotid artery disease was related to the presence of chromic subcortical ischaemic lesion location in the anterior thalamic radiation, forceps minor and forceps major, and the severity of cerebrovascular lesions damage. Also, the main findings were not confounded by the presence of MTA.

## 5.1 Introduction

Cognitive disorder following brain structural damage could be explained from two perspectives (Moeller, Willmes et al. 2015). The first perspective is to assume that cortical and subcortical grey matter act independently for specific cognitive functions therefore brain structural damage within specific areas lead to corresponding cognitive dysfunction. For example, historical evidences were provided to associate language production with Broca's area and to link Wernicke's area with language perception (Geschwind 1970). This concept may help researchers understand the specialisation of cortical and subcortical brain regions, however, it blinds researchers to understand cognitive function from a network perspective. Given the disadvantage of the first concept, the second perspective is derived from the hypothesis that brain networks consist of structurally or functionally interconnected areas which coordinate cognition. Information is processed in grey matter and passes along the brain network via white matter. Therefore, the cognitive role of a specific cortical or subcortical brain area is not only determined by its anatomical structure but also by its relationships with other cortical or subcortical brain areas connecting by white matter (Li, Liu et al. 2009). Emerging evidence has emphasised the role of understanding cognition from a perspective of structural and functional network (Bressler and Menon 2010). Li and colleagues have shown that the efficiency of structural connectivity was a key basis for intelligence (Li, Liu et al. 2009). The concept of functional cognitive networks including attention network, language network, memory network, recognition network, and working memory-executive function network was first reported by Mesulam in 1990 (Mesulam 1990). Additionally, a recent review has summarised that the functional integrity

of fronto-parietal network and the structural integrity of superior longitudinal fasciculus contributed to normal numerical cognition (Moeller, Willmes et al. 2015). All evidence highlight the role of structural and functional networks integrity in cognition. The network-based concept emphasises that cognitive impairment is not only associated with the focal effects of damaged brain regions, more importantly, it is also related to structural pathways disconnected by brain damage, or dysfunction of anatomically intact brain regions but functionally or structurally connected with damaged brain regions (Bartolomeo 2011).

Brain damage resulting from ischaemic stroke seems to be focal although diffuse brain damage, such as confluent white matter lesions, are also associated with ischaemic stroke (Carter, Shulman et al. 2012). The types of cerebrovascular lesions which associate with VCI have been explained in details in Chapter 1. Volumes and location of cerebrovascular lesions are two morphological substrates of VCI (Jellinger 2013). The association between VCI and volumes and location of cerebrovascular lesions including large vessel infarcts, lacunar infarcts and white matter lesions has been explained in Chapter 1. In short, VCI may not be directly linked with volumes of cerebrovascular lesions. A pathological review has suggested that location of cerebrovascular lesions might be more important than the volume of cerebrovascular lesions (Jellinger 2008). Multiple brain regions have been proposed as strategic locations in VCI (Tatemichi, Desmond et al. 1995) (Jellinger 2007). For instance, cerebrovascular lesions located in the angular gyrus of the dominant or bilateral hemisphere were related to cognitive deficit (Basso, Capitani et al. 1981) (Cummings and Benson 1988). Unilateral or more often bilateral thalamic infarcts were found to be related to memory dysfunction and ‘subcortical dementia’ (Castaigne, Lhermitte

et al. 1981) (Szirmai, Vastagh et al. 2002). Additionally, it has been found out that 48% of patients with VCI had infarcts in hippocampus and hippocampal infarcts were related to dementia (Dickson, Davies et al. 1994) (Markesbery 1998). Even though it has been suggested that hippocampus is less affected by cerebrovascular events than AD (Du, Schuff et al. 2002), hippocampal neuronal loss and atrophy exist in and increase the development of VCI (Kril, Patel et al. 2002) (Cordoliani-Mackowiak, Henon et al. 2003). Last but not least, infarcts located in the territories of left anterior cerebral artery and posterior cerebral artery were found to be frequently associated with post-stroke cognitive impairment (Liu, Miller et al. 1992).

Advanced MRI techniques provide a means to map the topographical distribution of brain damage and such that the association of lesion topography and cognition could be further investigated. Voxel-based lesion symptom mapping (VLSM) is a voxel-based statistical method that maps regions with significant lesion-deficit associations using non-parametric tests with correction of multiple comparisons. This technique was invented by Bates and colleague in 2003 to investigate the relationship between tissue damage and speech fluency and language comprehension in aphasic stroke patients (Bates, Wilson et al. 2003). Before using VLSM, patients were typically grouped either by the presence of lesions (lesion-defined method) or by cognitive status (performance-defined method) in the lesion-based analysis of cognitive process. Using lesion-defined methods, cognitive performance is compared between patients with brain tissue damage and those without (Chao and Knight 1998). This method provides the insights of the functional roles of a specific brain area, however, the results could be biased if a specific brain area contains sub-areas with each of

them contributing to cognitive performance. Also, this method may overlook the fact that brain areas outside the specific brain area are part of a distributed structural or functional brain network of cognitive process (Bates, Wilson et al. 2003). Using performance-defined methods, lesion prevalence maps are compared between patients with a specific cognitive deficit and those without. The main limitation of this method is that it only applies in situations where the cognitive outcome is binary rather than continuous (Adolphs, Damasio et al. 2000) (Dronkers 1996) (Bates, Wilson et al. 2003). Lesion-deficit relationship can be examined by VLSM without defining cognitive cut-offs or identifying a *priori* region of interest. Using VLSM, a statistical test (e.g. t-test) is performed to compare the cognitive scores in those patients with versus those without a lesion at each voxel (Figure 5. 1) (Baldo, Wilson et al. 2012). Then, voxels showing a significant relationship with cognitive performance are highlighted (Bates, Wilson et al. 2003). The statistical correction for multiple comparisons is also needed in VLSM due to the large number of voxels being sampled. According to Kimberg and colleagues, Bonferroni correction is too conservative while false discovery rate (FDR) correction is better for correcting multiple comparisons in VLSM (Kimberg, Coslett et al. 2007). Voxel-based technique has been utilised to investigate the relationship between general intelligence and brain lesions resulting from cerebrovascular diseases, anterior temporal lobectomy, surgical intervention, herpes simplex encephalitis and traumatic brain injury (Glascher, Tranel et al. 2009), which provides a clear understanding of the neural correlates of general intelligence. The cognitive profile of VCI, showing as executive dysfunction and slowing information process, has been linked to the frequent occurrence of cerebrovascular lesions located in the

frontal-subcortical circuits (Cummings 1989) (Chui 2007). Several studies have utilised voxel-based technique to investigate the relationship between cognitive decline and topography of lacunar infarcts and white matter lesions in patients with CADASIL which is considered as a model of 'pure' VCI (O'Sullivan 2010) (Duering, Zieren et al. 2011). No studies have investigated the association between VCI and topographical distribution of cerebrovascular lesions in elderly patients with symptomatic carotid artery disease.

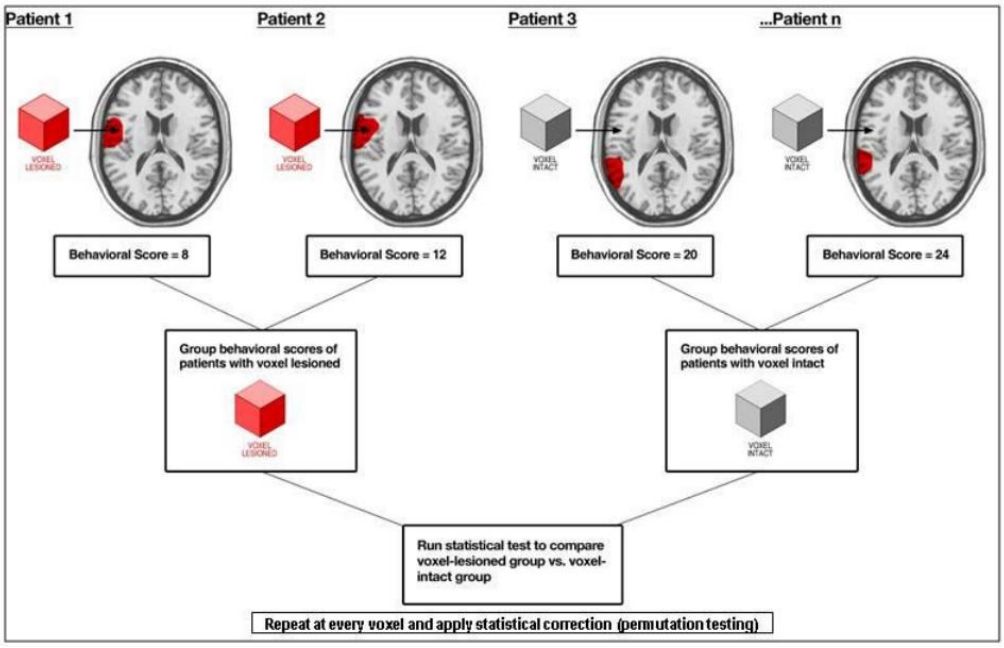



**Figure 5. 1 The steps of the analysis technique of VLSM. From (Baldo, Wilson et al. 2012)**

Measurements of the magnitude and directionality of water molecular motion have been used to identify abnormality within damaged brain tissues and normal appearing brain tissues in patients with multiple sclerosis (Horsfield, Lai et al. 1996) (Filippi, Rocca et al. 2012) and stroke (Lutsep, Albers et al. 1997)

(Schaefer, Ozsunar et al. 2003) (Sotak 2002). As introduced in Chapter 1, lower FA values indicate less fiber density or coherence and higher MD values imply less dense tissue in a voxel. These changes of diffusion parameters reflect the microscopic damage of brain tissue which may link to cognitive decline (Laukka, Lovden et al. 2013). There have been few studies investigating the association between severity of cerebrovascular lesions damage, indexed as diffusional changes, and cognitive impairment in patients with vascular pathology. Therefore the role of severity of cerebrovascular lesions damage in VCI remains unclear.

### 5.2 Aims and hypotheses

Hypotheses:

- The presence of cerebrovascular lesions in strategic brain areas contributes to cognitive impairment in patients with symptomatic carotid artery disease
- Severity of cerebrovascular lesions damage, indexed as increased MD, correlates with cognitive impairment in patients with symptomatic carotid artery disease
- The imaging-cognition findings are not confounded by the presence of medial temporal lobe atrophy (MTA)

Aims:

- Create the prevalence maps of infarctions and chronic subcortical ischaemic lesions and compare them between patients with normal and abnormal cognition

- Apply the VLSM technique to investigate the lesion-cognitive deficit relationship in patients with symptomatic carotid artery disease
- Investigate the association between severity of cerebrovascular lesions damage and global cognitive impairment using regression analysis
- Conduct all analyses in both the main cohort (n=108) and in the MTA negative (MTA-ve) sub-cohort (n=85) in order to examine whether the main findings are confounded by the presence of MTA

## 5.3 Methods

### 5.3.1 Subjects and assessments

The recruitment, clinical and cognitive assessment, MRI protocol of subjects included in this chapter was described in Chapter 3. The analyses of totally 108 subjects were included in this chapter.

### 5.3.2 Generation of frequency maps of infarctions and chronic subcortical ischaemic lesions

Lesion frequency maps were created by the following steps: (1) DTI and FLAIR images were registered into MNI template space by using FMRIB's Linear Image Registration Tool (FLIRT) (Jenkinson, Bannister et al. 2002). The registration results were visually checked for each participant in order to guarantee the quality of registration. Also, around 50% of the participants were randomly selected to conduct the registration step for the second time in order to make sure the registration results were consistent. (2) Lacunar infarctions, acute lesions, cortical infarctions and chronic subcortical ischaemic lesions were separately and manually outlined on co-registered images using the locally

developed NeuRoi software (http://www.nottingham.ac.uk/research/groups/clinicalneurology/neuroi.aspx). The details were described in Chapter 3; (3) Two kinds of lesion frequency maps were created by adding lesion masks together. The infarction frequency maps included acute lesions, cortical infarctions and lacunar infarctions. The chronic subcortical ischaemia frequency maps contained chronic subcortical ischaemic lesions only. Additionally, the total ischaemic lesion maps were created by combining the infarction and the chronic subcortical ischaemia frequency maps together. The frequency maps of infarctions and chronic subcortical ischaemic lesions were then superimposed onto the MNI template images and colour coded according to the local frequency of lesions. The spatial distribution of lesion probabilities was then compared between normal and abnormal cognition using a voxel-based Fisher test (controlled for multiple comparisons) as implemented in the NeuRoi software package (http://www.nottingham.ac.uk/research/groups/clinicalneurology/neuroi.aspx).

### 5.3.3 Strategic lesion topography

The contribution of lesion topography on global cognitive performance was investigated using non-Parametric voxel-based lesion-symptom mapping (VLSM) (http://www.neuroling.arizona.edu/resources.html) (settings: t-test, univariate analysis) (Bates, Wilson et al. 2003). Using VLSM, the association between ACE-R score and lesion status (presence or absence of a lesion in that voxel) was investigated at each voxel. Subjects were divided into two groups according to whether they had a lesion within a specific voxel. Then ACE-R score was compared between these two groups, yielding a t-statistic test for that

voxel. This procedure was repeated for each voxel. After that, the effect at each voxel was assessed for statistical significance using FDR for correcting multiple comparisons. Age and logTILL were treated as covariates of no interest therefore these two variables were controlled in the analysis. Correction for multiple comparisons was achieved by FDR at q=0.05 where q is expected proportion of false positives among supra-threshold voxels. Voxels reaching statistical significance were projected onto the MNI template and major white matter tracts as identified on a probabilistic white matter tract atlas provided within FSL [Johns Hopkins University-International Consortium for Brain Mapping (JHU-ICBM) tracts, maximum probability map] (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases) (Mori 2005) (Wakana, Caprihan et al. 2007) (Hua, Zhang et al. 2008).

### 5.3.4 Severity of cerebrovascular lesions damage

MD values were chosen to reflect the severity of cerebrovascular lesions damage. Give that only 15 directions were included in the DTI sequence, only MD was chosen because it is less affected by the diffusion directionality (Duering, Righart et al. 2015). The approach to derive MD values of cerebrovascular lesions was described below.

Each subject's MD map was co-registered with MNI 152 T1 2mm template images because the outlining process of infarctions and chronic subcortical ischaemic lesions were conducted on co-registered FLAIR images and DWI images (both were co-registered with MNI 152 T1 2mm template images). To avoid partial volume effect, co-registered MD images were thresholded at the level of $1.5\times10^{-9}$ $m^2$ $s^{-1}$ (Figure 5. 2). Then, each subject's infarction and chronic

subcortical ischaemic lesion maps were used to mask the co-registered MD map using the FSLmaths command line below:

*fslmaths (each subject's infarction/chronic subcortical ischaemic lesion map filename).hdr -mas (each subject's MD map filename).hdr (output filename of each subject's infarction/chronic subcortical ischaemic lesion MD maps)*

Then, averaged MD values were derived from each subject's infarction and chronic subcortical ischaemic lesions maps using the FSLstats command line below:

*fslstats (each subject's infarction/chronic subcortical ischaemic lesion MD maps) -M*

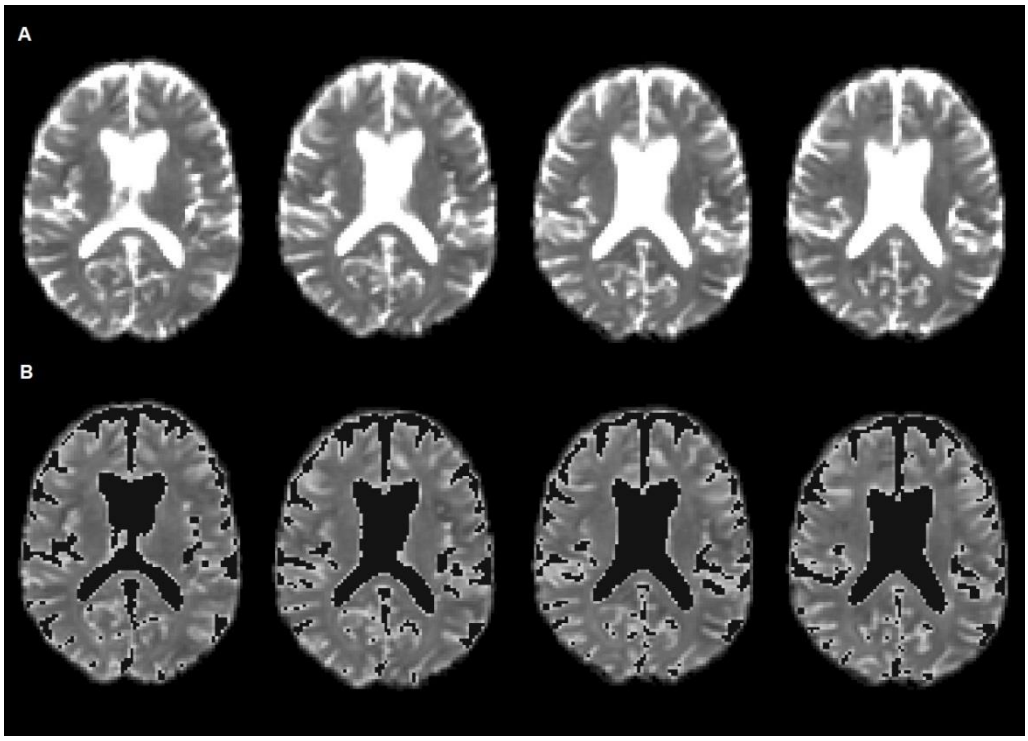



**Figure 5. 2 Correction of partial volume effects on MD maps. (A) Raw MD images. (B) Thresholded MD images.**

### 5.3.5 Statistical analysis

Regression analysis was used to investigate the relation between severity of cerebrovascular lesions damage and global cognitive performance. All analyses were conducted in SPSS version 21 (Chicago, IL, USA).

## 5.4 Results

The inclusion and exclusion criteria of participants included in this chapter and the procedure of imaging quality assurance have been described in Chapter 3. Totally 108 subjects were included in the analyses of this chapter. The cognitive profile of this study cohort was provided in Chapter 4 (Table 4. 1). The comparison of clinical characteristics between patients with normal and abnormal cognition was provided in Chapter 4 (Table 4. 3). In detail, cognitively

impaired subjects were older (P=0.027) and had more peripheral vascular disease (P=0.017), smokers (P=0.015) and MTA+ve (P<0.001) compared with patients with normal cognition.

### 5.4.1 Lesion mapping

Frequency maps of infarctions (Figure 5. 3) and chronic subcortical ischaemic lesions (Figure 5. 4) were created in the whole study cohort. According to visual inspection, infarctions including lacunar infarctions, acute lesions and chronic infarctions were more frequently located in subcortical brain areas, posterior limb of internal capsule and centrum semiovale in patients with normal cognition. In cognitively impaired patients, more scattered infarctions were located in subcortical areas and centrum semiovale. Additionally, patients with abnormal cognition appeared to have more cortical infarctions (Figure 5. 5). Chronic subcortical ischaemic lesions were more prevalent in periventricular and deep white matter but less frequent in subcortical brain areas in patients with normal and abnormal cognition (Figure 5. 6). However, the anatomical distribution of infarctions and chronic subcortical ischaemic lesions was not statistically different between cognitively intact and impaired patients using Fisher test (P>0.05 FDR).

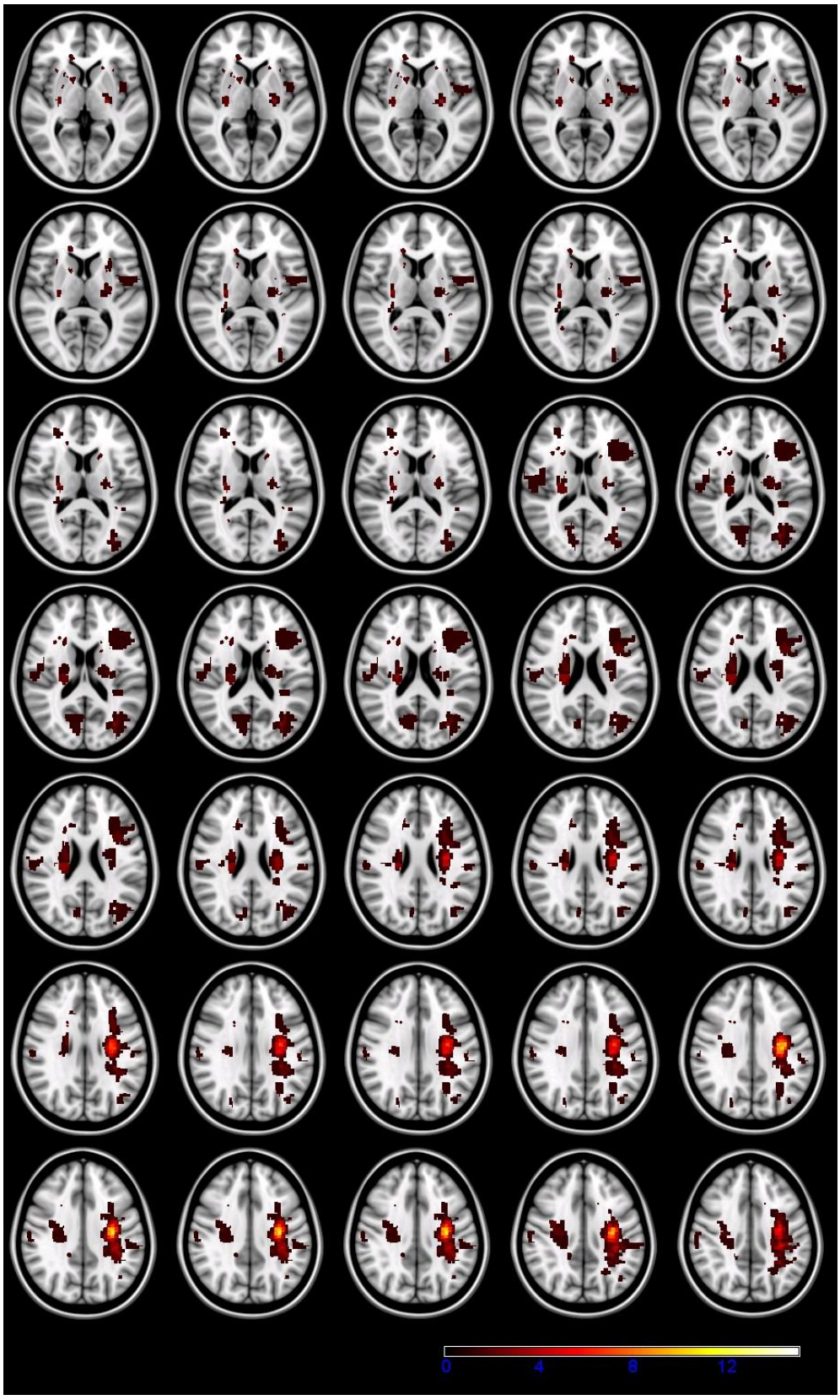



**Figure 5. 3 Frequency maps of infarctions in the cohort of 108 subjects. Infarctions include acute lesions, lacunar infarctions and chronic cortical infarctions. Maps were superimposed onto the 1×1×1 mm MNI 152 T1 template. The colour bars showed variations between the minimum and maximum number of lesions.**

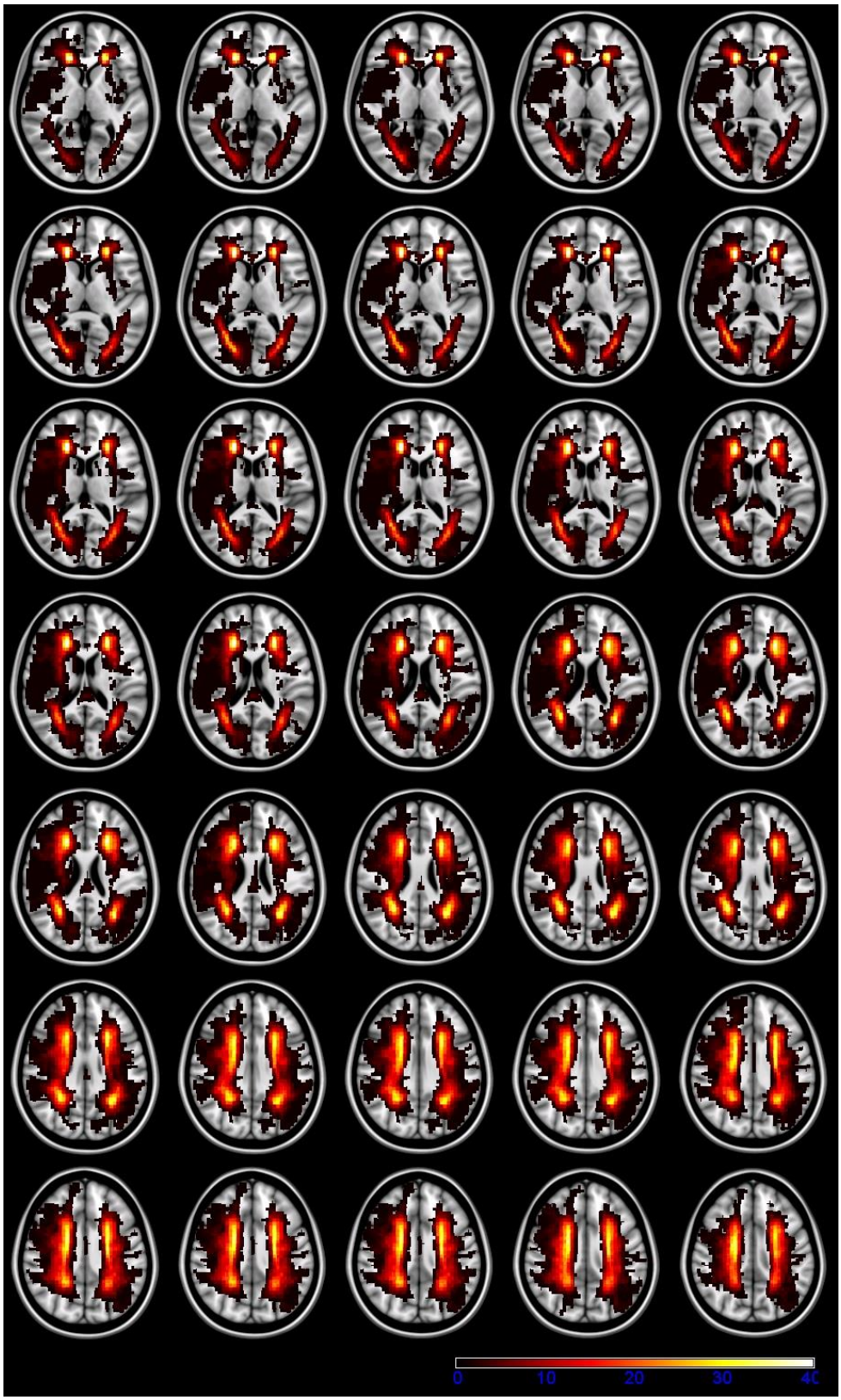



**Figure 5. 4 Frequency maps of chronic subcortical ischaemic lesions in the cohort of 108 subjects. Maps were superimposed onto the 1×1×1 mm MNI 152 T1 template. The colour bars showed variations between the minimum and maximum number of lesions.**

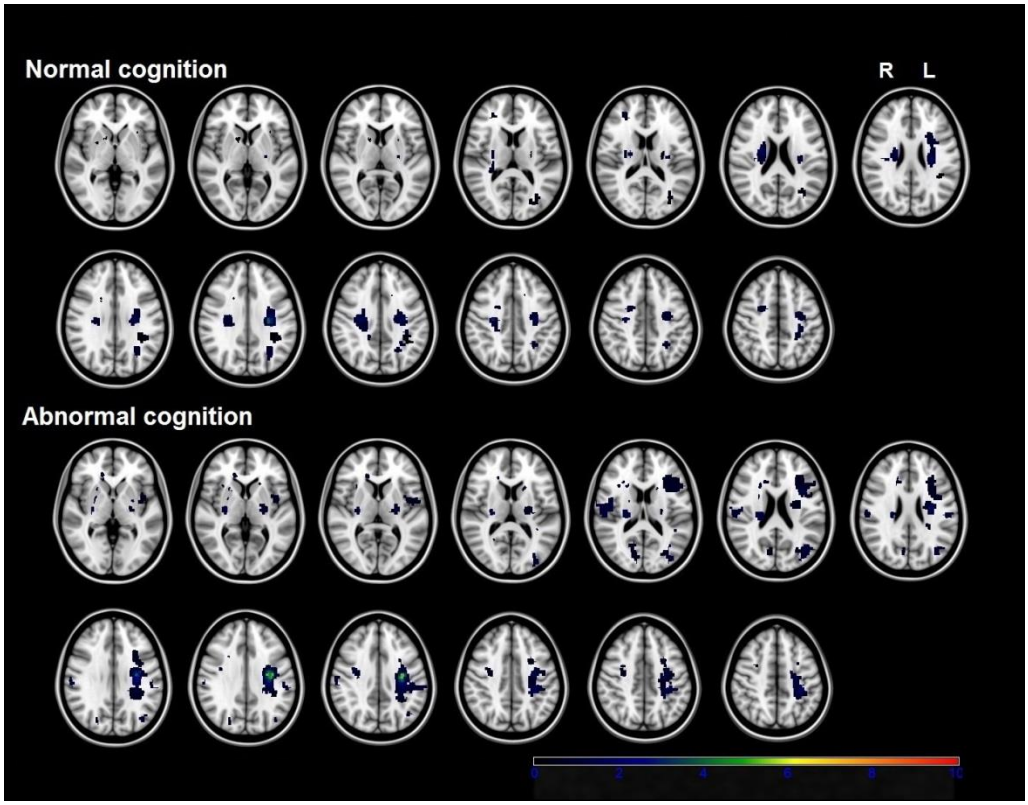



**Figure 5. 5 Infarction maps in patients with normal and abnormal cognition. Maps were superimposed onto the 1×1×1 mm MNI 152 T1 template. Infarctions included acute lesions, lacunar infarctions and cortical infarctions. The colour bar showed variations between the minimum and maximum number of lesions.**

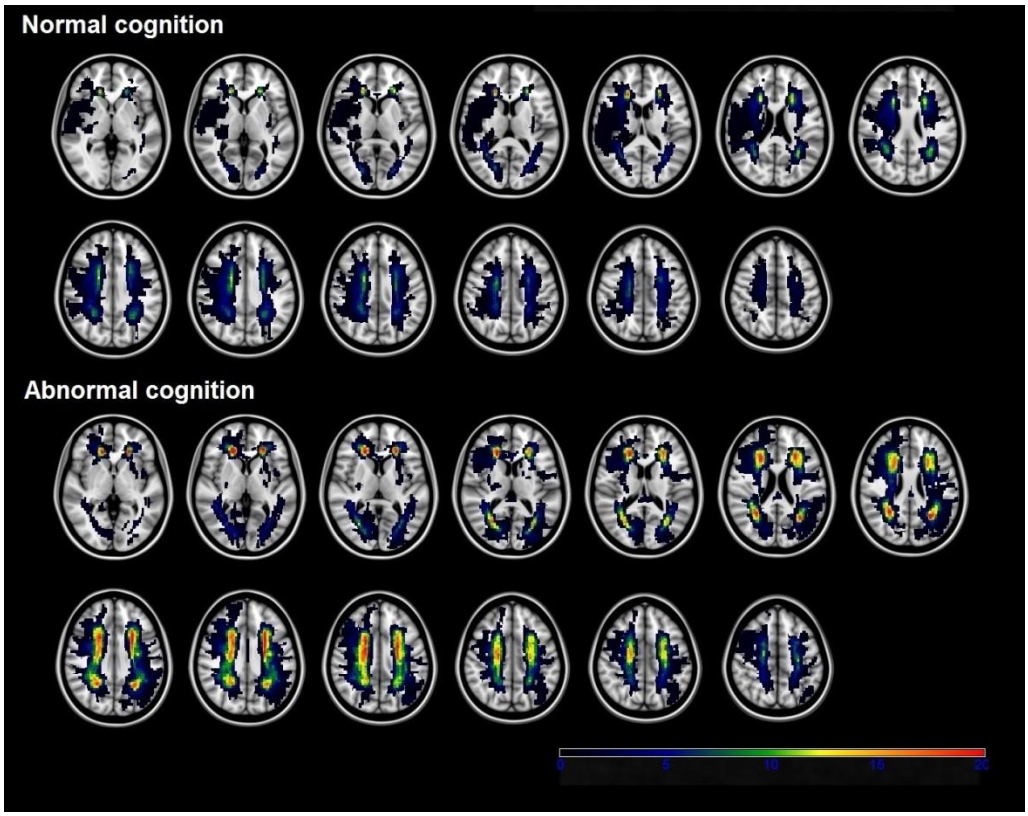



**Figure 5. 6 Chronic subcortical ischaemic lesion maps in patients with normal and abnormal cognition. Maps were superimposed onto the 1×1×1 mm MNI 152 T1 template. Chronic subcortical ischaemic lesions included subcortical hyperintensities on FLAIR images. The colour bar showed variations between the minimum and maximum number of lesions.**

### 5.4.2 The role of lesion topography in global cognition

VLSM analysis for the global cognitive impairment revealed significant clusters for chronic subcortical ischaemic lesions after controlling for age and logTILL. False discovery rate (FDR) with corrected $P<0.05$ was used for the correction for multiple comparisons. Significant clusters including totally 159 voxels were located in the bilateral frontal lobe white matter and the right occipital lobe white matter. The majority of significant clusters were mainly located within anterior thalamic radiation, forceps minor and forceps major (Table 5. 1and Figure 5. 7). The same analysis was conducted in the subgroup including patients without MTA (n=85). The strategic role of chronic subcortical ischaemic lesions was also

identified in the subgroup (Figure 5. 8).

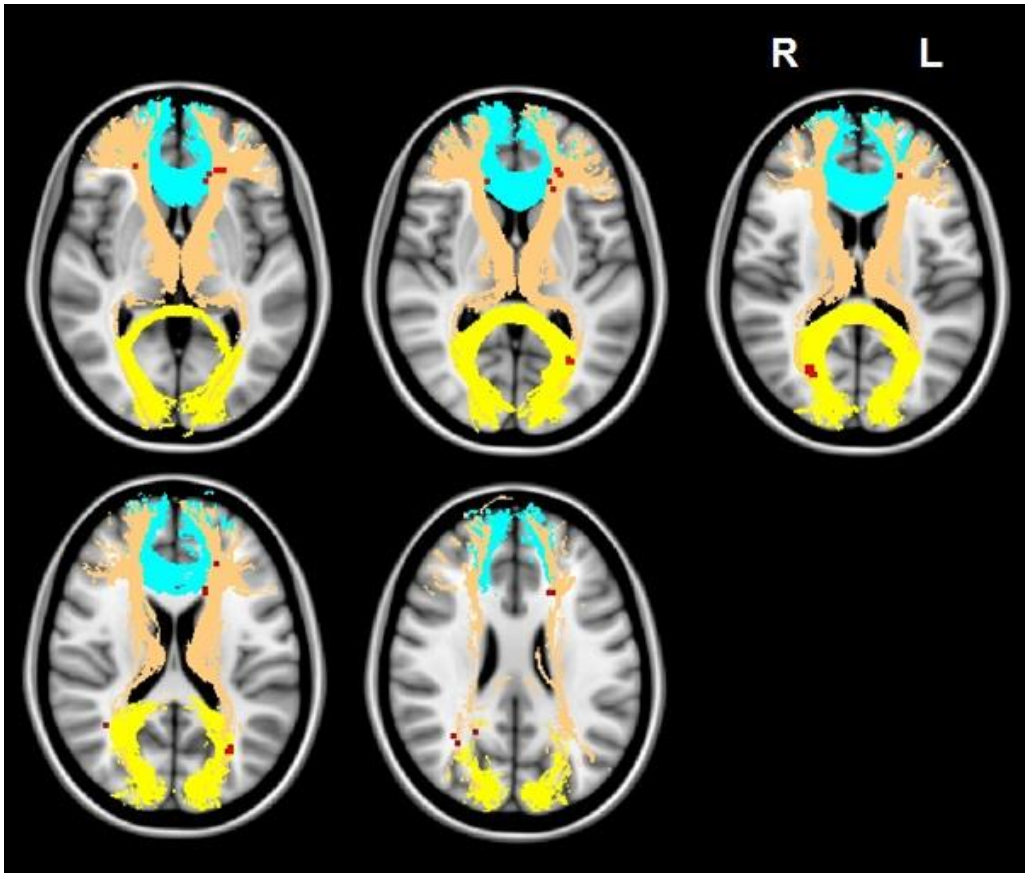



**Figure 5. 7 Topographical chronic ischemic lesion associations with global cognitive impairment in the main study cohort (n=108). Voxel-based lesion-symptom mapping revealed significant clusters of voxels (red) in the forceps major, forceps minor and bilateral anterior thalamic radiation. The presence of chronic subcortical ischaemic lesions in these voxels was related to global cognitive after controlling for age and logTILL, p< 0.05 (FDR corrected). Significant clusters were projected onto the MNI 152 standard space T1 template and white matter tracts extracted from JHU-ICBM white matter atlas (Yellow: forceps major; Brown: anterior thalamic radiation; Blue: forceps minor).**

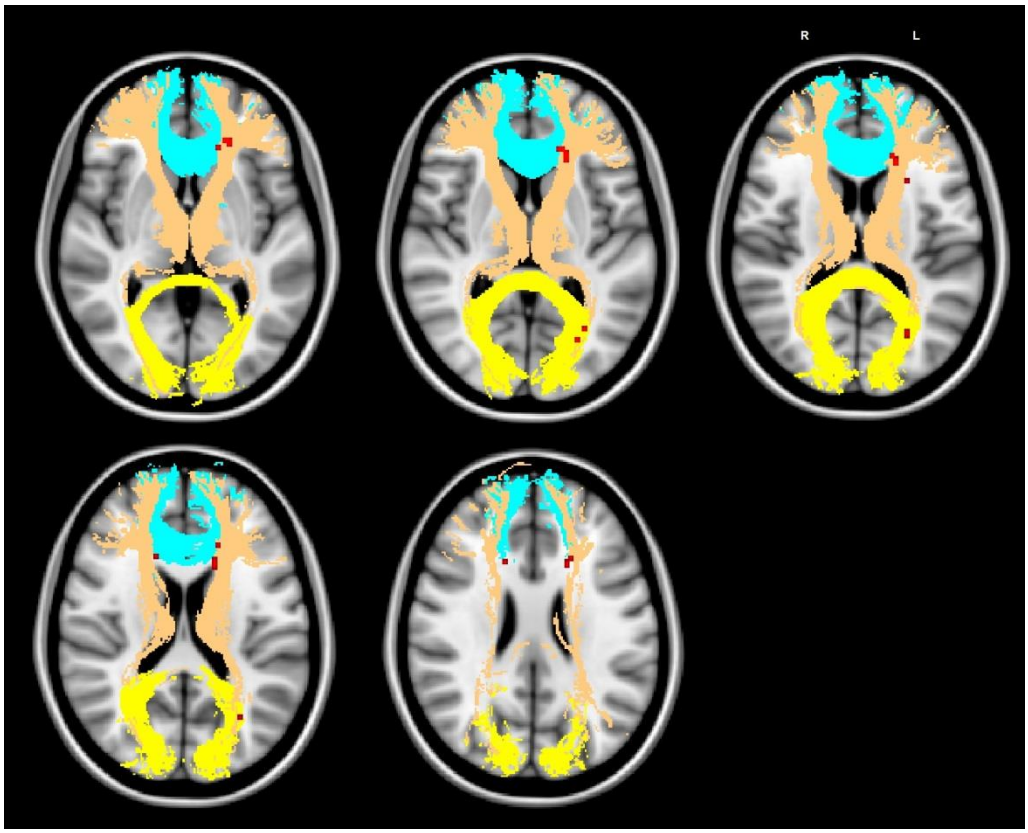



**Figure 5. 8 Topographical chronic ischemic lesion associations with global cognitive impairment in the subgroup including 85 MTA-ve subjects. Voxel-based lesion-symptom mapping revealed significant clusters of voxels (red) in the forceps major, forceps minor and bilateral anterior thalamic radiation. The presence of chronic subcortical ischaemic lesions in these voxels was related to global cognitive after controlling for age and logTILL, $p< 0.05$ (FDR corrected). Significant clusters were projected onto the MNI 152 standard space T1 template and white matter tracts extracted from JHU-ICBM white matter atlas (Yellow: forceps major; Brown: anterior thalamic radiation; Blue: forceps minor).**

**Table 5. 1 MNI coordinates of the local maxima of significant clusters resulting from VLSM methods**

| Locations | Cluster size (voxel) | MNI coordinates of peak voxel (mm) | | | T value |
|---|---|---|---|---|---|
| | | X | Y | Z | |
| Forceps minor | 31 | -24 | 38 | 2 | 3.42 |
| Forceps major | 22 | 22 | -64 | 18 | 3.26 |
| Left anterior thalamic radiation | 21 | -26 | 34 | 24 | 3.48 |
| Right anterior thalamic radiation | 11 | -26 | 8 | 44 | 3.18 |

### 5.4.3 The role of severity of cerebrovascular lesions damage in global cognition

Increased MD of infarctions (P=0.008) and chronic subcortical ischaemic lesions (P=0.002) were found in patients with abnormal cognition compared with patients with normal cognition.

.

When controlling for age, infarctions MD ($r$=-0.284, P=0.003) and chronic subcortical ischaemic lesions MD ($r$=-0.300, P=0.002) were associated with global cognitive performance. The significant correlation also existed when MTA was additionally controlled (Table 5. 2).

**Table 5. 2 The role of severity of cerebrovascular lesions damage in global cognition**

| | Normal cognition n=55 | Abnormal cognition n=53 | P[a] | *r* [b] | P | *r* [c] | P |
|---|---|---|---|---|---|---|---|
| **Infarctions MD $\times 10^{-9}$ $m^2$ $s^{-1}$, mean(SD)** | 0.29(0.38) | 0.49(0.39) | 0.008* | -0.284 | 0.003* | -0.246 | 0.011* |
| **Chronic subcortical ischaemic MD$\times 10^{-9}$ $m^2$ $s^{-1}$, mean(SD)** | 0.98(0.11) | 1.04(0.09) | 0.002* | -0.300 | 0.002* | -0.244 | 0.012* |

**a:** P value of comparison of MD values of infarctions and chronic subcortical ischaemic lesions between patients with normal and abnormal cognition

**b:** Correlation coefficient between MD of infarctions and chronic subcortical ischaemic lesions with global cognitive performance, controlled for age

**c:** Correlation coefficient between MD of infarctions with global cognitive performance, controlled for age and MTA

***:** Significant level at P value <0.05

## 5.5 Discussion

This study confirms that in patients with symptomatic carotid artery disease, global cognitive impairment is associated with (1) the presence of chronic subcortical ischaemic lesion location in the thalamic radiation and interhemispheric white matter tracts; (2) severity of cerebrovascular lesions damage.

The first hypothesis, which stated '*the presence of cerebrovascular lesions in strategic brain areas contributes to cognitive impairment in patients with symptomatic carotid artery disease*', has been upheld in this study. Chronic subcortical ischaemic lesions located in the anterior thalamic radiation, the forceps minor and the forceps major contributed to global cognitive impairment in patients with symptomatic carotid artery disease. This closely aligns with findings in patients with symptomatic atherosclerotic disease (Biesbroek, Kuijf et al. 2013) and in patients with CADASIL (Duering, Zieren et al. 2011) which is considered as a 'pure' model of VCI. It has been found that the presence of white matter lesions located in the superior longitudinal fasciculus and anterior thalamic radiation contributed to executive dysfunction in patients with symptomatic atherosclerotic disease using VLSM approach (Biesbroek, Kuijf et al. 2013). In patients with CADASIL, lacunar infarctions and white matter lesions located in the forceps minor and anterior thalamic radiation significantly contributed to impaired processing speed and presence of white matter lesions located in the forceps major contributed to memory impairment demonstrated by VLSM method (Duering, Zieren et al. 2011). These two studies mentioned above confirmed the contribution of lesion location in VCI by mainly investigating the

relationship between lesion location and performance of a specific cognitive domain rather than global cognitive performance (Selnes and Vinters 2006). Our study provided the first evidence of the contribution of lesion location on global cognitive impairment in elderly patients with advanced atherosclerosis.

The consistent findings from different study cohorts highlight that cerebrovascular lesion location rather than vascular aetiology determines the cognitive outcome in patients with vascular pathology. This in turn highlights the role of disconnection of cognitive network in VCI. For example, Duering and colleagues confirmed that the disruption of frontal-subcortical neuronal circuits by cerebrovascular lesions contributed to VCI (Duering, Zieren et al. 2011) (Duering, Gonik et al. 2013). Our study showed that the disruption of cognitive network connected by the anterior thalamic radiation, forceps minor and forceps major was related to global cognitive impairment. Forceps minor connects the frontal lobes and crosses the midline via genu of corpus callosum and forceps major connects the cortices of parietal, occipital and temporal lobes via the splenium of corpus callosum (Orrison 2008). The disruption of genu or splenium of corpus callosum has been found in normal ageing (Schulte, Sullivan et al. 2005), MCI and AD (Di Paola, Spalletta et al. 2010). The anterior thalamic radiation which are composed of fibre tracts projecting between the thalamus, striatum and frontal cortex, plays a key role in the prefrontal-subcortical circuits which has been demonstrated to be involved in executive function and processing speed (Pantoni and Gorelick) (Behrens, Johansen-Berg et al. 2003).

Different from previous studies in patients with vascular pathology, this study found a role of the presence of chronic subcortical ischaemic lesion location in forceps major in global cognitive impairment. This partial discrepancy could be

explained by the different underlying vasculopathy, but is more likely due to the variation of cognitive assessments. The two studies mentioned above focused on information processing speed which mainly reflects the frontal lobe function (Chui 2007). In contrast, in this study, we assessed the contribution of lesion location on global cognitive impairment including impairment of attention, memory, fluency, language and visuospatial ability. Therefore, the global cognitive role of forceps major identified in our study points to the affection of other cognitive domains such as memory. This hypothesis has been verified in the previous study showing that the presence of white matter lesion location within the forceps major affected memory performance in VCI (Duering, Zieren et al. 2011).

Secondly, this study verified the hypothesis that '*Severity of cerebrovascular lesions damage, indexed as increased MD, correlates with global cognitive impairment in patients with symptomatic carotid artery disease*'. More specifically, this study demonstrated increased MD of infarctions and chronic subcortical ischaemic lesions contributed to global cognitive impairment. It has been suggested that DTI parameters provide a better index of brain tissue damage severity than volumetric measurements shown on conventional MRI (Sundgren, Dong et al. 2004) (O'Sullivan, Morris et al. 2004) (Xu, Zhou et al. 2010). In this study, the damage severity of each type of cerebrovascular lesions, rather than their volumes (shown in Chapter 4), was found to be associated with global cognitive impairment. This further strengthens the point that DTI indices are better than conventional MRI volume measurements to predict global cognitive impairment in patients with vascular pathology, therefore probably providing new insights into the mechanisms of VCI.

Several limitations need to be considered. Firstly, this study was a lack of neuropsychometric information on premorbid cognition due to the study design and a lack of direct diagnostic tools for amyloid pathology, such as PET amyloid imaging. However, research investigators of ICAD study (Dr. Akram Hosseini and Richard Simpson) spoke to all participants and excluded patients without the capacity to consent and clinically apparent dementia before the MRI scans. Also, in the main study cohort (n=108), the clinical information of 53 cognitively impaired subjects was carefully reviewed by Dr. Akram Hosseini. 48 of them had first-ever stroke and no pre-existing cognitive impairment. 4 subjects had previous history of post-stroke cognitive impairment with subsequent partial improvement. One subject had amyloid angiopathy. Based on the recent published diagnostic criteria, all cognitively impaired subjects in this study cohort were considered as having probable VaMCI. Additionally, this chapter verified the imaging-cognitive relationship in the patients without MTA. It means the results are not substantially confounded by potentially comorbid AD pathology. Therefore, we are confident not to include patients with premorbid dementia and the results presented in this thesis are not confounded by potentially comorbid AD pathology.

Second, due to the lack of high resolution T1 weighted images, the assessment of MTA were conducted on re-constructed coronal FLAIR images. To overcome this limitation and to validate the reliability of using FLAIR images to assess MTA, MTA scale was also applied on all subjects who had coronal T1 images covering the hippocampal formation (n=50). The agreement of using MTA scale between FLAIR and coronal T1 images is high.

## 5.6 Conclusion

This study demonstrates the contribution of the presence of chronic subcortical ischaemic lesions and severity of cerebrovascular lesions damage on global cognitive impairment in patients with symptomatic carotid artery disease. The identification of strategic cerebrovascular lesion location and corresponding strategic white matter tracts highlight the role of disconnection of cognitive networks caused by disruption of strategic connecting white matter pathways in VCI.

# Chapter 6 Microscopic damage of non-lesional brain tissues in patients with symptomatic carotid artery disease


## Abstract

The main hypothesis of this chapter is that microscopic damage of non-lesion brain tissue including strategic brain regions, normal appearing white matter and whole white matter tract skeleton contributes to global cognitive impairment in patients with symptomatic carotid artery disease. Mean diffusivity (MD) values were extracted from hippocampus, thalamus, anterior thalamic radiation, forceps minor and forceps major, and were correlated with global cognitive performance in 108 subjects with symptomatic carotid artery disease. TBSS approach was applied to investigate the association between microscopic damage of white matter tract skeleton and global cognitive impairment. Microscopic damage of non-lesion brain tissues was related to global cognitive impairment, with white matter tract skeleton MD showing the strongest correlation. It suggests that white matter tract skeleton MD has the potential to be used as an imaging biomarker for VCI.

## 6.1 Introduction

The preceding chapter examined the contribution of cerebrovascular lesional damage in global cognitive impairment in patients with symptomatic carotid artery disease. Additionally, all main findings are verified in the subgroup only including patients without medial temporal lobe atrophy (MTA), meaning that the identified imaging-cognitive correlations are not confounded by the co-existing MTA. The results of Chapter 5 highlighted the role of cognitive network disconnection in vascular cognitive impairment (VCI). This chapter is to examine the role of microscopic damage of non-lesional brain tissues in global cognitive impairment in patients with symptomatic carotid artery disease. More specifically, the microscopic damage of strategic brain areas identified in previous studies, strategic white matter tracts identified in Chapter 5, normal appearing white matter (NAWM) and white matter tract skeleton will be investigated. The microscopic damage of these non-lesional brain tissues will be correlated with global cognitive performance in order to investigate their role in global cognitive performance in patients with symptomatic carotid artery disease.

Cognitive decline due to cerebrovascular lesions located in hippocampus and thalamus is defined as ‘strategic infarct dementia’ because these brain regions are considered as functionally important brain areas (Jellinger 2013). Microscopic damage of these strategic brain regions, in addition to cerebrovascular lesions located within these areas, has been found to be related to cognitive decline. For instance, increased MD of hippocampus was found to predict memory decline in healthy elderly subjects (Carlesimo, Cherubini et al. 2010). Hippocampal diffusional change might be superior to hippocampal

volume measurement to detect hippocampal abnormalities (Muller, Greverus et al. 2007) and to predict the conversion from MCI to dementia (Fellgiebel, Dellani et al. 2006). Additionally, a recent published study demonstrated that hippocampal MD might be superior to hippocampal volume and hippocampal functional connectivity to predict cognitive impairment in stroke patients (Kliper, Ben Assayag et al. 2016). The association between global cognitive impairment and thalamic diffusional changes has been identified in patients with multiple sclerosis (MS) (Benedict, Hulst et al. 2013). Also, DTI measurements of thalamus were found to correlate with executive dysfunction in patients with CADASIL (O'Sullivan, Singhal et al. 2004). The role of hippocampal and thalamic diffusional changes in cognitive impairment in patients with advanced atherosclerosis remains unclear.

Cytotoxic oedema occurs after cerebral ischaemia and this results in changes in membrane permeability and decreased extracellular volume fraction (Iadecola and Anrather 2011). These will lead to reduction of white matter integrity at the primary lesion as a consequence of tissue damage or at remote brain regions caused by anterograde Wallerian degeneration and/or retrograde axonal degeneration (Sykova, Svoboda et al. 1994) (Dacosta-Aguayo, Grana et al. 2014). Wallerian degeneration is the process of anterograde degeneration of the axons when a nerve fiber is cut or crushed (Brun, Gustafson et al. 1990) while retrograde axonal degeneration assumes white matter damage through axonal damage and myelin breakdown (Reisberg, Franssen et al. 2002). White matter pathway damage, which is secondary to cerebrovascular lesions, may lead to cognitive impairment. This could be explained by the fact that cognitive abilities are known to be the results of information communication among distributed

brain areas which constitute large-scale structural brain networks. Cognitive deficits could be caused by disruption of the integrity and organisation of white matter pathway connections among those distributed brain regions (Bressler and Menon 2010).Therefore, it is plausible that VCI in patients who had cerebrovascular lesions might be explained by the white matter structural disconnection.

Diffusion tensor imaging (DTI) is suited for investigating the contribution of disconnection on cognitive deficits as it provides indices of white matter connections within large-scale structural brain networks. FA and MD are two common diffusion metrics reflecting the microscopic integrity of white matter. Decreased FA reflects the decreased degree of anisotropic diffusion within a region. Increased MD indicates increased diffusivity due to the breakdown of axonal cell membranes or myelin sheaths (Bennett and Madden 2014).

A large number of studies have demonstrated the role of disconnection in normal ageing (Sullivan and Pfefferbaum 2006) (Sullivan and Pfefferbaum 2007) (Wozniak and Lim 2006) (Malloy, Correia et al. 2007) (Andrews-Hanna, Snyder et al. 2007) (Minati, Grisoli et al. 2007) (Madden, Bennett et al. 2009) (Madden, Bennett et al. 2012) (Salat 2011) (Carmichael and Lockhart 2012) (Bennett and Madden 2014). Several studies have demonstrated that white matter integrity showed an anterior-posterior gradient in which more age-related white matter disruption in frontal brain regions relative to posterior brain areas (Pfefferbaum, Sullivan et al. 2000) (Sullivan, Adalsteinsson et al. 2001) (Sullivan and Pfefferbaum 2003) (Madden, Whiting et al. 2004) (Madden, Spaniol et al. 2007) (Madden, Bennett et al. 2009) (Salat, Tuch et al. 2005) (Bucur, Madden et al. 2008) (Bennett, Madden et al. 2010) (Burzynska, Preuschhof et al. 2010)

(Michielse, Coupland et al. 2010). In contrast, a superior-inferior gradient of ageing-related white matter disruption has also been suggested by several studies (Zahr, Rohlfing et al. 2009) (Sullivan, Rohlfing et al. 2010) (Sullivan, Zahr et al. 2010). Across all these studies mentioned above, it was confirmed that white matter integrity declined in normal ageing, however, more studies are needed to identify a specific pattern of ageing-related difference of white matter integrity. Another question is whether ageing-related white matter disruption is more predominant in specific white matter tracts or in whole-brain white matter. To date, this question has not been entirely and clearly answered even though some studies have attempted to provide an answer. Penke and colleagues proposed a global effect of white matter disconnection in normal ageing (Penke, Munoz Maniega et al. 2010) while several studies, such as (Lovden, Laukka et al. 2013), (Li, Yang et al. 2012) and (Johnson, Diaz et al. 2015), suggested a combination of global and tract-specific effects of white matter disruption in ageing. As shown in normal ageing, the cognitive decline in MCI and AD could also be explained by disconnection syndromes (Matthews, Filippini et al. 2013) (Alves, Knochel et al. 2015). So far, there has been no firm conclusion regarding the pattern of white matter abnormalities in MCI and AD even though some attempts have been made by several research groups. For instance, Damoiseaux and colleagues demonstrated that the disruption of white matter integrity was primarily located in the temporal lobe in AD as opposed to that the white matter abnormality was primarily located in the frontal, parietal and subcortical brain areas in normal ageing (Damoiseaux, Smith et al. 2009). Different from this finding, Sexton and colleagues concluded that white matter disruption in AD

existed in all brain regions except internal capsule and parietal white matter (Sexton, Kalu et al. 2011).

In addition to the direct injury of cerebrovascular lesions, disconnection of large-scale cognitive networks could also explain VCI (Catani and ffytche 2005) (Lanna, Alves et al. 2012). Secondary neurodegeneration of cerebrovascular lesions could cause white matter microscopic damage (Duering, Righart et al. 2015), therefore leading to cognitive deficits. The cognitive deficit in VCI, which is characterised as dysfunction of attention and executive function, has been related to disconnection of frontal-subcortical circuits (Cummings 1989) (Chui 2007). Interestingly, widespread microscopic damage of white matter has been shown in VCI in several studies. For example, Kim and colleagues demonstrated increased MD and decreased FA in all white matter in patients with subcortical VaD compared with normal healthy subjects. Similar pattern of white matter microscopic damage has also been identified when comparing it between patients with subcortical VaD and those with pure AD (Kim, Kwon et al. 2015). A similar pattern of white matter microscopic damage has also been found by another research group (Kim, Park et al. 2011). The identification of posterior white matter microscopic damage in VCI could be explained from two perspectives: the involvement of cholinergic pathways and long association white matter tract fibers (Kim, Park et al. 2011).

Conventional ROI analysis of DTI has been criticised for the limitation to pre-specified regional hypothesis. In addition, partial volume effects can also be problematic with ROIs locating close to the ventricles. Voxel-based morphometry (VBM) approach is relatively operator-independent therefore overcoming some problems of ROI-based methods (Ashburner and Friston

2000). VBM is most commonly carried out in the Statistic Parametric Mapping (SPM) software developed by Welcome Department of Imaging Neuroscience, University College London (Ashburner and Friston 2000) (Mechelli, Price et al. 2005). Misalignment and smoothing are two main limitations of VBM-style analyses (Smith et al., 2006). Recently, these analysis approaches have largely been replaced by whole brain analysis approach, such as tract-based spatial statistics (TBSS) method which brings together the strengths of each method and at the meantime overcomes the problems of each method (Alves, Oertel Knochel et al. 2015). TBSS was developed by the Centre for Functional MRI of the Brain (FMRIB), University of Oxford (Smith et al., 2006). In TBSS, All subject's FA maps are firstly aligned to either standard space or a pre-defined target FA map. Then, the aligned FA maps are averaged to create a mean FA map. After that, the mean FA map is used to generate the white matter tract skeleton which represents each white matter tract as a line running down the centre of the white matter tract. Afterwards, each individual's FA map is projected onto the average FA white matter tract skeleton derived from the whole group. Finally, voxelwise cross-subject statistical analysis is applied using univariate linear modelling. Permutation-based approach is commonly used in TBSS to correct for multiple comparisons. The misalignment issue existed in VBM approach is minimised by TBSS approach and it is more sensitive and robust than VBM approach. However, it may be complex to interpret the TBSS results in regions of crossing tracts (Smith, Jenkinson et al. 2006).

### 6.2 Aims and hypotheses

Hypotheses:

- Microscopic damage of hippocampus and thalamus is related to global cognitive impairment in patients with symptomatic carotid artery disease.

- Microscopic damage of strategic white matter identified in Chapter 5 is associated with global cognitive impairment in patients with symptomatic carotid artery disease.
- Microscopic damage of widespread white matter including normal appearing white matter (NAWM) and white matter tract skeleton is associated with global cognitive impairment in patients with symptomatic carotid artery disease.

Aims:

- Obtain MD values from hippocampus and thalamus and correlate these MD values with global cognitive performance using regression analysis
- Obtain MD values from anterior thalamic radiation, forceps minor and forceps major and correlate these MD values with global cognitive performance using regression analysis
- Obtain total ischaemic lesion volumes within strategic white matter tracts and investigate the association between microscopic damage of these white matter tracts and total ischaemic lesion volumes within these white matter tracts
- Obtain MD values from NAWM and associate these MD values with global cognitive performance using regression analysis
- Apply TBSS approach to investigate the association between microscopic damage of white matter tract skeleton and global cognitive impairment

## 6.3 Methods

### 6.3.1 Subjects and assessments

The recruitment, clinical and cognitive assessment, MRI protocol of subjects included in this chapter was described in Chapter 3. Totally 108 subjects were included in this chapter. The study cohort used in this chapter was the same as that in Chapter 5.

### 6.3.2 Assessments of severity of brain tissue damage

MD values were chosen to reflect the damage severity of hippocampus and thalamus. The reason to choose MD value was stated in Chapter 5. The steps for extracting MD values from hippocampus and thalamus were as follows: (1) MD images that co-registered with MNI template images and thresholded at the level of $1.5\times10^{-9}$ $m^2$ $s^{-1}$ (details mentioned in Chapter 5) were chosen to extract MD values from bilateral hippocampi and thalamus; (2) Regions of interest (ROIs) were manually drawn within thalamus (Figure 6. 1) and hippocampus (Figure 6. 2) on three consecutive slices of co-registered and thresholded MD images. Due to the lack of high resolution T1-weighted images, all ROIs had to be manually drawn on MD images. Thus, ROIs could not cover the whole hippocampus and thalamus because of the relatively low spatial resolution of MD images; (3) The averaged MD values of hippocampus and thalamus were obtained by using the following FSLmaths and FSLstats command lines:

*fslmaths (each subject's hippocampus/thalamus mask filename).hdr* -mas *(each subject's MD map filename).hdr (output filename of each subject's hippocampus/thalamus MD maps)*

*fslstats (each subject's hippocampus/thalamus MD maps) -M*

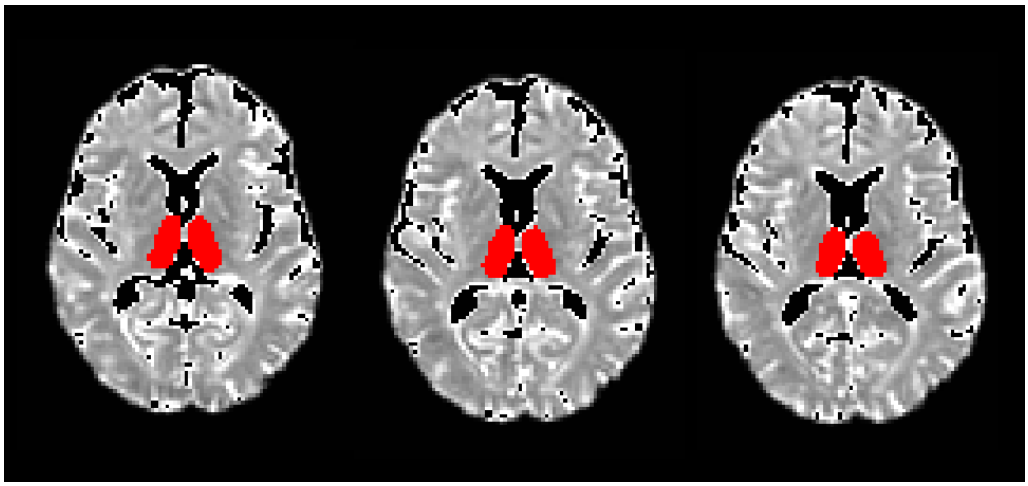


**Figure 6. 1 Thalamus ROIs on co-registered MD maps**

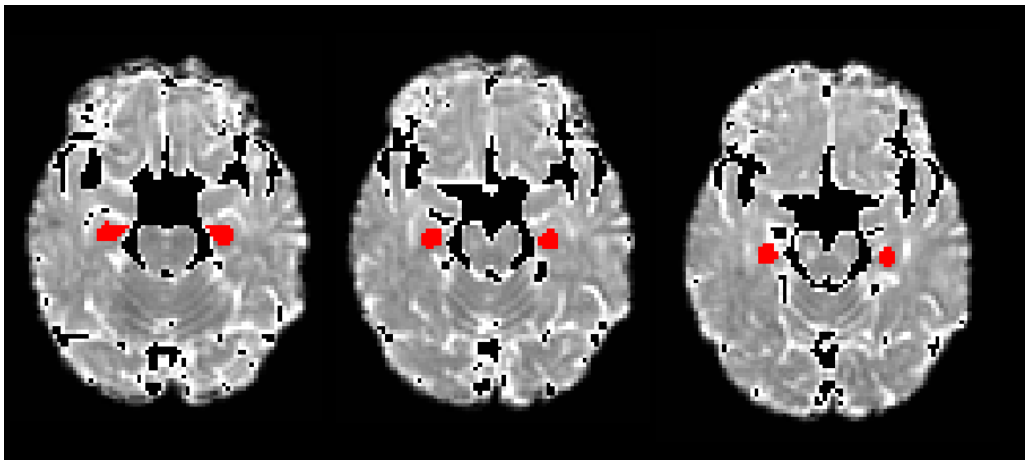


**Figure 6. 2 Hippocampal ROIs on MD maps**

### 6.3.3 Microscopic damage of strategic white matter tracts

Anterior thalamic radiation, forceps minor and forceps major were demonstrated as strategic white matter tracts for global cognitive performance in Chapter 5. To investigate the role of microscopic damage of strategic white matter tracts in global cognition, MD values of anterior thalamic radiation, forceps minor and forceps major were extracted following these steps:

Firstly, the atlases of anterior thalamic radiation, forceps minor and forceps major were extracted from the Johns Hopkins University (JHU) DTI-based white-matter atlases (Mori 2005) (Wakana, Caprihan et al. 2007) (Figure 6. 3). Since the atlases are the probabilistic atlases, the extracted atlases were set to a liberal threshold of 10% (Morgan, Mishra et al. 2009). Secondly, atlases of anterior thalamic radiation, forceps minor and forceps major were masked onto the co-registered and thresholded MD maps (details mentioned in 6.3.2 and Chapter 5) using the FSLmaths command line:

*fslmaths (subject's co-registered MD map filename).hdr –mas (mask of each strategic white matter tract filename).hdr (subject's strategic white matter tract MD output filename)*

After that, the MD values of strategic white matter tracts of each subject were computed using the FSLstats command line:

*fslstats (subject's strategic white matter tract MD output filename) –M*

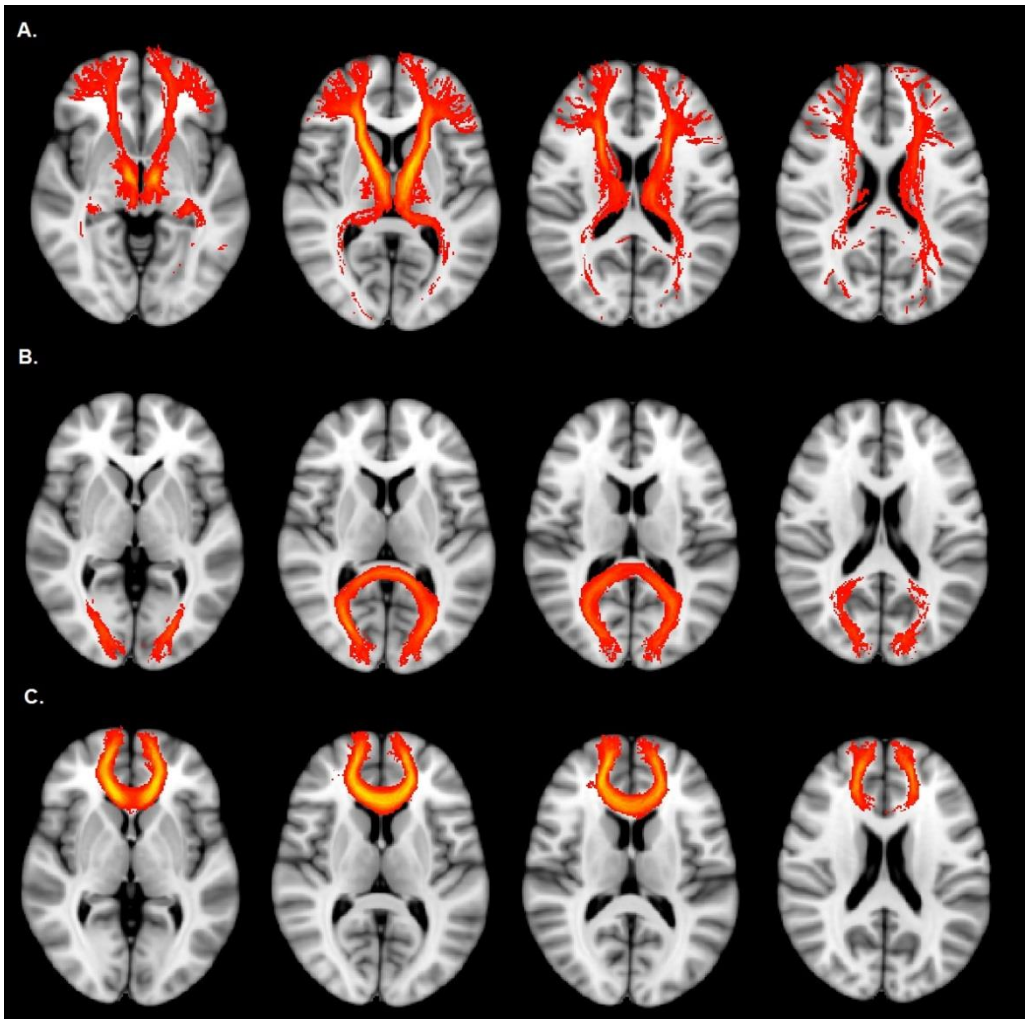



**Figure 6. 3 White matter tracts overlaid on MNI152 standard space. (A) Anterior thalamic radiation (B) Forceps major and (C) Forceps minor.**

### 6.3.4 Total ischaemic lesion volumes within strategic white matter tracts

The following steps were performed to investigate the role of cerebrovascular lesion volumes in microscopic damage of specific white matter tracts (Figure 6. 4). Firstly, each subject's total ischaemic lesion map (described in Chapter 5), co-registered with MNI template space, was masked by the atlas of each white matter tracts by using the FSLmaths command line:

*fslmaths (each subject's total ischaemic lesion map filename).hdr –mas (white matter tract mask filename) (each subject's total ischaemic lesion map within white matter tract filename)*

Then, the volumes of remained total ischaemic lesions within each white matter tract were computed by using the FSLstats command line:

*fslstats (each subject's total ischaemic lesion map within white matter tract filename).hdr –V*

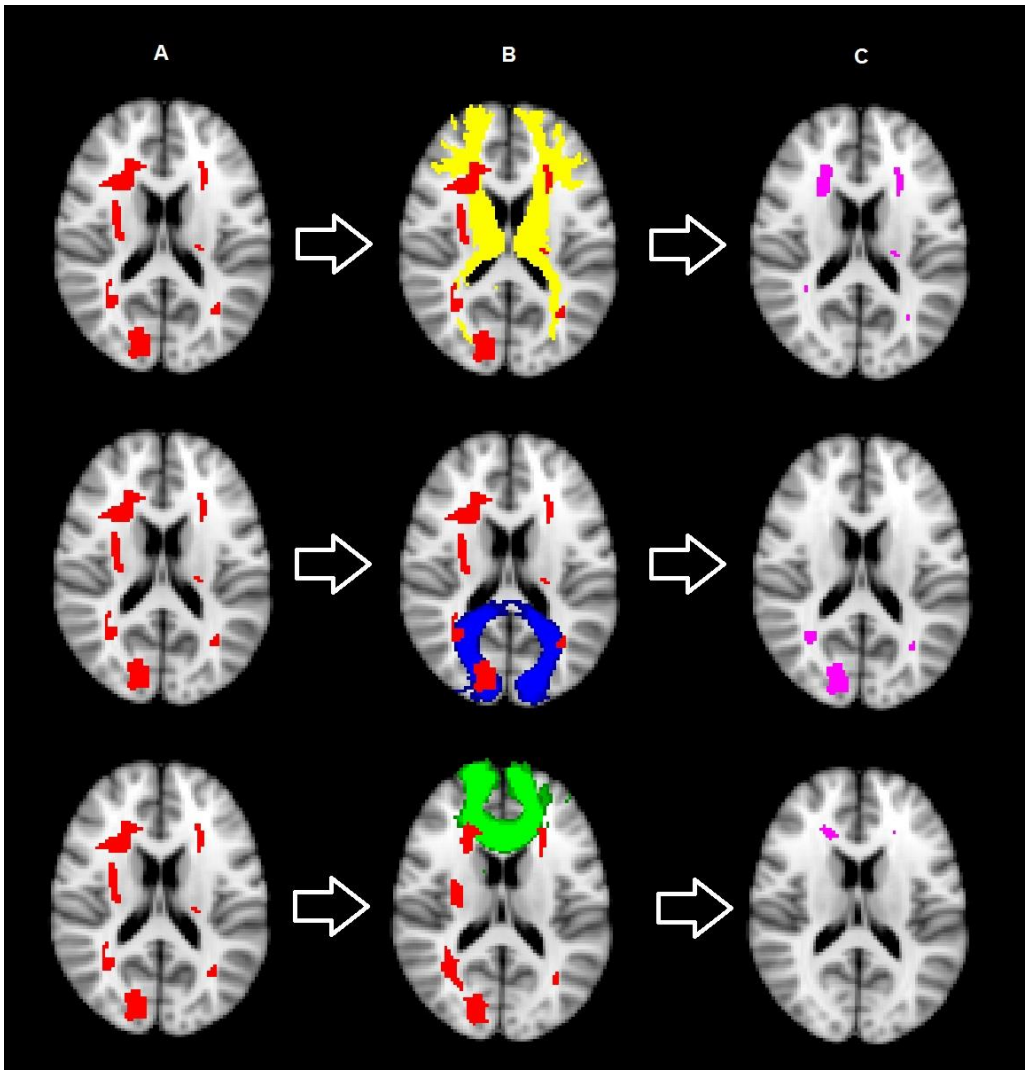



**Figure 6. 4 An example of calculating total ischaemic lesion volumes within each white matter tracts. Each subject's total ischaemic lesion map (described in Chapter 3) was overlaid onto each specific white matter tracts (B) (Yellow: anterior thalamic radiation; Blue: forceps major; Green: forceps minor). Then, fslmaths command line was used to mask out the total ischaemic lesions not locating in each white matter tract. The volumes of remained total ischaemic lesions (C) (Pink) were calculated using fslstats command line.**

### 6.3.5 Microscopic damage of normal appearing white matter (NAWM)

Before extracting MD values of NAWM, all images including b0 and MD maps were needed to be co-registered with MNI standard space as all types of cerebrovascular lesions were outlined on co-registered FLAIR and diffusion images. The details were described in Chapter 5. NAWM map of each subject was obtained by following the steps:

Firstly, given that no high resolution T1-weighted images were included in the MRI protocol, the segmentation of grey matter, white matter and CSF has to be conducted using b0 diffusion images. The segmentation process was performed using Statistical Parametric Mapping (SPM) 8 (http://www.fil.ion.ucl.ac.uk/spm/) (Figure 6. 5). Secondly, each subject's total ischaemic lesion map was subtracted from each subject's white matter map using the FSLmaths command line:

*fslmaths (subject's white matter map filename).hdr –sub (subject'st total ischaemic lesion map filename).hdr (subject's NAWM output filename)*

Then, the FSLstats command line was used to extract averaged MD value of each subject's NAWM map:

*fslstats (subject's NAWM output filename) –M*

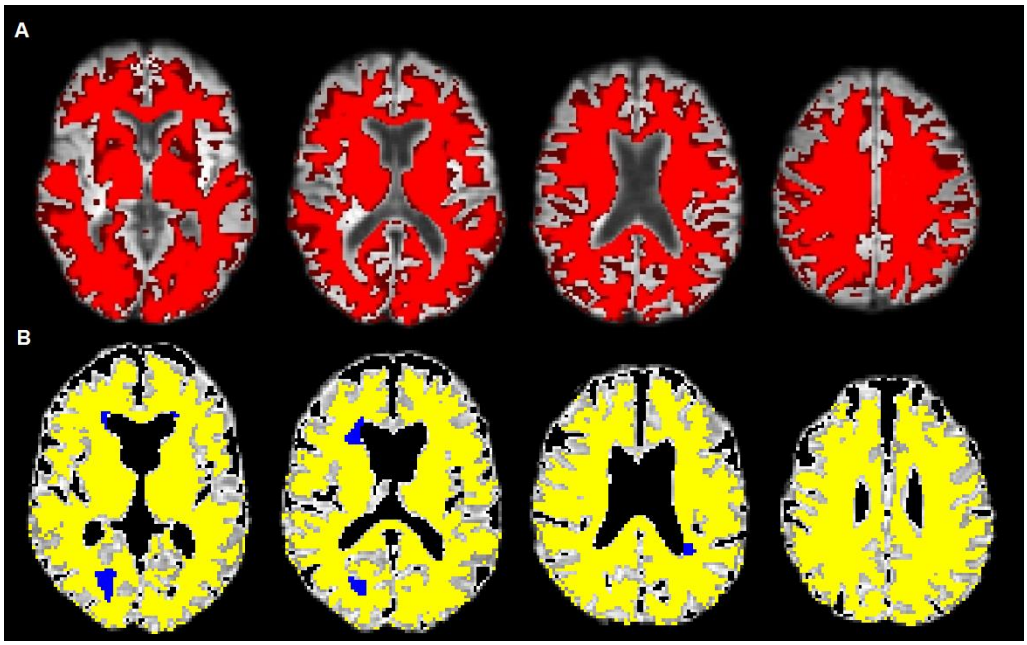



**Figure 6. 5 An example of segmented white matter and NAWM. (A) Segmented white matter masks (Red) overlaid on thresholded b0 images. (B) Segmented NAWM masks (Yellow) and total ischaemic lesion masks (Blue) overlaid on co-registered and thresholded images.**

### 6.3.6 Tract-based spatial statistics (TBSS)

Generally four steps were included in the TBSS analysis pipeline (Smith et al., 2006):

- Nonlinear alignment and identifying the target for alignment

The first step was to identify a common registration target image and align each subject's FA map to this target using nonlinear registration. The perfect registration was not required in this step. Instead, the aim of this step was to allow sufficient alignment so the skeletonisation step will proceed accurately.

- Creating the mean FA image and its skeleton

Once all subjects' FA maps were aligned to the identified target, all data were then co-registered with the MNI 152 1×1×1 $mm^3$ space. Using the MNI 152 standard space made the display and interpretation of results easier. After that, a

mean FA map was created by averaging all the transformed FA images, following a white matter tract skeleton map was created. The white matter tract skeleton represented each white matter tract as a single line running down the centre of the tract.

- Projecting each individual's FA map onto the skeleton

The white matter tract skeleton map was projected onto each subject's aligned FA images, resulting in the white matter tract skeleton map created for each subject. Each subject's skeleton map was thresholded at an FA value of 0.2 (default setting in TBSS) to exclude non-tract voxels. The higher FA values to choose for thresholding, the lower number of voxels within the skeleton remain (Figure 6. 6).

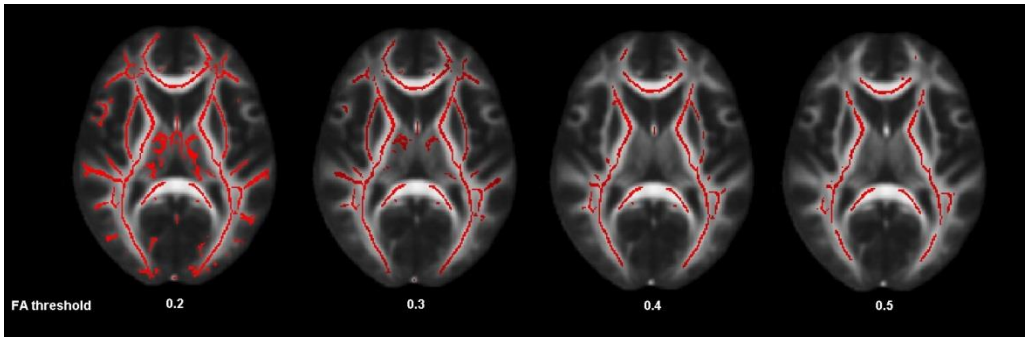



**Figure 6. 6 An example of different FA threshold values resulting different white matter skeleton outputs**

All the steps mentioned above were also applied on MD maps using the command line:

*tbss_non_FA (MD images directory filename)*

- Statistics and thresholding

Finally, all data were fed into voxel-wise cross-subject statistical analysis using a standard general linear model design matrix. A permutation-based method (n=5000) was chosen to correct for multiple comparisons. Bonferroni correction was not an appropriate method as the final skeletonised FA data contained some intrinsic spatial smoothness which may lead to an over-conservative correction.

After conducting all the steps mentioned above, the MD values of each subject's white matter tract skeleton map were computed using the FSLstats command line:

*fslstats (subject's MD white matter tract skeleton output filename) –M*

### 6.3.7 Statistical analysis

Linear regression analyses were chose to investigate the association between microscopic damage of strategic brain locations, strategic white matter tracts, NAWM, white matter tract skeleton and global cognitive performance. All analyses were conducted in SPSS version 21 (Chicago, IL, USA).

Using TBSS, the difference of FA and MD was compared between patients with normal and abnormal cognition. Additionally, the correlation analyses between FA/MD values and global cognitive performance were also performed using TBSS method. Age and logTILL were controlled in TBSS analyses.

The correlation coefficient between neuroimaging features of white matter microscopic damage and global cognitive performance were compared using VassarStats (http://www.vassarstats.net/index.html), an online statistical calculator, to identify the neuroimaging feature with the strongest correlation with global cognition.

## 6.4 Results

The inclusion and exclusion criteria of participants included in this chapter and the procedure of imaging quality assurance have been described in Chapter 4. Totally 108 subjects were included in the analyses of this chapter. The cognitive profile, comparison of clinical characteristics between patients with normal and abnormal cognition of this study cohort was provided in Chapter 4 (Table 4. 1) (Table 4. 3).

### 6.4.1 The role of hippocampal and thalamic damage in global cognition

Patients with abnormal cognition had increased MD of hippocampus than patients with normal cognition (effect size: 0.025, P=0.004). Also, there was a significant correlation between hippocampal MD and global cognitive performance when age was controlled ($r$=-0.289, P=0.003) (Table 6. 1).

Thalamic MD was increased in patients with abnormal cognition compared with patients with normal cognition (effect size: 0.148, P<0.001). Additionally, there was a significant association between thalamic MD and global cognitive performance ($r$ =-0.321, P=0.001) when controlling for age (Table 6. 1).

**Table 6. 1 The role of hippocampal and thalamic damage severity in global cognition**

| | Normal cognition n=55 | Abnormal cognition n=53 | P[a] | *r* [b] | P |
|---|---|---|---|---|---|
| **Averaged hippocampal MD $\times 10^{-9}$ $m^2$ $s^{-1}$, mean(SD)** | 0.75(0.04) | 0.78(0.04) | 0.004* | -0.289 | 0.003* |
| **Averaged thalamic MD$\times 10^{-9}$ $m^2$ $s^{-1}$, mean(SD)** | 0.85(0.02) | 0.88(0.03) | <0.001* | -0.321 | 0.001* |

**a:** P value of comparison of FA and MD values of hippocampus and thalamus between patients with normal and abnormal cognition
**b:** Correlation coefficient between FA/MD of hippocampus and thalamus with global cognitive performance, controlled for age
*: Significant level at $P < 0.05$

### 6.4.2 The role of total ischaemic lesion volumes within strategic white matter tracts in global cognition

Patients with abnormal cognition had larger normalised volumes of total ischaemic lesions within forceps minor (P=0.013), forceps major (P=0.002) and anterior thalamic radiation (P=0.006) compared to patients with normal cognition (Table 6. 2).

Normalised total ischaemic lesion volumes within forceps major was significantly correlated with global cognitive performance when age was controlled (*r*=-0.206, P=0.034). No significant association was identified between global cognitive performance and normalised total ischaemic lesion volumes within forceps minor or anterior thalamic radiation (Table 6. 2).

**Table 6. 2 The role of TILL within specific white matter tracts in global cognition**

| | **Normal cognition n=55** | **Abnormal cognition n=53** | **P[a]** | ***r*[b]** | **P** |
|---|---|---|---|---|---|
| **Normalised volumes of total ischaemic lesions within forceps minor, % mean (SD)** | 0.05(0.06) | 0.11(0.16) | 0.013* | -0.175 | 0.072 |
| **Normalised volumes of total ischaemic lesions within forceps major, % mean (SD)** | 0.08(0.11) | 0.17(0.18) | 0.002* | -0.206 | 0.034* |
| **Normalised volumes of total ischaemic lesions within anterior thalamic radiation, % mean (SD)** | 0.25(0.26) | 0.44(0.40) | 0.006* | -0.124 | 0.203 |

**a:** P value of comparison of normalised volumes total ischaemic lesions within specific white matter tracts between patients with normal and abnormal cognition

b: correlation between global cognition and normalised volumes total ischaemic lesions within specific white matter tracts, controlled for age

***:** Significant level at P value <0.05

### 6.4.3 The role of microscopic damage of strategic white matter tracts in global cognition

Patients with abnormal cognition had increased MD of forceps minor (P<0.001), forceps major (P<0.001) and anterior thalamic radiation (P<0.001) compared with patients with normal cognition (Table 6. 3).

When both age and total ischaemic lesion volumes within each specific white matter tracts were controlled, global cognitive performance was negatively correlated with MD values of forceps minor (*r*=-0.321, P=0.001), forceps major (*r*=-0.294, P=0.002) and anterior thalamic radiation (*r*=-0.395, P<0.001) (Table 6. 3).

**Table 6. 3 The role of microscopic damage of forceps minor, forceps major and anterior thalamic radiation in global cognition**

| | Normal cognition n=55 | Abnormal cognition n=53 | P[a] | *r* [b] | P |
|---|---|---|---|---|---|
| Forceps minor MD $\times 10^{-9}$ $m^2$ $s^{-1}$, mean(SD) | 0.85(0.03) | 0.89(0.04) | 0.000002* | -0.321 | 0.001* |
| Forceps major MD$\times 10^{-9}$ $m^2$ $s^{-1}$, mean(SD) | 0.85(0.03) | 0.88(0.04) | 0.000289* | -0.294 | 0.002* |
| Anterior thalamic radiation MD$\times 10^{-9}$ $m^2$ $s^{-1}$, mean(SD) | 0.84(0.03) | 0.88(0.03) | 0.000001* | -0.395 | 0.000027* |

**a:** P value of comparison of FA and MD values of forceps minor, forceps major and anterior thalamic radiation between patients with normal and abnormal cognition

b: correlation between global cognition and FA/MD of forceps minor, forceps major and anterior thalamic radiation, controlled for age and volumes of TILL within specific white matter tract

***:** Significant level at P value <0.05

### 6.4.4 The role of total ischaemic lesion volumes within white matter tracts in the microscopic damage of white matter tracts

The microscopic damage of forceps minor, forceps major and anterior thalamic radiation, indexed as increased MD, was significantly correlated with normalised total ischaemic lesion volumes within each white matter tract (Table 6. 4).

**Table 6. 4 Correlations between microscopic damage of strategic white matter tracts and TILL within white matter tracts**

| Forceps minor | | |
|---|---|---|
| | MD | |
| | $R^2$ | P |
| TILL Volumes | 0.188 | 0.000003* |
| Forceps major | | |
| | MD | |
| | $R^2$ | P |
| TILL Volumes | 0.246 | 0.0000001* |
| Anterior thalamic radiation | | |
| | MD | |
| | $R^2$ | P |
| TILL Volumes | 0.284 | 0.0000001* |

*: Significant level at P value <0.05

### 6.4.5 The role of microscopic damage of NAWM in global cognition

Patients with abnormal cognition had increased MD ($P<0.001$) of NAWM compared with patients with normal cognition. NAWM MD was significantly correlated with global cognitive performance when both age and logTILL were controlled ($r$=-0.225, P=0.020) (Table 6. 5).

**Table 6. 5 The role of NAWM microscopic damage in global cognition**

| | Normal cognition n=55 | Abnormal cognition n=53 | P[a] | *r* [b] | P |
|---|---|---|---|---|---|
| NAWM MD $\times 10^{-9}$ $m^2$ $s^{-1}$, mean(SD) | 0.75(0.03) | 0.78(0.04) | 0.000207* | -0.225 | 0.020* |

**a:** P value of comparison of FA and MD values of NAWM between patients with normal and abnormal cognition
**b**: correlation between global cognition and FA and MD of NAWM, controlled for age and logTILL
***:** Significant level at P value <0.05

### 6.4.6 The role of microscopic damage of white matter tract skeleton in global cognition

Decreased FA (Figure 6. 7) and increased MD (Figure 6. 8) were found in cognitively impaired subjects in widespread white matter including forceps major, forceps minor, external capsule, superior longitudinal fasciculus, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, fornix, cingulum, body of corpus callosum, compared with patients with abnormal cognition when age and logTILL were controlled. Increased MD of anterior thalamic radiation and anterior and posterior limb of internal capsule was additionally found in patients with abnormal cognition.

Increased MD of the body of corpus callosum, cingulum, forceps minor, forceps major, anterior thalamic radiation, anterior and posterior limb of internal capsule, superior longitudinal fasciculus, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus and fornix were significantly and negatively correlated with impaired global cognition after controlling for age and logTILL (Figure 6. 9). There was a positive correlation between impaired global cognitive decline and decreased FA of the body of corpus callosum, cingulum, forceps major, forceps minor, superior longitudinal fasciculus, inferior longitudinal fasciculus and inferior fronto-occipital fasciculus when treating age and logTILL as covariates of no interest (Figure 6. 10).

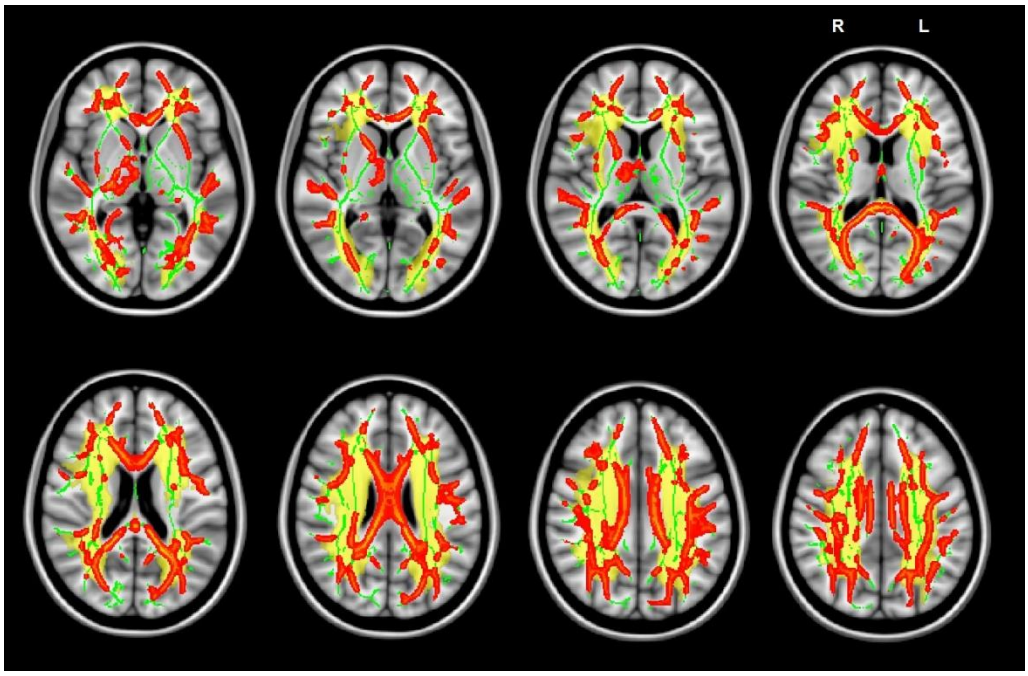



**Figure 6. 7 TBSS results for difference of FA between patients with normal and abnormal cognition. Significant skeletal voxels (Red) where cognitively impaired patients FA < cognitively intact patients FA. All analyses controlled for age and logTILL. Significant level was set at P < 0.05 (corrected for multiple comparisons). Mean TBSS white matter tract skeleton (Green) and total ischemic lesion distribution map (Yellow) were overlaid on the MNI 152 template.**

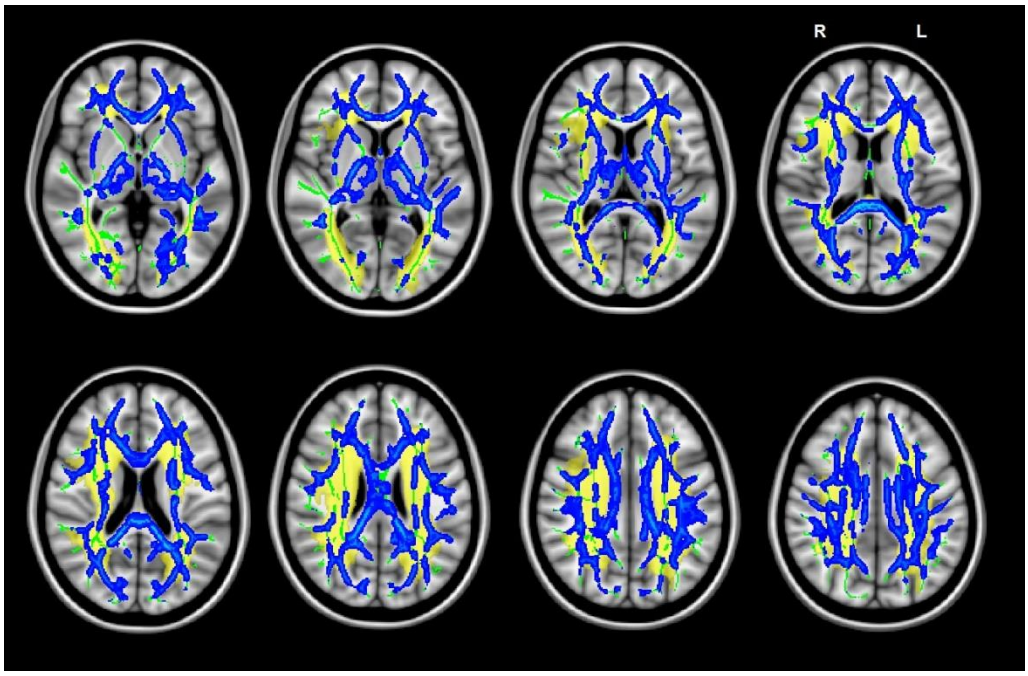



**Figure 6. 8 TBSS results for difference of MD between patients with normal and abnormal cognition. Significant skeletal voxels (Blue) where cognitively impaired patients MD > cognitively intact patients MD. All analyses controlled for age and logTILL. Significant level was set at P < 0.05 (corrected for multiple comparisons). Mean TBSS white matter tract skeleton (Green) and total ischemic lesion distribution map (Yellow) were overlaid on the MNI 152 template.**

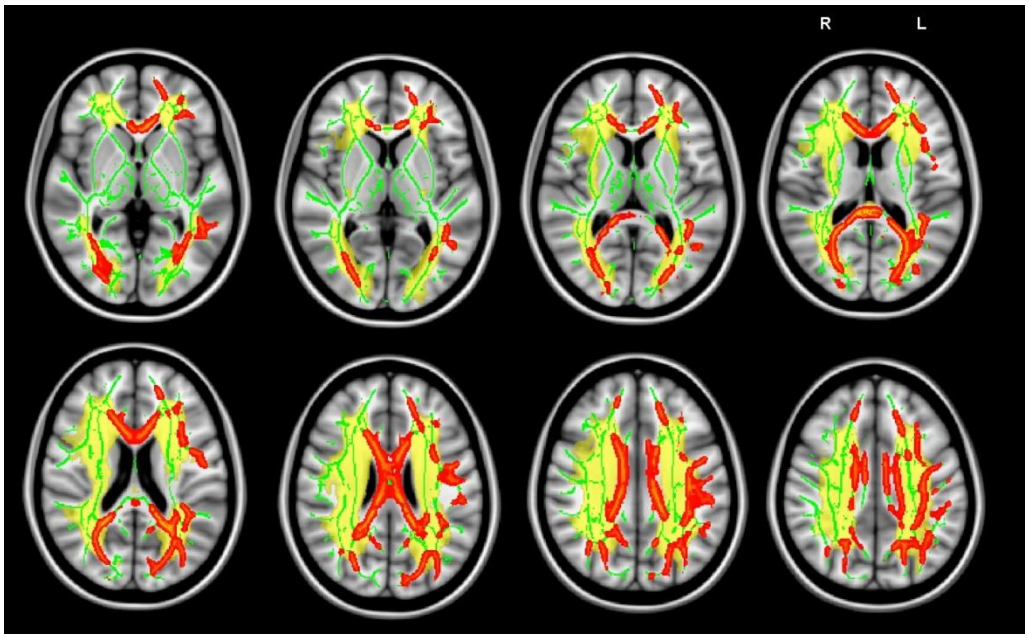



**Figure 6. 9 TBSS results for correlations between FA and global cognition. Significant skeletal voxels (Red) where decreased FA correlated with global cognitive decline. All analyses controlled for age and logTILL. Significant level was set at P < 0.05 (corrected for multiple comparisons). Mean TBSS white matter tract skeleton (Green) and total ischemic lesion distribution map (Yellow) were overlaid on the MNI 152 template.**

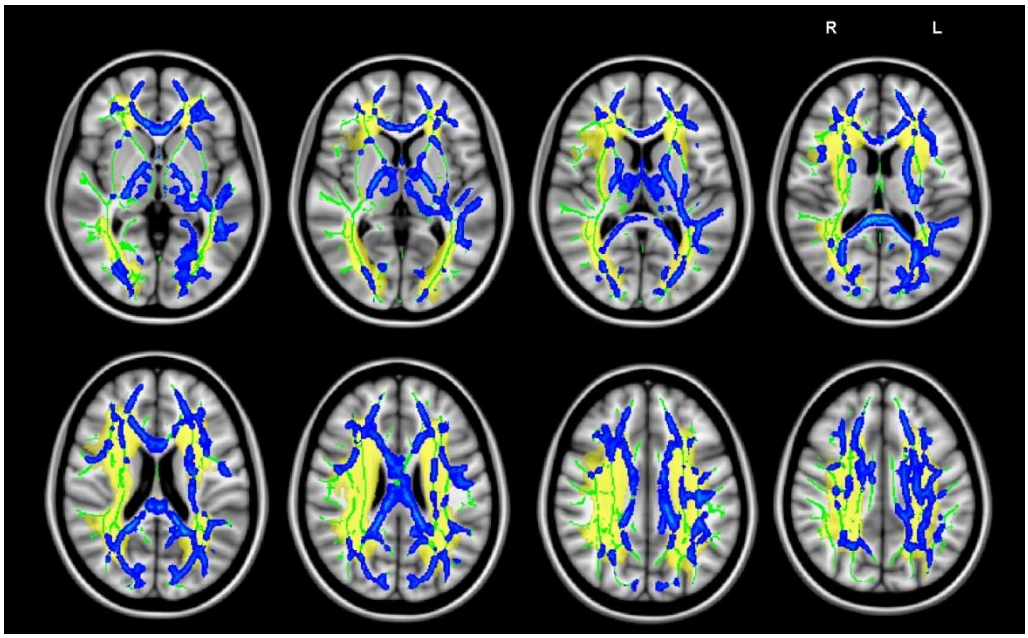



**Figure 6. 10 TBSS results for correlations between MD and global cognition. Significant skeletal voxels (Blue) where increased MD correlated with global cognitive decline. All analyses controlled for age and logTILL. Significant level was set at P < 0.05 (corrected for multiple comparisons). Mean TBSS white matter tract skeleton (Green) and total ischemic lesion distribution map (Yellow) were overlaid on the MNI 152 template.**

FA and MD values were extracted from each subject's white matter tract skeleton. There was significantly difference of FA and MD values extracted from the white matter skeleton between patients with normal and abnormal cognition (Table 6. 6).

A significant correlation was identified between white matter tract skeleton FA and MD and global cognitive performance when age and logTILL were controlled (Table 6. 6).

**Table 6. 6 The role of microscopic damage of white matter tract skeleton on global cognition**

| | Normal cognition n=55 | Abnormal cognition n=53 | P[a] | $R^{2\,b}$ | P |
|---|---|---|---|---|---|
| white matter skeleton FA, mean(SD) | 0.32(0.01) | 0.30(0.02) | 0.000003* | 0.326 | 0.001* |
| white matter skeleton MD $\times 10^{-9}$ $m^2$ $s^{-1}$, mean(SD) | 0.77(0.03) | 0.83(0.05) | 0.0000001* | -0.526 | 0.0000001* |

**a:** P value of comparison of FA and MD values of white matter skeleton between patients with normal and abnormal cognition
**b**: correlation between global cognition and FA and MD of white matter tract skeleton, controlled for age and logTILL
*****: significant level at P value < 0.05

**Table 6. 7 MNI coordinates of the local maxima of significant skeletal voxels results from TBSS methods**

| Significant skeletal voxels where cognitively impaired MD > cognitively intact MD | | | | | |
|---|---|---|---|---|---|
| Locations | Cluster size (voxel) | MNI coordinates of peak voxel (mm) | | | P value (FWE-corrected) |
| | | X | Y | Z | |
| Forceps major | 4049 | -17 | -44 | 7 | 0.011 |
| Forceps minor | 5776 | -10 | 39 | -18 | 0.006 |
| Left anterior thalamic radiation | 6703 | -8 | 57 | -14 | 0.007 |
| Right anterior thalamic radiation | 4671 | -14 | -52 | 18 | 0.001 |
| Left inferior fronto-occipital fasciculus | 7496 | -14 | 37 | -16 | 0.008 |
| Right inferior fronto-occipital fasciculus | 6229 | 30 | -60 | 11 | 0.012 |
| Left inferior longitudinal fasciculus | 4400 | -39 | -49 | 11 | 0.012 |
| Right inferior longitudinal fasciculus | 3423 | 30 | -67 | 12 | 0.017 |
| Left superior longitudinal fasciculus | 8278 | -21 | -20 | 41 | 0.007 |
| Right superior longitudinal fasciculus | 6397 | -12 | -17 | 30 | 0.011 |
| Fornix | 1330 | -1 | -1 | 8 | 0.010 |
| Left cingulum | 2728 | -12 | -19 | 30 | 0.006 |
| Right cingulum | 1000 | -4 | 30 | 2 | 0.007 |
| TBSS results---Clusters showing significantly lower FA values in patients with abnormal cognition | | | | | |
| Locations | Cluster size (voxel) | MNI coordinates of peak voxel (mm) | | | P value (FWE-corrected) |
| | | X | Y | Z | |
| Forceps major | 4331 | 27 | -77 | 4 | 0.012 |
| Forceps minor | 3599 | 14 | 28 | 15 | 0.015 |
| Left anterior thalamic radiation | 3348 | -21 | -79 | 17 | 0.009 |
| Right anterior thalamic radiation | 2610 | 28 | -75 | 9 | 0.018 |
| Left inferior fronto-occipital fasciculus | 4665 | -19 | -83 | 14 | 0.014 |
| Right inferior fronto-occipital fasciculus | 4839 | 27 | -77 | 4 | 0.016 |
| Left inferior longitudinal fasciculus | 2925 | -22 | -80 | 14 | 0.018 |
| Right inferior longitudinal fasciculus | 3369 | 30 | -71 | 4 | 0.011 |
| Left superior longitudinal fasciculus | 6327 | -21 | -79 | 16 | 0.009 |

| | | | | | |
|---|---|---|---|---|---|
| Right superior longitudinal fasciculus | 3721 | 33 | -64 | 12 | 0.019 |
| Fornix | 682 | 1 | -7 | 12 | 0.009 |
| Left cingulum | 2085 | -9 | 20 | 20 | 0.008 |
| Right cingulum | 873 | 14 | 23 | 23 | 0.009 |

Significant skeletal voxels where increased MD correlated with global cognitive performance

| Locations | Cluster size (voxel) | MNI coordinates of peak voxel (mm) | | | P value (FWE-corrected) |
|---|---|---|---|---|---|
| | | X | Y | Z | |
| Forceps major | 4247 | -2 | -39 | 12 | 0.016 |
| Forceps minor | 4706 | -11 | 24 | 15 | 0.017 |
| Left anterior thalamic radiation | 5977 | -22 | 14 | 13 | 0.015 |
| Right anterior thalamic radiation | 3713 | 31 | -4 | 13 | 0.012 |
| Left inferior fronto-occipital fasciculus | 7238 | -29 | -2 | 38 | 0.016 |
| Right inferior fronto-occipital fasciculus | 4446 | 32 | -59 | 11 | 0.022 |
| Left inferior longitudinal fasciculus | 4822 | -39 | -49 | 11 | 0.017 |
| Right inferior longitudinal fasciculus | 2256 | 30 | -67 | 12 | 0.025 |
| Left superior longitudinal fasciculus | 7029 | -21 | -20 | 41 | 0.016 |
| Right superior longitudinal fasciculus | 2510 | -12 | -17 | 30 | 0.025 |
| Fornix | 1112 | 0 | -4 | 14 | 0.023 |
| Left cingulum | 2007 | -18 | -37 | 30 | 0.016 |
| Right cingulum | 655 | -20 | 4 | 28 | 0.022 |

TBSS results---Clusters showing significant correlation between FA and global cognitive performance

| Locations | Cluster size (voxel) | MNI coordinates of peak voxel (mm) | | | P value (FWE-corrected) |
|---|---|---|---|---|---|
| | | X | Y | Z | |
| Forceps major | 4154 | -16 | -83 | -7 | 0.009 |
| Forceps minor | 3097 | -16 | 53 | -5 | 0.014 |
| Left anterior thalamic radiation | 2903 | -23 | -21 | 37 | 0.012 |
| Right anterior thalamic radiation | 871 | -22 | -35 | 45 | 0.021 |
| Left inferior fronto-occipital fasciculus | 3659 | -16 | -84 | -9 | 0.013 |
| Right inferior fronto-occipital fasciculus | 2444 | 30 | -60 | 11 | 0.015 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Left inferior fasciculus | longitudinal | 2375 | -29 | -2 | 38 | 0.011 |
| Right inferior fasciculus | longitudinal | 1964 | 32 | -59 | 11 | 0.013 |
| Left superior fasciculus | longitudinal | 5542 | -30 | -17 | 30 | 0.012 |
| Right superior fasciculus | longitudinal | 1238 | -15 | -20 | 32 | 0.022 |
| Left Cingulum | | 2053 | -16 | -23 | 31 | 0.012 |
| Right cingulum | | 699 | 14 | -17 | 31 | 0.014 |

### 6.4.7 Comparisons of correlation coefficient between neuroimaging features of white matter microscopic damage and global cognitive performance

As demonstrated in the previous parts, global cognitive performance was significantly correlated with all neuroimaging features of white matter microscopic damage when controlling for age and logTILL. The correlation between global cognitive performance and white matter tract skeleton MD was statistically stronger than that between global cognitive performance and forceps minor FA (P=0.002), forceps minor MD (P=0.02), forceps major MD (P=0.019), anterior thalamic radiation FA (P=0.015), anterior thalamic radiation MD (P=0.043), NAWM FA (P=0.001), NAWM MD (P=0.004) and white matter tract skeleton FA (P=0.037). The correlation between global cognitive performance and white matter tract skeleton MD was not statistically stronger than that between global cognitive performance and forceps minor FA (P=0.200).

## 6.5 Discussion

This study confirms that in patients with symptomatic carotid artery disease, global cognitive impairment is associated with microscopic damage of non-lesional brain tissues, which includes functionally important brain regions, strategic white matter tracts and widespread white matter tract skeleton.

The first hypothesis, which stated '*Microscopic damage of hippocampus and thalamus is related to global cognitive impairment in patients with symptomatic carotid artery disease*' has been upheld in this study. Increased MD of thalamus and hippocampus was found to be associated with global cognitive impairment. The contribution of thalamic damage severity on global cognitive impairment supports the findings from previous studies showing diffusion changes of thalamus correlated with cognitive performance in CADASIL (O'Sullivan, Singhal et al. 2004) (Molko, Pappata et al. 2001). The diffusional changes of thalamus could be caused by either the direct pathological changes within thalamus or secondary degeneration due to the disruption of white matter tracts connecting from thalamus to other brain regions. The role of anterior thalamic radiation in global cognitive performance revealed by VLSM methods demonstrated in Chapter 5 in turn verifies the role of secondary degeneration of white matter tracts connecting thalamus in the abnormalities of thalamus. Thus, the thalamic-cognition relationship identified in this study points to the fact that disconnection of neural circuits which connected through thalamic radiation contributes to global cognitive impairment in patient with symptomatic carotid artery disease.

The results of increased hippocampal MD contributing to global cognitive impairment is closely in line with that of a study in a group of patients with mild stroke (Kliper, Ben Assayag et al. 2016). Increased MD of hippocampus, a brain region which is considered to be vulnerable to neurodegenerative pathology and conditions, has been found to be a marker of MCI (Muller, Greverus et al. 2005). Our study, in combination with the previous study (Kliper, Ben Assayag et al. 2016), provide the evidence that hippocampal MD is significantly related to VCI. The abnormality of hippocampus and its contribution to global cognitive impairment could be explained from three aspects. Firstly, the increased MD of hippocampus may be induced by the cerebrovascular lesion damage within hippocampus. However, there were no obvious infarctions or chronic subcortical ischaemic lesions showing in our study cohort by visual inspection (Chapter 5). Secondly, the hippocampal abnormality might be explained by the microscopic damage of white matter tracts connecting with hippocampus. Thirdly, given that the neurodegenerative pathology is frequently found in hippocampus and medial temporal lobe (Braak and Braak 1991), the possibility of neurodegenerative pathology leading to hippocampal abnormality and accordingly causing cognitive impairment could not be completely ruled out. The relatively old age of this study cohort would also increase the chance of having co-existing neurodegenerative pathology.

Secondly, this study verified the hypothesis that '*Microscopic damage of strategic white matter tracts is associated with global cognitive impairment in patients with symptomatic carotid artery disease*'. The microscopic damage of forceps minor, forceps major and anterior thalamic radiation, indexed as increased MD, was associated with global cognitive impairment. This significant

association still existed when controlling for age and the total ischaemic lesion volumes within specific white matter tracts. In our study, a modest effect of total ischaemic lesion volumes within specific white matter tracts in global cognitive performance was identified. This result is partly in line with that in a previous study showing cumulative volumes of cerebrovascular lesions including lacunar infarctions and white matter hyperintensities within specific white matter tracts were more predictive for VCI than total volumes of lesions within the brain (Duering, Zieren et al. 2011). The observed microscopic damage of strategic white matter tracts might be explained by secondary degeneration caused by the cerebrovascular lesions. This hypothesis is supported by the finding of a significant association between microscopic damage of strategic white matter tracts and total ischaemic lesion volumes within these white matter tracts. Additionally, this assumption was consistent with the findings from a study highlighting the fact that acute infarctions induced degeneration of connecting white matter tracts (Duering, Righart et al. 2015). Thus, it seems to be justified to conclude that VCI is linked with microscopic damage of strategic white matter tracts which mainly induced by cerebrovascular lesion burden within the specific white matter tracts.

Thirdly, this study demonstrated that microscopic damage of widespread white matter including NAWM and white matter tract skeleton was associated with global cognitive impairment. The finding of association between NAWM MD and global cognitive impairment was in line with that of a study showing microscopic damage of NAWM integrity was strongly associated with cognitive decline in patients with subcortical ischaemic vascular disease (Xu, Zhou et al. 2010). However, in our study, the correlation between microscopic damage of

NAWM and global cognitive performance was not statistically stronger than that between global cognitive performance and total ischaemic lesion volumes (P=0.142), damage severity of infarctions (P=0.161) or damage severity of chronic subcortical ischaemic lesions (P=0.192). Thus, microscopic damage of NAWM might not be a better index than volume measurement to predict global cognitive impairment in vascular cognitive impairment.

Widespread white matter tract microscopic damage, indexed as increased MD, was found to be strongly related to global cognitive impairment in patients with symptomatic carotid artery disease. The observed widespread white matter microscopic damage could be a summation of lesional and non-lesional damage. This finding was well agreed with a previous study showing damage of all white matter was associated with cognition in patients with pure subcortical VaD (Kim, Park et al. 2011) (Kim, Kwon et al. 2015). The identified pattern of white matter microscopic damage was different from that in ageing showing an anteroposterior gradient of damage (Sullivan and Pfefferbaum 2006) (Voineskos, Rajji et al. 2012) and that in AD showing as microscopic damage of white matter within in frontal and temporal brain regions (Kim, Kwon et al. 2015). The different pattern of microscopic damage between VCI and AD is also partly supported by the evidence from a previous study using graph theoretical analysis to investigate the effect of amyloid pathology and SVD on white matter network disruption (Kim, Im et al. 2015). In their study, they have demonstrated that the disruption of white matter network in patients with SVD was characterised as 'regular' topology showing as increased network segregation but decreased network integration. In contrast, the disruption of white matter network in

patients with amyloid pathology showed a ‘random’ topology which was indicated by increased network integration but decreased network segregation.

The correlation between white matter tract skeleton MD and global cognitive impairment was found to be statistically stronger than that between NAWM MD, strategic white matter tracts MD and global cognitive impairment. Additionally, this correlation was also statistically stronger than that between global cognitive performance and total ischaemic lesion volumes (P=0.002), infarction damage severity (P=0.002) and chronic subcortical ischaemic lesion severity (P=0.003).Therefore, it is reasonable to summarise that white matter tract skeleton microscopic integrity is a better index to show VCI.

Several limitations need to be considered. A short DTI sequence with low diffusion direction was used in this study. However, the TBSS analyses were conducted for both FA and MD maps and the results of white matter disruption on both FA and MD maps were similar. The MD values were chosen to reflect microscopic damage of brain tissue as it is reliable and sensitive measure of white matter microstructural damage after stroke and it is less affected by the directionality of different white matter tracts (Duering, Righart et al. 2015). Therefore, the main findings in this study are unlikely to be confounded by the low diffusion direction in the DTI sequence.

### 6.6 Conclusions

Microscopic damage of non-lesional brain tissues is related to global cognitive impairment in patients with symptomatic carotid artery disease, suggesting that

white matter disconnection plays a role in VCI. White matter tract skeleton MD has the potential to be used as an imaging biomarker for VCI.

# Chapter 7 Functional disconnection of large-scale cognitive networks in patients with symptomatic carotid artery disease

## Abstract

The main hypothesis of this chapter is that functional disconnection of large-scale cognitive networks contributes to global cognitive impairment in patients with symptomatic carotid artery disease. A secondary hypothesis is that functional disconnection of large-scale cognitive networks mediates the associations between structural disconnection and global cognitive impairment. Independent component analysis (ICA) approach and seed-based analysis approach were used to respectively investigate functional connectivity within large-scale cognitive networks and that between a seed region with the rest of the brain. Functional connectivity metrics were compared between patients with normal and abnormal cognition, and correlated with global cognitive performance. Mediation analysis was used to investigate the association between functional connectivity, structural connectivity and global cognitive performance. Patients with abnormal cognition showed decreased functional connectivity within the default mode network, dorsal attention network, left fronto-parietal network and salience network. Also, decreased functional connectivity of bilateral hippocampi, dorsal lateral prefrontal cortex, ACC and PCC was found in patients with abnormal cognition. Additionally, large-scalr cognitive network functional connectivity mediated the association between structural connectivity and global cognitive performance.

## 7.1 Introduction

The preceding chapters examined lesional and non-lesional damage and their contributions on global cognitive impairment in patients with symptomatic carotid artery disease. All these results point to the role of structural disconnection in VCI. The current chapter attempts to explore the associations between functional disconnection of large-scale cognitive networks and global cognitive impairment in patients with symptomatic carotid artery disease. Additionally, this chapter will investigate what drives the cognitive-related functional disconnection of large-scale cognitive networks.

Localised ischaemic lesions have been found to result in alterations of functional networks and the alterations of functional connectivity extends beyond lesion areas (Ovadia-Caro, Villringer et al. 2013) (Rehme and Grefkes 2013). A focal lesion within a certain brain area may affect a specific functional network (Wang, Qin et al. 2014). Given that functional networks are connected with each other to maintain brain function, it is plausible that functional connectivity of brain networks without the presence of brain lesions within them may also be affected. This hypothesis has been verified by a study showing the functional connectivity of brain networks without a brain lesion was impaired, even though these brain networks are less affected than those containing brain lesions (Ovadia-Caro, Villringer et al. 2013).

The human brain is a complex network including structurally and functionally interacting regions. Functional connectivity, which describes as the relationship between the neuronal activation patterns of anatomically distinct brain regions, is found to be closely related to cognitive function (van den Heuvel and Pol 2010). Functional connectivity is usually computed from low-frequency (<0.1

Hz) resting-state fMRI data (Rehme and Grefkes 2013). Multiple resting-state networks (RSNs), which consist of brain regions that anatomically separated but showing a high level of functional connectivity, have been reported by resting-state fMRI studies (Beckmann, DeLuca et al. 2005). Up until now, resting-state networks including the default mode network (DMN), the dorsal attention network (DAN), the salience network, the executive control network, the visual network, the motor network and the left and right fronto-parietal networks, have been identified in neuroimaging studies. Among these eight networks, the DMN, the DAN, the two lateralised fronto-parietal networks, the executive control network and the salience network are found to be related to cognitive process (Beckmann, DeLuca et al. 2005) (Salvador, Suckling et al. 2005) (Damoiseaux et al., 2006) (De Luca, Beckmann et al. 2006) (van den Heuvel and Pol 2010).

The most fundamental resting-state network is the DMN which reflects a default state of neuronal activities of the human brain and is supposed to play a key role in a variety of cognitive functions. The core regions associated with the DMN are the ventral medial prefrontal cortex, posterior cingulate cortex, inferior parietal lobule, lateral temporal cortex, dorsal medial prefrontal cortex and hippocampal formation (Buckner, Andrews-Hanna et al. 2008). Empirical evidence has been provided to show that the strength of DMN functional connectivity decreases in normal ageing and neurodegenerative diseases including MCI and AD (Broyd, Demanuele et al. 2009). Additionally, decreased DMN functional connectivity has also been found in patients with vascular pathology, such as TIA (Li, Wang et al. 2013), ischaemic stroke (Tuladhar, Snaphaan et al. 2013), VCI no dementia (Sun, Qin et al. 2011) and subcortical VaMCI (Li, Wang et al. 2012). Therefore it is plausible that decreased functional

connectivity of DMN may be directly linked to cognitive deficits regardless of the underlying pathology (Siegel, Ramsey et al. 2016).

The dorsal attention network (DAN), which is involved in the goal-directed process and selection for stimuli and response, comprises the frontal eye fields and the intraparietal sulcus of each hemisphere (Vossel, Geng et al. 2014). A previous study has found that decreased DAN functional connectivity exists in MCI and AD and the decreased pattern correlated with disease progression (Zhang, Zheng et al. 2015). So far, no studies have provided the evidence of decreased functional connectivity of DAN contributing to cognitive impairment in patients with vascular pathology. Thus, it is not clear whether there is a specific pattern of DAN functional disconnection in patients with vascular pathology.

Brain areas showing functional connectivity within the executive control network include middle and superior prefrontal cortices, anterior cingulate cortex, paracingulate gyri, ventrolateral prefrontal cortex and thalamus (Beckmann, DeLuca et al. 2005). The executive control network is thought to be involved in guidance of thought and behaviour (Krmpotich, Tregellas et al. 2013). Executive function has been found to relate to lesions located in the frontal area and those disrupting the frontal basal ganglia-thalamocortical circuits (Royall, Lauterbach et al. 2002). The abnormality of functional connectivity within the executive control network has been found in AD while no study has identified the disconnection within this network in VCI (Agosta, Pievani et al. 2012).

The fronto-parietal networks show primarily lateralised activations. For example, the left fronto-parietal network includes the left lateral occipital complex, the left inferior parietal cortex, the left middle and superior frontal gyri and bilateral intraparietal sulcus. The complementary pattern of activations is seen in the right hemisphere (Beckmann, DeLuca et al. 2005). The fronto-parietal network is involved in category-based, object-based, feature-based and space-based attention selection (Scolari et al., 2015). It is still not clear about the role of functional connectivity within the fronto-parietal networks in cognition in patients with neurodegenerative pathology or vascular pathology.

Independent component analysis (ICA) approach is widely used to investigate the functional connectivity of large-scale cognitive networks in healthy subjects, normal ageing and patients with neurological diseases such as neurodegenerative diseases and cerebrovascular diseases (Beckmann, DeLuca et al. 2005) (Lee, Smyser et al. 2013). Seed-based analysis is another approach to investigate the functional connectivity of resting-state networks. It has been found that large-scale cognitive networks maps derived by ICA approach are similar with those derived by seed-based analysis method. However, it has been demonstrated that functional connectivity measurements derived by seed-based analysis approach are the sum of ICA-derived within large-scale cognitive network connectivity and between network connectivity (Joel, Caffo et al. 2011). Thus, we can get a more comprehensive understanding of the role of functional disconnection in VCI by using both ICA and seed-based analysis approach.

The hippocampus is one of the earliest brain regions affected by the accumulation of neurofibrillary tangles and thus is especially vulnerable to damage in AD (Mu and Gage 2011). An extensive review of the neuroimaging

literature concluded that hippocampal atrophy and microstructural damage reliably discriminated between AD patients and healthy subjects (Fellgiebel and Yakushev 2011). More importantly, hippocampal volume loss was correlated with cognitive decline in MCI and early AD, suggesting that it may be a predictor of conversion from MCI to AD (Allen, Barnard et al. 2007). Alteration of hippocampal functional connectivity and its correlation with cognitive decline were found in normal ageing, MCI and AD, meaning that the cognitive decline in these conditions may be explained by a perspective of hippocampal functional network damage (Wang, Zang et al. 2006, Cho, Yang et al. 2008, Clerx, Visser et al. 2012, Rowley, Fonov et al. 2013). Additionally, given that the hippocampus is a part of DMN (Buckner, Andrews-Hanna et al. 2008), the functional disconnection of hippocampus with the rest of brain could also reflect the decreased DMN functional connectivity.

The hippocampus has been considered as a key neuroanatomical structure of post-stroke cognitive impairment (Grysiewicz and Gorelick 2012). Results of studies investigating the relationship between hippocampal atrophy and cognitive dysfunction after ischemic stroke remains conflicting, meaning that hippocampal atrophy may not be an appropriate predictor of VCI (Sachdev, Chen et al. 2007). Based on work presented in Chapter 6, hippocampal microscopic damage existed in cognitively impaired patients with symptomatic carotid artery disease and hippocampal pathology indexed by increased hippocampal MD were independently associated with cognitive decline, showing that hippocampal pathology contributed to cognitive impairment in patients with advanced atherosclerosis. A recent study has shown inter-hemispheric hippocampi functional connectivity was related to global cognitive

impairment in patients with mild stroke (Kliper, Ben Assayag et al. 2016). However, there has been no study to investigate the functional connectivity of hippocampus with the rest of the brain and its contribution on global cognitive impairment in patients with VCI.

The dorsolateral prefrontal cortex (DLPFC) has been demonstrated as an important region in executive function and working memory (Curtis and D'Esposito 2003), attention (Fan, McCandliss et al. 2005), episodic memory (Murray and Ranganath 2007), decision making (Heekeren, Marrett et al. 2006) and reasoning (Goel and Dolan 2004). Abnormal activations and functional disconnection involving DLPFC are found in MCI and AD (Qi, Wu et al. 2010) (Liang, Wang et al. 2011) (Saykin, Wishart et al. 2004). However, the contribution of functional disconnection of DLPFC on cognition has not been well studied in patients with vascular pathology.

The posterior cingulate cortex (PCC) is a highly anatomically connected brain area (Hagmann, Cammoun et al. 2008). PCC shows a complex pattern of functional connectivity. For example, it is functionally connected with brain areas within the left and right fronto-parietal networks, the DAN, the salience network and the sensorimotor network (Leech and Sharp 2014). However, PCC is considered as a critical node in the DMN in general (Buckner, Andrews-Hanna et al. 2008). The abnormalities of PCC and the DMN are found in patients with a wide range of neurological and psychiatric diseases (Buckner, Andrews-Hanna et al. 2008). The functional connectivity between PCC and the rest of the brain regions within the DMN reduces in normal ageing and the decreased functional connectivity is related with cognitive impairment (Andrews-Hanna, Snyder et al. 2007). Empirical evidence are provided to demonstrate the abnormality of PCC

functional connectivity in neurodegenerative disease. The functional connectivity between PCC and hippocampus was particularly affected in AD (Greicius, Srivastava et al. 2004). This could be caused by the structural damage in PCC or hippocampus in AD. Several studies found that abnormality of PCC functional connectivity existed in patients with VCI in whom have no clear evidence of PCC structural damage (Leech and Sharp 2014). However, the mechanism of PCC functional disconnection contributing to cognitive deficits in VCI is still not clear.

### 7.2 Aims and hypotheses

Hypotheses:

- Functional disconnection of large-scale brain cognitive networks is associated with global cognitive impairment in patients with symptomatic carotid artery disease
- Functional disconnection of large-scale cognitive networks mediates the association between structural disconnection and global cognitive performance

Aims:

- Apply ICA and seed-based analysis approach on resting-state fMRI to investigate the functional disconnection of large-scale cognitive networks in patients with symptomatic carotid artery disease
- Investigate the role of functional disconnection of large-scale cognitive networks in global cognitive impairment
- Investigate the correlation between functional disconnection and microscopic white matter damage using regression analysis

## 7.3 Methods

### 7.3.1 Subjects and assessments

The recruitment, clinical and cognitive assessment, MRI protocol of subjects included in the main study cohort (n=108) were described in Chapter 3. Totally 72 out of 108 subjects (66.6%) were included in the analyses of this chapter.

### 7.3.2 Resting-state fMRI images quality assurance

An in-house pipeline created by Dr. Yue Xing and Thomas Welton was followed to examine the quality of resting-state fMRI data. This pipeline of quality assurance was recommended by a previous report of multicentre fMRI quality assurance protocol (Friedman and Glover 2006). The quality assurance was undergone on **pnjpuck** server. The outputs which were related to quality assurance of resting-state fMRI datasets include: (1) a graph of frequency spectrum of the resting-state fMRI dataset (Figure 7. 1). Resting-state fMRI focuses on low frequency oscillations (<0.1 Hz) in the BOLD signals (Biswal, Yetkin et al. 1995). The frequency spectrum of BOLD signal fluctuations allows to assess the frequency distribution of the resting-state fMRI dataset and find out the contribution of noise such as respiratory and cardiac oscillations. (2) a graph of the mean time course of the resting-state fMRI dataset (Figure 7. 2). The X-axis is the volume number and the Y-axis is the image intensity. (3) a static spatial noise image (Figure 7. 3). The first step to obtain this static spatial noise image is to separately sum all odd-numbered images and all even-numbered images. Then, the DIFF images, showing as the difference between the odd-numbered images and the even-numbered images, are taken as a raw measure of static spatial noise. The DIFF image will not display any brain structure if the

images exhibit no drift in amplitude or geometry (Friedman and Glover 2006) (Figure 7. 3).

The quality assurance pipeline was used in raw resting-state fMRI datasets. Then the raw resting-state fMRI datasets were fed into MELODIC (multivariate exploratory linear optimized decomposition into independent components) (Beckmann and Smith 2004) for pre-processing. The pre-processing steps in MELODIC include motion correction, slice timing correction, brain extraction, spatial smoothing and highpass temporal filtering. The raw resting-state fMRI datasets were decomposed into different spatial and temporal components using independent component analysis in MELODIC. All the components of each fMRI datasets were visually inspected by following a published procedure for artefact removal from fMRI data (Kelly, Alexopoulos et al. 2010). Components classified as noise were deleted using the command below (http://fsl.fmrib.ox.ac.uk/fslcourse/lectures/practicals/melodic/):

*fsl_regfilt -i filtered_func_data.nii.gz –d filtered_func_data.ica/melodic_mix -f 1,2,3 -o filtered_func_data_clean.nii.gz*

All resting-state fMRI analyses were conducted using the 'clean (denoised)' fMRI datasets.

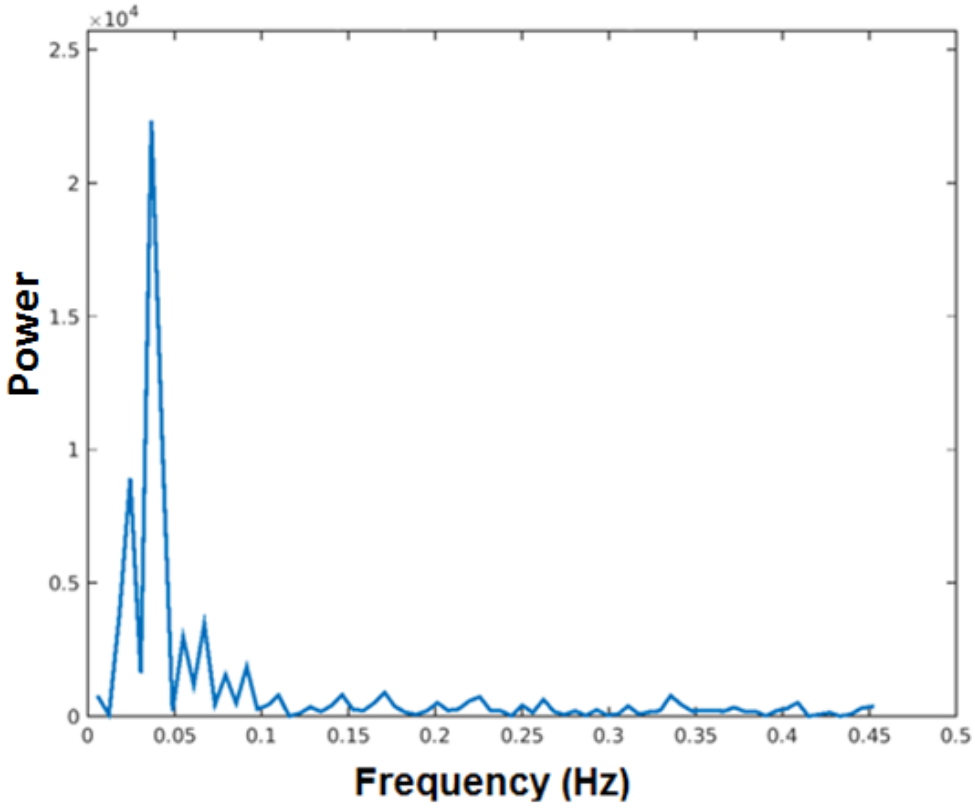



**Figure 7. 1 An example of the graph of frequency spectrum of an ICAD subject**

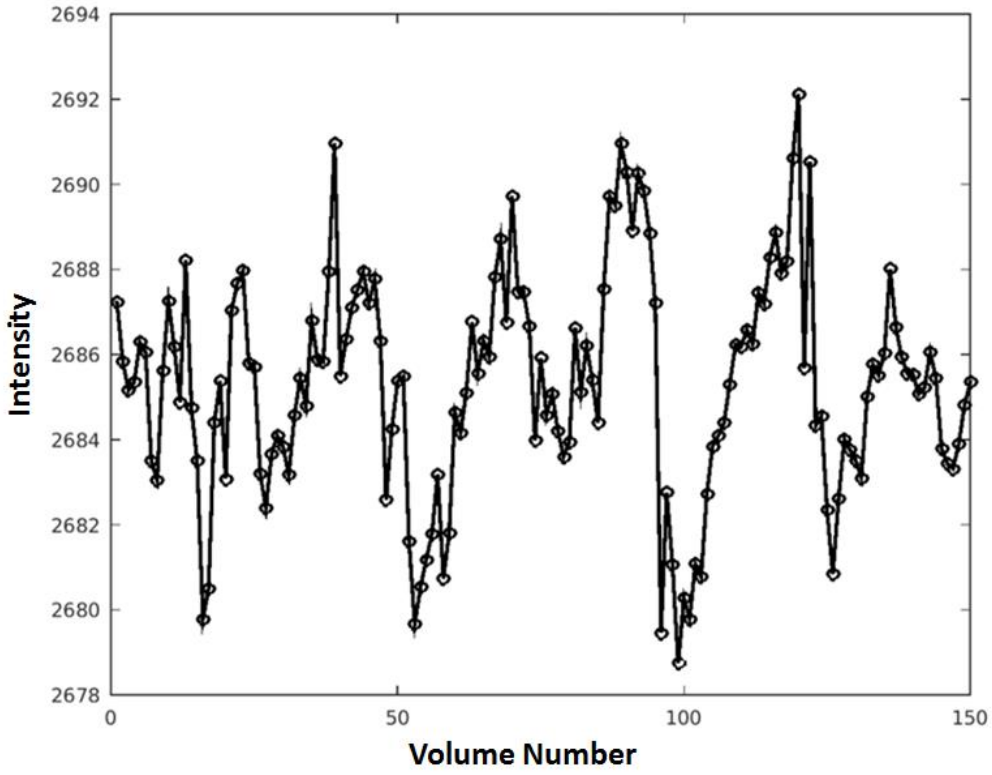



**Figure 7. 2 An example of the graph of mean timecourse of an ICAD subject**

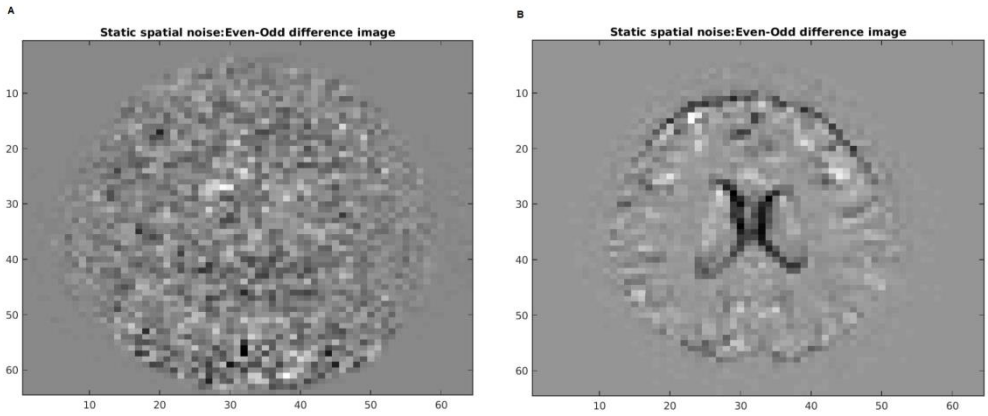



**Figure 7. 3 An example of the static spatial noise image of two ICAD subjects. (A) This ICAD subject's fMRI images in the time-series exhibit no drift in amplitude or geometry. (B) This ICAD subject's fMRI images in the time-series exhibit drift in amplitude or geometry as the static spatial noise image displays brain structure, which needed to be excluded for analysis.**

### 7.3.3 Independent component analysis (ICA)

'Clean' resting-state fMRI datasets were used for ICA analysis. Multi-session temporal concatenation probabilistic ICA implemented in MELODIC in FMRIB's Software Library (FSL) (Beckmann and Smith 2004) and followed by a dual regression approach was used to identify the spatial distribution of distinct resting-state networks and their difference between patients with normal and abnormal cognition (Damoiseaux, Rombouts et al. 2006, Smith, Fox et al. 2009, van den Heuvel and Hulshoff Pol 2010). The pre-processed fMRI data (details in Chapter 3) were registered to the standard stereotaxic anatomical Montreal Neurological Institute (MNI) 152 space using linear registration with a warp resolution of 2mm. Subsequently the fMRI data was decomposed into a set of independent components, with a free estimation for the number of components.

Components of interest were visually selected and compared with a recent published review paper (Barkhof, Haller et al. 2014).

Afterwards, a voxel-wise comparison of the resting-state networks was carried out using dual regression approach. The multi-session temporal concatenation ICA spatial maps were used in a linear model fit against each individual fMRI data, resulting in matrices that described the temporal dynamics for each component and subject separately. Then these time course matrices were used to estimate subject-specific spatial maps. After that, the difference of functional connectivity of identified networks between patients with normal and abnormal cognition was investigated, treating age as a covariate of no interest. Additionally, the correlation between the strength of RSNs functional connectivity and global cognitive performance was also investigated after controlling for age. The significant threshold was set at $P<0.05$ and family-wise error (FWE) correction for multiple comparisons was performed. The average Z score of each component of each subject was extracted as well.

### 7.3.4 Seed-based analysis

Seed-based analysis was conducted in order to investigate the functional disconnection beyond the large-scale cognitive networks. Analyses were conducted in the 'clean' resting-state fMRI datasets which went through the quality assurance (details in Chapter 3).

Seed-based analysis was carried out using FEAT (FMRI Expert Analysis Tool 6.0) in FSL (Jezzard, Matthews et al. 2001). The seed-based functional connectivity analysis was performed in order to investigate the correlation between activity in a region-of-interest (ROI) and that in the rest of brain

(Margulies, Bottger et al. 2010). Brain regions that showing decreased functional connectivity in patients with cognitive decline such as MCI and AD as demonstrated in previous studies were chosen for seed-based analyses. Totally six regions were selected for seed-based functional connectivity analysis. Square ROIs were separately drawn on three consecutive slices within bilateral hippocampus (Figure 7. 4), bilateral dorsal lateral prefrontal cortex (DLPFC) (Figure 7. 5), posterior cingulate cortex (PCC) (Figure 7. 6) and anterior cingulate cortex (ACC) (Figure 7. 7). Afterwards, the mean signal intensity of each ROI was calculated by averaging the time series of all voxels in the ROIs. In addition, several sources of spurious variances, such as signal averaged from the centre deep white matter, signal averaged over the ventricles, and six parameters obtained by rigid body head motion correction, were removed from the signal time-course of each voxel using the general linear model.

Higher-level analysis was carried out using FLAME (FMRIB's Local Analysis of Mixed Effects) (Jezzard, Matthews et al. 2001) in FSL. The functional connectivity of each ROI was compared between patients with normal and abnormal cognition. In addition, the strength of functional connectivity between each seed region and the rest of the brain was correlated with global cognitive performance. Mean relative displacement which is the net amount of motion between consecutive functional volumes and age were treated as further covariates of no interest in all higher-level analyses (Hlinka, Alexakis et al. 2010). *Z* (Gaussianised T/F) statistical images was estimated by clusters firstly determined by $Z>2.3$ and a corrected cluster significance threshold of $P<0.05$. If no significant clusters were identified, then clusters were determined by $Z>1.96$ and a corrected cluster significant threshold of $P<0.05$ (Jezzard, Matthews et al.

2001). Z score of functional connectivity of each seed was extracted from each subject.

An online database, Neurosynth (www.neurosynth.org) (Yarkoni, Poldrack et al. 2011), was used to perform an automated term-based meta-analysis of fMRI studies of navigation, revealing common activation across these fMRI studies in seed regions. The maps of functional activation of these seeds obtained from Neurosynth were visually compared with those obtained from this study.

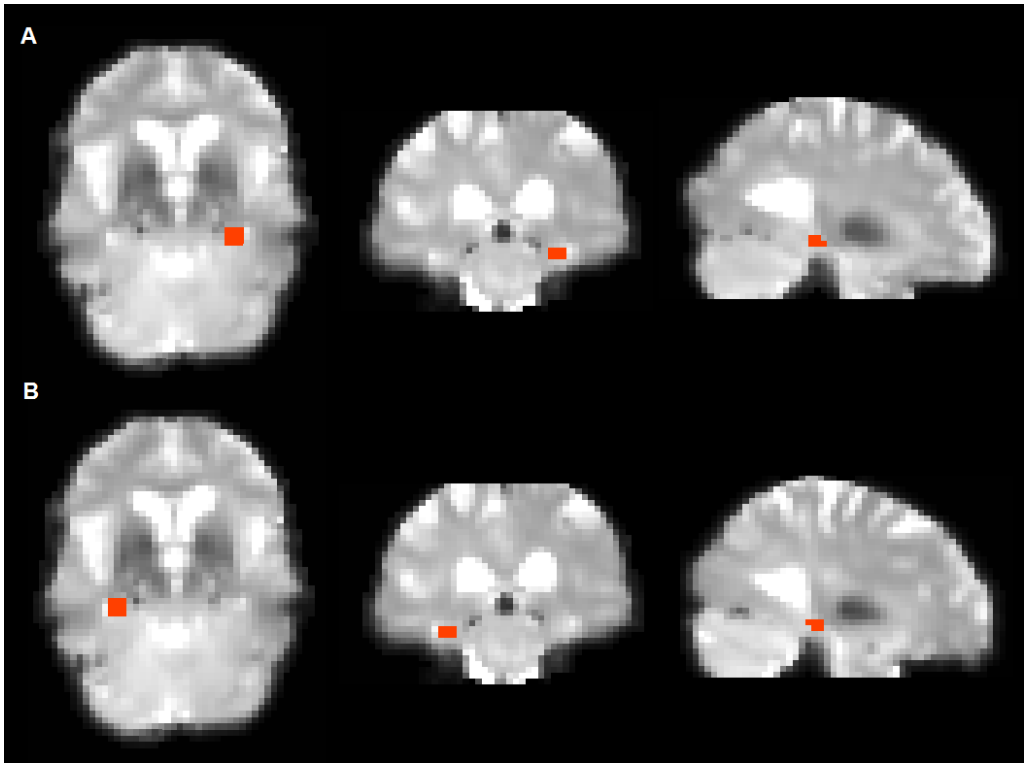



**Figure 7. 4 An example of (A) left hippocampal seeds and (B) right hippocampal seeds.**

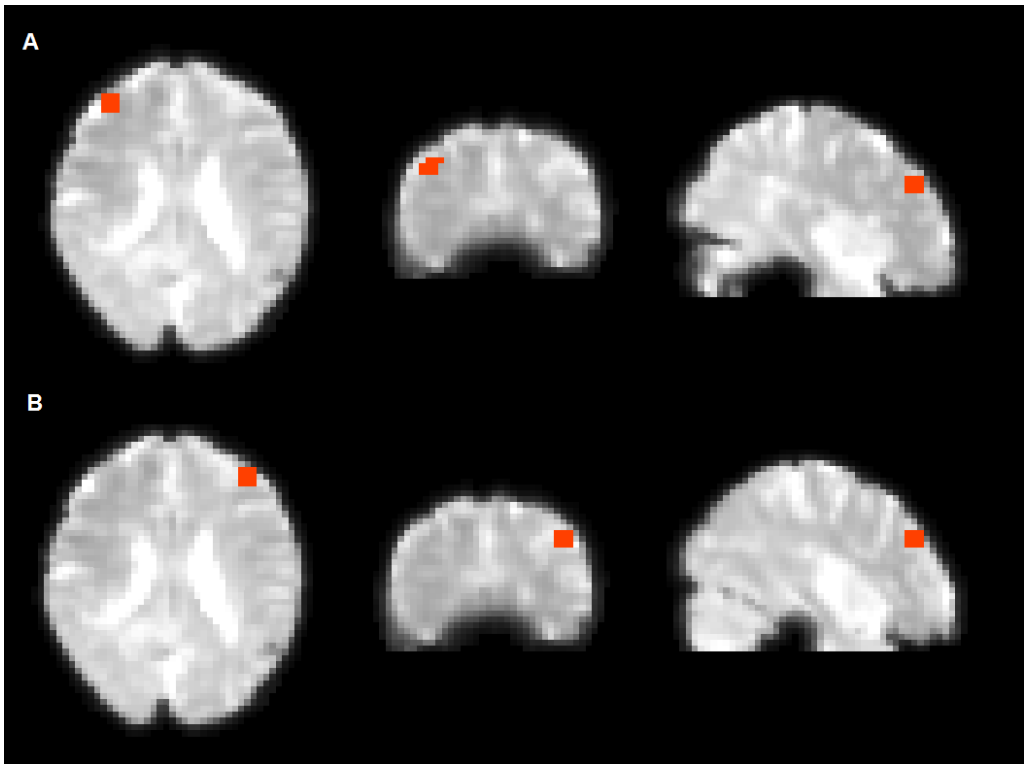



**Figure 7. 5 An example of the seeds of (A) right dorsal lateral prefrontal cortex and (B) left dorsal lateral prefrontal cortex.**

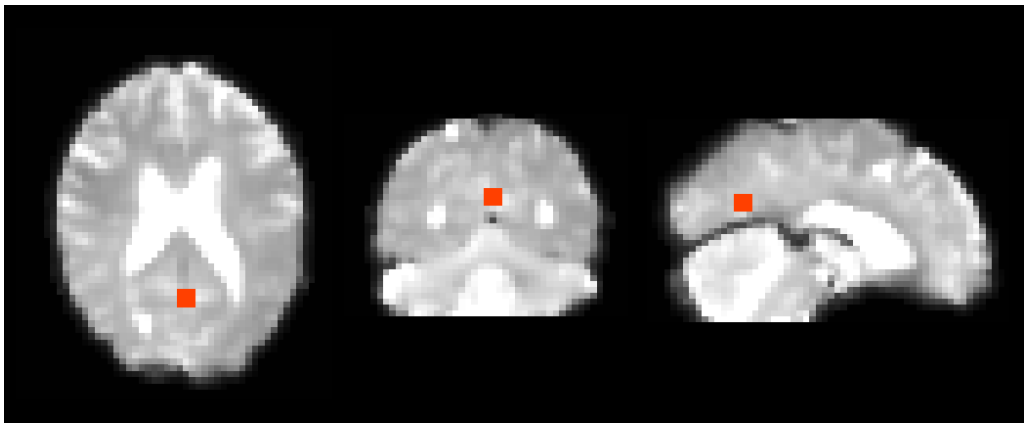


**Figure 7. 6 An example of the seeds of posterior cingulate cortex.**

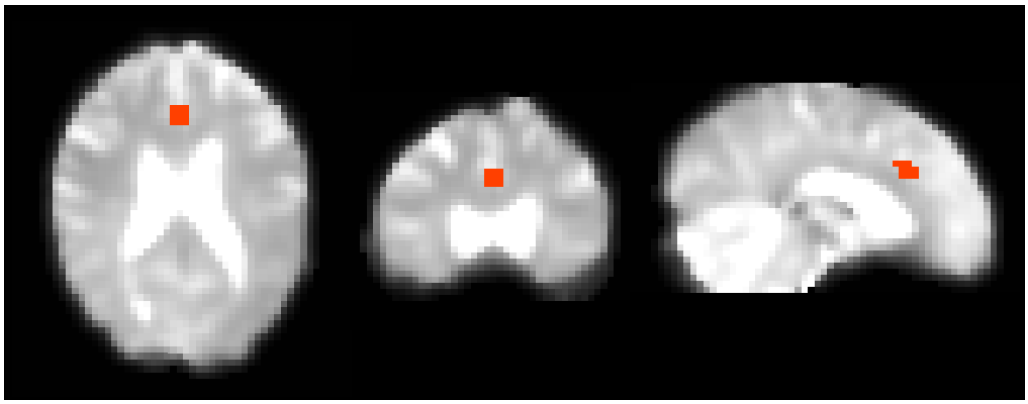


**Figure 7. 7 An example of the seeds of dorsal anterior cingulate cortex.**

### 7.3.5 Microscopic damage of white matter tracts

The approach to extract MD values from strategic white matter tracts was introduced in Chapter 6.

### 7.3.6 Statistical analysis

In order to investigate the correlation between RSNs functional disconnection and underlying structural disconnection, Z scores of brain regions showing significant difference of RSNs functional connectivity identified by using ICA approach were correlated with microscopic damage of white matter tracts underlying or connecting to these regions. Regression analysis in SPSS version 21 was used for correlation analysis. The interrelationship between functional disconnection and microscopic structural damage was investigated using regression analysis. All regression analysis was conducted in SPSS version 21 (Chicago, IL, USA).

The PROCESS marco for SPSS was used to conduct the mediation analysis in order to investigate whether functional disconnection of RSNs mediate the effect of microscopic damage of white matter tracts on cognition. The significance of

indirect effect is tested by bootstrapping with 1000 replications. The percentage of the mediator accounting for the total effect was reported.

### 7.4 Results

The resting-state fMRI sequence had not been added into the ICAD MRI protocol until February 2012. Therefore, subjects recruited before February 2012 did not have resting-fMRI scans. Among subjects who were recruited February 2012 afterwards (115 subjects), 25 (21.7%) of them did not have the resting-state fMRI scan. 18 out of 90 subjects (20%) were needed to be excluded due to poor fMRI data quality. Thus, totally 72 subjects had resting-fMRI scan with good quality and were included in the analysis of this Chapter.

In the cohort including 72 subjects who completed resting-state fMRI scan, 31 subjects (43.1%) had global cognitive decline, 12 subjects (16.7%) had impaired performance of attention & orientation, 31 subjects (43.1%) had impaired memory performance, 25 subjects (34.7%) had impaired of fluency, 22 subjects (30.6%) had visuospatial impairment and 10 subjects (13.9%) had impaired language performance (Figure 7. 8). The cognitive profile of the cohort including 72 subjects is illustrated in Table 7. 1.

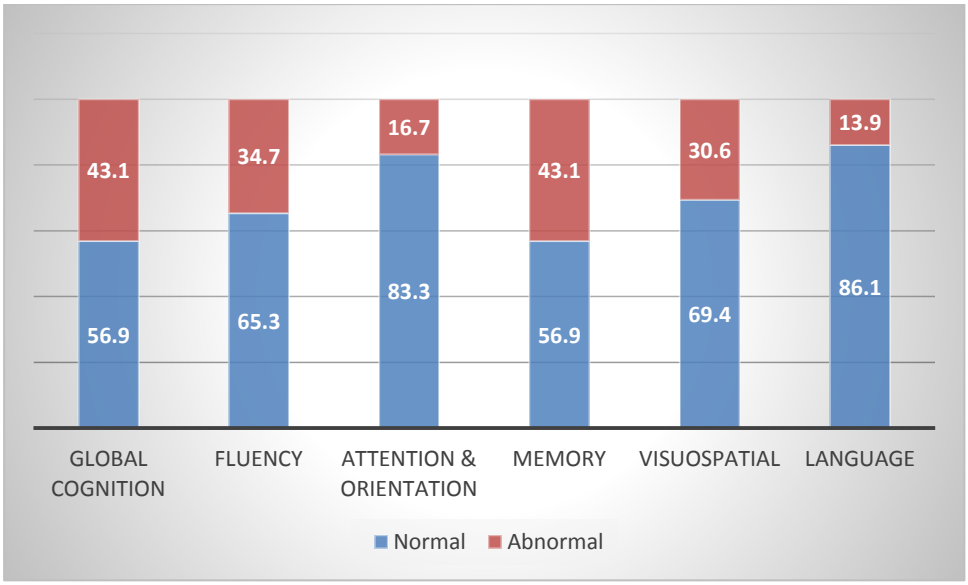



**Figure 7. 8 The percentage of subjects with normal and abnormal performance in global cognition and each cognitive domain**

**Table 7. 1 Cognitive profile of the cohort including 72 subjects**

| | Minimum | Maximum | Mean ± SD |
|---|---|---|---|
| ACE-R | 52 | 98 | 82.4 ± 9.9 |
| Memory | 7 | 26 | 18.1 ± 5.1 |
| Attention & orientation | 8 | 18 | 16.9 ± 1.8 |
| Fluency | 5 | 14 | 9.6 ± 2.4 |
| Visuospatial | 9 | 16 | 14.5 ± 1.5 |
| Language | 17 | 26 | 23.5 ± 2.3 |

In the study sub-cohort including 72 subjects, patients with abnormal cognition had more subjects who ever smoker before compared with patients with normal cognition (P=0.001) (Table 7. 2). All the cognitively impaired subjects in this study cohort were considered as having probable VaMCI.

**Table 7. 2 Comparison of demographic characteristics of between patients with normal and abnormal cognition in the study cohort including 72 subjects who completed the resting-state fMRI scan**

| Characteristics | The whole study cohort<br>n=72 | Normal cognition<br>n=41 | Abnormal cognition<br>n=31 | P value [a] |
|---|---|---|---|---|
| **Age, mean (SD) (years)** | 74.2 (10.1) | 72.3 (10.6) | 76.7 (9.1) | 0.062 |
| **Female, n (%)** | 27 (37.5) | 13 (31.7) | 14 (45.2) | 0.243 |
| **Atrial fibrillation, n (%)** | 16 (22.2) | 8 (19.5) | 8 (25.8) | 0.576 |
| **Peripheral vascular disease, n (%)** | 12 (16.7) | 4 (9.8) | 8 (25.8) | 0.109 |
| **Ischemic heart disease, n (%)** | 21 (29.2) | 10 (24.4) | 11 (35.5) | 0.433 |
| **Hypertension, n (%)** | 57 (79.2) | 31 (75.6) | 26 (83.9) | 0.559 |
| **Smoking, n (%)** | 45 (62.5) | 19 (46.3) | 26 (83.9) | 0.001* |
| **Diabetes mellitus, n (%)** | 17 (23.6) | 8 (19.5) | 9 (29.0) | 0.407 |
| **Symptomatic type (Stroke), n (%)** | 36 (50.0) | 20 (48.8) | 16 (51.6) | 0.812 |
| **Symptomatic side (Left), n (%)** | 31 (43.1) | 19 (46.3) | 12 (38.7) | 0.632 |

| | | | | |
|---|---|---|---|---|
| **Ipsilateral degree of carotid stenosis, n (%)** | | | | 0.688 |
| **30-49%** | 37 (51.4) | 20 (46.3) | 17 (54.8) | |
| **50-69%** | 23 (31.9) | 13 (31.7) | 10 (32.3) | |
| **>70%** | 12 (16.7) | 8 (19.5) | 4 (12.9) | |
| **Contralateral degree of carotid stenosis, n (%)** | | | | 0.265 |
| **0-29%** | 51 (70.8) | 30 (73.2) | 21 (67.7) | |
| **30-49%** | 5 (6.9) | 4 (9.8) | 1 (3.2) | |
| **50-69%** | 11 (15.3) | 6 (14.6) | 5 (16.1) | |
| **>70%** | 5 (6.9) | 1 (2.4) | 4 (12.9) | |
| **Time from symptom to MRI, mean (SD) (days)** | 37.2 (36.2) | 34.8 (32.1) | 40.4 (41.3) | 0.533 |

a: Comparison between patients with normal and abnormal cognition

*: Statistical significant at the level of P<0.05

MTA was significantly correlated with global cognitive performance in the study sub-cohort including 72 subjects ($r=-0.497$, $P<0.001$).

### 7.4.1 ICA networks

Using probabilistic ICA with temporal concatenation, a set of 17 independent components was obtained using the Laplace approximation to the Bayesian evidence of the model order (Minka 2001) (Beckmann and Smith 2004). 6 resting-state functional networks including default mode network (DMN), dorsal attention network (DAN), left fronto-parietal network, right fronto-parietal network, executive control network and salience network that related to cognition were identified (Figure 7. 9). Brain activities in the DMN included PCC and adjacent precuneous, bilateral medial temporal cortex, bilateral medial prefrontal cortex and bilateral inferior parietal cortex. Brain activities in the DAN encompassed the frontal eye fields and intraparietal sulcus in each hemisphere, anterior and posterior cingulate cortex and adjacent precuneous. Left fronto-parietal network showed brain activities in bilateral intraparietal sulcus, left lateral occipital complex, left inferior parietal cortex, left middle and superior frontral gyri, and bilateral inferior and middle temporal gyri. The complementary pattern of brain activities was seen in the right hemisphere in the right fronto-parietal network. Areas of brain activities in the executive control network included ventrolateral prefrontal cortex, bilateral middle and superior prefrontal cortices and anterior cingulate cortex. Brain activities in the salience network encompassed the anterior cingulate cortex and bilateral insula.

Four major resting-state networks showed significant decreased functional connectivity in patients with abnormal cognition after controlling for age. More specifically, in the probable VaMCI group, DMN connectivity was significantly reduced in the posterior cingulate cortex (PCC), precuneous and left inferior parietal gyrus. The decreased functional connectivity within PCC, precuneous and left inferior parietal lobule was significantly correlated with global cognitive performance ($r=0.485$, $P<0.001$, Figure 7. 10). Patients with probable VaMCI also demonstrated decreased functional connectivity of the DAN, with significant clusters located in the PCC. DAN functional disconnection within the PCC was also moderately associated with global cognitive performance ($r=0.387$, $P=0.001$, Figure 7. 11). Probable VaMCI subjects showed decreased functional connectivity in the left fronto-parietal network with a significant cluster in the anterior cingulate cortex (ACC).The functional disconnection within left fronto-parietal network was moderately correlated with global cognitive performance ($r=0.492$, $P<0.001$, Figure 7. 12). Lastly, patients with probable VaMCI had decreased functional connectivity of the salience network, with significant clusters located in the left middle frontal gyrus and this functional disconnection is correlated with global cognitive performance ($r=0.406$, $P<0.001$, Figure 7. 13).

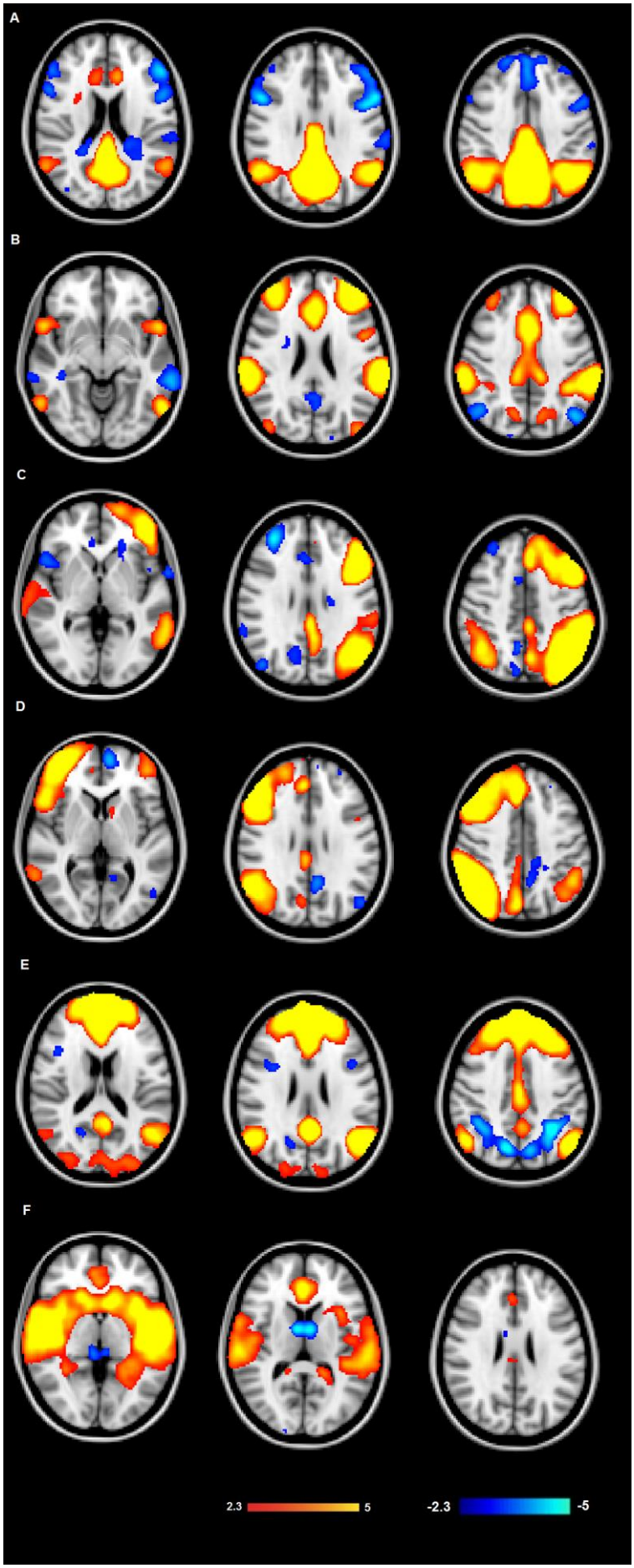

A
B
C
D
E
F
2.3
5
-2.3
-5

**Figure 7. 9 Resting state networks in the fMRI sub-cohort. (A) Default mode network (B) Dorsal attention network (C) Left fronto-parietal network (D) Right fronto-parietal network (E) Executive control network (F) Salience network. Red to Yellow: Positive correlations. Blue to light blue: Negative correlations.**

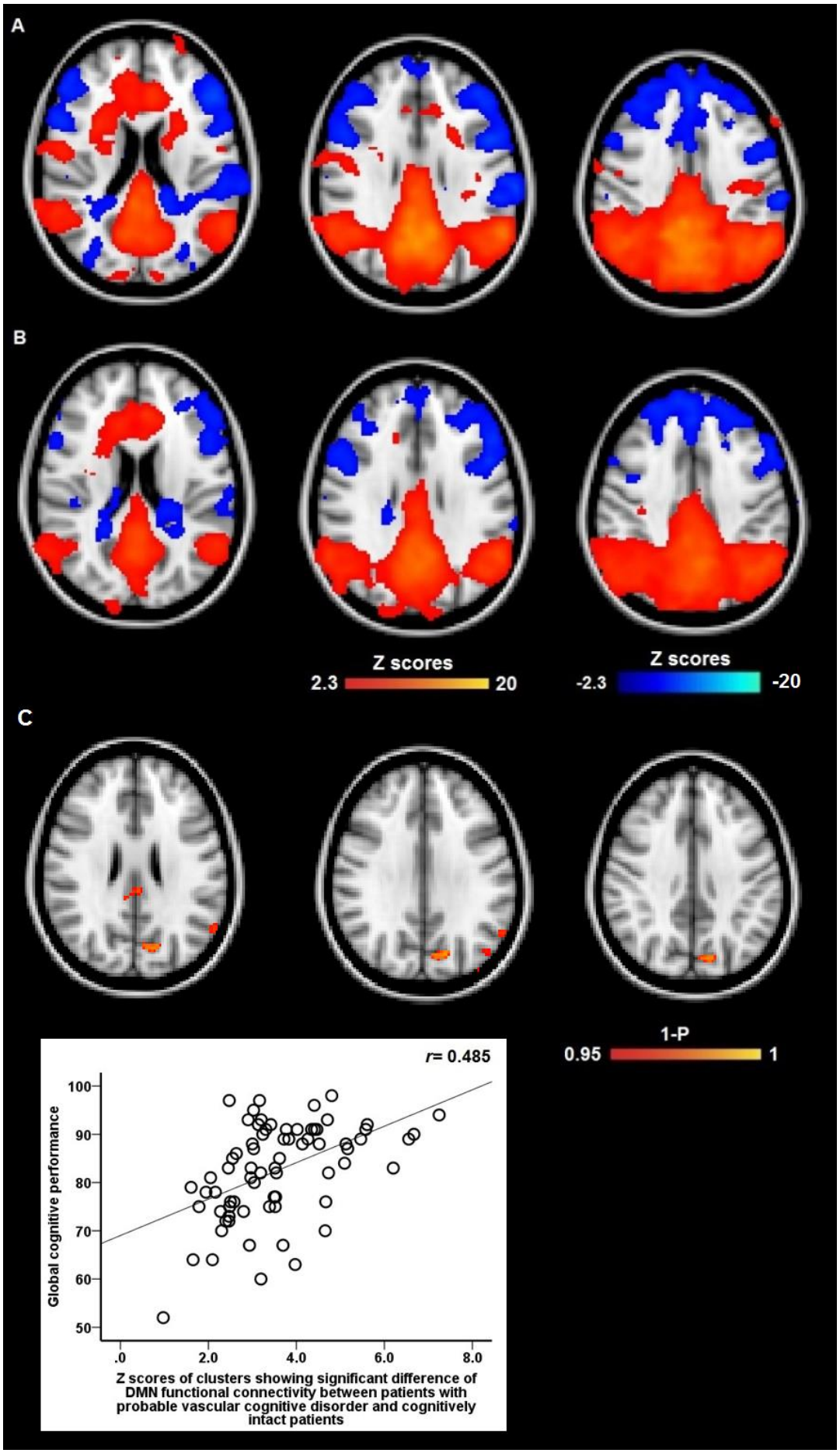

A
B
Z scores
2.3
20
Z scores
-2.3
-20
C
1-P
0.95
1
r= 0.485
Global cognitive performance
100
90
80
70
60
50
.0
2.0
4.0
6.0
8.0
Z scores of clusters showing significant difference of DMN functional connectivity between patients with probable vascular cognitive disorder and cognitively intact patients

**Figure 7. 10 DMN functional connectivity. DMN functional connectivity in (A) patients with normal cognition, (B) patients with normal cognition, and (C) difference between patients with normal and abnormal cognition after controlling for age. Red clusters in (A) and (B) showed positive correlations and Blue clusters in (A) and (B) showed negative correlations. Red clusters in (C) showed decreased functional connectivity in patients with abnormal cognition. Scatterplot showing the significant correlation between global cognitive performance and Z scores of significant clusters showing decreased DMN functional connectivity in patients with probable VaMCI. Significant level at P <0.05 (FWE corrected).**

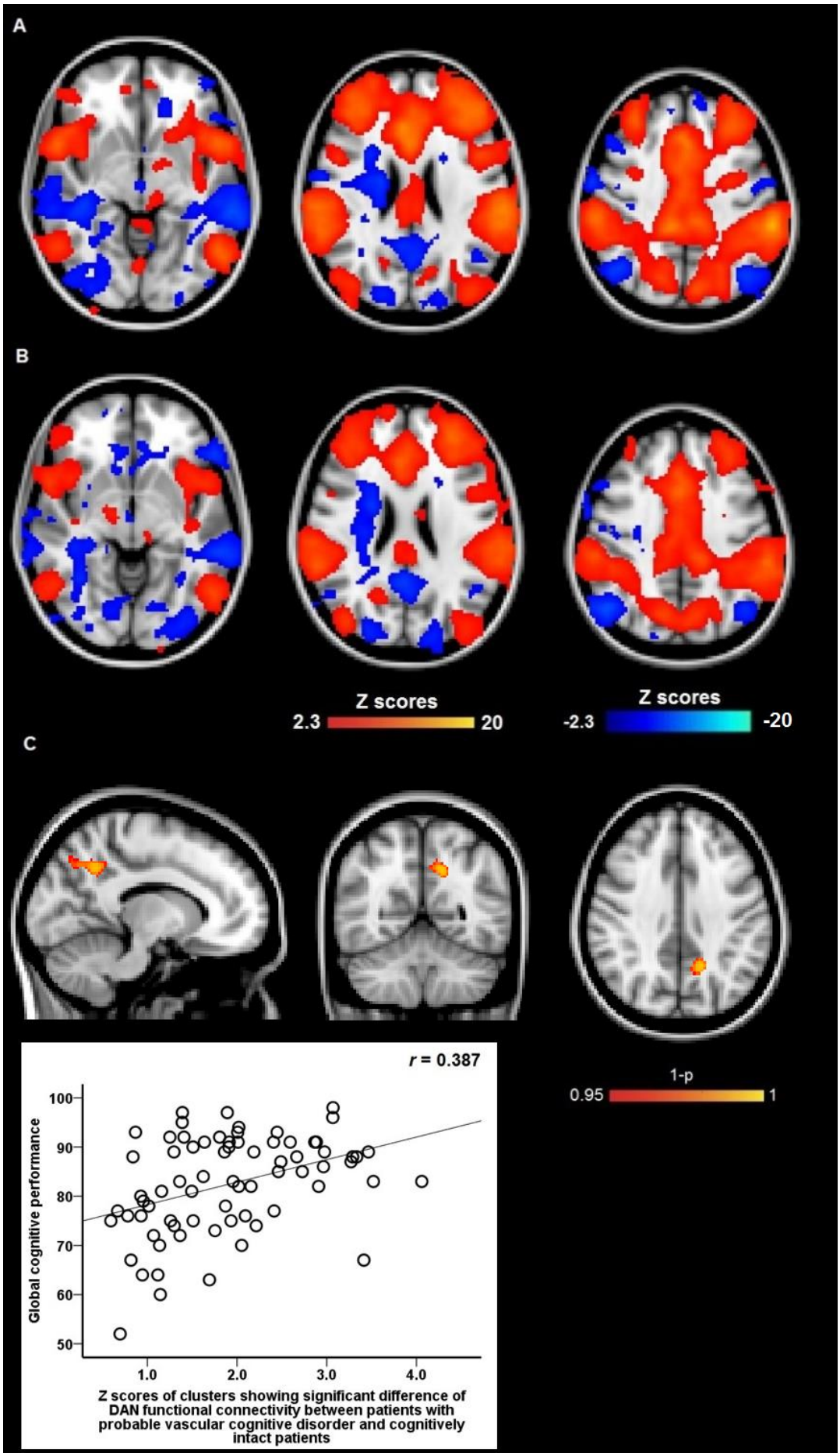

A
B
Z scores
2.3
20
Z scores
-2.3
-20
C
1-p
0.95
1
r = 0.387
Global cognitive performance
100
90
80
70
60
50
1.0
2.0
3.0
4.0
Z scores of clusters showing significant difference of DAN functional connectivity between patients with probable vascular cognitive disorder and cognitively intact patients

**Figure 7. 11 DAN functional connectivity. DAN functional connectivity in (A) patients with normal cognition, (B) patients with normal cognition, and (C) difference between patients with normal and abnormal cognition after controlling for age. Red clusters in (A) and (B) showed positive correlations and Blue clusters in (A) and (B) showed negative correlations. Red clusters in (C) showed decreased functional connectivity in patients with abnormal cognition. Scatterplot showing the significant correlation between global cognitive performance and Z scores of significant clusters showing decreased DAN functional connectivity in patients with probable VaMCI. Significant level at P <0.05 (FWE corrected).**

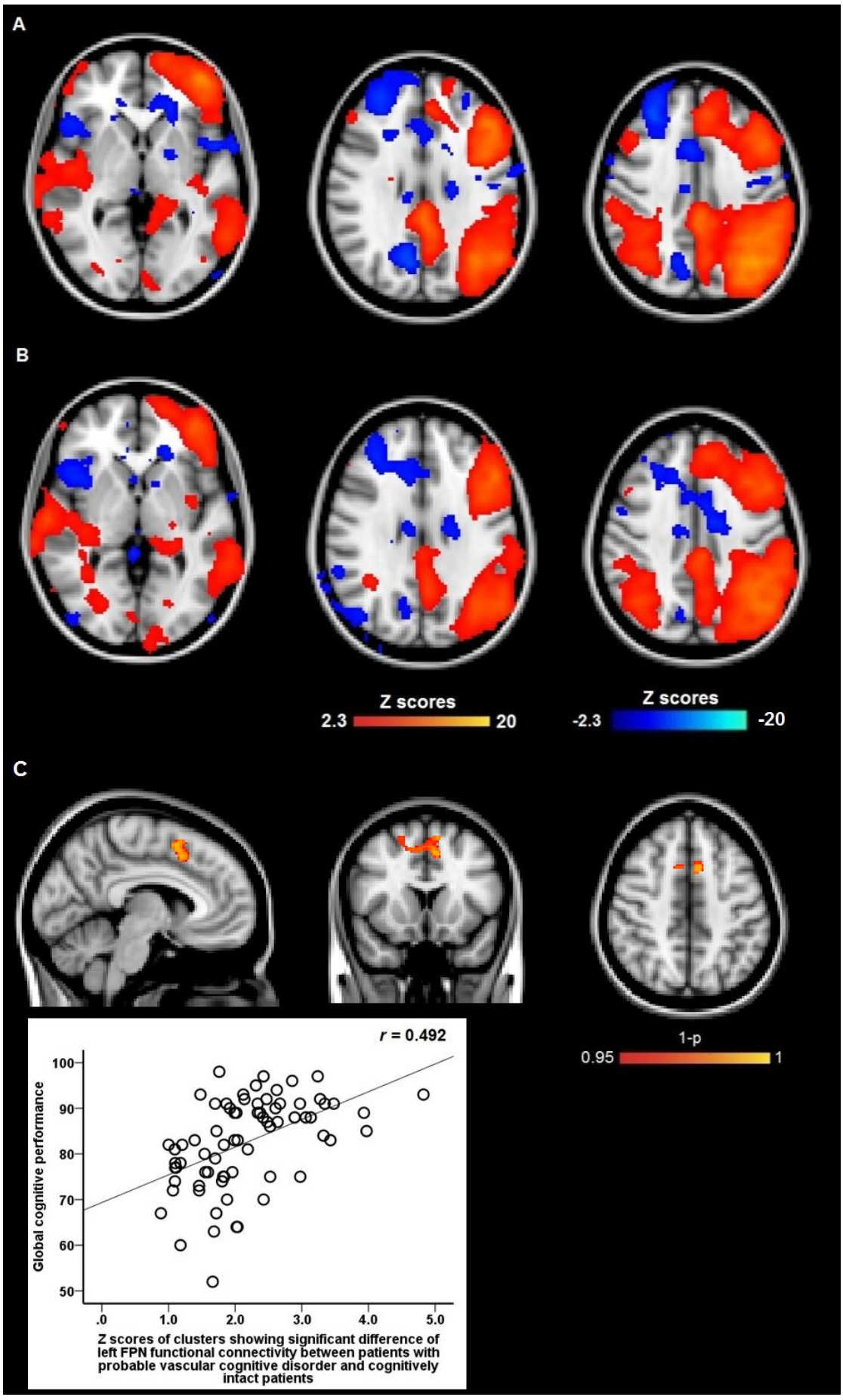

A
B
Z scores
2.3
20
Z scores
-2.3
-20
C
1-p
0.95
1
r = 0.492
Global cognitive performance
100
90
80
70
60
50
.0
1.0
2.0
3.0
4.0
5.0
Z scores of clusters showing significant difference of left FPN functional connectivity between patients with probable vascular cognitive disorder and cognitively intact patients

**Figure 7. 12 Left fronto-parietal network functional connectivity. Left fronto-parietal network functional connectivity in (A) patients with normal cognition, (B) patients with normal cognition, and (C) difference between patients with normal and abnormal cognition after controlling for age. Red clusters in (A) and (B) showed positive correlations and Blue clusters in (A) and (B) showed negative correlations. Red clusters in (C) showed decreased functional connectivity in patients with abnormal cognition. Scatterplot showing the significant correlation between global cognitive performance and Z scores of significant clusters showing decreased left fronto-parietal network functional connectivity in patients with probable VaMCI. Significant level at P <0.05 (FWE corrected).**

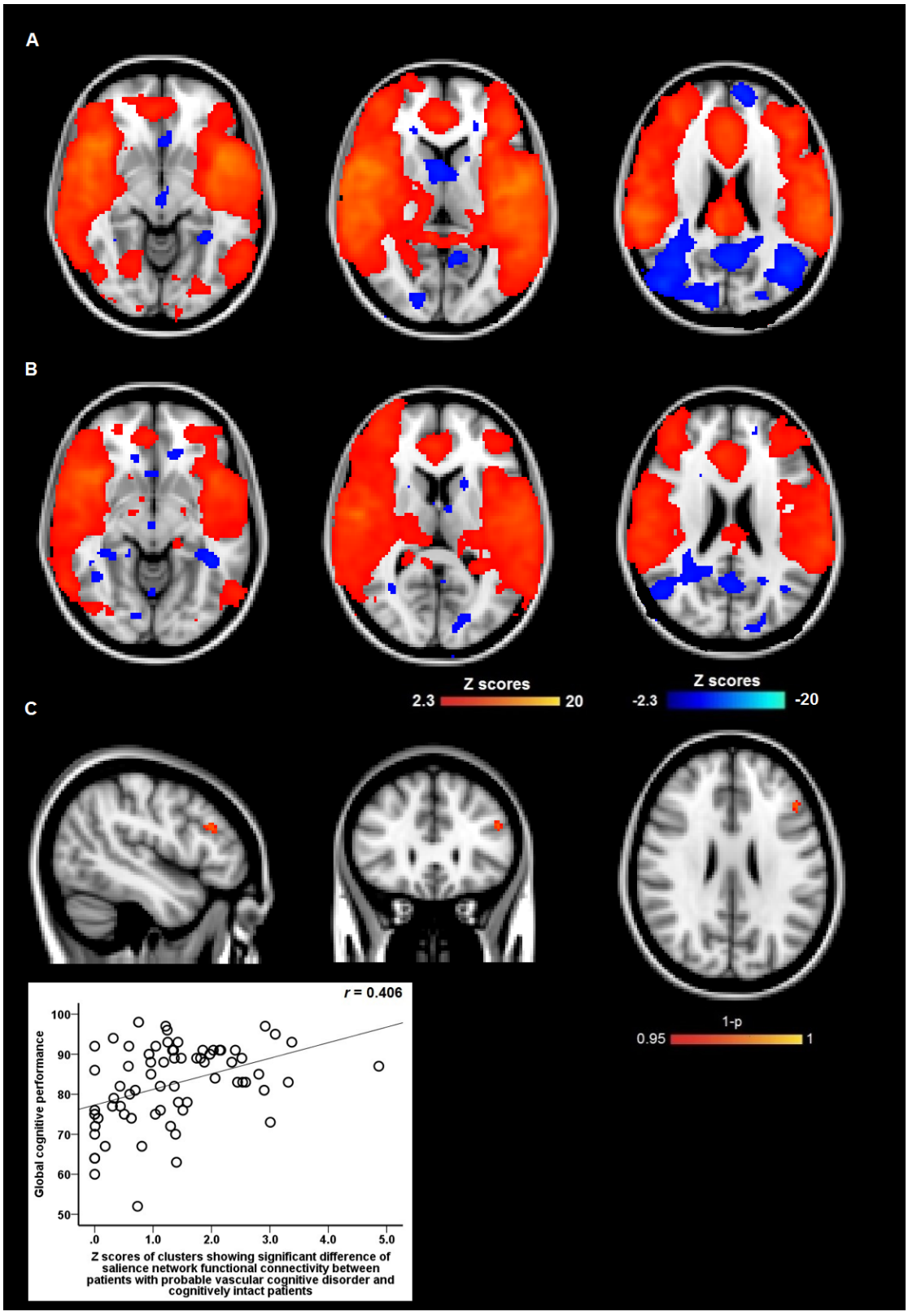

A
B
Z scores
2.3
20
Z scores
-2.3
-20
C
1-p
0.95
1
r = 0.406
Global cognitive performance
100
90
80
70
60
50
.0
1.0
2.0
3.0
4.0
5.0
Z scores of clusters showing significant difference of salience network functional connectivity between patients with probable vascular cognitive disorder and cognitively intact patients

**Figure 7. 13 Salience network functional connectivity. Salience network functional connectivity in (A) patients with normal cognition, (B) patients with normal cognition, and (C) difference between patients with normal and abnormal cognition after controlling for age. Red clusters in (A) and (B) showed positive correlations and Blue clusters in (A) and (B) showed negative correlations. Red clusters in (C) showed decreased functional connectivity in patients with abnormal cognition. Scatterplot showing the significant correlation between global cognitive performance and Z scores of significant clusters showing decreased salience network functional connectivity in patients with probable VaMCI. Significant level at P <0.05 (FWE corrected).**

### 7.4.2 Seed-based functional connectivity

As stated in the Method session, bilateral hippocampi, bilateral DLPFC, PCC and dorsal ACC were chose as seed regions to investigate the functional connectivity between them with the rest of brain. Therefore, totally six seed regions were selected to carry out seed-based analyses.

#### 7.4.2.1 Hippocampal functional connectivity obtained from Neurosynth

The Neurosynth database search of hippocampal functional connectivity was conducted in November, 2015 when the database had 822 studies and 31,168 activations (Figure 7. 14). The left-right orientation of the obtained activation map was swapped for radiological display convention. Hippocampus is functionally connected with ACC, PCC, bilateral medial frontal gyri, bilateral thalamus, bilateral parietal cortices and bilateral medial temporal gyri.

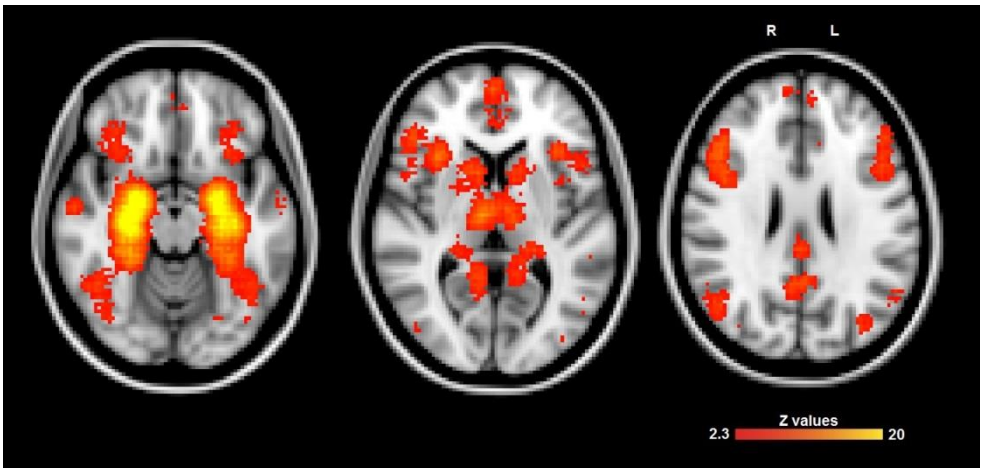



**Figure 7. 14 Hippocampal functional connectivity constructed from the Neurosynth database (FDR corrected, P<0.01).**

### 7.4.2.2 The role of hippocampal functional connectivity in global cognition

In both the groups of patients with normal and abnormal cognition (Figure 7. 15), left hippocampus was functionally connected with the right hippocampus, bilateral middle temporal gyri, bilateral parietal cortices and anterior cingulate cortex, PCC. Additionally, left hippocampus also functionally connected with bilateral thalamus in patients with normal cognition.

When controlled for age and mean relative displacement, patients with abnormal cognition showed decreased functional connectivity between left hippocampus and left inferior parietal lobule, ACC, left postcentral gyrus and left superior frontal gyrus. In addition, the functional connectivity between left hippocampus and left inferior parietal lobule, ACC, left postcentral gyrus and left superior frontal gyrus was significantly correlated with global cognitive performance ($r$=0.274, P=0.020, Figure 7. 15,Table 7. 3).

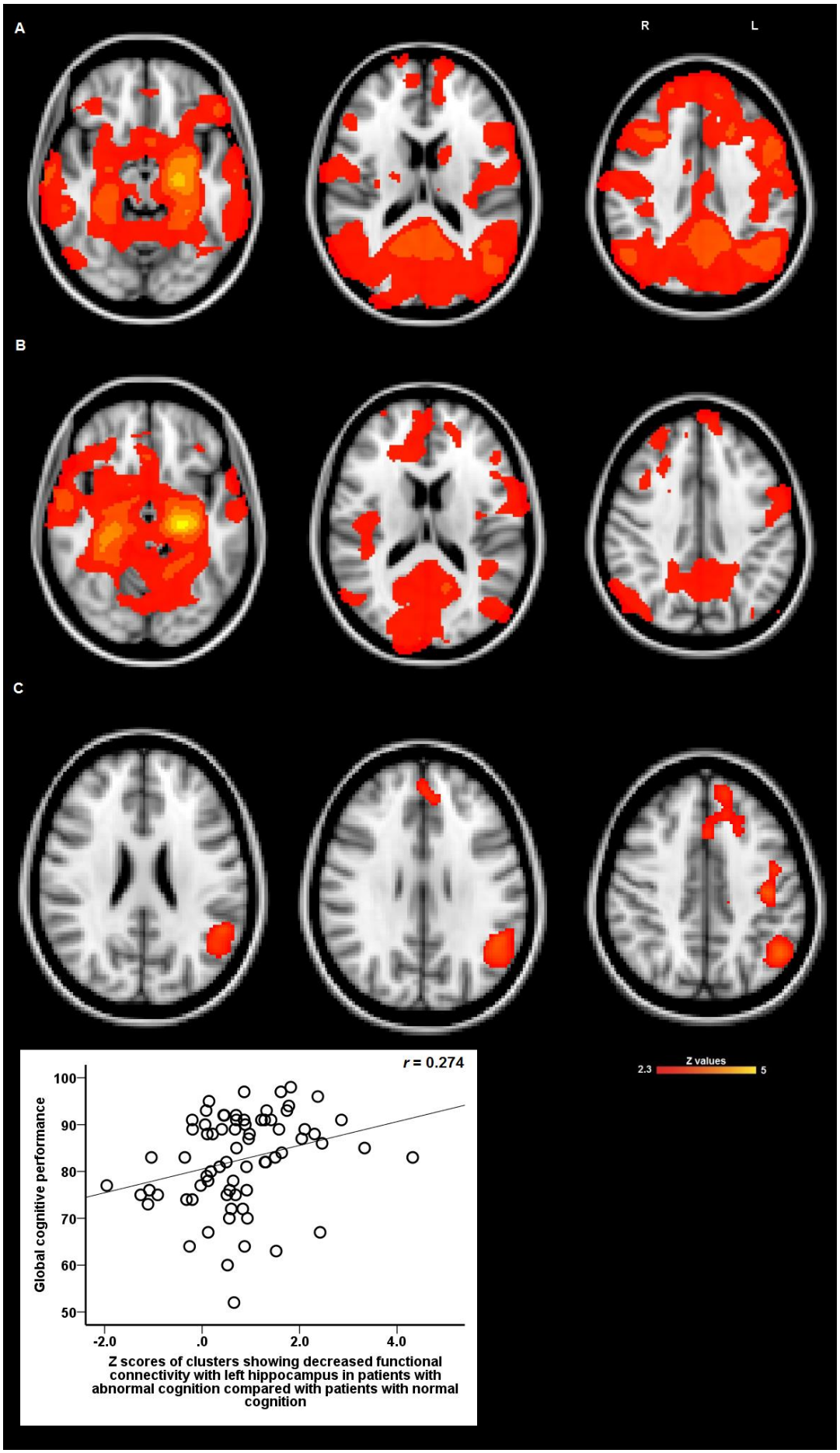

A
R
L
B
C
Z values
2.3
5
r = 0.274
Global cognitive performance
100
90
80
70
60
50
-2.0
.0
2.0
4.0
Z scores of clusters showing decreased functional connectivity with left hippocampus in patients with abnormal cognition compared with patients with normal cognition

**Figure 7. 15 Left hippocampal functional connectivity associated with global cognitive performance. Left hippocampal functional connectivity in (A) normal cognition, (B) abnormal cognition and (C) group difference of left hippocampal functional connectivity between patients with normal and abnormal cognition. Functional connectivity between clusters (Red-Yellow) in (C) and left hippocampus significantly decreased in patients with abnormal cognition. Analysis was controlled for age and mean relative displacement. The scatter plot showed the correlation between left hippocampal functional connectivity and global cognitive performance. Z scores were colour-coded from red to yellow. The Z scores maps were overlaid on MNI 2mm template.**

In patients with normal and abnormal cognition, right hippocampus was functionally connected with left hippocampus, anterior cingulate cortex and PCC and bilateral parietal cortices. Additionally, patients with normal cognition also showed functional connectivity between right hippocampus and bilateral thalamus, bilateral parietal cortices (Figure 7. 16).

When treating age and mean relative displacement as covariates of no interest, patients with abnormal cognition showed decreased functional connectivity between right hippocampus and left superior frontal gyrus, left middle frontal gyrus, ACC, right middle temporal gyrus, right superior temporal gyrus, right inferior parietal lobule, right postcentral gyrus and right middle frontal gyrus compared with patients with normal cognition (Figure 7. 16, Table 7.1). In contrast, there was no significant correlation between right hippocampal functional connectivity and global cognitive performance.

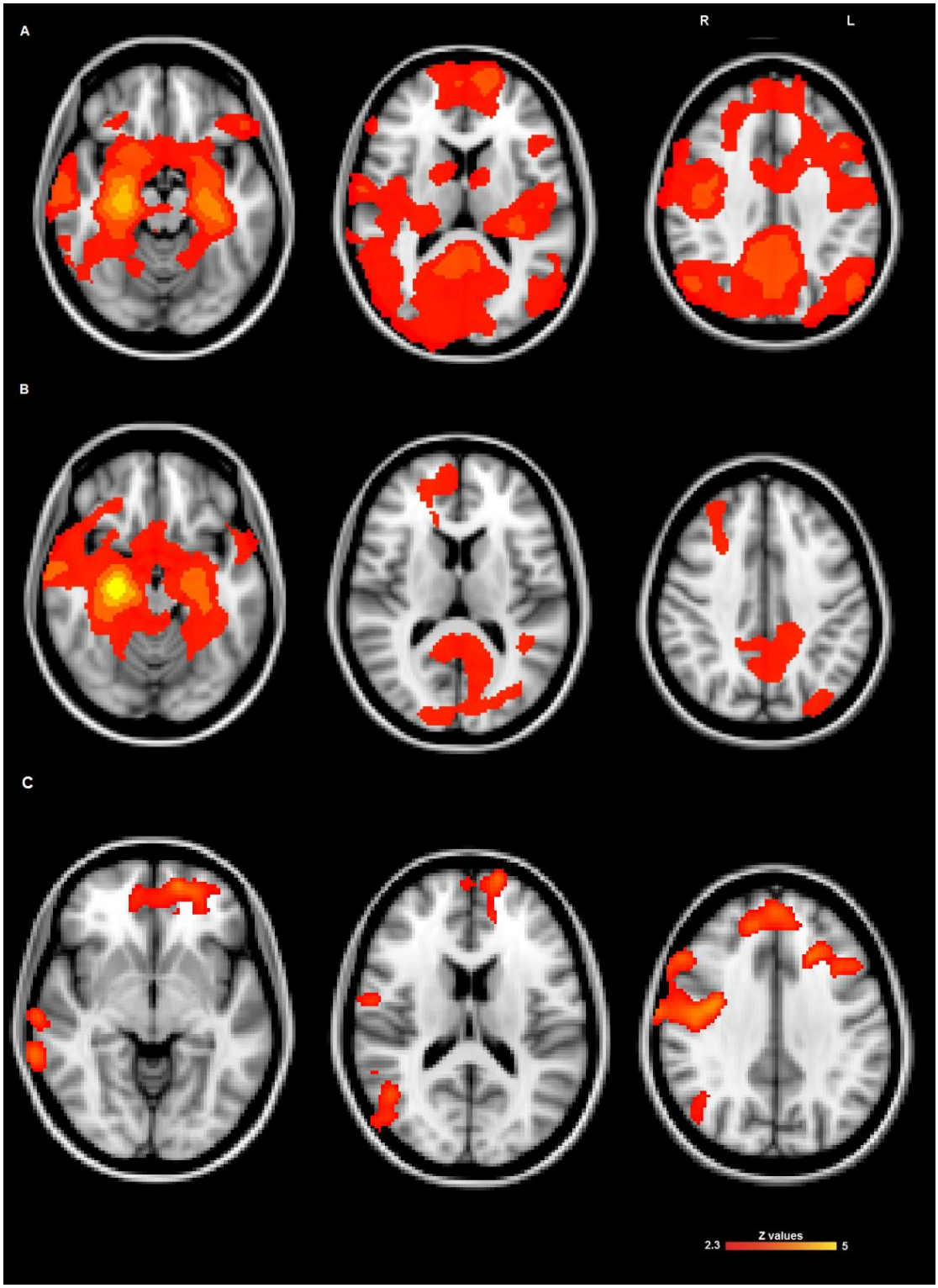

A
R
L
B
C
Z values
2.3
5

**Figure 7. 16 Right hippocampal functional disconnection associated with global cognitive impairment. Right hippocampal functional connectivity in (A) normal cognition, (B) abnormal cognition and (C) group difference of right hippocampal functional connectivity between patients with normal and abnormal cognition. Functional connectivity between clusters (Red-Yellow) in (C) and right hippocampus significantly decreased in patients with abnormal cognition. All analyses were controlled for age and mean relative displacement. Z scores were colour-coded from red to yellow. The Z scores maps were overlaid on MNI 2mm template.**

#### 7.4.2.3 DLPFC functional connectivity obtained from Neurosynth

The term 'DLPFC' was used to search in the Neurosynth database in November, 2015 and 362 studies and 13,036 activations were found (Figure 7. 17). The left-right orientation of the obtained activation map was swapped for radiological display convention. According to visual inspection, DLPFC was functionally connected with ACC, PCC and bilateral parietal cortices.

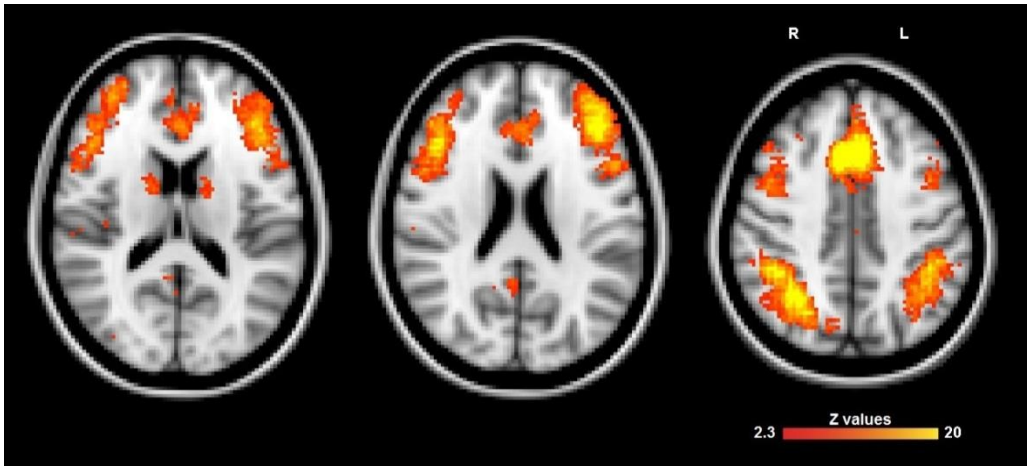



**Figure 7. 17 DLPFC functional connectivity constructed from the Neurosynth database (FDR corrected, P<0.01).**

#### 7.4.2.4 The role of DLPFC functional connectivity in global cognition

Left DLPFC functionally connected with PCC, anterior cingulate cortex, bilateral parietal cortices, bilateral middle frontal gyri and frontal pole in patients with normal and abnormal cognition (Figure 7. 18). Decreased functional connectivity between left DLPFC and right hippocampus, precuneous, ACC, PCC, left inferior frontal gyrus and right middle frontal gyrus was found in patients with abnormal cognition compared with patients with normal cognition when age and mean relative displacement were controlled. This left DLPFC functional disconnection was significantly correlated with global cognitive performance ($r$=0.405, P<0.001, Figure 7. 18, Table 7. 3).

The strength of functional connectivity in an extended network with the left DLPFC positively correlated with global cognitive performance after controlling for age and mean relative displacement. These areas were located in the bilateral superior frontal gyrus, precuneous, PCC, ACC, left middle occipital gyrus and left inferior parietal lobule (Figure 7. 19).

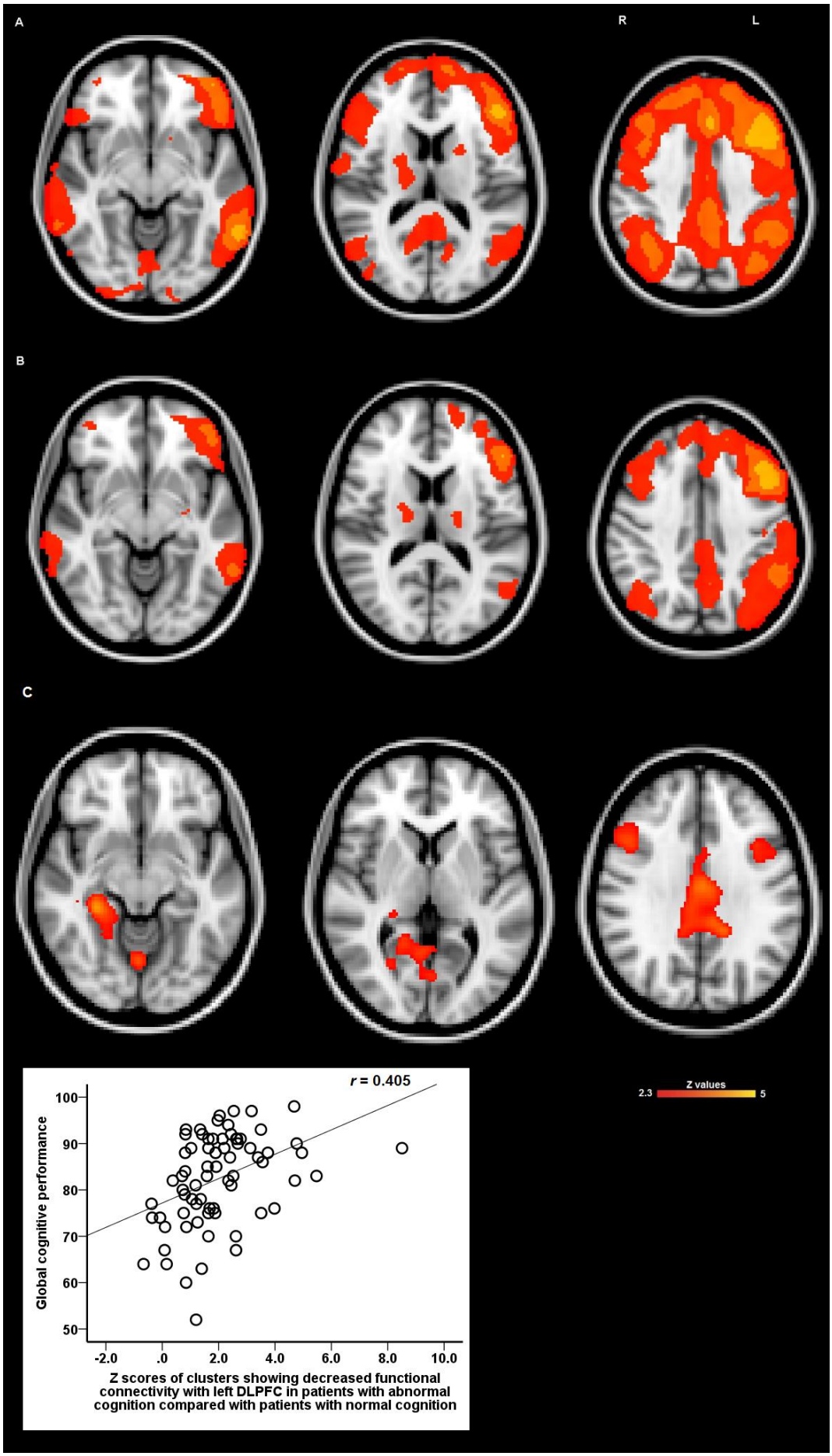

A
R
L
B
C
r = 0.405
Global cognitive performance
100
90
80
70
60
50
-2.0
.0
2.0
4.0
6.0
8.0
10.0
Z scores of clusters showing decreased functional connectivity with left DLPFC in patients with abnormal cognition compared with patients with normal cognition
Z values
2.3
5

**Figure 7. 18 Left DLPFC functional connectivity associated with global cognitive performance. Left DLPFC functional connectivity in (A) normal cognition, (B) abnormal cognition and (C) group difference of left DLPFC functional connectivity between patients with normal and abnormal cognition. Functional connectivity between clusters (Red-Yellow) in (C) and left DLPFC significantly decreased in patients with abnormal cognition. All analyses were controlled for age and mean relative displacement. The scatter plot showed the correlation between left DLPFC functional connectivity and global cognitive performance. Z scores were colour-coded from red to yellow. The Z scores maps were overlaid on MNI 2mm template.**

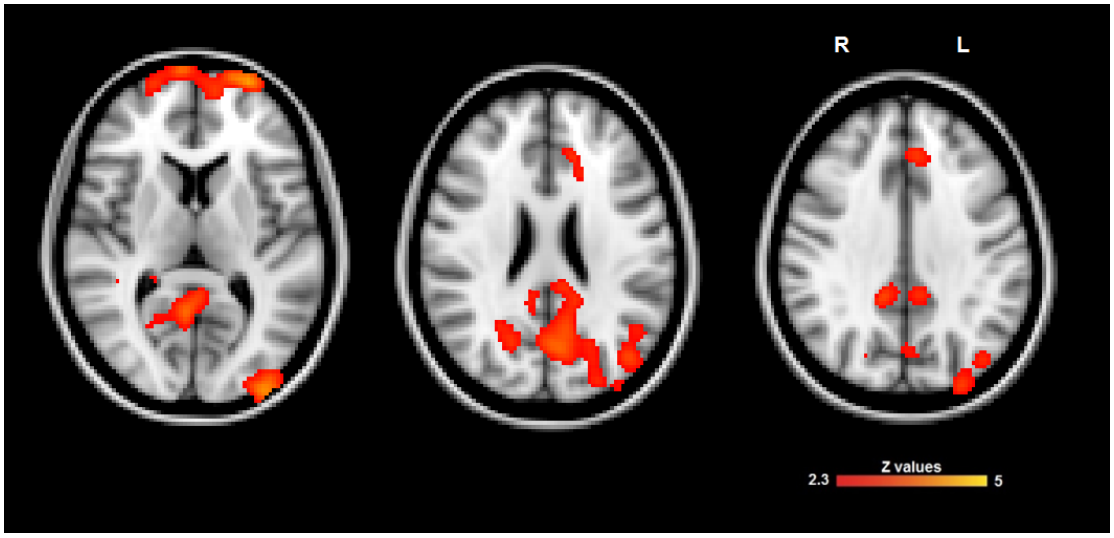



**Figure 7. 19 Correlation between the strength of left DLFPC functional connectivity and global cognitive performance. The left DLPFC functional connectivity was positively correlated with global cognitive performance (P<0.05, corrected). All analyses controlled for age and mean relative displacement. Z scores were colour-coded from red to yellow. The Z scores maps were overlaid on MNI 2mm template.**

In patients with normal and abnormal cognition, right DLPFC was functionally connected with PCC, frontal pole, right parietal lobe and bilateral middle frontal gyri. Additionally, patients with normal cognition also showed functional connectivity between right DLPFC and bilateral thalamus, left parietal lobe (Figure 7. 20). Patients with abnormal cognition showed decreased functional connectivity between right DLPFC and right hippocampus, right middle temporal gyrus, PCC, ACC, precuneous, right superior temporal gyrus, right insula, left superior frontal gyrus, left DLPFC compared with patients with normal cognition when age and mean relative displacement were controlled. The right DLPFC functional disconnection was significantly related to global cognitive performance ($r$=0.395, P=0.001, Figure 7. 20, Table 7. 1).

The functional connectivity between right DLPFC and PCC, precuneous, left DLPFC and left superior frontal gyrus was positively correlated with global cognitive performance when controlling for age and mean relative displacement (Figure 7. 21).

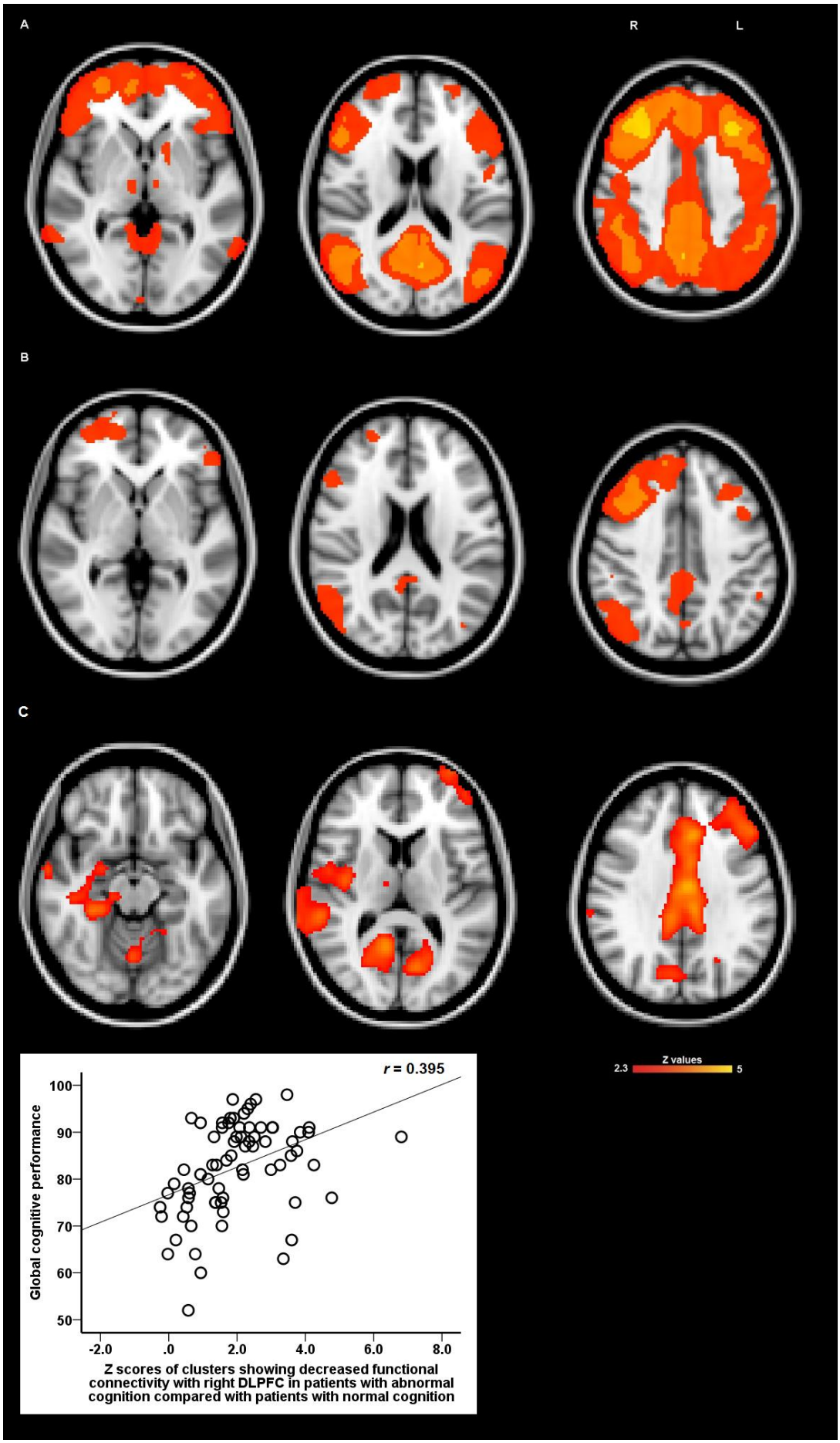

A
R
L
B
C
Z values
2.3
5
r = 0.395
Global cognitive performance
100
90
80
70
60
50
-2.0
.0
2.0
4.0
6.0
8.0
Z scores of clusters showing decreased functional connectivity with right DLPFC in patients with abnormal cognition compared with patients with normal cognition

**Figure 7. 20 Right DLPFC functional connectivity associated with global cognitive performance. Right DLPFC functional connectivity in (A) normal cognition, (B) abnormal cognition and (C) group difference of right DLPFC functional connectivity between patients with normal and abnormal cognition. Functional connectivity between clusters (Red-Yellow) in (C) and right DLPFC significantly decreased in patients with abnormal cognition. All analyses were controlled for age and mean relative displacement. The scatter plot showed the correlation between right DLPFC functional connectivity and global cognitive performance. Z scores were colour-coded from red to yellow. The Z scores maps were overlaid on MNI 2mm template.**

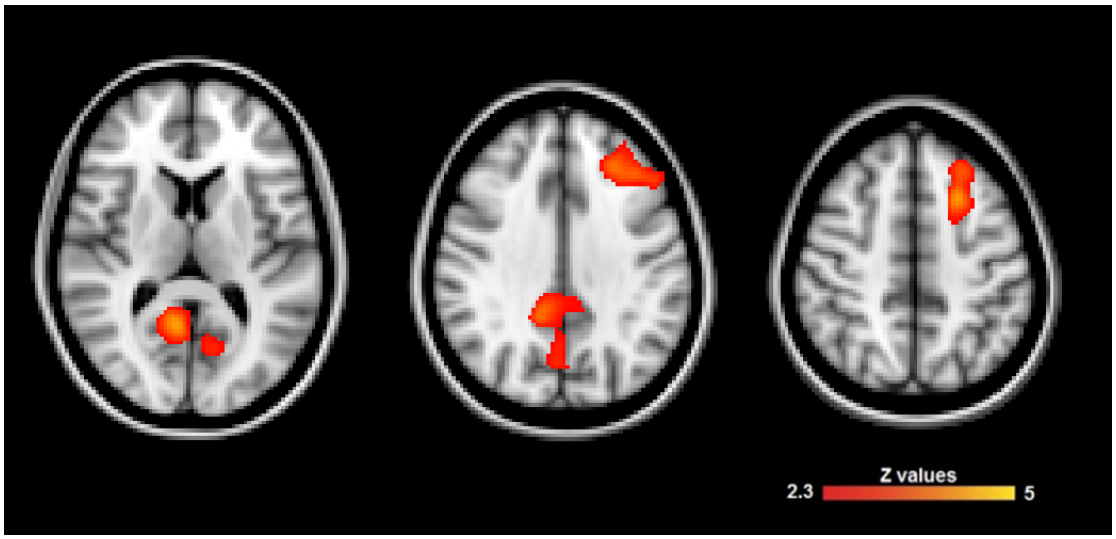



**Figure 7. 21 Correlation between the strength of right DLPFC functional connectivity and global cognitive performance. The functional connectivity between right DLPFC and significant clusters was positively correlated with global cognitive performance (P<0.05, corrected). All analyses were controlled for age and mean relative displacement. Z scores were colour-coded from red to yellow. The Z scores maps were overlaid on MNI 2mm template.**

### 7.4.2.5 PCC functional connectivity obtained from Neurosynth

The Neurosynth database search of PCC functional connectivity was conducted in November, 2015 when the database had 726 studies and 26914 activations (Figure 7. 22). The left-right orientation of the obtained activation map was swapped for radiological display convention. PCC was found to connect with bilateral hippocampi, ACC, bilateral medial temporal gyri, bilateral thalamus, bilateral medial frontal gyri and bilateral inferior parietal cortices.

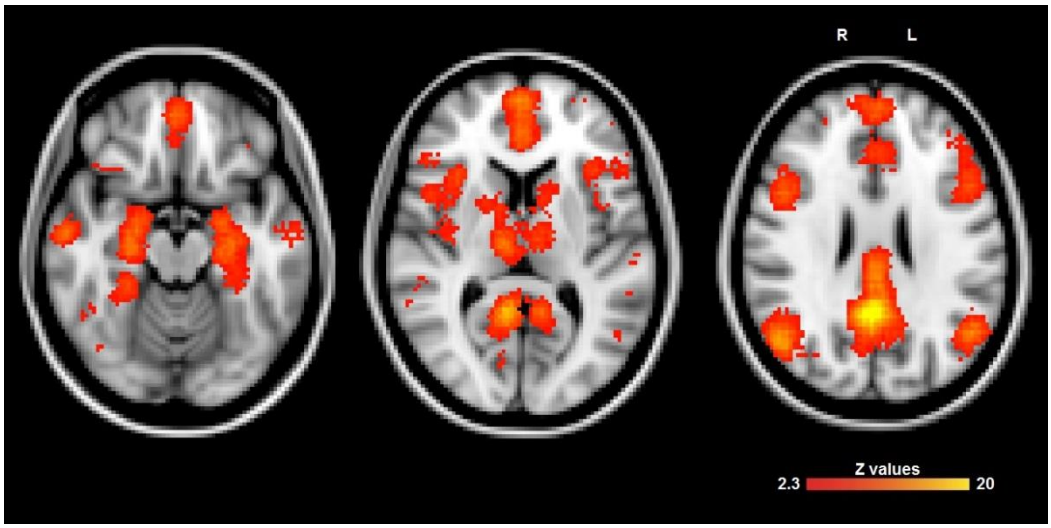



**Figure 7. 22 PCC functional connectivity functional connectivity constructed from the Neurosynth database (FDR corrected, P<0.01).**

#### 7.4.2.6 The role of PCC functional connectivity in global cognition

PCC was functionally connected with bilateral hippocampi, left middle temporal gyrus, bilateral parietal cortices, bilateral thalamus, bilateral middle frontal gyri, anterior cingulate cortex and frontal pole in patients with normal cognition. In patients with abnormal cognition, PCC functionally connected with bilateral hippocampi, bilateral parietal cortices, anterior cingulate cortex and frontal pole (Figure 7. 23).

Compared with patients with normal cognition, cognitively impaired patients had decreased functional connectivity between PCC and right superior frontal gyrus and right middle frontal gyrus when controlling for age and mean relative displacement .The functional disconnection between PCC and right superior frontal gyrus and right middle frontal gyrus was significantly related to global cognitive performance ($r=0.546$, $P<0.001$, Figure 7. 23,Table 7. 3).

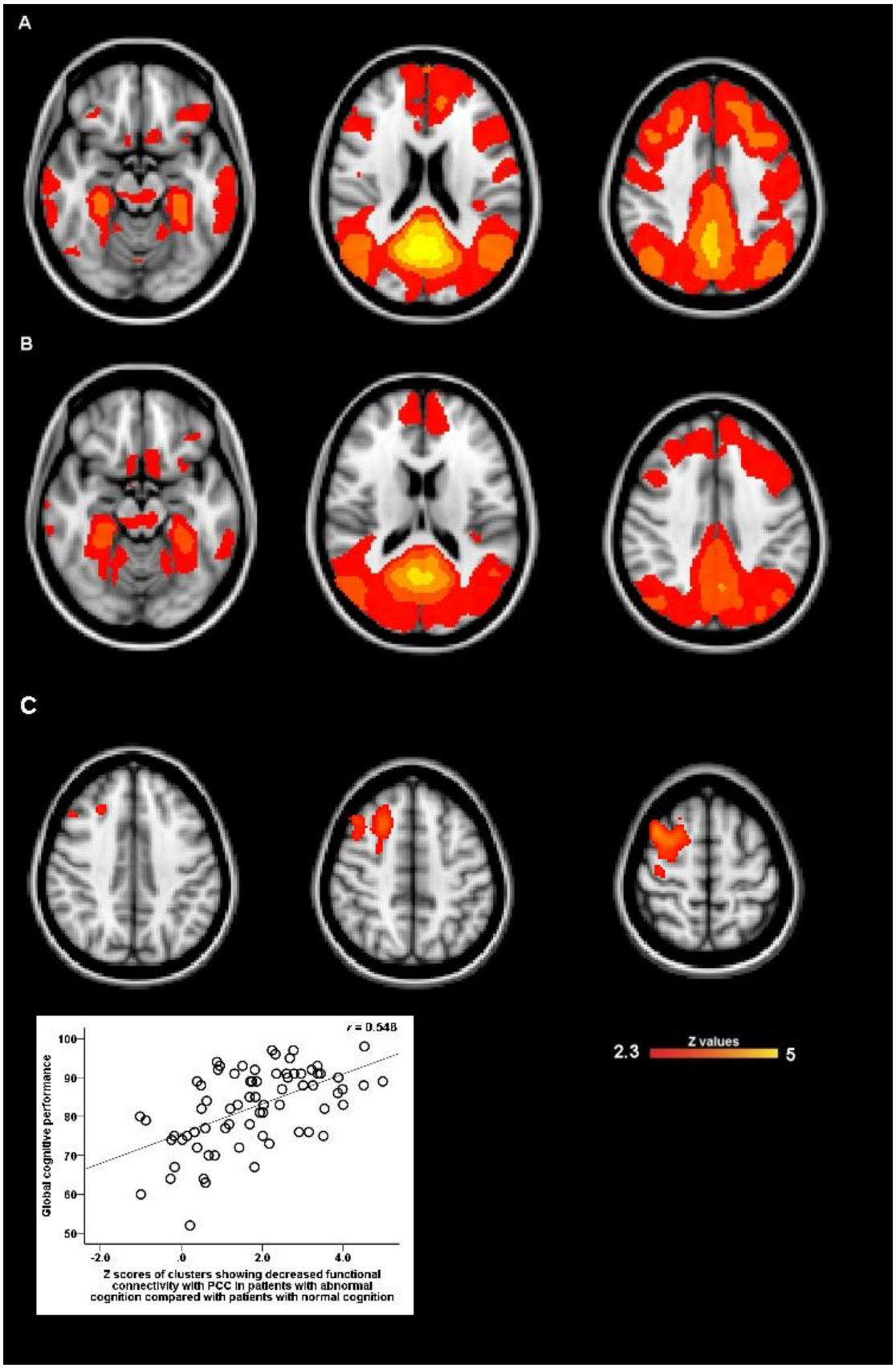

A
B
C
r = 0.548
Global cognitive performance
100
90
80
70
60
50
-2.0
.0
2.0
4.0
Z scores of clusters showing decreased functional connectivity with PCC in patients with abnormal cognition compared with patients with normal cognition
2.3
Z values
5

**Figure 7. 23 PCC functional connectivity associated with global cognitive performance. PCC functional connectivity in (A) normal cognition, (B) abnormal cognition and (C) group difference of PCC functional connectivity between patients with normal and abnormal cognition. Functional connectivity between clusters (Red-Yellow) in (C) and PCC significantly decreased in patients with abnormal cognition. All analyses were controlled for age and mean relative displacement. The scatter plot showed the correlation between PCC functional connectivity and global cognitive performance. Z scores were colour-coded from red to yellow. The Z scores maps were overlaid on MNI 2mm template.**

### 7.4.2.7 ACC functional connectivity obtained from Neurosynth

The term 'anterior cingulate' was used to search in the Neurosynth database in November, 2015 and 1677 studies and 60241 activations were found (Figure 7. 24). The left-right orientation of the obtained activation map was swapped for radiological display convention. According to visual inspection, ACC was functionally connected with bilateral insula and bilateral superior frontal gyri.

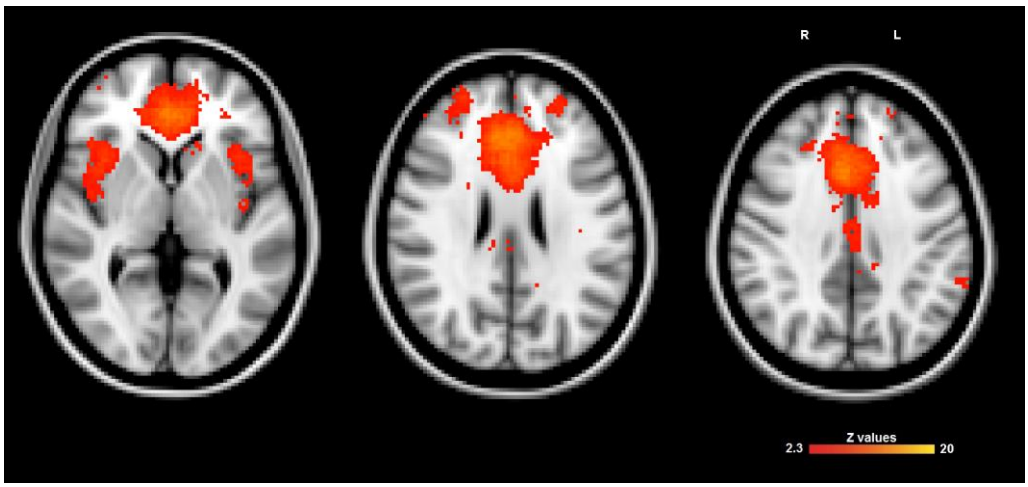



**Figure 7. 24 ACC functional connectivity obtained from the Neurosynth database (FDR corrected, P<0.01).**

#### 7.4.2.8 The role of ACC functional connectivity in global cognition

ACC was functionally connected with bilateral insula, bilateral superior temporal gyri, bilateral lateral occipital cortices, PCC and bilateral middle frontal gyri in patients with normal cognition. In patients with abnormal cognition, ACC was only functionally connected with left insula and bilateral middle frontal gyrus (Figure 7. 25).

Patients with abnormal cognition showed decreased functional connectivity within ACC, and between ACC and right middle temporal gyrus, right putamen, right thalamus and PCC compared with patients with normal cognition when controlling for age and mean relative displacement (Figure 7. 25). The ACC functional disconnection was significantly correlated with global cognitive performance ($r$= 0.369, P=0.001, Figure 7. 25).

The strength of functional connectivity between ACC and PCC, left superior temporal gyrus was significantly associated with global cognitive performance when age and mean relative displacement was controlled (Figure 7. 26).

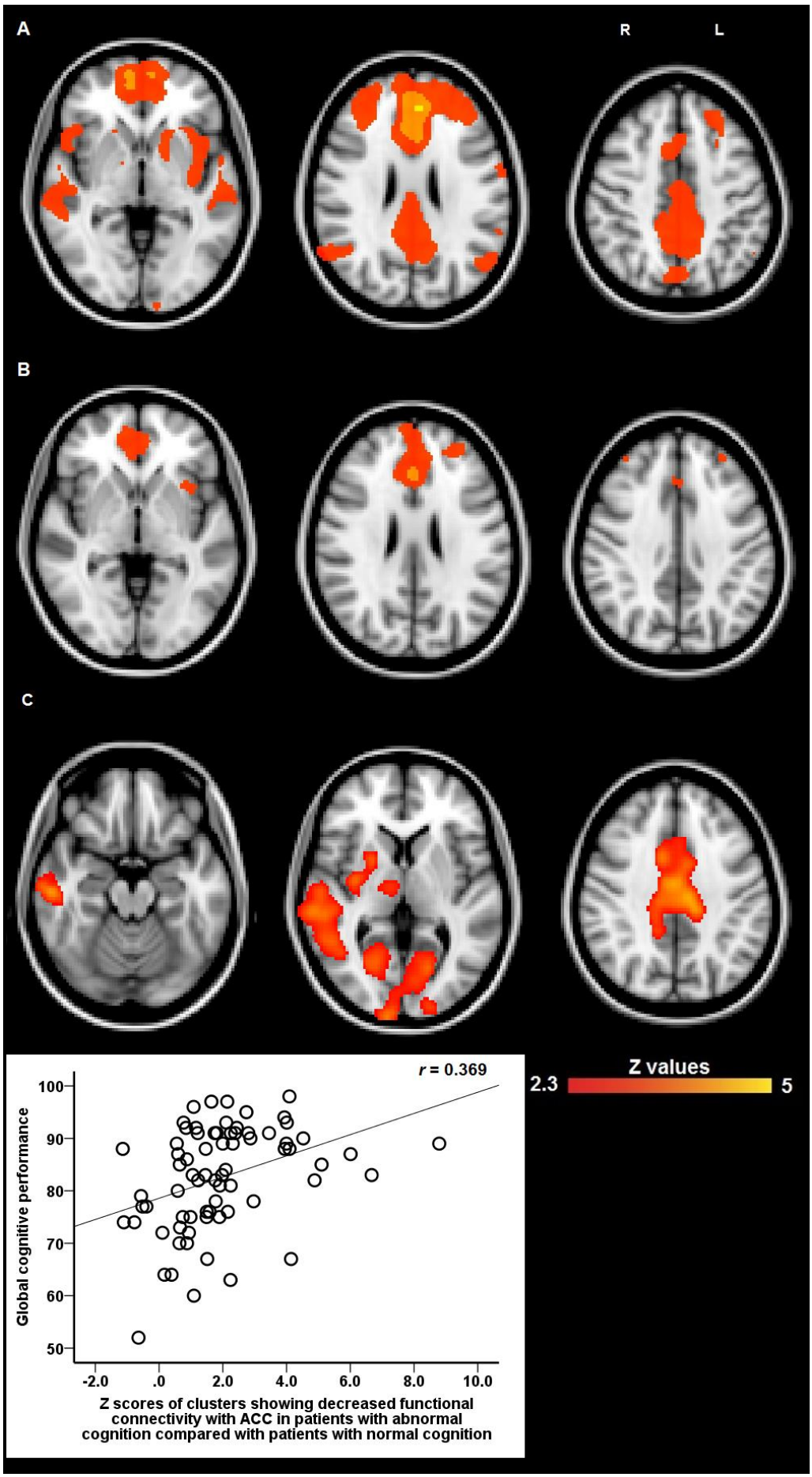

A
R
L
B
C
r = 0.369
Global cognitive performance
100
90
80
70
60
50
-2.0
.0
2.0
4.0
6.0
8.0
10.0
Z scores of clusters showing decreased functional connectivity with ACC in patients with abnormal cognition compared with patients with normal cognition
Z values
2.3
5

**Figure 7. 25 ACC functional connectivity associated with global cognitive performance. ACC functional connectivity in (A) normal cognition, (B) abnormal cognition and (C) group difference of ACC functional connectivity between patients with normal and abnormal cognition. Functional connectivity between clusters (Red-Yellow) in (C) and ACC significantly decreased in patients with abnormal cognition. All analyses were controlled for age and mean relative displacement. The scatter plot showed the correlation between ACC functional connectivity and global cognitive performance. Z scores were colour-coded from red to yellow. The Z scores maps were overlaid on MNI 2mm template.**

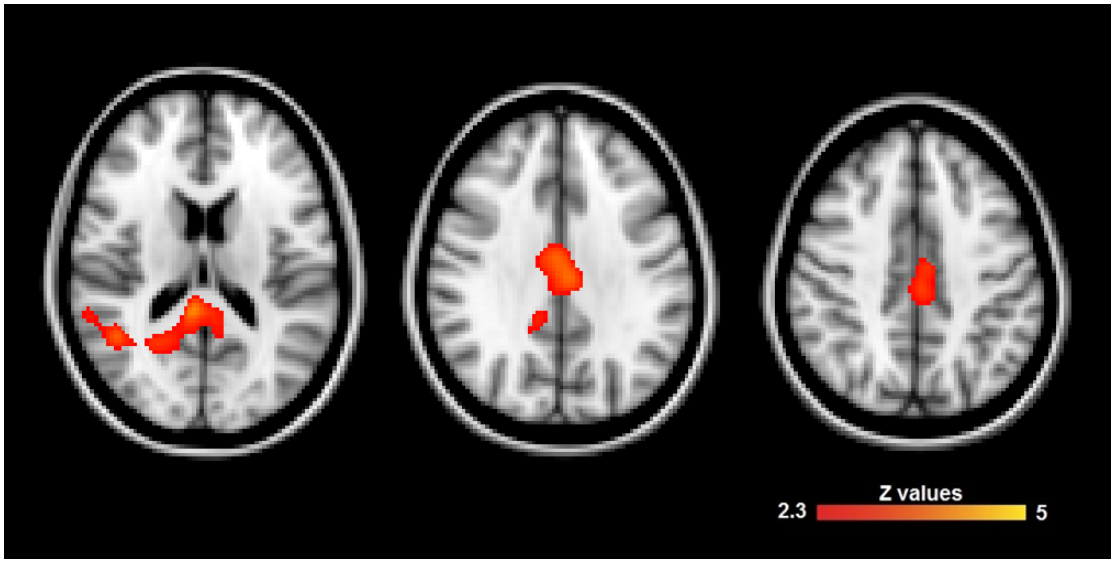



**Figure 7. 26 Correlation between the strength of ACC functional connectivity and global cognitive performance. The functional connectivity between ACC and significant clusters was positively correlated with global cognitive performance (P<0.05, corrected). All analyses controlled for age and mean relative displacement. Z scores were colour-coded from red to yellow. The Z scores maps were overlaid on MNI 2mm template.**

**Table 7. 3 Brain regions showing decreased functional connectivity within resting-state networks and between seed regions with the rest of the brain**

| | Coordinates (mm) | | | P value |
|---|---|---|---|---|
| | X | Y | Z | |
| **ICA analysis results** | | | | |
| **Default mode network** | | | | |
| PCC | -2 | -34 | 24 | 0.020 |
| Precruneous | -10 | -74 | 24 | 0.029 |
| Left inferior parietal lobule | -58 | -60 | 26 | 0.040 |
| **Dorsal attention network** | | | | |
| PCC | -14 | -54 | 36 | 0.019 |
| **Left fronto-parietal network** | | | | |
| ACC | -6 | 14 | 46 | 0.017 |
| **Salience network** | | | | |
| Left middle frontal gyrus | -46 | 28 | 26 | 0.034 |
| | **Coordinates (mm)** | | | **Z value** |
| | X | Y | Z | |
| **Seed-based analysis results** | | | | |
| **Left hippocampus functional connectivity** | | | | |
| Left inferior parietal lobule | -50 | -58 | 28 | 3.06 |
| ACC | -2 | 46 | 30 | 2.85 |
| Postcentral gyrus | -40 | -18 | 42 | 3.13 |
| Left superior frontal gyrus | -20 | 26 | 42 | 2.50 |
| **Right hippocampal functional connectivity** | | | | |
| Left superior frontal gyrus | -16 | 62 | 14 | 3.22 |
| Left middle frontal gyrus | -14 | 56 | -6 | 3.61 |
| ACC | 2 | 50 | -6 | 2.51 |
| Right middle temporal gyrus | 68 | -44 | -6 | 3.26 |
| Right superior temporal gyrus | 66 | -20 | -4 | 2.73 |
| Right inferior parietal lobule | 46 | -60 | 26 | 2.87 |
| Right postcentral gyrus | 54 | -14 | 26 | 3.51 |
| Right middle frontal gyrus | 14 | 42 | 36 | 3.31 |
| **Left DLPFC functional connectivity** | | | | |
| Right hippocampus | 30 | -32 | -10 | 3.80 |
| Precuneous | 16 | -50 | 2 | 3.05 |
| ACC | 0 | 12 | 26 | 2.72 |
| PCC | 0 | -32 | 26 | 3.13 |
| Left inferior frontal gyrus | -40 | 10 | 30 | 2.62 |
| Right middle frontal gyrus | 48 | 18 | 30 | 3.03 |
| **Right DLPFC functional connectivity** | | | | |

| | | | | |
|---|---|---|---|---|
| Right hippocampus | 24 | -34 | -10 | 3.93 |
| Right middle temporal gyrus | 62 | -10 | -10 | 2.98 |
| PCC | -4 | -16 | 30 | 4.02 |
| ACC | -8 | 26 | 30 | 3.96 |
| Precuneous | 12 | -56 | 8 | 4.02 |
| Right superior temporal gyrus | 60 | -36 | 8 | 3.13 |
| Right insula | 40 | -10 | 8 | 3.25 |
| Left superior frontal gyrus | -32 | 64 | 8 | 3.14 |
| Left DLPFC | -50 | 28 | 30 | 3.29 |
| **PCC functional connectivity** | | | | |
| Right superior frontal gyrus | 22 | 22 | 46 | 3.05 |
| Right middle frontal gyrus | 44 | 22 | 46 | 2.91 |
| **ACC functional connectivity** | | | | |
| Right middle temporal gyrus | 58 | -20 | -18 | 3.86 |
| Right putamen | 24 | 4 | 2 | 3.39 |
| Right thalamus | 12 | -14 | 2 | 2.60 |
| ACC | 8 | 12 | 32 | 3.51 |
| PCC | 12 | -44 | 34 | 2.87 |

### 7.4.3 Correlation between functional disconnection of RSNs, microscopic damage of underlying white matter tracts, and total ischaemic lesion volumes within specific white matter tracts

To further investigate the possible cerebrovascular nature of functional disconnection of large-scale cognitive networks, we extracted the MD values of white matter tracts which underlie or connect to the identified hubs of functional disconnection in order to investigate the association between microscopic damage of white matter tracts and functional disconnection of large-scale cognitive networks (Figure 7. 27).

Among 72 subjects who had resting-state fMRI scans, 71 of them had the measurement of microscopic damage of white matter tracts and 54 of them had the volumetric measurement of cerebrovascular lesions.

The normalised total ischaemic lesion (TILL) volumes within network specific white matter tracts were unrelated to functional disconnection (decreased DMN functional connectivity and TILL volumes within cingulum: P=0.224 and those within left superior longitudinal fasciculus: P=0.220; decreased DAN functional connectivity and TILL volumes within left cingulum: P=0.240; decreased left fronto-parietal network functional connectivity and TILL volumes within right anterior thalamic radiation: P=0.786; decreased salience network functional connectivity and TILL volumes within left anterior thalamic radiation: P=0.326). Conversely, we found significant negative correlations between key white matter tract microscopic integrity and associated decreased RSN functional connectivity. Decreased DMN functional connectivity was significantly associated with MD of cingulum (r=-0.353, P=0.003) and left SLF (r=-0.323, P=0.006). Decreased DAN functional connectivity was significantly related to left cingulum MD (*r*=-0.302, P=0.011). There was a significant correlation between decreased left fronto-parietal functional connectivity and cingulum MD (*r*=-0.364, P=0.002). No significant correlation was identified between decreased salience network functional connectivity and left anterior thalamic radiation MD (Figure 7. 28).

There was a significant indirect effect of cingulum MD and left SLF MD on global cognition through DMN functional disconnection. DMN functional disconnection accounted for 36.19% of the total effect of cingulum MD on global cognition and 32.47% of the total effect of left SLF on global cognition, respectively (Figure 7. 29). DAN functional disconnection mediated the effect of left cingulum MD on global cognition and it accounted for 25.81% of the total effect (Figure 7. 29). Moreover, there was a significant indirect effect of cingulum MD on global cognition through left FPN functional disconnection which accounted for 38.08% of the total effect (Figure 7. 29).

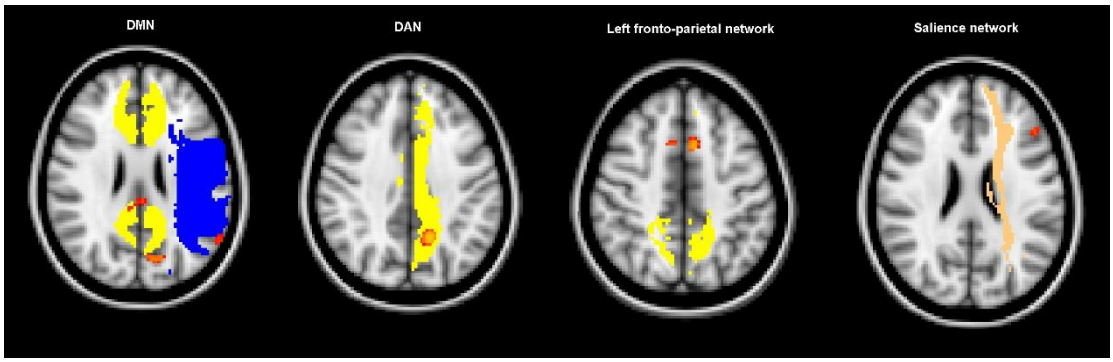



**Figure 7. 27 White matter tracts overlaid with significant clusters showing functional disconnection of the RSNs. Clusters (Red) showing significant difference of RSNs functional connectivity between patients with abnormal cognition and normal cognition were overlaid with specific white matter tracts which underlie or connect to the identified significant clusters (white matter tracts: yellow: cingulum; blue: left superior longitudinal fasciculus; brown: left anterior thalamic radiation).**

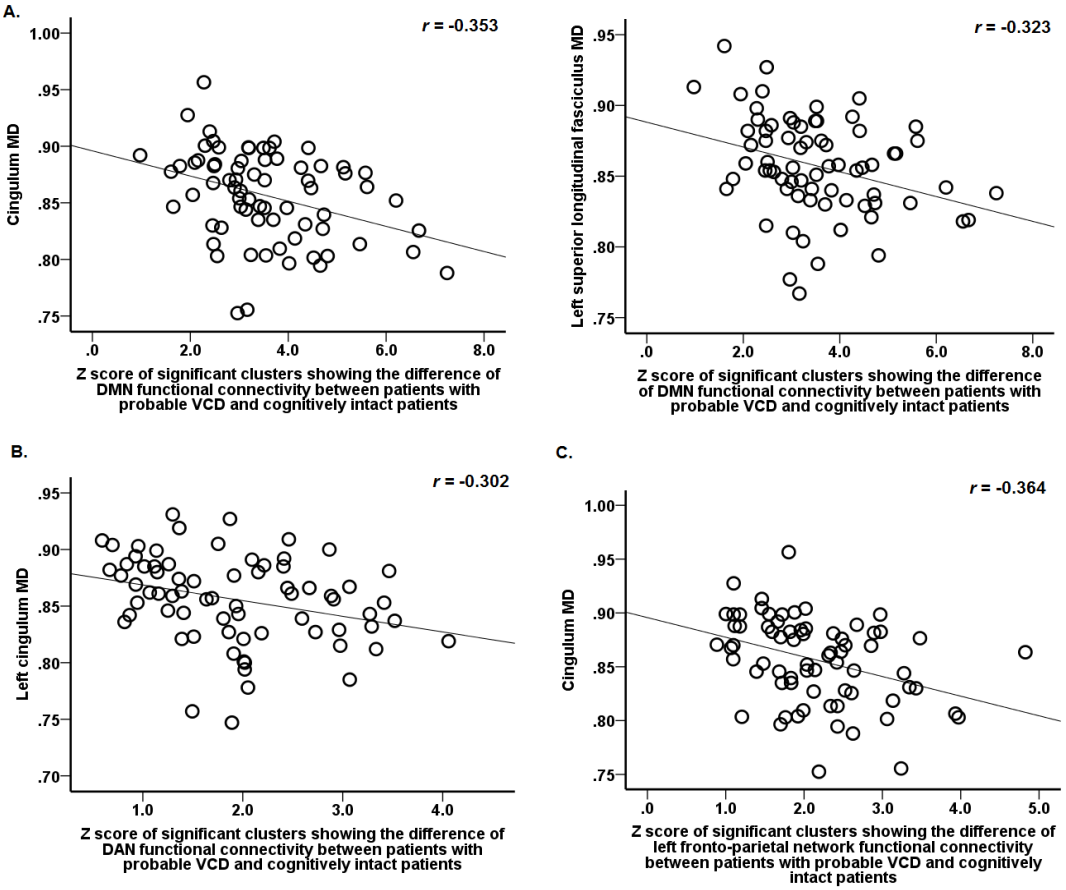



**Figure 7. 28 Scatter plots showed the correlation between decreased RSNs functional connectivity and specific white matter tract microscopic damage. (A) Scatter plots showed the correlation between decreased DMN functional connectivity and cingulum MD and left superior longitudinal fasciculus MD. (B) Scatter plot showed the correlation between decreased DAN functional connectivity and left cingulum MD. (C) Scatter plot showed the correlation between decreased left fronto-parietal network functional connectivity and cingulum MD.**

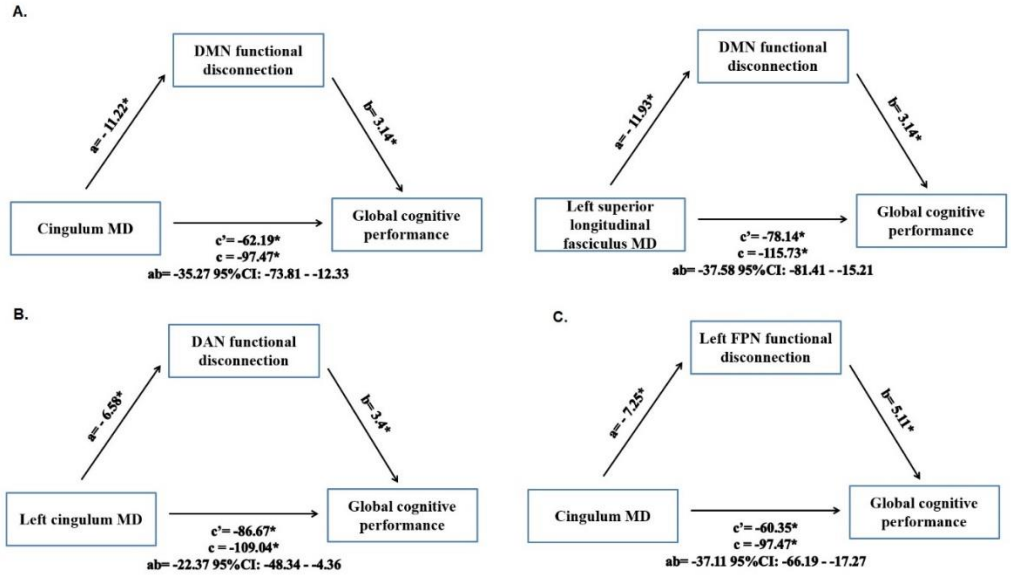



**Figure 7. 29 Mediation model of relationship among (A) DMN functional disconnection, cingulum MD, left SLF MD and global cognitive performance; (B) DAN functional disconnection, left cingulum MD and global cognitive performance and (C) left FPN functional disconnection, cingulum MD and global cognitive performance. Values (a, b, c ,c') presented were unstandardized regression coefficients. Path c represented the indirect effect. Path c' represented the direct effect. ab: indirect effect. *: significant correlation at P<0.05.**

## 7.5 Discussion

This study confirms that functional disconnection of large-scale brain cognitive networks and decreased functional connectivity between specific brain regions with the rest of brain are related to global cognitive impairment in patients with symptomatic carotid artery disease. There is a significant indirect effect of microscopic damage of cingulum and left superior longitudinal fasciculus on global cognitive performance through large-scale cognitive network functional

disconnection. This result suggests that the alterations of large-scale cognitive network functional connectivity underpin association between white matter structural disconnection and global cognition in patients with probable vascular mild cognitive impairment.

The first hypothesis, which stated '*Functional disconnection of large-scale brain cognitive networks is associated with global cognitive impairment in patients with symptomatic carotid artery disease'*, has been upheld in this study. By means of ICA followed by dual regression analysis approach, patients with probable VaMCI was found to exhibit decreased functional connectivity within the large-scale cognitive networks including DMN, DAN, left fronto-parietal network and salience network compared with patients with normal cognition. The identified decreased functional connectivity of DMN which mainly located in the PCC was in line with that in patients with subcortical VCI (Yi, Wang et al. 2012), in normal ageing subjects with amyloid pathology (Sheline, Raichle et al. 2010) and in patients with MCI and AD (Binnewijzend, Schoonheim et al. 2012). The consistent finding of DMN functional disconnection in PCC across patient cohorts with different underlying pathologies highlights that decreased PCC functional connectivity within DMN is not specific to one particular type of pathology. Additionally, our study demonstrated decreased functional connectivity of precuneous and left inferior parietal lobule within DMN in patients with probable VaMCI. Further research are needed to investigate

whether the functional disconnection of precuneous and left inferior parietal lobule is specific to VCI.

Our study provided the first evidence of functional disconnection of DAN, left fronto-parietal network and salience network in patients with probable VaMCI. More specifically, we found DAN functional disconnection that mainly located in the PCC in probable VaMCI patients, which was partly in line with a previous study showing decreased functional connectivity of left frontal eye fields, left inferior parietal lobule, medial dorsal frontal cortex and PCC within DAN in patients with amnestic MCI and AD (Zhang, Zheng et al. 2015). The pattern of functional disconnection within left fronto-parietal network showing as decreased functional connectivity in ACC was different from that in AD showing as functional disconnection in left inferior temporal gyrus (Agosta, Pievani et al. 2012). Functional disconnection of salience network which mainly located in the PCC was shown in patients with probable VaMCI in our study. This was in contrast with the finding of a previous study showing increased functional connectivity of salience network which mainly located in ACC, left prefrontal cortex and left caudate in AD patients (Agosta, Pievani et al. 2012).

Apart from the identified functional disconnection of DMN, DAN, left fronto-parietal network and salience network in patients with probable VaMCI, we also showed a mild to moderate correlation between the functional disconnection of these network and global cognitive performance in all subjects. Altogether, all the evidence identified in this experiment by using ICA analysis approach point

to the role of functional disconnection of large-scale brain networks in patients with probable VaMCI.

Seed-based analyses were conducted in order to investigate the role of brain functional disconnection beyond the large-scale cognitive networks in global cognitive impairment in patients with symptomatic carotid artery disease. We have shown that patients with probable VaMCI exhibited decreased functional connectivity of bilateral hippocampi with the rest of the brain. The hippocampal functional disconnection in patients with vascular pathology has been demonstrated in previous study of which showing inter-hemispheric hippocampal functional connectivity was related to global cognitive impairment in patients with mild stroke (Kliper, Ben Assayag et al. 2016). However, the Kliper and colleagues' study only investigated the role of inter-hemispheric hippocampal functional disconnection in cognition and did not consider the contribution of functional connectivity between hippocampus and the rest of the brain on cognition. Instead, we provided the evidence that the functional connectivity between bilateral hippocampi and the rest of the brain was decreased in patients with probable VaMCI.

Patients with probable VaMCI had decreased bilateral DLPFC functional connectivity as shown in this study. DLPFC, which is part of DAN, is considered as a key region in working and episodic memory, attention and executive function (Spreng, Sepulcre et al. 2013). Previous study demonstrated decreased DLPFC functional connectivity in MCI (Liang, Wang et al. 2011). Our study

provided the first evidence of decreased bilateral DLPFC functional connectivity in patients with probable VaMCI. Additionally, we found that the strength of functional connectivity between bilateral DLPFC and the rest of the brain was related to global cognitive performance. The identification of bilateral DLPFC functional disconnection in turn verified the cognitive profile of VCI showing as frontal dysfunction related cognitive deficits such as impairment of attention, executive function and information processing (Chui 2007).

Our study showed the decreased functional connectivity of cingulate cortex including ACC and PCC in patients with probable VaMCI and the contribution of cingulate functional disconnection in global cognitive impairment in patients with symptomatic carotid artery disease. PCC was considered as a key node of the DMN (Leech and Sharp 2014) therefore the abnormality of PCC functional connectivity could reflect DMN functional disconnection which has been verified in this experiment using ICA analysis approach. The PCC functional disconnection was also found in a group of cognitively impaired patients with subcortical ischaemic vascular disease (Sun, Qin et al. 2011). The pattern of PCC functional disconnection identified in Sun and colleague study, showing as decreased functional connectivity between PCC and left middle temporal gyrus, ACC, left middle frontal gyrus, right caudate, right middle frontal gyrus, was similar with the pattern of PCC functional disconnection identified in our study. Our findings, in combination of those from Sun et al. (2011), strengthened the role of PCC functional disconnection in VCI. However, PCC functional

disconnection was not specific in VCI provided by the evidence from studies showing decreased PCC functional connectivity in MCI and AD (Wang, Liang et al. 2012) (Zhang, Wang et al. 2009).

According to our results, large-scale cognitive network functional disconnection was mainly related to microscopic damage of underlying white matter tracts rather than the burden of cerebrovascular lesions within the specific underlying white matter tracts. Our findings could be explained by the fact that structural connectivity is correlated with functional connectivity therefore the microscopic damage of white matter could alter functional connectivity (Honey, Sporns et al. 2009). The correlation between functional and structural connectivity has also been identified in ageing (Burzynska, Wong et al. 2015). More importantly, we demonstrated that there was a significantly indirect effect of specific white matter tracts on global cognition through large-scale cognitive network functional disconnection in patients with symptomatic carotid artery disease. Specifically, we identified that the functional disconnection of DMN, DAN and left fronto-parietal network were mediators for the association between cingulum MD and global cognition in addition to that DMN functional disconnection was a mediator for the association between left SLF and global cognition. The results from this study, together with those from our previous study which demonstrated structural disconnection in probable VaMCI (Meng, Hosseini et al. 2016) (Chapter 6 and Chapter 7) suggested that RSNs functional disconnection mediated the effects of structural disconnection on cognition in

probable VaMCI, thus meaning that the alteration of large-scale cognitive network functional connectivity could explain the association between structural disconnection and cognition in probable VaMCI. Further research that develop interventions for VCI could focus on the efficacy of the interventions to improve large-scale cognitive network functional connectivity as a possible mean to mitigate structural disconnection and thus enhance cognition.

### 7.6 Conclusion

Functional disconnection within and beyond large-scale brain networks is related to global cognitive impairment in patients with symptomatic carotid artery disease. Large-scale cognitive network functional disconnection mediates the association between structural disconnection and cognition in probable vascular cognitive impairment.

# Chapter 8 Neuroimaging in fluency impairment in patients with vascular cognitive impairment


## Abstract

Given that executive function and processing speed are predominantly impaired in vascular cognitive impairment (VCI), the aim of this chapter is to provide preliminary findings of the role of cerebrovascular lesional damage, non-lesional microscopic damage and functional disconnection of large-scale cognitive networks in fluency. Fluency impairment in patients with symptomatic carotid artery disease was associated with (1) lacunar infarction volumes, chronic subcortical ischaemic lesion volumes and total ischaemic lesion volumes; (2) presence of subcortical ischaemic lesions in anterior thalamic radiation and superior longitudinal fasciculus; (3) damage severity of infarctions and chronic subcortical ischaemic lesions; (4) thalamic MD; (5) MD of anterior thalamic radiation, superior longitudinal fasciculus and white matter tract skeleton; (6) executive control network functional disconnection, ACC functional disconnection and bilateral DLPFC functional disconnection.

The preceding chapters demonstrated the cerebrovascular lesional damage, non-lesional microscopic damage and functional disconnection of large-scale cognitive networks were associated with global cognitive impairment in patients with symptomatic carotid artery disease. Given that executive function and processing speed are predominantly impaired in vascular cognitive impairment (VCI), this chapter will summarise preliminary findings of the role of cerebrovascular lesional damage, non-lesional microscopic damage and functional disconnection of large-scale cognitive networks in fluency performance measured by ACE-R. However, the cut-off score of fluency has not been confirmed in previous studies and it is still not clear whether using ACE-R to measure the performance of cognitive domains in patients with symptomatic carotid artery disease is accurate, the results provided in this chapter are preliminary and needed to be interpreted with caution.

## 8.1 The role of cerebrovascular lesional damage in fluency

This sub-chapter included the analyses of the associations between cerebrovascular lesion burden, lesion topography, lesion damage severity and fluency impairment in patients with symptomatic carotid artery disease.

### 8.1.1 The role of cerebrovascular lesion burden in fluency

The association between cerebrovascular burden and fluency was investigated in the cohort including 108 subjects. As stated in Chapter 4, 40 subjects (37%) had impaired fluency impairment. The method to measure cerebrovascular

lesion volumes was described in Chapter 4.

There was a significant association between volumes or presence of lacunar infarctions, chronic subcortical ischaemic lesions, total ischaemic lesions and fluency performance (Table 8. 1). More specifically, there was significant difference of the presence of lacunar infarctions between patients with normal and abnormal fluency (P=0.003).The normalised volumes of lacunar infarctions were significantly correlated with fluency performance when age was controlled ($r$=-0.272, P=0.005). The normalised volumes of chronic subcortical ischaemic lesions were significantly different between patients with normal and abnormal fluency (P=0.005). Patients with abnormal fluency had larger volumes of chronic subcortical ischaemic lesions when age was controlled ($r$=-0.258, P=0.007) compared with patients with normal fluency. Also, total ischaemic lesion volumes were significantly different between patients with normal and abnormal fluency (P<0.001). There was significant correlation between the total ischaemic lesion volumes and fluency performance when age was controlled ($r$=-0.300, P=0.002).

**Table 8. 1 The role of lesion characteristics in fluency**

| | Normal fluency n=68 | Abnormal fluency n=40 | P[f] | *r* [g] | P |
|---|---|---|---|---|---|
| **Presence of lacunar Infarctions[a], n (%)** | 9(13.2) | 15(37.5) | 0.003* | - | - |
| **Presence of acute lesions[b], n (%)** | 25(36.8) | 21(52.5) | 0.110 | - | - |
| **Presence of cortical infarctions[c], n (%)** | 1(1.5) | 3(7.5) | 0.109 | - | - |
| **Normalised lacunar infarction volumes,%, mean(SD) × $10^3$** | 0.7(2.4) | 3.7(7.5) | 0.002* | -0.272 | 0.005* |
| **Normalised acute lesion volumes,%, mean(SD) × $10^2$** | 3.3(7.6) | 5.9(10.5) | 0.169 | -0.175 | 0.072 |
| **Normalised chronic subcortical ischaemic lesion[d] volumes,%, mean(SD) × 10** | 8.5(10.8) | 17.0(16.3) | 0.005* | -0.258 | 0.007* |
| **logTILL[e]** | -5.6(1.5) | -4.6(1.2) | <0.001* | -0.300 | 0.002* |

**a:** acute lesions are defined as hyperintensities on b1000 images but hypointensities on MD maps.

**b:** lacunar infarctions are defined as small (<2cm) hypointense subcortical areas on FLAIR images.
**c:** cortical infarctions are defined as cortical FLAIR hypointensities.
**d:** chronic subcortical ischaemic lesions are defined as subcortical FLAIR hyperintensities.
**e:** logTILL=log(total ischaemic lesion volumes/total brain volumes).
**f:** P value of comparison between patients with normal and abnormal fluency performance.
**g**: Correlation between fluency and normalised lesion volumes, controlled for age
***:** Significant at P <0.05.

### 8.1.2 Cerebrovascular lesion mapping for fluency

The method to generate frequency maps of infarctions and chronic subcortical ischaemic lesions for patients with normal and abnormal fluency was described in Chapter 5. Cerebrovascular lesion maps were created in the cohort including 108 subjects.

Based on visual inspection, patients with abnormal fluency had more extended pattern of infarctions (Figure 8. 1). Chronic subcortical ischaemic lesions were more prevalent in the periventricular and deep white matter but less frequent in subcortical brain areas in both normal and abnormal fluency subgroups (Figure 8. 2). The anatomical distribution of infarctions and chronic subcortical ischaemic lesions was not statistically different between patients with normal and abnormal fluency ($P>0.05$ FDR).

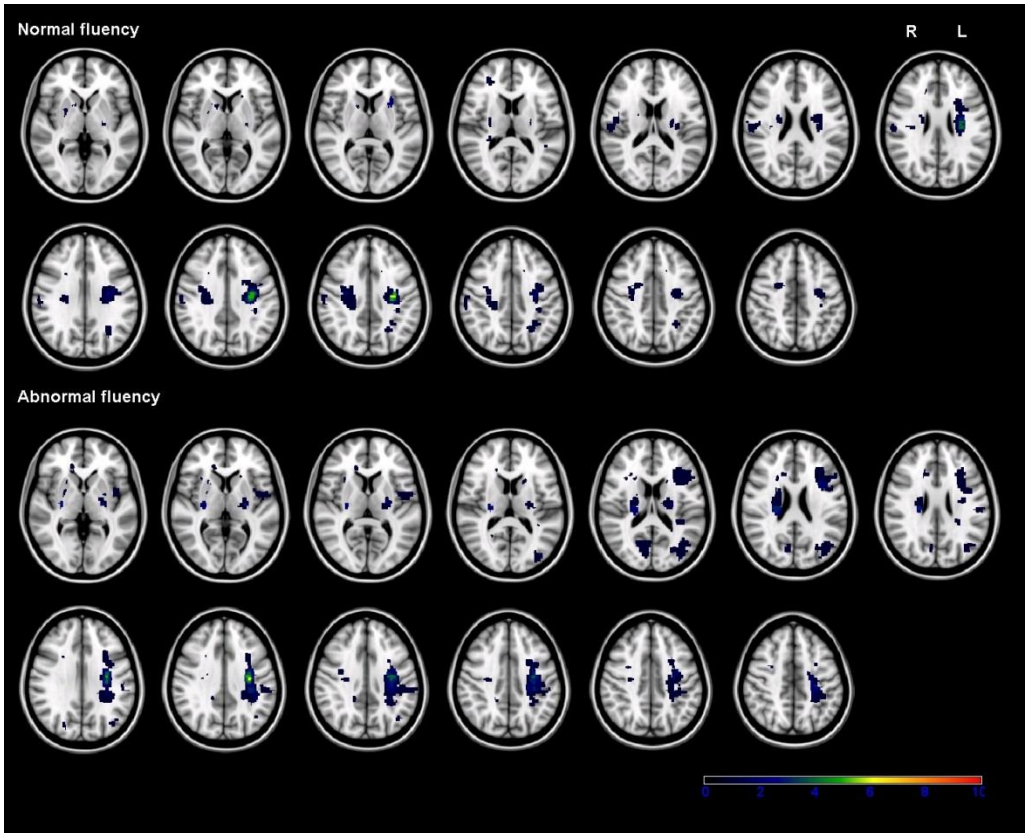



**Figure 8. 1 Infarction maps in patients with normal and abnormal fluency. Maps were superimposed onto the 1×1×1 mm MNI 152 T1 template. Infarctions included acute lesions, lacunar infarctions and cortical infarctions. The colour bars showed variations between the minimum and maximum number of lesions.**

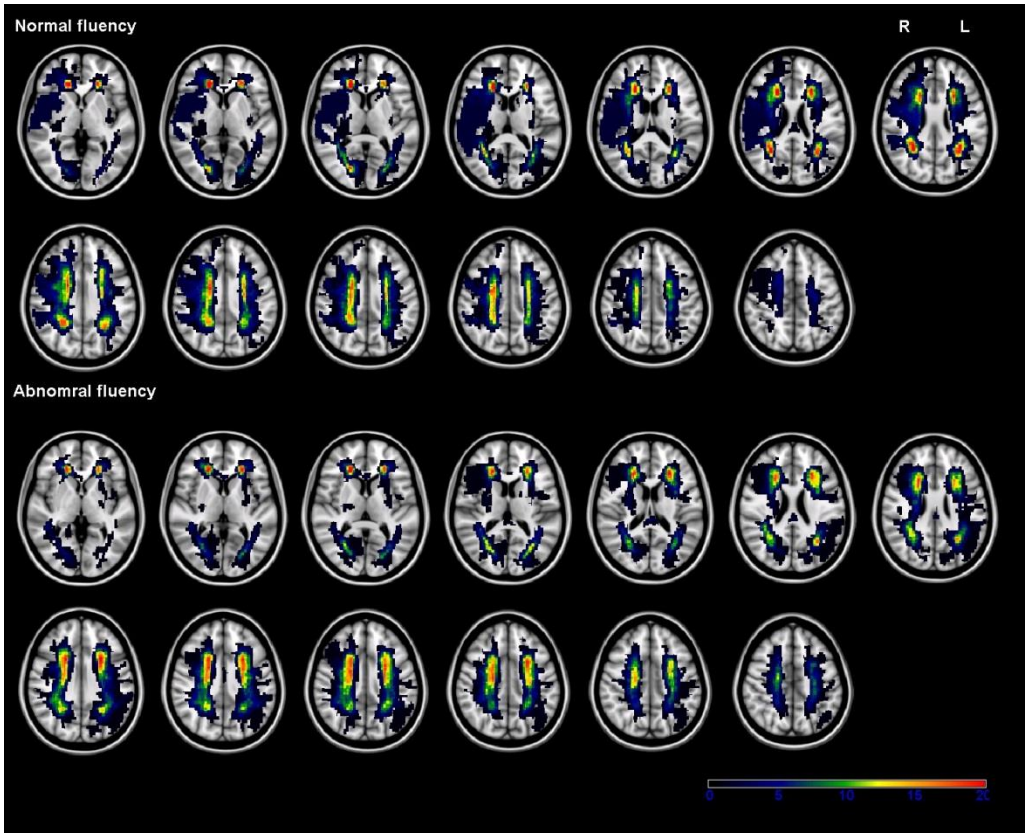



**Figure 8. 2 Chronic subcortical ischaemic lesion maps in patients with normal and abnormal fluency. Maps were superimposed onto the 1×1×1 mm MNI 152 T1 template. Chronic subcortical ischaemic lesions included subcortical hyperintensities on FLAIR images. The colour bars showed variations between the minimum and maximum number of lesions.**

### 8.1.3 The role of cerebrovascular lesion topography in fluency

VLSM method was applied to investigate the association between lesion topography and fluency in the cohort including 108 subjects. The details about applying VLSM method were provided in Chapter 5.

VLSM identified totally 1351 voxels in which chronic subcortical ischaemic lesions were significantly related to fluency after controlling for age, logTILL and correcting for multiple comparisons (FDR corrected $P<0.05$). These significant clusters mainly located in bilateral frontal lobe and projected on anterior thalamic radiation and superior longitudinal fasciculus (Figure 8. 3). No significant clusters of infarctions were identified for fluency.

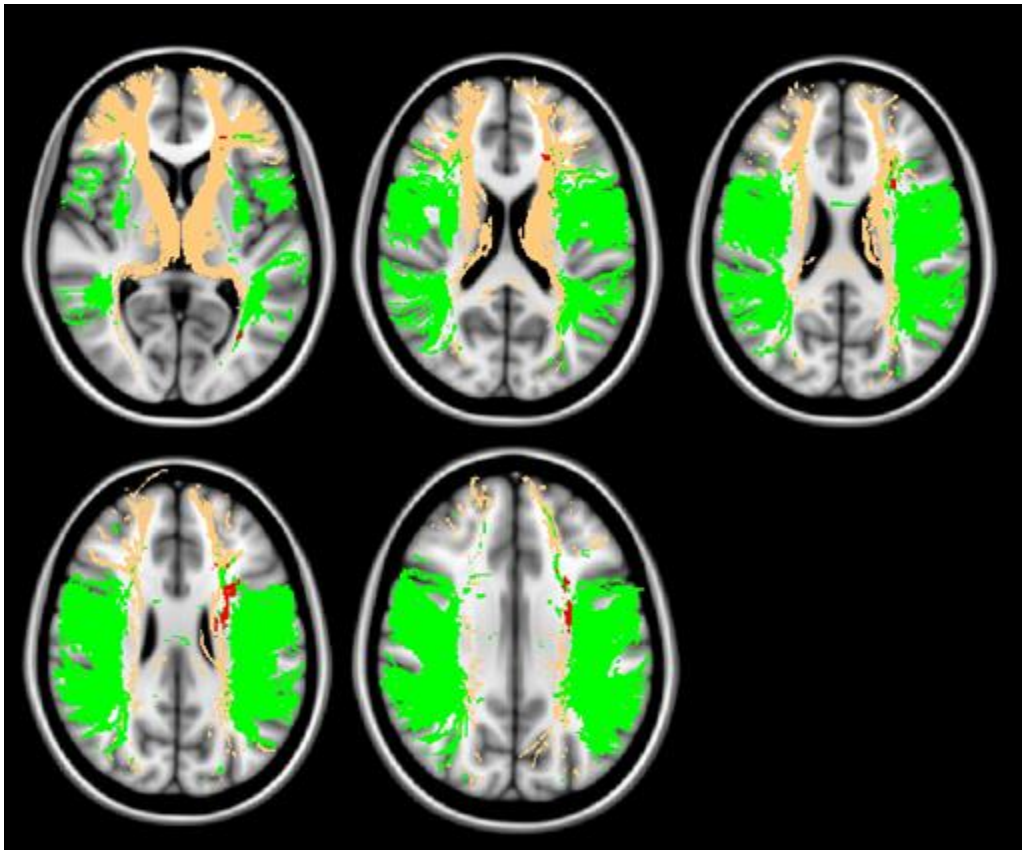


**Figure 8. 3 Topographical chronic ischemic lesion associations with fluency. Voxel-based lesion-symptom mapping revealed significant clusters of voxels (red) in bilateral anterior thalamic radiation and superior longitudinal fasciculus. The presence of chronic subcortical ischaemic lesions in these voxels was related to fluency after controlling for age, the performance of other cognitive domains and logTILL, p< 0.05 (FDR corrected). Significant clusters were projected onto the MNI 152 standard space T1 template and white matter tracts extracted from JHU-ICBM white matter atlas (Brown: anterior thalamic radiation; Green: superior longitudinal fasciculus).**

### 8.1.4 The role of severity of cerebrovascular damage in fluency

Infarction MD was significantly different between patients with normal and abnormal fluency (P=0.006) and correlated with fluency performance when age was controlled (r=-0.357, P<0.001). Patients with abnormal fluency had increased MD of chronic subcortical ischaemic lesions (P=0.026). There was a significant association between chronic subcortical ischaemic lesions MD and fluency performance when age was controlled (r=-0.211, P=0.029) (Table 8. 2).

**Table 8. 2 The role lesion damage severity in fluency**

| | Normal fluency n=68 | Abnormal fluency n=40 | P[a] | r [b] | P |
|---|---|---|---|---|---|
| **Infarctions MD $\times 10^{-9}$ $m^2$ $s^{-1}$, mean(SD)** | 0.31(0.39) | 0.52(0.37) | 0.006* | -0.357 | <0.001* |
| **Chronic subcortical ischaemic MD$\times 10^{-9}$ $m^2$ $s^{-1}$, mean(SD)** | 1.00(0.10) | 1.04(0.11) | 0.026* | -0.211 | 0.029* |

**a:** P value of comparison of FA and MD values of infarctions and chronic subcortical ischaemic lesions between patients with normal and abnormal fluency
**b:** Correlation coefficient between FA/MD of infarctions and chronic subcortical ischaemic lesions with fluency performance, controlled for age
*: Significant level at P<0.05

## 8.2 The role of non-lesional damage in fluency

The sub-chapter included the analyses of the associations between impairment of fluency and microscopic damage of strategic brain regions, strategic white matter tracts, NAWM and white matter tract skeleton.

### 8.2.1 The role of microscopic damage of strategic brain regions in fluency

The method to extract hippocampal and thalamic MD was described in Chapter 5. The association between microscopic damage of hippocampus and thalamus and fluency impairment was investigated in the cohort including 108 subjects.

Microscopic damage of thalamus but not hippocampus had an effect on fluency. More specifically, averaged thalamic MD was significantly different between patients with normal and abnormal fluency (P=0.007) and was significantly correlated with fluency performance when controlled for age (r=-0.242, P=0.012). No association between hippocampal MD and fluency performance was identified (Table 8. 3).

**Table 8. 3 The role of hippocampal and thalamic damage severity on fluency**

| | Normal fluency n=68 | Abnormal fluency n=40 | P[a] | r[b] | P |
|---|---|---|---|---|---|
| **Averaged hippocampal MD $\times 10^{-9}$ $m^2$ $s^{-1}$, mean(SD)** | 0.76(0.04) | 0.77(0.04) | 0.142 | -0.115 | 0.237 |
| **Averaged thalamic MD$\times 10^{-9}$ $m^2$ $s^{-1}$, mean(SD)** | 0.86(0.03) | 0.87(0.03) | 0.007* | -0.242 | 0.012* |

**a:** P value of comparison of FA and MD values of hippocampus and thalamus between patients with normal and abnormal fluency
**b:** Correlation coefficient between FA/MD of hippocampus and thalamus with fluency performance, controlled for ag

### 8.2.2 The role of microscopic damage of strategic white matter tracts in fluency

According to the VLSM results, anterior thalamic radiation and superior longitudinal fasciculus were identified as strategic white matter tracts for fluency, meanwhile, forceps major and anterior thalamic radiation were considered as strategic white matter tracts for memory. The method to extract MD values of anterior thalamic radiation, superior longitudinal fasciculus and forceps major was described in Chapter 6.

The microscopic damage of strategic white matter tracts had an effect on fluency performance. More specifically, patients with abnormal fluency had increased MD of anterior thalamic radiation ($P<0.001$) and superior longitudinal fasciculus ($P<0.001$) compared with patients with normal fluency. Anterior thalamic radiation MD ($r=-0.347$, $P<0.001$) and superior longitudinal fasciculus($r=-0.334$, $P<0.001$) was significantly correlated with fluency performance when age and normalised total ischaemic lesion volumes within specific white matter tracts were controlled (Table 8. 4).

**Table 8. 4 The role of microscopic damage of anterior thalamic radiation and superior longitudinal fasciculus on fluency**

| | Normal fluency n=68 | Abnormal fluency n=40 | P[a] | r [b] | P |
|---|---|---|---|---|---|
| **Anterior thalamic radiation MD×$10^{-9}$ $m^2$ $s^{-1}$, mean(SD)** | 0.85(0.03) | 0.88(0.04) | <0.001* | -0.347 | <0.001* |
| **Superior longitudinal fasciculus MD×$10^{-9}$ $m^2$ $s^{-1}$, mean(SD)** | 0.84(0.03) | 0.87(0.03) | <0.001* | -0.334 | <0.001* |

**a:** P value of comparison of FA and MD values of anterior thalamic radiation and superior longitudinal fasciculus between patients with normal and abnormal fluency

b: correlation between fluency and FA/MD of anterior thalamic radiation and superior longitudinal fasciculus, controlled for the performance of other domains, age and TILL within specific white matter tract

*: significant level at P <0.05

### 8.2.3 The role of microscopic damage of NAWM in fluency

The approach to extract NAWM MD values was described in Chapter 6.

There was significant difference of NAWM MD between patients with normal and abnormal fluency (P=0.024) while there was no significant correlation between NAWM MD and fluency performance when age and logTILL were controlled (Table 8. 5).

**Table 8. 5 The role of NAWM microscopic damage on fluency**

| | Normal fluency n=68 | Abnormal fluency n=40 | P[a] | r [b] | P |
|---|---|---|---|---|---|
| NAWM MD $\times 10^{-9}$ $m^2$ $s^{-1}$, mean(SD) | 0.76(0.04) | 0.77(0.04) | 0.024* | -0.101 | 0.305 |

**a:** P value of comparison of FA and MD values of NAWM between patients with normal and abnormal fluency
**b**: correlation between fluency and FA and MD of NAWM, controlled for age and logTILL

### 8.2.4 The role of microscopic damage of white matter tract skeleton in fluency

TBSS approach, described in Chapter 6, was applied to investigate the role of microscopic damage of white matter tract skeleton in fluency.

Increased MD of white matter tract skeleton, mainly in the frontal lobe, was significantly correlated with fluency impairment when controlled for age and logTILL (Figure 8. 4).

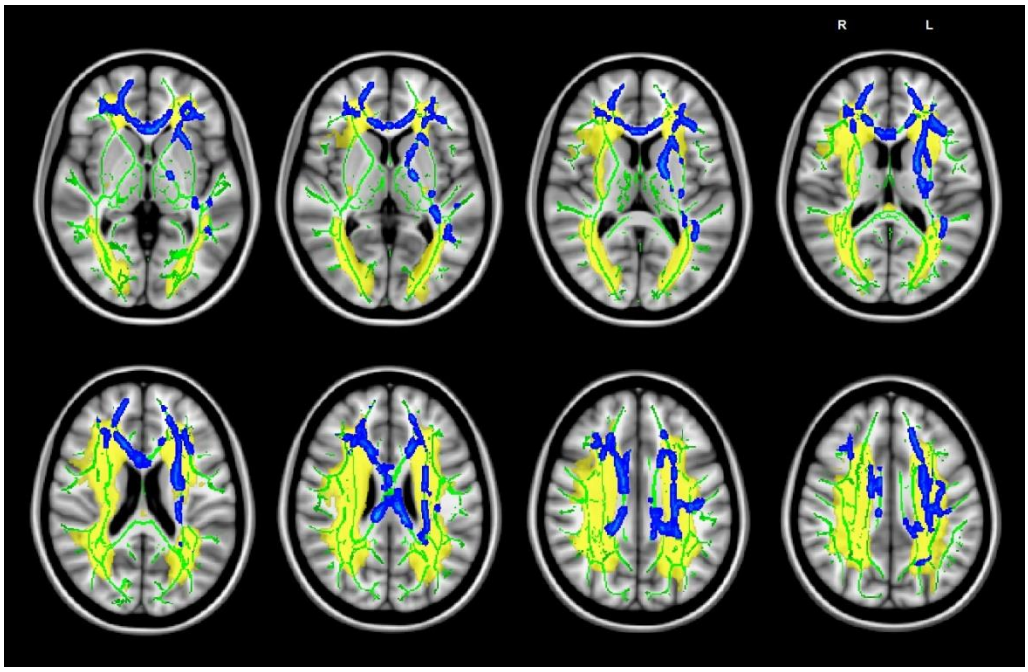



**Figure 8. 4 TBSS results for correlations between MD and fluency. Significant skeletal voxels (Blue) where increased MD correlated with fluency performance. All analyses controlled for the age and logTILL. Significant level was set at P < 0.05 (corrected for multiple comparisons). Mean TBSS white matter tract skeleton (Green) and total ischemic lesion distribution map (Yellow) were overlaid on the MNI 152 template.**

After extracting MD values from white matter tract skeleton, patients with abnormal fluency showed increased MD of white matter tract skeleton compared with patients with normal fluency (P<0.001). Also, there was a mild correlation between white matter tract skeleton MD and fluency when age and logTILL were controlled (r=-0.341, P<0.001) (Table 8. 6).

**Table 8. 6 The role of microscopic damage of white matter tract skeleton in fluency**

| | Normal fluency n=68 | Abnormal fluency n=40 | P[a] | r [b] | P |
|---|---|---|---|---|---|
| white matter skeleton MD $\times 10^{-9}$ $m^2$ $s^{-1}$, mean(SD) | 0.79(0.04) | 0.83(0.05) | <0.001* | -0.341 | <0.001* |

**a:** P value of comparison of FA and MD values of white matter skeleton between patients with normal and abnormal fluency
b: correlation between fluency and FA and MD of white matter tract skeleton, controlled for age and logTILL
*: significant level at P value < 0.05

## 8.3 The role of functional disconnection of large-scale cognitive networks in fluency

### 8.3.1 The role of functional disconnection in fluency

ICA and followed by dual regression approach was used to investigate the difference of resting-state network functional connectivity between patients with normal and abnormal performance of fluency. The details were described in Chapter 7. Seed regions, including bilateral hippocampus, bilateral DLPFC, PCC and ACC were selected to conduct seed-based analysis for fluency. The details were described in Chapter 7. Analyses were conducted in the cohort including 72 subjects.

Patients with abnormal fluency had decreased functional connectivity of executive control (EC) network, with significant clusters located in the PCC (Figure 8. 5). However, there was no significant correlation between functional disconnection of EC network and fluency performance.

When ACC was selected as a seed region, the functional connectivity between ACC and right superior temporal gyrus, bilateral lingual gyrus, left inferior occipital gyrus, right middle frontal gyrus, right precentral and postcentral gyrus and precuneous was decreased in patients with abnormal fluency compared with patients with normal fluency after age and mean relative displacement were controlled. The ACC functional disconnection was significantly correlated with fluency performance ($r$=0.366, P=0.002, Figure 8. 6).

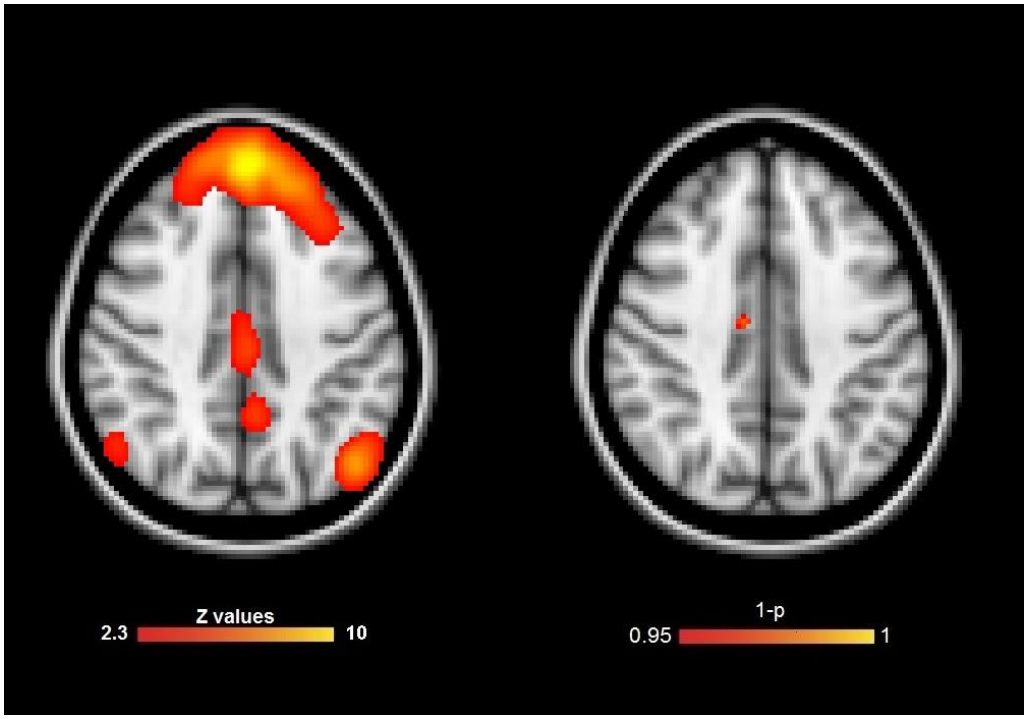



**Figure 8. 5 Functional disconnection of RSNs associated with fluency. Left: functional connectivity of EC network in the whole study cohort. Right: Significantly decreased fc of EC network in patients with abnormal fluency vs normal fluency. Colour bars for the images in the left side and right show the range of Z values and P values respectively. All tests age-corrected.**

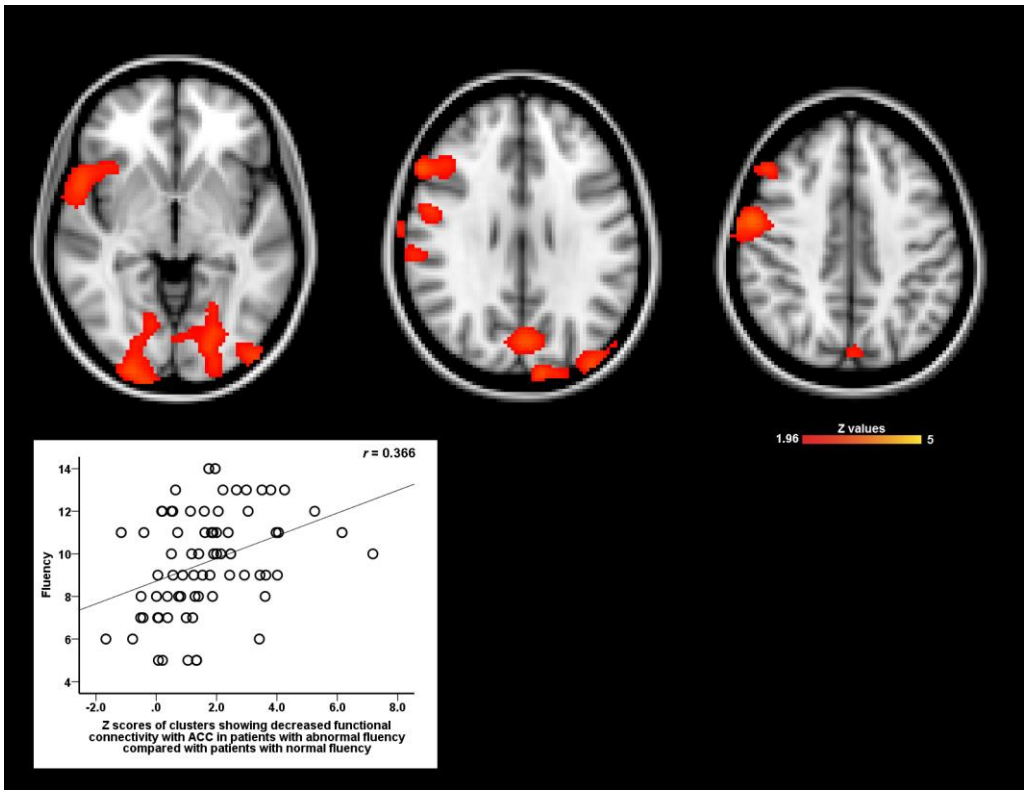



**Figure 8. 6 ACC functional connectivity associated with fluency performance. Decreased functional connectivity between ACC and significant clusters (Red) was found in patients with abnormal fluency compared with patients with normal fluency. The scatter plot showed the correlation between ACC functional connectivity and fluency performance. All analyses controlled for age and mean relative displacement. Z scores were colour coded from red to yellow. The Z score maps were overlaid on MNI 2mm template.**

When left DLPFC was chose as a seed, there was no group difference of left DLPFC functional connectivity between patients with normal and abnormal fluency after age and mean relative displacement were adjusted. However, the strength of functional connectivity between left DLPFC and precuneous, left middle occipital gyrus was significantly correlated with fluency performance after controlling for age and mean relative displacement (Figure 8. 7).

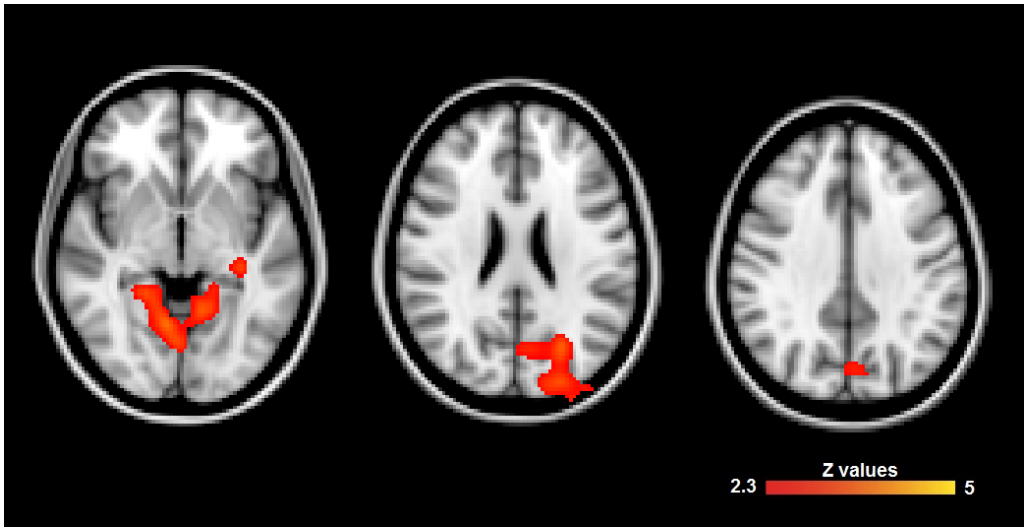



**Figure 8. 7 Left DLPFC functional connectivity associated with fluency performance. The strength of functional connectivity between left DLPFC and significant clusters (red) was significantly correlated with fluency performance. The analysis was controlled for age and mean relative displacement. Z scores were colour-coded from red to yellow. The Z scores maps were overlaid on MNI 2mm template.**

When right DLPFC was selected as a seed, patients with abnormal fluency had decreased functional connectivity between right DLPFC and precuneous, left middle temporal gyrus compared with patients with normal fluency when age and mean relative displacement was controlled. The right DLPFC functional disconnection was significantly related to fluency performance ($r$=0.437, P<0.001, Figure 8. 8). Additionally, the strength of functional connectivity between right DLPFC and precuneous, left superior parietal lobule was associated with fluency performance after adjusted for age and mean relative displacement (Figure 8. 9).

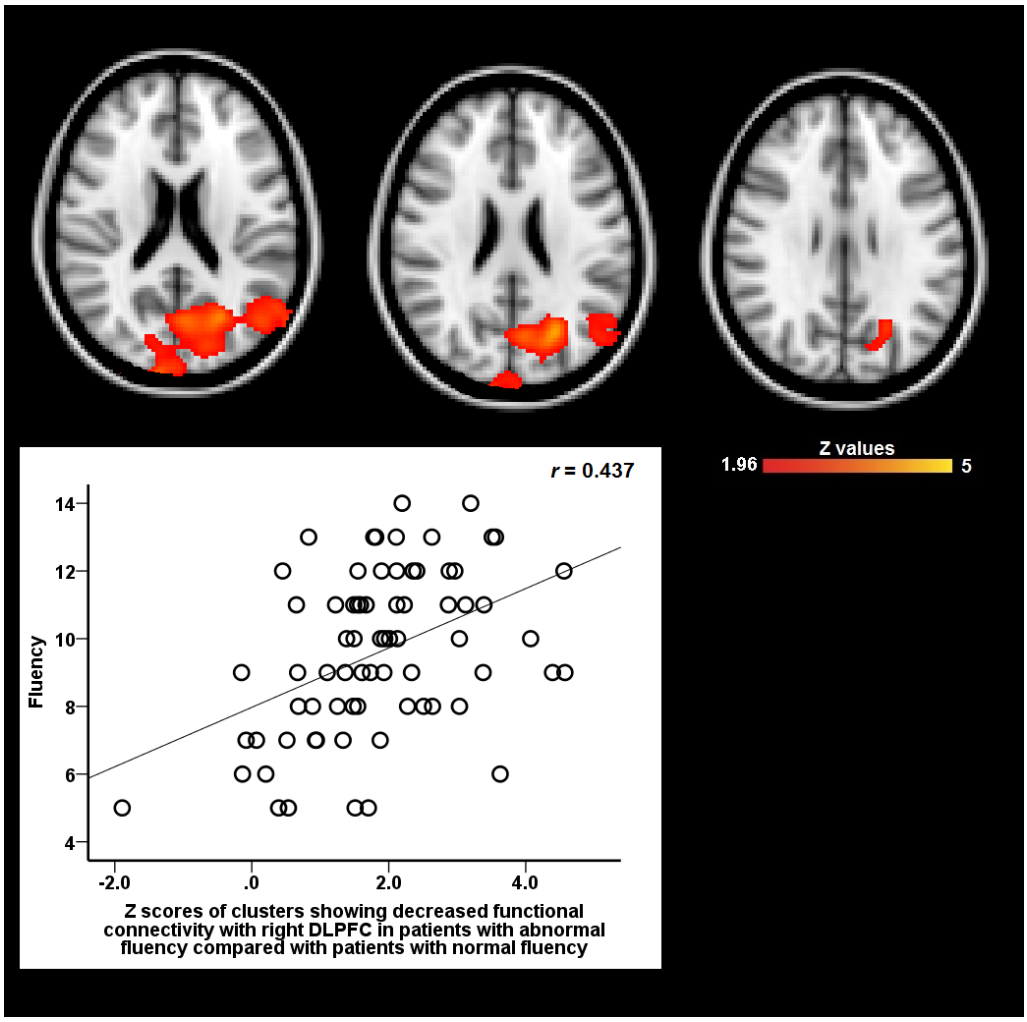



**Figure 8. 8 Right DLPFC functional connectivity associated with fluency performance. Patients with abnormal fluency had decreased functional connectivity between right DLPFC and significant clusters (red) compared with patients with normal fluency. The scatter plot showed significant correlation between right DLPFC functional connectivity and fluency performance. The analysis was controlled for age and mean relative displacement. Z scores were colour-coded from red to yellow. The Z scores maps were overlaid on MNI 2mm template.**

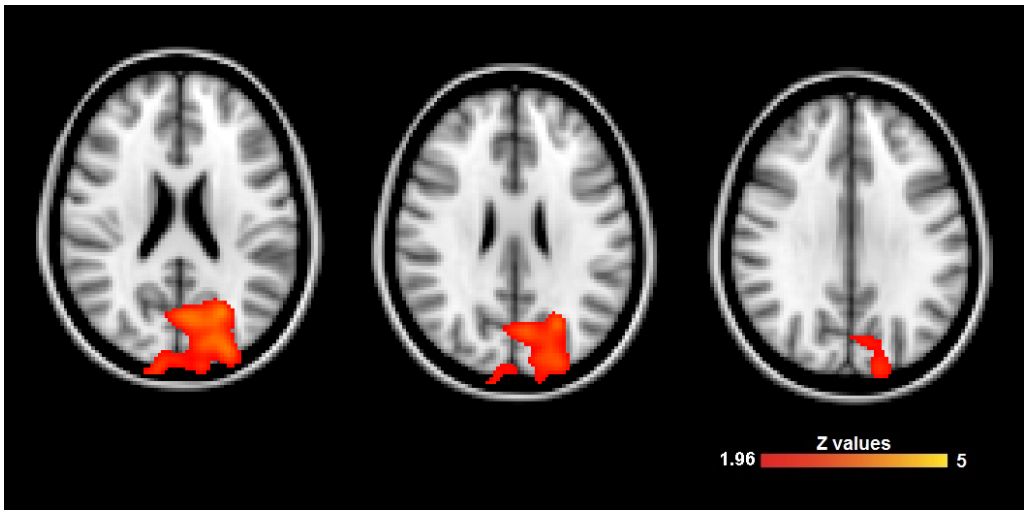



**Figure 8. 9 Correlation between the strength of right DLPFC functional connectivity associated with fluency performance. The strength of functional connectivity between right DLPFC and significant clusters (red) was significantly correlated with fluency performance. The analysis was controlled for age and mean relative displacement. Z scores were colour-coded from red to yellow. The Z scores maps were overlaid on MNI 2mm template.**

### 8.3.2 Correlation between fluency related RSNs functional disconnection, microscopic damage of underlying white matter tracts, and total ischaemic lesion volumes within specific white matter tracts

The identified clusters showing significant decreased EC functional connectivity in patients with abnormal fluency were overlaid with cingulum. The normalised total ischaemic lesion (TILL) volumes within network specific white matter tracts were unrelated to functional disconnection (fluency related decreased EC functional connectivity and TILL volumes within cingulum: P=0.061). There was no significant correlation between EC functional disconnection and cingulum MD.

## 8.4 Discussion

In this study, fluency impairment in patients with symptomatic carotid artery disease is associated with (1) lacunar infarction volumes, chronic subcortical ischaemic lesion volumes and total ischaemic lesion volumes; (2) presence of subcortical ischaemic lesions in anterior thalamic radiation and superior longitudinal fasciculus; (3) damage severity of infarctions and chronic subcortical ischaemic lesions; (4) thalamic MD; (5) MD of anterior thalamic radiation, superior longitudinal fasciculus and white matter tract skeleton; (6) executive control network functional disconnection, ACC functional disconnection and bilateral DLPFC functional disconnection.

Volumes of lacunar infarctions, chronic subcortical ischaemic lesions and total ischaemic lesions were found to associate with fluency performance,

independent of the effect of age and the total volumes of brain tissues. Our results are in line with those from previous studies showing the significant interrelation between lacunar infarction volumes and executive function in VCI (Liem, van der Grond et al. 2007) and that between numbers of lacunar infarctions and executive function in patients with atherosclerotic disease (Geerlings, Appelman et al. 2009). Additionally, the significant relationship between chronic subcortical ischaemic lesion load and fluency was consistent with that identified in previous studies (Liem, van der Grond et al. 2007) (Geerlings, Appelman et al. 2009). The total ischaemic lesion volumes were found to be related with fluency performance, which might be explained by the cumulative effects of lacunar infarctions and chronic subcortical ischaemic lesions on fluency.

Chronic subcortical ischaemic lesions located in the frontal areas were found to be associated with fluency impairment. This finding was consistent with a previous study showing the dysfunction of letter fluency performance was related to lesions located in the anterior brain regions while dysfunction of category fluency performance was associated with lesions located in the posterior brain regions in left-hemispheric stroke patients (Baldo, Schwartz et al. 2006). The identified strategic locations were found to be located in the anterior thalamic radiation and superior longitudinal fasciculus. The identification of the strategic role of anterior thalamic radiation in fluency performance is supported by the fact that the anterior thalamic radiation carries white matter fibre tracts from the thalamus to the prefrontal cortex which is thought to be involved in executive function (Zoppelt, Koch et al. 2003). The strategic role of superior longitudinal fasciculus, which connects the frontal lobe to the occipital and part of the parietal and temporal lobes (Bruni, Montemurro et al. 2009), in fluency

identified in this study is in line with the results from previous studies. For instance, it has been shown that integrity of superior longitudinal fasciculus was correlated with executive function in normal ageing (Sasson, Doniger et al. 2013). Additionally, the role of superior longitudinal fasciculus in executive function has been found in patients with symptomatic atherosclerotic disease (Biesbroek, Kuijf et al. 2013). Previous study has demonstrated that white matter lesions cause executive dysfunction via the cortical disconnection mediated by specific white matter tracts in VCI (Smith, Salat et al. 2011). The results present in this thesis verified the role of disconnection in VCI and additionally highlighted that disconnection of cognitive networks which connect by anterior thalamic radiation and superior longitudinal fasciculus was related to impaired fluency performance in patients with advanced atherosclerosis.

A modest association was identified between microscopic damage of anterior thalamic radiation and superior longitudinal fasciculus and fluency impairment in patients with symptomatic carotid artery disease. Meanwhile, there was a significant association between total ischaemic lesion volumes within anterior thalamic radiation and its microscopic damage (Chapter 6). Therefore, it is plausible that the association between fluency impairment and microscopic damage of anterior thalamic radiation may be partly influenced by the total ischaemic lesion volumes within anterior thalamic radiation. In contrast no significant correlation was identified between total ischaemic lesion volumes within superior longitudinal fasciculus and fluency impairment. Thus, the contribution of microscopic damage of superior longitudinal fasciculus was not caused by the total ischaemic lesion volumes within it. However, the contribution of microscopic damage of superior longitudinal fasciculus on

fluency impairment might be explained by the evidence from a previous study showing that diffusional measures of superior longitudinal fasciculus was correlated with executive function in healthy subjects (Sasson, Doniger et al. 2013). It means that microscopic damage of superior longitudinal fasciculus directly may contribute to fluency impairment.

As shown in our results, microscopic damage of white matter tract skeleton which mainly locate in the frontal lobe was associated with fluency performance. This finding agreed with that from a study showing that white matter disconnection in frontal lobe explains executive dysfunction in CADASIL (Craggs, Yamamoto et al. 2014). It makes sense that disconnection of frontal white matter contributes to VCI, given the reason that the cognitive profile of VCI is closely related to frontal dysfunction (Chui 2007).

Patients with abnormal fluency showed decreased EC functional disconnection which located in the PCC. The functional disconnection of EC network has been identified in patients with amnestic MCI and the strength of the EC network functional disconnection located in the ACC and right DLPFC was correlated with executive function (Wu, Soder et al. 2014). The functional disconnection of bilateral DLPFC and ACC identified in our studies also indirectly reflected the functional disconnection of EC network because bilateral DLPFC and ACC was part of the EC network. Altogether, this study showed the functional disconnection of executive function related network in patients with abnormal fluency. However, we did not identify any association between EC network functional disconnection and underlying white matter tract microscopic damage, meaning that the EC network functional disconnection could not be explained by underlying structural disconnection. Future research thus are needed to

investigate what drives the functional disconnection in patients with abnormal fluency.

### 8.5 Conclusion

This chapter showed preliminary results of the association between lesional damage, non-lesional damage, functional disconnection and impairment of fluency in patients with symptomatic carotid artery disease. Future studies are needed to use comprehensive neuropsychological battery to measure the performance of each cognitive domain and investigate the role of neuroimaging measures on performance of cognitive domains in patients with vascular cognitive impairment.

# Chapter 9 Discussion

## 9.1 Summary of the results

The primary aim of this study was to use advanced neuroimaging techniques to understand vascular cognitive impairment in a group of patients with symptomatic carotid artery disease.

In patients with symptomatic carotid artery disease, global cognitive performance is related to (1) the presence of chronic subcortical ischaemic lesion location in the thalamic radiation and interhemispheric white matter tracts; (2) total ischaemic lesion volumes; (3) microscopic damage of infarctions, chronic subcortical ischaemic lesions, hippocampus, thalamus, thalamic radiation, interhemispheric white matter tracts and widespread white matter; (4) functional disconnection of large-scale brain networks and that between specific brain regions and the rest of the brain. All the findings mentioned above highlight the role of structural and functional disconnection in VCI in patients with symptomatic carotid artery disease. The functional disconnection mediates the association between structural disconnection and global cognitive impairment. Therefore, it seems to be justified to conclude that disconnection is one of the mechanisms of global cognitive impairment in patients with symptomatic carotid artery disease.

## 9.2 Future directions

This study provides the evidence of the role of disconnection in VCI and provides evidence that white matter tract skeleton MD has the potential to be

served as an imaging biomarker to investigate cognitive impairment. Future research is necessary to evaluate whether white matter tract skeleton MD could predict the conversion from VCI to VaD in a longitudinal study.

From a perspective of imaging analysis, a logical next step would be to investigate structural connectivity and functional connectivity using graph theory analysis approach as it could provide more comprehensive information about the crucial hub of structural and functional networks. Also, a relatively new approach which called as edge density imaging has emerged as a promising method to map white matter nexus in the human brain. Twelve rich club nodes including bilateral precuneous, right superior parietal lobe, bilateral superior frontal lobes, left insula, bilateral thalamus, bilateral putamen and bilateral caudate have been shown to have the highest density of white matter edge (Owen, Chang et al. 2015). This technique could elucidate the importance of brain regions on cognition. However, due to the lack of high resolution of T1 weighted images, the masks of these brain regions could not be accurately extracted thus the correlation of MD values of thesis regions and global cognitive impairment could not be investigated.

Additionally, given the results of the mediation effect of functional disconnection on the association between structural disconnection and cognition, it seems to be justified to hypothesise that pharmacological and non-pharmacological interventions which could enhance large-scale cognitive network functional connectivity may have beneficial effects on improving cognition in VCI patients.

So far there are no effective treatments available for VCI. Current levels of evidence about treatments for VCI are inadequate to enable the effective treatment of people with this condition. Even though some treatments seem to show promising benefits for VCI patients, one of the main challenges in designing trials is to work out what can be measured in order to see whether the treatment has worked. Usually clinical trials into treatments for dementia will use cognitive assessments to see if there have been any improvements or slowing down of cognitive decline. However, these improvements reflected by cognitive assessments may only be temporary and thus more measures are needed to investigate whether the treatment is working. In fact, it will only be possible to develop targeted and effective treatments for vascular dementia patients when there is better understanding of what happens to the brain due to the effect of the treatments. Also, the clear understanding of the mechanisms could also prove to be the first step to modifying current treatments. Thus, there is an urgent need to conduct research to have a better understanding of the mechanisms of treatments that show promising benefit for vascular dementia patients, eventually aiming at providing robust evidence for testing the treatments in well-designed randomised controlled trials.

Non-pharmacological cognitive intervention has been increasingly recognised as an important adjunct and in some cases as an alternative to pharmacological treatment for individuals with dementia and those at risk of dementia (Woods, Aguirre et al. 2012). Cognitive stimulation and cognitive training are two major types of non-pharmacological cognitive interventions. Cognitive stimulation includes a range of activities and discussions (usually in a group) aimed at general enhancement of cognitive and social functioning. It has been

recommended in numerous guidelines and endorsed by many leading organisations in the UK and beyond (i.e. The UK Department of Health NICE guidelines on dementia, 2006; Alzheimer's Research UK, The Scottish Intercollegiate Guidelines Network, and the World Alzheimer Report, 2011). Cognitive training typically involves guided practice on a set of standardised tasks designed to reflect particular cognitive functions such as memory, attention or problem-solving (Clare and Woods 2004). Cognitive stimulation benefits cognition in people with mild to moderate dementia (Woods, Aguirre et al. 2012). However, the results regarding the efficacy of cognitive training for people with mild AD or vascular dementia are limit (Bahar-Fuchs, Clare et al. 2013). The lack of significant effects achieved in cognitive training studies must be interpreted in the context of methodological limitations including the low to moderate overall quality of trials, issues related to power, choice of control condition and choice of outcome measures, which may have constrained the possibility of demonstrating significant gains (Woods, Aguirre et al. 2012). The methodological limitations of cognitive training studies can be caused by the incomplete understanding of how cognitive training might work to preserve or improve cognition. Actually, the mechanisms of the beneficial effects of cognitive stimulation in improving cognition also remain unclear.

Before testing the efficacy of any non-pharmacological cognitive interventions in VCI patients in well-designed randomised controlled trials, it is essential to have a clear understanding about the mechanisms underlying the therapeutic effects of the interventions. Currently there has been no studies to clearly clarify the mechanisms of non-pharmacological cognitive interventions to improve cognition in patients with VCI. One hypothesised mechanism is that non-

pharmacological cognitive intervention is able to enhance brain plasticity. Brain plasticity, which is the capacity to modify the structure or function of the nervous system as a result of experience, seems to underlie the improvement of cognition induced by cognitive interventions with a restorative principle (such as cognitive training and cognitive stimulation) (Ylvisaker, Hanks et al. 2002). Promising results have been shown regarding the effect of cognitive interventions in promoting brain plasticity in normal ageing subjects and patients with neurological diseases. For instance, beneficial changes at functional and structural level in the ageing brain can occur due to cognitive training (Cao, Cao et al. 2016) (Chapman, Aslan et al. 2015). It has been found that cognitive training will lead to increased functional connectivity within three distributed networks including the default mode network (responsible for processing information regarding the self, thinking about others and remembering the past and thinking about the future), central executive network (responsible for decision making and working memory) and salience network (responsible for detecting internal and external events salient to homeostasis) in normal ageing subjects. Additionally, the increases of global and region cerebral blood flow of the default mode network and central executive network is also identified in ageing subjects. At the structural level, increased brain volume (i.e. prefrontal cortex and insular), cortical thickness and density, and increased integrity of white matter tracts (i.e. left uncinate) induced by cognitive training in normal ageing subjects. The effect of cognitive interventions in enhancing brain plasticity has also been found in patients with neurological diseases. For example, better functional brain network modularity was found after cognitive training in patients with acquired brain injury (Arnemann, Chen et al. 2015). Multiple

sclerosis patients showed enhanced brain plasticity at a structural or functional level due to the beneficial effects of cognitive interventions (De Giglio, Tona et al. 2016) (Parisi, Rocca et al. 2014) (Bonavita, Sacco et al. 2015).

Advanced MRI may address the knowledge gap between the observed cognitive improvement and the mechanisms underlying the improved cognition induced by cognitive interventions, providing a powerful tool to investigate brain plasticity related to cognitive improvement in patients with VCI. So far, no research has been conducted to investigate whether enhanced brain plasticity, measured by advanced MRI techniques, underlies the beneficial effects of cognitive interventions in patients with vascular dementia. It means that there is an urgent need to use advanced imaging techniques to have a clear understanding of the mechanisms of cognitive interventions in improving cognition in VCI, eventually aiming to develop and deliver hypothesis-driven cognitive interventions for VCI patients. Functional connectivity metrics may be sensitive markers of neural network dysfunction prior to irreversible structural brain damage.

In addition to providing a very important advance for understanding the mechanisms of therapeutic effects of cognitive interventions in VCI, advanced MRI techniques also are able to predict who will respond to cognitive interventions optimally. The identification of patients who are likely to respond to cognitive intervention using advanced neuroimaging approaches will be paramount in improving cognitive outcome in patients with VCI. To data, no studies has addressed this issue with the use of neuroimaging techniques to identify potential imaging biomarkers for predicting the effect of cognitive interventions in patients with vascular dementia. However, a previous study has

shown functional brain network modularity is able to predict response to cognitive training in patients with acquired brain injury (Arnemann, Chen et al. 2015), meaning that neuroimaging techniques has the potential to be used as a marker that could guide cognitive intervention efforts.

In order to test the hypothesis, a feasibility study will be designed as follows: Patients at the early-phase of VCI will be recruited. Participants will undergo MRI scans (anatomical MRI, resting-state fMRI, diffusion tensor MRI) and cognitive assessments at the baseline. Then, cognitive stimulation therapy will be given in a group with five participants by a therapist, 45 minutes per session, twice per week and totally seven weeks. Three months after the enrolment at baseline, participants will be invited to undergo the same MRI scans and cognitive assessment again. The MRI scan will include structural images (T1 MPRAGE, Axial FLAIR) that show any cerebrovascular lesions, diffusion tensor images that show microstructural integrity of white matter, resting-state fMRI scans that which show the functional connectivity of cognitive networks. Also, two task-based fMRI scans will be conducted when participants are asked to perform attention and memory tasks separately during the scanning. The aim of conducting the task-based fMRI scans is to investigate the alteration of fMRI activation patterns during the attention or memory tasks induced by the non-pharmacological cognitive intervention. The total scanning time will be expected to be 30-45 minutes.

## 9.3 Conclusion

Disconnection provides a plausible explanation for vascular cognitive impairment in patients with symptomatic carotid artery disease. White matter tract skeleton mean diffusivity has the potential to serve as an imaging marker for vascular cognitive impairment. Functional disconnection of large-scale cognitive networks underpins the association between structural disconnection and global cognitive impairment.

# Appendix

## List of conference abstracts, papers and prizes

### ● Journal Paper

1. **Dewen Meng**, Akram A. Hosseini, Richard J.Simpson, Christopher R. Tench, Robert A. Dineen, Dorothee P. Auer. Lesion topography and microscopic white matter tract damage contribute to cognitive impairment in symptomatic carotid artery disease. **Radiology** 2016 (Epub ahead of print).

2. Akram A. Hosseini, **Dewen Meng**, Richard J. Simpson, Dorothee P. Auer. Mesiotemporal atrophy and hippocampal diffusivity distinguish amnestic from non-amnestic vascular cognitive impairment. Under review.

3. Stefan Pszczolkowski, Rebecca Gallagher, Zhe K. Law, **Dewen Meng**, David J. Swienton, Paul S. Morgan, Nikola Sprigg, Robert A. Dineen. Automatic segmentation of haematoma and perihaematoma oedema in MRI of acute spontaneous intra-cranial haemorrhage: Preliminary results for the TICH-2 MRI sub-study. Under review.

4. **Dewen Meng**, Akram A. Hosseini, Richard J. Simpson, Robert A. Dineen, Dorothee P. Auer. On the role and nature of functional disconnection in probable vascular cognitive impairment. Preparation for submission .

### ● Conference abstracts

1. **Dewen Meng**, Akram A. Hosseini, Richard J. Simpson, Robert A.Dineen,

Dorothee P. Auer. Hippocampal disconnection predicts cognitive impairment in patients with cerebrovascular disease. **International Society for Magnetic Resonance in Medicine** (Italy, 2014).

2. **Dewen Meng**, Akram A. Hosseini, Richard J.Simpson, Christopher R. Tench, Robert A. Dineen, Dorothee P. Auer. Topography of chronic ischaemic brain lesions, but not volume or acute lesions contribute to cognitive impairment in patients with symptomatic carotid artery disease. **European Stroke Conference** (Austria, 2015).

3. **Dewen Meng**, Akram A. Hosseini, Richard J. Simpson, Robert A. Dineen, Dorothee P. Auer. Functional disconnection of posterior cingulate cortex contributes to cognitive impairment in patients with symptomatic carotid artery disease. **American Society of Neuroradiology** (US, 2016).

4. Akram A. Hosseini, **Dewen Meng**, Richard J. Simpson, Dorothee P. Auer. Does hippocampal damage explain memory loss in vascular cognitive impairment? **ABN Annual meeting** (UK, 2015).

5. Rebecca G. Gallagher, Xinzhong Ruan, **Dewen Meng**, Ian S. Scott, Christopher P. Fox, Dorothee P. Auer. Primary CNS lymphoma versus GBM: a diagnostic dilemma. **British Society of Neuroradiologists** (UK, 2015).

## ● Prizes and awards

1. ISMRM merit award (magma cum laude) (Italy, 2014)
2. The investigator award (Austria, 2015)
3. Guarantor of Brain travel prize (2014 and 2015)

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