

# **Profile, Determinants and Mechanisms of Cerebral Injury and Cognitive Impairment following Stroke**

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## Abstract

One in three people over a life time will develop a stroke, dementia or both but little is known about stroke - related cognitive impairment despite current epidemiologic transition in sub - Saharan Africa. The CogFAST Study was established in Newcastle to unmask risk factors, pathological substrates and cellular mechanisms underlying cerebral injury and cognitive impairment following stroke. The overall aim of this thesis was to establish a comparative cohort in Nigerian African stroke survivors and explore mechanisms in post - mortem brains accrued from the Newcastle cohort.

Two hundred and twenty Nigerian African stroke survivors were screened three months after index stroke out of whom 143 eligible participants underwent cognitive assessment in comparison with 74 stroke - free healthy controls. We found a high frequency (49.3%) of early vascular cognitive impairment and significant association with older age and low education. Pre-stroke daily fish intake and moderate – to - heavy physical activity were inversely associated. The frequency of vascular cognitive impairment no dementia (vCIND) in the cohort (39.9%) was relatively higher than earlier report from Newcastle (32%) but neuroimaging studies revealed significant findings of MTLA and correlative white matter changes in tandem with previous reports from the Newcastle cohort.

Given these, we investigated neurodegenerative hippocampal Alzheimer pathology and synaptic changes, as well as frontal and temporal white matter abnormalities in post - mortem brain tissue from the Newcastle cohort. We found increased Alzheimer pathology in the post - stroke groups but largely this did not differ between the demented (PSD) and non - demented (PSND) sub - groups. However, we found significantly higher hippocampal expression of synaptic markers (vesicular glutamate transporter – 1 and Drebrin) but lower expression of microglial, astrocytic and axonal injury markers in PSND compared to PSD subjects. The protective effect of educational attainment, pre-stroke physical activity and fish intake have public brain health implications.

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## Abbreviations

AD	Alzheimer's disease
ADDTc	Alzheimer's Disease Diagnostic and Treatment Centers
AHA	American Heart Association
ANOVA	Analysis of Variance
APOE	Apolipoprotein E
APP	Amyloid Precursor Protein
APP	Amyloid Precursor Protein
ASA	American Stroke Association
BBB	Blood Brain Barrier
CA	Cornu Ammonis
CAMCOG	Cambridge Cognitive Examination
CD68	Cluster of differentiation 68
CDR	Cognitive Drug Research
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
CogFAST	Cognitive Functions After Stroke
CRT	Choice Reaction Time
CSID	Community Screening Instrument for Dementia
CVD	Cerebrovascular disease
DALY	Disability Adjusted Life Years
DLB	Dementia with Lewy Bodies
dMBP	degraded Myelin Basic Protein
DSM -IV	Diagnostic and Statistical Manual of Mental Disorders, fourth revision
DSM -V	Diagnostic and Statistical Manual of Mental Disorders, fifth revision
DTI	Diffusion Tensor Imaging
FTD	Frontotemporal Dementia
GBD	Global Burden of Disease
GFAP	Glial Fibrillary Acidic Protein
HIC	High Income Countries
ICD -10	International Classification of Diseases – tenth revision
ICV	Intracranial Volume

IHC	Immunohistochemistry
LACI	Lacunar Infarct
LFB	Luxol Fast Blue
LMIC	Low and Middle Income Countries
MMSE	Mini Mental State Examination
MTLA	Medial Temporal Lobe Atrophy
NFT	Neurofibrillary Tangle
NINDS - AIREN	National Institute of Neurological Disorders and Stroke- Association Internationale pour la Recherche et l'Enseignement en Neurosciences
NU - 1	Northwestern University – 1 Antibody
OCSP	Oxford Community Stroke Project
PACI	Partial Anterior Circulation Infarct
PSD	Post Stroke Dementia
PSD - 95	Post Synaptic Density - 95
SMI – 32	anti – nonphosphorylated neurofilament H
SNAP -25	synaptic markers synaptosomal - associated protein 25
SRT	Simple Reaction Time
SSA	Sub - Saharan Africa
SY- 38	Synaptophysin
TACI	Total Anterior Circulation Infarct
TBV	Total Brain Volume
VaD	Vascular dementia
VaMCI	Vascular Mild Cognitive Impairment
VCI	Vascular Cognitive Impairment
VCIHS	Vascular Cognitive Impairment Harmonization Standard
vCIND	Vascular Cognitive Impairment No Dementia
VGLUT -1	Vesicular Glutamate Transporter - 1
VV	Ventricular Volume
WHO	World Health Organization
WM	White Matter
WMH	White Matter Hyperintensity

## List of Abstracts and Original Publications

### *List of Abstracts presented at meetings*

1. **Akinyemi RO**, Ogunniyi A, Allan L, Kalaria RN. Profile of cognitive impairment among Nigerian stroke survivors: a pilot study (presented at the 5th VASCOG Meeting, Lille, September 2011).
2. **Akinyemi R**, Gemmell E, Burke M, Slade J, Oakley A, Polvikoski T, Kalaria RN. Quantification of hippocampal neurodegenerative pathology in post – stroke cognitive impairment compared to other dementias. (presented at the 5th VASCOG Meeting, Lille, September 2011).
3. **Akinyemi RO**, Allan L, Owolabi MO, Akinyemi JO, Ajani A, Ogunniyi A, Kalaria RN. Profile and Predictors of Vascular Cognitive Impairment in African Stroke Survivors: The CogFAST – Nigeria Study (6<sup>th</sup> VASCOG Meeting, Toronto Canada, June 2013).
4. **Akinyemi RO**, Firbank M, Ogbole GI, Allan L, Owolabi MO, Akinyemi JO, Yusuf BP, Ogunseyinde O, Ogunniyi A, Kalaria RN. Medial temporal lobe atrophy predicts cognitive impairment among African survivors three months after stroke: The CogFAST- Nigeria Study. (6th VASCOG Meeting, Toronto Canada, June 2013).

### *List of Publications*

1. **Akinyemi RO**, Mukaetova-Ladinska EB, Attems J, Ihara M, Kalaria RN. Vascular risk factors and neurodegeneration in ageing related dementias: Alzheimer's disease and vascular dementia. *Curr Alzheimer Res.* 2013 Jul;10 (6):642 -
2. Kalaria RN, **Akinyemi R**, Ihara M. Does vascular pathology contribute to Alzheimer changes? *J Neurol Sci.* 2012 Nov 15; 322(1-2):141-7.
3. Ndung'u M, Härtig W, Wegner F, Mwenda JM, Low RW, **Akinyemi RO**, Kalaria RN. Cerebral amyloid  $\beta(42)$  deposits and microvascular pathology in ageing baboons. *Neuropathol Appl Neurobiol.* 2012 Aug;38(5):487-99.
4. **Akinyemi RO**, Allan L, Owolabi MO, Akinyemi JO, Ajani A, Ogunniyi A, Kalaria RN. Profile and Factors associated with Vascular Cognitive Impairment in African Stroke Survivors: The CogFAST – Nigeria Study. (*in review*)
5. **Akinyemi RO**, Firbank M, Ogbole GI, Allan L, Owolabi MO, Akinyemi JO, Yusuf BP, Ogunseyinde O, Ogunniyi A, Kalaria RN. Medial temporal lobe atrophy predicts cognitive impairment among African survivors three months after stroke: The CogFAST- Nigeria Study. (*in review*)
6. **Akinyemi RO**, Slade J, Oakley A, Polvikoski T, Kalaria RN. Quantification of hippocampal Alzheimer pathology in post – stroke dementia compared to other dementias and ageing controls (*in preparation*)

# Chapter 1. Introduction

## 1.1. The Global Burden of Stroke and Dementia

### 1.1.1. Global Burden of Stroke

Stroke is a disease that has been recognized since antiquity. Hippocrates (circa 400 BC) and others used the word “apoplexy” to describe sudden non-traumatic brain events, while the terminology ‘stroke’ is believed to have been introduced only late in the seventeenth century (Cole, 1869).

Stroke had been traditionally defined as “a rapidly developing focal or global disturbance of cerebral function resulting only from a vascular cause with dysfunction lasting more than 24hrs or leading to death (Aho *et al.*, 1980) . However, in a recent updated definition of stroke for the 21st century, “Central nervous system infarction is defined as brain, spinal cord, or retinal cell death attributable to ischemia, based on neuropathological, neuroimaging, and/or clinical evidence of permanent injury. Central nervous system infarction occurs over a clinical spectrum: Ischemic stroke specifically refers to central nervous system infarction accompanied by overt symptoms, while silent infarction by definition causes no known symptoms. Stroke also broadly includes intracerebral haemorrhage and subarachnoid haemorrhage”(Sacco *et al.*, 2013). This new definition is both time and tissue-based and builds on various advances in knowledge about stroke and has potential implications for measuring the epidemiology and impact of stroke and its associated morbidities in the future.

Stroke remains a major public health problem as the leading cause of adult disability and second leading cause of death worldwide (Donnan, 2008; Feigin *et al.*, 2013). Every year, 16 million people experience a stroke and 5.7 million die from it. Stroke is associated with 43.7 million lost disability – adjusted life years [DALY] annually around the world (Strong *et al.*, 2007) In 2010, there were 16.7 million first ever stroke, 33 million stroke survivors, 5.9 million stroke – related deaths and 102 million lost DALYs (Feigin *et al.*, 2013) . Stroke has huge economic impact, especially in developing nations where people in the prime of life are often affected.

Whereas stroke incidence rates declined by 42 % in high-income countries over a period of four decades (1970 – 2008), they doubled (100% increase) in low- and middle-income countries (Feign *et al.*, 2009). For instance, in a time –trend analysis of stroke in the United Kingdom over a ten year period (1999 – 2008), stroke incidence fell by 30%, from 1.48/1000 person-years in 1999 to 1.04/1000 person years in 2008. .Over the same period, 56-day mortality after first stroke reduced from 21% to 12% resulting in an increased prevalence of 12.5%, from 6.40/1000 in 1999 to 7.20/1000 in 2008. . Over this study period, a stable increase in the prescription of cardiovascular medications, especially lipid lowering agents and antihypertensive agents was also reported (Lee *et al.*, 2011b). This is in sharp contrast to the situation in LMIC where population ageing, changes in diet, physical inactivity, associated increase in vascular risk factors, especially hypertension, diabetes and obesity, as well as low awareness, under-detection and under-treatment of these factors are driving forward the burden of stroke (Strong *et al.*, 2007; Akinyemi *et al.*, 2009).

Stroke mortality and disability rates are disparately higher in low- and middle-income countries with 87% of global stroke deaths occurring in these regions (Kim and Johnston, 2013). Gaping differences in the availability of, and access to effective acute and chronic stroke care – including rehabilitation and secondary prevention may explain this wide disparity between HIC and LMICs (Norrving and Kissela, 2013).

Recent epidemiologic data from Africa suggest a rising burden of stroke (Connor *et al.*, 2007). In the Hai district (rural) and Dar-es-salaam (urban) Tanzania, age-standardized incidence rates of stroke were 108.6/100,000 and 315.9/100,000 respectively (Walker *et al.*, 2010). Nigeria, the most populous black nation with 167 million people, particularly exhibits a high burden. A recent community-based survey in Lagos, southwestern Nigeria put the prevalence rate at 114 per 100,000 in the general population and 224 per 100,000 in persons above the age of 65 years(Danesi *et al.*, 2007) . Stroke accounts for up to 45% of all neurological admissions and up to 17% of medical deaths in Nigeria. Thirty - day case fatality rate is as high as 40% (Ogun *et al.*, 2005). Stroke affects predominantly persons in the late middle age with a mean age of occurrence of about 60 years(Owolabi and Agunloye, 2013).

The INTERSTROKE Study provides the most comprehensive and global evidence of traditional and emerging risk factors for stroke. Undertaken in 22 countries – including high, middle and low income nations – and using a case- control study design, 10 key

risk factors were found to be responsible for up to 90% of the population-attributable risk for stroke. These were hypertension, diabetes, smoking, history of cardiac disease, alcohol use, poor diet (low intake of fruit and vegetables), elevated waist-to-hip ratio, physical inactivity, elevated apolipoprotein B to A1 ratios and depression .

Hypertension alone accounted for 54% of the population-attributable risk (O'Donnell *et al.*, 2010b).

Hypertension is the most common risk factor noted in 79% - 98% of Nigerian stroke patients. Ischaemic stroke accounts for 49% - 64% among Nigerian stroke patients compared to 80% - 93% recorded among Caucasians. Cerebral and subarachnoid haemorrhages constitute the remaining percentages, cerebral haemorrhages accounting for at least 75% of the remainder (Ogun *et al.*, 2005; Owolabi, 2011).

### ***1.1.2. Global Burden of Dementia***

Dementia is characterized by significant decline in cognitive functioning that is sufficiently severe to impair independent social and occupational functioning. It is a growing public health problem worldwide and the World Health Organization Mental Health Gap (mhGAP) document considers dementia a priority mental health disorder earmarked for scaled-up action on account of its huge economic cost and social burden (World Health Organization, 2010).

In a very recent systematic review and meta analysis of the global prevalence of dementia, the age-standardized prevalence rate of dementia in persons older than 60 years ranged between 5 and 7 %. Prevalence rates were higher in Latin America (8.5%) but lower in sub-Saharan Africa (2 - 4%). It was estimated that 35.6 million people worldwide lived with dementia in 2010, while the numbers are expected to double every 20 years to 65.7 million in 2030 and 115.4 million in 2050, the growth being driven by population growth and demographic ageing (Prince *et al.*, 2013).

Furthermore, much of the projected increase in the number of people with dementia will occur in the low and middle income countries, especially those in Southeast Asia and Latin America. In 2010, 57.7 % of all dementia cases lived in the LMIC regions but this is projected to increase to 63.4% and 70.5% in 2030 and 2050 respectively (Prince *et al.*, 2013). (Table 1.1)

Major dementia subtypes include Alzheimer’s disease (AD), vascular dementia (VaD), Dementia with Lewy bodies (DLB) and frontotemporal dementia (FTD). Given the difficulties in diagnosing vascular dementia (VaD), methodological and other differences, there is considerable variation in its epidemiology. Much of the information available today on VaD accrued from studies done in the developed world. A systematic analysis of previous studies obtained a prevalence ranging from 4.5 to 39% in clinical studies while in memory clinic- and population-based series VaD constituted 8 – 15.8% of dementias with standardized incidence rates between 0.42 and 2.68 per 100,000 world population. This, however, increased with age with rates being up to six times higher above age 85. Analysis of data from 12 centres for which neuroimaging findings were available indicate that 26% of cases of dementia fulfilled the National Institute of Neurological Disorders and Stroke-Association Internationale pour la Recherche et l’Enseignement en Neurosciences (NINDS-AIREN) criteria for VaD (Roman, 2008).

Region	Over 60 population (million, 2010)	Crude estimated prevalence % (2010)	No of people living with dementia (millions)		
			2010	2030	2050
Western Europe	97.3	7.2	6.9	10.0	13.4
North America	63.7	6.9	4.4	7.1	11.0
Latin America	52.0	6.1	3.1	7.0	15.0
Asia	406.6	3.9	15.9	33.0	60.9
Africa	71.1	2.6	1.9	3.9	8.7

**Table 1.1: Total population > 60, estimated prevalence of dementia (2010) and estimated number of people with dementia in 2010, 2030 and 2050.** (source: Prince et al. *Alzheimer’s & Dementia* 2013; 9: 63 -75)

There are limited reports on the epidemiology of VaD in sub-Saharan Africa (Ogunniyi and Akinyemi, 2010). Prevalence estimates of VaD are low ranging from 0.6% to 2.1% for ages over 65 years although VaD accounts for 1/3 of Chinese patients with dementia (Kalaria *et al.*, 2008). Data available from the Ibadan – Indianapolis Dementia Project showed an overall dementia prevalence rate of 2.29% among > 65 year old community-dwelling Yoruba Nigerians compared with 8.24% among African Americans. The prevalence of VaD among the Yoruba cohort was 0.72% (Ogunniyi *et al.*, 2000). Related data from the Assuit region of Egypt showed an overall dementia prevalence rate of 5.93% and 1.25% for VaD (Farrag *et al.*, 1998)(Farrag *et al.*, 1998). More recent data from selected francophone African countries still show similarly very low prevalence of VaD (Guerchet *et al.*, 2009; Guerchet *et al.*, 2010; Paraiso *et al.*, 2011; Mbelesso *et al.*, 2012). The possibility of under-diagnosis of VaD in the developing regions exists due to lack of neuroimaging facilities, bias of existing survey instruments and diagnostic criteria, and higher stroke mortality. The population structure of most sub-Saharan Africa shows the elderly constituting less than 5% in most countries (Dotchin *et al.*, 2013). Progress in the field however has led to changes in the annotation of the vascular dementia construct to encompass milder cognitive changes without full dementing features. The subsequent section will discuss this in more detail.

## **1.2. Vascular Cognitive Impairment**

The term '*vascular cognitive impairment*' (VCI) describes a spectrum of cognitive disorders arising as a consequence of diseases related to blood vessels in the brain (O'Brien *et al.*, 2003). Synonymous with '*vascular cognitive disorder*' (Sachdev, 1999), the VCI construct encompasses a whole range of levels of cognitive decline from mild deficits in one or more cognitive domains to a broad dementia syndrome (Moorhouse and Rockwood, 2008). It also integrates the interactions between vascular risk factors, cerebral vascular disorders of varying aetiologies and their accompanying cellular and molecular changes associated with the cerebral injury underlying the processes of cognitive dysfunction (Erkinjuntti and Gauthier, 2009). Thus VCI is an umbrella term that includes VCI –no dementia, vascular dementia and cognitive impairment of mixed (degenerative and vascular) origin (Moorhouse and Rockwood, 2008).



### ***1.2.1. Historical aspects***

The link between cerebral vascular disorders (CVD) and cognitive dysfunction has been recognized since the days of Willis (1672) who first described “*dullness of mind.....*” “*forgetfulness*”.....and “*foolishness*” as a sequelae of apoplexy (stroke)(Roman, 1999).

During the 18<sup>th</sup> and early 19<sup>th</sup> centuries, “brain congestion” most probably attributed to high blood pressure was most frequently diagnosed for conditions including stroke, anxiety and cognitive decline. Emil Kraepelin and Alois Alzheimer, during this period, used the concept of “arteriosclerotic dementia” to describe dementia resulting from chronic strangulation of blood supply to the brain. Alzheimer’s description of the neuropathology found in Auguste D also had evidence of significant small vessel disease (Alzheimer, 1907). Subsequent developments in the understanding of VCI could be attributed to the works of Otto Binswanger, who with Alzheimer separated vascular dementia from dementia paralytica attributable to neurosyphilis. Binswanger also described subcortical arteriosclerotic encephalopathy which is now better known to be due to small vessel disease (Kalaria and Erkinjuntti, 2006).

In the 1960s, the seminal clinico - neuropathological studies in Newcastle by Tomlinson and colleagues challenged the historical concepts of ‘hardening of the arteries’ and provided concrete evidence that senile dementia of the Alzheimer type was the commonest cause of dementia (Tomlinson *et al.*, 1970). They also provided evidence that destruction of brain tissue by ischaemic injury producing multiple infarctions is associated with cognitive impairment when a certain threshold is exceeded (Tomlinson *et al.*, 1968). Following the works of Tomlinson and colleagues, Hachinski described ‘multi-infarct dementia’ in a landmark 1974 paper stating that “...*when vascular disease is responsible for dementia it is through the occurrence of multiple small or large cerebral infarcts*”. He subsequently introduced the Hachinski Ischaemic Score (Hachinski *et al.*, 1974; Hachinski *et al.*, 1975).

However, over the last two decades, the evolution of the concept of VCI has witnessed the publication of several sets of diagnostic criteria for vascular dementia (Chui *et al.*, 1992; Roman *et al.*, 1993; World Health Organization, 1993; American Psychiatric Association, 1994). These have expanded the concept to include variations in the size and distribution of brain vascular pathologies. Now included are multiple cortical and/or subcortical infarcts, strategic single infarcts, non-infarction white matter lesions, haemorrhages and hypoperfusion as possible causes of vascular dementia.

It however became necessary to introduce the ‘vascular cognitive impairment’ (VCI) construct because the vascular dementia construct and its diagnostic criteria were heavily biased towards memory impairment, not recognizing the early manifestation of executive dysfunction. The ‘vascular dementia’ construct was insufficient to describe the heterogeneity of clinical profiles and pathologies seen in cognitive impairment and dementia associated with cerebral vascular disorders. Generally, the dementia construct was heavily biased by the predominance of impairment in the memory domain in AD whereas the pattern in VCI differs. It thus became necessary to introduce a construct which captured both the severity and specific cognitive domains affected (Sachdev, 1999; O'Brien *et al.*, 2003; Hachinski *et al.*, 2006b).

### ***1.2.2. Diagnostic Criteria***

The five commonly used sets of criteria for vascular dementia (VaD) are: the Hachinski Ischaemic Score (HIS)(Hachinski *et al.*, 1975) , the National Institute of Neurological Disorders and Stroke-Association Internationale pour la Recherche et l’Enseignement en Neurosciences (NINDS-AIREN) criteria (Roman *et al.*, 1993), the State of California Alzheimer’s Disease Diagnostic and Treatment Centers (ADDTC) criteria (Chui *et al.*, 1992) , the DSM-IV criteria (American Psychiatric Association, 1994) and the ICD-10 criteria (World Health Organization, 1993), with the latter two being more general and not as well operationalised as the first two. The DSM –V criteria were only recently released (American Psychiatric Association, 2013) while the eleventh revision of the ICD is still in progress.

The Hachinski Ischaemic Score (HIS) was developed as a checklist of vascular risk factors and clinical features which contribute to cerebrovascular disease (Hachinski *et al.*, 1975). Although the HIS and its modifications have been widely used to diagnose VaD, it was not designed for this purpose. The HIS recorded less than 70% accuracy

(Swanwick *et al.*, 1996), moderate sensitivity (0.43) but high specificity (0.88) in confirmatory neuropathological studies (Gold *et al.*, 1997) and remains the most widely used scale (with > 2200 citations) for identifying the cerebrovascular component of cognitive impairment and dementia. Recent efforts have been made to optimize the HIS and enhance its clinical utility (Hachinski *et al.*, 2012) .

The State of California Alzheimer Disease Diagnostic and Treatment Centers criteria for ischaemic vascular dementia (IVD) was developed in 1992 (Chui *et al.*, 1992). It excludes dementia resulting from haemorrhagic cerebral injury and requires cognitive deficit in more than one domain (of any type), at least one infarct outside the cerebellum on neuroimaging, evidence of at least 2 strokes from history, clinical signs or neuroimaging or a single stroke with evident temporal link to the onset of dementia. *Possible* VaD is diagnosed in the absence of a definite temporal link to dementia onset or clinical and neuroimaging evidence of extensive sub-cortical white matter disease. These criteria recorded a sensitivity of 0.63 and specificity of 0.64 in the neuropathological confirmatory study (Gold *et al.*, 1997).

The National Institute of Neurological Disorders and Stroke-Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) criteria for vascular dementia was published in 1993 (Roman *et al.*, 1993). These criteria require impairment in memory plus at least 2 other cognitive domains, cerebrovascular disease evident through clinical signs and relevant neuroimaging abnormality, temporal relationship of dementia onset within 3 months of stroke or an acute deterioration of cognitive function. A diagnosis of *Possible* VaD is made if neuroimaging evidence or a clear temporal association is absent. Neuropathological validation studies yielded sensitivity and specificity of 0.58 and 0.80 respectively in a geriatric cohort (Gold *et al.*, 1997). The specific requirement for neuroimaging abnormality probably accounts for its high specificity and low sensitivity

The International Classification of Diseases – tenth revision (ICD -10) was published in 1993 by the World Health Organization (World Health Organization, 1993) and requires patchy distribution of cognitive deficits, focal neurological signs and clinical or laboratory evidence of significant cerebrovascular disease (CVD) as well as evidence of a temporal link. The WHO is currently revising the International Classification of

Diseases towards the ICD -11 due for release in 2015

([http://www.who.int/classifications/icd/revision/en/retrieved 11 -09-2013](http://www.who.int/classifications/icd/revision/en/retrieved%2011-09-2013))

The DSM – IV required the presence of impairment in memory in addition to one other cognitive domain for dementia, focal neurological symptoms or signs, or neuroimaging evidence of vascular injury and historical temporal relationship.

The ‘Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM –V) has just been released (American Psychiatric Association, 2013), improving upon the DSM –IV (American Psychiatric Association, 1994) which had, hitherto, been widely used in routine clinical practice and epidemiological studies.

These criteria share a number of conceptual weaknesses haven been developed largely based on consensus without gold standard neuropathological confirmation (Kalaria *et al.*, 2004; Hachinski *et al.*, 2006b; Jellinger, 2008a; Deramecourt *et al.*, 2012) although a general consensus is yet to be achieved on the neuropathological criteria for VCI.

They also demonstrate differential utility and accuracy with DSM –IV tending to give the highest rate of VaD diagnosis, the NINDS –AIREN the lowest and the ICD -10 and ADDTC intermediate rates. DSM –IV proved useful for epidemiological studies because of its high sensitivity and absence of neuroimaging requirement while the NINDS-AIREN criteria has been useful in clinical trials because of high specificity (Wetterling *et al.*, 1996; Pohjasvaara *et al.*, 1997; Chui *et al.*, 2000).

Other specific shortcomings of these criteria include:

[1] The emphasis on ‘dementia’ prejudices against the growing understanding that these impairment lie on a continuum rather than occurring as discrete entities(Viswanathan *et al.*, 2009). This precludes the recognition of early and potentially reversible mild cognitive impairment. Besides, the term ‘dementia’ is primarily used in older individuals whereas cognitive disorders can affect individuals at all ages (Sachdev, 2000) . These led to repeated calls to abandon the VaD construct in favour of an overarching description of vascular cognitive impairment (VCI) to overcome this limitation (Hachinski and Bowler, 1993; O'Brien *et al.*, 2003).

[2] The majority of these criteria sets require memory impairment as necessary for the diagnosis of VaD, thus using a definition of dementia based on clinical features of AD. Substantial evidence have, however, accrued suggesting that disturbance in frontal-

executive functions, rather than memory, is the predominant feature of VaD and memory impairment may in fact be absent in some cases with significant cognitive deficits or its characteristics may be different from those seen in AD (Looi and Sachdev, 1999; Ballard *et al.*, 2003b; Ballard *et al.*, 2004a).

[3] These criteria sets differ significantly on the requirements for levels of cognitive impairment, number and location of strokes, neuroimaging abnormalities and neurological features, such that when applied to the same data set, the prevalence estimates vary as much as fourfold between criteria (Wetterling *et al.*, 1996; Pohjasvaara *et al.*, 1997) (Chui *et al.*, 2000)).

[4] These criteria do not take due cognizance of the fact vascular cognitive disorder is not just one disease but one of diverse aetiologies (Kalaria *et al.*, 2004; Jellinger, 2013) .

[5] These criteria do not recognize the co-existence of vascular and neurodegenerative pathologies and their additive/synergistic effect (Kalaria, 2002b; Kalaria *et al.*, 2004; Iadecola and Davisson, 2008) which is sometimes referred to as ‘mixed’ dementia. Furthermore, there is increasing recognition that vascular and neurodegenerative processes may interact, so that risk factors for CVD also increase the risk of AD (Skoog, 2000; Kalaria *et al.*, 2012a; Kalaria and Ihara, 2013).

In response to the aforementioned failings of the VaD criteria and in an attempt to move the field forward, the last decade has witnessed efforts to harmonize the standards of assessment of various aspects of vascular cognitive impairment in order to set the pace for establishment of globally acceptable criteria for clinical practice and research (Hachinski *et al.*, 2006b). Recently, recommendations for diagnosis of VCI have been published (Gorelick *et al.*, 2011) while the recently published fifth revision of the Diagnostic and Statistical Manual (DSM- V) and the ICD -11 in preparation reflect significant changes that attempt to address the shortcomings.

## **The 2006 NINDS-CSN Harmonization standards**

The NINDS and the Canadian Stroke Network convened a workshop in 2006 to harmonize the standards of assessment of various aspects of cognitive impairment due to vascular factors and made a number of recommendations to guide the conduct of studies. Whilst the harmonization standards do not currently include specific criteria, research using these standardized methods of assessment should yield valid results suitable for consideration in the generating diagnostic criteria with excellent clinimetric properties. The assessment recommendations covered different aspects including clinical assessment, neuropsychological, neuroimaging, neuropathological, genetic evaluation as well as animal models (Hachinski *et al.*, 2006a). Validation studies of the recommended standards are now being published (Pendlebury *et al.*, 2012) including Chinese (Wong *et al.*, 2013)(Wong *et al.*, 2012) and Korean versions (Yu *et al.*, 2013).

## **AHA/ASA 2011 Criteria**

Recently, the American Stroke Association (ASA) and the American Heart Association (AHA) have published a joint statement on vascular contribution to cognitive impairment and dementia (Gorelick *et al.*, 2011). The statement provided an overview of the evidence on vascular contributions to cognitive impairment and dementia. Definitions of vascular cognitive impairment (VCI), neuropathology, basic science and pathophysiological aspects, role of neuroimaging and vascular and other associated risk factors, and potential opportunities for prevention and treatment were reviewed and criteria for VCI were advanced building on the earlier work of the NINDS-CSN Group (Hachinski *et al.*, 2006a). The proposed criteria recognized the occurrence of vascular cognitive disorder as a continuum and thus included criteria for VaD (probable and possible) as well as VaMCI (probable and possible). Similar to previous criteria, impairment in at least one and two cognitive domains was required for VaMCI and VaD respectively, although this domain does not necessarily have to include memory. The criteria require objective assessment of cognitive functions covering at least four major domains of executive function, memory, language and visuospatial function. A clear temporal relationship between vascular event (eg

stroke) and onset of cognitive impairment is required while functional impairment must be independent of motor/sensory deficit resulting from the vascular event.

However, the co- occurrence of mixed vascular and degenerative pathologies was not adequately teased out as cases with such co-existence were recommended to be classified as possible VaD or possible VaMCI without a clear statement on how such degenerative pathologies can be diagnosed in live as well as the required diagnostic threshold, since the utilization of advanced neuroimaging techniques such as Pittsburgh Compound B (PIB) may not be universally available. Besides, there is also the questionable category of ‘unstable VaMCI’ to capture those subjects whose cognition improves as symptoms revert to normal. The instability connoted by this term would suggest that the cognitive trajectory can move in either directions of improvement or worsening.

### **The 2013 DSM – V Neurocognitive Disorders Criteria**

In the DSM –V, a major change from the DSM – IV is the introduction of the diagnosis of ‘mild neurocognitive disorder’. This accords recognition to the concept of ‘mild cognitive impairment’ (MCI)(Petersen *et al.*, 1999) or ‘cognitive impairment no dementia’ (CIND) as diagnostic entity of scientific, clinical and epidemiological significance. With the emergence of different biological markers such as CSF –beta amyloid and tau, platelet tau(Arai, 1996) (Schoonenboom *et al.*, 2004) (Mukaetova-Ladinska *et al.*, 2013a)and positron emission tomography (PET) evidence of  $\beta$  – amyloid deposition (Klunk *et al.*, 2004) there is fast progress in the understanding of neurobiology of cognitive disorders. Thus, it is becoming increasingly possible to identify individuals in ‘pre – clinical stage’ of disease with the possibility of interventions that can retard or reverse the progression of the disease. This argument was a major justification for the inclusion of ‘mild neurocognitive disorder’ in the DSM – V (Ganguli *et al.*, 2011). The other diagnosis ‘Major neurocognitive disorder’ covers the ‘dementia’ construct in previous versions. Other neurocognitive disorders associated with traumatic brain injury, Parkinson’s disease, Huntington’s disease, prior diseases, HIV and substance abuse have also been incorporated.

### **1.3.Post – Stroke Vascular Cognitive Impairment**

Cognitive changes are common, though not universal in patients with stroke.. This results in increased care giver burden, higher cost of care, impaired quality of life and higher mortality (Jaracz and Kozubski, 2003). Given the discussion of diagnostic criteria in the last section, what is the evidence regarding the frequency of post – stroke VCI ?

#### ***1.3.1. Prevalence***

Up to 64% of stroke survivors have some degree of cognitive impairment while up to a third develop frank dementia (Hachinski *et al.*, 2006b). The prevalence rates of post-stroke vascular cognitive impairment vary with study design, study population, test batteries and diagnostic criteria. It also varies between hospital-based and population-based studies as well as whether post-stroke vascular cognitive impairment no dementia (vCIND) or post-stroke dementia (PSD) is measured. A review of the extant literature on post-stroke cognitive impairment (1950- 2013) reveals vCIND rates ranging between 21.8 % and 71.4% and PSD rates between 2.9% and 41.5 % in twenty-eight hospital-based studies while in six community-based studies, vCIND rates range from 6.1% to 37.5% and PSD rates vary between 7.0% and 19.3% (Table 1.2).

In a systematic review and meta analysis of 22 hospital-based and eight community-based studies, Pendlebury and Rothwell (2009) obtained a pooled prevalence of post-stroke dementia ranging from 7.4% (4.8 -10.0) in population – based studies of first – ever stroke in which pre-stroke dementia was excluded to 41.3% (29.6 – 53.1) in hospital –based studies of recurrent stroke in which pre-stroke dementia was included. The analysis demonstrated much heterogeneity in the prevalence figures of post-stroke dementia arising from marked variation in patient characteristics (including cut off age), inclusion criteria, test batteries and diagnostic criteria and inclusion/exclusion of pre-stroke dementia and recurrent strokes versus first-ever strokes (Pendlebury and Rothwell, 2009b). Generally, however, up to a 9 –fold increased risk of dementia is



### Hospital-based Studies

No	Authors	Location	Date of cohort collection	Study design	Sample size	Mean age (yrs)	% female	Stroke type	Diagnostic criteria	% vCIND	% PSD	Risk factors
1	(Tatemichi <i>et al.</i> , 1990)	Columbia, USA	1988-90	X	251	70	53	IS	DSM-IIIIR	ND	26.3	Age, previous stroke, previous MI, cortical atrophy, large vessel disease
2	(Gorelick <i>et al.</i> , 1993)	Chicago, USA	1987-90	X	147	72	49	multiple IS	DSM-III	ND	41.5	Low education, low income, family history of dementia, proteinuria, worse neurological status
3	(Andersen <i>et al.</i> , 1996)	Farsoe and Aalborg, Denmark	1991 -92	L	142	72	ND	IS,H	Mattis Dementia Rating Scale	ND	26.1	Older age, female gender, stroke severity
4	(Censori <i>et al.</i> , 1996)	Bergamo, Italy	1993 -94	X	125	65	35	IS	NINDS-AIREN	ND	13.6	Older age, DM, aphasia, MCA territory infarction, frontal lesion
5	(Pohjasvaara <i>et al.</i> , 1997)	Helsinki, Finland	1993 -95	X	451	71	49	IS	DSM III	ND	31.8	Dysphasia, major dominant stroke syndrome , history of prior cerebrovascular disease , and low educational level .
6	(Inzitari <i>et al.</i> , 1998)	Florence, Italy	1993-94	X	339	71	48	IS, H		ND	16.8	Older age, female gender, stroke severity, atrial fibrillation, aphasia
7	(Linden <i>et al.</i> , 2004)	Gothenburg , Sweden	1993-94	X	149	80	65	IS,H	DSM-IIIIR	ND	28	not stated
8	(Barba <i>et al.</i> , 2000)	Madrid, Spain	1994-95	X,L (FU at 3,6,24 mths)	326	69	47	IS, H	DSM -IIIIR, DSM IV	ND	30	Age, nephropathy, atrial fibrillation, previous mental decline, and stroke severity
9	(Hoffmann, 2001)	Durban, South Africa	1992-98	X	1000		44	IS, H	Clinical judgement	63.5 ( 2 weeks post-stroke)	ND	increasing age, black race, overweight body habitus, and recent infection
10	(Henon <i>et al.</i> , 2001)	Lille, France	1995-96	L (6 months, yrly × 3 yrs	202	72	46	IS, H	ICD-10; DSM - IIIIR	ND	28.5	ageing, pre-existing cognitive decline, severity of deficit at admission, diabetes mellitus, and silent infarcts.
11	(Madureira <i>et al.</i> , 2001)	Lisbon, Portugal	1995-97	X	237	59	45	IS, H	DSM IV	55	6	female gender (P=0.01), older age (P=0.01) and lower education level
12	(Lowery <i>et al.</i> , 2002)	Newcastle, UK	ND	L (FU at 1, 6, 12 mths)	360	74	52	IS,H	DSM IV	ND	23	age > 75 years
13	(Ballard <i>et al.</i> , 2002)	Newcastle, UK	1999 -2000	X	150	ND	ND	IS,H	DSM IV, CAMCOG, COGDRAS-D	32	ND	ND
14	(Stephens <i>et al.</i> , 2004)	Newcastle, UK	1999 – 2003	X	384	80	48	IS,H	DSM -IIIIR	24	8.6	ND
15	(Lin <i>et al.</i> , 2003)	Taiwan	1995-99	X	283	64	34	IS	ICD-10	ND	9.2	age > 65 yrs, low occupational attainment, prior stroke, left carotid vascular territory, moderate to severe stroke
16	(Sachdev <i>et al.</i> , 2004a)	Sydney, Australia	1997-2000	X,L	170	72.2	39	IS,	Consensus	39.4	19.1	premorbid functioning and stroke volume

No	Authors	Location	Date of cohort collection	Study design	Sample size	Mean age (yrs)	% female	Stroke type	Diagnostic criteria	% vCIND	% PSD	Risk factors
17	(Zhou <i>et al.</i> , 2005)	Chongqing, China	1999-2000	X	434	68	47	IS	DSM IV	37.1	27.2	age, low educational level, everyday drinking, prior stroke, dysphasia, atrial fibrillation, and left carotid territory infarction
18	(Klimkowitz <i>et al.</i> , 2002)	Cracow, Poland	2000-01	X	220	66	55	IS,H	DSM IV/IQCODE	ND	31.4	age, DM, neurological deficit on admission
19	(Tang <i>et al.</i> , 2004)	Hong Kong, China	2000-02	X	280	71	55	IS,H	DSM IV	ND	20	Premorbid level of cognitive function, severity of stroke, leukoaraiosis, level of education, and bilateral lesions.
20	(Tang <i>et al.</i> , 2006)	Hong Kong, China	2000-02	X	280	71	55	IS,H	DSM IV	21.8	ND	female sex, education, National Institutes of Health Stroke Scale dysarthria score, urinary incontinence, and atrial fibrillation
21	(de Koning <i>et al.</i> , 2000)	Rotterdam-Rijnmond, Netherlands	1993-96	X	300	70	40	IS,H	DSM -IIIR	ND	23.7	ND
22	(de Koning <i>et al.</i> , 2005)	Rotterdam-Rijnmond, Netherlands	2000-02	X	121	70	38	IS,H	DSM -IV	ND	28.9	ND
23	(Rasquin <i>et al.</i> , 2005a)	Maastricht, Netherlands	2000-01	L	156	68	45	IS	DSM IV	72.4 (at 1 month post-stroke)	9.6	Psychiatric symptoms
24	(Fatoye <i>et al.</i> , 2007)	Ile Ife, Nigeria	2006	X	109		41.3	IS, H	MMSE	17.4	ND	ND
25	(Khedr <i>et al.</i> , 2009)	Assiut, Egypt	ND	X	81	58	33	IS,H	DSM IV	ND	21	increasing age, low educational level, large vessel and lacunar infarction, severity of stroke, prolonged P300 latency, smoking, hypertension, elevated Hcy levels.
26	(Delgado <i>et al.</i> , 2010)	Santiago, Chile	ND	X, L	164	72	ND	IS, H	DSM IV	39	22	Higher functional impairment at hospital admission, left-hemisphere large-vessel infarction, and a larger amount of white matter changes.
27	(Dong <i>et al.</i> , 2012)	Singapore	2009 -2011	X,L	239	60	32.4	IS,H	DSM IV	54.8	2.9	older age, lower education, smoking, previous heart disease, previous stroke, stroke severity
28	(Yu <i>et al.</i> , 2013)	Korea (multi-centre study)	2007- 2008	X - multicentre	353	64	39	IS,H	DSM IV (Korean VCI HS Protocol)	49.9	12.7	older age, small vessel disease, poor functional status

**Table1. 2 : Profile of post - stroke cognitive impairment and dementia from hospital-based studies (1950 -2013)**

X= cross-sectional; L=longitudinal;IS=ischaemic;H=haemorrhagic;ND=not documented; DSM =Diagnostic Statistical Manual ; ICD -10= International Classification of Disease - 10; MMSE = Minimal State Examination; NINDS AIREN = National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherche et l'Enseignement en Neurosciences ; IQCODE = informant questionnaire on cognitive decline in the elderly; VCI = Vascular Cognitive Impairment; CAMCOG = Cambridge Cognitive Examination; COGDRAS = cognitive drug research computerized assessment system;

Population-based studies												
No	Author	Location	Date of cohort collection	Study Design	Sample size	Mean age (yrs)	% female	Stroke type	Diagnostic criteria	% vCIND	% PSD	Risk factors
1	(House <i>et al.</i> , 1990)	Oxford , UK	1990	X,L	122	71	55		MMSE	21	ND	Age
2	(Kokmen <i>et al.</i> , 1996)	Rochesta, Minnesota, USA	1960 -84	L, RPS	971	ND	50	IS	Review of medical records	ND	7	age, male gender, repeat stroke
3	(Ivan <i>et al.</i> , 2004)	Framingham, USA	1982 -2001	L	212	79	61	IS,H	DSM III, MMSE, DSM IV	ND	19.3	age < 80 years, ApoE3/E3, high school graduate
4	(Srikanth <i>et al.</i> , 2004)	Melbourne, Australia	1998-99	X,L	99	69	41	IS,H	DSM -IV	37.5	12.5	Stroke, age, baseline cognitive ability.
5	(Das <i>et al.</i> , 2012)	Kolkota, India	2006-2010	L	281	63.1	ND	IS.H	DSM -IIIR	6.1	13.9	older age, cortical atrophy
6	(Douiri <i>et al.</i> , 2013b)	London, UK	1995 -2010	P,L	4212	70	47	IS, H	MMSE	22	ND	age, black race, occupational attainment, small vesle disease, lacunar infarction

**Table 1.3: Profile of post-stroke cognitive impairment and dementia from community-based studies (1950 -2013)**

X= cross-sectional; L=longitudinal;IS=ischaemic;H=haemorrhagic;ND=not documented; DSM =Diagnostic Statistical Manual ; ICD -10= International Classification of Disease - 10; MMSE = Minimental State Examination; NINDS AIREN = National Institute of Neurological Disorders and Stroke and Association Internationale pour la Recherche et l'Enseignement en Neurosciences ; IQCODE = informant questionnaire on cognitive decline in the elderly; VCI = Vascular Cognitive Impairment; CAMCOG = Cambridge Cognitive Examination; COGDRAS = cognitive drug research computerized assessment system;

seen in patients with stroke versus controls in the first year after stroke (Tatemichi *et al.*, 1994) (Kokmen *et al.*, 1996; Desmond *et al.*, 2002).

Efforts to quantify the burden of post-stroke cognitive impairment without dementia (CIND) have gained momentum in the last two decades compared to earlier periods of study. Table 1.2 shows generally higher rates of post stroke – CIND than PSD thus revealing the high burden of this previously under- recognized and under-diagnosed cognitive dysfunction among stroke survivors. However, it is noted that the highest rates were reported in subjects who were assessed within the first month after the ictus (72.4% (Rasquin *et al.*, 2005); 63.5% (Hofmann, 2001), a period during which acute post- stroke delirium might not have resolved fully (Desmond DW, 1996). Nonetheless, generally higher rates of dementia are reported within 3 months after stroke than at 1 year or more later although attrition of patients as a result of mortality need to be considered (Leys *et al.*, 2005).

### ***1.3.2. Clinical Features of VCI***

As previously discussed, vascular cognitive impairment (VCI) is a continuum. It is characterized typically by the manifestations of disturbance of the subcortico-frontal circuitry which include early deficits of attention, slowing of information processing speed, mental inflexibility and impairment of working memory, planning, organization and goal-directed behaviours. Memory impairment may be equally or less prominent but tends to occur later (Looi and Sachdev, 1999; Reed *et al.*, 2007). Furthermore, the memory problem is more of a deficit of retrieval of stored information rather than of storage (Cummings, 1994; Moorhouse and Rockwood, 2008). Language and visuospatial/ visuoconstructional deficits also occur (Shibata *et al.*, 2007).

Among stroke survivors, the pattern of cognitive impairment is generally similar between vCIND and PSD but more severe in PSD across multiple domains, and more especially the memory domain (Ballard *et al.*, 2002; Ballard *et al.*, 2003b; Sachdev *et al.*, 2004a). In the study of 384 stroke survivors in Newcastle, a sub- group of subjects without clinically apparent cognitive impairment demonstrated increased simple and choice reaction time

signalling slowing of mental speed despite lack of apparent impairment in cognitive domains (Stephens *et al.*, 2004).

Due to disruption of thalamocortical, striatocortical and prefrontal-basal ganglia pathways, and the consequent impact on cortical and limbic brain structures, post-stroke VCI may also be associated with behavioural and psychological disturbances (O'Brien *et al.*, 2003).

### ***1.3.3 Natural History of VCI***

The classical description of multi-infarct dementia was that of an acute step-wise or fluctuating decline in cognition, with intervening periods of stability (Hachinski *et al.*, 1974; Hachinski *et al.*, 1975; Hachinski, 1983). This pattern is usually temporally related to the cerebrovascular event and not difficult to establish clinically. Cognitive impairment is usually at its peak soon after a stroke and may show significant improvement over the next three months whilst associated delirium clears. Further improvement may occur beyond the three months, but its rate is much slower. Some individuals, however, remain stable whilst others experience further decline in cognitive functions.

Findings from the Newcastle study showed improvement in global cognition in 50% of subjects, 41% exhibited stable cognition, while 9% developed incident dementia between 3 and 15 months after stroke (Ballard *et al.*, 2003a; Kalaria, 2012b).

Over an average follow up period of 3.8 years, 24 % of survivors developed incident dementia giving an incidence rate of 6.3 cases per 100 person years (Allan *et al.*, 2011). Other studies of post-stroke cognitive impairment have similarly documented improvement in 10 - 31 % of subjects and further decline and incident dementia in 21 – 36% of survivors (Desmond DW, 1996; Rasquin *et al.*, 2002; Tham *et al.*, 2002; Sachdev *et al.*, 2009). Younger stroke cohorts are more likely to experience improvement (Tham *et al.*, 2002) while individuals with vCIND progress to dementia at a rate of about 8% per year (Sachdev *et al.*, 2009).

#### ***1.3.4. Determinants of Post-Stroke VCI***

In the systematic review by Pendlebury and Rothwell (Pendlebury and Rothwell, 2009b), significant predictors of dementia after stroke were identified and categorized into patient- and stroke-related variables. Patient-related variables include increasing age, low level of education, dependency before stroke and pre-stroke cognitive decline without dementia.

Age is a strong risk factor for all types of dementia and several studies of post-stroke cognitive impairment also show this consistently (Kalaria, 2012b). Educational and occupational attainment have been described as surrogates of cognitive reserve (Stern, 2009). Several studies on stroke survivors have demonstrated a strong association between low educational attainment and risk of dementia (Sharp and Gatz, 2011; Meng and D'Arcy, 2012). Pre-stroke cognitive decline has been associated with post-stroke cognitive decline and is considered to be due to possible Alzheimer neuropathology which becomes unmasked or exacerbated after stroke (Leys *et al.*, 2005) . However, findings from the large Rotterdam Study were contrary and suggested that pre-stroke cognitive function may not be a major determinant of post-stroke dementia (Reitz *et al.*, 2008).

Co - morbidities like arterial hypertension, diabetes mellitus, atrial fibrillation, myocardial infarction] cardiac arrhythmias, congestive cardiac failure also significantly predict post-stroke cognitive impairment have demonstrated variable association with post-stroke cognitive decline. For instance, the recent meta-analysis reported that vascular risk factors like diabetes and atrial fibrillation but not hypertension, ischaemic heart disease, cholesterol levels, previous transient ischaemic attack or previous smoking were significant predictive factors of post-stroke dementia (Pendlebury and Rothwell, 2009a).

However, the combined effect of aggregated vascular risk factors may be more important than individual risk factors especially in putting the brain at risk over a prolonged period of time as recently demonstrated in the Newcastle cohort wherein aggregated number of vascular risk factors was a strong predictor of dementia and death in post - stroke subjects (Sachdev *et al.*, 2006; Allan *et al.*, 2011).

Stroke-related variables that predict cognitive decline include more severe clinical deficit at stroke onset, stroke recurrence, supratentorial lesions, left hemispheric lesions, multiple

infarcts, strategic infarcts and territorial infarcts. The meta analysis found multiple lesions or recurrent stroke events over the course of time to be strongly associated with the development of incident dementia, the risk rising up to 30% (Pendlebury and Rothwell, 2009a) . Lacunar strokes increased the risk of post-stroke dementia by seven times that of other stroke subtypes in the 24 –year-population –based Dijon Stroke Registry (Bejot *et al.*, 2011).

Besides, complications arising during the acute stage of stroke care such as infection, incontinence, aspiration, hypoxic-ischaemic episodes and early occurrence of seizures have also been shown to be risk factors for subsequent cognitive decline (Moroney *et al.*, 1996; Hoffmann, 2001).

NOS 3 gene and ApoE genes (especially the  $\epsilon 4$  allele) have been independently associated with cognitive decline in stroke survivor (Jones *et al.*, 2011; Morris *et al.*, 2011).

### ***1.3.5. Pathological Substrates***

Pioneering studies from Newcastle (Blessed *et al.*, 1968; Tomlinson *et al.*, 1968; Tomlinson *et al.*, 1970) and more recent evidence (Jellinger, 2008b; Jellinger, 2013) have shown that the volume, location and number of cerebrovascular lesions are important pathogenic factors in the development of post-stroke VCI. Besides, neurodegenerative changes, deep white matter lesions, hippocampal microinfarct/sclerosis, basal ganglia and thalamic microinfarcts and lacunes, global atrophy and medial temporal lobe atrophy are also important substrates of VCI especially in patients with small infarct volumes (Kalaria *et al.*, 2004) (Bastos-Leite *et al.*, 2007).

Estimates of the proportion of PSD patients with presumed Alzheimer pathology are up to 60% in some series, while up to 30% have a history of dementia before stroke and significant medial temporal lobe atrophy respectively (Pohjasvaara *et al.*, 1999; Cordoliani-Mackowiak *et al.*, 2003; Leys *et al.*, 2005).

Neurochemical studies in VaD have shown abnormalities in key neurotransmitter systems, particularly cholinergic deficits (Roman and Kalaria, 2006) (Keverne *et al.*, 2007) which

may cause cerebral hypoperfusion, a critical factor in the pathogenesis of VaD. Other neurochemical deficits in VaD include reduction in vasopressin and histamine due to lesions in the supraoptic and tuberomammillary nuclei (Ishunina *et al.*, 2004) as well as glutamatergic deficits (Kirvell *et al.*, 2010).

### ***1.3.6. Neuroimaging features***

Evidence of significant vascular pathology in stroke is usually examined through structural neuroimaging techniques including computed tomography (CT) scan and magnetic resonance imaging (MRI) - based techniques although the latter is more sensitive. Newer MRI-based methods include diffusion tensor imaging (DTI).

Cerebrovascular diseases are aetiologically and morphologically heterogeneous, consequently, the neuroimaging features are diverse. In stroke, there may be single or multiple infarcts involving the cortical regions of the frontal, temporal, parietal or occipital lobes, the infratentorial structures, the hippocampus or sub-cortical structures such as the thalamus or the basal ganglia. Other imaging biomarkers which are particularly important for small vessel strokes include white matter hyperintensities, lacunar infarcts, microbleeds and enlarged Virchow Robin spaces (Knopman, 2007; Mills *et al.*, 2007).

Cortical infarcts, multiple anterior infarcts and MCA territorial infarct have been associated with cognitive impairment in stroke patients (Tay *et al.*, 2006; Nys *et al.*, 2007; Jaillard *et al.*, 2010). Lacunar infarcts result from occlusion of perforating arteries and may appear as cystic lesions with signal intensity similar to that of CSF and may present as clinical stroke syndromes or remain silent (Derouesne and Poirier, 1999).

Medial temporal lobe atrophy at baseline is a significant determinant of the course of cognition after stroke predicting further cognitive decline, dementia and death (Henon *et al.*, 1998; Firbank *et al.*, 2007; Firbank *et al.*, 2012) while amygdala volume correlated with cognitive functions in an Australian stroke cohort (Sachdev *et al.*, 2007a; Sachdev *et al.*, 2007b).

White matter hyperintensities connote areas of high signal intensities best seen on T2 weighted MRI sequences or fluid attenuated inversion recovery (FLAIR) sequences on magnetic resonance imaging (MRI). They may be ‘periventricular’, seen around the



margins of the lateral ventricles (PVH) or ‘deep’, occurring in the deep parts of the corona radiata and centrum semiovale (DWMH) (Mills *et al.*, 2007). They represent pathologic ischaemic demyelination predominantly (Young *et al.*, 2008) though seepage of CSF from the lateral ventricle into interstitial spaces may contribute to PVH. Using positron emission tomography (PET) imaging, WMH corresponds to areas of hypometabolism, reduced cerebral blood flow and increased oxygen extraction which are further evidence of the ischaemic origin (Yamaji *et al.*, 1997; Takahashi *et al.*, 2000). White matter hyperintensities correlate with measures of executive function (Burton *et al.*, 2004; Wen *et al.*, 2004; Jokinen *et al.*, 2012). Assessing white matter integrity in a cohort of Singaporean stroke survivors compared to controls using diffusion tensor imaging (DTI), subjects with vCIND and PSD revealed an increased mean diffusivity in the white matter and decreased generalized fractional anisotropy in the vCIND group and these changes predominated in the frontal lobes (Jin Thong *et al.*, 2013). Using arterial spin labelling MRI to determine cerebral blood flow, a recent study on a sub-cohort from the Newcastle study found evidence of reduced grey matter/white matter cerebral blood flow in PSD relative to control subjects and non – demented stroke subjects (Firbank *et al.*, 2011). More recently, the imaging of amyloid with compounds such as the radiolabeled Pittsburgh compound B ( $^{11}\text{C}$  - PiB) has received much interest and is being proposed as a biomarker of MCI associated with AD (Albert *et al.*, 2011). Amyloid imaging has recently been used to support the diagnosis of pure subcortical vascular dementia (Lee *et al.*, 2011a) as well as post-stroke dementia (Mok *et al.*, 2010) suggesting that the latter may be due to a combination of AD pathologic changes and vascular pathology.

### **1.3.7 Treatment**

The primary approach to the treatment of VCI has been the management of vascular risks and pharmacologic treatment. There is established evidence for the occurrence of cholinergic deficit in VCI (Roman and Kalara, 2006; Keverne *et al.*, 2007) based upon which trials of cholinesterase inhibitors were conducted (Bullock *et al.*, 2004; Erkinjuntti *et al.*, 2004). Trials have confirmed the usefulness of Donepezil (Black *et al.*, 2003; Roman *et al.*, 2010), which showed consistent modest cognitive improvements while galantamine and

rivastigmine have less robust effect (Craig and Birks, 2005; Birks *et al.*, 2013). Besides pharmacotherapy, there is only limited evidence in support of non - pharmacological approaches such as cognitive stimulation and acupuncture (Quayhagen *et al.*, 2000). Management of vascular risk factors follows the general principles of life style modification and use of specific pharamacologic agents following appropriate evidence-based treatment guidelines (Fraser *et al.*, 2012).

### ***1.3.8. Prevention***

This entails general preventive measures including lifestyle modification: cessation of smoking, moderation of alcohol intake, physical activity, weight control, early detection and proper management of hypertension, diabetes and dyslipidaemia and other vascular risk factors. These are discussed in details in the next section.

## **1.4. Vascular Risk Factors in Cognitive Decline and Dementia**

Several vascular risk factors have been found, in the last two decades, to be associated with cognitive decline and dementia (both degenerative and vascular). These include hypertension, diabetes mellitus, hypercholesterolemia, obesity and metabolic syndrome, smoking, atherosclerosis and apolipoprotein E (*APOE*). Clinically, the risk of cerebral vascular disease can be assessed by the profile of vascular risk factors as demonstrated in the Hachinski Ischaemic Score (Hachinski *et al.*, 1974) and the Framingham stroke risk score (Wang *et al.*, 2003). These were outcomes of clinico-epidemiologic investigations (Gorelick *et al.*, 2011).

### **1.4.1. Hypertension**

The literature is replete with studies on the relationship between blood pressure and cognitive function, including evidence from experimental, clinical and epidemiological studies. Hypertension appears to be the strongest vascular disease risk factor for dementia (Kennelly *et al.*, 2009). Hypertension in mid-life predisposes to cognitive decline and dementia while in late life, the relationship appears to be J-shaped (Knopman, 2009). Although initial cross-sectional studies found both positive (Starr *et al.*, 1993) (Seux *et al.*, 1998) and negative correlations between blood pressure and cognitive decline, longitudinal studies have been more informative showing positive (Skoog *et al.*, 1996; Kivipelto *et al.*, 2001; Knopman *et al.*, 2001; Bohannon *et al.*, 2002)(Skoog *et al.*, 1996, Kivipelto *et al.*, 2001, Knopman *et al.*, 2001), U-shaped (Bohannon *et al.*, 2002) (Bohannon *et al.*, 2002), negative (Verghese *et al.*, 2003) or no correlation (Morris *et al.*, 2001) with cognitive decline and dementia. In a recent meta-analysis, hypertension was significantly associated with increased risk of incident VaD (odds ratio, OR: 1.59, CI: 1.29-1.95,  $p < 0.001$ ) and prevalent VaD (OR: 4.84, CI: 3.52-6.67,  $p < 0.001$ )(Sharp *et al.*, 2011). Long-standing increase in blood pressure may increase risk of dementia by inducing small-vessel disease, WM changes and cerebral hypoperfusion through the disruption of vasoregulatory functions or atherosclerotic disease (Kalaria, 2010; Waldstein *et al.*, 2010).

Antihypertensive therapy, particularly with Angiotensin Converting Enzyme inhibitors /Angiotensin Receptor Blockers, has been shown to reduce the incidence of cognitive decline and dementia (Takeda *et al.*, 2009; Wang *et al.*, 2009). In a Cochrane review of three trials, Mc Guinness *et al* showed that BP reduction resulted in 11% reduction in the relative risk of dementia in patients with no prior cerebrovascular disease (McGuinness *et al.*, 2006) while Haag *et al* (2009) also showed that antihypertensive use was associated with an 8% reduction of risk of dementia per year of use in persons  $\leq 75$  years of age (Haag *et al.*, 2009) . A recent clinic-pathological study also revealed that persons treated for hypertension in mid-life were less demented clinically and had less AD pathology than either non-hypertensive patients or hypertensive patients who were not treated (Hoffman *et al.*, 2009) . However, blood pressure lowering in later-life may not prevent the development of cognitive decline and dementia in elderly hypertensives with no previous evidence of cerebrovascular disease (McGuinness *et al.*, 2009b) .

#### ***1.4.2. Diabetes Mellitus and Metabolic Syndrome***

A relationship between cognitive decline and diabetes mellitus (DM) has been well established. In a review of 33 prospective studies, Brands *et al* found that Type 1 DM (T1DM) was associated with reduced mental speed and mental inflexibility while learning and memory were spared (Brands *et al.*, 2005) . Similarly, in the Atherosclerosis Risk In Communities study (ARIC) study, a significant association between type 2 DM (T2DM) and cognitive decline characterized by dysexecutive syndrome and impaired recent memory was found among 10,963 middle-aged people followed up over 6 years (Knopman *et al.*, 2001) . The increased risk of dementia in DM is attributed to cerebrovascular disease (Kalaria, 2009b; Ahtiluoto *et al.*, 2010) and the association of AD with DM may be clearer if milder cases are included in the analysis (Kopf and Frolich, 2009). For VaD, it was recently shown that a history of pre-morbid DM is associated with earlier onset and faster decline of cognitive decline and more neuropsychiatric symptoms in a cohort of elderly subjects (Murthy *et al.*, 2010) .

DM causes ischaemic cerebrovascular disease, primarily lacunar infarcts, and is positively associated with AD pathology through hyperinsulinemia (causing increased secretion but reduced extracellular degradation of amyloid  $\beta$ ), impaired insulin signalling, oxidative

stress, inflammatory mechanisms and coupling of neuronal components by advanced glycation end products (Luchsinger and Gustafson, 2009). Besides, elevated morning cortisol has been recently implicated in cognitive impairment associated with T2DM (Reynolds *et al.*, 2010). Features of the insulin resistance syndrome and adiposity have also been associated with low cognitive function (Kalmijn *et al.*, 1995; Kalmijn *et al.*, 2000) and with AD (Kuusisto *et al.*, 1997) while adipocytokines such as leptin have been implicated in the neurodegenerative pathway (Benoit *et al.*, 2004). A 27- year old prospective population-based study of 10,276 subjects showed that people with high BMI had a significantly raised risk of dementia (Whitmer *et al.*, 2005) while a longer 36-year longitudinal study showed that people who were obese at mid-life had a 3 –fold increased risk of dementia (AD, VaD) at old age (Whitmer *et al.*, 2007). In contrast, a faster rate of decline of body mass index in late-life is suggested to be a pre-clinical marker of dementia, especially in subjects who were overweight (Hughes *et al.*, 2009).

#### ***1.4.3. Cholesterol and Statins***

The relationship between cholesterol and cognitive decline and dementia is less robust, and sometimes with conflicting findings. Generally, however, hypercholesterolemia in mid-life tends to show positive association with dementia including VaD and AD (Notkola *et al.*, 1998; Kivipelto *et al.*, 2002; Solomon *et al.*, 2009), while on the other hand, cholesterol levels assessed in late life reveal less significant association with AD (Tan *et al.*, 2003; Reitz *et al.*, 2004) (Panza *et al.*, 2006). Statins have a broad range of properties including antioxidant activity, immunomodulation and regulation of inflammatory processes, all of which could prevent neuronal death. Simvastatin has been found to reduce the levels of A $\beta$ 42 and A $\beta$ 40 in vitro in the brain of guinea pigs (Fassbender *et al.*, 2001), but the results of clinical studies do not show robust support for the protective effect of statins (Shepherd *et al.*, 2002). Recent Cochrane database reviews have shown that statins given in late life to individuals at risk of vascular disease have no effect in preventing or treating AD or dementia (McGuinness *et al.*, 2009a; McGuinness *et al.*, 2010b). Similarly, statin use does not reduce VaD risk (Muangpaisan and Brayne, 2010).

#### ***1.4.4. Smoking, Atherosclerosis and Homocysteine***

Smoking predisposes to oxidative stress, atherosclerosis, plaque formation and silent brain infarctions (Howard *et al.*, 1998; Armani *et al.*, 2009). In the Honolulu-Asia Ageing Study [HAAS], the association between mid-life smoking and late- life dementia was assessed in 3,734 Japanese- American men, and following adjustment for age, education and Apo E, the risk of AD in smokers increased with pack-years of smoking. Neuropathologic data from 218 men in an autopsied sub-sample of the cohort showed increased number of neuritic plaques with higher smoking levels (Tyas *et al.*, 2003) . A recent Finnish study has also demonstrated that heavy smoking in mid-life is associated with >100% increased risk of AD and VaD after over twenty years (Rusanen *et al.*, 2010).

Results from the Rotterdam study including 6,647 participants followed up for 9 years revealed that atherosclerosis, predominantly of the carotid arteries, was associated with an increased risk of dementia [AD and VaD] (van Oijen *et al.*, 2007). Atherosclerosis predisposes to small and large infarcts and cerebral hypoperfusion leading to vascular and degenerative changes associated with cognitive decline and both AD and VaD. Other complications relating to atherosclerosis include coronary heart disease and congestive heart failure, and these have also been shown to have significant association with dementia, and AD through the causation of multiple cerebral emboli and reduced cerebral perfusion (Zuccala *et al.*, 2005; Dolan *et al.*, 2010).

Although homocysteine is an established risk factor for cardiovascular disease, its role in dementia has been controversial (Seshadri, 2006). In a recent prospective study from Gotheburg, mid-life homocysteine and late - life dementia were assessed in 1368 women after 35 years of follow up. The highest total homocysteine tertile was related to a hazard ratio of 1.7 for developing any dementia, 2.1 for AD and 2.4 for AD without cerebrovascular disease thus showing that high homocysteine in mid-life is an independent risk factor for the development of late-life AD in women (Zylberstein *et al.*, 2009).

#### ***1.4.5. Influence of Apolipoprotein E (APO E)***

Apolipoprotein E (ApoE) is a major constituent of very low density lipoproteins (VLDL) and plays a key role in the transport of cholesterol among various cells of the body. APOE  $\epsilon$ 4 allele has the most consistent but varying modulating influence on vascular risk factors, lacunar infarcts and amyloid accumulation, and increases disease risk in a dose-dependent manner in both AD and VaD (Duron and Hanon, 2008; McGuinness *et al.*, 2010a). In a recent study of genetic association of dementia subtypes, associations were found between the APOE  $\epsilon$ 4 allele and mixed dementia, stroke – related dementia and subcortical ischemic vascular dementia (Jones *et al.*, 2011).

#### ***1.4.6. Implications for Treatment Interventions***

With the growing understanding of the contributions of vascular pathology to the burden of dementia, there is now an increasing call for the revision of the classification of dementias in order to reflect the strong interplay between vascular and degenerative pathologies and the influence of vascular factors and vascular brain disorders on the neurobiology, natural history and the threshold and profile of clinical manifestations (Viswanathan *et al.*, 2009). Whereas the current classification scheme of clinical dementia is based on trying to separate vascular and degenerative pathologies, it is advocated that a new scheme be adopted based on the spectrum nature of the dementias, apportioning weights to the relative contributions of the vascular risk factors and vascular brain disorders based on available evidence such as relative or attributable risks derived from population-based studies (Kalaria, 2012a)..

#### ***1.4.7. Prevention through diet, nutritional intervention and physical exercise***

Maintenance of healthy blood vessels and adequate cerebral blood flow is central to the goal of preventing brain vascular disorders and ameliorating vascular risk factors, and consequently preventing and/or reducing the incidence of VCI. Appropriate diet, healthy nutrition and adequate physical exercise are important non-pharmacologic, lifestyle-related

interventions that could help in maintaining normal vascular tone, adequate cerebral blood flow and normal cognitive ageing.

The Mediterranean diet consists of whole grains, fish and olive oil and moderate consumption of alcohol and is a typical example of a healthy and brain-friendly diet (Sofi *et al.*, 2008). The protective effect of fish intake against cerebral vascular disease risk (including stroke and VCI) and AD is fairly well established (van Gelder *et al.*, 2007; Chowdhury *et al.*, 2012; Kieffe-de Jong *et al.*, 2012).

Overall, diets rich in polyphenols such as resveratrol, omega-3 fatty acids, docosahexaenoic and eicosapentaenoic acids as well as flavinoids and the B vitamins, especially folate, B6 and B12 are known to enhance cognitive function in older age (Kidd, 2008). Similarly, poorer cognitive function and an increased risk for vascular dementia are associated with lower consumption eg. milk or dairy products (Crichton *et al.*, 2010) while polyphenols supplementation (eg. grape juice) have been shown to reduce inflammation, blood pressure and vascular pathology in individuals with cerebrovascular disease (Krikorian *et al.*; Krikorian *et al.*, 2010). Rich sources of polyphenols include wine, milk, cocoa, coffee, grape seed, blueberries, strawberries, tea, curcumin, pomegranate, fruits and vegetables. Polyphenols exert an anti-oxidant effect by preventing the accumulation of reactive oxygen-species (Craggs and Kalaria, 2010) which could trigger atherosclerosis and chronic neurodegeneration that contribute significantly to the pathology of AD and other dementias (Zandi *et al.*, 2004). Resveratrol has also been implicated in the regulation of the cell cycle, mitochondrial energy production, vascular reactivity, oncogene suppression and activation of sirtuins (silent information regulator-related enzymes), as anti-ageing inhibitors (Marques *et al.*, 2009).

There is a large body of evidence on the beneficial effects of physical exercise on brain health across the life span (Dishman *et al.*, 2006). Among humans, exercise improves quality of sleep (Driver and Taylor, 2000), depression (Dunn *et al.*, 2005) and cognitive functioning including memory and executive functioning (van Gelder *et al.*, 2004). In a prospective study of a cohort of community-dwelling elderly people, higher physical activity was found to reduce the risk of dementia (Scarmeas *et al.*, 2009). In a related study, increased physical activity in mid-life was found to be associated with less neocortical atrophy in late life (Rovio *et al.*, 2008). In the largest clinical trial of its kind, 170 physically active volunteers with memory problems exhibited significant improvement



in cognition after a 24-week intervention (Lautenschlager *et al.*, 2008). A most recent systematic analysis of 24 longitudinal studies including 1378 VaD subjects, also points out the beneficial effect of physical activity in prevention of VaD, reducing the risk for developing dementia by 38% (Aarsland *et al.*, 2010). Higher aerobic fitness levels have been shown to be associated with larger hippocampus, increased blood flow, oxygen delivery and better spatial memory (Colcombe *et al.*, 2006).

Physical exercise, apart from enhancing cerebral blood flow, activates the expression of brain neurotrophins. In rats and mice, chronic physical exercise has been accompanied by increased expression of brain-derived neurotrophic factor (BDNF) (Neeper *et al.*, 1995; Neeper *et al.*, 1996), nerve growth factor (Ang *et al.*, 2003) and galanin (Tong *et al.*, 2001) which enhance neuronal and synaptic plasticity. Reduction of infarct volume with concomitant increased expression of BDNF following treadmill exercise training has been shown in a rat model of focal ischaemia by middle cerebral artery occlusion (Ding *et al.*, 2004) while reduction of caspase-dependent apoptosis in hippocampal neurons has similarly been reported in a gerbil model of transient global ischaemia following treadmill exercise training (Lee *et al.*, 2003).

## **1.5. The Cognitive Functions After Stroke Study**

### ***1.5.1. The Cognitive Functions After Stroke (CogFAST) Study- Newcastle***

In an effort to understand the contribution of vascular factors to dementia and neurodegeneration, and the risk factors and substrates of dementia resulting from cerebrovascular disease, 400 stroke survivors who were 75+ years old and free of dementia at three months after stroke were recruited from six regional stroke registers in the Northeast Region of England into the Cognitive Functions After Stroke (CogFAST) Programme, a longitudinal cohort study which started in 1999.

The subjects received a comprehensive baseline clinical and neurological assessment, neuropsychological evaluation and neuroimaging (MRI) (25% of the cohort). Subjects were followed up and received annual clinical and cognitive evaluation to ascertain their progression. Multiple assessments including neuroimaging (MRI), genetic and cardiovascular biomarkers were undertaken.

Early in the study, participants were also approached for brain donation and over 50 brains have been donated and stored in the Newcastle Brain Tissue Resource (NBTR) so far. The aim was to identify critical pathological substrates and mechanisms which distinguished those cases who developed delayed PSD from those who maintained normal cognitive functioning at the time of death.

The study has contributed significantly to the body of knowledge on the natural history of post-stroke cognition. These include:

- the very early occurrence and predominance of executive dysfunction (impaired working memory, prolonged simple and choice reaction time etc) in stroke survivors and the progressive worsening of multi-domain cognitive functions as dementia sets in (Ballard *et al.*, 2002; Ballard *et al.*, 2003a; Ballard *et al.*, 2003b; Stephens *et al.*, 2004)

- The cognitive trajectory of stroke survivors between 3 and 15 months (improvement in 50% of cases and incident dementia in about 10% (Ballard *et al.*, 2003a).
- the predictive role of vascular risk factors aggregate on the progression of cognitive decline and long term outcomes of dementia and death (Rowan *et al.*, 2005; Allan *et al.*, 2011).
- MRI findings of white matter hyperintensities (WMH) correlated significantly with cognitive processing speed and attention measures (Burton *et al.*, 2004)
- MRI studies on the CogFAST cohort showed that medial temporal atrophy rather than white matter hyperintensities predicted cognitive decline, progression to dementia, and death in stroke survivors (Firbank *et al.*, 2007; Firbank *et al.*, 2012).
- The APOE epsilon4 allele was associated with the progression of cognitive decline (Rowan *et al.*, 2005)
- Identification of a single nucleotide polymorphism (SNP) at codon 298 of the nitric oxide synthase (NOS) gene [NOS3 gene rs1799983 polymorphism (TT genotype)] which increased the risk of dementia in the cohort (Morris *et al.*, 2011).
- Median survival rate from the incidence of stroke was 6.72 years. Over this period, 23.9% of the subjects developed dementia and 75% of PSD cases at autopsy met neuropathological criteria for VaD (Allan *et al.*, 2011).
- By arterial spin labelling MRI, reduced global cerebral blood flow (cortex to WM ratio) best predicted PSD while hippocampal volume was reduced in PSD and AD subjects (Firbank *et al.*, 2011).
- In a study examining sub-cortical changes in the cohort, the number of caudate lacunes was higher in the PSND group, compared with AD and controls while putaminal volume was smaller in the stroke and AD groups compared with controls. In the whole stroke group, putamen lacunes were correlated with impairment in memory while WMH and hippocampal volume both correlated with global dysfunction (Lopes *et al.*, 2012).
- More recently, data from the CogFAST programme using three dimensional morphometric techniques have shown that neuronal volume significantly decreases in subjects who experience cognitive decline post stroke compared to those who remain cognitively stable (Gemmell *et al.*, 2012).

- Most recently, it was discovered that post-stroke subjects who maintained cognitive functions over time demonstrated sustained levels of (Hu C and D) markers of neuronal maintenance compared to post - stroke subjects who became demented (Burke et al, PhD Thesis, 2013).

In all, the CogFAST Newcastle Study has significantly advanced knowledge on the neurobiology of post-stroke cognitive dysfunction through clinico – neuroimaging – neuropathological correlative approaches. Findings suggest that about 25% of persons who suffer a stroke progress to develop post - stroke dementia (predominantly VaD), the risk being higher with poor baseline performance on cognitive testing, baseline medial temporal atrophy, depression and higher aggregate number of vascular risk factors. The unique finding of hippocampal atrophy and reduced cerebral blood flow in demented post-stroke subjects provide eloquent support for a vascular basis of neurodegeneration among other vital contributions to knowledge.

### ***1.5.2. The Cognitive Functions After Stroke (CogFAST) Study - Nigeria***

Cross – cultural studies in dementia contribute very significantly to the understanding of disease variation and the influence of genes, epigenetic, environmental and cultural factors on the mechanisms, natural history and phenomenology of disease (Osuntokun *et al.*, 1992; Kalaria *et al.*, 1997; Hendrie, 2006; Hendrie *et al.*, 2006). Recent scientific evidence suggests that the incidence of stroke is rising in the developing world including countries of sub- Saharan Africa, attributable to epidemiologic transition and changing lifestyles (Walker *et al.*, 2010; Feigin *et al.*, 2013). As stroke care also improves in these regions, the number of stroke survivors is likely to increase with attendant increased burden of long term consequences of stroke which includes cognitive impairment. Currently, knowledge gap exists on cognitive impairment and dementia in sub- Saharan African stroke survivors. Previous dementia studies (focusing largely on AD) generally suggest prevalence rates between 2 and 3% (Ogunniyi *et al.*, 2000; Ogunniyi and Akinyemi, 2010; Prince *et al.*, 2013).

The CogFAST – Nigeria study was therefore set up to evaluate the profile and risk factors of stroke - related VCI in a previously un - investigated indigenous African population

given the on - going epidemiologic transition on the continent and the enhanced predisposition of ethnic black persons to cognitive impairment from cerebrovascular disorders (Douiri *et al.*, 2013b) with worse outcome (Chang *et al.*, 2013; Roth *et al.*, 2011). The CogFAST – Nigeria study was established building on the principles and approaches of the COGFAST –Newcastle study to generate complementary data in a multidisciplinary manner as far as possible in a different population with different genetic signature, culture and lifestyle practices. In addition, scientific evidence of vascular brain injury suitable for planning appropriate preventive and therapeutic interventions applicable to sub - Saharan Africa was anticipated. Our aim is to generate the full spectrum of clinical, neuroimaging, neuropsychological, genetic, possibly pathological and lifestyle factors results from older Nigerian stroke survivors. The pilot and baseline data from the CogFAST-Nigeria project have been presented at the 5<sup>th</sup> and 6<sup>th</sup> VASCOG Meetings in Lille, France and Toronto, Canada in September 2011 and June 2013 respectively.

## **1.6. Mechanisms of Post - Stroke Cognitive Impairment**

Mechanistic approaches stemming from multiple streams of evidence from animal, epidemiological, neuropathologic, cellular and molecular studies are required to develop evidence-based therapeutic and neuroprotective interventions to combat the early and delayed cognitive consequences of stroke. Although accumulating evidence has provided a durable link between cerebral vascular disease and cognitive impairment, and pathological substrates are more clearly delineated, the underlying mechanisms are not yet fully understood (Moskowitz *et al.*, 2010; Gorelick *et al.*, 2011; Kalaria, 2012b; Kalaria, 2012a).

### ***1.6.1. Neuropathological substrates of VCI***

Cerebrovascular pathology may be divided into two broad categories: large vessel diseases (infarcts, artery –to –artery embolism, occlusion of extra-cranial or intracranial arteries, haemorrhages) and small vessel diseases that make up to 25% of all cerebral vascular lesions: white matter lesions –leukoaraosis, lacunar infarcts, microinfarcts, cerebral embolic disease, microbleeds, hereditary disorders –cerebral autosomal dominant arteriopathy

subcortical infarcts and leucoencephalopathy (CADASIL), cerebral autosomal recessive arteriopathy subcortical infarcts and leucoencephalopathy (CARASIL), specific arteriopathies or haemodynamic mechanisms resulting in cerebral hypoperfusion (pulse wave encephalopathy and chronic heart failure) (Erkinjuntti and Kalaria, 2006; Knopman, 2006).

Small vessel disease (SVD) abnormalities which may lead to diffuse white matter disease and microinfarcts are common in VCI. SVD microangiopathy consists of arteriolosclerosis, lipohyalinosis, fibrinoid necrosis and microatheromas. Microinfarcts, especially when multiple, have been identified with very robust association with cognitive impairment especially in elderly subjects with CVD (Ballard *et al.*, 2000; Vinters *et al.*, 2000; White *et al.*, 2002; Kalaria, 2012a).

<b>Pathological Substrate</b>	<b>Strength of association with VCI</b>
Microinfarcts	+++
Demyelination and oligodendrocyte changes	+++
Cribriform change, perivascular spacing	+++
Alzheimer pathology	+++
Hippocampal atrophy and sclerosis	+++
Atheromatous and occlusive disease	++
Lacunar infarcts	++
Cerebral amyloid angiopathy	++
Astrogliosis and microgliosis	++
Intracerebral haemorrhages	++
Atheromas	+
Macroscopic infarcts	+

**Table 1.4.: Neuropathological Substrates Associated with VCI.** (+ -mild; ++ =moderate; +++ - strong) *Adapted from Kalaria et al., 2004; Kalaria, 2012*

Lacunar infarcts demonstrate a moderate association with VCI and may be confused with lacunar haemorrhages or dilated perivascular space (Kalaria *et al.*, 2004; Kalaria, 2012a). White matter changes attributable to loss of myelin and axonal damage often result from oligoemia/ chronic hypoxic state with associated expression of hypoxia-inducible factors (Fernando *et al.*, 2006; Simpson *et al.*, 2009).

Apart from large infarcts, microinfarcts, diffuse white matter disease and periventricular lesions being the predominant neuropathological substrates of VCI, hippocampal atrophy and sclerosis (Gemmell *et al.*, 2012) may also be seen. In the general absence of significant degenerative neuropathology, this is believed to provide evidence for a vascular basis for hippocampal neurodegeneration in tandem with growing neuroimaging evidence of medial temporal lobe atrophy in vascular dementia (Bastos-Leite *et al.*, 2007; Scher *et al.*, 2011) including CADASIL which is a classical model of 'pure vascular dementia syndrome (O'Sullivan *et al.*, 2009)..

### ***1.6. 2. Overlap of AD and VCI***

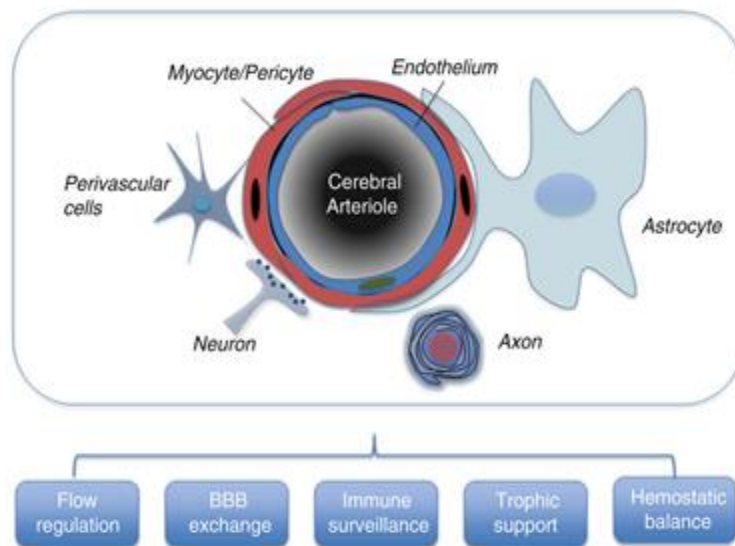
AD and VCI demonstrate a lot of similarity in risk factors, pathomechanisms, clinical features and neuropathological substrates (Kalaria, 2002a) . In the very first documented case of AD reported by Alzheimer, the descriptions of autopsy findings in the index patient, Auguste D, showed significant vascular pathology (Alzheimer, 1907) . In a series of 300 autopsy cases of AD from the Newcastle Prospective Dementia series, there were 98% cerebral amyloid angiopathy, 100% microvascular degeneration, 31% infarcts of all sizes and 7% intracerebral haemorrhages (Kalaria and Ballard, 1999) while in another series, Brun and Englund reported periventricular white matter lesions similar to Binswanger's disease in up to 60 % of patients with AD (Brun and Englund, 1986). Generally, it is recognized that with co-existent pathological substrates of AD and cerebrovascular lesions, it takes fewer AD pathologies to produce the same degree of dementia (Snowdon *et al.*, 1997; Esiri *et al.*, 1999) .

Animal models of combined AD and VCI have also been developed. A combined mouse model of AD and VCI expressed higher inflammatory response and more hippocampal AD pathology as well as larger infarcts and reduced threshold of manifesting cognitive

impairment (Whitehead *et al.*, 2005; Whitehead *et al.*, 2007). Ageing baboons have also demonstrated co- existence of beta - amyloid and microvascular pathologies (Ndung'u *et al.*, 2012).

### 1.6.3. The Neurovascular Unit

An intimate developmental, structural and functional relationship exists among the cerebral microvascular cells (endothelium, pericytes and adventitial cells), neurons and glial cells (astrocytes, microglia and oligodendrocytes). The ‘neurovascular unit’ aptly describes this relationship and ‘neurovascular coupling’ maintains the delicate functional relationship between the neural and the vascular components that ensure a coordinated response to ageing or injury (Girouard and Iadecola, 2006; Iadecola, 2010).



**Figure 1.1. The Neurovascular Unit.** Microvascular cells (endothelial, pericytes), neurons and glial cells (astrocytic foot process), in close structural and functional relationship. (Adapted from Iadecola *Acta Neuropathologica* 2010; 120: 287 – 296).

To maintain the integrity of the cerebral circulation and thus ensure a consistent flow of blood and supply of energy substrates commensurate to the needs of the different parts of



the brain, mechanisms are in place which include cerebrovascular autoregulation, functional hyperaemia and the blood brain barrier (BBB)(Kalaria, 1996; Kalaria, 2009a).

Cerebrovascular autoregulation ensures that cerebral blood flow (CBF) is maintained independently of alterations in mean arterial blood pressure provided it is within the range of 50 -160 mmHg. Functional hyperaemia ensures that cerebral blood flow is increased to an activated brain region through the release of several vasoactive agents (including nitric oxide, prostanoids, adenosine, K<sup>+</sup> ions, carbon monoxide, cytochrome p450 metabolites) released from neurons, astrocytes and vascular cells. The BBB limits the entry of potentially toxic substances into the brain through its impermeable membrane (Kalaria, 1996; Girouard and Iadecola, 2006; Kalaria, 2009a).

#### ***1.6.4. The ageing cerebral microvasculature***

Ageing as a biological phenomenon has measurable impact on the structural and functional integrity of the vasculature, and neurovascular unit. The brain depends on a continuous but regulated supply of oxygen and nutrient - laden blood through its dense network of the macro and microvasculature. The macrovasculature consisting of vessels arising from the circle of Willis stiffens with ageing and acquires atheromas. The main branches emanating from the circle including the anterior, middle and posterior cerebral arteries and the basilar artery may develop up to 30% stenosis(Ferrer *et al.*, 2008). The pial arteries which divide into smaller penetrating arteries give rise to arterioles and capillaries deeper in the brain which undergo arteriosclerosis, basement membrane thickening and numerous endothelial cell changes with ageing (Ogata *et al.*, 2011).

Microemboli and thrombi originating from the cardiovascular system may cause further structural distortions of the ageing microvasculature and alteration of the rheology of the blood making the brain more vulnerable to reduced perfusion of cerebral surfaces and deeper structures (Kalaria *et al.*, 2012b).

Therefore, one major effect of ageing on cerebrovascular disorders and vascular risk factors is the breakdown of cerebrovascular defence mechanisms resulting in reduction of regional blood flow causing focal cerebral hypoperfusion and breach of the BBB thus enabling potentially toxic substances and metabolites to gain access into the brain (Kalaria, 1996).

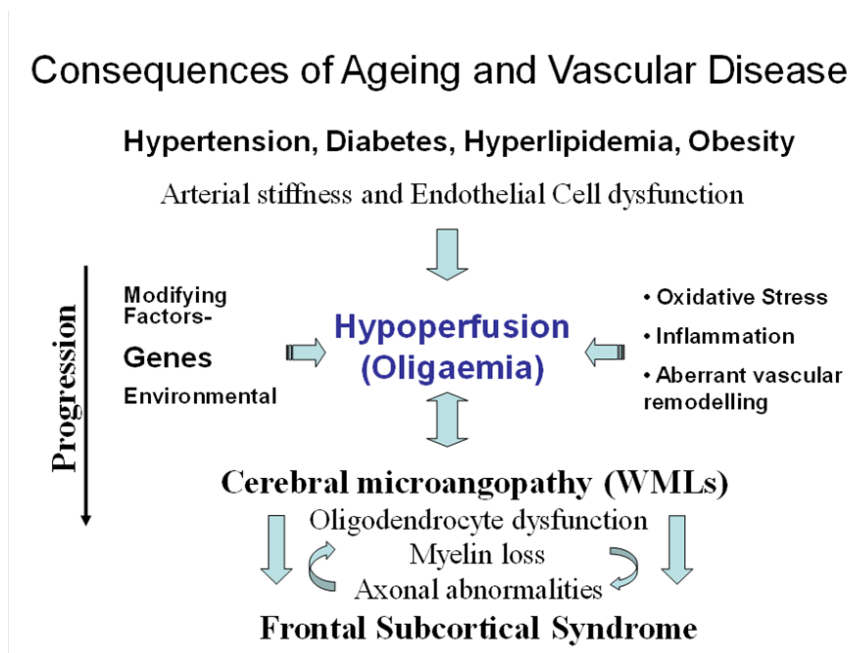
### ***1.6.5. Cerebrovascular Mechanisms***

The structural and functional changes associated with cerebral vascular disease may cause a reduction in cerebral blood flow at rest and during brain activation. When this reduction reaches a critical level, it is described as the “*Critically Attained Threshold of Cerebral Hypoperfusion (CATCH)*” (de la Torre and Mussivand, 1993; de la Torre, 2002). This hypothesis suggests that a critical reduction of cerebral perfusion results in the breakdown of the cerebrovascular defence system, uncoupling of the neurovascular unit, reduced clearance of by-products of brain activity, accumulation of substances like amyloid- $\beta$  and activation of neuroinflammatory response (Iadecola, 2010) (Figure 1.2).

The link between hypoxia/ischaemia, accumulation of amyloid- $\beta$  and cognitive dysfunction has been demonstrated in experimental models and epidemiologic studies of ageing, AD and VaD. Studies have shown accumulation of amyloid- $\beta$  in rodent models of brain ischaemia (Kitaguchi *et al.*, 2009) as well as in brain tissue of human subjects with VaD (Lewis *et al.*, 2006). Apart from this, there is a direct cognitive effect of microvascular pathology as seen in subjects with DM type II and AD (Kalaria, 2009b) and Cerebral Autosomal Dominant Arteriopathy Subcortical Infarcts and Leucoencephalopathy (CADASIL) subjects with VaD (Yamamoto *et al.*, 2009).

As part of the compromise of cerebrovascular defence mechanisms occasioned by cerebral vascular disease, there could be disruption of the endothelium and the blood brain barrier (BBB) causing a transport disequilibrium across the barrier. In rats, amyloid- $\beta$  peptides cross a defective BBB into the brain by passive diffusion and through a shared influx transporter, the advanced glycation end product receptor (RAGE) (Deane *et al.*, 2009) while the low density lipoprotein receptor related protein (LRP) is a major efflux transporter which expression is reduced in ageing rodents, non-human primates and AD patients (Shibata *et al.*, 2000; Sagare *et al.*, 2007). These alterations compromise the homeostasis of the cerebral microenvironment and reduce the synthesis of neuronal proteins involved in memory formation causing reduced formation and consolidation of new memories and alteration of synaptic plasticity (Klann and Dever, 2004; Ihara and Kalaria, 2007).

Oxidative stress in the brain and cerebral blood vessels has been found to play a critical role in the processes associated with cerebrovascular dysfunction, with NADPH oxidase being a major source of reactive oxygen species (ROS) involved (Iadecola, 2004; Miller *et al.*, 2005). Reactive oxygen species (ROS) alter vascular regulation through processes involving the formation of peroxynitrite from the reaction between nitric oxide (NO) and superoxide radical. Peroxynitrite exerts biological effects through several processes which include cysteine oxidation, tyrosine nitration, altering protein function and damage to lipid membranes and DNA (Pacher *et al.*, 2007) (Girouard *et al.*, 2007). Oxidative stress and reactive oxygen species resulting from mitochondrial dysfunction have, consequently, been strongly implicated in brain ageing, AD and VaD (Bennett *et al.*, 2009; Massaad *et al.*, 2010).



**Figure 1.2: Hypothetical scheme for cerebrovascular mechanisms of vascular cognitive impairment.** Vascular risk factors and ageing produce arterial stiffness and endothelial dysfunction resulting in cerebral hypoperfusion which activates oxidative stress, inflammation and consequent white matter changes including oligodendrocyte loss, axonal abnormalities and myelin loss. (Adapted from Kalara *et al* 2010, 2012a)

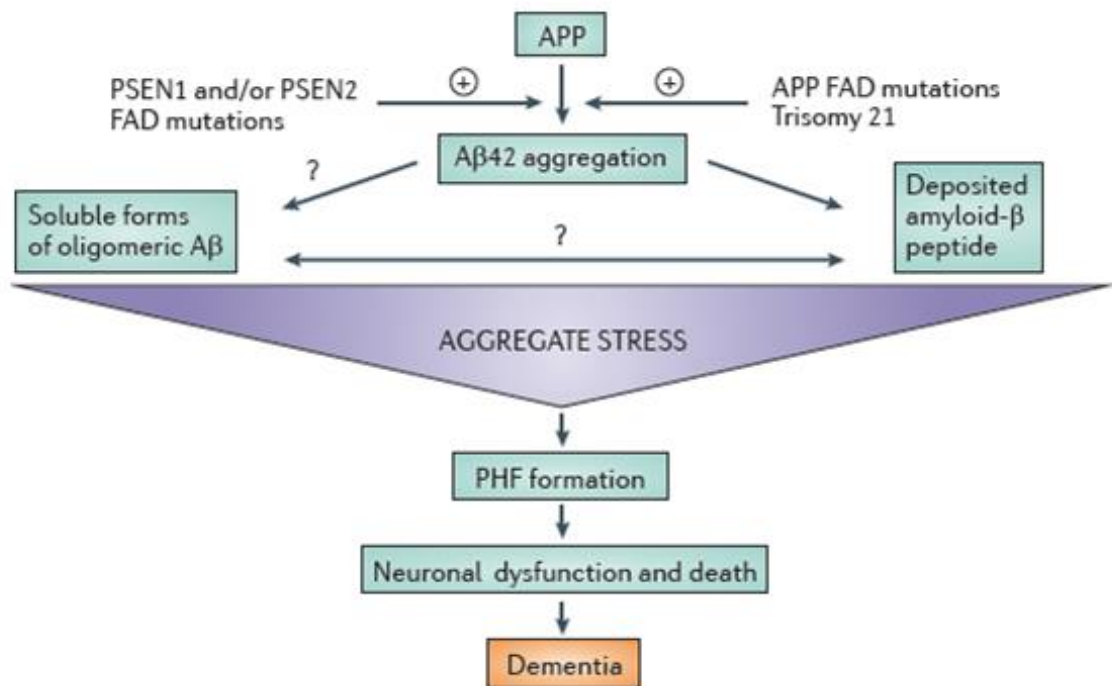
Cerebrovascular disorders (CVDs) also cause cognitive impairment and dementia through their impact on cholinergic neurotransmission which plays a major role in normal cognition, particularly in the domains of attention, emotion and memory (Roman and Kalaria, 2006; Keverne *et al.*, 2007) . CVDs may affect the population of cholinergic neurons in the basal forebrain including the nucleus basalis of Meynert (nBM). Ischaemic injury resulting from cerebral hypoperfusion leads to widespread disconnection of cholinergic innervations from the neuronal population of the nBM to other parts of the brain including the neocortex, and this has been documented in both AD and VaD (Roman and Kalaria, 2006; Keverne *et al.*, 2007) . Loss of glutamatergic synapses, assessed by vesicular glutamate transporter 1 (VGLUT 1) in the temporal cortex but preserved in the frontal cortex has been documented in post- stroke VCI. The preservation in the frontal cortex of non-demented subjects supports a role for glutamatergic synapses in the maintenance of cognition after stroke (Kirvell *et al.*, 2010).

#### ***1.6.6. Neurodegenerative Mechanisms***

Neurodegeneration is central to the pathobiology of age-associated diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS). These are commonly characterized by the accumulation of abnormal protein products within neuronal cells and progressive loss of selective anatomically and physiologically related neurons by apoptotic and autophagic mechanisms (Bredesen *et al.*, 2006; Lin and Beal, 2006). AD is characterized by the accumulation of amyloid plaques in extracellular compartments of the brain parenchyma and vessel walls, and hyperphosphorylated tau proteins intracellularly. In the amyloid cascade hypothesis, amyloid precursor protein (APP) a transmembrane protein is sequentially cleaved by  $\alpha$ ,  $\beta$  and  $\gamma$  secretases. Cleavage by  $\alpha$  and then  $\gamma$  secretase channels APP into the non-amyloidogenic pathway, while cleavage by  $\beta$  and then  $\gamma$  secretase breaks down APP in the amyloidogenic pathway producing the amyloid  $\beta$  (1 – 40) or (1 – 42) molecule (depending on the site of  $\gamma$  cleavage). Amyloid  $\beta$  (1 – 42) is less soluble and more toxic. Amyloid monomers aggregate to form soluble oligomers, toxic species, that are believed to mediate perturbation of synaptic connections and network dysfunction (Hardy and Higgins, 1992;

Hardy and Selkoe, 2002; Palop *et al.*, 2006) ; Ultimately, insoluble amyloid fibrils are formed and deposited in the brain parenchyma and walls of cerebral vessels (Small, 1998).

However, within the cell membrane are neuronal sorting proteins which control the traffic of proteins between the cell membrane and cytoplasmic compartments such as the Golgi apparatus or nucleus. The neuronal sortilin-related receptor protein SORL1 (aka LR11/SORLA) is a 250kDa type -1 glycoprotein and transmembrane receptor protein involved in the regulation of the intracellular trafficking and processing of amyloid precursor protein (APP)(Rogaeva *et al.*, 2007). It channels amyloid precursor protein (APP) from amyloidogenic into non-amyloidogenic pathways (Andersen *et al.*, 2005) . Reduction in the expression of SorL1 therefore leads to the formation of more amyloid, first in soluble forms which aggregate to be laid down as insoluble amyloid deposits (Andersen *et al.*, 2005; Offe *et al.*, 2006) . Recent meta –analysis have confirmed the association of the SorL1 (SorLA) gene with late –onset sporadic AD (Jin *et al.*, 2013).



**Figure 1.3: The Amyloid Cascade Hypothesis.** APP is sequentially cleaved by  $\beta$  and  $\gamma$  secretases to generate amyloid  $\beta$  1- 40/42 monomers which form intermediate toxic soluble oligomers and insoluble amyloid fibrils that are laid down in brain parenchyma and vessel wall. The dynamics of how the soluble species change to the insoluble forms and trigger tau hyperphosphorylation are, however, not yet clearly defined.(Adapted from Hardy and Higgins, 1992; Selkoe and Hardy, 2002)

Reduced expression of SorL1 was initially demonstrated in brain tissue from subjects with mild cognitive impairment (MCI) and Alzheimer's disease (AD), showing correlation with the degree of cognitive impairment rather than the severity of amyloid or neurofibrillary pathology (Sager *et al.*, 2007). But a recent report from the same group did not validate the initial findings as similar levels of SorL1 were expressed in MCI and control groups and reduction was reported only in 29% of AD subjects (Sager *et al.*, 2012). They suggested that the relationship between SorL1 and AD was more complex and warranted further investigation. The role of SorL1 and its relationship to markers of amyloid, tau and synaptic pathology are, however, yet unexplored in vascular cognitive impairment.

#### **1.6.7.1. Effect of cerebrovascular disease on the neurovascular unit and amyloid balance.**

A delicate balance is normally maintained between the production, metabolism and clearance of amyloid. However, in the presence of cerebral vascular disease (CVD), this balance could be altered resulting in accumulation of amyloid.

As part of the compromise of cerebrovascular defence mechanisms caused by cerebral microvascular disease, the endothelium and the blood brain barrier (BBB) could be disrupted resulting in transport disequilibrium across the barrier. These microvascular abnormalities may be accelerated by ageing but further degenerative changes could also be accentuated by amyloid deposition. Amyloid deposition in the microvasculature impedes 'functional hyperemia' during brain activation, uncouples the neurovascular unit and induces 'vasoconstriction' causing reduction of perivascular drainage and amyloid clearance (Iadecola, 2010).

Besides, hypoxia resulting from hypoperfusion due to cerebrovascular disease can induce hypoxia-inducible factor (HIF) 1 $\alpha$  which stimulates upregulation of Beta-site APP cleaving enzyme 1 (BACE1) expression, the major  $\beta$  – secretase enzyme, leading to increased generation of amyloid  $\beta$ . Amyloid  $\beta$  overproduction leads to deposition of amyloid in plaques and vessel wall. The vasoconstrictive effect of this further potentiates the reduction of cerebral blood flow by worsening the pre- existing arteriosclerosis and

BBB disruption leading to a vicious cycle of increasing amyloid accumulation (Velliquette *et al.*, 2005).

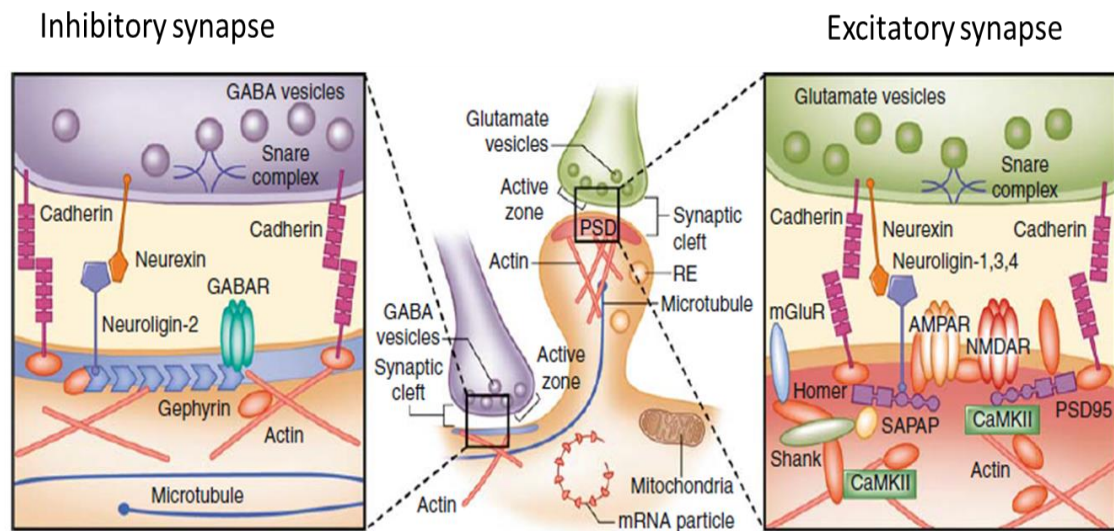
Therefore, cerebral hypoperfusion induced by cerebral vascular disease may either initiate and/or accelerate the neurodegeneration cascade causing amyloid deposition, synaptic and neuronal dysfunction leading to cognitive impairment (Kalaria, 2000; Ihara and Kalaria, 2007). Previous studies on rat and mice, including (transgenic APP mice) models of chronic cerebral hypoperfusion have shown acceleration of amyloid deposition, cortical microinfarcts and hippocampal atrophy (Kalaria *et al.*, 1993b; Kalaria, 2000) ; (Kitaguchi *et al.*, 2009; Nishio *et al.*, 2010; Yamada *et al.*, 2011; Okamoto *et al.*, 2012).

### ***1.6.7. Synaptic integrity and cognitive functioning***

The regulation of neural communication depends upon alterations in the structure and chemistry of synapses, the formation of new synapses and elimination of old ones. This underscores the concept of ‘synaptic plasticity’ which underpins the neurobiology of learning and memory (Kandel, 2001; Di Maio, 2008) .

#### **1.6.7.1. Molecular architecture of synapses**

The mammalian synapse consisting of the pre – synaptic axon terminal delimited by the pre –synaptic terminal button and the post –synaptic region (often on dendrites) described as the post – synaptic density. The pre – and post – synaptic membranes are separated by a gap of 20 to 25 nm, known as the *synaptic cleft* and held together at the appropriate separation by cell adhesion molecules including N – cadherin, neuroligin, neurexin and other scaffolding proteins and receptors (Sheng and Hoogenraad, 2007; van Spronsen and Hoogenraad, 2010). Synapses may be excitatory (e.g. glutamatergic) or inhibitory (e.g. GABAergic) (Figure 1.4) and specific morphological or functional alterations in different elements of the synapse lead to cognitive and behavioural dysfunction associated with ageing and different disorders of the brain (van Spronsen and Hoogenraad, 2010; Morrison and Baxter, 2012).



**Figure 1.4. Molecular architecture of inhibitory and excitatory synapses.** Inhibitory shaft is present along the dendritic shaft while excitatory synapse is present on mushroom shaped dendritic spine. (from van Spronsen and Hoogenraad. *Curr Neurol Neurosci Rep* (2010) 10: 207 -214)

#### 1.6.7.2. Pre – synaptic structure and function.

Action potential travels down the axonal membrane of the pre –synaptic axonal terminal. Synaptic vesicles ‘dock’ and make contact with the ‘active zone’ of the pre –synaptic membrane, fuse with the membrane and then release neurotransmitters which diffuse into the synaptic cleft. This process involves  $Ca^{2+}$  dependent mechanisms and the interaction of several proteins which form the soluble N – ethylmaleimide – sensitive factor attachment protein receptor (SNARE) complex (Sudhof, 2004). The SNARE complex mediates vesicle fusion and exocytosis and are responsible for ‘docking’ of the synaptic vesicle. Members of the SNARE complex include syntaxin -1, synaptosomal – associated protein of 25 kDa (SNAP – 25) and synaptobrevin (Deak *et al.*, 2004). Pre – synaptic functioning may be influenced by the amount of neurotransmitters stored in the released vesicles, the structure of the fusion pores,  $Ca^{2+}$  ion dynamics and glutamate recycling mechanisms (van Spronsen and Hoogenraad, 2010; Penzes and Vanleeuwen, 2011).



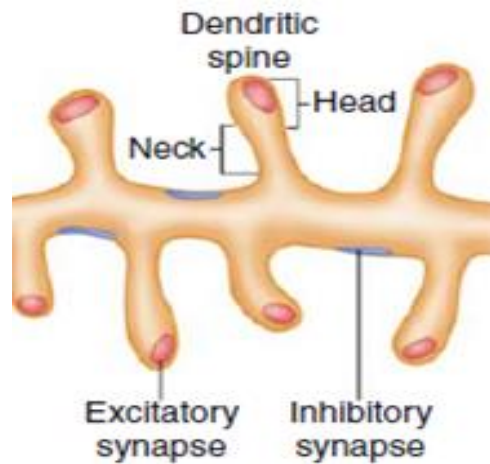
### **1.6.7.3. Post - synaptic structure and function**

Following release, neurotransmitters bind to receptors on the post – synaptic membrane which may be ligand - gated ion channels (ionotropic receptors) or G protein - coupled (metabotropic) receptors. Beyond the post – synaptic membrane is a complex of interlocked proteins known as the post – synaptic density. This includes cell adhesion molecules,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-Methyl-D-aspartic acid or N-Methyl-D-aspartate (NMDA) glutamate receptors,  $Ca^{2+}$  /Calmodulin dependent kinase II, actin and other signaling proteins (Sekino *et al.*, 2007). The post – synaptic density is an electron – dense structure consisting of various scaffolding proteins of which PSD – 95 is a prototype. Post – synaptic density plays a central role in excitatory synaptic plasticity working in synergy with microtubular structural proteins to regulate the morphogenesis and function of dendritic spines (Kojima and Shirao, 2007; Sekino *et al.*, 2007; van Spronsen and Hoogenraad, 2010).

### **1.6.7.4. Dendritic spine morphology structure and function.**

Dendritic spines are small membraneous protrusions on the post- synaptic side of excitatory synapses (Kojima and Shirao, 2007).

Dendritic spines may have different shapes but generally consist of a bulbous head and a thin neck that connects them to the dendritic shaft. Spine contents include neurotransmitter receptors, ion channels, scaffolding proteins, actin, actin – binding cytoskeletal proteins and intracellular signaling molecules (Kojima and Shirao, 2007; van Spronsen and Hoogenraad, 2010). Dendritic spines are extremely dynamic structures that change size and shape continuously in relation to the changing dynamics of synaptic connections (Yuste and Bonhoeffer, 2001).



**Fig 1. 5. Dendritic spines are located along dendritic shafts of excitatory synapses.** Their dynamic characteristics are determined by a balance of actin cytoskeleton, scaffolding proteins, adhesion molecules and receptors. (from van Spronsen and Hoogenraad. *Curr Neurol Neurosci Rep* (2010) 10: 207 -214)

Spine morphology changes rapidly in tandem with neuronal activity and glutamate receptor activation such that induction of long –term potentiation (LTP) causes spine head enlargement while long – term depression causes spine head shrinkage (Yuste and Bonhoeffer, 2001; Kasai *et al.*, 2003). The continuous dynamic relationship of the actin cytoskeleton and its binding proteins such as gelsolin, cofilin, adducin, profilin, fascin, neurabin , myosin and drebrin is the major factor in dendritic spine morphogenesis. Drebrin A is a neurone specific side actin - binding protein that plays a critical role in spine morphogenesis, morphology and function (Sekino *et al.*, 2007).

<b>Marker</b>	<b>Localisation</b>	<b>References</b>	<b>Described Changes</b>
Synaptosomal –associated protein 25 kDa (SNAP – 25)	First identified in hippocampal mossy fibers. Part of SNARE complex (Soller et al.,1993) Plays a role in pre-synaptic vesicular traffick and processing (Sudhof et al., 1995)	Greber et al., 1999 Sze et al., 2000 Downes et al.,2008 Mukaetova – Ladinska et al., 2009. Connelly et al., 2011 Honer et al., 2012 Beerl et al, 2012 Mukaetova – Ladinska et al., 2013	↓ Down syndrome and AD ↔ AD and control subjects ↓ In Downs Syndrome ↓ Lewy variant of AD ↓ selective in FTLT ↓ dementia by SNAP -25 – syntaxin interaction ↓ In AD vs controls ↓ DLB visual cortex compared to AD and controls
Synaptophysin (SY -38)	Labels synaptic vesicle membranes. Part of SNARE complex. Role in transmitter release (Clare et al., 2010)	Heinonen et al., 1995 Kirvell et al., 2006 Downes et al.,2008 Mukaetova – Ladinska et al., 2009. Mukaetova – Ladinska et al., 2013	↓ AD ↓ AD ↓ Down Syndrome ↔ In Lewy variant of AD ↓ In DLB visual cortex compared to AD and Ctrs
Vesicular Glutamate Transporter -1 (VGLUT -1)	Loads glutamate, the principal excitatory neurotransmitter of cortical and hippocampal neurons, into vesicles (Freneau et al.,2004)	Kirvell et al.,2006 Kashani et al., 2008 van der Hel et al.,2009 Kirvell et al., 2010	↓ VGLUT-1 in parietal and occipital cortex in AD ↓ VGLUT-1 in prefrontal cortex in AD VGLUT- 1 predominant in human hippocampus ↓ VGLUT-1 in frontal cortex in AD and VaD but preserved in post –stroke no dementia
Post-synaptic density – 95 (PSD – 95)	Neuronal scaffolding protein localized at PSD of excitatory synapses(Hart et al.,1996; Aoki et sl.,2001)	Gyls et al.,2004 Love et al.,2006 Leuba et al., 2008 Sultana et al, 2010 Li and Xin, 2013	↓ PSD – 95 in AD brain ↓ PSD- 95 in human temporal cortex ↑ PSD -95 in AD ↓ PSD – 95 in hippocampus of MCI subjects ↓ PSD – 95 expression in Wistar Rat stroke model exposed to nitrogen dioxide
Drebrin A	Neurone –specific F- actin binding protein, regulates dendritic spine structure and function (Hayashi et al., 1996)	Harigaya et al., 1996 Hatanpaa et al.,1999 Counts et al., 2006 Counts et al., 2012	↓ Drebrin in hippocampus in AD ↓ Drebrin in normal ageing and AD ↓ Drebrin in MCI and AD temporal cortex but ↑ Drebrin in MCI frontal cortex ↓ Drebrin in hippocampus of MCI and AD

**Table 1.5. Pre – and Post – synaptic markers, their cellular localization and described changes in previous human studies**

#### **1.6.7.5. Synaptic changes in cognitive impairment and dementia**

Synaptic, molecular, morphological and functional alterations are hallmarks of disease processes causing cognitive dysfunction in several brain disorders and these often occur before neuronal loss and other obvious pathological changes are seen.

Correlation between synaptic loss and AD was first established in 1987 through the seminal ultrastructural electron microscopic studies undertaken by Davies and colleagues (Davies *et al.*, 1987). Their findings were subsequently substantiated by other investigators (DeKosky and Scheff, 1990; Scheff *et al.*, 1990) who further showed that synaptic loss was the best correlate of AD severity. This view was subsequently accepted by the larger community of AD researchers with Selkoe declaring AD as a synaptic failure (Selkoe, 2002). Subsequent studies investigated the relationship between the traditional neuropathologic substrates of AD ie neurofibrillary tangles and amyloid deposits and severity of synaptic decline but the findings revealed that synaptic markers only correlated with the degree of severity of dementia and not necessarily the traditional AD pathologies (Callahan and Coleman, 1995; Blennow *et al.*, 1996). However, soluble amyloid oligomers have since shown clear relationship with synaptic dysfunction in different models (Selkoe, 2002; Mucke and Selkoe, 2012).

To date, several deficits in different pre- and post – synaptic markers have been reported in AD as well as in mild cognitive impairment (Table.1.5). These include synaptophysin (Heinonen *et al.*, 1995; Ishibashi *et al.*, 2006; Kirvell *et al.*, 2006; Kashani *et al.*, 2007; Downes *et al.*, 2008); syntaxin (Sze *et al.*, 2000; Clare *et al.*, 2010) (Mukaetova-Ladinska *et al.*, 2013b) (Mukaetova-Ladinska *et al.*, 2009; Honer *et al.*, 2012), SNAP – 25 (Shimohama *et al.*, 1997; Greber *et al.*, 1999; Bailey and Lahiri, 2006; Connelly *et al.*, 2011; Beeri *et al.*, 2012; Honer *et al.*, 2012); vesicular glutamate transporter (Fremeau *et al.*, 2004a; Fremeau *et al.*, 2004b) (Kirvell *et al.*, 2006; Kashani *et al.*, 2007; van der Hel *et al.*, 2009; Kirvell *et al.*, 2010); PSD –95 (Gyls *et al.*, 2004; Love *et al.*, 2006) (Leuba *et al.*, 2008; Sultana *et al.*, 2010; Li and Xin, 2013) as well drebrin (Harigaya *et al.*, 1996; Hayashi *et al.*, 1996; Hatanpaa *et al.*, 1999; Counts *et al.*, 2006; Counts *et al.*, 2012).

Synaptic alterations have similarly been reported in other dementia phenotypes including frontotemporal dementia and dementia with Lewy bodies (DLB) (Ferrer, 1999; Clare *et al.*, 2010); (Mukaetova-Ladinska *et al.*, 2013b).

Few studies, however, have been reported on synaptic dysfunction in vascular dementia. Perdahl and colleagues reported no significant change in the expression of synapsin -1 in subjects with multi –infarct dementia (MID) (Perdahl *et al.*, 1984) and similarly no change in the level of synaptophysin was seen in MID in another study (Heinonen *et al.*, 1995). However, decreased synaptic proteins were reported in the frontal, hippocampal and occipital regions of patients with Binswanger’s disease (Zhan *et al.*, 1993a; Zhan *et al.*, 1993b; Zhan *et al.*, 1994) while more recently reduced expression of VGLUT-I was reported in subjects with vascular dementia (Kirvell *et al.*, 2010).

#### ***1.6.8. Role of white matter pathology***

White matter (WM) is composed largely of myelinated and unmyelinated axons, glial cells (microglia, astrocytes, oligodendrocytes) and blood vessels. The integrity of WM is critical to the regulation and efficiency of neuronal communication as well as maintenance of cognitive functioning. Loss of WM integrity which often manifests as hyperintensity on MRI occurs in the settings of ageing, cerebrovascular diseases and their associated dementias (Pantoni and Garcia, 1995; Moskowitz *et al.*, 2010). White matter lesions consist of demyelination, axonal loss, enlarged perivascular spaces, astrogliosis, microglial activation and oligodendrocyte shrinkage or loss (Pantoni and Garcia, 1995; Fernando *et al.*, 2006; Simpson *et al.*, 2007a). In addition, arteriolar wall may be thickened by the accumulation of hyaline material (hyalinosis) or completely disrupted by fibrinoid material (fibrinoid necrosis) causing microhaemorrhages (Fisher, 1968). In addition, hypoxia-inducible genes and markers (hypoxia –inducible factors -1  $\alpha$  and  $\beta$  are upregulated suggesting hypoxia – ischaemia (Simpson *et al.*, 2009).

Disruption of the blood brain barrier associated with the elaboration of matrix metalloproteinases (2, 3 and 9) leads to perivascular oedema and microhaemorrhages. This is a distinct feature of neuroinflammation (Rosenberg *et al.*, 2001; Candelario-Jalil *et al.*, 2009; Rosenberg, 2009). Matrix metalloproteinases are also detected in inflammatory cells, reactive astrocytes and microglia and can also be quantified from the cerebrospinal

fluid (Rosenberg, 2009). Matrix metalloproteinases have also been demonstrated in animal models of chronic hypoperfusion where ischaemic demyelination is a major pathophysiological mechanism (Nishio *et al.*, 2010; Coltman *et al.*, 2011; Horsburgh *et al.*, 2011).

Axonal damage is a component of white matter damage and a key predictor of outcome in neurological disorders including neurotrauma, metabolic encephalopathies, multiple sclerosis, leucodystrophies and other white matter disorders (Medana and Esiri, 2003). Demyelination and axonal damage cause attenuation of cortico-cortical and cortico-subcortical connections “disconnection syndromes” (Catani and ffytche, 2005) which manifest in impairment of executive functioning and slowed information processing speed. MRI-based neuroimaging modalities including diffusion tensor imaging, tissue segmentation, magnetic resonance spectroscopy are useful for probing microstructural integrity of white matter (Black *et al.*, 2009).

In AD, there is robust evidence from neuropathological and neuroimaging studies using (Pittsburgh Compound B) showing the activation of microglia by the presence and accumulation of amyloid fibrils (Cagnin *et al.*, 2001; Edison *et al.*, 2011).. In studies examining the preservation of cognitive functions despite high amyloid burden, compensatory mechanisms of early cellular response associated with activation of glial cells and neuronal nuclear hypertrophy have also been implicated (Erten-Lyons *et al.*, 2009).

#### ***1.6.9. The ‘Cognitive Reserve’ Hypothesis***

Several clinico - pathologic studies have demonstrated that the level of brain pathology does not always correlate with the degree of cognitive impairment (Erten-Lyons *et al.*, 2009; Nelson *et al.*, 2009; Chetelat *et al.*, 2010; Nelson *et al.*, 2012) . Although the concept owes its origins to the writings of Satz (Satz *et al.*, 1993), who described ‘*brain reserve*’ as the physiologic element that supports brain function, Stern has probably devoted more attention to develop the concept of ‘*cognitive reserve*’ such that while ‘*brain reserve*’ represents the hardware, ‘*cognitive reserve*’ represents the ‘software’ (Stern, 2002; Stern, 2009; Stern, 2012; Stern, 2013). Stern’s concept of ‘*cognitive reserve*’ suggests that the brain enlists compensatory processes, such that a greater amount of pathology is

required for cognitive impairment to develop (Stern, 2002; Stern, 2012). Several epidemiological studies have linked persons with low educational and low occupational attainment with increased risk of dementia (Stern *et al.*, 1996; Stern *et al.*, 1999; Valenzuela and Sachdev, 2006; Brayne *et al.*, 2010; Valenzuela *et al.*, 2011; Prince *et al.*, 2012). In another recent study, (Wilson *et al.*, 2013) found higher noradrenergic neuronal density in the locus ceruleus correlated significantly with reduced risk of cognitive decline (Wilson *et al.*, 2013a; Wilson *et al.*, 2013b). Apart from education, other factors that may enhance ‘cognitive reserve’ include environmental enrichment, physical activity, and social networking (Satz *et al.*, 2011).

## **1.7. Hippocampal formation and its connections**

### ***1.7.1. Hippocampal circuitry and function***

The hippocampal formation is one of the most studied regions of the brain. Lorento de No provided early description of the hippocampus using the Cornu Ammonis nomenclature (Lorento de No, 1934; Duvernoy, 2005). There is a huge volume of robust evidence from neuroimaging, neuropathological and molecular studies confirming the role of the hippocampal formation in the processes of memory formation and consolidation, as well as its structural and functional changes in ageing and disease (Brickman *et al.*, 2011; Small, 2011; Small *et al.*, 2011). The hippocampal formation consists of the hippocampus (CA regions, dentate gyrus), the subiculum and the entorhinal cortex. The term “*Ammonshornsklerose*” was first introduced by Sommer to define loss of pyramidal cells in the Sommer sector of the Cornu Ammonis (Sommer, 1880).

#### **1.7.1.1. Anatomy**

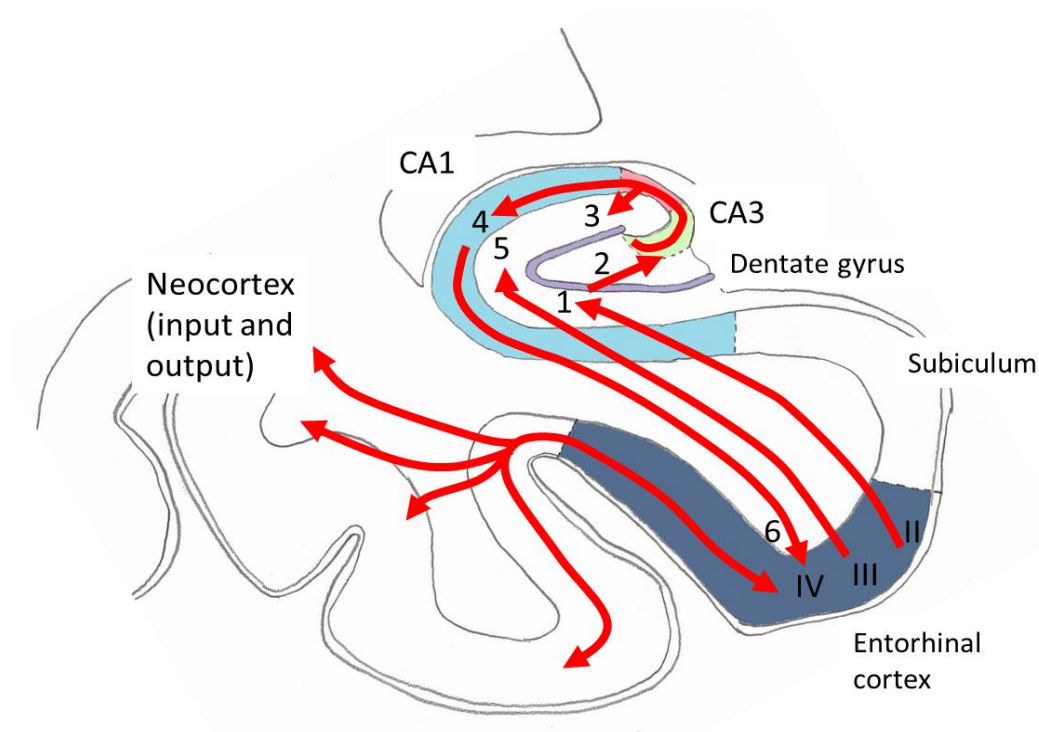
The hippocampal formation spans the posterior –anterior extent of the base of the temporal lobe of the brain and consists of multiple sub-regions – the entorhinal cortex, the subiculum, the dentate gyrus and the Cornu Ammonis (CA) subfields. The CA region is a three layered strip of archicortex consisting of polymorphic, pyramidal cells and molecular layers (Duvernoy, 2005). The pyramidal cells are then divided up into three or four separate fields (CA1, CA2, CA3, CA4). The CA1 occupies the largest proportion of the CA between

the dentate gyrus and the subiculum. The CA2 is a distinct, compact subfield between CA1 and CA3 while CA3 and CA4 occupy the segment aborting the dentate gyrus. Each hippocampal region has a unique molecular anatomy as recent gene expression studies show (Zhao *et al.*, 2001; Datson *et al.*, 2004). This distinctive molecular anatomy provides a substrate for regional vulnerability of the different subfields to different toxic agents (Small *et al.*, 2011).

#### **1.7.1.2. Connections**

The entorhinal cortex is the gateway into the hippocampal formation. It receives monosynaptic projections from several cortical and subcortical regions including the amygdala, olfactory and auditory cortices, prefrontal cortex, parahippocampal and perirhinal cortices (Goldman-Rakic *et al.*, 1984). Then the layer II of the entorhinal cortex (EC) connects to the dentate gyrus through the *Perforant Pathway*, and the DG connects to CA3 through Mossy fibres. CA3 “auto – associates” with other CA3 neurons or connects with CA1 through “Schaffers collaterals” while the CA1 pyramidal neurons connect to the subiculum. This is described as the “*tri-synaptic pathway*”. Besides, layer III of the entorhinal cortex may connect directly with CA3, and layer III of the EC can project to CA1 directly and the subiculum (Insausti *et al.*, 1987; Insausti and Amaral, 2008; Insausti *et al.*, 2013). Outflow impulses out of the hippocampal formation is provided dominantly by the subiculum and CA1 sub-region which connect with deep layers of the EC. The EC then re- connects – through the parahippocampal gyrus – with neocortical sites that provide the original input (Amaral, 1993). However, anatomical tracing studies have also provided evidence that the outflow from the subiculum and CA1 can bypass the EC and ‘monosynaptically’ connect with a broad range of brain areas including the amygdala, the pre-frontal and orbitofrontal cortices, the cingulate cortex and the temporal cortex (Rosene and Van Hoesen, 1977) (Goldman-Rakic *et al.*, 1984).





**Figure 1. 6: Hippocampal sub - regions and connections.** The CA1, CA2, CA3, subiculum and entorhinal cortex are highlighted. 1 = the Perforant Pathway; 2 = Mossy fibres; 3 = Schaffers collaterals; 4 = outflow from CA1; 5 = direct EC – CA1 fibres; 6 = EC – Neocortical connections

### 1.7.1.3. Function

There is robust evidence from electrophysiological, functional MRI and gene expression profiling studies suggesting an anterior –posterior functional specialization of the hippocampus in its longitudinal axis (Small *et al.*, 2011). While the anterior regions are involved with goal –oriented activities, stress, emotion and sensori – motor coordination, the posterior regions are more involved with cognitive processing and memory (Bast and Feldon, 2003) (Moser and Moser, 1998; Bast *et al.*, 2009; Fanselow and Dong, 2010). The hippocampus through the presence of ‘*place cells*’ also plays a major role in facilitating spatial localization. The neuroplastic effect of spatial localization was demonstrated in London taxi drivers (Maguire *et al.*, 2000).

#### **1.7.1.4. Regional Vulnerability**

The hippocampal formation, due to regional variation in the molecular anatomy of the different sub-fields, shows variation in the vulnerability of its different sub- regions to different diseases. While the CA1 sub-field is most susceptible to vascular disease, the EC is differentially susceptible to AD and the DG is differentially susceptible to ageing (Small *et al.*, 2011).

#### **1.7.2. Hippocampal changes in cognitive impairment and dementia**

Neuroimaging and neuropathological studies have advanced our knowledge on global and sub - regional hippocampal changes in the continuum of cognitive impairment and dementia. Hippocampal atrophy was previously considered a signature of degenerative pathology, particularly AD (Soininen and Scheltens, 1998; Almkvist and Winblad, 1999) but growing evidence suggests it may also have a vascular basis. Hippocampal atrophy predicted cognitive performance in a cohort of CADASIL subjects (O'Sullivan *et al.*, 2009), showed significant association with midlife vascular risk factors and vascular brain injury (DeBette *et al.*, 2011) and vascular dementia in the Honolulu – Asia Aging Study (HAAS) (Scher *et al.*, 2011). Hippocampal atrophy has also been associated with DLB (Barber *et al.*, 1999) and a positive family history of AD (Okonkwo *et al.*, 2012). Pathologically, hippocampal atrophy may be due to hippocampal sclerosis (Dawe *et al.*, 2011; Nelson *et al.*, 2013) or neuronal atrophy (Gemmell *et al.*, 2012). Impairment of episodic memory and even executive functions are significant neurocognitive correlates of hippocampal atrophy (Mormino *et al.*, 2009; Gemmell *et al.*, 2012; Oosterman *et al.*, 2012).

In degenerative dementias, particularly AD, the hallmark lesions of neurofibrillary tangles and extracellular amyloid plaques are seen in the hippocampal formation although the temporal evolution and hierarchical progression differs. Whereas amyloid deposition originates in the neocortex and only gets to the hippocampus later in its course (Thal *et al.*, 2002b), neurofibrillary pathology usually involves the hippocampal formation early after starting off in the entorhinal cortex (Braak and Braak, 1991). These have been incorporated into a recent revision of the diagnostic criteria for AD, especially as this hierarchical progression permits the detection and diagnosis of pre-clinical stages of AD (Montine *et al.*, 2012).

The cognitive impact of hippocampal pathologies depends on the type of pathology, the sub - regional location and severity, and their influence on the hippocampal circuitry (Lace *et al.*, 2009). For instance, neuritic plaques and tangles show better correlation with cognitive scores than cored or diffuse plaques while neocortical amyloid correlates better than archicortical amyloid (Nelson *et al.*, 2009; Nelson *et al.*, 2012). Tau pathology in the perforant pathway particularly correlates with the onset and severity of dementia (Thal *et al.*, 2000; Lace *et al.*, 2009). Furthermore, the CA1 sub - region, in particular, demonstrates high susceptibility to insult from hypoxia/ischaemia, hypoglycemia, whereas the CA2 region is more resistant (Duvernoy, 2005).

## **1.8. Aims and Outline of the Thesis**

In view of the foregoing background, the overall aim of this thesis was to establish a comparative cohort in Nigerian African stroke survivors to investigate the profile and determinants of post - stroke cognitive impairment (VCI) and further explore the mechanisms of cerebral injury and cognitive impairment following stroke in post - mortem brains collected from the Newcastle cohort who had come to autopsy. The specific objectives of this thesis were:

1. To determine the profile and determinants of cognitive impairment in a cohort of Nigerian African stroke survivors three months after stroke.
  - *Hypothesis:* Given the general low prevalence of vascular dementia often reported from Africa, a low frequency, multi - domain cognitive impairment would be seen in a cohort of Nigerian African stroke survivors at three months post – stroke.
2. To determine the neuroimaging features associated with cognitive impairment in Nigerian African stroke survivors
  - *Hypothesis:* Neuroimaging features including medial temporal lobe atrophy and white matter hyperintensities would be associated with cognitive impairment in Nigerian African stroke survivors.
3. To quantify hippocampal Alzheimer pathology in demented and non - demented post stroke cases in comparison with other dementias and ageing controls.

- *Hypothesis:* Hippocampal Alzheimer pathology would be differentially expressed in demented and non – demented stroke survivors in comparison with other dementia subjects and normal ageing controls.
- 4. To determine hippocampal synaptic changes in post-mortem brains collected from the Newcastle cohort.
  - *Hypothesis:* Differences in the expression of hippocampal synaptic markers would distinguish demented from non – demented post – stroke cohorts in relation to normal ageing controls and other dementia subjects.
- 5. To assess frontal and temporal white matter abnormalities (glial activation, demyelination and axonal damage) in the post- stroke cohorts compared to normal ageing controls and other dementias..
  - *Hypothesis:* Markers of glial activation, demyelination and axonal damage would be differentially expressed in the white matter of demented and non-demented post – stroke cohorts compared to normal ageing controls and other dementia subjects.

The following chapter describes general materials and methods used in the study, chapters three to seven contain results, and the final chapter contains an overall discussion.

Chapters three and four are devoted to the profile, determinants and neuroimaging features associated with post-stroke cognitive impairment in the Nigerian African cohort. Stroke survivors were assessed with a combination of clinical, neuropsychological and neuroimaging approaches.

Chapters five, six and seven report our findings in the post - mortem brain tissue from the Newcastle cohort, which were investigated given the significant findings from the neuroimaging studies in the Nigerian African cohort that were in tandem with previous reports from the Newcastle cohort. Using immunohistochemical and neurochemical approaches and a panel of different markers, we investigated neurodegenerative hippocampal Alzheimer pathology and synaptic changes, and frontal and temporal white matter abnormalities in demented and non - demented post - stroke subjects compared to normal controls and Alzheimer’s disease subjects. Finally, the complementary findings from the two major arms of the project are discussed in the context of extant literature, public health perspectives and anticipated future work.

## **Chapter 2. Materials and Methods**

### **2.1. Introduction**

This chapter describes the methods used in the analysis of the aims of the project reported in this thesis. These include a new clinico epidemiological study on cognitive function in a cohort of African stroke survivors in southwestern Nigeria and a post - mortem clinico - pathological study in Newcastle, United Kingdom. It details the design, sites, subjects, protocol, procedure and flow of the Cognitive Function After STroke (CogFAST) – Nigeria Study as well as the identification of the laboratory cohort, the diagnostic criteria for the CogFAST - Newcastle cohort, VaD, AD and definition of control subjects. Finally, the immunohistochemical procedures, image acquisition and analysis as well as statistical analysis of clinical, neuropsychological, neuroimaging and laboratory data from the overall project are described.

### **2.2. The CogFAST- Nigeria Study**

#### ***2.2.1 Introduction***

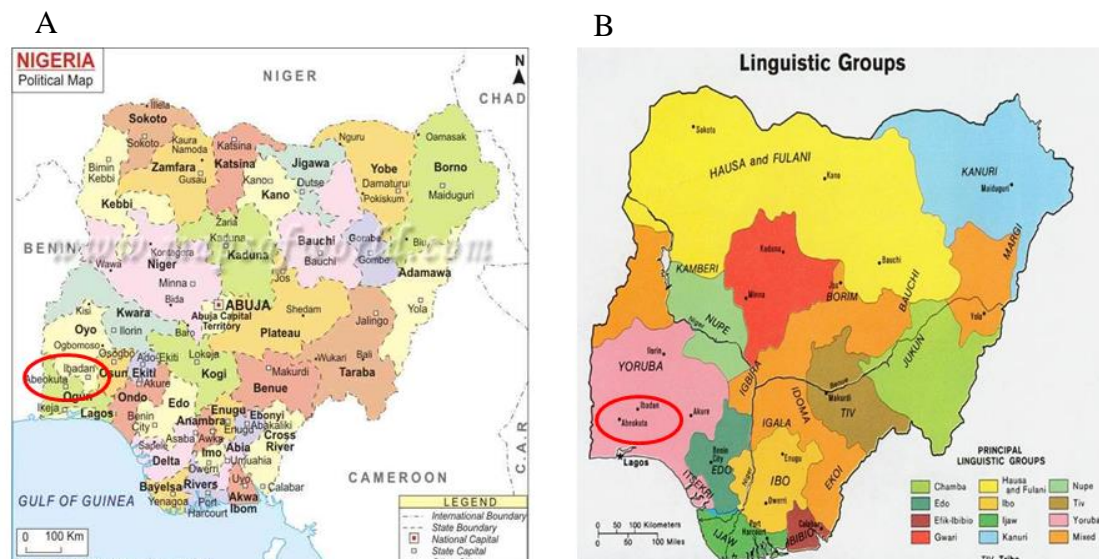
Despite a growing burden of stroke in sub-Saharan Africa (Connor *et al.*, 2007; Strong *et al.*, 2007; Owolabi, 2011) with all its accompanying motor and non-motor consequences, there is scanty information on the cognitive sequelae of stroke on the continent. The Cognitive Functions After Stroke- Nigeria (CogFAST–Nigeria) Study is an extension of the highly successful CogFAST-Newcastle Study (Allan *et al.*, 2011) to undertake a pioneering investigation of post-stroke cognitive impairment in the most populous nation of people of the Black race, Nigeria.

### 2.2.2. Study Design

The CogFAST- Nigeria Study had a mixed study design with a combination of case - control and longitudinal cohort approaches. Stroke survivors were recruited and assessed three months after the ictus. They were subsequently rolled into a follow up programme with assessments conducted at 9 months, 15 months and 27 months post – stroke respectively. Stroke – free healthy volunteers of comparable age, gender and levels of education were also recruited from representative members of the community, spouses and caregivers of stroke survivors without any somatic, mental or neurological illness in order to generate normative neuropsychological data with which data accrued from the stroke survivors were compared.

### 2.2.3. Study Sites

Stroke patients were recruited from the services of two specialist hospitals - Federal Medical Centre Abeokuta and the University College Hospital, Ibadan both cities in southwestern Nigeria. These hospitals are the two major referral specialist centres in the two respective cities and staffed with one neurologist (FMC Abeokuta) and three neurologists (UCH Ibadan).



**Figure 2.1. Map of Nigeria showing the study area in southwestern part of the country [A] Political map of Nigeria showing Abeokuta and Ibadan north of Lagos [B] An ethno – linguistic map showing the Yoruba – speaking southwestern region of the country within which the study centres are located.**

Abeokuta, located 60 miles north of Lagos, is the capital city of Ogun State in southwestern Nigeria and has a population of 593,140 (2005 Nigeria census). The principal inhabitants of the city are the Yoruba people of Niger-Kordofonian ancestry (Campbell and Tishkoff, 2010). The Federal Medical Centre, Abeokuta is a 250-bed regional tertiary centre which was established in April 1993. It receives patients from Ogun and neighbouring states, and relates with two secondary care level facilities and smaller hospitals and community care clinics within and outside the Abeokuta metropolis. In 2010 there were 6,410 admissions and 101,435 outpatient visits. There are about 50 stroke discharges annually. Many stroke cases are seen by complementary medicine practitioners while fatal and mild strokes often remain unaccounted for within the community (Ogun *et al.*, 2005).

Ibadan, the capital city of Oyo State also in southwestern Nigeria, is the third largest city in Nigeria by population, and the largest in geographical area. It had a population of 2,550,593 in 2005 spread over 11 local government areas. The principal inhabitants of the city are also Yoruba people. The University College Hospital Ibadan was established in 1952 as the first teaching hospital in Nigeria. The hospital is a tertiary institution with a number of affiliated community care centers where the hospital offers secondary and primary health care. About 150 stroke patients are discharged annually. However, the lack of an effective health insurance system and out – of – pocket payment by patients often precluded patients from following up care transfers to tertiary centres where there is a concentration of specialist expertise to manage conditions like stroke.

Three other centers (Catholic Mission Hospital, Oluyoro Ibadan, Oluwaseun Physiotherapy Clinic, Ibadan and State Hospital, Ijaiye Abeokuta) were also included in the study. These were smaller secondary healthcare centers with fewer stroke patients and who were usually referred to the specialist centres. These smaller centres were, however, included to ensure representativeness of different levels and types of healthcare facilities in the region. The three smaller facilities are community-based secondary healthcare facilities providing largely general medical care and services.

## 2.2.4 Study Subjects

### Stroke participants

Eligible cases were stroke patients aged 45 years or older. This cut off age was adopted for the purpose of this study in order to better reflect the relative youthfulness of the Nigerian population. For instance, recent life expectancy estimates showed: total population (46.94 years) male (46.16 years); female (47.76 years) (2011 estimates) and only 4% of Nigerians are older than 60 years ([www.population.gov.ng/](http://www.population.gov.ng/)]. In contrast, current life expectancy in Newcastle upon Tyne (2012 estimates) is 76.2 years (male) and 81.0 years (female) (<http://www.ons.gov.uk/ons/publications>). The cut off age for the CogFAST – Newcastle Study was 75 years (Ballard *et al.*, 2002; Allan *et al.*, 2011).

Stroke patients diagnosed clinically by the most senior physician (neurologist) in the specialist centres were admitted to the medical wards and then logged onto the study register. They were subsequently screened for eligibility within three months after the ictus. Subjects were approached regarding participation in the study at discharge from hospital or during initial outpatient visit after stroke (patients who presented primarily in the outpatient clinic). They were subsequently invited for assessment by word of mouth or through mobile telephone contact. Between the point of discharge and 3 months posts - stroke, each potential subject was screened for eligibility based on the criteria of: 45 years of age or older, duration after stroke within 3 months and clinically confirmed stroke based on history, physical examination and neuroimaging as much as possible.. Stroke was defined according to the WHO clinical definition (World Health Organization, 1988) and classified using the WHO Definition, the Oxford Community Stroke Project Classification (Bamford J, 1991) and neuroimaging (CT scan and/ or MRI) findings when available. Some patients did not have neuroimaging due to limited access and high cost in Nigeria. The WHO clinical definition/criteria have been shown to have a sensitivity of 73 % for haemorrhage, 69% for infarction and an overall accuracy of 71% in Nigeria (Ogun *et al.*, 2002).

Exclusion criteria were: [1] subarachnoid haemorrhage [2] significant physical illness and motor impairment that precluded paper and computer-based neuropsychological evaluation (eg. visual impairment, moderate-severe aphasia, hemiparesis affecting the dexterous hand (MRC power grade <3) [3] any co – morbid psychiatric or neurologic illness [4] any



systemic disease that could impair cognition e.g. chronic liver disease, chronic kidney disease [5] inability or failure to give consent.

### **Stroke-Free Controls**

For comparison with the neuropsychological data from stroke survivors, apparently healthy subjects who were free of clinically - evident stroke were recruited from a pool of community-dwelling volunteers who were unrelated to the stroke subjects and were participating in a community health literacy programme. Control subjects were also recruited from among the spouses and, unrelated caregivers of stroke survivors as well as from among patients attending the general outpatient clinic for routine physical assessment. Individuals with background dementia (DSM IV criteria) or scoring less than 20 on the Community Screening Instrument for Dementia and psychiatric disorders e.g. schizophrenia, major depression, manic-depressive disorder; background neurological disorders e.g. Parkinson's disease, (evidence from case records, informant or self report) or who were unable to provide consent and/or informant were excluded from being controls.

#### ***2.2.5 Ethical Approval***

The local research ethics committees of the Oyo State Ministry of Health (University College Hospital, Ibadan , Catholic Mission Hospital, Oluyoro Ibadan, Oluwaseun Physiotherapy Clinic, Ibadan ) and the Federal Medical Centre Abeokuta) and State Hospital, Ijaiye Abeokuta granted approval for the study while written informed consent was obtained from each subject.

#### ***2.2.6 The Study Protocol***

The study instrument consisted of a screening proforma, case questionnaire, control questionnaire, informant/caregiver interview and cognitive assessment battery. These we

developed following a rigorous process of reviewing tools which had been used in previous studies on stroke epidemiology and risk factors, ageing and dementia, and post-stroke cognitive dysfunction, with particular attention paid to validated tools previously used in the study population and other developing countries (Gureje *et al.*, 1995; Ogunniyi *et al.*, 2000; Baiyewu *et al.*, 2005; Hendrie *et al.*, 2006; Truelsen *et al.*, 2007; Akinyemi *et al.*, 2008; O'Donnell *et al.*, 2010b). To assess cognitive function in our subjects, the Vascular Neuropsychological Battery was developed in step with the 60 – minute protocol suggested by the NINDS – CSN Harmonization Standards and in strict adherence to the recommendations of the group (Hachinski *et al.*, 2006a). Thus, relevant validated tools previously used in the CogFAST - Newcastle Study (Ballard *et al.*, 2002; Ballard *et al.*, 2003b) and the Ibadan – Indianapolis Dementia Study were adopted, or refined to reflect the peculiarities of the Yoruba language, culture and belief systems prevalent in the study area. These were also all factored into the subsequent training of the staff of the project. Translations and back –translations from English to Yoruba language were undertaken where necessary. All instruments were reviewed by a team of neurologists, neuropsychologist, research nurses and a biostatistician before pilot tests were conducted on a purposive sample of stroke survivors.

The screening proforma contained an essential checklist of items to determine the eligibility of stroke survivors on the registers for recruitment into the study and is usually administered within one to two weeks of the 3 months baseline assessment. The case and control questionnaires contained items assessing demographic information, vascular risk factors, other – comorbidities, lifestyle (smoking, alcohol use, physical exercise), diet, details of index stroke as well as findings of relevant laboratory investigations including (neuroimaging [CT scan/MRI]. Administration of the case questionnaire (including general and neurologic examination) and cognitive assessment were performed on separate days (not more than 48hrs apart) to avoid excessive fatigue from a single session. Cognitive assessment was undertaken in a quiet room and usually in the early working hours of the day. Subjects were educated to avoid any stimulants within 12 hours of cognitive assessment.

### **2.2.6.1 Cognitive Assessment Battery**

An ideal neuropsychological battery should be robust, brief, valid, reliable, cost effective, sensitive enough to detect deficits but specific enough to avoid false positives. In addition, it should be easy to administer, be available in multiple forms, have cross cultural capability and have no floor or ceiling effect (Blake *et al.*, 2002; Hachinski *et al.*, 2006a). However, in reality no single tool satisfies all these requirements. New tools being devised, nonetheless, are to aspire to attain these ideal characteristics.

The cognitive assessment tools utilized in this study consisted of the Community Screening Instrument for Dementia (CSID) – cognitive part (Hall *et al.*, 2000; Ogunniyi *et al.*, 2000) the mini-mental state examination (MMSE) (Folstein *et al.*, 1975) and the Vascular Neuropsychological Battery (V – NB) (Hachinski *et al.*, 2006b). The CSID and the MMSE are tests of general cognitive functioning while the V –NB consists of battery of tests assessing functioning in specific domains of cognition including executive function, language, memory and visuospatial/visuoconstructive domains.

### **2.2.6.2. Community Screening Instrument for Dementia (CSID)**

The CSID is a paper and pencil test of global cognitive performance which adaptability, validity and utility in populations from different cultural, educational and socio-economic backgrounds have been established. From previous validation studies on the CSID, the maximum total cognitive score was 33 while a cut off score of 28.5 was used to define cognitive impairment (Hall *et al.*, 2000; Ogunniyi *et al.*, 2000). It has a sensitivity of 87% and specificity of 83% for the clinical diagnosis of dementia and has been used reliably and widely to assess cognition in the Yoruba speaking population of southwestern Nigeria wherein the present study was conducted. The CSID includes sub-scores for attention, orientation, calculation, short and long term memory, language comprehension and expression, praxis and abstract thinking. A raw score method was used for scoring resulting in score range of 0 – 30 with higher scores indicating better cognitive function.

In the current study, three items were excluded from the CSID. These were two items assessing constructional ability (overlapping circles and interlocking pentagons). They were deleted because the loss of motor function and dexterity in the dominant hand occasioned by stroke could impair performance on these tasks. Moreover, these two items were strongly influenced by education. Also the item assessing the ‘name of local mayor’ was dropped because of confusion between the real names and title names of local mayors in the African setting. Experience had shown that some respondents to this item mentioned the ‘real name’ of the mayor while others mentioned the ‘designated title’ and these were not exactly the same. With this minor revision of the CSID scores, the highest possible score came down to 30 while the cut off for cognitive impairment became 25.5.

#### **2.2.6.3. Mini - Mental State Examination (MMSE)**

The Mini - Mental State Examination (MMSE) is a 30 – item test of general cognitive functioning that includes items for assessment of orientation, memory, attention, language and constructional abilities (Folstein *et al.*, 1975). In the validated Yoruba version of MMSE, the attention item required the subject to state the days of the week backwards (from Friday to Monday) (Gureje *et al.*, 1995). This was a substitute for spelling WORLD backwards in the English version. While a cut -off score of 24 or less is most frequently used to define the presence of dementia in the English version, this was found inappropriate in sample with limited education. A cut-off score of 13 or less for Yoruba-speaking Nigerians with no education and 16 or less for subjects with one or more years of formal education were used in defining possible dementia (Gureje *et al.*, 1995).

#### **2.2.6.4. Vascular Neuropsychological Battery (V-NB)**

In 2006, the National Institute of Neurological Disorders and Stroke (NINDS) in collaboration with the Canadian Stroke Network (CSN) published the Harmonization Standards for a comprehensive characterization of vascular cognitive impairment (Hachinski *et al.*, 2006a). Specific recommendations were made for clinical, neuropsychological, neuroimaging, neuropathological and animal models in the characterization and development of criteria for the definition of vascular cognitive

impairment. The neuropsychological sub-section contained recommendations of cognitive tests for 60 –min, 30 – min and 5 - min assessment of the core domains of executive function, language and visuospatial functioning. We, therefore, devised the Vascular Neuropsychological Battery (V –NB) after the NINDS –CSN Harmonization Standards 60 –minute neuropsychological protocol with relevant modifications to ensure adaptability to the language and culture of our predominant Yoruba study population.

The V- NB consists of multiple test items examining specific cognitive domains (executive function, memory/learning, language, visuospatial function/visuoconstructive skills) (Table 2. 1).

Executive function/activation and mental speed were assessed using the category (animal) fluency test, phonemic (letter) fluency test, verbal reasoning and visual reasoning tests. The number of animals listed in the first 15 sec of the animal fluency test provided an assessment of mental speed while all the tests differently assessed mental flexibility and divergent thinking.

The verbal reasoning and visual reasoning tests were adapted from the Cambridge Cognitive Examination (CAMCOG), a cognitive test section of the CAMDEX assessment (Cambridge Mental Disorders of the Elderly Examination) designed to assess a broad range of cognitive functions (Blessed *et al.*, 1991). It demonstrates excellent psychometric properties and has been used widely in assessing vascular cognitive impairment (de Koning *et al.*, 2000; Ballard *et al.*, 2002; de Koning *et al.*, 2005)

Memory/learning was assessed with the 10- item word list learning test and delayed recall of stick design, (Gureje *et al.*, 1995; Baiyewu *et al.*, 2005). The word list learning is a 3 - trial, 10 – item test with free recall taken after each learning trial and after a brief delay. The total number of words recalled across the three trials make up the total score (range: 0 – 30) while the delayed recall is scored ( 0 -10)`, higher scores indicating better performance. Language was assessed through the 15 – item Boston Naming Test (Hachinski *et al.*, 2006a). In a prior validation study of the CERAD battery among Yoruba Nigerians, subjects were requested to name line drawings of common and uncommon objects. Four of the low frequency items from the standard CERAD-NB were replaced with items felt to be more culturally appropriate (i.e. guitar for harmonica, blacksmith tongs for

ice cube tongs, mosquito netting for hammock, and 'ayo' (Nigerian board game) for dominoes(Gureje *et al.*, 1995).

Visuospatial/visuoconstructive functioning was assessed through the Stick Design Test (Baiyewu *et al.*, 2005) and the Modified Token Test (IU Token Test) (Unverzagt *et al.*, 1999; Akinyemi *et al.*, 2008) . The Stick Design Test is a non- graphomotor test of visuospatial /visuoconstructive ability. The respondent is requested to use match sticks to reproduce four different graphical shapes with particular attention to the correctness of relative orientation of the match heads. Thereafter the respondent reproduces the four shapes without any cues to assist. The test is particularly useful in older adults with limited formal education (Baiyewu *et al.*, 2005). The Modified Token test consists of a piece of laminated paper with a spectrum of squares and circles of varying sizes (small and large) and colours (black, yellow, green and red). The interviewer reads aloud a series of commands that request the subject to point to the figures in different combinations. The test is scored based on correct identification of shapes in the required sequence (range 0 – 24) with higher scores indicating better performance (Unverzagt *et al.*, 1999).

Test items from the Cognitive Drug Research (CDR) computerized assessment battery were also included in the V-NB for the evaluation of attention, processing speed and executive function. [The constituent tests included Simple Reaction Time (SRT), Choice Reaction Time (CRT), Digit Vigilance (DV) and Spatial Working Memory (SWM) (Ballard *et al.*, 2002; Wesnes, 2002). The instructions were translated and back –translated from English into Yoruba Language by experienced linguists. Several of these tests were also previously validated and successfully utilized to evaluate cognitive functions in a cohort of Nigerian subjects with Parkinson’s disease (Akinyemi *et al.*, 2008). The cognitive assessment battery was pilot - tested on 42 stroke survivors between January and June 2010 in order to further evaluate the feasibility of the methods, acceptance and adaptability in persons living with stroke.

<b>Cognitive Domain</b>	<b>Test</b>	<b>Reference</b>
Executive Function /Activation	Category (Animal) Fluency Test	<i>Gureje et al., 1995;</i> <i>Blessed et al, 1991</i> <i>Ballard et al, 2002</i>
	Phonemic (Letter) Fluency Test	<i>Blessed et al, 1991</i> <i>Ballard et al, 2002</i>
	Verbal Reasoning (Similarities Test)	<i>Blessed et al, 1991</i> <i>Ballard et al, 2002</i>
	Ideational Fluency Test	<i>Blessed et al, 1991</i> <i>Ballard et al, 2002</i>
Language/ Lexical Retrieval Memory/ Learning	Boston Naming Test (2nd version)	<i>Gureje et al., 1995;</i> <i>Akinyemi et al., 2008</i>
	Word List Test (Learning, Recall, Recognition) Delayed Recall of Stick Design	<i>Gureje et al., 1995;</i> <i>Akinyemi et al., 2008</i> <i>Gureje et al., 1995;</i> <i>Akinyemi et al., 2008</i>
Visuospatial/ Visuoconstruction	Stick Design Test	<i>Baiyewu et al., 2005</i>
	Modified Tokens Test (IU Token Test)	<i>Unverzagt et al., 1999;</i> <i>Akinyemi et al., 2008</i>
General Cognitive Functioning	Community Screening Instrument for Dementia (CSID)	<i>Hall et al, 1993</i> <i>Hall et al, 2000</i>
	Minimental State Examination (MMSE)	<i>Folstein, 1975</i> <i>Gureje et al, 1995</i>

**Table 2. 1. The Vascular Neuropsychological Battery** devised after the 60 –minute Vascular Cognitive Impairment Harmonization Standards – Neuropsychological Protocol proposed by the NINDS – CSN (Hachinski et al, 2006). Multiple test items assessing each cognitive domain were selected in consonance with the recommendations of the Harmonization standards and utility of such tests in previous cognitive evaluations in the environment of the study population.

## ***2.2.7 Study Procedure***

### **2.2.7.1 The Pilot Study**

An initial pilot study was designed to test the feasibility of the methods and procedures of the study. Logistic issues relating to the general conduct of the study such as clarity and comprehensibility of instructions on the study instruments, cultural acceptability of methods, for instance computer based testing in a low literate population; correct operation of equipment and duration of assessments were evaluated (Lancaster *et al.*, 2004; Thabane *et al.*, 2010; Leon *et al.*, 2011).

### **2.2.7.2. Baseline Evaluation**

Baseline evaluation was performed at three months post-stroke in tandem with the design of Desmond *et al* (Desmond DW, 1996) to enable the resolution of acute post-stroke delirium. The evaluation included comprehensive medical history, assessment of neurological impairment and disability using the modified Rankin Scale (Wilkinson *et al.*, 1997), Stroke Levity Score (Owolabi and Platz, 2008) and Barthel Index (Wilkinson *et al.*, 1997), depressive symptoms (using the Centre for Epidemiologic Studies Depression Scale (Andresen *et al.*, 1994) and four – item Geriatric Depression Scale (Almeida and Almeida, 1999), and blood screens. The Stroke Levity Score is an assessment of stroke severity based on maximum power (0-5) in the dexterous hand + maximum power in the weaker lower limb + mobility score-1 (if aphasia present). It has shown excellent correlation with the National Institutes of Health Stroke Scale (NIHSS) (Owolabi and Platz, 2008). Confirmation of cardiovascular risk factors (hypertension, diabetes mellitus, atrial fibrillation, dyslipidaemia, cigarette smoking, alcohol use) were based on self- report, use of relevant medications and review of medical notes. Nutritional lifestyles and physical activity were assessed according to the design of the INTERSTROKE Study (O'Donnell *et al.*, 2010b). Dietary patterns were assessed in all subjects with a food frequency questionnaire assessing frequency of different types of food taken by the subjects in the prior year before the onset of stroke (and separately in the three months period after stroke)



with appropriate addition of common local foods and delicacies to relevant categories. Physical activity was assessed at work and during leisure time and subjects were stratified into sedentary, mild, moderate and heavy physical activity categories (O'Donnell *et al.*, 2010b). Cognitive assessment was performed by experienced interviewers who received further two weeks training on the study instrument and had to achieve an inter – rater reliability of at least 90% in mock assessments done with volunteers from the hospital community before conducting cognitive assessment on the study subjects.



**Figure2.2. Study Flow chart showing basic study procedure**

### **2.2.7.3. Follow up evaluations**

This thesis will describe the baseline data only but follow up evaluation of the cohort is ongoing. Evaluations are undertaken at 9 months, 15 months and 27 months respectively after stroke to assess the evolution of cognition and other functions in the cohort. This consists of cognitive assessment with the CSID, MMSE and V –NB and dementia status

according to the DSM IV criteria. Also assessed are motor functions, functional status, cardiovascular status, quality of life and caregiver/informant assessment.

#### **2.2.7.4. Neuroimaging assessment (Magnetic Resonance Imaging)**

Brain magnetic resonance imaging (MRI) was performed on a subset of recruited stroke survivors 3 months after the stroke event. Two MRI scanners used operated between 0.2 and 0.35 T. Medial temporal lobe atrophy and white matter changes were assessed using the Schelten's scales (Scheltens *et al.*, 1992; Scheltens *et al.*, 1993). All images were transferred to computer workstation with Clear canvas DICOM viewer and evaluated by two experienced radiologists. All ratings were performed by consensus agreement.

Assessment of brain volumes – total intracranial volume, total brain volume and ventricular volume - was performed by creating a mask using the brain extraction tool (Bet) from the FSL software ([www.fmrib.ox.ac.uk/fsl/](http://www.fmrib.ox.ac.uk/fsl/)).

#### **2.2.8. Cognitive Diagnosis**

To make a cognitive diagnosis on a subject, all available datasets including cognitive scores, functionality and disability scores (the Barthel Index and modified Rankin score) coupled with the physician's assessment were assembled and discussed by the research team for consensus diagnosis. Final cognitive diagnosis was made based on the VCI criteria proposed by the American Stroke Association/American Heart Association Vascular Cognitive Impairment (VCI) Guidelines (Gorelick *et al.*, 2011) and the DSM IV criteria (American Psychiatric Association, 1994).

##### **2.2.8.1. Operational definitions of Vascular Cognitive Impairment**

Failure on a test was defined as individual mean score that was at least 1.5 standard deviations below the mean score of the control group. Impairment in a domain was defined as failure on at least 50% of tests examining that particular domain (Dong *et al.*, 2012).

Vascular Mild Cognitive Impairment (Vascular MCI) or Vascular Cognitive Impairment No Dementia (Vascular CIND) and Post- Stroke Dementia (PSD) were defined according to the American Stroke Association/American Heart Association Vascular Cognitive Impairment (VCI) Guidelines (Gorelick *et al.*, 2011).

Vascular MCI (Vascular CIND) was defined as impairment in at least 1 cognitive domain (executive function, memory/learning, language, visuospatial/ visuoconstructive skills) and normal or mild impairment of instrumental activities of daily living independent of motor/sensory symptoms. PSD (in accord with the DSM IV criteria), was defined as impairment in  $\geq 2$  cognitive domains that were of sufficient severity to affect the subject's activities of daily living independent of motor/sensory symptoms (Gorelick *et al.*, 2011).

### ***2.2.9. Data Management and Statistical Analysis***

Data were analyzed using the Statistical Package for Social Sciences version 19.0 (SPSS Chicago Inc.). Categorical variables were examined and summarized in percentages while continuous variables were described using measures of central tendency (mean, median and semi-interquartile range) and compared using the student's t-test, analysis of variance (ANOVA) and Kruskal-Wallis Test. Correlations were examined using Pearson's correlation coefficient while logistic regression models were fitted to determine univariate and multivariate relationships between cognitive status and patient – related variables including demographic, lifestyle and vascular risk factors; stroke disability and depression symptoms. Unadjusted and adjusted ORs with 95% CIs were estimated. For multivariate analysis, variable groups were entered incrementally so that the mediating effect of each could be evaluated. Level of statistical significance was set at  $p < 0.05$ . For this sample and its associated sub - sample, appropriate power calculation was performed using the G\*Power software (Faul et al, 2007), a significance level,  $\alpha$  – level = 0.05 and assuming a moderate effect size Cohen's  $d = 0.5$ . This was to determine the strength of associations and conclusions that would be derived from the analysis of the dataset.

## **2.3 The CogFAST- Newcastle Study**

### ***2.3.1. Introduction***

The Cognitive Functions After Stroke (CogFAST) Newcastle Study is a Medical Research Council (MRC) funded study which began in 1999 with the main aim of investigating disease mechanisms and risk factors associated with the long term consequences of stroke (Ballard *et al.*, 2003b; Allan *et al.*, 2011). Delayed PSD is dementia that occurs in stroke patients over months to years, not as an immediate result of the stroke itself. The aim was to identify distinct pathological features which distinguished those cases who developed delayed PSD from those who maintained normal cognitive functioning at the time of death.

### ***2.3.2. Study Subjects***

Three hundred and fifty five elderly post-stroke (older than age 75) who were not demented at baseline (3 months after the index stroke) were recruited. Each of these cases received annual clinical evaluation and cognitive testing performed by assistant psychologists using the Mini – Mental State Examination (MMSE), the Cambridge Cognitive Examination (CAMCOG) tests and the Clinical Dementia Rating (CDR) Scale (Allan *et al.*, 2011).

### ***2.3.3. Cognitive Assessment***

#### **2.3.3.1. Mini – Mental State Examination (MMSE)**

The MMSE test as described above was used in its original English versions (Folstein *et al.*, 1975). A cut off score of less than 24 is most frequently used to indicate presence of cognitive impairment/dementia (Crum *et al.*, 1993). Participants who scored less than 24 on the MMSE at screening were excluded from the study.

### **2.3.3.2. Cambridge Cognitive Examination – revised (CAMCOG - R)**

The CAMCOG - R is the cognitive test section of the revised CAMDEX assessment (Cambridge Mental Disorders of the Elderly Examination) and was designed to assess cognitive performance for the detection and grading of dementia with a commonly used cut – off point of 79/80 (Roth *et al.*, 1986; Blessed *et al.*, 1991). It assesses a wide range of cognitive domains including orientation, language, memory, praxis, attention, abstract thinking, perception and calculation. The revised CAMCOG includes additional evaluation of executive function.

### **2.3.3.3. Clinical Dementia Rating (CDR) Scale**

The Clinical Dementia Rating (CDR) Scale is a numeric scale used to evaluate the severity of symptoms of dementia with a structured-interview protocol. Subjects' cognitive and functional performance are assessed in six domains: memory, orientation, judgment and problem solving, community affairs, homes and hobbies, and personal care. Scores in each of these domains are combined to obtain a composite score ranging from 0 to 3; 0 – normal, 0.5 – questionable (MCI), 1 – mild dementia, 2 – moderate dementia, 3 – severe dementia (Hughes *et al.*, 1982).

### **2.3.3.4. Clinical Definition of CogFAST Groups**

Stroke survivors in the longitudinal CogFAST Study were classified based on the performance at the last cognitive assessment before death. They were classified as post-stroke non-demented (PSND) if CAMCOG score was > 80 and Clinical Dementia Rating (CDR) was less than 1, but as post-stroke demented (PSD) if CAMCOG score was < 80 and CDR score was  $\geq 1$ .

#### **2.3.4. Brain Tissue Preparation**

At post-mortem examination, each brain was bisected into two hemispheres; the left hemisphere was fixed in 10% buffered formalin and coronal slices were taken and embedded in paraffin wax after standard processing with dehydrating agents. The right hemisphere was stored at  $-80^{\circ}\text{C}$ .

##### **2.3.4.1. Diagnostic Neuropathological Evaluation**

Post-mortem reports were retrieved for all the cases used in this study. Primary neuropathological diagnoses were made from brain tissue sampled at several coronal levels to check for pathological changes consistent with AD, VaD and mixed AD\_VaD in accordance with established pathologic diagnostic criteria (Hyman and Trojanowski, 1997; Kalaria *et al.*, 2004), and following macroscopic and microscopic post-mortem examination of the brain tissue.

Haematoxylin and Eosin was utilized as a standard stain for a general neuropathologic structural evaluation of the brain, and for the detection of infarcts and rarefactions. Gallyas and Bielschowsky's silver impregnation stains and AT8 immunohistochemistry were used to evaluate 'CERAD' neuritic plaques and neurofibrillary tangles according to the methods of Braak (Braak and Braak, 1991) and the Consortium to Establish A Registry for Alzheimer's Disease (CERAD) Staging (Mirra *et al.*, 1991). In addition, Thal staging was performed (Thal *et al.*, 2002b) and there were additional stains including  $\alpha$ -synuclein, ubiquitin and TDP - 43 immuno-histochemistry.

Vascular lesions (cortical and sub-cortical infarcts, border-zone infarcts, strategic infarcts, lacunar infarcts ( $< 15$  mm), microinfarcts ( $< 5$  mm) and mild, moderate and severe cerebral amyloid angiopathy were recorded (Kalaria *et al.*, 2004). In addition, a new vascular severity scale devised within our group was used to assess vascular pathology (Deramecourt *et al.*, 2012).

Final diagnoses were assigned during monthly clinicopathologic consensus meetings. A final diagnosis of VaD was made if there was clinical evidence of dementia (DSM IV) and pathologic evidence of multiple or cystic infarcts, lacunes, micro-infarcts, small vessel disease in the absence of severe degenerative pathology (Braak Stage < III) (Kalaria *et al.*, 2004). Subjects were assigned mixed AD\_VaD if there was pathologic evidence of cerebrovascular disease in the presence of significant AD pathology (Braak Stage V or VI) and moderate to severe CERAD scores. A diagnosis of AD was assigned when there was significant Alzheimer pathology – Braak V –VI, moderate to severe CERAD score and absence of significant vascular pathology. Control subjects were historical subjects that had no significant evidence of cognitive impairment upon scrutiny of their medical records and whose post-mortem brain tissue was considered devoid of sufficient vascular or degenerative pathologies beyond the threshold for assigning a specific pathologic diagnosis.

### ***2.3.5. Identification of Laboratory Sample***

#### **2.3.5.1. CogFAST Groups**

This consisted of cases from the prospectively assessed CogFAST cohort who had come to autopsy. The demographic, cognitive and diagnostic neuropathologic characteristics of the two broad categories of the cohort - post-stroke demented (PSD) and post-stroke non – demented (PSND) are described in Chapter 5. The final cognitive diagnosis was based on the last clinical assessment performed before death.

#### **2.3.5.2. Controls, VaD and AD groups**

Appropriate controls, and neuropathologically diagnosed cases of VaD and AD were selected from available archival records and after rigorous matching with the CogFAST cases with respect to age, gender, length of fixation and post-mortem delay. The

neuropathological diagnostic criteria have been described in Section 2.2.3.1. Full details of these groups are also described

### ***2.3.6. Brain Tissue Acquisition***

Ninety-four human post-mortem formalin-fixed, paraffin-embedded brain tissue block samples were retrieved from the Newcastle Brain Tissue Resource (NBTR), Institute for Ageing and Health, Newcastle University. Ethical approval for the use of brain tissue for our study was granted by the local research ethics committee (Newcastle upon Tyne Hospitals National Health Service Trust, UK).

The sample consisted of CogFAST demented, PSD (n =15), CogFAST non-demented, PSND (n=23), controls (n =12), AD (n= 14), mixed AD\_VAD (n=13) all matched for age, gender, length of fixation and post –mortem delay. Informed consent from all subjects for autopsy and tissue donation were obtained from next of kin and post mortem examinations were undertaken at the Newcastle General Hospital (NGH).

Autopsy was performed within 24 and 92 hrs after death and the brain fixed in 10% buffered formalin for 6 - 34 week. Subjects ranged from 78 – 96 years in age. There were no significant differences between groups of subjects with respect to age, gender, length of fixation and post –mortem delay. Brains with fixation period less than 40 weeks were selected for the project in order that differential staining intensity arising from prolonged fixation might be obviated. Also, cases with significant history of death arising from cardiovascular morbidities such as myocardial infarction were excluded from the study.

Much of the work in this study was carried out on the hippocampal formation. Additional analyses were also performed on the entorhinal cortex and the temporal neocortex (BA 36) as required. Analyses of neuroinflammation and axonal damage in the WM were performed on frontal and temporal WM of a sub-cohort of the cases.



### ***2.3.7. Regions of Interest***

#### **2.3.7.1. The Hippocampal formation and Entorhinal Cortex**

The hippocampal formation occupies the posterior-anterior extent of the base of the temporal lobes. It consists of multiple sub-regions of the hippocampus and the adjacent entorhinal cortex which include the dentate gyrus, the CA1, CA2 and CA3 subfields, and the subiculum (Eichenbaum, 2004; Duvernoy, 2005; Small *et al.*, 2011). The entorhinal cortex is the gateway into the hippocampal formation and receives monosynaptic inputs from numerous cortical and sub-cortical region and then connects in a ‘*trisynaptic pathway*’ with the sub-fields and the subiculum. The CA1 subfield and the subiculum provide the main outflow out of the hippocampus to different subcortical and cortical regions including the dorsolateral prefrontal cortex and the temporal cortex. Functionally, the hippocampus plays a vital role in the integration of declarative and episodic memory as well as in visuospatial orientation .Subfields of the hippocampus have differential susceptibility to insult including anoxia, hypoxia, ischaemia and carbon monoxide poisoning, especially the CA1 subfield (Small *et al.*, 2011). From the Newcastle Brain Map (Perry and Oakley, 1993), the temporal lobe tissue block was selected from coronal level 18 – 20 and from which the hippocampal subfields were defined (Figure 2.3a)

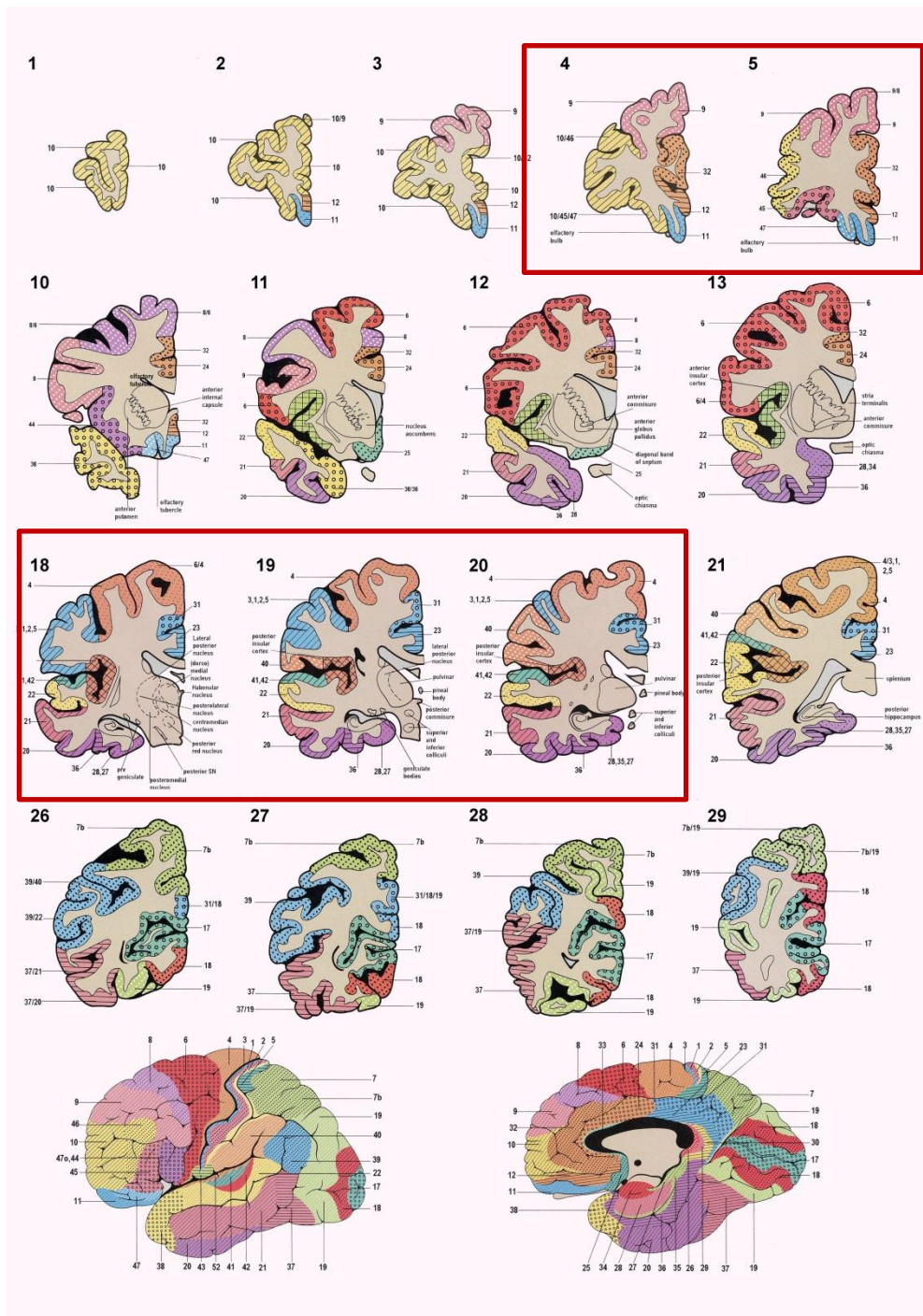
#### **2.3.7.2. Frontal and Temporal White Matter**

The frontal lobe is a vital region of the brain for planning, goal setting, solving complex problems and working memory, all of which are encompassed as executive function (Hoffmann, 2013) while the temporal lobe is vital for memory and language functions (Squire *et al.*, 2004; Insausti *et al.*, 2013)

White matter (WM) is composed largely of myelinated and unmyelinated axons, glial cells (microglia, astrocytes, oligodendrocytes) and blood vessels. Loss of WM integrity integrity occurs in the setting of ageing, cerebrovascular disease and other dementias (Ihara *et al.*, 2010a; Horsburgh *et al.*, 2011). The frontal lobe is particularly susceptible to cerebral

vascular disorders during which ‘disconnections’ occur in the white matter as a result of loss of white matter integrity from hypoxic- ischaemic damage (O’Sullivan *et al.*, 2001; Hoffmann, 2013).

Frontal lobe sections at the level of the olfactory bulbs (corresponding to coronal levels 4-6) and temporal lobe sections at the level of the anterior hippocampus (corresponding to coronal levels 18 – 20 ) were selected from the Newcastle Brain Map (Perry and Oakley, 1993) for the white matter components of this study (Figure 2.3b).



**Figure 2.3a. Newcastle Brain Map.** Schematic representation of coronal slices from human brain, cut anterior to posterior. Large bold numbers indicate coronal levels of the brain. Brodmann areas are colour coded and numbered at each level. Sections were cut from blocks (highlighted in red )taken from the coronal levels 4 – 6 for frontal white matter and levels 18 – 20 for the temporal white matter , hippocampal formation and entorhinal cortex.(Perry and Oakley, 1993).

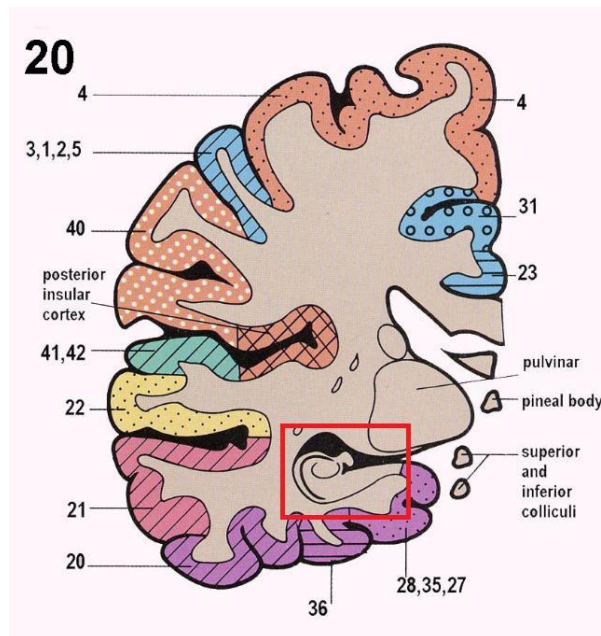


**Figure 2.3b. Newcastle Brain Map.** Schematic representation of coronal slices from human brain, cut anterior to posterior. Large bold numbers indicate coronal levels of the brain. Brodmann areas are colour coded and numbered at each level. Sections were cut from blocks (highlighted in red) taken from the coronal levels 4 – 6 for frontal white matter and levels 18 – 20 for the temporal white matter, hippocampal formation and entorhinal cortex. (Perry and Oakley, 1993).

### 2.3.8. Immunohistochemistry

#### 2.3.8.1. Standard Procedure for Immunohistochemistry

Paraffin embedded brain tissue blocks taken from relevant coronal levels of the Newcastle Brain Map and containing the hippocampal formation, entorhinal cortex, frontal and temporal white matter were cut into 10 $\mu$ m serial sections using a rotary microtome. Sections were mounted on slides coated with 2% APES (3-aminopropyltriethoxysilane) solution in acetone, and dried in a pre-heated oven at 60<sup>0</sup>C for 30 minutes. The sections were de-paraffinized in two sequential solutions of Xylene for 15 minutes and then rehydrated using decreasing concentrations of ethanol (100%, 95%, 70% and 50%) to deionized water.



**Figure 2.4.** Newcastle coronal brain map reference level 20. Hippocampus block (red box) including entorhinal cortex was used for analysis.

Antigen retrieval was performed using heat in the form of microwaving sections for 10 minutes or pressure cooking them for 2 minutes in a solution of 0.01M citrate buffer (PH 6.0). The buffer was brought to boil in the microwave, slides were added, buffer was microwaved at 450W for 10 min, and the solution was then allowed to cool for 20 minutes

following which slides were transferred to deionized water. Non-specific reaction was quenched with 0.9% hydrogen peroxide (unless otherwise stated.) in 5Mm Tris buffered Saline (TBS) solution (pH 7.6) for 15 min in order to remove endogenous peroxidase. Non-specific antigens were blocked using normal horse serum (anti-mouse antibody) , normal goat serum (anti-rabbit antibody) or normal rabbit serum (anti-goat antibody) for up to 45 mins. The slides were then incubated with the primary antibody diluted to specific concentration with buffer, and for a specific length of time in room temperature or overnight at 4°C. After washes in buffer, biotinylated secondary antibody was applied to the sections with the blocking serum for 30 minutes, followed by the addition of the avidin biotin complex (ABC) for 30 min to remove excess secondary antibody. Finally, the slides were immersed in a 0.025% diaminobenzidine (DAB) solution for a variable short period of time to visualize the positive antibody reaction. Sections were then rinsed in water and counterstained in haematoxylin as indicated. Sections were then dehydrated back through graded alcohols, cleared in xylene and mounted with glass coverslips using DPX mounting medium (Sigma, UK). After each step, with the exception of the blocking stage, sections were rinsed in buffer (TBS or PBS) three times for five minutes each. Dual labeling followed a similar procedure as described above. The single antibody was labeled initially and then the procedure was repeated for the second antibody or the two antibodies were applied simultaneously during a single procedure. All immunohistochemical protocols included a positive control and a negative control for which the primary antibody is omitted.

<b>Antibody</b>	<b>Nature /Source</b>	<b>Antigen retrieval</b>	<b>Assay buffer</b>	<b>Block</b>	<b>Dilution</b>	<b>Secondary Antibody</b>	<b>SABC</b>	<b>Chromogen</b>
G48	Monoclonal Signet 9220-10	Concentrated formic acid	TBS	1.5% NoHoS x 1hour	1:1000 O/N	Anti-mouse	Yes	DAB (5 min)
T -42	Polyclonal	Concentrated formic acid	TBS	1.5% NoGoS x 1 hour	1:5000 O/N	Anti-rabbit	Yes	DAB ( 3min)
T- 40	Polyclonal	Concentrated formic acid	TBS	1.5% NoGoS x 1 hour	1:5000 O/N	Anti-rabbit	Yes	DAB ( 3min)
APP (22C11)	Monoclonal Chemicon MAB 348	Microwave/ Citrate buffer	TBS	1.5% NoHoS x 1hour	1:2000 O/N	Anti-mouse	Yes	DAB (5 min)
NU-1	Monoclonal	Concentrated formic acid	TBS	-	1:3200 O/N	Anti-mouse	Yes	DAB ( 2 min)
AT8	Monoclonal Innogetics Autogen Bioclear INNX 90206	Microwave/ Citrate buffer	TBS	1.5% NoHoS x 1hour	1: 1000 2Hrs Room Temperature	Anti-mouse	Yes	DAB (4 min)

Drebrin	Monoclonal D029-3 Clone M2F6	Microwave/ Citrate buffer	TBS	1.5% NoHoS x 1hour	1:200 O/N	Anti-mouse	Yes	DAB (3min)
PSD-95	Polyclonal	Microwave/ Citrate buffer	TBS	3% NoGoS x 1hour	1: 750 O/N	Anti-rabbit	Yes	DAB (5 min)
VGLUT-1	Polyclonal Synaptic Systems Lot 135303/ 28	Microwave/ Citrate buffer	TBS	3% NoGoS x 1hour	1:1000 O/N	Anti-rabbit	Yes	DAB (5 min)
SY-38	Monoclonal Cymbus, UK	Microwave/ Citrate buffer	TBS	1.5% NoHoS x 1hour	1:150 O/N	Anti-mouse	Yes	DAB (4 min)
GFAP	Polyclonal Dako Cytomation Lot 00045904	Microwave/ Citrate buffer	TBS	1.5% NoGoS x 1hour	1: 4000 O/N	Anti-rabbit	Yes	DAB (2 min)
CD68	Monoclonal PGMI Lot 00027860	Microwave/ Citrate buffer	TBS	1.5% NoHoS x 1hour	1:400 O/N	Anti-mouse	Yes	DAB (4 min)
dMBP	Polyclonal AB5864 Lot 2138534	Microwave/ Citrate buffer	TBS	1.5% NoGoS x 1hour	1: 2000 O/N	Anti-rabbit	Yes	DAB (3 min)

**Table 2. 2: Table of primary antibodies used for Immunohistochemistry in this Project**



### **2.3.8.2. Fluorescent immunohistochemistry**

Six micron sections were cut on a rotary microtome for fluorescent staining. The sections were de - paraffinized and rehydrated followed by antigen retrieval by heat. Quenching with hydrogen peroxide was not required but blocking with normal horse serum was applied in all cases. Selected antibodies were then incubated overnight at 4<sup>0</sup>C with the sections using a four - fold concentration of the primary antibody (in comparison to single – labeling IHC). Sections were then incubated with appropriate secondary antibody at 1: 200 concentration, anti –rabbit IgG Dylight 549 (red) or anti-mouse IgG Dylight 488 (green) in the dark. Excess antibody was removed and sections were washed in PBS in the dark. They were then mounted with Vectashield (Vector Labs, UK). This procedure was performed with PBS buffer adjusted to pH 7.5.

### **2.3.8.3. Image Acquisition and Analysis**

The stained sections were examined and imaged using a Zeiss Axioplan 2 research grade microscope coupled to an Infinity 2 camera. Set at X10 magnifications for the hippocampal sub-regions CA1, CA2 and CA3, the frontal and temporal white matter, and X5 for the subiculum and EC. Five images were taken at random from the CA1, CA3 and subiculum, 3 images from CA2 and 4 x 3 from the EC from the pial surface to the white matter. Between 15 and 20 images were taken from the frontal and temporal white matter. An appropriate number of images was taken to ensure an adequate, complete and unbiased number of images to cover the regions as much as possible.

### **2.3.8.4. In Vitro Image Analysis**

Using the software Image Pro-Plus 4.0 (Media Cybernetics, USA), the images were analyzed using histogram-based analysis and obtaining the variables: per area, a measure of the number of pixels stained within the area of interest (AOI) and expressed as a percentage. The integrated optical density (IOD) was also determined, and thus making possible the calculation of mean immunoreactivity (IR) for each image and then for each sub-region of the hippocampus and other brain regions analyzed

### **2.3.8.5. Confocal fluorescent image capture**

All confocal fluorescent imaging was performed at the Newcastle University Bio Imaging Unit using a Leica TCS SP2 AOBS (UV) microscope (Leica, Germany) and with the assistance of the technical staff of the unit. Images may be acquired at different lens magnifications up to X 100 on the confocal microscope with the highest attainable resolution with fluorescent imaging. A laser beam travels through the 'Z' plane acquiring images at known focal depths. Individual 'z' planes may be re-constructed into a two – or three - dimensional image. The confocal microscope provides a clearer fluorescent signal compared to standard fluorescent microscopy with less background noise.

### ***2.2.8. Statistical analysis for in vitro imaging analysis dataset***

Data was analyzed with Statistical Package for the Social Sciences (SPSS version 19.0) (IBM, USA). Normality of data was tested using the Shapiro – Wilk or Kolmogorov-SmirnovTest depending on the number of cases in the dataset. When data were normally distributed, one –way ANOVA with Tukey's or Bonferenni post - hoc test were used as required. However, when data were non – normally distributed, non - parameteric Kruskal - Wallis test was performed to tease out significant differences between groups. Differences in percentage mean area, mean pixel intensity and total immunoreactivity of disease groups and sub- regions were examined. Correlations were examined with Pearson's correlation or Spearman's correlation for normally and non-normally distributed data respectively. For this sample and its associated sub – samples, appropriate power calculation was performed using the G\*Power software {Faul, 2007 #1877}, a significance level,  $\alpha$  – level = 0.05 and assuming a moderate effect size Cohen's d = 0.4. This was to determine the strength of associations and conclusions that would be derived from the analysis of the dataset.

### **2.3.9. Immunoblotting**

At post – mortem, brains were cut coronally in slices in both right and left hemispheres. For the brains obtained from subjects the CogFAST – Newcastle study, alternate slices were fixed in formalin and frozen respectively. However, for other categories of brain including brains from subjects who had suffered AD during life, whole hemispheres were frozen and fixed in accordance with standard convention.

For the immunoblotting experiments, whole hippocampal formation containing the CA fields, the dentate gyrus and EC samples (Newcastle Brain Map level (18 -20) were obtained from the Newcastle Brain Tissue Resource. One hundred and twenty five (125) micrometer frozen sections from coronal levels 18 - 20 (Newcastle Brain Map) were cut with a vibratome. The whole hippocampus (CA regions and dentate gyrus) was then sub - dissected into microvials at 0 - 4<sup>0</sup>C. Approximately 100 mg of tissue was homogenized in 2 mls of ice-cold buffer (50 mM Tris – HCL, 5mM EGTA, 10 mM EDTA, pH 7.4, containing protease and phosphatase inhibitors – 2µg/ml leupeptin hemisulphate, 2µg/ml aprotonin, 1µg/ml E64, 2µg/ml pepstatin A and 20µg/ml phenylmethylsulphonyl fluoride) using an Omni TH homogeniser in a Class 3 safety cabinet prior to being aliquoted into 200 µl aliquots and subsequently frozen on ice and stored at -70°C.

#### **2.3.9.1. Determination of Protein Concentration**

Relative protein concentration across samples was assessed in triplicate using the DC Kit protein assay (Bio- Rad) in order to ensure that protein concentration was equal across all samples. The Bio-Rad DC Protein Assay is a colorimetric assay for protein concentration following detergent solubilization. The reaction is similar to the well - documented Lowry assay, but with the following improvements: The reaction reaches 90% of its maximum colour development within 15 minutes thereby saving valuable time, and the colour changes not more than 5% in 1 hour or 10% in 2 hours after the addition of reagents.

A standard curve of known protein standards was prepared using Bovine Serum Albumin (BSA; Sigma) diluted in BSA buffer (0.2 M TEAB and 2% SDS diluted in deionised water at 1:50). Sample homogenates were diluted 1: 20 in deionised water

(MilliQ, Millipore) Twenty-five microliter of solution A (Bio-Rad DC Protein Assay Kit) was added into necessary wells followed by 5ul of the sample and 200 microlitres of solution B (Bio-Rad DC Protein Assay Kit) and mixed well with repeat pipetting.

Following an incubation period of 15 minutes, the plate was read at 595 nm using an Omega plate reader . Protein concentrations were then calculated for samples relative to protein standards and equalised across samples for 100 µl preparations. Aliquots were thawed and mixed with concentrated sample buffer [0.0625 M Tris-HCl, containing 2% sodium dodecyl sulphate (SDS), 5% β – mercaptoethanol, 10% glycerol and 0.002% bromophenol blue) and stored at -20<sup>0</sup>C.

### **2.3.9.2. Immunoblotting Procedure**

Whole hippocampal homogenate samples were defrosted on ice before tissue samples of equal concentration were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. 10 – 20 µl of each 2 µg/µl sample was pipetted into wells of a 30 lane 1.5mm 8 - 12 % polyacrylamide gels [ratio of acrylamide to bis-acrylamide constant (17.5:1 acrylamide: bis - acrylamide) depending on the molecular weight of protein to be investigated: 8% w/v for proteins 100 -200kDa, 10% w/v for proteins 40 – 100 kDa and 12% w/v for 30kDa. Ten microliter of pre-stained molecular weight marker L: Spectra multicolour broad range protein ladder (Thermo Scientific) was loaded at each end (to demonstrate distance moved by known MW protein standards, ranging from 10-250kDa). The gel was electrophoresed at 120 V for 45 minutes to 1 hour in SDS running buffer.

Following electrophoresis the separated proteins were transferred from gels to 0.45 mm nitrocellulose membranes using an electro - blotting trans - blot for 120 minutes at 0.35 A. Ponceau S solution (0.5% w/v Ponceau S, 5% (w/v) trichloroacetic acid) was used to confirm successful protein transfer before membranes were cut depending on the molecular weights of proteins of interest. Ponceau S solution was washed from membranes by a flash wash of deionised water (MilliQ) followed by two 10 minute washes in tris-buffered saline combined with 0.2% Tween 20 under gentle agitation.

Membranes were blocked against non - specific binding using 5% non - fat dried milk (Marvel) in TBS -T for 1 hour under gentle agitation. Primary antibody incubation was achieved by exposing the membranes to primary antibodies suspended in a 5% dry milk (Marvel)/TBS -T solution at previously optimised concentrations overnight (Table 6.3). Membranes were then washed for 15 minutes (three 5 minute washes) in 1% non-fat dried milk (Marvel) in TBS-T.

<b>Target</b>	<b>Band Size (kDa)</b>	<b>Species</b>	<b>Dilution for IHC</b>	<b>Dilution for WB</b>	<b>Manufacturer</b>
SY -38	38	mouse/monoclonal	1:150 O/N	1:2000 O/N	Dako
SNAP-25	25	mouse/monoclonal	1:1000 O/N	1:2000 O/N	SMI, Affinity, UK
VGLUT-1	60	mouse/monoclonal	1:1000 O/N	1:1000 O/N	Synaptic Systems
Drebrin	120	Rabbit/Polyclonal	1:200 O/N	1:1000 O/N	Cell Signalling
PSD -95	95	Rabbit/Polyclonal	1:750 O/N	1:1000 O/N	Abcam
beta tubulin III	55	mouse/monoclonal		1:1000 O/N	Sigma – Aldrich

**Table 2.3: Panel of antibodies tested for the pre - and post – synaptic markers by immunoblotting**

Secondary antibody incubation was achieved by exposing membranes to horseradish peroxidase (HRP) conjugated secondary antibodies (anti – mouse or anti-rabbit depending on the primary antibody) suspended in a 5% non-fat dried milk (Marvel) in TBS-T solution at a dilution of 1: 2000 for 1 hour. To enable viewing of antibody binding, membranes were exposed to ECL2 enhanced chemiluminescence kit (Thermo Scientific) for 2 minutes on a glass plate. Bands were visualized with a LAS - 4000 Luminescent Image Analyzer Version 1.0 (Fuji Film Cooperation, Tokyo, Japan). Optical density of bands were measured using Image J. Variation in signal detection (including gel and blotting variation) across gels was corrected for using the protein

standard replicate loaded on gels. Data were expressed as optical density of the band per weight of protein loaded per sample.

## **Chapter 3. Profile and Determinants of Vascular Cognitive Impairment in Nigerian African Stroke Survivors**

### **3.1. Introduction**

It is estimated that one in three people all over the world will, over a lifetime, develop a stroke, dementia or both (Seshadri and Wolf, 2007; Gorelick *et al.*, 2011). In response to the growing burden of stroke and associated conditions including post-stroke cognitive dysfunction, a synergium was recently convened to develop strategies to '*prioritize stroke on the world agenda*' (Hachinski *et al.*, 2010).

Sub-Saharan Africa (SSA) is in epidemiologic transition and faces a growing burden of non-communicable diseases in addition to the persistent burden of infections, conflicts and poverty (Kalara *et al.*, 2008; Akinyemi *et al.*, 2009). Ageing populations, rapid urbanisation and lifestyle changes appear to be the main drivers of the burgeoning epidemic of vascular disorders (hypertension, diabetes, metabolic syndrome) which frequently culminate in stroke (Yusuf *et al.*, 2001b)

Although physical disability is most commonly associated with stroke, cognitive changes and other non-motor consequences are quite frequent in survivors (Pendlebury, 2009). Post stroke cognitive dysfunction encompasses a multi-domain impairment of attention and concentration, executive function, language, memory and visuospatial function with executive dysfunction being the earliest and predominantly affected domain (Ballard *et al.*, 2003a; Henon *et al.*, 2006; Erkinjuntti and Gauthier, 2009) (Gorelick *et al.*, 2011). Up to 64% of stroke survivors suffer some degree of cognitive impairment and about 30% develop dementia (Hachinski *et al.*, 2006b). In a recent meta- analysis, the pooled prevalence estimates of post - stroke dementia (PSD) within one year of stroke ranged from 7.4% (4.8 -10.0) in population-based studies of first-ever stroke excluding pre-stroke dementia to 41.3% (29.6 – 53.1) in hospital-based studies of all strokes including pre-stroke dementia (Pendlebury, 2009). However, there is wide variation in the definition and estimates of vascular cognitive impairment (VCI) owing to differences in diagnostic criteria, study populations and methodologies. These have led to recent harmonization efforts and criteria development (Hachinski *et al.*, 2006b).

Few recent studies have been undertaken utilizing the Vascular Cognitive Impairment Harmonization Standards (VCIHS) recommended protocol or other –related protocols that are enriched in assessing the executive function domain (Hachinski *et al.*, 2006a). In a study of 239 Singaporean stroke survivors assessed 3 - 6 months after stroke, 54.8% and 2.9% were diagnosed of vascular cognitive impairment no dementia (vCIND) and vascular dementia (VaD) respectively (Dong *et al.*, 2012) while a more recent study of 353 Korean stroke survivors assessed 3 months after stroke using the Korean version of the VCIHS found rates of 49.9 % and 12.7% for vCIND and VaD respectively (Yu *et al.*, 2013). It is noteworthy that the rates of vCIND were particularly high in these recent studies with robust cognitive assessment tools that were robustly sensitive to executive dysfunction.

The cognitive trajectory that ensues after stroke may be influenced by patient-related variables such as age at stroke occurrence, level of educational attainment (as a surrogate of cognitive reserve), physical activity, dietary and nutritional lifestyles. Cardiovascular risk factor load, stroke-related variables, acute stroke complications and quality of care –related issues are also important determinants (Pendlebury, 2009; Pendlebury and Rothwell, 2009a; Gottesman, 2010; Allan *et al.*, 2011; Kalaria, 2012b)

Little is known, however, about the burden or risk factors of post-stroke cognitive dysfunction in Africa as a whole and previous studies were all cross –sectional and patients were not assessed with appropriate standardized cognitive assessment tools sensitive to executive dysfunction. In a study of 1000 stroke survivors of mixed black and white ancestry, Hoffman reported a rate of cognitive dysfunction of 63.5% at two weeks after the index stroke even though some of the subjects might still have been delirious after the index stroke (Hoffmann, 2001) (Desmond DW, 1996). Fatoye *et al.* (2007) examined 109 Nigerian stroke survivors at various durations after the event, using only the MMSE. They found a post – stroke cognitive dysfunction rate of 17.4% (Fatoye *et al.*, 2007) while another study among 81 Egyptian stroke survivors found a post-stroke dementia frequency of 21%. No rates were cited for vCIND (Khedr *et al.*, 2009).

Patterned after the project in Newcastle (Ballard *et al.*, 2002; Ballard *et al.*, 2003b; Stephens *et al.*, 2004; Allan *et al.*, 2011) the aim of this chapter is to report the preliminary baseline frequency, pattern and factors associated with VCI in Nigerian African stroke survivors participating in the Cognitive Function After Stroke



(CogFAST) Nigeria Study. This chapter describes the first comprehensive, detailed and prospective African study of post stroke - related cognitive dysfunction.

## **3.2. Methods**

### ***3.2.1. A Pilot Study***

Prior to the recruitment of the main study sample, an initial pilot study was conducted over a six month period (January – June 2010) among 42 stroke survivors purposively recruited from among stroke patients who were already attending the medical outpatient clinics of the Federal Medical Centre Abeokuta, and the Catholic Hospital, Oluyoro Ibadan, Nigeria. The purpose of this was to test the feasibility of the methods and procedures including study instruments, cultural adaptability and acceptance (Lancaster *et al.*, 2004; Thabane *et al.*, 2010; Leon *et al.*, 2011).

### ***3.2.2. Study Design and Stroke Participants***

The study design was mixed cohort and case – control. Stroke patients ( $\geq 45$  years) presenting consecutively were recruited from the stroke registers on the medical wards of two specialist hospitals [Federal Medical Center Abeokuta and University College Hospital, Ibadan , southwestern Nigeria] between July 2010 and June 2012. In addition, three smaller centers (Catholic Mission Hospital, Oluyoro Ibadan, Oluwaseun Physiotherapy Clinic, Ibadan and Sacred Heart Hospital, Lantoro Abeokuta) were included to ensure representativeness of different levels and types of healthcare facilities in the region to which stroke patients might present. Subjects were approached regarding participation in the study at discharge from hospital or during initial outpatient visit after stroke. They were subsequently invited for assessment by word of mouth or mobile telephone.

Stroke was defined according to the World Health Organization (WHO) Clinical definition and classified using the WHO Clinical Criteria, the Oxford Community Stroke Project Classification and neuroimaging (CT scan and/ or MRI) findings (Ballard *et al.*, 2003a). However, neuroimaging was performed only in 61.5% of our cohort because of limited access and high cost. The WHO criteria have been shown to have a

sensitivity of 73 % for haemorrhage, 69% for infarction and an overall accuracy of 71% in Nigeria (Ogun *et al.*, 2002) Exclusion criteria were: [1] subarachnoid haemorrhage [2] significant physical illness and motor impairment that precluded paper and computer-based neuropsychological evaluation (eg. visual impairment, moderate-severe aphasia, hemiparesis affecting the dexterous hand (MRC power grade <3) [3] any co – morbid psychiatric or neurologic illness [4] any systemic disease that could impair cognition e.g. chronic liver disease, chronic kidney disease [5] inability or failure to give consent.

### ***3.2.3. Stroke-Free Controls***

For comparison with the neuropsychological data from stroke survivors, apparently healthy subjects who were free of clinically - evident stroke were recruited from a pool of community-dwelling volunteers (unrelated to the stroke subjects), spouses, unrelated caregivers of stroke survivors and patients attending outpatient clinic for refraction or routine physical assessment. Individuals with background dementia (DSM IV criteria), psychiatric disorders e.g. schizophrenia, major depression, manic - depressive disorder; background neurological disorders e.g. Parkinson's disease, (evidence from case records, informant or self report) or who were unable to provide consent and/or informant were excluded from being controls. Seventy -four stroke- free apparently healthy controls of comparable age, gender and education as the cohort of stroke survivors were recruited and included in the analysis.

### ***3.2.4. Ethical Approval***

The local research ethics committees of the Oyo State Ministry of Health (University College Hospital, Ibadan , Catholic Mission Hospital, Oluyoro Ibadan, Oluwaseun Physiotherapy Clinic, Ibadan ) and the Federal Medical Centre Abeokuta) and Sacred Heart Hospital Abeokuta granted approval for the study while written informed consent was obtained from each subject.

### **3.2.5. Baseline Evaluation**

Baseline evaluation was performed at three months post-stroke in tandem with the design of Desmond et al (1996) to enable the resolution of acute post-stroke delirium. As described in Chapter 2, the evaluation included comprehensive medical history, assessment of neurological impairment and disability (using the modified Rankin Scale, Stroke Levity Score and Barthel Index, depressive symptoms (using the Centre for Epidemiologic Studies Depression Scale and four – item Geriatric Depression Scale, and blood screens (Ballard *et al.*, 2003a; Owolabi and Ogunniyi, 2009) Confirmation of cardiovascular risk factors (hypertension, diabetes mellitus, atrial fibrillation, dyslipidaemia, cigarette smoking, alcohol use) were based on self- report, use of relevant medications and review of medical notes. Nutritional lifestyles and physical activity were assessed according to the design of the INTERSTROKE Study (O'Donnell *et al.*, 2010a). Dietary patterns were assessed in all subjects with a food frequency questionnaire assessing frequency of different types of food taken by the subjects in the prior year before the onset of stroke (and separately in the three months period after stroke) with appropriate addition of common local foods and delicacies to relevant categories. Physical activity was assessed at work and during leisure time and subjects were stratified into sedentary, mild, moderate and heavy physical activity categories.

### **3.2.6. Cognitive assessment**

An ideal neuropsychological battery should be robust, brief , valid, reliable, cost effective, sensitive enough to detect deficits but specific enough to avoid false positives, easy to administer, should be available in multiple forms , have cross cultural capability and have no floor or ceiling effect (Blake *et al.*, 2002; Hachinski *et al.*, 2006a). However, in reality no ideal tool exists. New tools being devised, nonetheless, are to aspire to attain these ideal characteristics.

The neuropsychological instrument used in this study consisted of the Community Screening Instrument for Dementia (CSID) – cognitive part, (Hall *et al.*, 2000) the mini-mental state examination (MMSE) (Gureje *et al.*, 1995) and the Vascular Neuropsychological Battery, (Hachinski *et al.*, 2006b). Subjects were assessed by experienced interviewers who received further two weeks training on the study

instrument and had to achieve an inter-rater reliability of at least 90% in mock assessments done with volunteers from the hospital community before conducting fieldwork.

The CSID and the MMSE are tests of general cognitive functioning while the V-NB consists of battery tests assessing functioning in specific domains of cognition. The CSID is a paper and pencil test of global cognitive performance which adaptability, validity and utility in populations from different cultural, educational and socio-economic backgrounds have been established (Hendrie *et al.*, 1995; Hall *et al.*, 2000). It has sensitivity of 87% and specificity of 83% and has been used reliably and widely to assess cognition in the Yoruba speaking population of southwestern Nigeria where the present study was conducted (Ogunniyi *et al.*, 2000). The schedule included sub-scores for attention, orientation, calculation, short and long term memory, language comprehension and expression, praxis and abstract thinking. A raw score method was used for scoring resulting in score range of 0 – 30 with higher scores indicating better cognitive function. The CSID has good two – week test – retest reliability (intra-class correlation = 0.79) and inter-rater reliability (kappa = 1 for 94% of the items) (Hall *et al.*, 2000).

The Vascular Neuropsychological Battery [V- NB] was modeled after the NINDS – CSN Harmonization Standards 60 –minute neuropsychological protocol (Hachinski *et al.*, 2006b) with minor modifications to ensure adaptability to the language and culture of the study population. The V- NB consists of multiple validated test items examining specific cognitive domains (executive function, memory/learning, language, visuospatial/visuoconstructive skills). Executive function/activation and mental speed were assessed using the category (animal) fluency test (Gureje *et al.*, 1995), phonemic (letter) fluency test, verbal reasoning and visual reasoning tests which were adapted from the Cambridge Cognitive Examination (CAMCOG) battery which was utilized for the CogFAST – Newcastle Study (Roth *et al.*, 1986; Ballard *et al.*, 2002). The number of animals listed in the first 15 sec of the animal fluency test provided an assessment of mental speed while all the tests differently assessed mental flexibility and divergent thinking (Hachinski *et al.*, 2006b). Memory/learning was assessed with the 10- item word list learning test and delayed recall of stick design (Gureje *et al.*, 1995; Baiyewu *et al.*, 2005). The word list learning is a 3 - trial, 10 – item test with free recall taken after each learning trial and after a brief delay. The total number of words recalled across the three trials make up the total score (range 0 – 30) while the delayed recall is scored (0 -

10), higher scores indicating better performance. Language was assessed through the 15 – item Boston Naming Test. In a prior validation study of the CERAD battery among Yoruba Nigerians, subjects were requested to name line drawings of common and uncommon objects. Four of the low frequency items from the standard CERAD-NB were replaced with items felt to be more culturally appropriate (i.e. guitar for harmonica, blacksmith tongs for ice cube tongs, mosquito netting for hammock, and 'ayo' (Nigerian board game) for dominoes (Gureje *et al.*, 1995).

Visuospatial/visuoconstructive functioning was assessed through the stick design test (Baiyewu *et al.*, 2005) and the Modified Token Test (IU Token Test) (Ball *et al.*, 2002; Akinyemi *et al.*, 2008). The stick design test is a non-graphomotor test of visuospatial /visuoconstructive ability. The respondent is requested to use match sticks to reproduce four different graphical shapes with particular attention to the correctness of relative orientation of the match heads. Thereafter the respondent reproduces the four shapes without any cues to assist. The test is particularly useful in older adults with limited formal education (Baiyewu *et al.*, 2005). Test items from the Cognitive Drug Research (CDR) computerized assessment battery were also included in the V-NB for the evaluation of attention, processing speed and executive function. [The constituent tests included Simple Reaction Time (SRT), Choice Reaction Time (CRT), Digit Vigilance (DV) and Spatial Working Memory (SWM) (Ballard *et al.*, 2003a) . The instructions were translated and back –translated from English into Yoruba Language by experienced linguists. Several of these tests were previously validated and successfully utilized to evaluate cognitive functions in a cohort of Nigerian subjects with Parkinson’s disease (Akinyemi *et al.*, 2008) .

### ***3.2.7. Operational definitions of cognitive dysfunction***

Failure on a test was defined as individual mean score that was at least 1.5 standard deviations below the mean score of the control group. Impairment in a domain was defined as failure on at least 50% of tests examining that particular domain.(Dong *et al.*, 2012) Vascular MCI and PSD were defined according to the American Stroke Association/American Heart Association VCI Guidelines (Gorelick *et al.*, 2011). Vascular MCI (Vascular CIND) (Gorelick *et al.*, 2011) was defined as impairment in at least 1 cognitive domain (executive function, memory/learning, language,

visuospatial/visuoconstructive skills) and normal or mild impairment of instrumental activities of daily living independent of motor/sensory symptoms. PSD, (Gorelick *et al.*, 2011) (in accord with the DSM IV criteria), was defined as impairment in  $\geq 2$  cognitive domains that were of sufficient severity to affect the subject's activities of daily living independent of motor/sensory symptoms (Gorelick *et al.*, 2011).

### **3.2.8. Statistical analysis**

Data were analyzed using the Statistical Package for Social Sciences version 17.0 (SPSS Chicago Inc.). Categorical variables were examined, analyzed and compared using the chi square test while continuous variables were described using measures of central tendency and compared using the student's t-test and analysis of variance (ANOVA). Logistic regression models were used to determine univariate and multivariate relationships between cognitive status and patient – related variables including demographic, lifestyle and vascular risk factors; stroke disability and depression symptoms. Unadjusted and adjusted ORs with 95% CIs were estimated. For multivariate analysis, variable groups were entered incrementally so that the mediating effect of each could be evaluated. Level of statistical significance was set at  $p < 0.05$ .

## **3.3. Results**

### **3.3.1. The Pilot Study**

The aim of the pilot study was to test the feasibility of the methods and procedures including study instruments, cultural adaptability and acceptance. A total of 42 stroke survivors (66.7 % males; [age; mean,  $57.0 \pm 9.3$ ; range, 45 – 75 years] were enrolled into the pilot study. Duration post-stroke ranged from 3 to 60 months, median, 5 months; while the average number of years of education was  $7.3 \pm 6.0$ ; range (0 – 18 years) (73.8 % had at least one year of formal education).

<b>Characteristic</b>	<b>Value</b>
Age (mean + SD) years	57.0 ± 9.3
Gender: N (% Female)	14 (33.3)
Education ( mean + SD) years	7.3 ± 6.0
Duration post-stroke (median, range) mths	5 ( 3 - 60)
<b><i>Stroke Type</i></b>	
Ischaemic (N, %)	21 (50.0)
Haemorrhagic (N, %)	4 (9.5)
Indeterminate	17 (40.5)
Modified Rankin Score (median, range)	2, (1 – 3)
<b><i>Vascular Risk Factors</i></b>	
Hypertension	32 (76.2)
Diabetes Mellitus	8 (19.1)
Dyslipidaemia	5(11.9)

**Table 3. 1. Demographic and Clinical Characteristics of the Pilot Sample (N = 42)**

### **3.3.1.1. Cognitive Assessment**

Subjects in this purposive sample of stroke survivors were willing and eager to participate in the new study after full explanation in the language best understood, particularly with the encouragement of a family member or caregiver who usually accompanied them for hospital consultations. Thus consent rate was 100 %. Thirty – nine (93%) of these completed the full range of cognitive assessments over a test duration ranging from 50 – 108 minutes. One patient had to discontinue on account of visual problems (diplopia) and another subject had post – stroke movement disorders which impaired his ability to cope with paper and pencil testing. The CAMCOG visual reasoning and ideational fluency tests were found most difficult for subjects to tackle. Sixteen stroke survivors and six healthy stroke – free volunteers performed the pilot on the CDR computerized tests. Although subjects who, ordinarily, were not used to the computer screen and keyboard, were taken aback when told they would perform some computer – based test, full explanations in the language best understood coupled with the encouragement of the accompanying family member/caregiver and an initial trial run prompted by the programme helped most subjects to surmount the fear and they could subsequently follow through on the various sub - tests.

### 3.3.1.2. Performance on the Cognitive Assessment Battery

Based on the CSID cut of score of 25.5(Section 2.6.2), the frequency of cognitive impairment in this cohort was 28.6% (12/42). Subjects who were cognitively impaired were slightly older, had slightly fewer years of education but shorter duration post-stroke (Table 3.2). However, subjects who were cognitively impaired achieved lower performance scores on the neuropsychological test items of the Vascular Neuropsychological Battery (V – NB): CAMCOG animal fluency CAMCOG letter fluency, Boston Naming Test, Word List Learning Stick Design and Modified Token Test (Table 3. 2).

Variable	Cognitive Category		p value
	normal (N =30)	impaired (N =12)	
	mean ( SD)	Mean (SD)	
Age (yrs)	56.7 (9.7)	57.7 (8.7)	0.764
Gender : n (% female)	8( 26.7)	4 (33.3)	0.469a
No of years of education (yrs)	7.9 (5.8)	5.7(6.6)	0.266
Duration post-stroke (mths)	8.9 (8.3)	7.3(6.2)	0.444b
Post stroke duration			0.483a
<= 6 mths : N, %	16 (38.1)	8 (19.0)	
> 6 mths: N, %	13 (31.0)	5 (11.9)	
CSID total score	27.6(1.1)	22.6(2.7)	< 0.001
MMSE total score	26.6(4.0)	20.3(5.2)	<0.001
CAMCOG animal fluency	12.9(4.9)	8.5 (3.7)	0.013
CAMCOG letter fluency	6.4(4.0)	2.3 (2.6)	0.005b
CAMCOG verbal reasoning	4.2 (2.3)	3.2 (2.3)	0.191
CAMCOG ideational fluency	2.1(1.1)	1.6 (0.9)	0.164
CAMCOG visual reasoning	2.1(1.1)	1.5 (1.7)	0.191b
Boston Naming Test	9.7(3.0)	7.0 (2.4)	0.014
Word List Learning	17.1(4.0)	11.7 (5.4)	0.001
Stick Design	10.5(2.6)	7.7 (3.4)	0.006
Modified Token Test	18.7(4.5)	14.2 (4.9)	0.012

**Table 3.2: Characteristics of cognitive categories of pilot sample (n = 42 )**



### 3.3.1.3. Performance on the CDR Computerized Battery

Table 3.3. shows the comparative performance of healthy controls, cognitively normal and cognitively impaired stroke survivors (based on CSID cut off score) with a trend of performance: normal control > cognitively normal stroke survivors> cognitively impaired stroke survivors on simple reaction time, choice reaction time, digit vigilance and spatial working memory .

Variable	Pilot Sample			p - value
	Control (N = 6) mean (SD)	Normal (N = 12 ) mean (SD)	Impaired (N = 4 ); mean (SD)	
SRT (reaction time) ms	400.55 (91.27)	628.00 (366.92)	713.18 (315.12)	0.008
CRT (reaction time)ms	579.55 (62.91)	835 (336.698)	911.29 (156.68)	0.001
SPM (reaction time) ms	1278.57 (516.54)	2521.09 (1055.06)	2599.75 (999.55)	< 0.001
VIR (reaction time)ms	432.85(35.64)	517.62 (68.4)	554.00 (49.82)	< 0.001
CRT (accur)%	98.18 (2.12)	97.65 (2.81)	96.57 (3.59)	0.378
SPM (accur)%	80.39 (23.33)	71.47 (25.20)	54.46 (33.6)	0.071
VIR (accur)%	96.06 (5.26)	76.81 (19.07)	58.09 (31.28)	< 0.001

**Table 3.3: CDR computerized neuropsychological assessment data for pilot sample**

### 3.3.2 The baseline assessment at 3 months post - stroke

We recorded 417 patients with a stroke, of whom 101 died in hospital, 31 were discharged against medical advice and 65 were lost to follow up. The remaining 220 were assessed for eligibility in the study, out of whom 145 met the selection criteria. The ineligible 75 subjects were excluded due to: [1] no consent (n = 25) [2] < 45 years of age (n= 32), [iii] severe aphasia and motor impairment precluding computer- assisted cognitive impairment (n = 18). The records of 2 stroke survivors were excluded from further analysis on account of incomplete assessment details. Figure 1 shows the flow chart of study participation in line with the STARD (Standards for Reporting of Diagnostic Accuracy) guidelines.(Bossuyt and Reitsma, 2003)

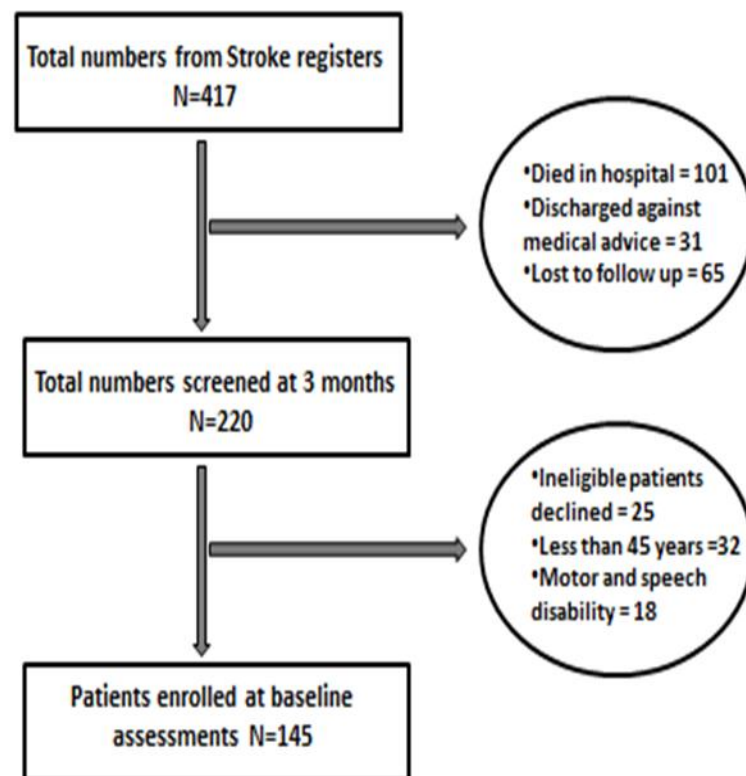


Figure 3. 1: Flow chart showing number of subjects screened and recruited at baseline.

### 3.3.2.1. Characteristics of study participants

#### *Sociodemographic profile*

One hundred and forty three (143) cases and 74 controls were recruited into this baseline assessment. Using the G\*Power software (Faul et al, 2007), a significance level,  $\alpha$  – level = 0.05 and assuming a moderate effect size Cohen's  $d = 0.5$ , the computed power ( $1 - \beta$ ) = 0.9352.

Table 3.4 shows the sociodemographic profiles of stroke survivors and the stroke-free healthy controls. Majority of stroke survivors were male 81 (56.6%) while their mean age at study onset was  $60.4 \pm 9.5$  years ( $58.8 \pm 8.6$  years for the control subjects). Subjects over 65 years of age were 46 (32.2%) cases and 11 (14.9%) controls. Majority of stroke survivors (85.3%) and controls (89.2%) had at least one year of formal education, most cases (82.6%) and controls (86.1%) lived in nuclear families while 93.4% of cases and 91.9% of controls had access to personal or proxy mobile phones. Stroke survivors and controls were largely of the Yoruba ethnic extraction and most of the subjects (80% of cases and 70% of controls) earned less than 50,000 naira (approximately 200 Pounds Sterling) per month.

#### *Cardiovascular risk and health behaviour profile*

A significantly higher proportion of the stroke survivors were hypertensive ( $p < 0.001$ ) and diabetic ( $p < 0.001$ ). Atrial fibrillation was reported in 5 (3.6%) stroke survivors and none among the control subjects. Physical inactivity was prevalent among both cases and control population while majority of cases and controls had never smoked. A significantly higher proportion of stroke survivors (18.9 %) were current smokers compared to 6.8% controls while a higher proportion of controls (14.9%) were current users of alcohol in comparison with stroke survivors (2.8%). Fish intake was quite common among cases and controls at least on a weekly basis while a slightly higher proportion of controls (54.1%) took fruits at least on a weekly basis compared to stroke survivors (41.3%). Depressive symptoms scores were significantly higher on the CESD ( $p = 0.015$ ) in stroke survivors compared with controls (Table 3.5).

<b>Characteristic</b>	<b>Stroke survivors N = 143</b>	<b>Stroke - free controls N = 74</b>	<b>Significance</b>
<i>Gender</i> : N (%) female	62 (43.4)	27 (36.5)	$\chi^2 = 0.951$ ; p = 0.329
<i>Age</i> : (mean $\pm$ SD) years	60.4 $\pm$ 9.5	58.8 $\pm$ 8.6	0.136*
<i>Educational Attainment</i> (years)			$\chi^2 = 5.24$ ; p = 0.264
None	21(14.7)	8(10.8)	
Primary (1- 6)	39(27.3)	13(17.6)	
Secondary ( 7 -12)	35(24.5)	18(24.3)	
Tertiary ( > 12)	48(33.6)	35(47.3)	
<i>Marital Status</i>			$\chi^2 = 2.11$ ; p = 0.348
Married	122 (85.3)	65(87.8)	
Widowed	17 (11.9)	9(12.2)	
Separated	4 (2.8)	--	
<i>Living Situation</i>			$\chi^2 = 4.74$ ; p = 0.093
Alone	9 (6.8)	8 (11.1)	
Nuclear family	109(82.6)	62(86.1)	
Extended	14(10.6)	2(2.8)	
<i>Mobile Phone Contact</i> (%)	93.4	91.9	
<i>Ethnicity</i>			$\chi^2 = 17.5$ ; p = 0.052
Yoruba	126 (88.1)	64 (86.5)	
Others	17 (11.9)	10 (13.5)	
<i>Average monthly income</i> (Naira)			$\chi^2 = 15.4$ ; p = 0.017
Less than 10 000	52 (36.4)	16 (21.6))	
10 001 - 25 000	28 (19.6)	15 (20.3)	
25 001 - 50 000	37 (25.9)	22 (29.7)	
50 001 - 100 000	13 (9.1)	10 (13.5)	
100 001 - 150 000	3 (2.1)	8 (10.8)	
150 001 and above	10 (7.0)	3 (4.1)	
<i>Occupation</i>			$\chi^2 = 14.3$ ; p = 0.157
Professional	26 (18.2)	8 (10.8)	
Skilled	33 (23.1)	30 (40.5)	
Semi -skilled	11 (7.7)	4 (5.4)	
Unskilled	47 (32.9)	21(28.4)	
Others	26 (18.2)	11 (14.9)	

**Table 3. 4. Sociodemographic characteristics of stroke survivors and stroke free – apparently healthy controls. \*student t - test**

Characteristic	Stroke survivors N = 143	Stroke – free controls N = 74	Significance
<i>Cardiovascular risk factors, n (%)</i>			
Hypertension	132 (93.6)	44 (59.5)	< 0.001
Diabetes Mellitus	34 (24.5)	3 (4.1)	< 0.001
Obesity (BMI > 30kg/m <sup>2</sup> )	23 (16.1)	14 (18.9)	0.131
Atrial fibrillation	5 (3.6)	--	--
<i>Health behavioural factors</i>			
<i>Physical Activity: n (%)</i> (Leisural)			Fisher's = 5.33; p = 0.070
Sedentary	118 (82.5)	69 (93.2)	
Moderate	11 (7.7)	1 (1.4)	
Strenuous	14 (9.8)	4 (5.4)	
<i>Smoking: n (%)</i>			$\chi^2 = 15.43$ ; p = 0.017
Never smoked	108 (75.5)	55 (74.3)	
Currently smoking	27 (18.9)	5 (6.8)	
Stopped smoking	8 (5.6)	14 (18.9)	
<i>Alcohol Use: n (%)</i>			$\chi^2 = 19.34$ ; p = 0.011
Never used	70 (49.0)	33 (44.6)	
Current user	4 (2.8)	11 (14.9)	
Stopped	45 (31.5)	22 (36.5)	
<i>Fish Intake: n (%)</i>			$\chi^2 = 1.96$ ; p = 0.582
Daily	59 (41.3)	24 (32.4)	
Weekly	58 (40.6)	35 (47.3)	
<i>Fruit Intake: n (%)</i>			$\chi^2 = 8.88$ ; p = 0.031
Daily	33 (23.1)	9 (12.2)	
Weekly	59 (41.3)	40 (54.1)	
<i>Depressive symptoms score</i>			
CESD Score	6.3 + 4.8	4.8 + 3.4	0.025*
GDS - 4	0.7 + 1.1	0.4 + 0.7	0.248+

**Table 3. 5: Cardiovascular and behavioural risk factors in stroke survivors and stroke – free apparently healthy controls**

### *Stroke characteristics*

Eighty-eight cases in our cohort (61.5%) had neuroimaging (CT and/or MRI) within three months of presentation while 42 cases (30%) had CT scan within one week. Subjects who had neuroimaging did not differ significantly from those who did not with respect to age [60.4 (9.4) vs 60.3(9.8),  $t = 0.061$ ;  $p = 0.952$ ], years of educational

attainment [9.3 (5.6) vs 9.5 (5.6),  $t = 0.178$ ,  $p = 0.859$ ], gender [ female (45.5 % vs 42.0%,  $\chi^2 = 0.160$ ,  $p = 0.689$ ] and mean modified Rankin score [ 2.32(1.1) vs 2.29(1.0),  $t = 0.150$ ,  $p = 0.881$ ].

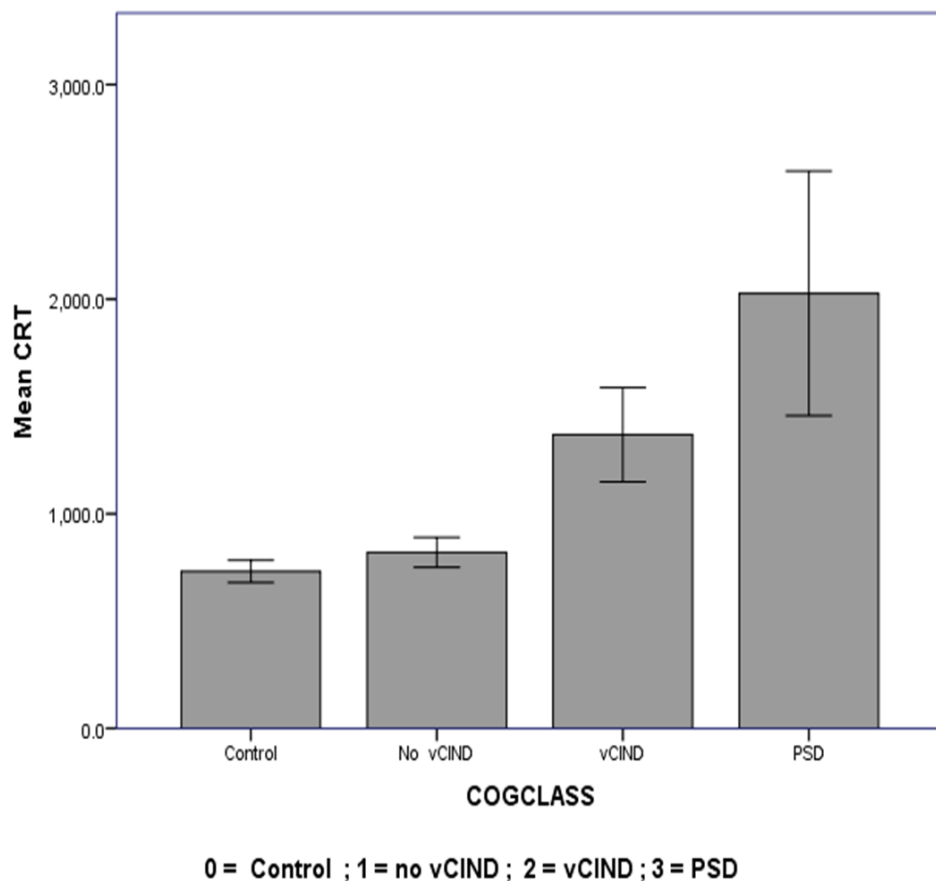
Using the WHO Stroke scale alone, stroke types were: ischemic stroke 113 (79%), haemorrhagic 16 (11.2%) and indeterminate 14 (9.8%). Table 3.6 shows the details of stroke types, subtypes and aetiologic classification in the subset of 88 cases who had neuroimaging (brain CT scan and/or MRI). However, in the 42 cases who had CT scan within one week of presentation, 28 cases (66.7%) were ischaemic and 12 cases (33.3%) were haemorrhagic thus showing a higher relative proportion of haemorrhagic stroke were diagnosed when neuroimaging was done closer to the time of the event rather than much later. In this subset of 42 cases, ischaemic stroke subtypes based on the OCSF classification and TOAST etiologic classification were: 46.4% partial anterior circulation, 32.2% lacunar infarction, 14.3 % total anterior circulation infarction and 7.1% posterior circulation infarction. The aetiologic classes based on the TOAST criteria were: 39 % large vessel disease, 50% small vessel disease, 7.1% cardioembolic and 3.6 indeterminate respectively. Across the total cohort of 143 stroke survivors in this study, most cases had mild disability (median modified Rankin score = 2, median stroke severity score = 12 and good functional score (median Barthel index = 18) at baseline (3 months after stroke).

Stroke Type (based on cases with neuroimaging (CT/MRI) (N = 88)	
Ischaemic	70 (79.5)
Haemorrhagic	18 (20.5)
Stroke Location	
Anterior circulation	83 (94.3)
Posterior circulation	5 ( 5.7)
Ischaemic stroke subtypes ( n = 70)	
OCSF Classification	
Partial anterior circulation infarct	31 (44.4)
Lacunar infarct	29 (41.4)
Total anterior circulation infarct	7 (10.0)
Posterior circulation infarct	3 (4.2)
TOAST Classification (Aetiologic)	
Small vessel disease	41 (58.6)
Large vessel disease	21 (30.0)
Cardioembolic	5 (7.1)
Indeterminate	3 (4.2)

**Table 3.6: Stroke characteristics in subjects**

### 3.3.2.2. Profile of cognitive performance across controls and cognitive groups

At three months following the index stroke, 74 (51.7 %) stroke survivors had no cognitive impairment (NCI), 57 (39.9%) had VCI no dementia (vCIND) while 12 (8.4%) had PSD. The vCIND group included 19/57 (33.3%) single domain non-amnestic, 11/57 (19.3%) multiple domain non - amnestic, 5/57 (8.8%) single domain amnestic and 22/57 (38.6%) multiple domain amnestic.. Table 3.7 shows the pattern of performance of the controls and different cognitive sub-groups of stroke survivors on the various test items assessing general cognitive functioning as well as domain – specific cognitive functioning while Figure 3.2 shows the pattern of performance on choice reaction time (CRT).



**Figure 3.2. The pattern of mean choice reaction time (CRT) (milliseconds) of control subjects and cognitive categories of stroke survivors.**

<b>Variable</b>	<b>Conotrol (N = 74) Mean(SD)</b>	<b>no vascular CIND (N=71) Mean(SD)</b>	<b>vascular CIND (N = 57) Mean(SD)</b>	<b>PSD ( N = 12) Mean(SD)</b>	<b>p - value ANOVA</b>
Age (years)	58.8 (8.6)	58.2 (9.3)	62.3 (8.9)	65.4 (10.2)	< 0.001
Education (years)	9.8 (6.7)	11.6 (4.4)	7.1 (5.8)	5.9 (5.3)	< 0.001
CSID total score	27.8 (2.8)	27.3 (1.8)	25.0 (2.7)	15.9 (5.0)	< 0.001
MMSE total score	27.4 (3.0)	27.8 (2.7)	23.0 (4.1)	13.1 (4.2)	< 0.001
Animal Fluency score	13.4 (4.0)	11.3 (3.2)	7.7 (3.8)	3.8 (3.7)	< 0.001
Animal fluency ( 0-15 sec)	5.4(1.7)	4.5(2.9)	3.6(1.6)	2.1 (1.7)	<0.001
FAS total score	25.0 (15.1)	20.1 (9.7)	6.6 (6.3)	0.9 (2.0)	< 0.001
Visual Reasoning test score	3.3 (1.6)	2.6 (1.5)	1.8 (1.2)	1.2 (0.7)	< 0.001
Word List Learning total score	17.2 (3.9)	17.2 (3.1)	12.7 (3.9)	7.6 (4.5)	< 0.001
Word List Recall score	5.7 (1.9)	5.2 (1.8)	2.4 (1.9)	1.2 (1.5)	< 0.001
Boston Naming Test score	12.1 (2.7)	12.0 (1.9)	8.2 (3.3)	6.7 (6.6)	< 0.001
Stick Design total score	11.5 (1.6)	11.8 (0.6)	8.0 (3.8)	6.5 (4.1)	< 0.001
Stick Design Recall total score	6.1 (3.0)	5.9 (2.1)	3.1 (2.6)	1.9 (2.2)	< 0.001
Modified Token Test total score	20.7 (3.0)	20.5 (2.1)	14.8 (4.9)	9.8 (3.8)	< 0.001
SRT (ms)	682.6 (617.6)	647.4 (403.3)	1268.1(1017.3)	2128.6(1421.8)	< 0.001
CRT (ms)	731.4 (204.5)	826.3 (286.7)	1360.1(766.0)	2026.9 (945.4)	< 0.001
DVRT (ms)	470.9 (65.9)	518.6 (83.6)	570.7 (76.5)	605.9 (71.4)	< 0.001
SPMRT (ms)	2201.6(1015.2)	2412.5(982.3)	3522.6(2090.6)	3802.4(2546.0)	< 0.001
VIGACC (%)	87.2 (14.3)	81.5 (19.5)	54.4 (24.2)	40.6 (26.1)	< 0.001
SPMOACC (%)	82.9 (18.3)	80.1 (20.2)	69.0 (25.6)	54.6 (31.3)	< 0.001
SPMSI	0.6 (0.4)	0.6 (0.4)	0.4 (0.3)	0.1 (0.3)	< 0.001

**Table 3.7: Demographic and cognitive performance profile of stroke survivors and control subjects.** Abbreviations: CIND = cognitive impairment no dementia; PSD = post - stroke dementia; SRT= simple reaction time; CRT = choice reaction time; DVRT= digit vigilance reaction time; SPMRT= spatial working memory reaction time; VIGACC = digit vigilance accuracy; SPMOACC = spatial working memory original stimuli accuracy; SPMSI = spatial working memory sensitivity index.



*Comparison of stroke survivors with no apparent cognitive impairment (no vCIND) and controls*

Compared to controls, stroke survivors without any apparent cognitive impairment (no vascular CIND) were of comparable age ( $t = -0.76$ ;  $p = 0.450$ ), number of years of educational attainment ( $t = 0.63$ ;  $p = 0.528$ ) and performance on cognitive test items assessing the domains of memory, language and visuospatial/visuoconstructive functioning (Table 3.7). However, they performed significantly worse than controls on cognitive measures of attention, mental speed and executive function [animal fluency,  $t = -3.17$ ;  $p = 0.002$ ; letter fluency (FAS),  $t = -2.20$ ;  $p = 0.03$ ; visual reasoning,  $t = -0.14$ ;  $p = 0.06$ ; CRT mean,  $t = -2.15$ ;  $p = 0.034$ ; and VIGRT,  $t = -3.58$ ;  $p < 0.001$ ] (Table 3.7).

*Comparison of stroke survivors with no vascular CIND and those with vascular CIND*

Stroke patients meeting the criteria for vascular CIND were significantly older ( $t = 2.54$ ;  $p = 0.012$ ) less educated ( $t = -5.02$ ;  $p < 0.001$ ) and more impaired in all cognitive domains compared to those without vascular CIND: executive function and mental speed (animal fluency  $t = -5.35$   $p < 0.001$ ; letter fluency,  $t = -8.18$ ;  $p < 0.001$ ; CRT mean,  $t = -5.22$ ;  $p < 0.001$ ; VIGRT mean,  $t = -3.42$ ;  $p = 0.001$ ); language (BNT,  $t = -8.15$ ,  $p < 0.001$ ), memory (WLL,  $t = -3.31$ ;  $p = 0.001$ ) and visuospatial/visuoconstructive functioning (stick design,  $t = -8.15$ ;  $p < 0.001$ ; modified token test,  $t = -8.79$ ;  $p < 0.001$ ) (Table 3.7).

*Comparison of survivors with vascular CIND and those with post-stroke dementia*

Stroke survivors with vascular CIND were comparable to those with PSD with respect to age ( $t = -1.03$ ,  $p = 0.306$ ), years of formal education ( $t = 0.671$ ,  $p = 0.504$ ) and cognitive performance in the domain of language (BNT,  $t = 1.22$ ;  $p = 0.227$ ) while the demented subgroup had worse performance in the cognitive domains of mental speed/ executive function (animal fluency,  $t = -3.20$ ;  $p = 0.002$ ; letter fluency,  $t = -3.08$ ,  $p = 0.003$ ; CRT mean,  $t = -2.49$ ;  $p = 0.016$ ; SPMSI,  $t = -2.98$ ;  $p = 0.014$ ), memory (word list learning,  $t = -4.05$ ;  $p < 0.001$ ; word list recall,  $t = -2.11$ ;  $p = 0.039$ ) and visuospatial/visuoconstructive functioning (stick design,  $t = -3.33$ ;  $p = 0.001$ ; modified token test,  $t = -3.33$ ;  $p = 0.001$ ).

### 3.3.2.3. Factors associated with cognitive dysfunction three months after stroke

We used various logistic regression models to estimate odds ratio (OR) for cognitive dysfunction (Table 3.8). Unadjusted odds of cognitive dysfunction were statistically significantly higher for older baseline age at stroke occurrence, female gender, alcohol use, and less than 6 years of educational attainment but were lower for daily pre-stroke fish intake and moderate to heavy pre-stroke physical activity. Multivariate regression analysis showed the ORs were attenuated and no longer significant for female gender, alcohol use and moderate to heavy pre-stroke physical activity. However, the ORs remained significant and similar for baseline age, but stronger for less than 6 years of educational attainment and daily pre-stroke fish intake.

Baseline characteristic	Univariate analysis OR (95% CI)	Multivariate analysis OR (95% CI)
Baseline age (years)	<b>1.06 (1.02 - 1.10)</b>	<b>1.05 (1.00 - 1.09)</b>
Female Gender	<b>2.27 (1.15-4.45)</b>	1.87 (0.80 - 4.40)
< 6 years of education	<b>4.84 (2.36 - 9.92)</b>	<b>5.09 (2.17 – 11.95)</b>
Hypertension	1.18 (0.30 – 4.58)	
Diabetes Mellitus	1.29 (0.59 – 2.79)	
Previous Stroke	1.38 (0.51 – 3.10)	
Smoking	1.25 (0.51 – 3.10)	
Alcohol use	<b>2.01 (1.01 – 4.00)</b>	1.19 (0.47 – 3.00)
Pre-stroke daily fish intake	<b>0.42 (0.20 – 0.88)</b>	<b>0.37 (0.15 – 0.89)</b>
Pre-stroke moderate to heavy physical activity	<b>0.17 (0.04 – 0.84)</b>	1.00 (0.99 -1.02)
modified Rankin score	1.03 (0.53 – 1.98)	
Barthel Index score	0.98 (0.90 – 1.06)	
CESD score	1.04 (0.96 – 1.12)	

**Table 3.8. Univariate and multivariate analysis of factors associated with cognitive impairment 3months after stroke.\*significant results are shown in bold (p < 0.05).**

### 3.4. Discussion

To our knowledge, this study is the first in sub-Saharan Africa to examine in detail the profile and factors associated with vascular cognitive impairment in a cohort of stroke survivors with varying degrees of cognitive dysfunction using a robust cognitive battery. Our principal findings are that at three months post-stroke, 51.7% of a cohort of Nigerian African stroke survivors were cognitively intact, 39.9% had VCI no dementia (vascular MCI) while 8.4% had PSD using the AHA/ASA criteria (Gorelick *et al.*, 2011) and a cognitive battery modeled after the NINDS-CSN Harmonization Standards Neuropsychological Battery (Hachinski *et al.*, 2006b). Furthermore, attention, mental speed and executive function deficits were found in stroke patients who were apparently categorized as cognitively normal in comparison with matched stroke-free controls. In patients who met criteria for vCIND, there was further worsening of executive functioning with additional significant impairment in the domains of language, memory and visuospatial/visuoconstructive function. Survivors who were demented at three months had further worsening of executive function, memory and visuospatial/visuoconstructive function. Significant factors associated with cognitive dysfunction at three months post-stroke included older baseline age, female gender, lower educational attainment and history of alcohol use while pre-stroke daily intake of fish and moderate-to-heavy physical activity were protective.

#### *Frequency of vCIND and PSD*

Our finding of vCIND frequency of 39.9% is close to some previous reports (Ballard *et al.*, 2002; Patel *et al.*, 2003; Sachdev *et al.*, 2006; Dong *et al.*, 2012; Pendlebury *et al.*, 2012; Yu *et al.*, 2013) but considerably higher than those reported by others (Hoffmann, 2001; Stephens *et al.*, 2004; Das *et al.*, 2012). However, our PSD rate of 8.4% is similar to the findings of 8.6% by Stephens *et al.* (Stephens *et al.*, 2004) and lies within the range of pooled post-stroke prevalence of 7.4% - 41.3% within the first year reported in a recent systematic review (Pendlebury, 2009). Although the mean age of our cohort is about 60 years similar to that of Dong *et al.* (Dong *et al.*, 2012) but younger than in other studies (Ballard *et al.*, 2003a).

It is noteworthy that recent studies that have adopted the Vascular Cognitive Impairment Harmonization Standards (VCIHS) and the NINDS/CSN Neuropsychological Battery similar to our study have reported relatively high rates of vCIND in particular. Vascular CIND rates of 43.0 %, 54.8 % and 49.9 % have been reported in studies among UK (Pendlebury *et al.*, 2012), Singaporean (Dong *et al.*, 2012) and Korean (Yu *et al.*, 2013) stroke cohorts respectively. This may reflect the high sensitivity of the VCIHS protocol to detecting vascular MCI, especially in the executive function domain. In addition, the high rate of vCIND found in this cohort of Black African stroke survivors may suggest the possibility of an increased susceptibility to the cognitive sequelae of stroke just as they have demonstrated worse cardiovascular outcomes in studies involving multiracial populations (Pendlebury, 2009). In a recent report on post-stroke cognitive impairment over a fifteen year period from the London Stroke Registry, the risk of post-stroke VCI was twice higher among black subjects compared to non-blacks (Douiri *et al.*, 2013b). Apart from this, a stroke injury may trigger/accelerate cognitive decline, (Kalara, 2012b) the age of occurrence notwithstanding (Owolabi, 2011). Poorer outcome may also reflect sub-optimal status of acute stroke care, rehabilitation and secondary prevention seen in many developing countries (Norrving and Kissela, 2013). Appropriate and effective management of vascular risk factors has been associated with a reduction of risk of long-term cognitive impairment in stroke survivors (Allan *et al.*, 2011; Douiri *et al.*, 2013a).

#### *Pattern of post-stroke cognitive dysfunction*

The heterogeneity of the prevalence of post-stroke MCI and dementia from different studies may be due to differences in patient characteristics (sample size, cut-off age, gender, population structure, ethnicity), study setting (hospital vs community-based), study design (first ever and/or recurrent strokes, timing of assessment), differences in study instruments and in the diagnostic criteria for post-stroke cognitive impairment and dementia (Hachinski *et al.*, 2006b; Pendlebury, 2009; Gottesman, 2010). It is important to point out that with the growing understanding of the early and prominent occurrence of executive dysfunction in subjects with VCI, recent studies that have used cognitive tools with robust items sensitive to executive dysfunction have reported more cases of MCI which hitherto might have been wrongly passed as normal.

Previous observations from Newcastle (Ballard *et al.*, 2002; Ballard *et al.*, 2003a; Stephens *et al.*, 2004) showed varying degrees of early cognitive impairment in stroke survivors. In this present study, stroke patients without significant cognitive impairment at three months after stroke still showed deficits in attention, mental speed and executive function compared to control subjects, thus confirming the early occurrence of executive dysfunction in VCI. Subjects with vCIND and PSD had persistence and progression of executive dysfunction in addition to multi-domain impairments of language, memory and visuospatial/visuoconstructive functioning in tandem with previous findings in literature (Ballard *et al.*, 2002; Ballard *et al.*, 2003a; Stephens *et al.*, 2004; Sachdev *et al.*, 2006; Pendlebury *et al.*, 2012). The prominent worsening of memory and visuospatial functioning in patients with PSD may suggest the presence of underlying neurodegenerative pathologies producing an additive/ synergistic effect with vascular pathologies (Deramecourt *et al.*, 2012; Kalaria, 2012b; Kalaria, 2012a). Like previous findings, (Pendlebury *et al.*, 2012) we also found non - amnesic (single and multi-domain) impairment more common than amnesic (single and multi-domain) impairment in the vCIND subgroup, a further reflection of the sensitivity of the NINDS/CSN Neuropsychological Battery for detecting non-memory domain impairments in subjects with vascular MCI (Hachinski *et al.*, 2006b).

#### *Factors associated with post- stroke cognitive dysfunction*

Our observation of older age, female gender and low educational attainment as significant associated factors of post-stroke cognitive dysfunction are comparable with findings in previous studies (Patel *et al.*, 2003; Henon *et al.*, 2006; Sachdev *et al.*, 2006; Pendlebury, 2009; Gottesman, 2010; Das *et al.*, 2012; Kalaria, 2012b). Older age and female gender are risk factors for AD thus suggesting a likely role for age-related neurodegeneration synergizing with stroke to cause cognitive impairment and dementia (Kalaria, 2012b). Lower educational attainment is associated with lower cognitive reserve and reduced resilience to dementing brain pathologies (Stern, 2009).

The protective effect of fish intake against cerebral vascular disease risk (including stroke and VCI) is established (Chowdhury *et al.*; Perez *et al.*, 2012). Although, omega – 3 fatty acids (docosahexaenoic acid and eicosapentaenoic acid) in fish have been largely implicated in the protection, other nutritional constituents of fish including vitamins D and B complex,

essential amino acids and trace elements in fish (for example, taurine, arginine, selenium, calcium, magnesium, potassium, and iodine) may have potentially favourable effects on inflammation, endothelial function, vascular tone, neuronal functioning and cell death (Chowdhury *et al.*; Robinson *et al.*, 2010). Recently, lower red blood cell omega-3 fatty acid levels were associated with smaller brain volumes, increased white matter hyperintensity and vascular pattern of cognitive impairment on tests of visual memory and executive function (Tan *et al.*, 2012). However, this finding should be interpreted with caution as we did not collect specific details on the different species of edible fish, methods of preparation -whether fried or boiled – and the exact quantity of fish intake. These are more specific details worthy of further exploration in future studies.

Unexpectedly, cardiovascular risk factors were not significantly associated with early post – stroke cognitive dysfunction in this study similar to findings in the Sydney Stroke Study at baseline assessment 3 – 6 months after stroke (Sachdev *et al.*, 2006). Pendlebury and Rothwell (2009) had also reported in their meta analysis the lack of association of hypertension, and ischemic heart disease with PSD. This paradox is explained by Allan *et al* (2011) wherein cardiovascular risk factor load predicted long term cognitive outcome and mortality in elderly stroke survivors. Follow up of our cohort is currently in progress to further explore the evolution of cognitive functions and the determining factors. Such longitudinal studies are needed to tease out the influence of cardiovascular risk factors, genetic architecture, epigenetic factors, social engagement and other cultural influences on the cognitive trajectory after stroke in populations of African ancestry.

This study is not without its limitations. The potential existence of familywise Type 1 error associated with multiple pairwise comparisons is acknowledged. The sample size was modest and complete characterization of all our stroke cases was limited by the scant availability and high cost of neuroimaging. Nonetheless, cases with and without neuroimaging were quite similar statistically, and our cohort shares similar demographic and phenomic characteristics with stroke cases described earlier in Nigeria (Owolabi and Agunloye, 2013) and sub-Saharan Africa (O'Donnell *et al.*, 2010a). Further studies with bigger cohorts and population-based samples are required to validate the present findings and explore other associations.

### 3.5. Chapter Summary

- Frequency of post –stroke VCI

Cognitive Category of Stroke Survivors	No (%)
no vCIND	74 (51.7)
vCIND	57 (39.9)
PSD	12 (8.4)

- Categories of vCIND

Category	No (%)
single domain non - amnesic	19 (33.3)
multiple domain non - amnesic	11 (19.3)
single domain amnesic	5 (8.8)
multiple domain amnesic	22 (38.6)

- Factors associated with post – stroke VCI at 3 months

*Univariate*

*Risk factors*

Baseline age

< 6 years of education

Female gender

Alcohol use

*Multivariate*

*Risk factors*

Baseline age

< 6 years of education

*Protective factors*

Pre – stroke daily fish intake

Pre – stroke moderate to heavy

physical activity

*Protective*

Pre – stroke daily fish intake

## **Chapter 4. Medial temporal lobe atrophy, white matter hyperintensities and cognitive impairment among Nigerian African stroke survivors.**

### **4.1. Introduction**

Although physical disability is most commonly associated with stroke, cognitive changes and other non-motor consequences are quite frequent in those who survive longer. Up to 64% of stroke survivors will develop a degree of cognitive impairment and about 30% succumb to dementia (Hachinski *et al.*, 2006b). In a recent meta- analysis, the pooled prevalence estimates of post-stroke dementia (PSD) within one year of stroke ranged from 7.4% in population-based studies of first-ever stroke excluding pre-stroke dementia to 41.3% in hospital-based studies of all strokes including pre-stroke dementia (Pendlebury and Rothwell, 2009b).

Post-stroke cognitive dysfunction characteristically encompasses a multi-domain impairment of attention and concentration, executive function, language, memory and visuospatial function, with executive function being the earliest and predominantly affected domain (Ballard *et al.*, 2003a; Erkinjuntti and Gauthier, 2009; Gorelick *et al.*, 2011).

In Chapter 3, we established the profile and determinants of VCI in our unique cohort of Nigerian African stroke survivors. We found a multi – domain pattern of cognitive impairment with early and dominant involvement of frontal executive function. For the clinical determinants of post - stroke VCI, older age, - surrogate of age – associated neurodegeneration, female gender and low education were robust findings. The work detailed in this chapter sought to relate these results to neuroimaging parameters that are currently associated with VCI within the context of the specific objectives of the thesis.

There is a large body of structural brain imaging evidence in VCI, which suggests that medial temporal lobe atrophy or global cerebral atrophy, white matter changes, lacunar infarcts, strategic infarcts and cerebral microbleeds contribute to vascular cognitive impairment, although the relative contributions of each varies across studies (Firbank *et al.*, 2007; Burton *et al.*, 2009; Ihle-Hansen *et al.*, 2012; Jokinen *et al.*, 2012; Kalaria, 2012a; Kalaria, 2012b; Poels *et al.*, 2012). However, the neuroimaging substrates of post - stroke VCI have never been examined among among sub - Saharan Africans. The aim of the study



reported in this chapter was to determine the neuroimaging correlates of vascular cognitive impairment (VCI) three months post-ictus in older Nigerian African stroke survivors participating in the Cognitive Function After STroke (CogFAST)-Nigeria Study. This is the first comprehensive, detailed and prospective African study of stroke-related cognitive dysfunction.

## **4.2. Materials and Methods**

### ***4.2.1. Study Design and Participants***

Stroke patients ( $\geq 45$  years) presenting consecutively were recruited from the stroke registers of two specialist hospitals, University College Hospital, Ibadan and Federal Medical Center Abeokuta (Akinyemi *et al.*, 2009), and three smaller secondary healthcare centres in South-western Nigeria between July 2010 and June 2012.

Stroke was defined according to the World Health Organization (WHO) definition (World Health Organization, 1988) and classified using the WHO criteria, the Oxford Community Stroke Project Classification (OCSP) (Bamford J, 1991) and neuroimaging (CT scan and/ or MRI) findings, when available. Neuroimaging was not performed on some patients due to limited access and prohibitive cost in Nigeria. The WHO criteria have been shown to have a sensitivity of 73% for haemorrhage, 69% for infarction and an overall accuracy of 71% in Nigeria (Ogun *et al.*, 2002). The cohort was comprehensively assessed 3 months after stroke, allowing time for the resolution of post-stroke delirium in accordance with the design of Desmond *et al* (Desmond DW, 1996). The evaluation included a medical history, assessment of neurological deficits and MRI scan, where possible ( $n = 58$ ).

Cardiovascular risk factors (CVRFs) including hypertension, diabetes mellitus, dyslipidaemia, smoking, excessive alcohol use, atrial fibrillation and previous stroke were ascertained from medical history and clinical records.

Exclusion criteria were: (i) subarachnoid haemorrhage, (ii) significant physical illness and motor impairment that precluded paper- and computer-based neuropsychological evaluation (e.g. visual impairment, moderate to severe aphasia, hemiparesis affecting the dexterous hand (MRC power grade  $<3$ ), (iii) any co-morbid psychiatric or neurologic illness, (iv) any systemic

disease that could impair cognition e.g. chronic liver disease and chronic kidney disease, (v)non-consent to take part in the study. The local research ethics committees granted approval for the study (University College Hospital, Ibadan and Federal Medical Centre Abeokuta), while written informed consent was obtained from each subject.

#### **4.2.2. Cognitive assessment**

The neuropsychological instrument consisted of the Community Screening Instrument for Dementia (CSID) – cognitive part (Hall *et al.*, 2000), the Mini-Mental State Examination (MMSE) (Gureje *et al.*, 1995) and the Vascular Neuropsychological Battery (Hachinski *et al.*, 2006b). The CSID is a paper and pencil test of global cognitive performance in which adaptability, validity and utility in populations from different cultural, educational and socio-economic backgrounds have been established (Hendrie *et al.*, 1995; Hall *et al.*, 2000). It has a sensitivity of 87% and specificity of 83%, and has been used reliably and widely to assess cognition in the Yoruba-speaking population of south-western Nigeria, where the present study was conducted (Ogunniyi *et al.*, 2000). The schedule includes sub-scores for attention, orientation, calculation, short- and long-term memory, language comprehension and expression, praxis and abstract thinking. Pre- stroke cognitive status was assessed using the CSID- informant part by trained interviewers.

The Vascular Neuropsychological Battery [V-NB] was devised by us after the NINDS-CSN Harmonization Standards 60-minute neuropsychological protocol (Hachinski *et al.*, 2006b), with minor modifications to ensure adaptability to the language and culture of the study population. The V-NB consisted of multiple test items examining specific cognitive domains (executive function, memory/learning, language, visuospatial/visuoconstructive skills). Other details are as described in Chapter 2.

Test items from the Cognitive Drug Research (CDR) computerized assessment battery were also included in the Vascular-Neuropsychological Battery for the evaluation of attention, processing speed and executive function [the constituent tests included Simple Reaction Time (SRT) – a measure of attention, Choice Reaction Time (CRT) – measuring processing speed, Digit Vigilance (DV) and Spatial Working Memory (SWM) – measuring attention and working memory, respectively (Ballard *et al.*, 2003a).

### ***4.2.3. Operational definitions of cognitive dysfunction***

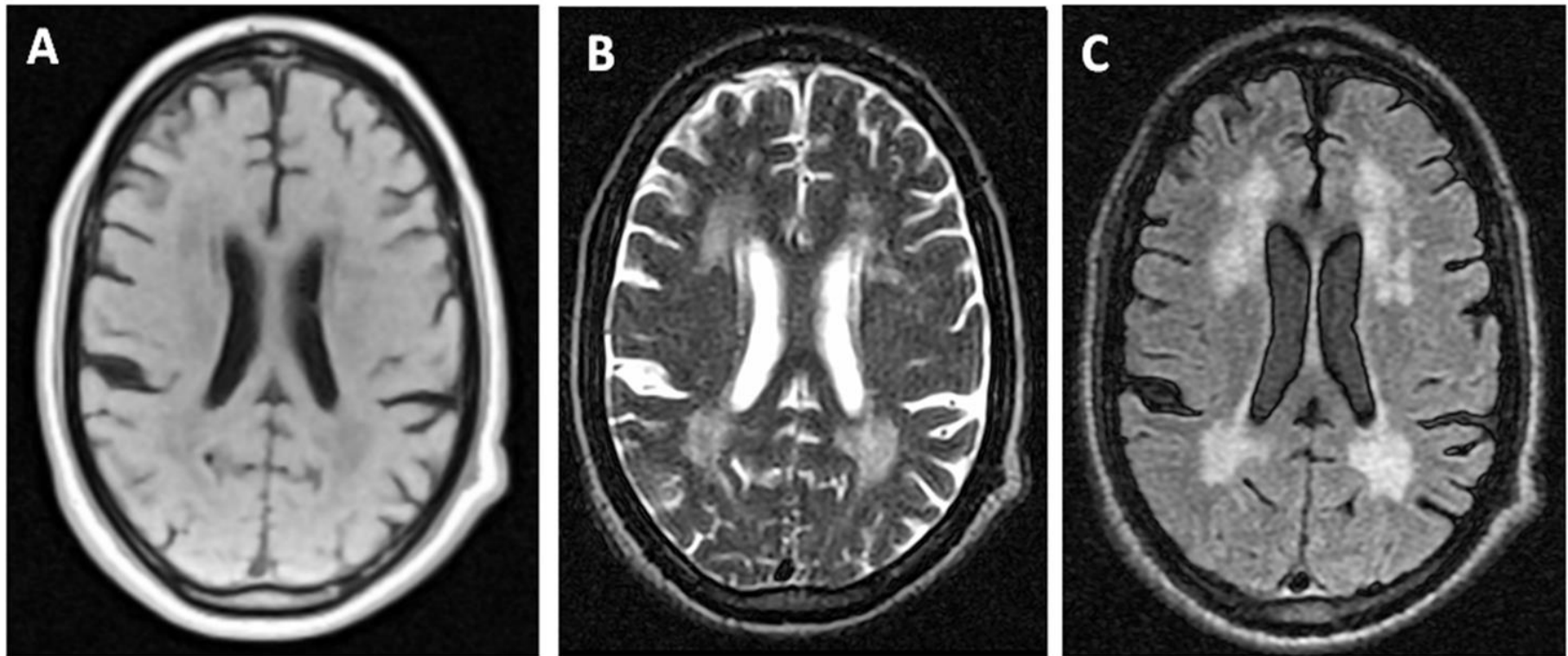
Vascular mild cognitive impairment (MCI) and PSD were defined according to the American Stroke Association/American Heart Association Vascular Cognitive Impairment Guidelines (Gorelick *et al.*, 2011). Vascular MCI (Vascular Cognitive Impairment No Dementia-CIND) (Gorelick *et al.*, 2011) was defined as impairment in at least one cognitive domain (executive function, memory/learning, language, visuospatial/visuoconstructive skills) and normal or mild impairment of instrumental activities of daily living, independent of motor/sensory symptoms. PSD (Gorelick *et al.*, 2011), in accordance with the DSMIV criteria, was defined as impairment in two or more cognitive domains that are of sufficient severity to affect the subject's activities of daily living, independent of motor/sensory symptoms. Other details of the operational definitions of vCIND and PSD as well details of cognitive diagnosis are as described in Chapter 2.

### ***4.2.4 MRI Protocol***

Patients underwent an MRI examination three months after the stroke event. The two MRI scanners operated between 0.2 and 0.35 T. Axial spin-echo T2-weighted (T2W) images (echo time, 80 to 120 ms; repetition time, 4000 to 6500 ms; slice thickness, 5 mm); and axial, sagittal and coronal spin-echo T1-weighted (T1W) images (echo time, 9 to 15 ms; repetition time, 350 to 500 ms; slice thickness, 5 mm) were acquired. These were complemented by fluid-attenuated inversion recovery (FLAIR) sequences (echo time, 90 to 120 ms; repetition time, 6000 to 9000 ms; inversion time, 2000 to 2200 ms; slice thickness, 5 mm) to allow for better separation and identification of WMHs and cerebrospinal fluid, as used in a previous study (Ogbole *et al.*, 2013). All images were transferred to computer workstation with Clear canvas DICOM viewer and evaluated by two experienced neuroradiologists . All ratings were performed by consensus agreement.

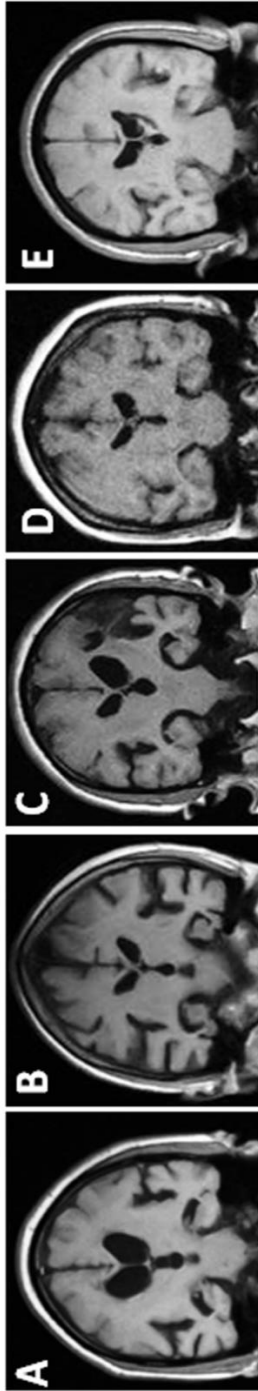
#### **4.2.5. Image Assessment**

White matter changes were assessed using the Scheltens visual rating scale for white matter hyperintensities (WMHs) (Scheltens *et al.*, 1993). Ratings were performed on MRI images on computer screen with T2 and FLAIR images. Periventricular WMH score was compiled as a summation of all three periventricular WMH scores in the frontal and occipital regions, as well as along the ventricles; the deep WMH score was a summation of all the deep WMH scores in the four regions assessed. The total WMH score for each patient was the sum of all ratings. Medial Temporal Lobe Atrophy (MTLA) was evaluated using the Scheltens MTLA visual rating scale (Scheltens *et al.*, 1992). Both sides were assessed and the score of the more affected side was used in cases of severe asymmetry. Total brain volume (TBV) was measured from the T1-weighted axial images. Slice-to-slice variations in intensity were first removed. This was performed by creating a mask using the brain extraction tool (Bet) from the FSL software ([www.fmrib.ox.ac.uk/fsl/](http://www.fmrib.ox.ac.uk/fsl/)). The mean intensity within the mask was determined on each slice, and the overall intensity for the whole slice scaled accordingly. We then used the segmentation tool in SPM8 ([www.fil.ion.ucl.ac.uk/spm/](http://www.fil.ion.ucl.ac.uk/spm/)) to generate gray and white matter segmentations. A brain mask was generated from the sum of gray + white matter. This brain mask was visually inspected, and manually edited, where necessary, to remove non - brain tissue; total brain volume was measured from the number of voxels in the mask. Total intracranial volume (ICV) was measured from the T2 weighted axial images in a similar fashion, correcting for slice intensity variations, using SPM to segment the brain, then manually editing the segmentation, where appropriate. Total intracranial volume was then taken as the sum of gray matter + white matter + CSF. Ventricular volume was measured from the T2-weighted axial images. We used a previously-created standard space template of probable location of the ventricles in older people (Firbank *et al.*, 2003). This template was transformed from standard space to each subject and used to mask the CSF segmentation from the previous step. The resulting ventricle segmentation was manually edited, and volume determined. All neuroimaging evaluations were undertaken with the assessors blind to clinical information.



**Figure 4.1.** Magnetic resonance imaging (MRI) T1- and T2-weighted (A and B), and fluid-attenuated inversion recovery (C ) axial images from a 70 - year old female Nigerian stroke survivor showing moderate white matter hyperintensities.





#### 4.2.6. Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences version 19.0 (SPSS Chicago Inc.). Categorical variables were examined and summarized in percentages, while continuous variables were described using measures of central tendency (mean, median and semi-interquartile range) and compared using the Student's t-test, analysis of variance (ANOVA) and Kruskal-Wallis Test. Correlations were examined using Pearson's correlation coefficient, while logistic regression models were fitted to determine univariate and multivariate relationships between cognitive status and patient-related demographic, cognitive and neuroimaging variables. Age and years of educational attainment were entered as dichotomous measures and other determinants as continuous measures in the regression models. Number of CVRFs was aggregated as 0 – none; 1 – 1 or 2 CVRFs and 2 - 3 or more CVRFs. Age and sex were included in the multivariate model, even if not significant. Unadjusted and adjusted odds ratios (OR) with 95% CIs were estimated. Level of statistical significance was set at  $p < 0.05$ .

**Figure 4. 2. Magnetic resonance imaging (MRI) T1- weighted coronal images showing different degrees of medial temporal lobe atrophy (MTLA) in Nigerian stroke survivors: [A] Grade 4 MTLA in a 70 year old male; [B] Grade 3 MTLA in an 81 year female; [C] Grade 2 MTLA in a 52 year male; [D] Grade 1 MTLA in an 48 year male; [E] Grade 0 MTLA in an 49 year female.**

## 4.3. Results

### 4.3.1. Participant characteristics

Out of 220 consecutively presenting stroke survivors assessed for eligibility in the study, 145 met the selection criteria (Figure 3.1). Seventy-five subjects were excluded due to: no consent ( $n = 25$ ),  $< 45$  years of age ( $n = 32$ ), severe aphasia and motor impairment precluding computer-assisted cognitive impairment ( $n = 18$ ). The records of two stroke survivors were excluded from further analysis on account of incomplete assessment details. Out of a total of 143 stroke survivors at baseline three months after stroke, 58 had a brain MRI performed at three months, in addition to clinical and neuropsychological assessment. Given a significance level,  $\alpha = 0.05$  and assuming a moderate effect size Cohen's  $d = 0.4$ , using the G\*Power software (Faul et al, 2007), the computed power ( $1 - \beta$ ) = 0.7599.

Table 4.1 shows the demographic, clinical and neuroimaging characteristics of those who had MRI and constitute the current study group.

Subjects who had brain MRI ( $n = 58$ ) did not differ significantly from those who did not ( $n = 85$ ) with respect to age [59.6(9.6) versus 60.8(9.5) years] ( $t = 0.75$ ,  $p = 0.453$ ); gender: female [28(49.1%) versus 34 (39.5%)] ( $\chi^2 = 1.28$ ,  $p = 0.302$ ) years of formal education [8.5(5.7) versus 9.9(5.5)],  $t = 1.45$ ;  $p = 0.150$ ; and stroke type [cerebral infarction/intracerebral haemorrhage/indeterminate (50/8/0 versus 64/11/8)],  $\chi^2 = 6.88$ ,  $p = 0.08$ ) and OCSP classification [LACI/PACI/TACI/POCI (23/20/5/2 versus 20/33/7/4)],  $\chi^2 = 8.39$ ,  $p = 0.211$ ). Five (8.6%) subjects had significant pre-stroke cognitive impairment from the informants' rating of subjects' cognitive function.

Based upon our operational definition, 26 (44.8%) stroke survivors had no vascular cognitive impairment (vCIND), while 24 (41.4%) and 8 (13.8%) had vCIND and post-stroke dementia (PSD), respectively.



Age at baseline: (mean, SD) years	60.1(10.7)
Sex : n (%) female	28(50%)
Total number of years of education :( mean, SD) years	8.6(5.6)
CSID total score (mean, SD)	24.8(4.6)
MMSE score (mean, SD)	23.5(5.9)
Executive function score (mean, SD)	10.6(4.6)
Memory score (mean, SD)	29.6(10.4)
Simple reaction time (mean, SD)	947.5(861.0)
Choice reaction time (mean, SD)	1170.6(763.4)
CESD Score (mean, SD)	6.5(5.4)
Stroke Type (diagnosed by CT and/or MRI)	
Ischaemic	50(86.2)
Haemorrhagic	8(13.8)
Cardiovascular risk factors, n (%)	
Hypertension	53(91.4)
Diabetes Mellitus	13(22.4)
Dyslipidemia	6(10.3)
Atrial fibrillation	1(1.7)
Ever smoked	15(25.9)
Ever taken alcohol	28(48.2)
Previous stroke	7(12.1)
*Imaging Volumetrics (mean, SD)	
ICV (mls)	1331.0(146.7)
TBV (mls)	1024.9(132.2)
Ven vol (mls)	44.7(19.3)
TBV/ICV	0.77(0.06)
Ven vol/TBV	0.04(0.02)
MTLA (L+R) total score	7.06(1.67)
Vascular lesions on MRI	
Large vessel infarct-right [n (%)]	3(5.1)
Large vessel infarct-left [n (%)]	3(5.1)
Frontal infarct –right [n (%)]	4(6.9)
Frontal infarct –left [n (%)]	3(5.1)
Parietal infarct – right [n (%)]	17(29.3)
Parietal infarct – left [n (%)]	13 (22.4)
Basal ganglia small vessel disease – right [n (%)]	15(25.9)
Basal ganglia small vessel disease – left [n (%)]	9(15.5)
**Total brain WMHs (median, semi-interquartile range) <sup>x</sup>	7.00(0- 13.75)
* *Periventricular WMHs(median, semi-interquartile range) <sup>x</sup>	3.00(0- 5.00)
* *Deep WMHs(median, semi-interquartile range) <sup>x</sup>	4.00(0- 9.25)

**Table 4. 1. Demographic, clinical and neuroimaging characteristics of subjects (n = 58).**

\*\*computed based on Schelten's WMH scale; data non - normally distributed<sup>x</sup>; \*volumetric analysis was done only in 54 cases.

### 4.3.2. Characteristics of cognitive sub – groups of subjects

Table 4.2 presents the demographic, cognitive and MRI imaging characteristics of cognitive sub-groups of stroke survivors, demonstrating the pattern of performance on tests of general cognitive functioning as well as in specific domains of memory (V –NB memory score), executive function (V –NB executive score), attention (SRT), information processing speed (CRT) and mental flexibility (SPMRT). There were statistically significant differences in performance (mean and standard deviation) across the spectrum of stroke survivors (Normal, vCIND and PSD) on each cognitive test.

Regarding neuroimaging metrics, total intracranial volume ( $F = 0.898$ ,  $p = 0.414$ ) and ventricular volume ( $F = 1.823$ ,  $p = 0.172$ ) were similar across the subgroups, whereas total brain volume ( $F = 7.686$ ,  $p = 0.001$ ) and the ratio of total brain volume to intracranial volume ( $F = 7.950$ ,  $p = 0.001$ ) were significantly reduced in cognitively impaired and demented stroke survivors. Medial temporal lobe atrophy (MTLA) scores were significantly increased in cognitively impaired and demented stroke survivors ( $F = 6.776$ ,  $p = 0.003$ ), while WMHs also showed a similar increasing trend, although this did not attain statistical significance ( $p > 0.05$ ).

<b>Variable</b>	<b>Normal (N =26)</b> <b>Mean(SD)</b>	<b>vascular CIND (N=24)</b> <b>Mean(SD)</b>	<b>PSD (N=8)</b> <b>Mean(SD)</b>	<b>p - value (ANOVA)</b>
Age (years)	54.9(7.8)	62.8(8.9)	68.3(15.6)	<b>0.001</b>
Education (years)	11.3(4.1)	6.9(6.2)	4.6(4.1)	<b>0.001</b>
CSID score	27.3(1.6)	24.9(3.3)	16.6(5.4)	< <b>0.001</b>
MMSE score	27.8(2.3)	22.3(4.2)	13.4(4.5)	< <b>0.001</b>
V – NB Memory score (total)	38.0(5.0)	24.5(7.6)	16.7(8.6)	< <b>0.001</b>
V – NB Executive score (total)	14.1(2.6)	8.7(3.4)	4.8(2.9)	< <b>0.001</b>
SRT (ms)	599.1(622.6)	1001.9(656.5)	1886.4(1283.3)	< <b>0.001</b>
CRT (ms)	826.3(442.9)	1296.8(794.8)	1899.2(920.9)	< <b>0.001</b>
SPMRT (ms)	2186.0(1068.8)	2827.2(1542.9)	3741.2(2850.9)	0.06
Log_ICV	6.12(0.05)	6.12(0.05)	6.10(0.04)	0.414
Log_TBVa	6.02(0.05)	6.00(0.04)	5.93(0.06)	<b>0.001</b>
Log_Venvol	4.56(0.17)	4.66(0.23)	4.66(0.13)	0.172
TBV/ICVb	0.79(0.04)	0.77(0.06)	0.70(0.09)	<b>0.001</b>
MTA total (L+ R) scorec	6.28(1.49)	7.79(1.58)	8.00(1.27)	<b>0.001</b>
Total WMH score	6.80(7.53)	11.52(11.78)	14.57(15.34)	0.273 $\beta$
Periventricular WMH score	2.38(2.21)	3.31(2.38)	3.85(2.73)	0.231
DeepWMH score	4.42(6.20)	8.21(10.11)	10.71(13.62)	0.492 $\beta$

**Table 4. 2: Characteristics of cognitive sub- groups of subjects ( N = 58).**

Statistical comparisons: <sup>a</sup>Normal vs vCIND, t = 1.209 p = 0.233; vCIND vs PSD, t = 3.160 p = 0.004; Normal vs PSD , t = 3.596 p = 0.001;

<sup>b</sup>Normal vs vCIND, t = 1.762 p = 0.085; vCIND vs PSD, t = 2.216 p = 0.036; Normal vs PSD, t = 4.053 p < 0.001; <sup>c</sup>Normal vs vCIND, t = -3.244 p = 0.002; vCIND vs PSD, t = -0.296 p = 0.770; Normal vs PSD, t = -2.608 p = 0.014;  <sup>$\beta$</sup> Kruskal-Wallis Test. Statistics: \*p value = significant p values are in bold.

### 4.3.3. Correlation of clinical, cognitive and neuroimaging variables

Age correlated significantly with total brain volume ( $r = -0.393$ ,  $p = 0.004$ ), MTLA total score ( $r = 0.525$ ,  $p < 0.001$ ) but not WMH total score ( $r = 0.206$ ,  $p = 0.144$ ). Number of years of educational attainment correlated significantly with total brain volume ( $r = 0.324$ ,  $p = 0.018$ ) but not MTLA ( $r = 0.263$ ,  $p = 0.065$ ) or total WMH ( $r = -0.012$ ,  $p = 0.935$ ). MTLA correlated significantly with total WMH score ( $r = 0.461$ ,  $p = 0.002$ ), total CSID score ( $r = -0.378$ ,  $p = 0.019$ ), memory ( $r = -0.702$ ,  $p < 0.001$ ) and executive function ( $r = -0.369$ ,  $p = 0.016$ ) but not total brain volume ( $r = -0.203$ ,  $p = 0.157$ ). Deep WMH frontal score correlated significantly with MTLA ( $r = 0.352$ ,  $p = 0.013$ ), executive function ( $r = -0.350$ ,  $p = 0.013$ ) choice reaction time ( $r = 0.345$ ,  $p = 0.015$ ) and memory ( $r = -0.333$ ,  $p = 0.021$ ). Deep WMH parietal score correlated with memory ( $r = -0.502$ ,  $p < 0.001$ ) and executive function ( $r = -0.315$ ,  $p = 0.026$ ), while deep WMH temporal score correlated with executive function ( $r = -0.303$ ,  $p = 0.033$ ) but not with memory ( $r = -0.226$ ,  $p = 0.123$ ). Pre-stroke informant cognitive score showed significant correlation with post-stroke memory score ( $r = -0.321$ ,  $p = 0.022$ ) and a trend with post-stroke general cognitive functioning CSID total score ( $r = -0.248$ ,  $p = 0.071$ ) but lacked correlation with structural MRI variables; MTLA ( $p = 0.438$ ), TBV ( $p = 0.137$ ) total WM score ( $p = 0.642$ ). Presence of hypertension correlated significantly with total WM score ( $r = 0.361$ ,  $p = 0.001$ ) and total deep WM score ( $r = 0.375$ ,  $p = 0.007$ ). Aggregated vascular risk factor load correlated significantly with the female gender ( $r = 0.372$ ,  $p = 0.005$ ) but showed a trend with age ( $r = 0.251$ ,  $p = 0.064$ ) and MTLA ( $r = -0.248$ ,  $p = 0.086$ ). Left parietal infarcts (mainly large vessel) were also significantly associated with cognitive dysfunction as an outcome ( $r = 0.780$ ,  $p = 0.002$ ).

### 4.3.4. Univariate determinants of cognitive outcomes

Table 4.3 presents univariate logistic regression analyses of statistical predictors of cognitive impairment in three different models. In model I (Normal vs vCIND), education  $< 7$  years, and MTLA rating were significantly associated with vCIND. In model II (vCIND vs PSD), TBV was significantly associated with PSD. In model III, [Normal vs (vCIND + PSD)], age  $> 60$  years, educational attainment  $< 7$  years, TBV and MTLA significantly differentiated normal (no vCIND) from cognitively impaired (vCIND + PSD) study subjects.

	Normal vs vCIND			vCIND vs PSD			Normal vs (vCIND + PSD)		
	OR	95%CI	*p -value	OR	95% CI	*p -value	OR	95% CI	*p – value
Age > 60 years	3.21	0.98-10.45	0.053	2.54	0.42-15.21	0.308	3.97	1.30-12.13	<b>0.016</b>
Female gender	2.24	0.72 – 6.95	0.163	1.19	0.23 – 6.17	0.835	2.34	0.81 – 6.74	0.116
Education < 7 years	6.67	1.92–23.18	<b>0.003</b>	3.50	0.37 –33.56	0.277	8.52	2.58-23.12	<b>&lt; 0.001</b>
Total WMH score	1.06	0.97-1.13	0.123	1.02	0.95-1.09	0.580	1.06	1.00-1.13	0.073
Periventricular WMH score	1.20	0.92-1.57	0.181	1.10	0.76-1.60	0.610	1.22	0.96-1.56	0.102
Deep WMH score	1.06	0.98-1.15	0.145	1.02	0.95-1.10	0.600	1.07	0.99-1.15	0.094
Log _ ICV	0.22	0.001-5462.00	0.809	0.02	0.001 - 10385.0	0.247	0.01	0.001 – 1144.1	0.439
Log _ TBV	0.04	0.01 –137.28	0.230	0.03	0.01 – 0.023	<b>0.024</b>	0.04	0.01 – 0.20	<b>0.025</b>
Log _ VenVol	13.44	0.52 – 347.02	0.117	1.22	0.020 – 74.90	0.924	18.6	0.81 – 429.36	0.067
MTLA rating	1.91	1.19 – 3.06	<b>0.007</b>	1.10	0.59 – 2.07	0.759	2.05	1.28 – 3.27	<b>0.003</b>

**Table 4.3 . Univariate logistic regression model of demographic and imaging determinants of cognitive dysfunction among subjects**Abbreviations: vCIND = vascular cognitive impairment no dementia; PSD = post - stroke dementia; CSID= Community Screening Instrument for Dementia; MMSE = Minimental State Examination; V – NB = Vascular Neuropsychological Battery; ICV = Intracranial volume; TBV = Total Brain Volume; VenVol = Ventricular Volume; MTLA = Medial Temporal Lobe Atrophy rating; WMH = White Matter Hyperintensity. OR = Odds Ratio; CI = Confidence Interval. \*p value = significant p values are in bold.

#### **4.3.5. Multivariate determinants of cognitive outcomes**

Multivariate logistic regression analyses were performed in the three models described above. Demographic and significant univariate neuroimaging predictors were fed into the models and following which, educational attainment < 7 years and MTLA rating remained significant independent statistical predictors of post-stroke vascular cognitive impairment no dementia (model I) and of post-stroke cognitive dysfunction (model III) accounting for up to 49% of the variance of cognitive outcome (Table 4.4).

#### **4.4. Discussion**

The principal finding was that the presence of MTLA on neuroimaging was independently associated with early post-stroke cognitive dysfunction in a sample of Nigerian African stroke survivors, apart from the demographic variable of lower educational attainment. In addition, MTLA showed significant correlation with WMH, general cognitive performance, executive function, and memory score. Non – demented (vCIND) stroke survivors had significantly higher total brain volume (TBV) than the demented (PSD) group but did not significantly differ in medial temporal lobe atrophy (MTLA) and severity of white matter hyperintensities (WMHs). Stroke survivors without apparent cognitive impairment (no vCIND) had higher TBV and lower WMHs scores compared to PSD, and significantly lower MTLA score compared to vCIND and PSD. Nevertheless, we acknowledge the potential influence of multiple pairwise comparisons on the results of our analysis and the corresponding interpretations.

Despite a modest sample size, the study is unique in being the first in sub-Saharan Africa to examine neuroimaging correlates of cognitive impairment. Our findings provide robust evidence in support of other previous studies showing the predictive role of MTLA in vascular cognitive impairment (Bastos-Leite *et al.*, 2007; Firbank *et al.*, 2007; Burton *et al.*, 2009). Although MTLA has often been interpreted as a signature of Alzheimer pathology (Burton *et al.*, 2009), some recent studies suggest it may also have a vascular basis resulting from cerebral hypoperfusion (Firbank *et al.*, 2012).

Variable	Normal vs vCIND			vCIND vs PSD			Normal vs (vCIND + PSD)		
	OR	95%CI	*p value	OR	95%CI	p value	OR	95%CI	*p value
<i>Nagelkerke R<sup>2</sup></i>	<i>R<sup>2</sup> = 0.414</i>			<i>R<sup>2</sup> = 0.470</i>			<i>R<sup>2</sup> = 0.490</i>		
Age > 60 years	1.06	0.19 – 5.92	0.945	0.50	0.05 – 5.45	0.579	0.79	0.15 - 4.27	0.787
Female gender	1.42	0.33 – 6.17	0.641	0.25	0.02 – 3.86	0.322	0.83	0.14 - 4.79	0.834
Education < 7 years	6.22	1.35 – 28.73	<b>0.019</b>	8.88	0.26 – 306.34	0.227	6.95	1.54 – 1.30	<b>0.012</b>
MTLA rating	2.02	1.05 – 3.87	<b>0.035</b>				2.25	1.16 – 4.35	<b>0.016</b>
Log _ TBV							0.01	0- 1996.50	0.260

**Table 4.4: Multivariate logistic regression model of significant univariate determinants of cognitive dysfunction among subjects**

Qui et al (Qiu *et al.*, 2012) recently reported a significant association between aggregated vascular risk factors and reduced hippocampal volume in a cohort of men, while hippocampal neuronal atrophy was found to correlate with post-stroke dementia in another cohort with insignificant degenerative pathology (Allan *et al.*, 2011; Gemmell *et al.*, 2012). Although we found no correlation between pre-stroke informant cognitive score and MTLA in this cohort, there was a trend towards significance in the relationship between aggregated vascular risk factors and MTLA as well as in the progression of WMH measures across the cognitive groups. Thus, the relationship between MTLA and cognitive impairment in our cohort may suggest a bi-directional causality mediated by cerebral vascular disease. However, the strength of this interpretation is limited by the moderate power  $(1 - \beta) = 0.76$  of our sample owing to limited availability and high cost of MRI in our study population. Further validation in a bigger cohort studied over time is necessary.

The finding of a significant correlation between MTLA and WMH agrees with others (Oosterman *et al.*, 2008) and further strengthens the case for a vascular basis in the pathomechanism of MTLA, WMH being a surrogate of small vessel disease (Kalaria, 2012a). We also found a significant association between MTLA and WMH, executive function, processing speed and memory in line with previous studies (Jokinen *et al.*, 2005; Oosterman *et al.*, 2012). Similarly, Jokinen et al found synergistic interactions of MTLA, white matter lesions, regional and cortical atrophy on cognitive performance in subjects with small vessel disease in the LADIS study (Jokinen *et al.*, 2012). Our findings, therefore, provide further evidence that global and regional cerebral atrophy, cortico-cortical and cortico-subcortical disconnections and slowing of neural impulse transmission consequent to white matter damage from microvascular pathologies do have robust impact on cognitive processes (Gorelick *et al.*, 2011; Kalaria, 2012a; Kalaria, 2012b).

Executive dysfunction is an early and prominent feature of vascular cognitive impairment of varying aetiologies and natural history (Erkinjuntti and Gauthier, 2009; Gorelick *et al.*, 2011) In previous studies, executive dysfunction had been found to correlate with both WMH (Jokinen *et al.*, 2005) and MTA (Oosterman *et al.*, 2008; Oosterman *et al.*, 2012) and is thought to further mediate their relationship with memory and visuospatial dysfunction in the context of cerebral vascular disorders.



Surrogates of cognitive reserve include number of years of educational attainment (Staff *et al.*, 2004) and total brain volume (Mori *et al.*, 1997). Our finding of older age and low educational attainment as significant predictors of post-stroke cognitive dysfunction are consistent with previous studies (Patel *et al.*, 2003; Henon *et al.*, 2006; Pendlebury, 2009; Gottesman, 2010; Das *et al.*, 2012; Kalaria, 2012b). Age is the strongest risk factor for age-associated cerebrovascular and neurodegenerative disorders implicating a likely role for age-related neurodegeneration, synergizing with stroke to cause cognitive impairment and dementia in this cohort (Kalaria, 2012b). Lower educational attainment is associated with lower cognitive reserve and reduced resilience to dementing brain pathologies (Stern, 2009), especially in the presence of an accompanying reduction in total, cortical or regional brain volume (Mok *et al.*, 2011). This study, nonetheless, has several limitations. Though sample size was modest, the significant findings, the first of its kind in sub-Saharan Africa, are worthy of note. The CogFAST – Nigeria project is still in progress in a longitudinal cohort approach with a view to confirming the current findings and unraveling new associations. We assessed white matter changes with the Scheltens' scale (Scheltens *et al.*, 1993). Generally, visual rating scales are not as sensitive as structural volumetric measures and this may also partly explain the lack of statistical significance in the findings of white matter changes in our cohort. Nevertheless, visual rating scales are cost effective, useful in clinical practice and have been proved to attain good reliability and correlation with volumetric measurements (Wahlund *et al.*, 2000; Bresciani *et al.*, 2005). A possibility of selection bias also exists because of our inability to obtain brain MRI for all the available subjects, although we demonstrated that those who had brain imaging did not differ significantly from those who did not have.

In conclusion, this study found an independent association of MTLA and early cognitive decline and dementia post-stroke in a sample of Nigerian African stroke survivors. This pioneering study underscores the importance of considering early- and long-term sequelae of stroke as care improves and early stroke mortality falls in the developing world (Feigin *et al.*, 2013). Acute and restorative services delivered to stroke survivors will need to be set up in anticipation of a rising number of people with long term motor- and non-motor consequences following stroke, including cognitive impairment. Further studies with larger samples and longitudinal design are also needed to unravel more associations.

#### 4.5. Chapter summary

Variable	Measure (n, %, mean, SD, median, range)
Left parietal infarct	17 (29.3%)
Right basal ganglia small vessel disease	15 (25.9%)
Left basal ganglia small vessel disease	9 (15.5%)
Total brain volume (ms)	1024.9 (132.2)
TBV/ICV	0.77 (0.06)
MTLA score	7.06 (1.67)
Total brain WMH	7.0 (0 - 13.75)
Periventricular WMH	3.0 (0 - 5.00)
Deep WMH	4.0 (0 - 9.25)

**Table 4. 5. Summary of neuroimaging findings (n= 58)**

- Total Brain Volume : N = vCIND > PSD
  - MTLA : N < vCIND < PSD
  - Total WMH : N < vCIND < PSD
  - Deep WMH : N < vCIND < PSD
- MTLA : < means less medial temporal lobe atrophy  
 WMH : < means less white matter hyperintensities
- ***Neuroimaging factors associated with post –stroke cognitive dysfunction***
    - Univariate*
    - Total brain volume
    - MTLA
    - Multivariate*
    - MTLA

## **Chapter 5. Hippocampal Alzheimer pathology in post – stroke dementia compared with other dementias and ageing controls**

### **5.1. Introduction**

The pathological hallmarks of Alzheimer's disease (AD) include amyloid beta (A $\beta$ ) protein deposits in the brain parenchyma (as amyloid plaques) and in walls of blood vessels (cerebral amyloid angiopathy) and neurofibrillary tangles (NFT) which comprise hyperphosphorylated tau protein (Querfurth and LaFerla, 2010). The hippocampus plays a very strategic role in the neurobiology of memory (Eichenbaum, 2001; Burgess *et al.*, 2002) and appears central to the hierarchical spread of amyloid and NFT pathologies (Braak and Braak, 1991; Thal *et al.*, 2002b; Thal *et al.*, 2006).

Whereas amyloid is deposited in the hippocampus in late stages, NFT occur quite early within the hippocampal formation during their natural histories, and both deposits appear to relate to the connections of the hippocampal circuitry (Lace *et al.*, 2009).

However, the precise relationship between NFT and amyloid deposits is still a subject of continuing debate (Querfurth and LaFerla, 2010).

Although Alzheimer pathology characteristically defines AD, it is often found in cognitively normal elderly and A $\beta$  deposits may particularly co - exist with other degenerative pathologies such as  $\alpha$  – synuclein and TDP – 43 in Lewy body diseases and frontotemporal lobar degeneration respectively (Tomlinson *et al.*, 1968; CFAS), 2001; Bennett *et al.*, 2006; Bennett *et al.*, 2012; Boyle *et al.*, 2013b).

The link between cerebrovascular disease, neurodegeneration and cognition has long been debated (de la Torre and Mussivand, 1993; Kalaria *et al.*, 1993b). Evidence for this link has been provided by experimental models (Kalaria *et al.*, 1993a; Kitaguchi *et al.*, 2009) and from epidemiological studies of ageing, AD and VaD (Schneider *et al.*, 2004; Petrovitch *et al.*, 2005; Okamoto *et al.*, 2012). Accumulation of Alzheimer pathology in primary vascular brain disorders occurs largely as a result of shared mechanisms of neurovascular unit dysfunction (Kalaria, 2000; Iadecola, 2004; Kalaria *et al.*, 2012a). Experimental evidence from animal studies has accrued on how cerebral hypoxia/ischaemia may exacerbate amyloid production (Kalaria *et al.*, 1993a; Whitehead *et al.*, 2005; Yamada *et al.*, 2011). This may occur through an initial

upregulation of amyloid precursor protein (APP) as has been shown in rat and mouse models of chronic cerebral hypoperfusion (Kalaria *et al.*, 1993b; Yamada *et al.*, 2011).

The upregulation of APP is mediated by hypoxia inducible factor –  $\alpha$  (HIF -1 $\alpha$ ) which stimulates the expression of the beta secretase enzyme that promotes amyloidogenic processing of APP (Zhang *et al.*, 2007). This is balanced by the activity of the neuronal sorting and intracellular trafficking membrane protein (LR11) or SorL1 which, on the other hand, regulates APP breakdown by channelling it into non – amyloidogenic pathways (Dodson *et al.*, 2008; Gustafsen *et al.*, 2013). While the association of the SorL1 gene with late – onset AD is now well established (Miyashita *et al.*, 2013), the cellular expression of SorL1 was recently shown to also be induced by hypoxia (Nishii *et al.*, 2013).

The introduction of Pittsburgh Compound B - positron emission tomography [PiB - PET] imaging has facilitated in vivo visualisation of amyloid deposition in the living brain (Klunk *et al.*, 2004). Whereas a small pilot PIB – PET study found significant deposition of amyloid (PIB binding) in 40% of subjects with post - stroke dementia (Mok *et al.*, 2010), more recent PiB - PET imaging studies examining the association between vascular brain injury and cerebral amyloid in cognitively normal and mild cognitively impaired elderly subjects failed to establish a relationship between vascular brain injury and amyloid  $\beta$ , as well as failing to detect a direct influence of amyloid -  $\beta$  on cognition (Marchant *et al.*, 2012; Marchant *et al.*, 2013).

In a large post - mortem study of subjects with cerebrovascular disease (CVD) and age – matched normal ageing controls, Aho *et al.*, (2006) using immunohistochemistry found no significant increase in amyloid load in the subjects with CVD compared to controls although there was a trend of increased deposition of A $\beta$  – 42 over A $\beta$  – 40 (Aho *et al.*, 2006). In contrast, in a post – mortem study of ageing controls and subjects with vascular dementia, evidence was found for the accumulation of total guanidine HCl extractable A $\beta$  – 42 peptide (over A $\beta$  – 40) in subjects with VaD as well as in the ageing controls (Lewis *et al.*, 2006) although the impact on cognition was not examined. Whereas amyloid load was only assessed semi – quantitatively in the Aho *et al.* study, more accurate quantitative determination including the use of fluorescence assay and mass spectrometry in the latter study showed that cerebral tissues retain

soluble potential toxic A $\beta$  peptides which does not aggregate as visible amyloid plaques in vascular cases.

Cross-sectional studies examining the relationship of A $\beta$  with cognitive function have also yielded conflicting results. Whereas some studies have reported significant correlation between metrics of AD pathology and cognitive performance (Bennett *et al.*, 2006; Mormino *et al.*, 2009) others have reported dissociation between Alzheimer pathologic load and cognitive status especially in subjects with presumed high cognitive reserve, mixed pathologies or non – AD subjects (Mufson *et al.*, 1999; Aizenstein *et al.*, 2008; Stern, 2009). Similarly, the morphologic variants and anatomical localization of the AD pathology may be important. Neuritic plaques and neurofibrillary tangles have stronger impact on cognition than diffuse plaques, and pathologies in the neocortical region exert more influence than those in the allocortical regions (Nelson *et al.*, 2009; Nelson *et al.*, 2012).

Neuroimaging studies in the longitudinal Newcastle post - stroke cohort had found that medial temporal lobe atrophy (MTLA) predicted progression to cognitive impairment, dementia and death (Firbank *et al.*, 2007; Firbank *et al.*, 2012) while neuropathological studies unmasked hippocampal neuronal atrophy as an important substrate of post - stroke dementia (PSD) in the cohort (Gemmell *et al.*, 2012). A similar predictive role was also found for MTLA in relation to cognitive impairment and dementia in the CogFAST – Nigeria cohort (Chapter 4).

Given the hypothesis ascribing MTLA to Alzheimer pathology (Barclay *et al.*, 1992; Henon *et al.*, 1998; Cordoliani-Mackowiak *et al.*, 2003; Firbank *et al.*, 2007), the study described in this chapter investigated Alzheimer pathology in hippocampal formation and entorhinal cortex in tissue obtained from the Newcastle post – stroke cohort who had come to autopsy. There were also comparative groups of ageing controls and other dementias all from brain tissue obtained from the Newcastle Brain Tissue Resource (NBTR).

We hypothesized that hippocampal Alzheimer pathology would be differentially expressed in demented and non - demented stroke survivors in comparison with other dementias and ageing controls. Using a very unique and comprehensive approach, we quantified the expression of a range of markers of different species of amyloid (total amyloid, A $\beta$  – 40, A $\beta$  – 42, soluble amyloid and APP), neuronal sorting protein (SorL1)

as well as, a marker of hyperphosphorylated tau pathology (AT8) across groups of diseases and across sub - regions of the hippocampal formation and entorhinal cortex.

## **5.2. Methods**

### **5.2.1. Subjects**

Ninety - four human post - mortem brain tissue block samples were retrieved from the NBTR. The samples consisted of post - stroke demented, PSD (n =15), post - stroke non - demented, PSND (n = 23), normal controls (n =12), AD (n = 14), mixed AD\_VAD (n = 13) Tables 5.1 and 5.2 provide details of the demographic, cognitive and pathologic characteristics of the subjects. There were no significant differences in the age (p = 0.786), gender distribution (p = 0.493), post -mortem delay (p = 0.902) and length of fixation of tissues (p = 0.589). Autopsies were performed between 24 and 92 hr after death and brains were fixed for between 6 - 34 weeks. Cognitive scores on the Mini - Mental State Examination (MMSE) and Cambridge Cognitive Examination (CAMCOG) proximate to death as well as APOE status were retrieved from clinical and research records of the subjects in the CogFAST- Newcastle cohort. The CogFAST - Newcastle Study and ancillary studies had ethical approval from the local Newcastle Ethical committees and participants gave written consent for brain tissue donation. Use of brain tissue was also approved by the local Ethical committees and the committee of the NBTR.

### **5.2.2. Immunohistochemistry**

Detailed description of the methodological approaches are already provided in Chapter 2. In brief, paraffin embedded blocks taken from the Newcastle Brain Map coronal levels 18-20 (Perry and Oakley, 1993) and containing the hippocampal formation and entorhinal cortex were cut into 10 $\mu$ m sections using a rotary microtome. Sub - regions of the hippocampal formation selected for analysis consisted of the CA1, CA2, CA3 and the subiculum. The adjacent entorhinal cortex was also included. The cut sections were serially immunostained in duplicates with primary antibodies to all amyloid - $\beta$  species (4G8, 1: 1000, Monoclonal, Signet 9220 -10), amyloid -  $\beta$  (42) (T - 42, 1 : 5000, Polyclonal, gift; H. Mori, Japan), amyloid -  $\beta$  (40) (T - 40, 1 : 5000, Polyclonal, gift; H. Mori, Japan), soluble amyloid oligomer (NU-1, Monoclonal, gift: C. Klein, US) anti - amyloid precursor protein clone 22C11 (APP, 1: 2000, Monoclonal, Chemicon), neuronal Sortilin A (SorL1, 1: 2000, Monoclonal, Abcam) and antibody to hyperphosphorylated tau (AT8, 1: 1000, Monoclonal, Innogetics) as described in

Variable	PSND	PSD
Number of cases (N)	23	15
Age, years (mean $\pm$ SD)	83.7 $\pm$ 3.9	87.3 $\pm$ 5.9
Gender (M/F)	13/10	6/9
PMD (Hours)(mean $\pm$ SD)	45.0 $\pm$ 21.9	45 $\pm$ 25.3
Fixation Length (Weeks) (mean $\pm$ SD)	11 $\pm$ 7.3	8 $\pm$ 2.9
MMSE score (mean $\pm$ SD)	27.1 $\pm$ 1.6	17.5 $\pm$ 3.7*
CAMCOG total score (mean $\pm$ SD)	89.1 $\pm$ 5.1	63.6 $\pm$ 13.5*
Braak Stage median (range)	2.0 (0 - 5)	3.0 (0 - 4)
CERAD score Median (range)	2.0 (0 - 2)	1.0 (0 - 3)
Thal Stage – median (range)	3.0 (2 - 4)	1.0 (0-3)+
Vascular Score total – median (range)	13.0 (7 – 16)	12.0 (7 – 18)
Vascular Score – hippocampus Median (range)	2.0 (1 - 3)	2.0 (1 - 3)
ApoE Genotypes (%)	€3/3(43.7; €3/4, 2/4(50.0) Others (6.3)	€3/3 (53.8); €3/4 (23.1) €2/3 (16.7) Others (6.4)
Time from stroke to death Mean $\pm$ SD (months)	60.7 $\pm$ 47.4	59 $\pm$ 21.8
Previous Stroke (% of cases)	Yes (52.6), No (42.1), Unknown (5.3)	Yes (30.8), No (61.5), Unknown (7.7)
Location of lesion (% cases)	Parietotemporal (17.4), deep WM (13.0), cerebellum (8.7%) unknown (60.9)	Parietotemporal (35.7), deep WM (21.4), cerebellum (7.1), unknown (35.7)
Side of Lesion %cases)	Left (8.7), Right (21.7), Both (26.1), None (26.1), Unknown (17.4)	Left (35.7), Right (21.4), Both (14.3), None (21.4), Unknown (7.1)
Vascular territory	MCA (30.4%), PCA(8.7), Unknown (60.9)	MCA(57.1), Vertebrobasilar (7.1), Unknown (35.7)

**Table 5.1. Characteristics of the CogFAST non – demented (PSND) and demented (PSD) subjects.\*p < 0.001; +p = 0.028); Abbreviations: MCA, middle cerebral artery; PCA, posterior cerebral artery.**

Variable	Controls	VaD	AD	AD_VaD
No	12	17	14	13
Age, years (mean $\pm$ SD)	79.1 $\pm$ 6.8	85.1 $\pm$ 6.4	83.5 $\pm$ 5.9	84.8 $\pm$ 5.7
Gender (M/F)	7/5	7/10	8/6	6/7
PMD (Hours) (mean $\pm$ SD)	26.0 $\pm$ 11	42.0 $\pm$ 14.1	45.0 $\pm$ 26.1	41.0 $\pm$ 24.5
Fixation Weeks) (mean $\pm$ SD)	15.0 $\pm$ 8.0	13.0 $\pm$ 12.1	10.0 $\pm$ 5.3	15.0 $\pm$ 10.4
Braak Stage median (range)	2.0 (1 - 4)	2.0 (1 - 4)	5.5 (4 - 6)	5.0 (1 - 6)*
CERAD score median(range)	NPD	1.0 (0 - 2)	3.0 (3 - 3)*	3.0 (1 - 3)*
Thal Stage – median (range)	NPD	2.0 (0 - 3)	4.0 (3 - 4)*	4.0 (3 - 4)*
Vascular Score total – median (range)	NPD	14.0 (12 - 18)	NPD	12.0 (7 - 15)

**Table 5. 2. Characteristics of the Control, VaD and AD groups. \*\*p < 0.001; Abbreviations: NPD = No pathological data available.**

Chapter 2. To minimize variability of immunohistochemical staining quality, positive and negative control sections were included in assays, and experiments were run in duplicates for each marker, using freshly prepared buffer solutions and tinctorial stains. Sections were further counterstained with haematoxylin following the assays of NU -1, APP, SorL1 and AT8 to enhance identification of neuronal and glial cellular structures. Sections were numbered randomly from 1 – 100 and then analysed blind to the diagnoses of the cases.

### **5.2.3. Microscopy and Image Analysis**

The stained sections were examined and imaged using a Zeiss Axioplan 2 research grade microscope coupled to an Infinity 2 camera. Magnification was set at X10 for the hippocampal sub - regions CA1, CA2 and CA3, and X5 for the subiculum and entorhina 1 cortex (EC). Five images were taken at random from the CA1, CA3 and subiculum, 3 images from CA2 and 4 x 3 from the EC from the pial surface to the white matter. Approximately 2820 images were taken.



Using the software Image Pro - Plus 4.0 (Media Cybernetics, Silver Spring, MD, USA), the images were analyzed using histogram-based analysis and obtaining the variables: per area, a measure of the number of pixels stained within the area of interest (AOI) and expressed as a percentage. The integrated optical density (IOD) was also determined, and the total immunoreactivity (IR) was derived for each image and then for each sub-region of the hippocampal formation and EC as described in Chapter 2.

#### ***5.2.4. Statistics***

Statistical analysis was carried out using the IBM SPSS software (version 19.0). The Kolmogorov - Smirnov Test was used to establish normality of data. Comparisons across groups of cases and across sub - regions were performed using parametric tests (ANOVA for group means and Tukey post – hoc analysis for between – group differences) and non - parametric tests Kruskal – Wallis and Mann – Whitney U tests) for non – normally distributed dataset. The relationship among markers, and with demographic, cognitive and pathological variables were assessed using Pearson's correlation coefficient (r) or Spearman's correlation (rho) as necessary depending on the normality of the dataset.

## 5.3. Results

### 5.3.1. Characteristics of Study Subjects

The demographic, cognitive and pathological characteristics of the non - demented (PSND) and demented (PSD) subjects from the CogFAST – Newcastle study are detailed in Table 5.1.while the details of the control, VaD, AD and mixed AD\_VaD groups are shown in Table 5.2. There were no significant differences across the groups with respect to age, gender, post - mortem delay and length of fixation period ( $p > 0.05$ ). However, the PSND group had significantly higher scores on the cognitive batteries MMSE and CAMCOG compared to the PSD group ( $p < 0.05$ ). Similarly, Braak stage, CERAD score and Thal stage were significantly higher in the AD, AD\_VaD groups compared to the VaD and post – stroke groups (PSND and PSD) ( $p < 0.05$ ). Hippocampal vascular scores were similar among the PSND, PSD, VaD and AD\_VaD groups. The distribution of the  $\epsilon 3$  and  $\epsilon 4$  alleles of Apo E were not significantly different between the PSND and PSD groups (Fisher's exact test = 2.13;  $p = 0.249$ ).

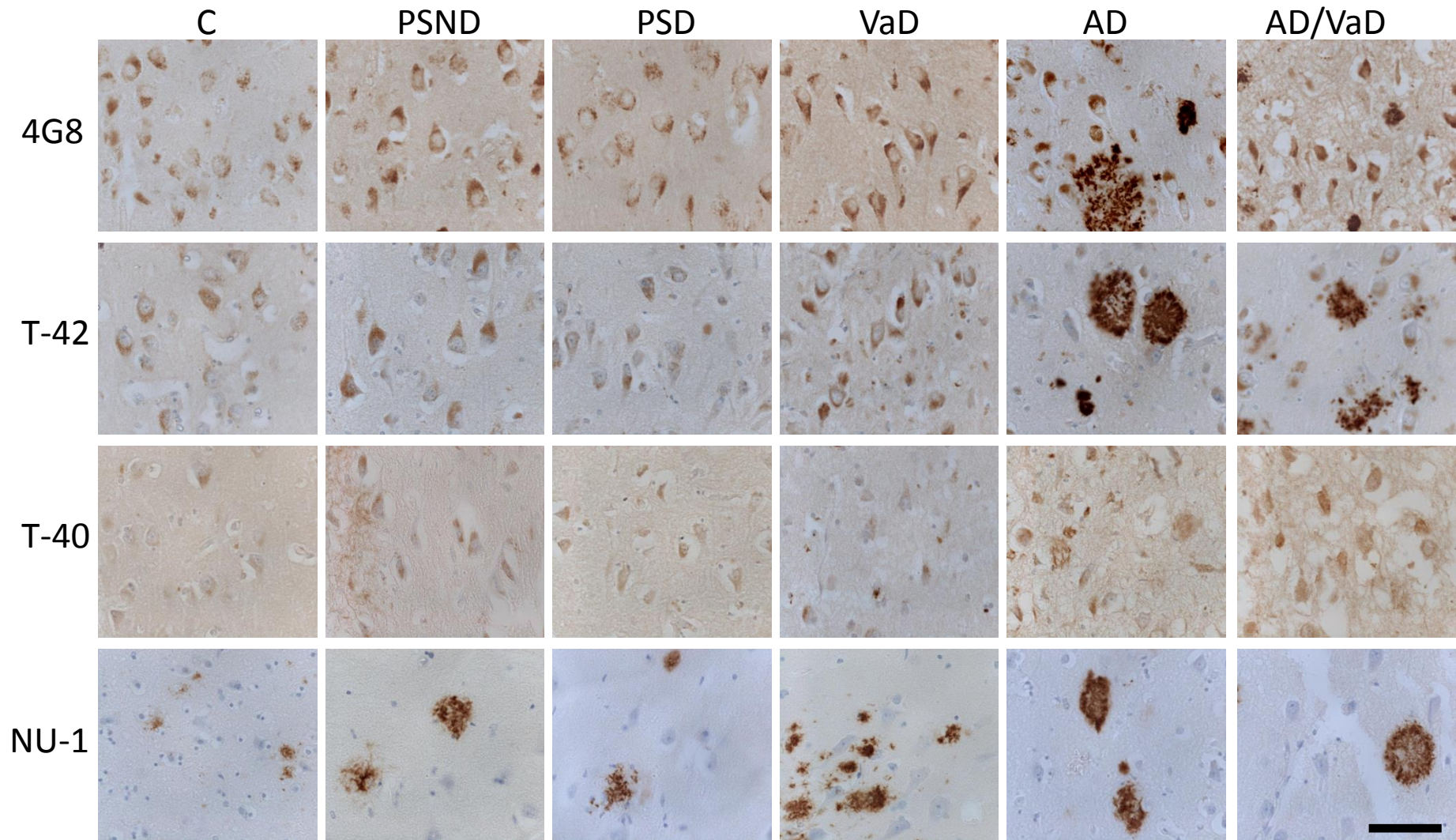
Given a total sample size of 94, a significance level,  $\alpha = 0.05$  and assuming a moderate effect size Cohen's  $d = 0.4$ , 6 sub - groups and 5 degrees of freedom, the computed Power ( $1 - \beta$ ) = 0.8424 using the G\*Power software (Faul et al, 2007).

### 5.3.2. Quantification of amyloid burden

A $\beta$  burden was quantified in this study using a panel of markers identifying different species as indicated in Section 5.2.2 ie 4G8, T – 42, T – 40 and NU -1. In quantifying amyloid burden with the Image Pro – Plus software, parenchymal, vascular as well as intraneuronal amyloid immunoreactivities were all incorporated in order to capture the total quantity of the different species of amyloid detected within the defined area of interest as previously performed (Lewis *et al.*, 2006). Figure 5.1 illustrates the immunostaining pattern with antibodies to various A $\beta$  species in serial sections across the CA1 sub – region of the hippocampal formation and entorhinal cortex.

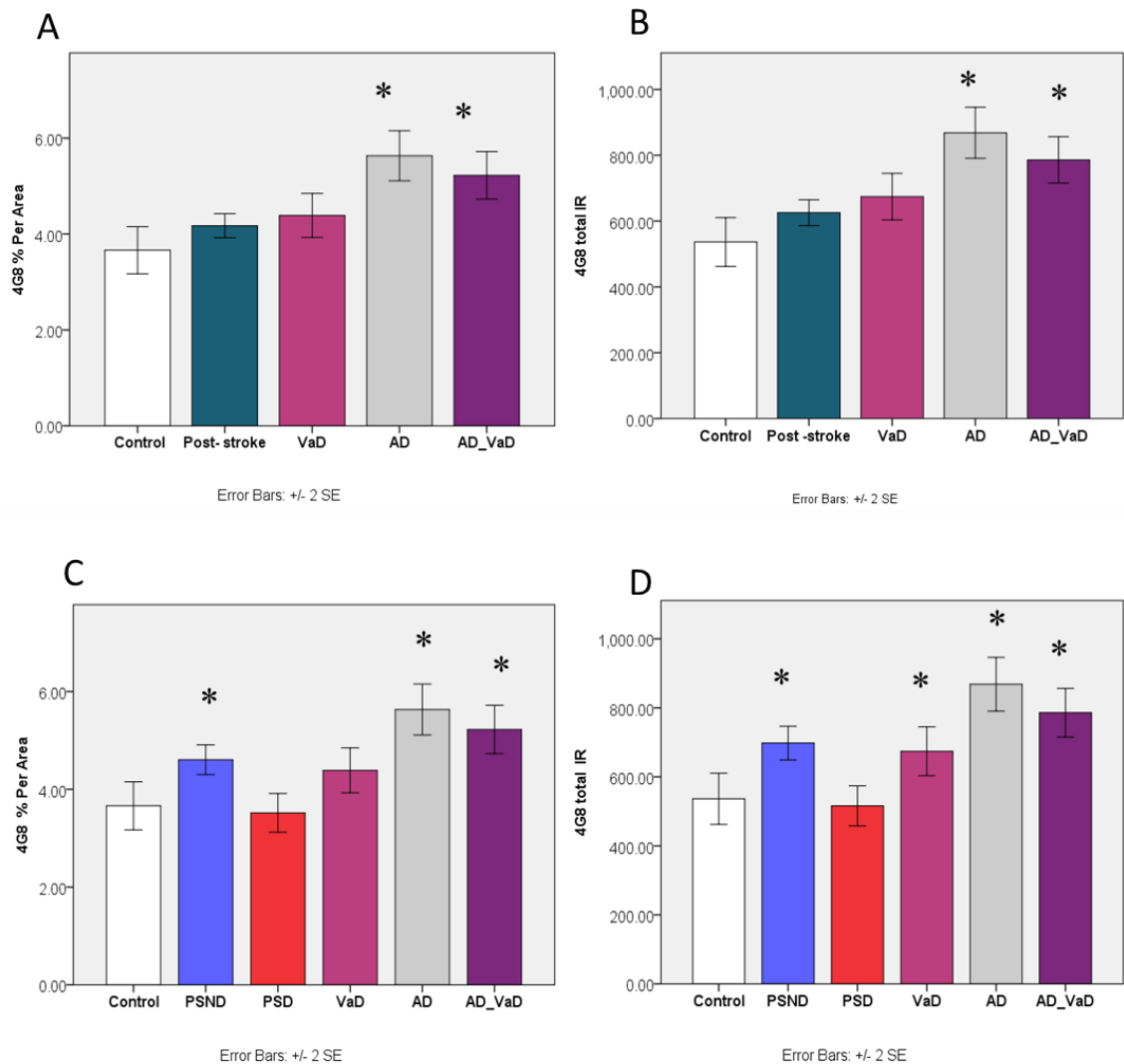
### **5.3.2.1. Amyloid immunohistochemistry with 4G8 antibody**

Distribution of 4G8 antibody immunostaining dataset assessed by Kolmogorov - Smirnov test showed non – normal distribution. Figure 5.2 shows the average distribution of 4G8 immunostaining per area and relative total immunoreactivity across the entire hippocampal formation and entorhinal cortex across all disease groups. Kruskal – Wallis Test shows significant variation of staining intensity per area and total immunoreactivity ( $p < 0.001$ ) across groups. Compared to the control group, there was a trend of progressively higher 4G8 immunoreactivity in the post- stroke groups (together) across to AD with statistical significance as shown in Figure 5.2 A and B respectively.



Bar: 100µm

**Figure 5.1: illustrative images of amyloid pathology in the CA1 sub – region across different markers and across disease groups and ageing controls.** There is higher expression of amyloid in the AD and AD\_VaD groups compared with the VaD, PSD and PSND and control groups. The level of amyloid immunoreactivity is similar between 4G8 and T – 42 but much lower in T – 40 and NU -1.



**Figure 5.2 :** Bar graph showing measures of total 4G8 immunostaining across the whole hippocampal formation and entorhinal cortex with the post – stroke groups together [A] 4G8 immunoreactivity percentage per area [B] total 4G8 immunoreactivity post – stroke group separated [C] 4G8 immunoreactivity percentage per area [D] , total 4G8 immunoreactivity in comparison with Control, , VaD, AD and AD\_VaD. Bars show  $\pm 2$  SEM \* Mann Whitney U test was used to compare means of each group. \*p < 0.05; ( in comparison with the control group).

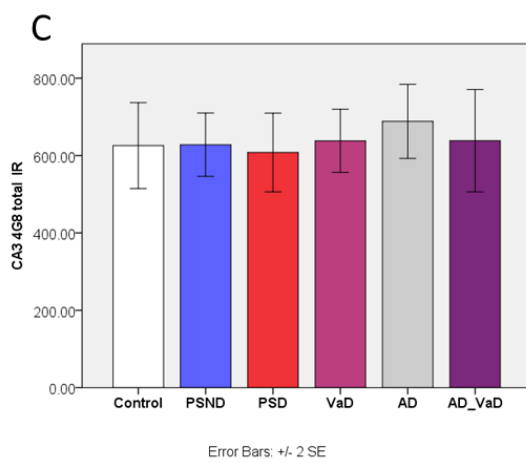
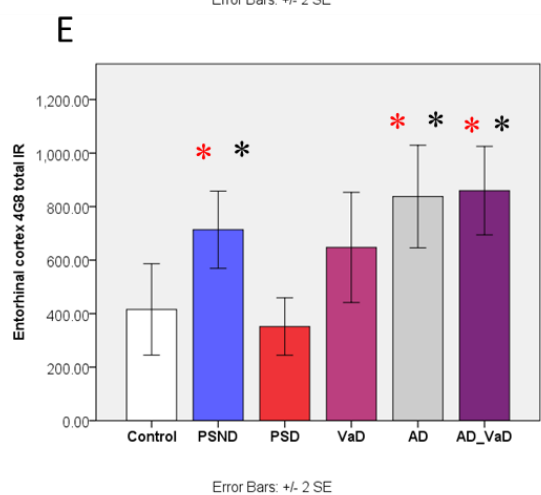
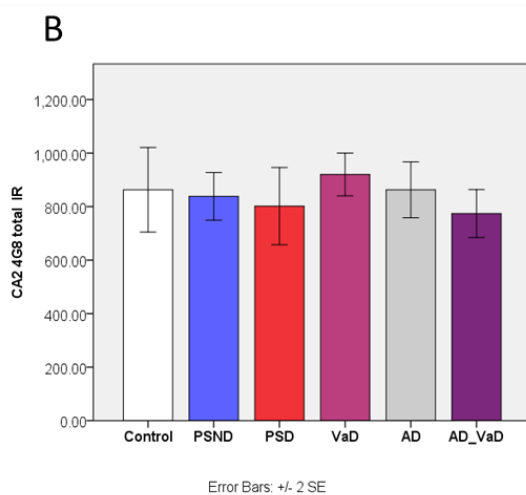
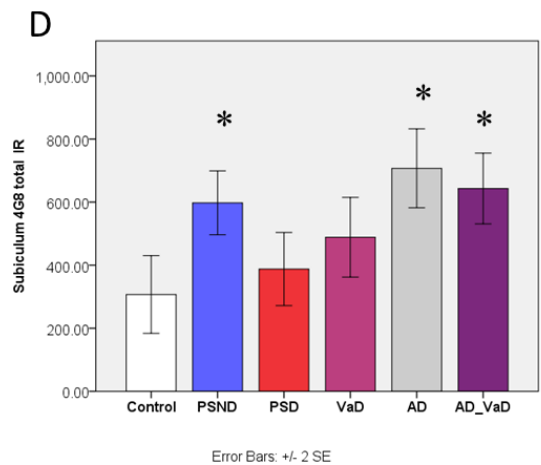
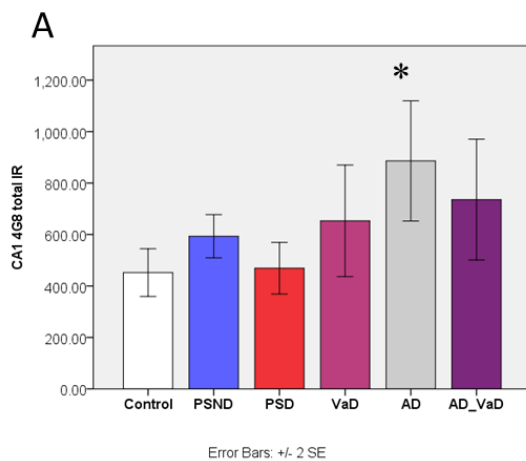
When the two post - stroke groups were divided into PSND and PSD groups, however, the significant variation across groups was still maintained (p < 0.001, Kruskal – Wallis Test). Further analysis for between group differences by Mann – Whitney U Test showed that with respect to the control group, 4G8 staining per area and total IR was significantly higher in the AD ( p < 0.001), AD\_VaD ( p < 0.001), VaD ( p = 0.013) and PSND (p < 0.001) groups. 4G8 IR was higher in the PSND group compared to the PSD group (p < 0.001) (Figure 5.2).

Further analysis of 4G8 staining per area and total IR was undertaken across sub – regions of hippocampal formation and the entorhinal cortex across disease groups in comparison with the control group (Figure 5.3).

In the CA1 region, 4G8 total IR varied significantly across groups ( $p = 0.008$ , Kruskal - Wallis Test). Between group differences assessed with Mann – Whitney U Test showed that compared to the control group, the IR was significantly higher in AD ( $p = 0.002$ ), showed a trend with AD\_VaD ( $p = 0.064$ ) and PSND ( $p = 0.076$ ) but not significantly different from PSD and VaD. In the CA2 and CA3 regions, 4G8 immunoreactivity showed no significant variation across disease groups. In the subiculum, 4G8 data immunostaining per area and total IR measures varied significantly across disease groups and controls ( $p < 0.001$ , Kruskal – Wallis Test). With respect to the control group, 4G8 immunoreactivity was significantly higher in the AD ( $p = 0.001$ ), AD\_VaD ( $p = 0.008$ ) and PSND ( $p = 0.015$ ) groups. but not significantly different from PSD and VaD.

In the entorhinal cortex, 4G8 total IR showed significant variation across the sub - region ( $p < 0.001$ , Kruskal – Wallis Test) with values significantly higher in AD ( $p = 0.022$ ), AD\_VaD ( $p = 0.016$ ) than in controls while PSD was significantly lower than PSND ( $p = 0.019$ ), AD\_VaD ( $p = 0.002$ ) and AD ( $p = 0.002$ ) (Figure 5.3).

Spearman's rank correlation analysis revealed no significant associations between post-mortem delay, length of fixation period and 4G8 immunoreactivity measures in the hippocampal sub- regions and entorhinal cortex. The semi - quantitative amyloid rating scales of CERAD score, Thal staging as well as Braak and tau stages showed significant positive correlation with the metrics of 4G8 total immunoreactivity in the entorhinal cortex, subiculum and CA1 sub - regions (Table 5.3) suggesting good agreement between the semi-quantitative and quantitative measures of amyloid and tau quantification.



**Figure 5. 3: Bar graph showing the distribution of 4G8 IR across hippocampal sub-regions [A] CA1 [B] CA2 [C] CA3 [D] Subiculum [E] Entorhinal cortex in Controls, PSND, PSD, VaD, AD and AD\_VaD. Bars show  $\pm 2$  SEM \* Mann Whitney U test was used to compare means of each group. \* $p < 0.05$ ; +  $p < 0.1$  (in comparison with the control group). \* (red) showed significant difference from PSD group.**

Spearman's rho		4G8 IRCA1	4G8 IRCA2	4G8 IRCA3	4G8 IRSB	4G8 IREC	Braak Stage	tau stage	Thal Stage	CERAD score
4G8 IRCA1	rho	1.000								
	p									
4G8 IRCA2	rho	.403**	1.000							
	p	.000								
4G8 IRCA3	rho	.444**	.665**	1.000						
	p	.000	.000							
4G8 IRSB	rho	.618**	.169	.243*	1.000					
	p	.000	.174	.048						
4G8 IREC	rho	.490**	.094	.220†	.758**	1.000				
	p	.000	.432	.061	.000					
Braak stage	rho	.420**	.009	.166	.404**	.429**	1.000			
	p	.001	.946	.222	.003	.001				
tau stage	rho	.316**	-.160	-.019	.334**	.474**	.582**	1.000		
	p	.008	.194	.878	.008	.000	.000			
Thal stage	rho	.442**	-.223†	.001	.633**	.653**	.680**	.685**	1.000	
	p	.000	.059	.995	.000	.000	.000	.000		
CERAD score	rho	.341*	.045	.095	.492**	.524**	.721**	.597**	.706**	1.000
	p	.011	.749	.492	.000	.000	.000	.000	.000	

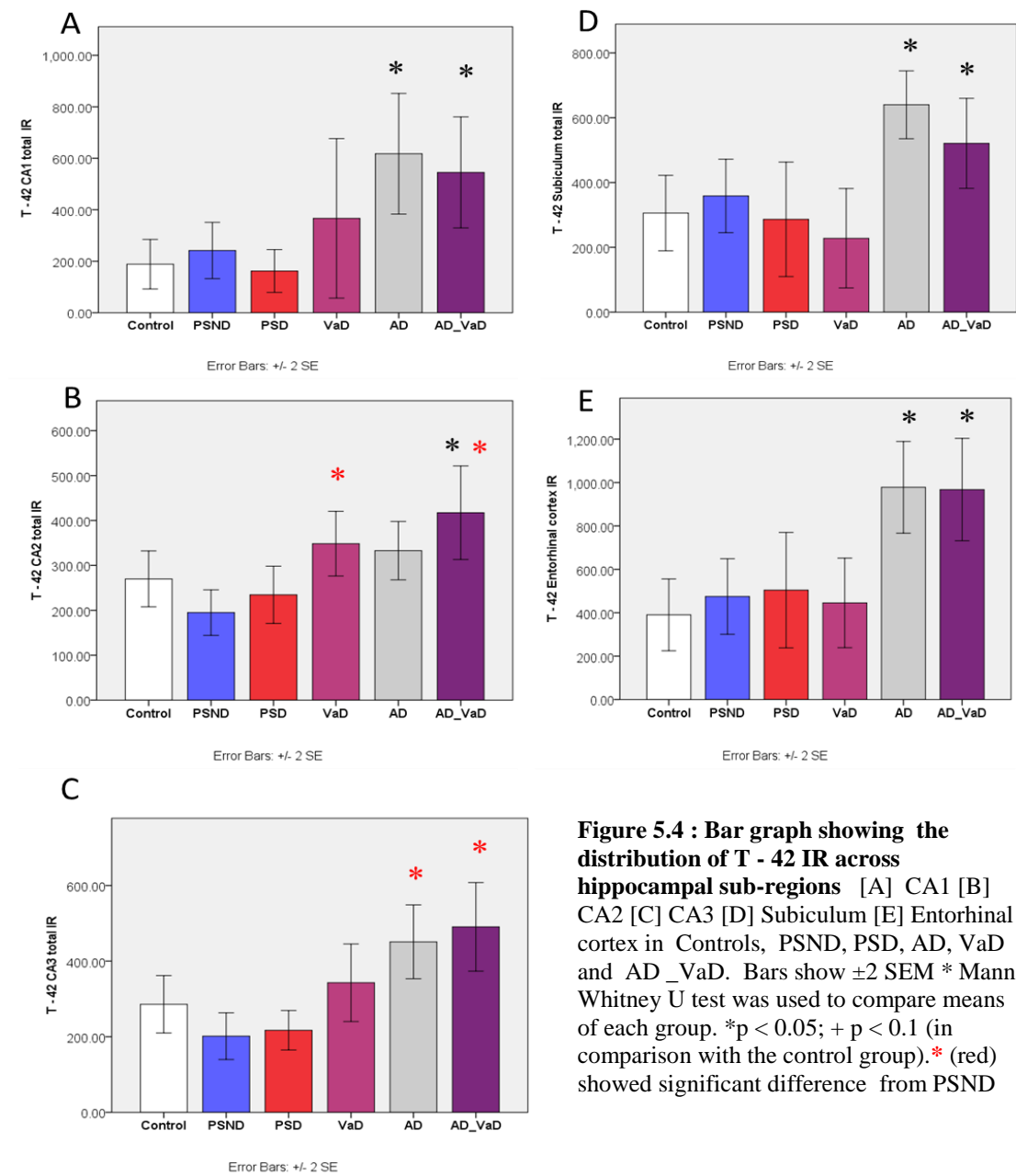
**Table 5. 3. Correlation Matrix of sub - regional 4G8 immunoreactivity, CERAD score, Thal stage, Braak stage and tau stage.** Statistical significance designated by the following p values: \*\* p < 0.01; \*p < 0.05 †p < 0.1. Abbreviations: IR, immunoreactivity; CERAD, Consortium to Establish a Registry for Alzheimer's Disease.

### 5.3.2.2. Amyloid immunohistochemistry with A $\beta$ (42) (T – 42 antibody)

In the CA1 region, T - 42 immunoreactivity varied significantly across groups (p < 0.001, Kruskal - Wallis Test). Between group differences assessed with Mann – Whitney U Test showed that compared to the control group, T – 42 immunoreactivity was significantly higher in AD (p = 0.002) and AD\_VaD (p = 0.005), but not significantly different from VaD, PSND and PSD (Figure 5.4A). In the CA2 region, immunoreactivity varied across disease groups (p = 0.001, Kruskal –Wallis Test) with the IR being significantly higher in AD\_VaD (p = 0.046) and VaD (p = 0.014) groups compared to the control and PSD groups respectively (Figure 5.4B). In the CA3 region, datum showed normal distribution by Kolmogorov – Smirnov test of normality and ANOVA showed significant variation of T -42 immunoreactivity across the regions (p < 0.001). Compared to the PSND group, immunoreactivity was significantly higher in AD (p < 0.001) and AD\_VaD (p = 0.002) groups but not significantly different from control PSD and VaD groups (Fig 5.4C).



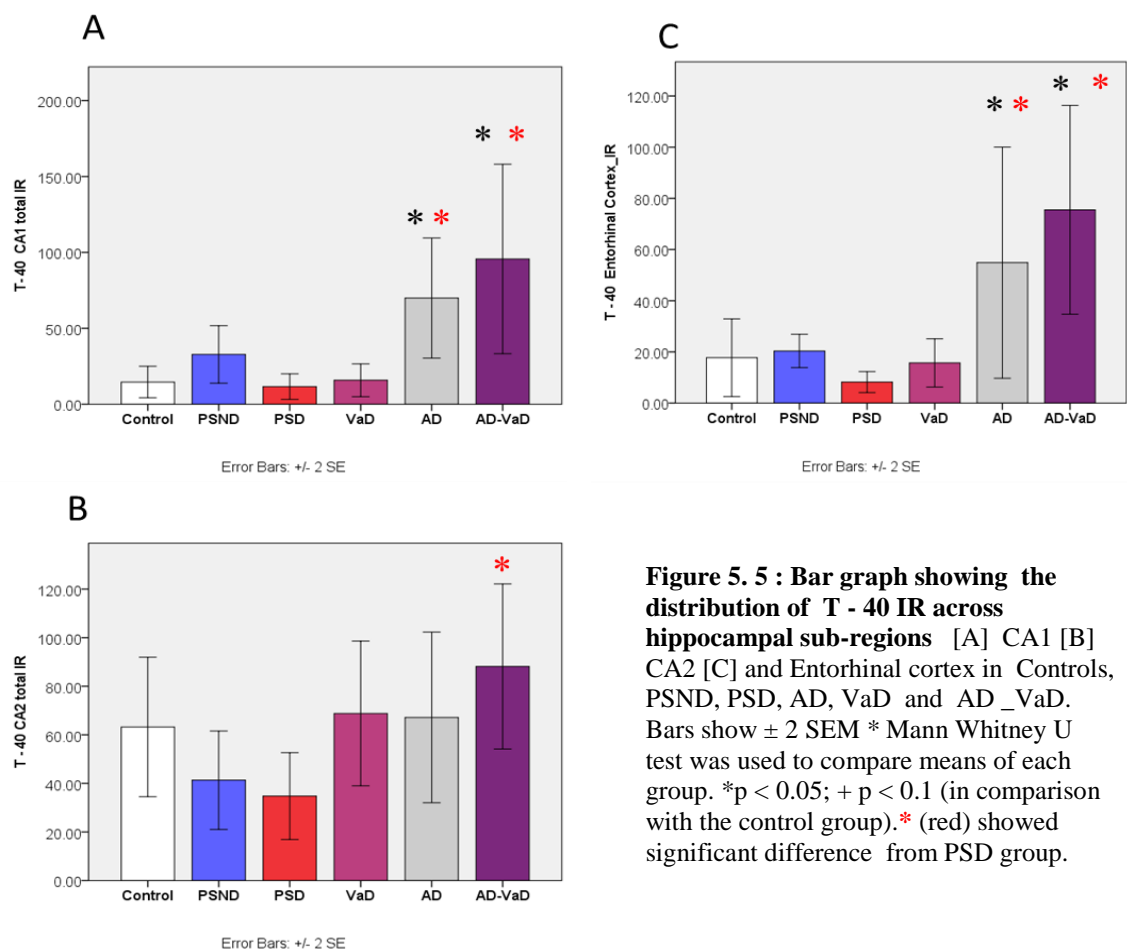
In the subiculum, T – 42 immunoreactivity varied significantly across groups ( $p < 0.001$ ) and was significantly higher in the AD ( $p = 0.001$ ) and AD\_VaD ( $p = 0.008$ ) groups compared to the control and VaD groups respectively. However, the VaD groups did not differ significantly from the PSND and PSD groups respectively (Figure 5.4D). Similarly, T – 42 immunoreactivity varied significantly across the entorhinal cortex ( $p < 0.001$ ) and was significantly higher in the AD ( $p = 0.001$ ) and AD\_VaD ( $p = 0.001$ ) groups but not different from the VaD, PSND and PSD groups.



**Figure 5.4 : Bar graph showing the distribution of T - 42 IR across hippocampal sub-regions [A] CA1 [B] CA2 [C] CA3 [D] Subiculum [E] Entorhinal cortex in Controls, PSND, PSD, AD, VaD and AD\_VaD. Bars show  $\pm 2$  SEM \* Mann Whitney U test was used to compare means of each group. \* $p < 0.05$ ; +  $p < 0.1$  (in comparison with the control group). \* (red) showed significant difference from PSND**

### 5.3.2.3. Amyloid immunoreactivity with A $\beta$ (42) (T – 40 antibody)

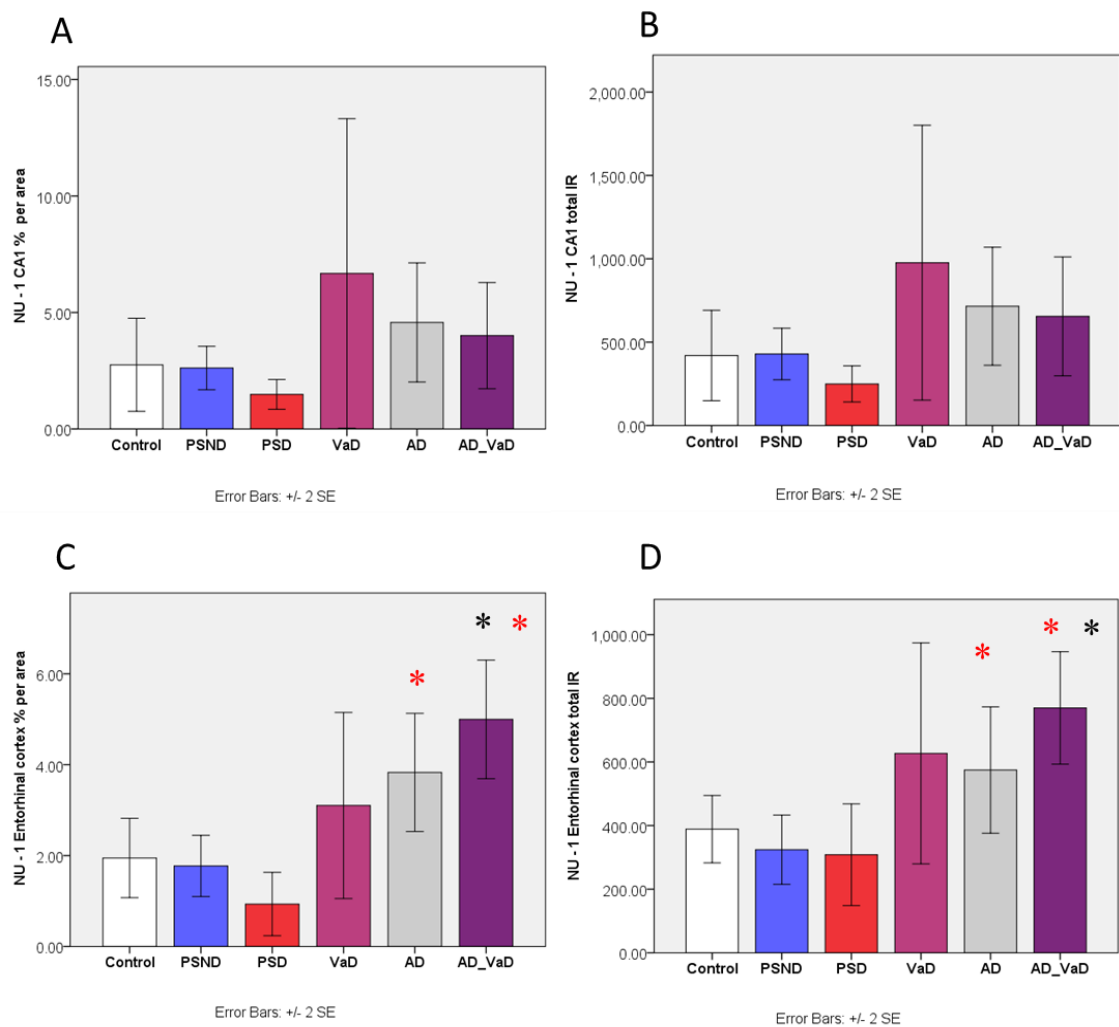
Figure 5.5 shows the immunostaining and distribution of T – 40 antibody across disease groups in the CA1, CA2 and entorhinal cortex regions. The IR varied significantly across the sub - regions ( $p = 0.001$ , Kruskal – Wallis Test). In CA1, the IR in the control group was not significantly different from that of PSND, PSD and, VaD but was significantly lower than AD ( $p = 0.004$ ) and AD – VaD ( $p = 0.010$ ). There was no difference between the PSND and PSD groups. In CA2, the control group IR was lower than AD, VaD and AD\_VaD although this did not attain statistical significance ( $p > 0.05$ ). However, VaD ( $p = 0.005$ ) and AD\_VaD ( $p = 0.006$ ) groups were significantly higher than the PSD group. The PSND group did not differ from the PSD group (Figure5.5). In the entorhinal cortex, T – 40 IR was significantly higher in the AD and AD\_VaD groups compared to the control and PSD groups ( $p < 0.05$ , Kruska – Wallis Test). There was no significant variation in the CA3 region and subiculum.



**Figure 5.5 : Bar graph showing the distribution of T - 40 IR across hippocampal sub-regions [A] CA1 [B] CA2 [C] and Entorhinal cortex in Controls, PSND, PSD, AD, VaD and AD\_VaD. Bars show  $\pm 2$  SEM \* Mann Whitney U test was used to compare means of each group. \* $p < 0.05$ ; +  $p < 0.1$  (in comparison with the control group). \* (red) showed significant difference from PSD group.**

### 5.3.2.4. Soluble amyloid immunoreactivity with NU- 1 antibody

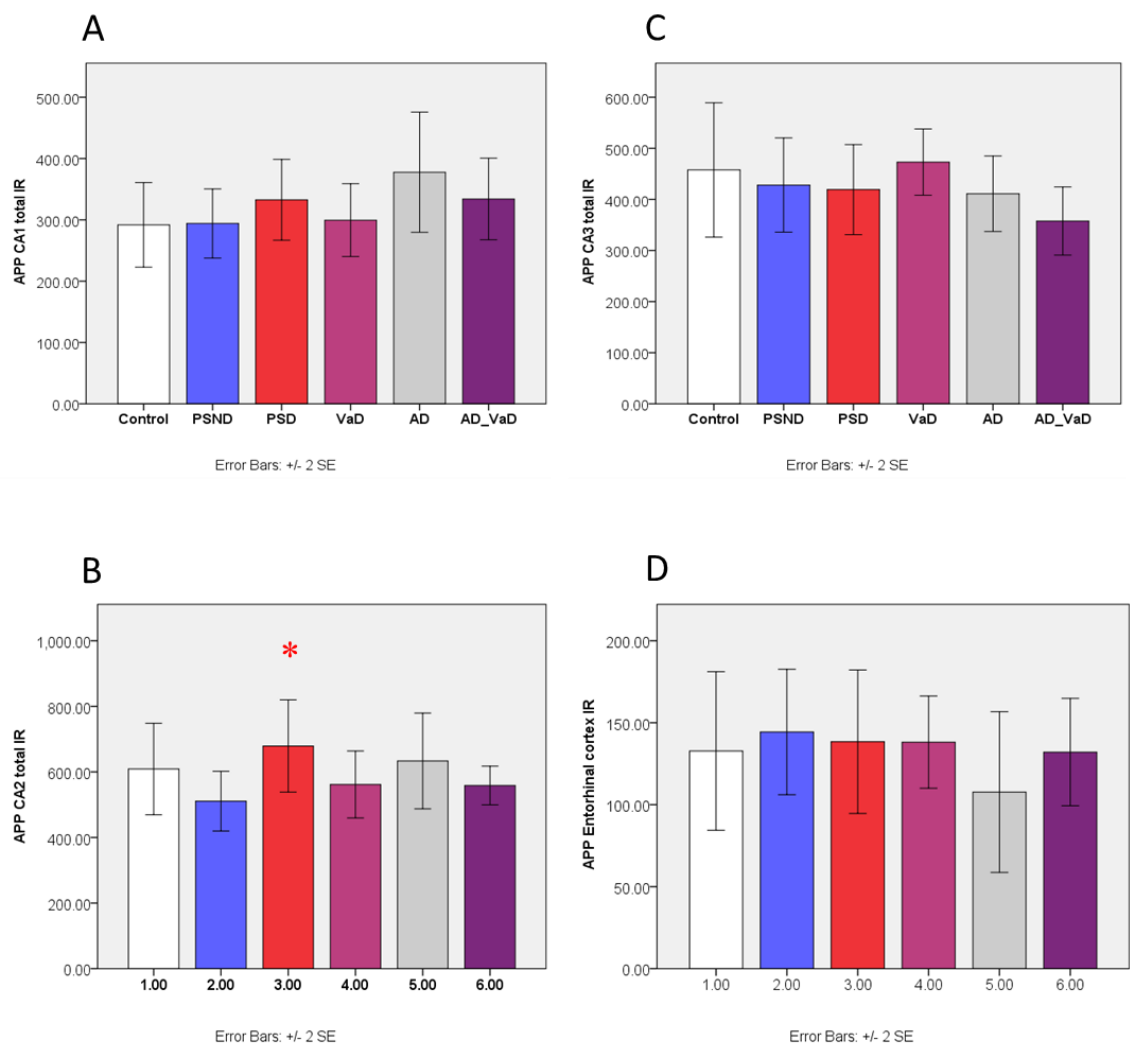
There were no significant differences in the percentage area and total immunoreactivity of NU-1 across the disease groups in the CA1, CA2 and CA3 regions ( $p > 0.05$ , Kruskal Wallis Test). However, in the subiculum and entorhinal cortex, the immunoreactivity varied significantly ( $p < 0.05$  Kruskal – Wallis Test). Mann – Whitney U analysis of between group differences showed significantly higher immunoreactivity in the AD\_VaD ( $p = 0.007$ ) group compared to the control group, and in the AD\_VaD ( $p = 0.008$ ) and AD ( $p = 0.040$ ) compared to the PSD group (Figure 5.6). The PSND and PSD groups showed no significant differences.



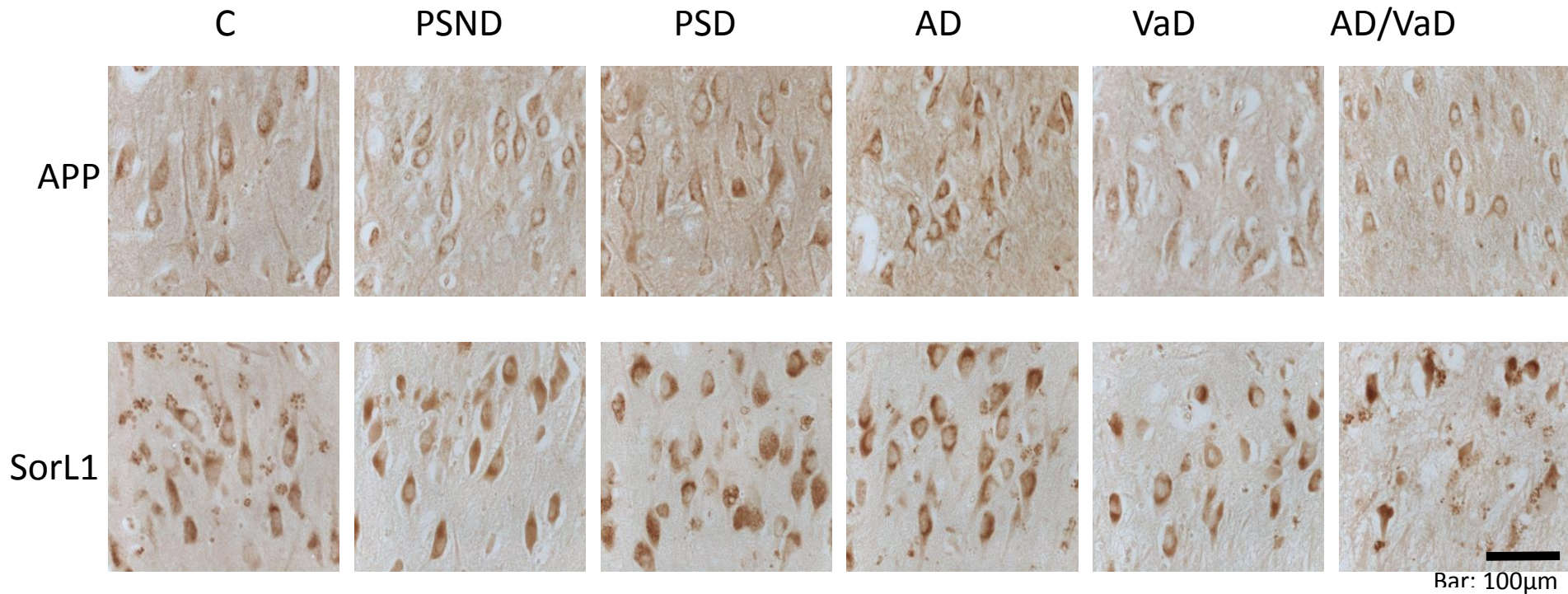
**Figure 5.6:** Bar graph shows percentage area and total immunoreactivity (IR) of NU-1 across the CA1 region [A] and [B] and Entorhinal cortex [C] and [D] respectively in Control, PSND, PSD, VaD, AD and AD\_VaD. Bars show  $\pm 2$  SEM \* Mann Whitney U test was used to compare means of each group.  $p < 0.05$  (in comparison with the \* control group and \* PSD group). There were no significant differences across groups in the CA1, CA2 and CA3 regions ( $p > 0.05$ , Kruskal Wallis Test).

### 5.3.2.5 Amyloid Precursor Protein (APP) immunoreactivity

Figures 5.7 and 5.8 show the immunoreactivity of APP across groups in the CA regions and entorhinal cortex. In the CA1 region, APP immunoreactivity was slightly increased in the AD, AD\_VaD and PSD groups compared with the control and PSND groups, even though the difference did not attain statistical significance ( $p > 0.05$ , Kruskal-Wallis Test). However, in the CA2, the APP immunoreactivity in PSD was significantly higher than in PSND ( $p = 0.043$ , Mann - Whitney U test). There were no significant differences in the CA3 sub – region, subiculum and entorhinal cortex.



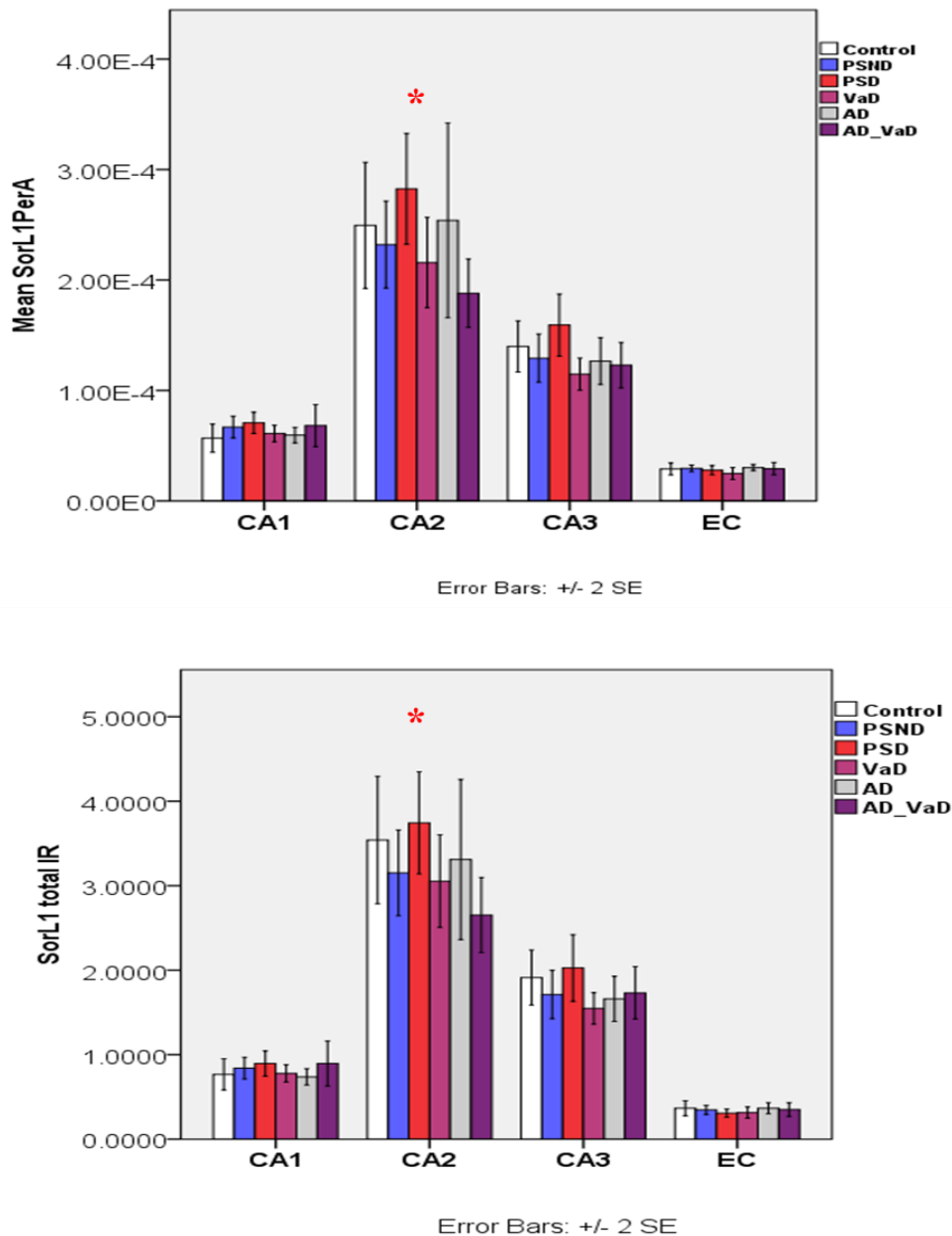
**Figure 5.7:** Bar graph showing the distribution of APP IR across hippocampal sub-regions [A] CA1 [B] CA2 [C] CA3 and [D] Entorhinal cortex in Controls, PSND, PSD, VaD, AD and AD\_VaD. Bars show  $\pm 2$  SEM \* $p < 0.05$  (PSD vs PSND).



**Figure 5.8: illustrative images of hippocampal pyramidal neurons in the CA2 sub – region expressing amyloid precursor protein (APP) (upper row) and SorL1 (lower row) across normal ageing controls and disease groups (PSND, PSD, AD, VaD, AD\_VaD). APP expression is higher in PSD and AD compared to other groups while SorL1 expression is higher in PSD compared to other groups.**

### 5.3.2.6. Intracellular neuronal trafficking and sorting protein (SorL1)

Given the pattern of APP expression, particularly the relationship between PSND and PSD, we investigated the expression of the intracellular neuronal trafficking and sorting protein, SorL1 (SorLA/LRII) which regulates cellular processes of APP trafficking and metabolism and enhance its channelling into non - amyloidogenic metabolic pathways (Dodson *et al.*, 2008).



**Figure 5.9 : Bar graph showing the distribution of % SorL1 per area and total immunoreactivity across hippocampal sub-regions CA1, CA2, CA3 and entorhinal cortex in Controls, PSND, PSD, AD, VaD and AD\_VaD. Bars show  $\pm$  2 SEM \*p < 0.05 (PSD vs AD\_VaD in CA2 sub- region).**

Figures 5.8 and 5.9 show the percentage expression of SorL1 per area and its total immunoreactivity across the regions CA1, CA2 CA3 and entorhinal cortex. The expression of SorL1 varied significantly across the sub - regions ( $p < 0.001$ , Kruskal – Wallis Test) being significantly higher in the CA2 ( $p < 0.001$ , Mann – Whitney U Test) compared to the CA1, CA3 and EC sub - regions and in CA3 than in CA1 and EC regions ( $p < 0.001$ , Mann – Whitney U Test). Within each region, the expression of SorL1 was similar across disease groups in the CA1 and EC regions while differences occurred across disease groups within the CA2 and CA3 regions. In the CA2 region, SorL1 expression was highest in the PSD group compared to other disease categories, the difference attaining significance with respect to AD\_VaD group ( $p < 0.05$ , Mann – Whitney Test).

### **5.3.3. Quantification of hyperphosphorylated tau (AT8) immunoreactivity**

Hyperphosphorylated tau was quantified in our cohort using the semi - quantitative approach of tau staging (Lace *et al.*, 2009) of disease groups and quantification of AT8 immunoreactivity in each of the sub - regions. Braak staging showed moderate correlation with tau staging ( $\rho = 0.576$ ;  $p < 0.001$ ) and tau staging varied significantly across disease groups ( $p < 0.001$ ) being significantly higher in AD and AD\_VaD as expected. The slight difference in tau stage between PSND and PSD did not attain statistical significance (Figures 5.10 a and b).

Figure 5.11 shows the quantification of AT8 IR across regions and disease groups. Across disease groups, AT8 immunoreactivity was highest in AD and AD\_VaD groups in comparison to each of the other groups of PSND, PSD, VaD and Controls ( $p < 0.05$ ). There was slightly higher AT8 immunoreactivity in PSD compared to the PSND group in the CA2 and CA3 sub – regions (Figure 5.12) although the difference was not statistically significant.. CAMCOG memory score correlated significantly with the mean % AT8 per area in the subiculum ( $\rho = - 0.425$ ,  $p = 0.024$ ).



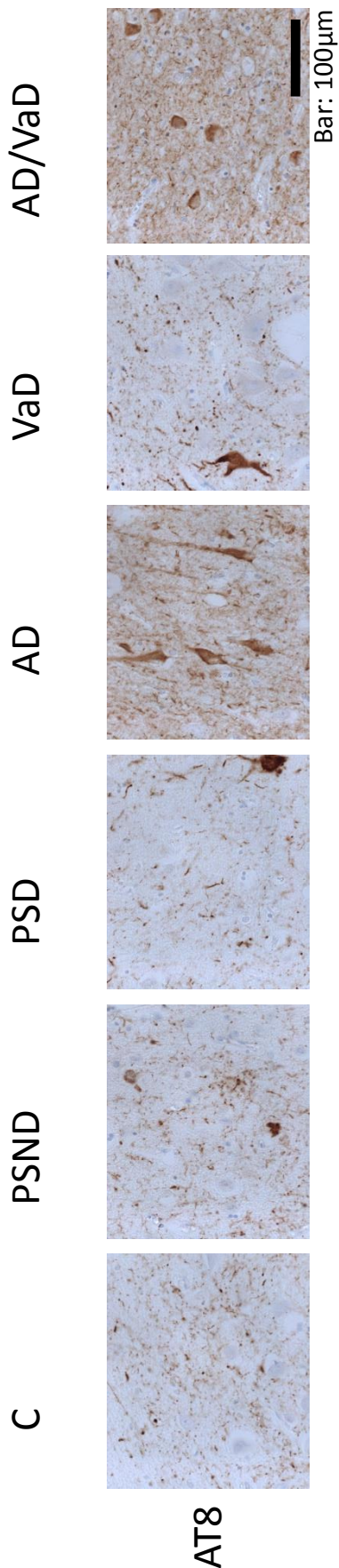
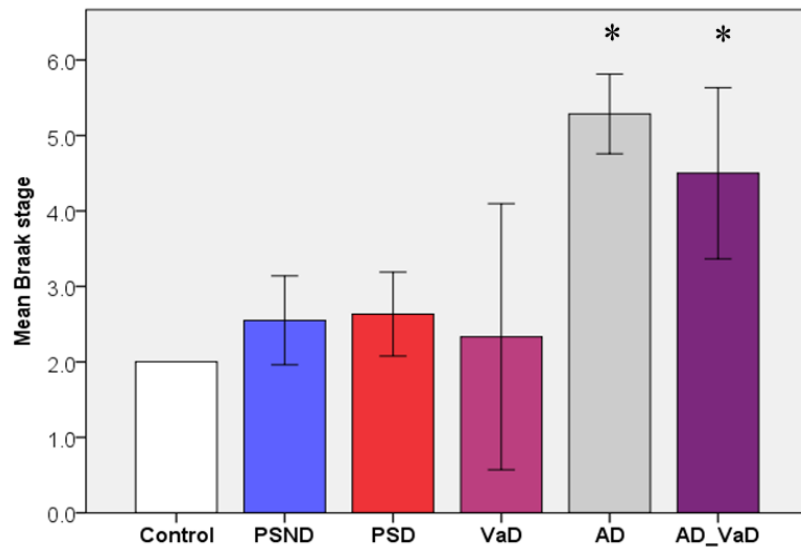
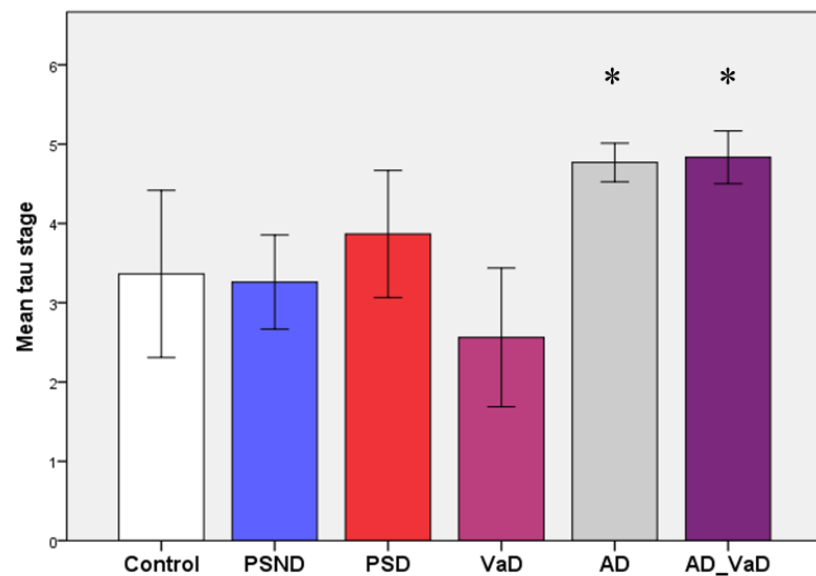


Figure 5.10a



Error Bars: +/- 2 SE

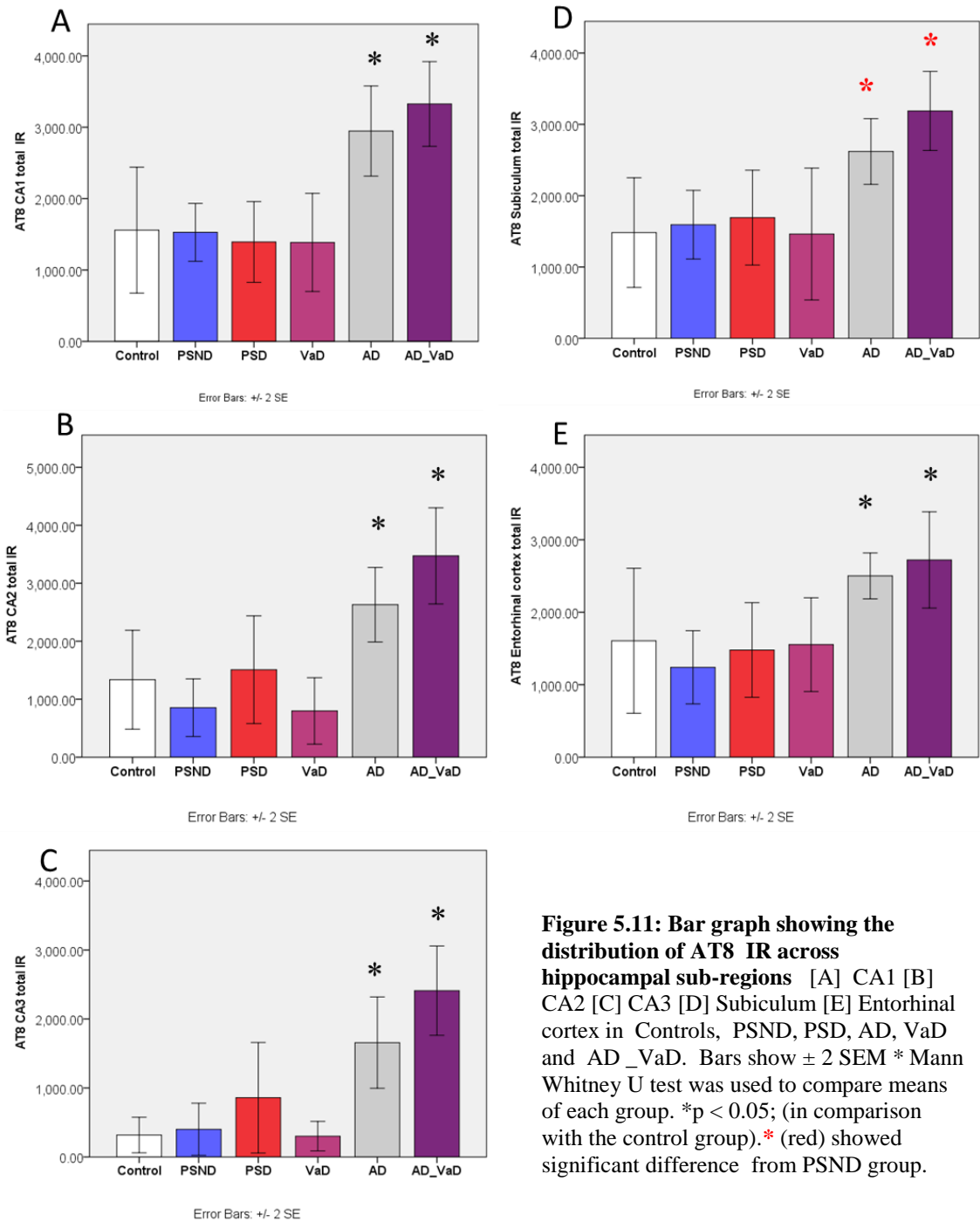


Error Bars: +/- 2 SE

**Figure 5.10b : Bar graph showing the mean Braak stage and mean tau stage of each group :**Controls, PSND, PSD, AD, VaD and AD\_VaD. Bars show  $\pm 2$  SEM . Braak stage and tau stage showed good correlation ( $\rho = 0.576$ ;  $p < 0.001$ ). \* $p < 0.05$  in comparison to control group.

**Figure 5.10a. Hyperphosphorylated tau (AT8) immunostaining** showing neuropil threads, pre – tangles and tangles in hippocampal sub – region CA1. AT8 immunostaining is relatively higher in AD and AD\_VaD compared to other groups.





**Figure 5.11: Bar graph showing the distribution of AT8 IR across hippocampal sub-regions [A] CA1 [B] CA2 [C] CA3 [D] Subiculum [E] Entorhinal cortex in Controls, PSND, PSD, AD, VaD and AD\_VaD. Bars show  $\pm 2$  SEM \* Mann Whitney U test was used to compare means of each group. \* $p < 0.05$ ; (in comparison with the control group). \* (red) showed significant difference from PSND group.**

**Figure 5. 12 (power point) double label amyloid and tau**

### 5.3.4. APOE ε4 genotype: influence on amyloid and tau accumulation in post – stroke sub - cohort

Given the unexpected finding of a trend of higher amyloid pathologic load in the PSND group compared to the PSD group across some sub-regions (CA1, subiculum and entorhinal cortex) and some amyloid markers we investigated the possible influence of APO E ε4 status in the post-stroke sub – cohort.

**Table 5.4: APOE ε4 allele genotype in the post - stroke sub - cohort**

Variable	PSND	PSD
	n= 16	n = 13
APOE ε4 +ve	8 (50.0 %)	3 (23.1 %)
APOE ε4 -ve	8 (50.0 %)	10 (76.9 %)

(Fisher's exact test = 2.13; p = 0.249)

There was a higher proportion of APO ε4 (50.0%) in the PSND group compared to the PSD group (23.1%), although the difference did not attain statistical significance (Table 5.4). Table 5.5. shows the total amyloid burden quantified by 4G8 immunoreactivity in each sub – region of the hippocampal formation and entorhinal cortex and demonstrating statistically significant higher amyloid load in APO E ε4 positive post-stroke tissue sample in the subiculum and entorhinal cortex respectively (p = 0.01) (Figure 5.13).

**Table 5.5. 4G8 sub - regional immunoreactivity and APO E ε4 status**

APOE ε4 present		CA1	CA2	CA3	SB	EC
No	Mean	506.60	754.93	566.87	396.77	386.73
	SEM	40.55	39.79	35.98	55.21	56.73
Yes	Mean	611.08	774.90	588.18	655.29	676.74
	SEM	101.93	87.66	74.47	78.21	102.57
All	Mean	541.42	762.42	574.54	493.71	491.13
	SEM	43.31	40.11	34.47	51.37	57.94
MW - U test	F	1.31	0.06	0.08	7.65	7.28
	p	0.26	0.82	0.77	<b>0.01*</b>	<b>0.01*</b>

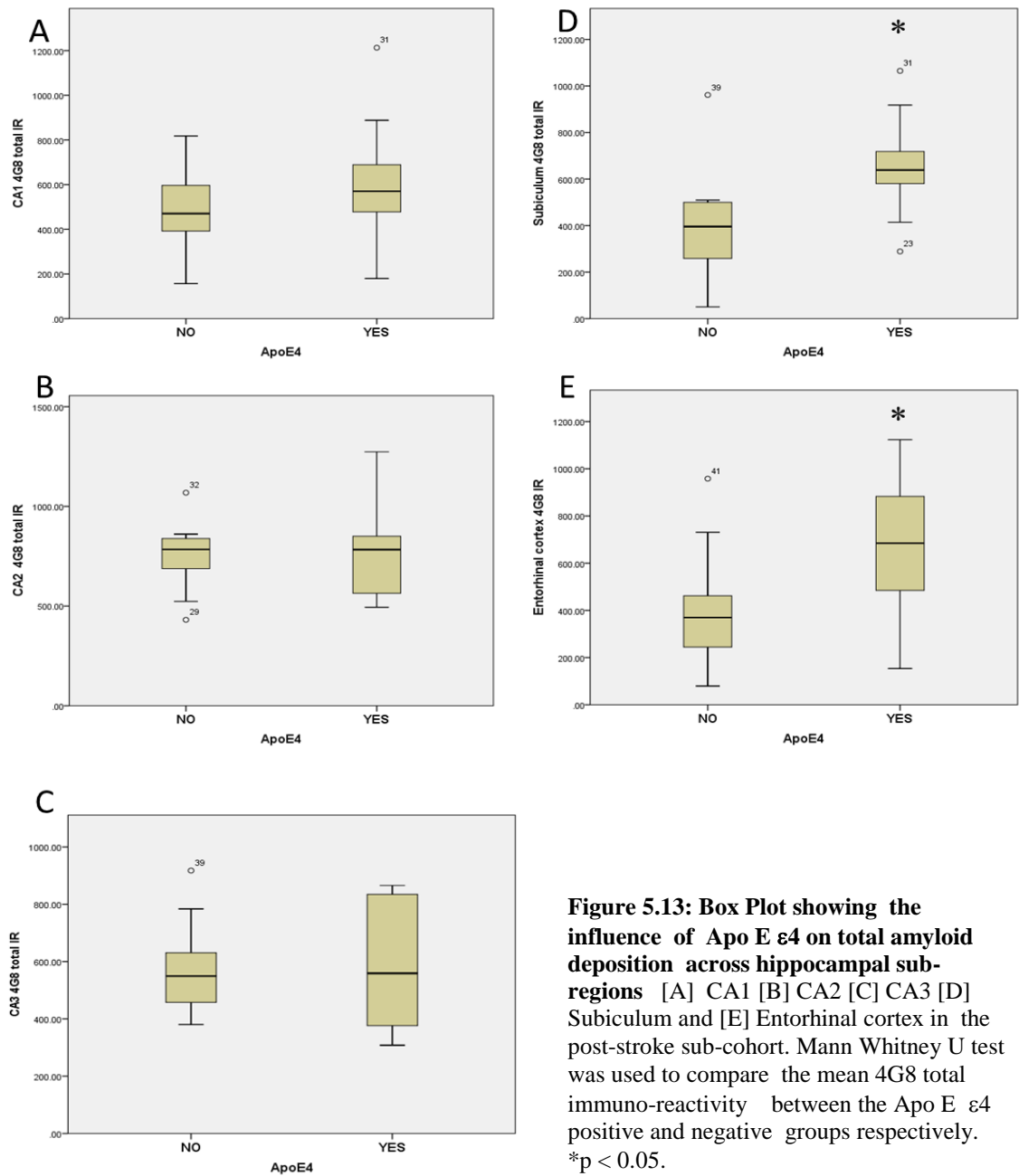


Figure 5.14 further illustrates the influence of APO E ε4 on 4G8 immunoreactivity (total amyloid deposition) in the subiculum showing significantly higher immunostaining [Figure 5.14A] in APOE ε4 - positive compared to APOE ε4 - negative post - stroke subjects. Furthermore, when the post – stroke group is split into PSD and PSND sub -groups, ApoE ε4 positivity appears to drive higher amyloid production in both groups although the small number of subjects in each group precluded the statistical power required to demonstrate significance [Figure 5.14B].

Figure 5.14C demonstrates that the A $\beta$  (42): A $\beta$  (40) ratio is slightly higher in the APO E  $\epsilon$ 4 positive subjects within the post-stroke group all together while Figure 5.14 D shows that the difference is more distinct (although not significant due to lack of statistical power from small number in each group) and suggesting that A $\beta$  (42) is the specie more related to APO 4  $\epsilon$ 4 positivity.

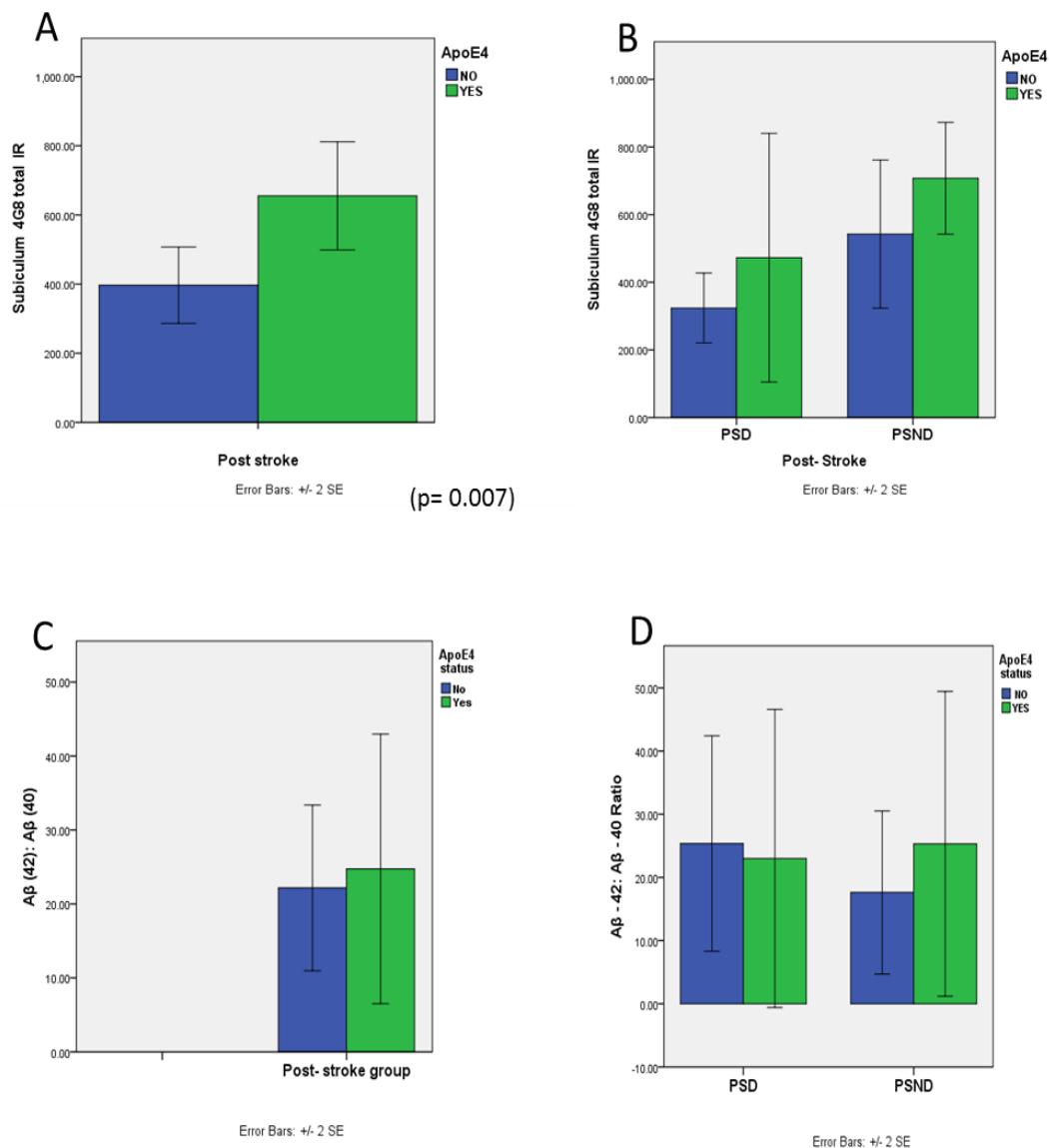
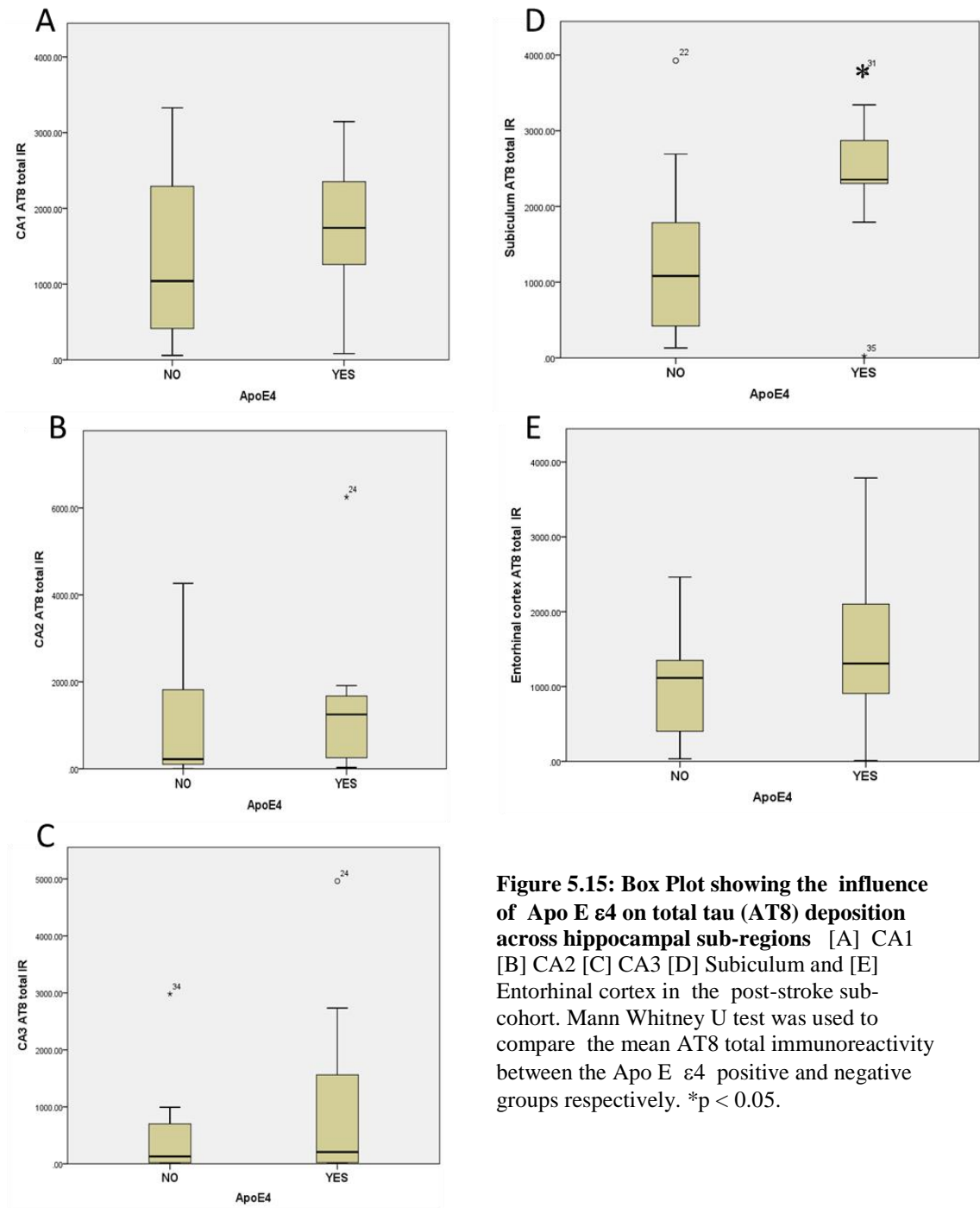


Figure 5.14: Bar graph showing 4G8 immunoreactivity in the subiculum in relation to the presence or absence of APOE  $\epsilon$ 4 allele in the post-stroke cases [A]. Bars show  $\pm$  2 SEM. [B] Presence of APO E $\epsilon$ 4 appeared to drive higher subicular amyloid deposition in both PSD and PSND groups. [C] Presence of APOE $\epsilon$ 4 allele increases A $\beta$  (42) deposition, and this appears relatively higher in the PSND group [D].

Presence of APOE  $\epsilon 4$  was also found to influence the amount of AT8 immunostaining in the post – stroke cohort, the burden of tau being significantly higher in APOE  $\epsilon 4$  positive cases compared to APOE  $\epsilon 4$  negative cases in the subiculum (Mann – Whitney U Test,  $p = 0.042$ ) ( Figure 5.15).



**Figure 5.15: Box Plot showing the influence of Apo E  $\epsilon 4$  on total tau (AT8) deposition across hippocampal sub-regions [A] CA1 [B] CA2 [C] CA3 [D] Subiculum and [E] Entorhinal cortex in the post-stroke sub-cohort. Mann Whitney U test was used to compare the mean AT8 total immunoreactivity between the Apo E  $\epsilon 4$  positive and negative groups respectively. \* $p < 0.05$ .**

### **5.3.5. Clinico - pathological correlations**

We explored relationships among the various markers of AD pathology used in this study and between cognitive scores of the post - stroke group utilizing Spearman correlation analysis.

#### **5.3.5.1. Correlations among pathology markers**

Table 5.6 below shows a correlation matrix of markers of AD pathology used in this study, and demonstrates significant correlations among different markers based on their immunostaining in the CA1 sub-region. Significant inter – marker correlations affirm the agreement and sensitivities of different markers in identifying their specific pathologic species in relation to other markers.

#### **5.3.5.2. A $\beta$ – 42: A $\beta$ - 40 Ratio**

We further explored the relationship of A $\beta$  – 42 and A $\beta$  – 40 based on the relative immunoreactivities of T – 42 and T – 40 in the CA1 sub- region. The ratio varied between 1.17 and 748.39 and the mean value was least in the control group and progressively increased in the PSND, PSD, VaD and AD groups to attain a maximum value in the AD\_VaD group. However, the variation across groups did not attain statistical significance (Table 5.7).

#### **5.3.5.3. AD pathologic measures and cognitive scores in the post-stroke group.**

Measures of general cognitive functioning (MMSE and CAMCOG total) and functioning in the memory domain (CAMCOG memory) which were available for the subjects in the post-stroke group only were correlated with two measures of AD pathologic burden: T – 42 immunoreactivity (being the predominant  $\beta$  – amyloid species deposited in the hippocampus) and AT8 immunoreactivity measures in the CA1, subiculum and entorhinal cortex : sub – regions which demonstrated the most consistent patterns of variation of immunoreactivity across the hippocampal formation.

Table 5.8 demonstrates significant correlation of AT8 immunoreactivity in the subiculum with CAMCOG memory ( $\rho = 0.419$ ,  $p = 0.024$ ), otherwise, there were no significant correlations of T – 42 immunoreactivity.



Spearman's rho		4G8 CA1 IR	T42 CA1 IR	T40 CA1 IR	NU1 CA1 IR	APP CA1 IR	SorL1 CA1 IR	AT8 CA1 IR
4G8 CA1 IR	p	1.000						
	rho	.						
T42 CA1 IR	p	.623 <sup>**</sup>	1.000					
	rho	.000	.					
T40 CA1 IR	p	.377 <sup>**</sup>	.634 <sup>**</sup>	1.000				
	rho	.001	.000	.				
NU1 CA1 IR	p	.730 <sup>**</sup>	.684 <sup>**</sup>	.265	1.000			
	rho	.000	.000	.051	.			
APP CA1 IR	p	.245 <sup>*</sup>	.293 <sup>**</sup>	.227 <sup>*</sup>	.166	1.000		
	rho	.025	.008	.046	.230	.		
SorL1 CA1 IR	p	-.056	-.234	-.133	-.038	-.218	1.000	
	rho	.661	.091	.343	.810	.113	.	
AT8 CA1 IR	p	.374 <sup>**</sup>	.656 <sup>**</sup>	.460 <sup>**</sup>	.068	.337 <sup>**</sup>	-.285 <sup>*</sup>	1.000
	rho	.000	.000	.000	.614	.002	.029	.

**Table 5. 6. Correlation matrix showing correlation among markers of AD pathology:** based on their immunostaining in the CA1 sub – region. Statistical significance designated by the following p values: \*\* p < 0.01; \*p < 0.05 Abbreviations: IR, immunoreactivity.

<b>95% Confidence Interval for Mean</b>						
<b>A<math>\beta</math> (42): A<math>\beta</math> (40) Ratio</b>						
	<b>Mean</b>	<b>Std. Error</b>	<b>Lower Bound</b>	<b>Upper Bound</b>	<b>Minimum</b>	<b>Maximum</b>
<b>Control</b>	16.38	3.87	5.65	27.12	7.89	29.72
<b>PSND</b>	19.42	4.65	9.69	29.15	1.95	78.11
<b>PSD</b>	27.42	7.38	11.60	43.24	2.34	90.33
<b>VaD</b>	41.86	13.33	13.06	70.65	3.04	148.46
<b>AD</b>	55.44	40.65	32.38	143.26	2.66	579.38
<b>AD_VaD</b>	76.44	67.28	73.47	226.34	1.17	748.39
<b>Total</b>	39.04	11.97	15.22	62.87	1.17	748.39

**Table 5.7. Ratio of A $\beta$  (42): A $\beta$  (40) across groups in the CA1 hippocampal sub-region** demonstrates the mean values  $\pm$  SEM, 95% Confidence Interval, minimum and maximum values of A $\beta$  - 42: A $\beta$  - 40 ratios across control and disease group

Spearman's rho		CAMCOG memory	CAMCOG total	MMSE	AT8 CA1 IR	AT8 IRSB	AT8 IREC	T42 CA1 IR	T42 SB IR	T42 ECIR
CAM_memory	rho	1.000								
	p	.								
CAMCOG score	rho	.814**	1.000							
	p	.000	.							
MMSE	rho	.790**	.951**	1.000						
	p	.000	.000	.						
AT8TOTAL IRCA1	rho	-.077	.029	.053	1.000					
	p	.657	.857	.754	.					
AT8TOTAL IRSB	rho	<b>-.419*</b>	-.189	-.047	.793**	1.000				
	p	<b>.024</b>	.301	.807	.000	.				
AT8TOTAL IREC	rho	-.308	-.064	-.031	.785**	.686**	1.000			
	p	.091	.720	.869	.000	.000	.			
T42CA1IR	rho	-.046	.109	.054	.656**	.493**	.567**	1.000		
	p	.792	.513	.759	.000	.000	.000	.		
T42SBIR	rho	-.080	.120	.090	.634**	.563**	.557**	.779**	1.000	
	p	.646	.471	.609	.000	.000	.000	.000	.	
T42ECIR	rho	-.033	.087	.082	.566**	.457**	.557**	.566**	.671**	1.000
	p	.849	.606	.641	.000	.000	.000	.000	.000	.

**Table 5.8 Correlation matrix showing association of neuropathologic measures of amyloid and tau pathology with cognitive scores in selected hippocampal sub-regions and entorhinal cortex** shows significant correlation of AT8 immunoreactivity in the subiculum with CAMCOG memory. p values: \*\* p < 0.01; \*p < 0.05. Abbreviations: IR, immunoreactivity; CAMCOG, Cambridge Cognitive Examination; MMSE, Minimental State Examination; SB, subiculum; EC, entorhinal cortex .

## 5.4. Discussion

The objective in this study was to assess whether the burden of amyloid and tau pathologies in the hippocampal formation and entorhinal cortex differentiated post-stroke demented from non - demented subjects. The analysis was also done in comparison with normal ageing controls and other dementias. We hypothesized that Alzheimer pathology would be differentially expressed in demented and non - demented post- stroke subjects in comparison to normal ageing controls and other dementias.

We found 4G8 antibody immunoreactivity (a measure of immunostaining of all  $\beta$  - amyloid species) in the hippocampal formation of normal ageing controls, post-stroke subjects and other dementias (AD, VaD and AD\_VaD). While 4G8 immunoreactivity was lowest in the control group as expected and highest in the AD and AD\_VaD groups, it was intermediate and similar in the post-stroke and VaD groups (Figure 5.2). Within the sub - regions of the hippocampal formation, differential expression of 4G8 immunoreactivity was found in the CA1, subiculum and entorhinal cortex (Figure 5.3). Among amyloid markers, the pattern of T - 42 immunoreactivity ( $A\beta$  (42)) was similar to that of 4G8, while T - 40 immunoreactivity ( $A\beta$  (40)) was reduced across groups. The  $A\beta$  (42):  $A\beta$  (40) ratio was greater than 10:1 across the control and disease groups and demonstrated an interestingly increasing trend from approximately 20 in the control group to approximately 80 in the AD\_VaD group. Immunoreactivity of the soluble amyloid marker, NU - 1 mirrored the pattern of 4G8 although the total load was much less.

Furthermore, the expression of APP was fairly uniform across the sub - regions and groups and only showed a significantly higher expression in PSD than PSND in the CA2 sub - region. The expression of the neuronal sorting protein, SorL1 was highest in the CA2 sub - region where the PSD group also had the highest expression compared to other disease categories. As expected, tau pathology showed highest expression in the AD and AD\_VaD disease groups and similar level expressed in the control, post-stroke and VaD groups. Interestingly, we found an association between ApoE  $\epsilon$ 4 allele positivity and higher load of amyloid and tau pathology in the subiculum and entorhinal cortex of post-stroke cases. ApoE  $\epsilon$ 4 allele positivity also appeared to be more related to  $A\beta$  (42) deposition.

Comparison between PSND and PSD revealed significantly higher 4G8 immunoreactivity in PSND compared to PSD in the CA1, subiculum and entorhinal cortex sub- regions. There were no statistically significant differences between PSND and PSD in T – 42, T - 40 and NU – 1 immunoreactivities although APP and SOrL1 immunoreactivities were significantly higher in PSD than PSND. Higher proportion of Apo E  $\epsilon$ 4 in PSND than in PSD was found to be responsible for the higher 4G8 immunoreactivity in PSND than PSD. All amyloid species (4G8, T – 42, T – 40 and NU – 1) were significantly higher in AD and AD\_VaD in comparison to the control group. Hyperphosphorylated tau immunoreactivity did not differ between PSND and PSD, but was significantly higher in AD and AD\_VaD compared to other groups. Nevertheless, we acknowledge the potential influence of multiple pairwise comparisons on the results of our analysis and the corresponding interpretations.

### **Amyloid accumulation, ageing and cerebrovascular disease**

Consistently across all the markers of amyloid pathology, we found evidence of amyloid accumulation in ageing controls, post - stroke groups and other dementias, the quantity increasing in that order. Our finding of amyloid accumulation in normal ageing controls concurs with the biological phenomenon of ageing – associated accumulation of amyloid that has been reported across species: in drosophilia (Rogers *et al.*, 2012), mice (Yamada *et al.*, 2011), non – human primates (Ndung'u *et al.*, 2012) and man (Tomlinson *et al.*, 1968; Katzman *et al.*, 1988; Bennett *et al.*, 2006; Lewis *et al.*, 2006; Boyle *et al.*, 2013b). This occurs as a result of ageing-related compromise of the neurovascular unit resulting in increased production of amyloid and reduced clearance through the perivascular space (Iadecola, 2004; Kalaria, 2009a; Kalaria *et al.*, 2012a). Amyloid accumulation in the post-stroke group as a whole mirrored that in the VaD group and was less than in the AD and AD\_VaD groups (Figure 5.2) in consonance with the findings of Lewis *et al.* (2006) showing enhancement of amyloid accumulation in VaD possibly triggered by cerebral hypoxia consequent to cerebral vascular disease (Lewis *et al.*, 2006; Kalaria, 2010). This is in tandem with previous findings of enhanced accumulation of amyloid in animal models of chronic cerebral hypoperfusion (Kalaria *et al.*, 1993a; Yamada *et al.*, 2011) as well as increased PIB uptake in a 40% of PSD subjects in a small pilot study (Mok *et al.*, 2010). In addition, this

suggests that beyond age - associated accumulation of amyloid, cerebral vascular disorders including stroke do exacerbate brain amyloid deposition as previously demonstrated in brain tissue from hypertensive (Petrovitch *et al.*, 2000) and diabetic subjects (Luchsinger, 2010). Although Marchant *et al* failed to establish a direct relationship between CVD and  $\beta$  – amyloid using PIB –PET approach in a cohort of non – demented elderly subjects (Marchant *et al.*, 2012), and further suggested that the PIB – PET amyloid measures did not influence cognition, the authors admitted that the limited statistical power of the study may have failed to detect any existent interaction. In addition, a neuropathological study of 484 post - mortem brains did not find a relationship between amyloid deposition and cerebrovascular lesions (Aho *et al.*, 2006). However, the validity of this study is weakened by its utilization of semi-quantitative assessment of amyloid burden. The previous study of (Lewis *et al.*, 2006) and this current work have utilized quantitative approaches which may be more sensitive to detect differences. Besides, findings from studies in non – demented elderly subjects may not necessarily simulate those in demented subjects with significant CVD as the mechanisms that produce cognitive decline and dementia may differ in different clinicopathological scenarios (Kalaria, 2012a; Kalaria, 2012b).

### **Sub-regional variation in hippocampal amyloid accumulation**

Across the sub-regions of the hippocampal formation and entorhinal cortex, amyloid deposition was significantly higher in the CA1, subiculum and entorhinal cortex compared to the CA2 and CA3 regions respectively. This differential pattern may be related to the spatial localization and role of these regions in the hippocampal circuitry (Lavenex and Banta Lavenex, 2013), differential susceptibility of these sub - regions to different pathologies (Small *et al.*, 2011) or the temporal evolution and hierarchical progression of cerebral amyloidosis (Thal *et al.*, 2006).

The entorhinal cortex has been described as the gateway into the hippocampal formation whereas the subiculum and CA1 regions constitute the outflow stations (Goldman-Rakic *et al.*, 1984; Suzuki and Amaral, 2004). Alzheimer pathology tends to spread along the hippocampal circuitry (Thal *et al.*, 2002a) (Lace *et al.*, 2009) and this may explain the

differential susceptibility and high  $\beta$  – amyloid load in these sub-regions. Besides, in the hierarchical evolution and natural history of amyloid and tau pathologies, these sub - regions are affected much earlier in the disease course, compared to other regions like CA2 and CA3 (Thal *et al.*, 2002b; Lace *et al.*, 2009). The later affectation of the CA2 region, in particular may reflect the natural course of disease or the existence of some underlying protective mechanisms operating in the early stages of disease and only giving way in the advanced stage of the disease (Caruana *et al.*, 2012).

The finding of a relatively higher ratio of A $\beta$  (42) compared to A $\beta$  (40) which further increases with the degree of accumulation of AD pathology is in concordance with previous findings (Selkoe, 2008) (Aho *et al.*, 2006) demonstrating the predominance of A $\beta$  (42) over A $\beta$  (40) in brain parenchymal amyloid deposits. We have also previously demonstrated a preponderance of A $\beta$  (42) over A $\beta$  (40) in parenchymal and vascular amyloid deposits in non – human primates including squirrel and rhesus monkeys and ageing baboons (Ndung'u *et al.*, 2012).

### **Differential amyloid deposition between PSND and PSD**

Largely, there were no significant differences in the quantity of amyloid deposited across hippocampal regions and markers in PSND compared to PSD groups. This suggests age - related deposition of amyloid in post - stroke survivors. However, there was unexpected finding of significant differences in 4G8 immunoreactivity in the entorhinal cortex between PSD and PSND subjects. Though not statistically significant, a similar pattern was observed with T – 42 in the subiculum, T – 40 in the entorhinal cortex, and NU – 1 in the CA1 sub-region. This suggest that amyloid accumulation alone in post-stroke subjects might not have played a primary role in determining progression of cognitive decline to dementia as indeed has been found in normal elderly subjects with huge quantities of amyloid pathology, yet preserved cognitive functioning (Bennett *et al.*, 2006; Chetelat *et al.*, 2010); (Esiri *et al.*, 1997). It may also imply that amyloid needs the synergy of other pathologies including tau pathology, vascular lesions, brain atrophy, white matter pathology, medial temporal lobe atrophy in order to produce significant cognitive decline and dementia (Mormino *et al.*, 2009). In a PIB- PET study of elderly subjects – normal, MCI and AD, the investigators found

that whereas amyloid load (PIB index) and hippocampal atrophy both predicted loss of episodic memory, amyloid deposition alone in the absence of hippocampal atrophy failed to predict episodic memory loss (Mormino *et al.*, 2009). A complementary study of our current cohort (Gemmell *et al.*, 2012) found that whereas pyramidal neuronal volume was preserved in the CA regions and entorhinal cortex of the control and PSND groups, subjects in all the demented groups had significant atrophy of these neurons. This would, therefore, suggest that a high amyloid load in the PSND group was insufficient to produce dementia because of preserved neuronal volume. This, indeed, may be a signature of brain/cognitive reserve, preserved synaptic integrity or some other compensatory mechanisms (Stern, 2009; Boyle *et al.*, 2013a).

The observation of similarity between the amyloid load in the PSD compared to the control group could imply that given similar quantities of amyloid pathology with respect to the control group, the PSD group was demented possibly because of the presence of CVD lesions which lowered the threshold for dementia in the PSD group (Snowdon *et al.*, 1997; Esiri *et al.*, 1999); the presence of neuronal atrophy (Gemmell *et al.*, 2012) or the slightly higher and more advanced tau pathology in the PSD group (higher tau stage).

### **Hyperphosphorylated Tau and NFT and accumulation**

The accumulation of hyperphosphorylated tau and neurofibrillary tangles in this study showed expected distribution with highest quantities in AD and AD\_VaD. However, we observed a relatively higher amount of AT8 immunostaining in the CA2 and CA3 sub – regions in the PSD compared to the PSND group. In spite of a lack of statistically significant difference, this may suggest relatively more advanced tau pathology in the PSD group compared to PSND.



## **APOE ε4 status and accumulation of amyloid and tau pathologies**

Further analysis of the post – stroke cohort suggested that the presence of the APOE ε4 allele is responsible for driving amyloid and tau accumulation in those who possessed the allele (which was present in 50%) of the PSND subjects. Despite the small samples, the load of amyloid was significantly higher in the PSND group than the PSD group. APOE ε4 has been associated with accumulation of amyloid and/or tau pathology ((Nagy *et al.*, 1995; Saito *et al.*, 2002) in AD but the relationship with post - stroke dementia has been conflicting and less well defined. Previous studies in the Newcastle cohort failed to establish a relationship between APOE ε4 with post- stroke cognitive impairment at three months after the stroke ((Rowan *et al.*, 2005) but predicted decline at 1 – year follow up ((Ballard *et al.*, 2004b), further pathological verification had hitherto been lacking. Some other studies had also reported both positive (Packard *et al.*, 2007; Liu *et al.*, 2012) (McGuinness *et al.*, 2010a) and negative associations (Gdovinova *et al.*, 2006) although these were largely clinical studies. It is indeed plausible that the APO E ε4 allele might have contributed to the higher quantity of amyloid in the PSND group compared to the PSD group, but studies will be needed to explore this relationship further.

## **SorL1**

We found a significantly increased expression of SorL1 in the CA2 hippocampal sub-region compared to other regions across all disease groups in this study. And within the CA2 sub - region, the highest expression of SorL1 was found in the PSD group while the lowest was in the AD\_VaD group. Considering the resilience of the CA2 sub-region to insult (Small *et al.*, 2011), this finding suggests that the expression of SorL1 may have a protective role in addition to its traditional role of intracellular trafficking of APP (Nishii *et al.*, 2013). In this context, the higher expression of SorL1 in PSD compared with PSND in the CA2 sub – region mirrored the finding of higher expression of APP in PSD compared to PSND also in the CA2 region of subjects in this cohort. APP production may be upregulated in response to hypoxia (Kalaria *et al.*, 1993, 1996, 2000, 2002, 2012). In a very recent report (Nishii *et al.*, 2013), SorL1 was found to be upregulated /induced by hypoxic stimulus mediated by hypoxia

–inducible factor (HIF –  $\alpha$ ) in haematopoietic stem and progenitor cells. It is therefore conceivable that SorL1 expression in the hippocampal formation and entorhinal cortex occurs in response to hypoxic stimulus which also upregulates APP production. Evidence of increased levels of APP and SorL1 has been demonstrated in the cerebrospinal fluid (CSF) of patients with AD (Alexopoulos *et al.*, 2012). The higher production of SorL1 in the CA2 may be a protective mechanism for the CA2 pyramidal neurons that channels the APP into non – amyloidogenic metabolic pathways resulting in a lower load of amyloid deposited until this protective function becomes compromised.

### **Correlation of cognitive scores with AD pathology**

In the post – stroke cohort with available cognitive scores, very limited correlation of tau pathology with CAMCOG memory score was established. In this cohort, there was dissociation of cognitive performance and hippocampal AD pathologic burden. The implication of this may be that in general, AD pathology probably does not contribute very strongly to the substrates of dementia after a stroke event as previously hypothesized (Henon *et al.*, 1997). And if there was any contribution at all, tau pathology probably contributed more than amyloid pathology. Recent reports also suggest that MTLA which has been widely ascribed to AD pathology, may have a vascular basis (Bastos-Leite *et al.*, 2007; O'Sullivan *et al.*, 2008). The lack of association may also be due to the presence of other pathologies such as  $\alpha$  – synuclein, (the determination of which is beyond the scope of the current study), presence of robust cognitive reserve and other lifestyle and psychosocial might also offer reasons for the dissociation between clinical and cognitive measures in the current study. In conclusion, non – AD pathologic mechanisms appear to play a more dominant role in the neurobiology of post - stroke cognitive impairment. Further studies are, however, advocated to further unravel the mechanisms in order to develop better preventive and therapeutic interventions.

## 5.5. Chapter Summary

Marker	Neuropathological Categories					
	Control	PSND	PSD	VaD	AD_VaD	AD
4G8	↑	↑↑	↑	↑↑	↑↑	↑↑↑
T - 42	↑	↑	↑	↑	↑↑	↑↑
T - 40	↑	↑	↑	↑	↑↑	↑↑
NU -1	↑	↑	↑	↑	↑	↑
APP	↑	↑	↑↑	↑	↑	↑
SO <sub>r</sub> L1	↑	↑	↑↑	↑	↑	↑
AT8	↑	↑	↑	↑	↑↑	↑↑↑
Aβ 42: Aβ 40 ratio	↑	↑	↑↑	↑↑↑	↑↑↑↑	↑↑↑↑

**Table 5.9. Summary of findings on measures of hippocampal AD pathologic quantification**

**Annotation:** (↑ = mild increase; ↑↑ = moderate increase, ↑↑↑ = severe increase  
↑↑↑↑ = highest increase)

- **Correlations**
  - 4G8, T – 42, T – 40, NU -1 and AT8 demonstrate statistically significant inter - correlation with one another.
  - Hyperphosphorylated tau (AT8) immunoreactivity showed significant negative correlation with CAMCOG Memory Score in the CA1 sub- region.
  
- APOE ε4 positivity was significantly associated with higher 4G8 (total amyloid) immunoreactivity in the subiculum and entorhinal cortex.

## **Chapter 6. Quantification of hippocampal synaptic integrity in post - stroke dementia compared with AD and normal ageing controls.**

### **6.1. Introduction**

Progression of cognitive decline in patients with post – stroke vascular cognitive impairment no dementia (vCIND) to vascular dementia (VaD) has been related to the development of memory impairment, apart from worsening in other domains of cognition (Ballard *et al.*, 2002; Ballard *et al.*, 2003a; Ballard *et al.*, 2003b; Sachdev *et al.*, 2004a; Sachdev *et al.*, 2004b; Stephens *et al.*, 2004; Sachdev *et al.*, 2006; Moorhouse and Rockwood, 2008; Gorelick *et al.*, 2011). Neuroimaging studies in the longitudinal Newcastle cohort found that medial temporal lobe atrophy (MTLA) predicted progression to cognitive impairment, dementia and death (Firbank *et al.*, 2007; Firbank *et al.*, 2012) while neuropathological studies unmasked hippocampal neuronal atrophy as an important substrate of post-stroke dementia (PSD) in the cohort (Gemmell *et al.*, 2012). The hippocampus plays a major role in the neurobiology of learning and memory (Amaral, 1993; Kandel, 2001; Small *et al.*, 2011).

Given a similar predictive role of MTLA in relation to cognitive impairment and dementia in the CogFAST – Nigeria cohort (Chapter 4) and the hypothesis ascribing MTLA to Alzheimer pathology (Henon *et al.*, 1997; Henon *et al.*, 1998; Leys *et al.*, 2005; Firbank *et al.*, 2007), we investigated Alzheimer pathology in hippocampal tissue from subjects in the Newcastle post – mortem cohort compared with other dementias (Chapter 5). However, our findings showed that, largely, hippocampal Alzheimer pathology burden did not differ between demented and non - demented post – stroke survivors. Hence, Alzheimer pathology does not seem to provide a clear mechanistic distinction between non - demented (PSND) and demented (PSD) stroke survivors.

Given the above rationale, in this study we sought to establish if alterations in synaptic integrity within the hippocampus could provide a mechanistic insight into the different cognitive trajectories that ensue after stroke. It has been previously established that synaptic dysfunction correlates with cognitive profile and often precedes neuronal loss and the appearance of measurable pathologies such as amyloid plaques and tangles (Hardy and

Selkoe, 2002; Selkoe, 2002; Ihara and Kalaria, 2007; Mucke and Selkoe, 2012). Improved understanding of such neurochemical changes in vascular cognitive impairment (VCI) is critical to the development of effective therapeutic and neuroprotective (preventive) interventions to prevent or delay onset or progression of disease.

We hypothesized that differences in the expression of hippocampal synaptic markers would distinguish demented from non – demented post-stroke cohorts in relation to normal ageing controls and other dementia subjects. Using immunohistochemistry, we evaluated the expression level of the pre - synaptic markers [ synaptic markers synaptosomal - associated protein 25 (SNAP-25); synaptophysin (SY – 38); vesicular glutamate transporter - 1 (VGLUT - 1)] and the post – synaptic markers [post synaptic density - 95 (PSD – 95) and Drebrin ] in hippocampal tissue of post – stroke cases compared with AD and normal ageing controls and using Western Blot, we quantified the expression of these markers in hippocampal homogenates obtained from frozen brain tissue samples.

## **6. 2. Methods**

### ***6.2.1. Study Subjects***

Samples from a subset of 42 subjects consisting of 10 with AD, 11 post-stroke non - demented (PSND), 11 post - stroke demented (PSD) and 10 controls were evaluated. Table 6.1 shows the demographic characteristics of the subjects. There were no significant differences in age, gender, postmortem delay and length of fixation period across the groups ( $p > 0.05$ , ANOVA). However the PSND and PSD groups differed significantly on cognitive scores ( $p < 0.001$ , student t – test) while neuropathological variables also showed significant variation across the cohort ( $p < 0.001$ , ANOVA). The CogFAST Study and ancillary studies had ethical approval from the local Newcastle Ethical committees and participants gave written consent to brain tissue donation. Use of brain tissue was also approved by the local Ethical committees and the committee of the Newcastle Brain Tissue Resource (NBTR).

Variable	Clinical Diagnosis					p value
	Controls	PSND	PSD	AD	All	
No (n)	10	11	11	10	42	
Age (years)	81.3 ± 7.8	84.1 ± 4.5	84.9 ± 6.1	81.6 ± 6.9	82.9 ± 6.4	0.405
Gender : (No , % female)	5 (50.0)	3(27.3)	5(45.5)	6(60.0)	19(45.2)	0.493*
MMSE score	ND	27.9 ± 1.4	15.1 ± 3.1	ND		< 0.001+
CAMCOG total	ND	92.9 ± 4.8	55.7 ± 10.1	ND		< 0.001+
CAMCOG memory	ND	21.9 ± 2.7	9.2 ± 8.3	ND		< 0.001+
Post-mortem delay (hours)	26.4 ± 10.7	22.4 ± 7.3	24.9 ± 9.5	21.5 ± 10.9	23.9 ± 9.5	0.678
Fixation period (weeks)	9.2 ± 6.5	10.2 ± 5.9	9.9 ± 6.0	7.8 ± 5.3	9.3 ± 5.7	0.799
CERAD Score	0.2 ± 0.4	0.9 ± 0.8	1.4 ± 1.1	2.6 ± 1.0	1.4 ± 1.2	<0.001
Braak Stage	1.7 ± 0.8	2.3 ± 0.8	2.9 ± 1.6	5.5 ± 0.7	3.2 ± 1.8	<0.001
Vascular Score	ND	1.7 ± 0.8	1.8 ± 0.5	ND		0.826

**Table 6.1: Demographic, cognitive and pathological characteristics of the subjects,** \*chi - square test; + students't – test; all others ANOVA. Subjects do not vary significantly with age, gender, post – mortem delay or length of fixation period. CAMCOG = Cambridge Cognitive Examination; CERAD = Consortium to Establish a Registry for Alzheimer's disease; ND = No data available.

### 6.2.2. Neuropathological examination

Hippocampal sections (corresponding to coronal levels 18 – 20) were selected from the Newcastle Brain Map (Perry and Oakley, 1993). Formalin fixed paraffin blocks were retrieved from the NBTR and processed as described in Chapter 2. Cresyl Violet and Haematoxyline and Eosin stains were used to evaluate general cellular and neuropathologic changes in accordance with standard protocol (Kalaria *et al.*, 2004; Deramecourt *et al.*, 2012).

### ***6.2.3. Quantitative immunohistochemistry***

Ten micron hippocampal sections were immunostained with primary synaptic antibodies to pre – synaptic markers : synaptosomal - associated protein 25 (SNAP - 25, 1: 1000, monoclonal); synaptophysin (SY – 38, 1: 150, monoclonal); vesicular glutamate transporter - 1 (VGLUT – 1, 1 : 1000, polyclonal;) and post – synaptic markers [post synaptic density - 95 (PSD – 95, 1 : 750, polyclonal) and Drebrin ( 1: 200, monoclonal) as described in Chapter 2. To minimize variability of immunohistochemical staining quality, control sections were included in assays, and experiments were run in two batches for each marker and using freshly prepared buffer solutions and tinctorial stains. Sections were further counterstained with haematoxylin following the assays of PSD – 95 and drebrin to enhance identification of neuronal cellular structures. Sections were numbered randomly from 1 – 50 and then analysed blind to the diagnoses of the cases.

#### **6.2.3.1. Image Acquisition and Analysis**

The stained sections were examined and imaged using a Zeiss Axioplan 2 research grade microscope coupled to an Infinity 2 camera. Set at X10 magnifications for the hippocampal sub-regions CA1, CA2 and CA3 and dentate gyrus. At least five images were taken at random from the CA1, CA3 and dentate gyrus and three images from CA2. Tissue sections were assigned random numbers and all analysis were performed blind to the pathological diagnosis of the subject.

Images were first qualitatively assessed with respect to overall quality and consistency of staining characteristics across cases and in relation to known characteristics such as post – mortem delay and length of fixation period. Image Pro-Plus 4.0 (Media Cybernetics, USA) software was used for further analysis of the images. The histogram - based method was used to determine the percentage of area stained over the total area analyzed (PA) and the integrated optical density (IOD). The area of interest was manually determined and a threshold of optimal staining with good signal – background ratio was established with a Red

– Green – Blue histogram –based method: the red and green spectra were fixed from 0 to 255 while the blue limit was manually determined. The mean total immunoreactivity (IR) for each image was derived and then for each sub - region analyzed.

#### **6.2.4. Immunoblotting**

Immunoblotting was performed as previously described (see section 2.3.9). Frozen sections containing whole hippocampal formation containing the CA fields and the dentate gyrus samples (Newcastle Brain Map level 18 -20) were obtained from the Newcastle Brain Tissue Resource, sub-dissected, homogenized and stored at -70°C . Relative protein concentration across samples was assessed in triplicate using the DC Kit protein assay (Bio- Rad). Appropriate aliquots of sodium dodecyl sulphate were then added to each sample to prepare samples with equalized protein concentration across all samples. 20 micrograms of protein was loaded into each gel and run on 10% sodium dodecyl acrylamide gel (8% gel for drebrin).. Whole hippocampal homogenate samples were loaded into wells and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by protein transfer onto nitrocellulose membranes and transfer confirmed with Ponceau stain. Membranes were blocked against non - specific binding using 5% non - fat dried milk (Marvel) in TBS -T and incubated overnight at 4<sup>0</sup>C with primary antibodies at previously optimized concentrations of each synaptic marker (Table 2.3). Beta – tubulin was employed as a loading control. Secondary antibody incubation was achieved by exposing membranes to horseradish peroxidase (HRP) conjugated secondary antibodies (anti – mouse or anti - rabbit depending on the primary antibody) suspended in a 5% non - fat dried milk (Marvel) in TBS -T solution. Membranes were exposed to the enhanced chemiluminescence kit (Thermo Scientific) for 2 minutes on a glass plate and bands were visualized with a LAS - 4000 Luminescent Image Analyzer Version 1.0 (Fuji Film Cooperation, Tokyo, Japan). Optical density of bands were measured using Image J. Variation in signal detection (including gel and blotting variation) across gels was corrected for using the protein standard replicate loaded on gels. Data were expressed as optical density of the band per weight of protein loaded per sample.



### ***6.2.5. Statistical analysis***

Statistical analysis was carried out using the IBM SPSS software (version 19.0). The Shapiro – Wilk Test was used to establish normality of data. Comparisons across groups were done using parametric tests (ANOVA for group means and Tukey post – hoc analysis for between – group differences) non - parametric tests (Kruska – Wallis and Mann – Whitney U tests) for non – normally distributed dataset.

## **6.3. Results**

### ***6.3.1. Immunohistochemistry***

Sections immunostained with the five synaptic markers were first qualitatively assessed to evaluate the anatomical distribution of the markers within the hippocampal sub –regions. As the length of fixation period affected the intensity of staining of markers, particularly SNA P-25, SY -38 and PSD – 95, it made it impracticable to fully quantify with Image Pro Plus. Besides, the distribution of immunoreactivity pattern of markers (SNAP – 25, SY – 38 , and VGLUT - 1) was widespread across the hippocampal sub –regions with limited distinction between background staining and specific immunoreactivity. In such instances, it was deemed that large number of images would be required at very high magnifications in order to detect very distinct differences between groups. In such instances, quantification of immunoreactivity was performed limited to representative subsets of cases with more accurate quantification of all markers in all cases performed by immunoblotting analysis of hippocampal homogenates from frozen tissue sections of the same subjects. Figure 6.1 shows the immunoreactivity pattern of the synaptic markers across groups.

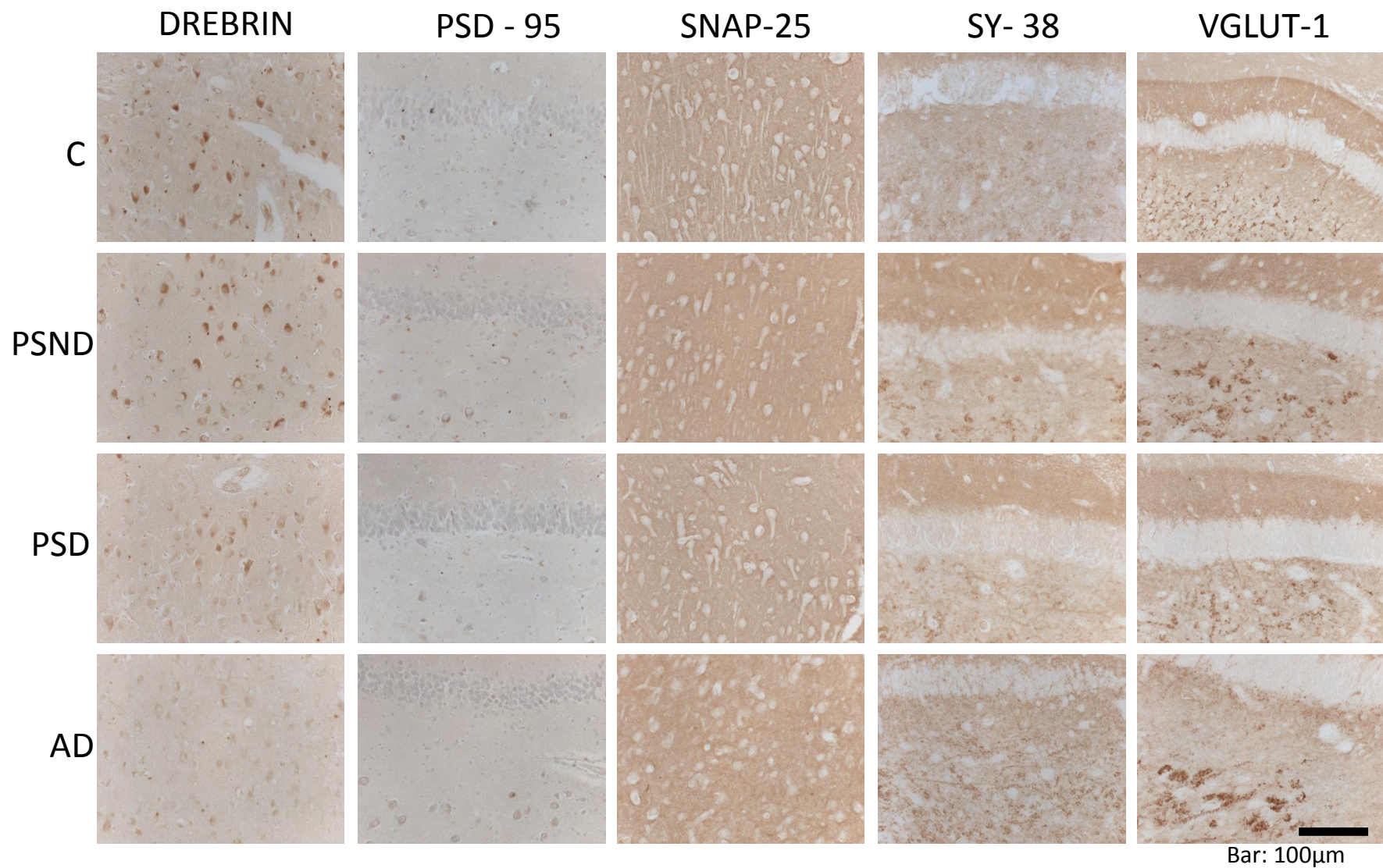
We computed the statistical power of this sub – sample of 42 cases of four groups using the G\*Power software (Faul et al, 2007). Given a significance level,  $\alpha = 0.05$  and assuming a moderate effect size Cohen's  $d = 0.4$ , 4 sub-groups and 3 degrees of freedom, the computed Power  $(1 - \beta) = 0.5230$ .

***Limitation with tissue availability:***

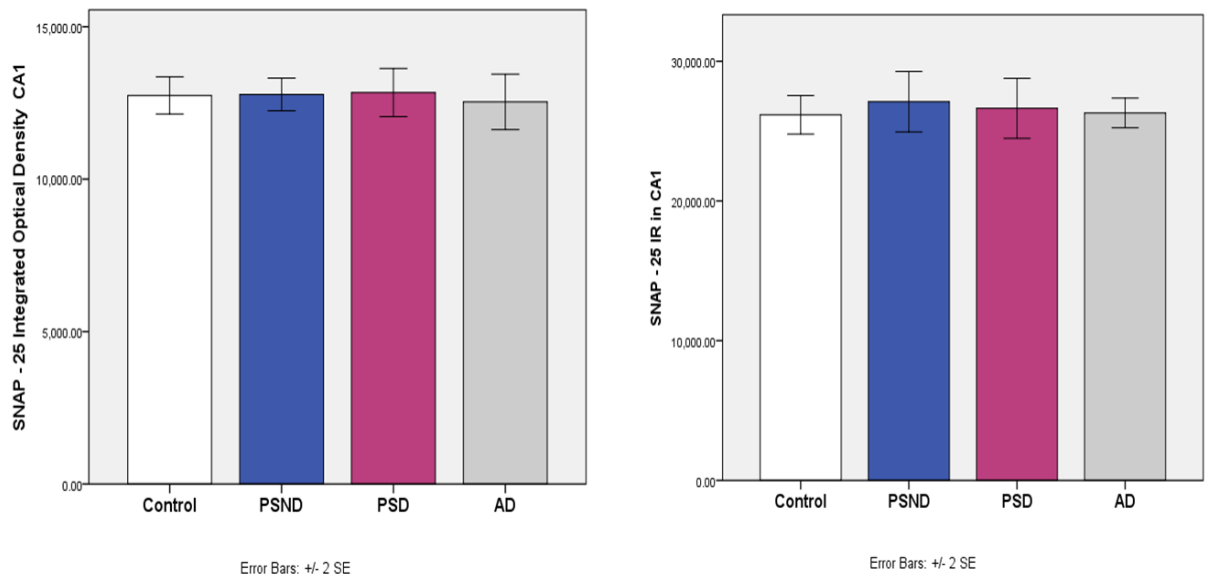
Pilot experiments had shown that the immunoreactivity of some synaptic and white matter pathology markers varied with certain parameters such as fixation period. We were therefore constrained to use only cases which parameters fell within certain acceptable ranges such as fixation period less than 24 weeks. Also, we had to ensure that cases were well matched for demographic variables – age and gender. Furthermore, we had to select cases that had both paraffin – embedded brain tissue blocks and frozen tissue for synchrony of findings from immunohistochemistry and immunoblotting synaptic markers experiments. All the aforementioned reasons limited the number of cases we could possibly select from an already limited pool of tissue available in the Newcastle Brain Tissue Resource (NBTR).

**6.3.1.1. SNAP -25**

SNAP - 25 showed strong uniform staining in the neuropil of all the hippocampal regions excluding pyramidal neuronal cell bodies of the CA and the granule cells of the dentate gyrus. It was difficult to distinguish differences in specific IR signals from background even at high magnifications (Figure 6.1) to detect significant differences. Figure 6.2 shows the integrated optical density (IOD) and immunoreactivity of SNAP – 25 across groups of a representative sub – cohort in the CA1 hippocampal sub - region. The measures of SNAP – 25 were similar in other hippocampal sub – regions.



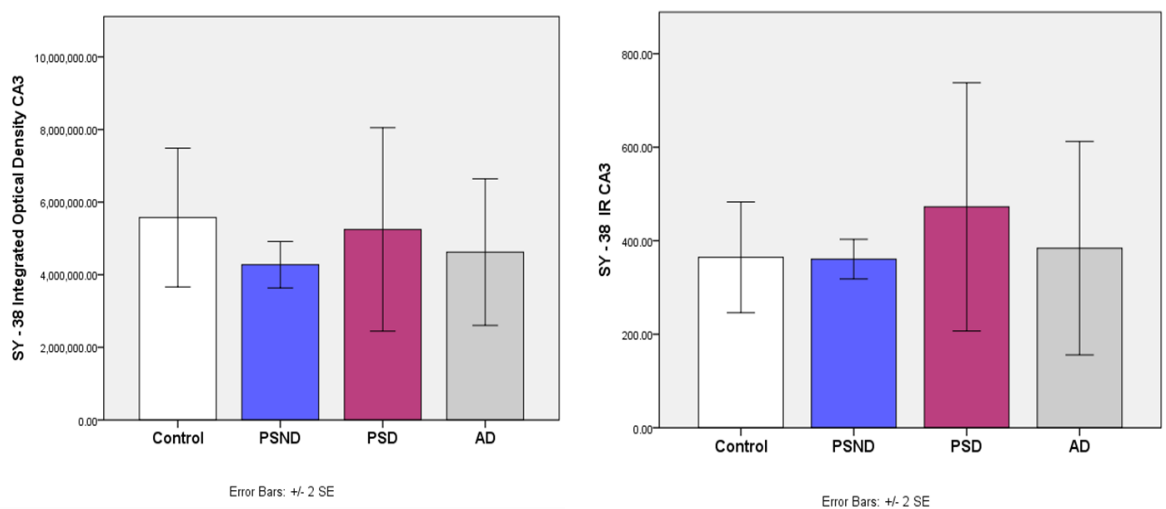
**Figure 6.1. Representative images showing variation of Drebrin, PSD – 95, SNAP – 25, SY -38, VGLUT -1 immunostaining across different groups : C, PSND, PSD, and AD. Hippocampal sub –region CA1 (Drebrin and SNAP – 25) and CA3/4 (PSD – 95, SY – 38 and VGLUT – 1).**



**Figure 6.2.** Bar graph showing the relative expression of SNAP - 25 across diagnostic categories with no significant difference ( $p = 0.808$ , Kruskal Wallis Test)

### 6.3.1.2. Synaptophysin

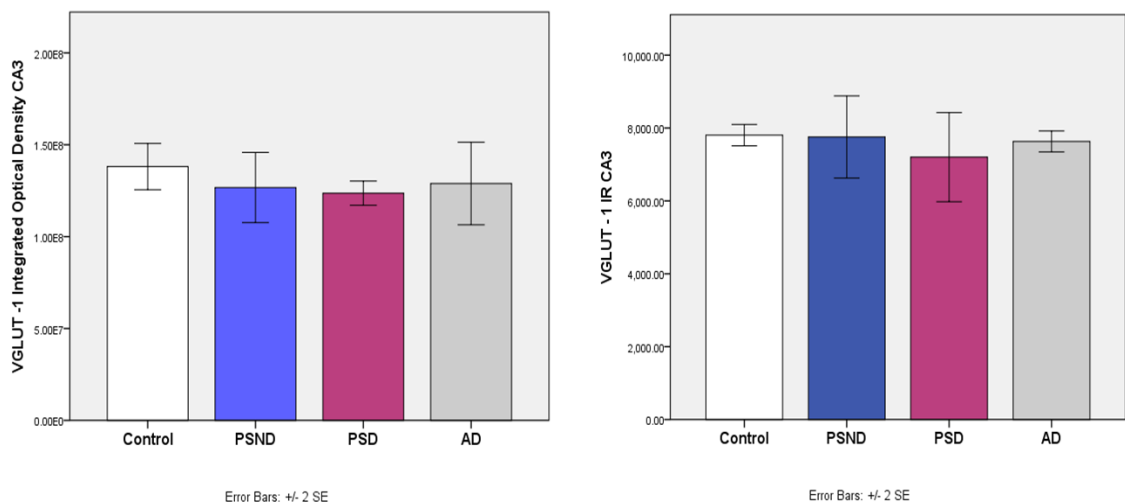
Synaptophysin demonstrated expected non - uniform staining of the neuropil that was very intense particularly in the outer molecular layer (OML) of the dentate gyrus, and the stratum moleculare and radiatum of the CA. Neuronal cell bodies were not stained. Furthermore, punctate immunoreactive deposits were seen in the CA3/4 sub-region (Figure 6.1). Quantification of immunoreactivity by Image – Pro Plus was performed on a subset of cases from each group and this revealed no significant differences across groups ( $p > 0.05$ ) even after excluding long – fixed cases (Figure 6.3).



**Figure 6.3.** Bar graph showing the relative expression of –SY - 38 integrated optical density (IOD) and immunoreactivity (IR) in the CA3 sub – region . There was no difference across diagnostic categories ( $p = 0.537$ , Kruskal Wallis Test).

### 6.3.1.3. VGLUT -1

VGLUT- 1 demonstrated intense immunoreactivity in the neuropil of the hippocampus particularly in the stratum pyramidale layer but excluding the bodies of pyramidal neurons. Punctate deposits were seen around pyramidal neuronal cell bodies and along axons in the CA3/4 sub- region in particular (Figure 6.1). Quantification by Image Pro Plus was performed on a subset of cases from each group and this revealed slightly reduced ( $p > 0.05$ ) expression of VGLUT-1 in PSD compared to Control and PSND in the CA3/4 sub – region. There were no differences in the intensity of immunoreactivity across groups in other sub – regions (Figure 6.4).



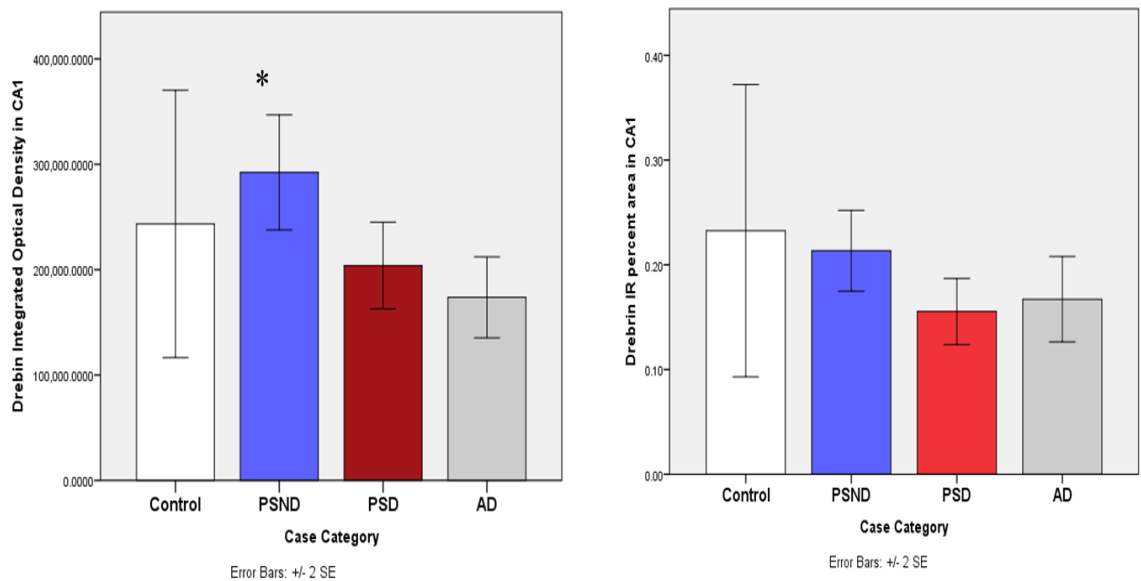
**Figure 6.4: Bar graph showing the relative expression of VGLUT - 1 integrated optical density (IOD) and immunoreactivity (IR) in the CA3 sub – region. There was no difference across diagnostic categories ( $p = 0.537$ , Kruskal Wallis Test)**

### 6.3.1.4. PSD - 95

PSD – 95 demonstrated low staining intensities across the hippocampal sub – regions. The CA1 region yielded very low specific staining .The inner and outer molecular layers of the dentate gyrus as well as the CA3/4 sub – region showed low and varying intensity of immunostaining across cases. Thus, rigorous quantification of PSD - 95 immunoreactivity by Image – Pro Plus was precluded and further quantification was undertaken by protein immunoblotting.

### 6.3.1.5. Drebrin

Drebrin immunoreactivity in the hippocampus showed specific and moderately intense staining of pyramidal neurons of the CA and cells of the outer molecular layer of the dentate gyrus. The signal – noise ratio was more distinct compared to PSD – 95, the other post –synaptic marker (Fig 6.1) and this enabled quantification of immunoreactivity by Image Pro Plus. The integrated optical density of Drebrin (IOD) in the CA1 sub - region varied significantly ( $p = 0.028$ , Kruskal Wallis Test) and was higher in PSND than PSD ( $p = 0.027$ ) and AD ( $p = 0.004$ ) (Mann - Whitney U), The percentage area was also slightly higher in the control and PSND, but this did not attain statistical significance ( $p = 0.183$ , Kruskal Wallis Test )(Figure 6.5).



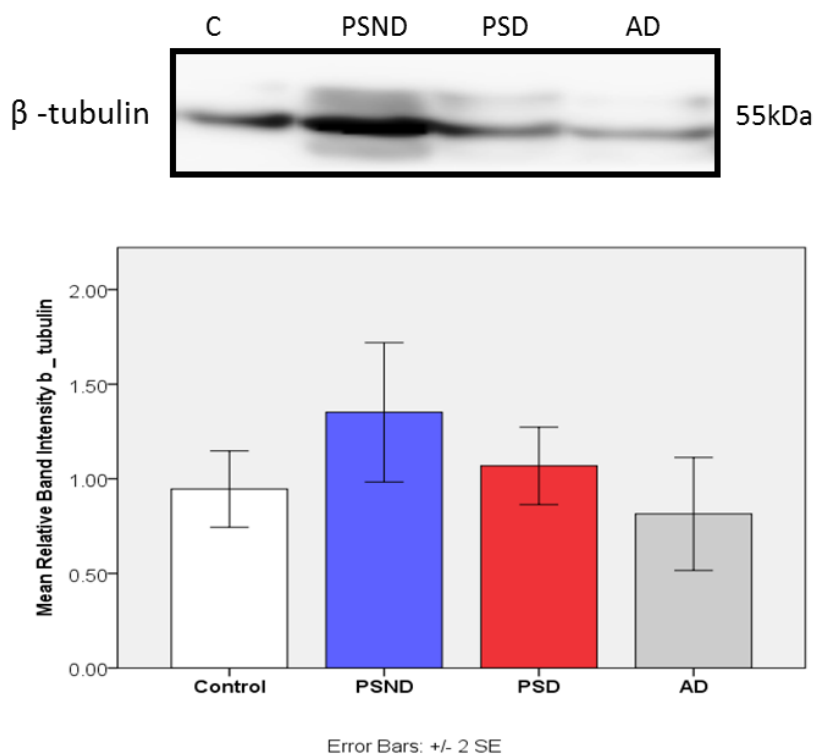
**Figure 6. 5: Bar graphs displaying mean Drebrin immunoreactivity, a) integrated optical density in CA1, b) percent area in CA1 \*  $P < 0.05$  Drebrin IR displays no significant difference in CA2, CA3 and DG.**

### 6.3.2. Immunoblotting Analysis

To determine the potential changes in hippocampal pre- and post- synaptic markers, we used immunoblotting techniques and antibodies to  $\beta$  – tubulin III (Figure 6.4), synaptophysin (Figure 6.5), SNAP -25 (Figure 6.6), VGLUT - 1(Figure 6.7), PSD -95 (Figure 6.8) and Drebrin (Figure 6.9) as described in Section 6.2.4

#### 6.3.2.1. $\beta$ - tubulin

$\beta$  – tubulin III levels were quantitatively determined as described in previous studies (Nieto *et al.*, 1989; Sze *et al.*, 1997; Kirvell *et al.*, 2006). This marker has been used as a neuron –specific protein as a control to confirm normalization of the sample loading. The antibody recognized a distinct band at 55 kDa (Fig 6.6). Densitometric analysis revealed a slight variation in the expression of  $\beta$  – tubulin although this was not statistically significant ( $p > 0.05$ , Kruskal Wallis) (Fig 6.4).



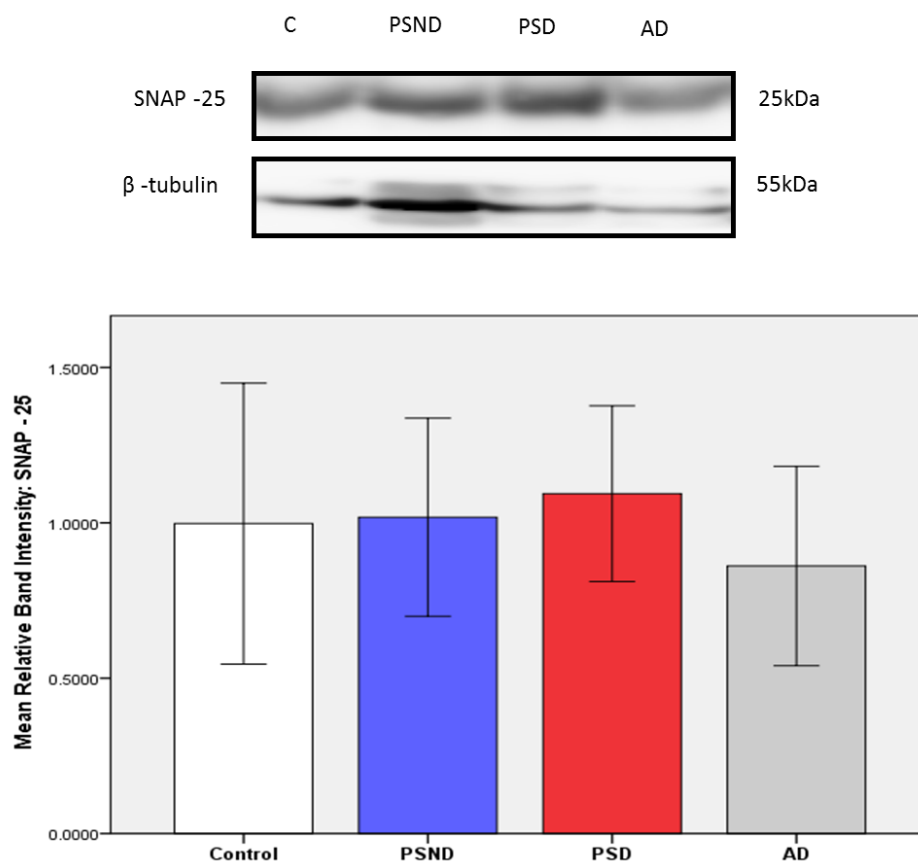
**Figure 6.6:** Bar graph showing the relative expression of  $\beta$  – tubulin across diagnostic categories. with no significant difference ( $p > 0.05$ , Kruskal Wallis Test)

### 6.3.2.2. Pre-synaptic Markers

The expression of synaptosomal – associated protein kDa 25 (SNAP -25), synaptophysin and vesicular glutamate transporter 1 (VGLUT - 1) were similarly quantified by densitometric analysis and normalized to the expression of  $\beta$  - tubulin.

#### 6.3.2.2.1 SNAP -25

SNAP -25 recognized a distinct band at 25kDa consistent with previous observations (Downes *et al.*, 2008). The expression of SNAP – 25 was similar across diagnostic groups ( $p = 0.476$ , Kruskal Wallis Test) (Fig 6.7).



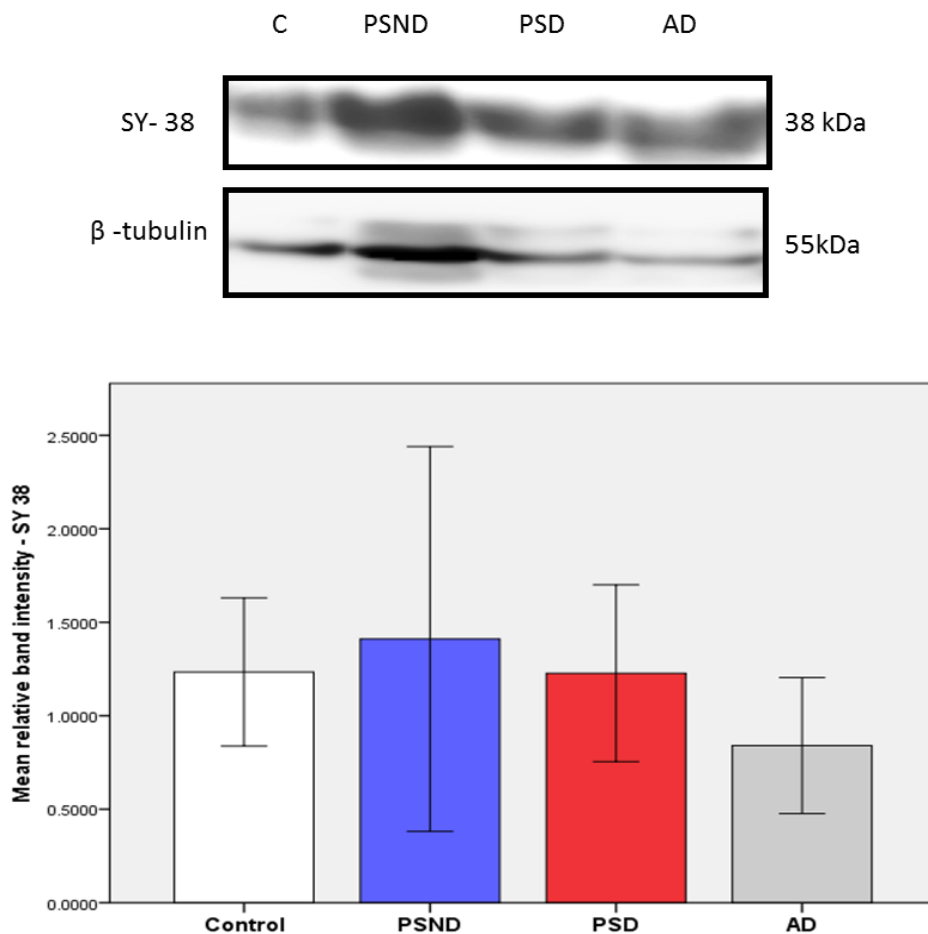
**Figure 6.7:** Bar graph showing the expression of SNAP -25 relative to  $\beta$  – tubulin across diagnostic categories with no significant difference ( $p = 0.476$ , Kruskal Wallis Test)



SNAP - 25 showed significant correlation with synaptophysin ( $r = 0.450$ ,  $p = 0.007$ ,  $n = 35$ ) but not with age ( $r = -0.169$ ,  $p = 0.284$ ,  $n = 42$ ) and cognitive function scores: CAMCOG total ( $r = -0.161$ ,  $p = 0.567$ ,  $n = 15$ ), CAMCOG memory ( $r = -0.095$ ,  $p = 0.736$ ,  $n = 15$ ).

### 6.3.2.2.2. Synaptophysin

Synaptophysin recognized a distinct band at 38kDa (Fig 6.8) consistent with extant literature (Downes *et al.*, 2008).



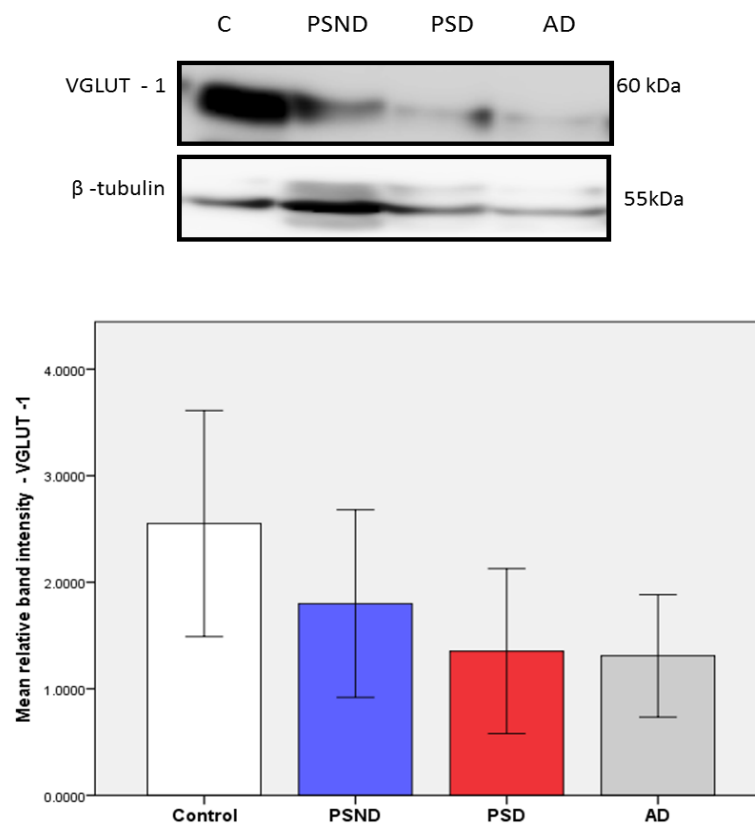
**Figure 6.8: Bar graph showing the relative expression of SY -38 relative to  $\beta$  - tubulin across diagnostic categories with no significant difference ( $p = 0.537$ , Kruskal Wallis Test)**

Densitometric analysis showed that synaptophysin expression did not vary significantly across disease groups ( $p = 0.537$ , Kruskal - Wallis Test). Synaptophysin demonstrated significant correlation with SNAP -25 ( $r = 0.450$ ,  $p = 0.007$ ,  $n = 35$ ) and VGLUT-1 ( $r =$

-0.414,  $p = 0.013$ ,  $n = 35$ ) but not with age ( $\rho = -0.006$ ,  $p = 0.973$ ,  $n = 35$ ) or CAMCOG total ( $\rho = 0.225$ ,  $p = 0.459$ ,  $n = 13$ ) but showed a trend with CAMCOG memory scores ( $\rho = 0.530$ ,  $p = 0.063$ ,  $n = 13$ ).

### 6.3.2.2.3. VGLUT -1

VGLUT- 1 is a specific marker of neurotransmitter - laden vesicles at excitatory glutamatergic synapses. In this Western Blot experiment, it was recognized as a band at about 60 kDa (Fig 6.9).



**Figure 6.9:** Bar graph showing the relative expression of VGLUT -1 across diagnostic categories with significant difference ( $p = 0.039$ , Kruskal Wallis Test).

VGLUT - 1 showed statistically significant differential expression across groups, highest in the control group and lowest in PSD and AD groups ( $p = 0.039$ , Kruskal – Wallis Test) (Fig 6.9). Further analysis for within group differences with Mann - Whitney U Test revealed no significant difference in VGLUT – expression between Control and PSND ( $p = 0.223$ ) whereas significant differences occurred between

Control and PSD ( $p = 0.020$ ) as well as between Control and AD ( $p = 0.019$ ) while the expression in PSD was not different from AD ( $p = 0.387$ ). VGLUT-1 expression correlated significantly with synaptophysin ( $\rho = 0.414$ ,  $p = 0.013$ ,  $n = 35$ ) and drebrin ( $\rho = 0.564$ ,  $p < 0.001$ ,  $n = 36$ ) but not with age ( $\rho = 0.033$ ,  $p = 0.836$ ,  $n = 42$ ). VGLUT-1 expression, also, correlated significantly with MMSE score ( $\rho = 0.540$ ,  $p = 0.038$ ,  $n = 15$ ), CAMCOG memory ( $\rho = 0.604$ ,  $p = 0.022$ ,  $n = 14$ ) and CAMCOG total ( $\rho = 0.518$ ,  $p = 0.048$ ,  $n = 15$ ) in the post-stroke subset of the cohort.

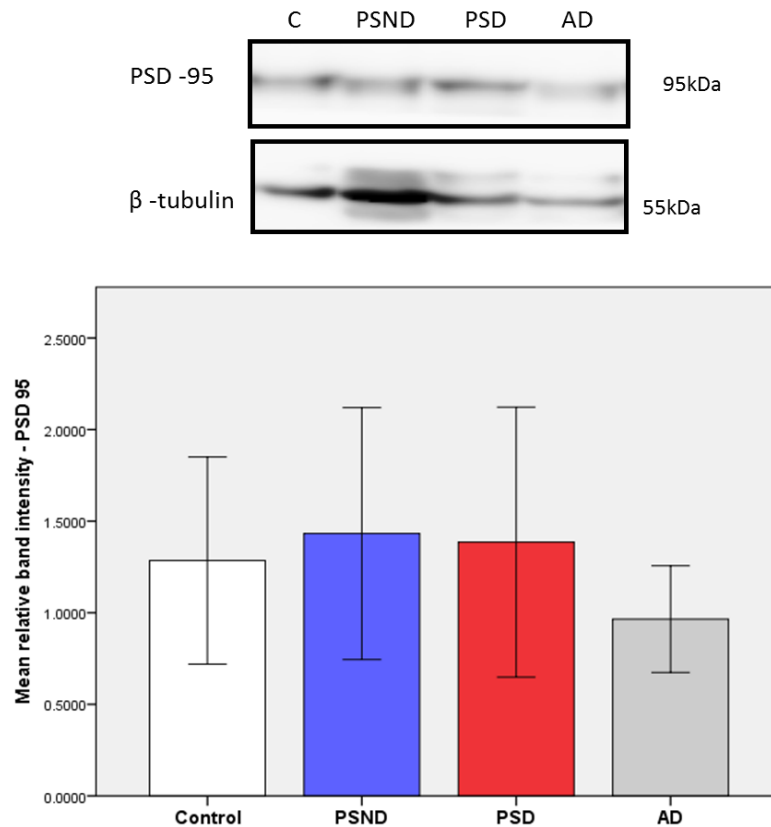
### **6.3.2.3. Post – synaptic markers**

The expression of the post-synaptic markers, PSD – 95 and Drebrin were similarly quantified by densitometric analysis and normalized to the expression of tubulin.

#### **6.3.2.3.1. PSD – 95**

This scaffolding protein is expressed in excitatory synaptic terminals. We detected it as a specific band at 95 kDa however, its expression relative to  $\beta$  – tubulin did not vary significantly across the groups ( $p = 0.694$ , Kruskal – Wallis Test) (Fig 6.10).

PSD - 95 did not correlate with age ( $\rho = 0.157$ ,  $p = 0.384$ ,  $n = 33$ ) or cognitive measures: CAMCOG memory ( $\rho = 0.377$ ,  $p = 0.283$ ,  $n = 10$ ) and CAMCOG total ( $\rho = 0.103$ ,  $p = 0.777$ ,  $n = 10$ ).

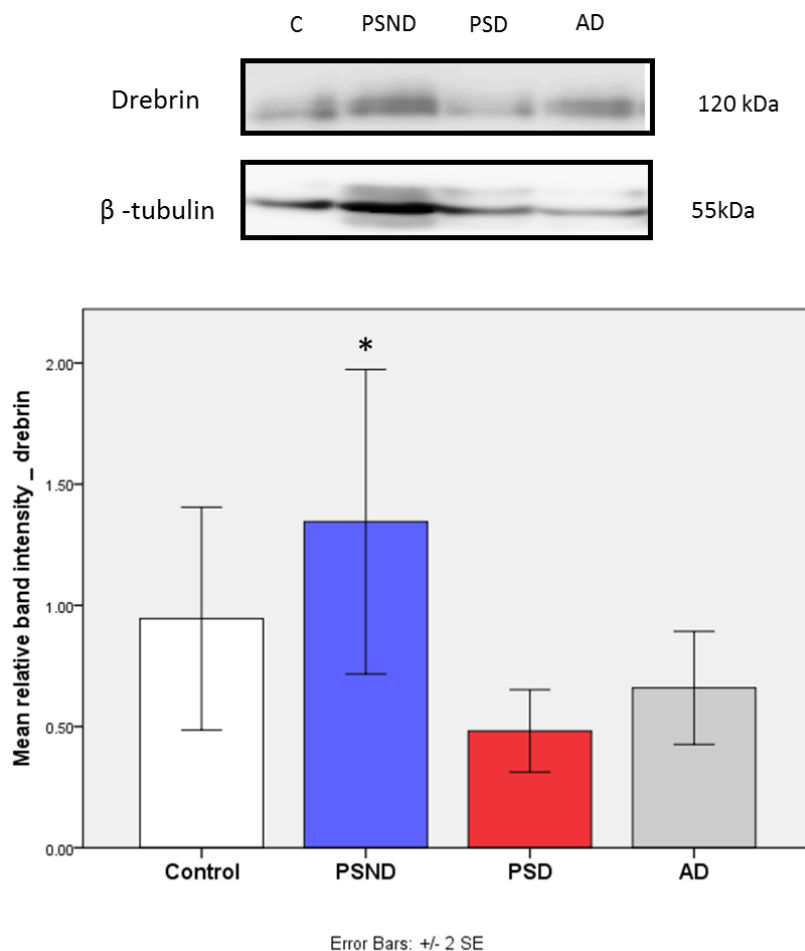


**Figure 6.10: Bar graph showing the relative expression of PSD - 95** across diagnostic categories with no significant difference ( $p = 0.694$ , Kruskal Wallis Test)

### 6.3.2.3.2. Drebrin

Drebrin is an F - actin binding protein which is localized to post - synaptic dendritic spines at excitatory synapses. It localized to a band at 120 kDa (Harigaya *et al.*, 1996; Counts *et al.*, 2012) and its expression level varied across groups ( $p = 0.05$ , ANOVA) (Figure 6.11).

Post - hoc analysis by Fisher's LSD showed that the relative intensity of drebrin expression was not significantly different between PSND and control ( $p = 0.197$ ) but higher than PSD ( $p = 0.009$ ) and AD ( $p = 0.049$ ). Drebrin showed significant correlation with VGLUT-1 ( $r = 0.564$ ,  $p < 0.001$ ,  $n = 36$ ). There was a significant negative correlation with hippocampal vascular score ( $r = - 0.721$ ,  $p = 0.028$ ,  $n = 9$ ) and robust positive correlation with the cognitive parameters: MMSE ( $r = 0.662$ ,  $p = 0.014$ ,  $n = 13$ ), CAMCOG memory ( $r = 0.683$ ,  $p = 0.014$ ,  $n = 12$ ) and CAMCOG total ( $r = 0.825$ ,  $p = 0.001$ ,  $n = 12$ ) in the post - stroke sub - cohort.



**Figure 6.11: Bar graph showing expression of Drebrin relative to  $\beta$  – tubulin across diagnostic categories with partial significant difference ( $p = 0.05$ , ANOVA).**

#### 6.4. Discussion

Given that synaptic integrity is critical to the effectiveness of neural communication, and its compromise is an early feature of brain disorders causing cognitive impairment and dementia (Scheff and Price, 2003; Di Maio, 2008; van Spronsen and Hoogenraad, 2010), we attempted to differentiate PSD and PSND subjects. Expression of synaptic markers (SNAP -25, synaptophysin, VGLUT -1, PSD – 95 and Drebrin) by immunohistochemical assessment showed no difference between PSND and PSD except for Drebrin where expression was higher in PSND than PSD. Quantification of synaptic markers by immunoblotting showed a non – statistically significant higher expression in PSND than PSD in synaptophysin, VGLUT -1 and PSD – 95 but significantly higher in Drebrin. Expression was lower in AD compared to the post – stroke groups across most markers, and comparable between the control and PSND across most markers.

The major findings here are that there was a significant reduction in the expression of VGLUT -1 and Drebrin in PSD and AD subjects in comparison with control and PSND subjects. In addition, both markers showed significant positive correlation with cognitive performance measures on the CAMCOG total and memory domain as well as with each other. However, one – way analysis of variance revealed no significant differences in the level of expression of SNAP -25, synaptophysin and PSD – 95 across the various subject groups. The anatomical distribution of immunoreactivity of pre – synaptic markers (SNAP – 25, SY – 38 and VGLUT -1) was as expected in the neuropil excluding neuronal cell bodies. Of particular interest were the punctate concretions of SY – 38 and VGLUT – 1 immunoreactivity within the CA3/4 sub – regions in keeping with changes to the hippocampal circuitry and re – organization of synaptic networks occasioned by disease processes as previously reported by others (Kirvell *et al.*, 2006; Kashani *et al.*, 2007; Kashani *et al.*, 2008; van der Hel *et al.*, 2009; van Spronsen and Hoogenraad, 2010). On the other hand, drebrin and PSD -95 were more localized to pyramidal neuronal cell bodies due to their location in the post – synaptic density (Keith and El-Husseini, 2008).

The reduction of VGLUT- 1 expression in the hippocampus of AD subjects is consistent with, and extends the previous findings of down regulation of VGLUT -1 expression in the frontal, parietal and occipital cortices of AD subjects (Kirvell *et al.*, 2006; Kirvell *et al.*, 2010). These results also corroborate the earlier findings of a dominant expression of VGLUT-1 in the human hippocampus as previously observed in hippocampal tissue sections of epileptic subjects with and without hippocampal sclerosis (van der Hel *et al.*, 2009).

In a previous study, Kirvell and colleagues (Kirvell *et al.*, 2010) had demonstrated a higher concentration of VGLUT – 1 in the frontal cortex of subjects who developed a stroke but did not progress to dementia. Their data also showed significant correlation between VGLUT-1 concentration and cognitive scores, especially the memory subscale of CAMCOG. Our data confirm and also extend these findings by demonstrating significant up - regulation of VGLUT-1 in the hippocampus of non - demented post – stroke subjects (PSND) and evidence of significant positive correlation with cognitive performance on the MMSE, CAMCOG memory and CAMCOG total scales.

Taken together, these previous results (Kirvell *et al.*, 2006; Kashani *et al.*, 2007; Kashani *et al.*, 2008; Kirvell *et al.*, 2010) and ours provide robust evidence in support of the role of glutamatergic synapses in the neurobiology of VCI and AD. Maintaining the glutamatergic system appears critical to the maintenance of cognitive functions after stroke in particular and this may be a molecular signature of cognitive reserve (Stern *et al.*, 1999; Stern, 2009; Stern, 2013) or an evidence of compensatory mechanisms that sustain cognition after vascular cerebral injury.

Drebrin is a neuron – specific, post – synaptic, actin – binding protein which is critical to dendritic spine morphogenesis, morphology and functioning. Our finding of a significant downregulation of Drebrin in the AD group is in tandem with previous studies demonstrating down regulation of drebrin in the hippocampus and temporal cortex of subjects with AD (Harigaya *et al.*, 1996; Hatanpaa *et al.*, 1999; Counts *et al.*, 2006; Counts *et al.*, 2012) Counts *et al.*, 2006, 2012) and these suggest that drebrin expression is an important predictor of deteriorating cognition in AD.

Our results demonstrate for the first time an upregulation of hippocampal drebrin expression in non – demented post stroke (PSND) subjects compared to demented stroke (PSD) and AD in agreement with previous report of drebrin upregulation in the frontal cortex of MCI subjects (Counts *et al.*, 2006). In contrast, a very recent study found reduced expression of drebrin in the hippocampus of subjects with mild cognitive impairment in comparison with controls (Counts *et al.*, 2012). This contradistinction suggests that different synaptic plasticity mechanisms operate in different brain regions (DeCarli *et al.*, 2012) and these may also differ between vascular and degenerative brain disorders.

These findings collectively underscore the critical role of dendritic spine dynamics in the neurobiology of cognition (Hara *et al.*, 2012). Furthermore, differences in the expression level of drebrin appear to be a possible predictor teasing out demented from non – demented post-stroke subjects. Previous neuropathological data from the Newcastle post-stroke cohort had shown that whereas control and non – demented stroke subjects (PSND subjects maintained pyramidal hippocampal neuronal volumes, demented post –stroke subjects (PSD) and other dementias (mixed AD/VaD, and VaD) showed significant shrinkage of hippocampal pyramidal neurons in the CA1 AND CA2 sub –regions (Gemmell *et al.*, 2012). It seems therefore that there may be an association

between the upregulation of hippocampal drebrin and the maintenance of neuronal volume. Perhaps, maintenance of neuronal volume and metabolic activity preserves synaptic connections and the dendritic network surrounding the neuron (Hara *et al.*, 2012; Kalaria, 2012b).

The correlation between VGLUT – 1 (a pre – synaptic marker) and Drebrin (a post – synaptic marker) respectively may reflect functional synergy as the two markers are both involved in the cascade of glutamatergic neurotransmission. It had been shown previously in cultured hippocampal neurons that accumulation of drebrin within dendritic spines depends on AMPA receptor activation by glutamate (Takahashi *et al.*, 2004). The magnitude of AMPA receptor activation depends on the quantity of glutamate released at the pre - synaptic terminal, and this in turn depends on the level /quantity of available vesicular transporters. Substantiating this idea, Hsieh *et al.* (2006) showed reduced AMPA receptors and loss of dendritic spines (and consequently drebrin) with increased  $\beta$  – amyloid levels in neuronal cell culture (Hsieh *et al.*, 2006).

SNAP – 25 and SY- 38 are ubiquitously distributed in the brain as they generally mark synaptic membranes (Geddes *et al.*, 1990a; Geddes *et al.*, 1990b). Although reduced expression of SNAP -25 (Sze *et al.*, 2000; Minger *et al.*, 2001) and SY – 38 (Heinonen *et al.*, 1995; Kirvell *et al.*, 2006; Downes *et al.*, 2008) in AD have been reported previously, the mild reduction of their expression in AD in our cohort did not attain statistical significance. Expression of SNAP -25 and SY – 38 in the post-stroke groups (PSND and PSD) were not significantly different from each other, or from controls. This may suggest that synaptic changes of significance in vascular disorders relate more to specific neurotransmitter systems rather than universal synapses such as SNAP -25 and synaptophysin , especially in the early stages of disease.

For the postsynaptic density – 95 (PSD – 95), there was a slight reduction in its expression in AD which did not attain statistical significance. Previous studies have shown conflicting results of PSD - 95 dysregulation in AD. In some instances (Gyls *et al.*, 2004; Love *et al.*, 2006) a down regulation was reported while Leuba *et al.* (2008)(Leuba *et al.*, 2008) reported an upregulation. Reduced expression of PSD – 95 has also been reported in MCI subjects (Sultana *et al.*, 2010) and in Wistar Rat stroke models exposed to nitrogen dioxide (Li and Xin, 2013). Contrary to expectations, we found no significant difference between controls, non – demented post-stroke and



demented post – stroke groups. We had expected to see a pattern similar to that of drebrin since they were both post-synaptic markers and related to post – synaptic transmission. However, drebrin plays a more strategic role in the regulation of dendritic morphogenesis and function than PSD – 95 (Harigaya *et al.*, 1996; Kojima and Shirao, 2007; Sekino *et al.*, 2007).

Correlation between SY – 38 and VGLUT-1 and between SY – 38 and SNAP -25 could imply mechanistic synergy among the three pre – synaptic markers (Geddes *et al.*, 1990a; Geddes *et al.*, 1990b). However, the absence of similar correlation between PSD – 95 and Drebrin, both being post –synaptic markers may be due to differential modes of action of both markers and their susceptibility to different regulatory factors (Keith and El-Husseini, 2008).

The positive correlation between hippocampal vascular score and SNAP -25, and the negative correlation with drebrin have not been reported before. It is intuitive that direct relationship with SNAP -25 may be a compensatory response or release of this marker in response to vascular damage. A possible explanation for the negative correlation between vascular score and drebrin may be that it is due to the effect of chronic hypoperfusion on the neuronal cell. Evidence is growing in favour of a vascular basis of neurodegeneration (Kalaria, 2012b; Kalaria, 2012a). Neuroimaging studies have shown evidence of medial temporal atrophy of vascular origin (den Heijer *et al.*, 2003; Bastos-Leite *et al.*, 2007; Qiu *et al.*, 2012). The hippocampal neuronal atrophy described in Gemmell *et al.* (2012) has also been suggested to be of vascular origin as the cases had little or minimal neurodegenerative pathology (Gemmell *et al.*, 2012). Neuronal atrophy with accompanying reduction/loss of dendritic network and constituent dendritic spines could then account for a reduction in the expression of drebrin.

It is of note in this study that we observed some alterations (although not significant) in the expression of  $\beta$  – tubulin across diagnostic groups. Although, as in previous studies (Nieto *et al.*, 1989; Sze *et al.*, 1997; Sze *et al.*, 2000; Kirvell *et al.*, 2006),  $\beta$  – tubulin was included as a loading control to ensure equal amount of protein was loaded for each sample. Densitometric quantification of the expression of each marker in the samples was then normalized to the expression of  $\beta$  – tubulin in the specific experiment.

Although a protein that is ubiquitously expressed, reduced expression of  $\beta$  – tubulin and other loading control markers may sometime occur because of the impact of pathologic

processes causing loss of neuronal cells and cerebral volume rather than just the effect of a disease – specific process. It is significant that similar alteration of  $\beta$  – tubulin has been reported in an immunoblotting experiment on AD cases without negating the integrity or interpretation of the results (Kirvell *et al.*, 2006)

Nevertheless, these results require independent validation in larger samples and other regions of the brain while we also acknowledge the potential influence of multiple pairwise comparisons and the impact on our conclusions. Overall, the combination of findings from this current study provides support for the conceptual framework that synaptic dysfunction is an early process in the evolution of cognitive impairment and may precede the appearance of gross pathologies. In addition, synaptic dysfunction correlates well with ante-mortem cognitive performance (especially the most proximate to death) and enables to distinguish different cognitive trajectories in longitudinally followed up patients. In our cohort, down regulation of VGLUT -1 and Drebrin differentiated between demented and non – demented stroke survivors.

## 6.5 Chapter Summary

Marker	Neuropathological Categories			
	Control	PSND	PSD	AD
SNAP -25	↔	↔	↔	↓
Synaptophysin	↔	↔	↓	↓
VGLUT -1	↑	↔	↓	↓
PSD – 95	↔	↔	↔	↓
Drebrin	↔	↑	↓	↓

**Table 6.2. Summary of findings on hippocampal synaptic markers**

**Annotation:** (↔ = little or no change; ↑ = increase; ↓ = decrease )

### ▪ *Correlations*

- Inter - marker correlations were significant between :
  - Synaptophysin and VGLUT -1
  - Synaptophysin and SNAP – 25
  - VGLUT -1 and Drebrin
- Correlation between markers and cognitive scores
  - VGLUT -1 showed significant positive correlation with MMSE score, CAMCOG memory and CAMCOG total scores.
  - Drebrin showed significant positive correlation with MMSE score, CAMCOG memory and CAMCOG total scores.

## **Chapter 7. Assessment of white matter pathology in post - stroke demented subjects compared with AD and normal ageing controls**

### **7.1. Introduction**

Diffuse white matter (WM) changes are common in stroke survivors. WM pathology was evident upon MRI in the Nigerian post-stroke survivors similar to WM burden previously documented in the Newcastle elderly cohort (Burton *et al.*, 2004). The changes were associated with significant cognitive deficits, predominantly attentional impairment and executive dysfunction. There were increased reaction time (reduced information processing speed) and impairment of cognitive flexibility, functions subserved by the frontal lobe (Hoffmann, 2013). Episodic memory dysfunction was also prominent in both series with a predictive value on progression of cognitive decline from vCIND to PSD (Ballard *et al.*, 2002; Ballard *et al.*, 2003a; Stephens *et al.*, 2004). The temporal lobe, particularly the hippocampus, is vital to the neurobiology of memory formation and storage (Suzuki and Amaral, 2004). Cortico – cortical and cortico – subcortical connections present within the WM provide important anatomical connections underlying complex brain networks associated with cognitive control mechanisms. Disruption of these connections have been implicated in ‘disconnection syndromes’ with consequent cognitive and behavioural deficits (Geschwind, 1965a; Geschwind, 1965b; Catani and ffytche, 2005).

The integrity of WM is critical to the regulation and the efficiency of neuronal communication and maintenance of cognitive functioning (Nave, 2010). Loss of WM integrity demonstrated by hyperintense signal on T2W MRI occurs in the settings of ageing, cerebrovascular diseases and their associated dementias (VCI and AD and have been associated with increased risk of stroke, dementia and death (DeBette and Markus, 2010). Clinically, white matter hyperintensities (WMHs) cause reduced psychomotor speed, impairment of working memory and cognitive inflexibility, otherwise semantically referred to as executive dysfunction (DeCarli *et al.*, 1996) (Nordahl *et al.*, 2006) (DeBette and Markus, 2010). Imaging studies have revealed WM abnormalities due to ageing (de Leeuw *et al.*, 2001) as well as in dementing disorders particularly vascular cognitive impairment and Alzheimer’s disease (AD). Particularly, WMHs contribute significantly to cognitive deficits after stroke (Burton *et al.*, 2004; Jokinen *et al.*, 2005; Lawrence *et al.*, 2013). More recent techniques including diffusion tensor

imaging (DTI) and magnetization transfer imaging (MTI) are now being used to study microstructural integrity of the WM in greater detail, including ordinarily normal appearing WM (Gunning-Dixon *et al.*, 2009; Kennedy and Raz, 2009a; Kennedy and Raz, 2009b; Vernooij *et al.*, 2009).

Increased mean WM diffusivity and decreased generalized fractional anisotropy have been observed in subjects with post - stroke VCI with the predominant changes being in major axonal bundles in the frontal regions and anatomically related cortical and sub - cortical structures (Jin Thong *et al.*, 2013). In another study on a cohort of subjects with lacunar stroke and confluent WM changes, diffusivity of normal appearing WM and lacunar infarct count were found to predict executive dysfunction while radial diffusivity (suggesting demyelination) rather than axial diffusivity ( suggesting axonal damage) was an important predictor of overall cognitive impairment (Lawrence *et al.*, 2013).

Neuropathological correlates of WMH include myelin loss, axonal damage and gliosis (Kalaria and Ballard, 1999; Kalaria *et al.*, 2004; Kalaria, 2012a). However, the exact mechanisms of cerebral injury leading to post - stroke cognitive impairment are not yet fully understood even though additive interactions between vascular pathology (including WM disease) and neurodegenerative pathology are increasingly substantiated (Haight *et al.*, 2013; Kalaria and Ihara, 2013). The molecular mechanisms underlying WM disease are complex, but there is a clear demonstration of the role of hypoxia, immune modulation (such as activation of glial cells), apoptosis, impaired regulation of ion imbalances across membranes and mitochondrial dysfunction resulting in demyelination and/or axonal damage (Pantoni and Garcia, 1995; Fernando *et al.*, 2004; Fernando *et al.*, 2006; Simpson *et al.*, 2007a; Simpson *et al.*, 2007b; Simpson *et al.*, 2009). Ischaemic demyelination is a major pathophysiological mechanism but the role of axonal degeneration is not very clear yet (Ihara *et al.*, 2010b; Horsburgh *et al.*, 2011).

Neuroinflammation characterized by microglial and astrocytic activation is believed to contribute to the pathogenesis of dementing disorders. For instance, there is robust evidence from neuropathological and neuroimaging studies using Pittsburgh Compound B (PIB) showing the activation of microglia by the presence and accumulation of amyloid fibrils (Cagnin *et al.*, 2001; Edison *et al.*, 2008). In studies examining the preservation of cognitive functions despite high amyloid burden, compensatory

mechanisms of early cellular response associated with activation of glial cells and neuronal nuclear hypertrophy have also been implicated (Erten-Lyons *et al.*, 2009). This has not been explored, particularly in the setting of stroke-related cognitive impairment in comparison with other dementing disorders.

Neuroinflammation is associated with demyelination and the severity of WM changes quantified by different techniques in animal and human studies has been found to correlate with the level of degraded myelin basic protein (dMBP) in vascular dementia and models of chronic hypoperfusion (Whitehead *et al.*, 2005; Whitehead *et al.*, 2007; Ihara and Tomimoto, 2011). Axonal damage has also been well studied in head injury, immune-mediated demyelinating brain disorders such as multiple sclerosis, viral and parasitic infections but is less well studied in dementing disorders (Medana and Esiri, 2003). Axonal damage may be quantified by measurements of proteins transported by axonal transport such as the amyloid precursor protein (APP) (Akiguchi *et al.*, 2004; Buttner *et al.*, 2006) as well as the neurofilament protein SMI 32 (Lindner *et al.*, 2009; Craggs *et al.*, 2013).

The objective of the study described in this chapter was to investigate the contribution of microglial and astrocytic activation, demyelination and axonal damage to the pathobiology of post-stroke cognitive dysfunction. We hypothesized that markers of glial activation, demyelination and axonal damage will be differentially expressed in the WM of PSND and PSD compared to AD and controls.

## **7.2. Methods**

### **7.2.1. Subjects**

Forty seven subjects consisting of twelve subjects with post-stroke dementia (PSD), twelve subjects with stroke but no dementia (PSND), twelve subjects with Alzheimer's disease (AD) and eleven controls were assessed in the study. Subjects selected Table 7.1 provides details of the demographic, cognitive and pathological characteristics of the subjects. There were no significant differences in the age ( $p = 0.786$ ), gender distribution ( $p = 0.493$ ), post-mortem delay ( $p = 0.902$ ) and length of fixation of tissues ( $p = 0.589$ ). Autopsies were performed between 5 and 67 hours after death and brains were fixed for between 2 and 32 weeks. Subjects with as short fixation period as

possible (< 24 weeks) were chosen for this study from the bigger cohort described in Section 5.2.1. The CogFAST Study and ancillary studies had ethical approval from the local Newcastle Ethical committees and participants gave written consent to brain tissue donation. Use of brain tissue was also approved by the local Ethical committees and the committee of the Newcastle Brain Tissue Resource (NBTR).

Variable	Clinical Diagnosis				p –value
	Control	PSND	PSD	AD	
Number	11	12	12	12	
Age (years)	82.7 ± 9.9	84.3 ± 5.2	85.8 ± 6.7	84.5 ± 5.9	0.786
Gender: n (% F)	6 (54.5)	5 (41.7)	5 (41.7)	7 (58.3)	0.493*
PM delay (hours)	22.9 ± 6.8	22.7 ± 6.8	21.4 ± 7.8	24.2 ± 2.8	0.902
Fixation (weeks)	9.2 ± 4.1	10.4 ± 5.7	9.4 ± 6.0	7.3 ± 6.3	0.589
CAMCOG total	ND	91.5 ± 5.2	53.4 ± 13.5	ND	< 0.001 <sup>+</sup>
CAMCOG exec	ND	15.9 ± 4.0	5.5 ± 4.8	ND	< 0.001 <sup>+</sup>
CAMCOG mem	ND	21.50 ± 2.6	9.3 ± 7.5	ND	< 0.001 <sup>+</sup>
Braak Stage	1.8 ± 1.0	2.3 ± 0.9	2.9 ± 1.6	5.4 ± 0.8	< 0.001
CERAD score	0.2 ± 0.4	1.2 ± 0.8	1.4 ± 1.1	2.5 ± 1.2	< 0.001
Vasc score - fron	2.7 ± 0.3	4.3 ± 0.8	4.6 ± 1.1	2.2 ± 0.3	< 0.001
Vasc score – temp	2.3 ± 0.2	3.3 ± 1.5	3.4 ± 1.2	2.1 ± 0.2	0.137

**Table 7.1: Demographic, cognitive and pathological characteristics of the subjects,** \*chi - square test; + students’ t – test; all others ANOVA. Subjects do not vary significantly with age, gender, post – mortem delay or length of fixation period. CAMCOG = Cambridge Cognitive Examination; CERAD = Consortium to Establish a Registry for Alzheimer’s disease; ND = No data available; Vasc score – fron = Vascular Score – frontal; Vasc score – temp = Vascular score temporal.

### 7.2.2. Neuropathological examination

Frontal lobe sections at the level of the olfactory bulbs (corresponding to coronal levels 4 - 6) and temporal lobe sections at the level of the anterior hippocampus (corresponding to coronal levels 18 – 20) were selected from the Newcastle Brain Map (Perry and Oakley, 1993). Formalin fixed paraffin blocks [frontal and temporal] were retrieved from the NBTR and processed as described in Chapter 2. Haematoxylin and Eosin (H & E) and Luxol Fast Blue (LFB) stains were used to evaluate general neuropathologic changes and WM changes in accordance with standard protocol developed by our group (Kalaria *et al.*, 2004; Deramecourt *et al.*, 2012).

### **7.2.3. Quantitative immunohistochemistry**

Ten micron tissue sections obtained from the frontal and temporal blocks were immunostained with primary antibodies to microglia (CD68, 1: 400, Monoclonal, PGMI), astrocytes (GFAP, 1 : 4000, Polyclonal, Dako), anti - amyloid precursor protein clone 22C11 (APP, 1: 2000, Monoclonal, Chemicon) anti – nonphosphorylated neurofilament H (anti - SMI -32, 1: 1000, Covance, CA, USA) and degraded myelin (dMBP, 1: 2000, Polyclonal, AB5864) as described in Chapter 2. To minimize variability of immunohistochemical staining quality, control sections were included in assays, and experiments were run in duplicates for each marker by the same team (investigator assisted by technicians) and using freshly prepared buffer solutions and tinctorial stains. Sections were further counterstained with haematoxylin following the assays of CD68, GFAP and dMBP to enhance identification of neuronal and glial cellular structures. Sections were numbered randomly from 1 – 50 and then analysed blind to the diagnoses of the cases.

#### **7.2.3.1 Quantification of glial cellular activation**

Microglia and astrocytic activation were quantified by a combination of two approaches: [a] determination of percent area of region of interest, integrated optical density and derived total immunoreactivity using the Image Pro Plus (Version 4.0; Media Cybernetics; Silver Springs, MD, USA). [b]determination of glial (microglia and astrocytes) cellular count per unit area.

Images of 14 – 20 randomly selected regions of interest (ROI) within the WM of frontal and temporal sections were captured with a Zeiss Axioplan 2 research grade microscope coupled to an Infinity 2 camera at X10 magnification. Using the software Image Pro-Plus 4.0 (Media Cybernetics, Silver Spring, MD, USA), the images were analyzed using histogram-based analysis and obtaining the variables: per area, a measure of the number of pixels stained within the area of interest (AOI) and expressed as a percentage. The integrated optical density (IOD) was also determined, and the mean total immunoreactivity (IR) derived as described in Chapter 2. For determination of glial cellular number per area, a grid of 0.5mm<sup>2</sup> area was placed randomly over the ROI of

five random images. The numbers of cell bodies of microglia and astrocytes respectively, were counted and summed up for each case.

### **7.2.3.2 Assessment of White Matter Severity**

#### **7.2.3.2.1. White Matter Rating Scale**

A semi-quantitative WM rating scale [0 -3] (Deramecourt *et al.*, 2012; Smallwood *et al.*, 2012) was used to assess severity of WM damage by examining H & E and LFB stained sections using the Zeiss Axioplan 2 research grade microscope at X 5 and X 10 magnification. This was then correlated with the Myelin Index. On this scale, 0 = normal, 1 – mild, 2 = moderate and 3 = severe (Appendix 7.1)

#### **7.2.3.2.2. Myelin Index**

The Myelin Index was determined as a measure of normality of myelin. To determine the myelin index, images of sections stained with LFB were taken and converted into monochrome images and analysed using the gray scale of Image Pro Plus as follows. The WM was outlined manually using the semi-automatic trace tool taking care to exclude cortical tissue or areas of infarcted brain tissue. The lower and upper range of grey values within the entire WM was determined corresponding to the staining intensity which normally fell within the range 0 – white and 255 – black. This range of grey levels was divided up into four quartiles and the median grey level – a measure of staining intensity - value for each quartile further determined. The percentage area of each quartile was also determined and multiplied by the median grey level for that quartile. The sum total of this product for each of the four quartiles gave the total myelin index. This was an adaptation of the method described previously (Ihara *et al.*, 2010a)(Appendix7.2)



### **7.2.3.3. Semi-quantitative rating of dMBP immunoreactivity.**

Degraded myelin basic protein (dMBP) immunoreactivity was determined semi – quantitatively on a 4 – point Likert scale where 0 = none; 1 - mild; 2 = moderate and 3 = severe (Appendix 7. 3).

### **7.2.3.4. Assessment of Axonal damage**

Axonal damage was assessed by semi-quantitative rating of two markers on a 4 – point Likert scale where 0 = none; 1 - mild; 2 = moderate and 3 = severe (Appendix 7. 4).

Amyloid Precursor Protein (APP) is transported through axonal transport and tends to accumulate in conditions where there is compromise of axonal transport. It's particularly useful as a marker of acute axonal damage (Hortobagyi *et al.*, 2007). SMI - 32 is a non - phosphorylated neurofilament protein that is expressed by large pyramidal neurones. It is also found in association and commissural fibers which connect different cortical brain areas together. It has been described as useful marker of chronic axonal degeneration (Lindner *et al.*, 2009).

### **7.2.3.5. Vascular score**

Vascular score was determined for all groups according to the previously described methods (Deramecourt *et al.*, 2012).

## **7.2.4. Statistical analysis**

Statistical analysis was carried out using the IBM SPSS software (version 19.0). The Shapiro – Wilk Test was used to establish normality of data. Comparisons across groups were done using parametric tests (ANOVA for group means and Tukey post –hoc analysis for between – group differences) non- parametric tests Kruska – Wallis and Mann – Whitney U tests) for non –normal distribution. The relationship between myelin index (MI) and WM severity score, glial markers and axonal markers and demographic, cognitive and pathological variables were assessed using Spearman's correlation ( $\rho$ ).

### 7.3. Results

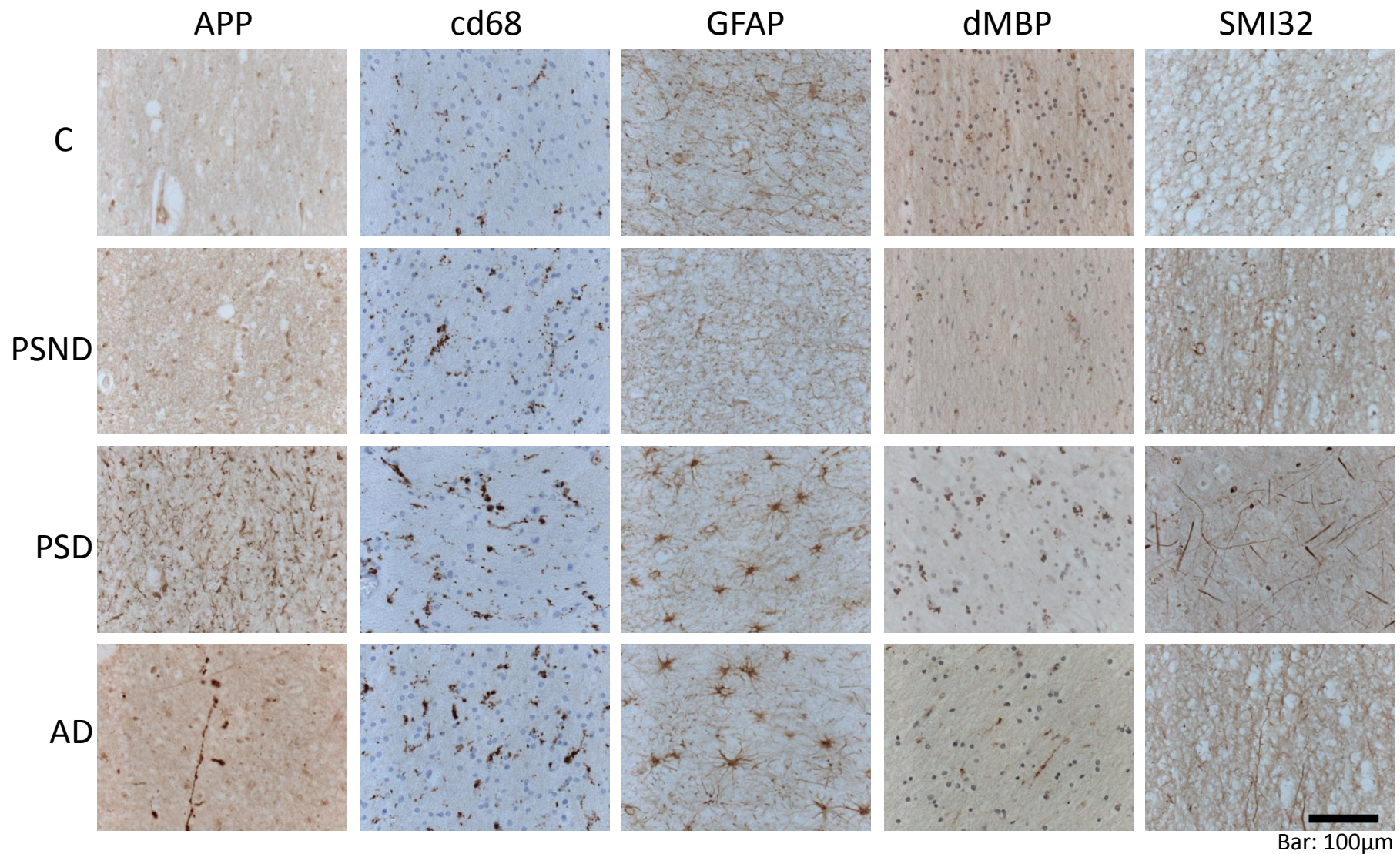
We found that the immunoreactivities of the selected markers were expectedly localised in cells and axonal structures as expected (Figure 7.1).

#### 7.3.1. *Glial Cell Markers*

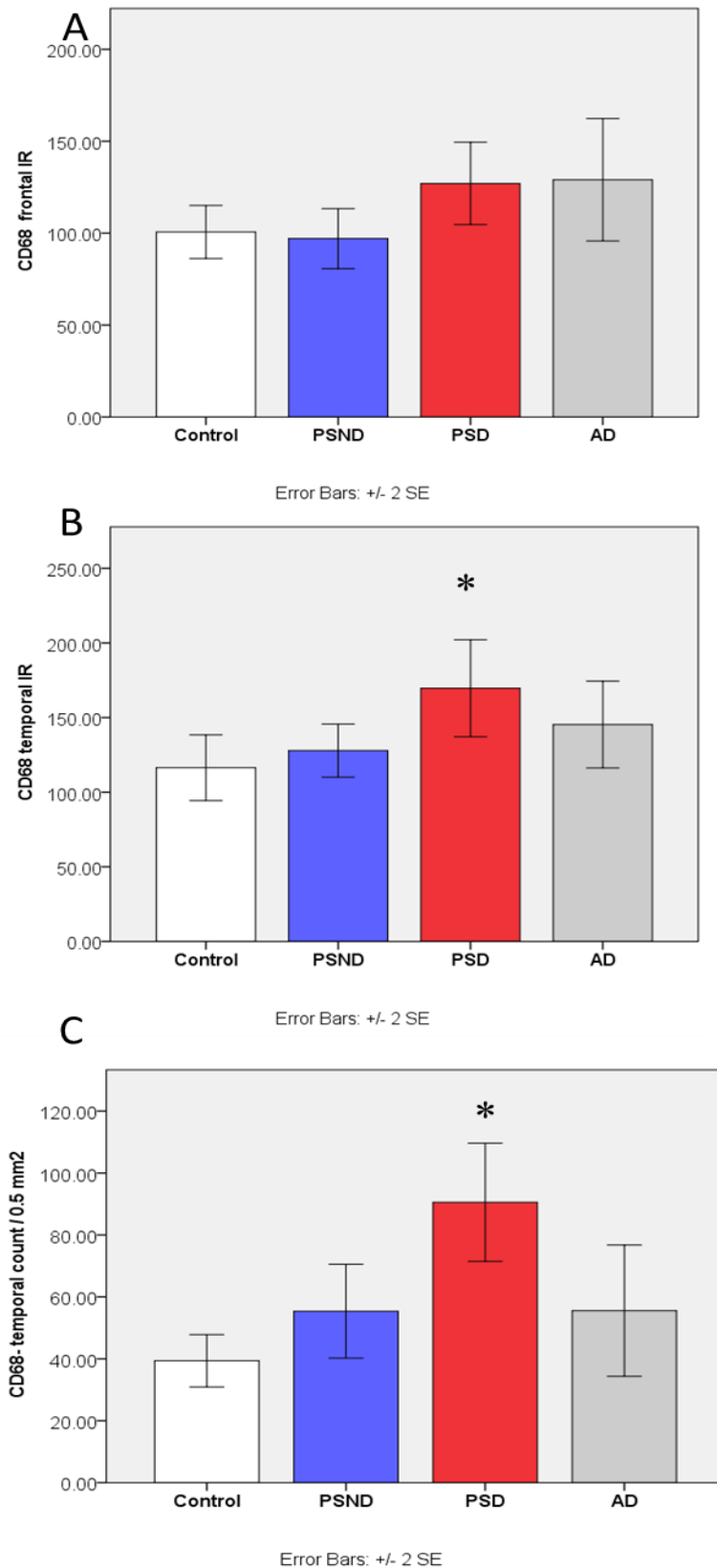
##### 7.3.1.1 CD 68

The results showed normal distribution by Shapiro – Wilk Test. CD68 immunoreactivity (IR) was not significantly associated with age, postmortem delay and length of fixation across the groups in both frontal and temporal WM ( $p > 0.05$ ). In the frontal WM, CD68 immunoreactivity was relatively higher in the AD and PSD groups compared to the PSND and control groups, although the difference did not attain statistical significance ( $p = 0.129$ , ANOVA)(Fig 7.2a) . However, in the temporal WM, there was significant variation in the CD68 per area ( $p = 0.031$ ), integrated optical density,IOD ( $p = 0.019$ ), total immunoreactivity, IR ( $p = 0.045$ ) and microglia cell count per  $0.5\text{mm}^2$  area of tissue ( $p = 0.002$ ) (ANOVA). Furthermore, post- hoc Tukey's test showed that CD68 IR was significantly higher in PSD compared to the control group ( $p = 0.019$ ) while microglial cell count was significantly higher in the PSD group compared to other groups ( $p < 0.05$ ) (Fig 7.2b and c). CD68 immunoreactivity showed significant positive correlation between the frontal and temporal WM ( $r = 0.497$ ,  $p < 0.001$ ). Also, there were significant positive correlation with CERAD score ( $r = 0.480$ ,  $p = 0.020$ ) and negative correlations with CAMCOG total ( $r = - 0.562$ ,  $p = 0.024$ ) and CAMCOG memory ( $r = - 0.516$ ,  $p = 0.041$ ) scores.

We computed the statistical power of this sub – sample of 47 cases of four groups using the G\*Power software (Faul et al, 2007). Given a significance level,  $\alpha = 0.05$  and assuming a moderate effect size Cohen's  $d = 0.4$ , 4 sub - groups and 3 degrees of freedom, the computed power ( $1 - \beta$ ) = 0.6569.



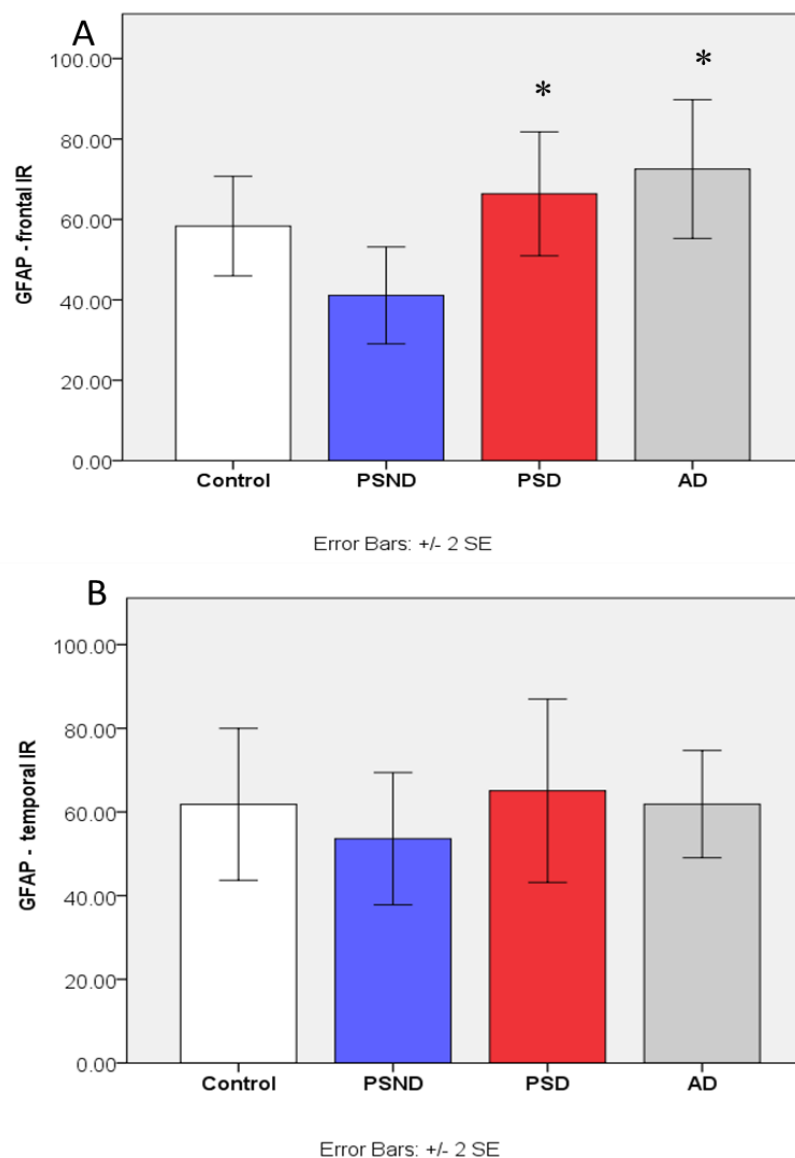
**Figure 7.1. Representative images showing variation of APP, cd68, GFAP, dMBP and SMI32 immunostaining across different groups : C,PSND, PSD, and AD. There is relative higher expression of APP, cd68, GFAP and SMI32 in PSD and AD compared to C and PSND**



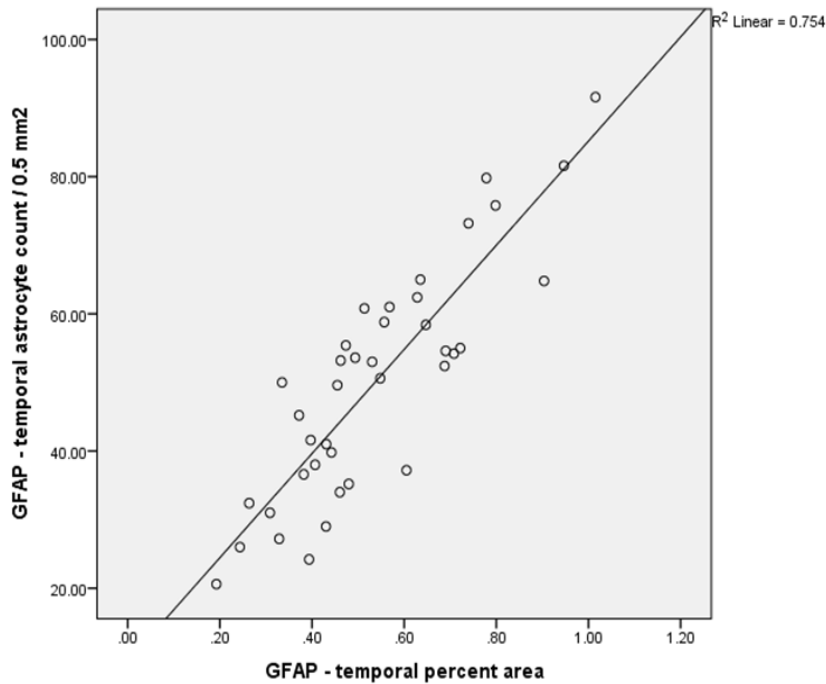
**Figure 7. 2. Bar graph shows CD68 immunoreactivity** in the frontal [A] and temporal [B] white matter and [C] microglial count per 0.5 mm<sup>2</sup> in the temporal white matter in Control, PSND, PSD and AD groups. SPSS generated p values using ANOVA, p = 0.045 [B] and p = 0.002 [C]. post – hoc Tukey’s test was used to compare means of each group. \*p < 0.05. Bars show ± 2 SEM.

### 7.3.1.2 GFAP

GFAP dataset showed normal distribution and immunoreactivity measures (per area, IOD and total IR) were not significantly associated with age and post – mortem delay but were affected by fixation period; GFAP frontal PA ( $r = - 0.431$ ;  $p = 0.005$ ). The immunoreactivity was higher in AD and PSD compared to PSND and control in the frontal WM ( $p = 0.034$ , ANOVA). Tukey’s post – hoc analysis showed that GFAP frontal IR was significantly lower in PSND ( $p = 0.027$ ) (Fig 7.3a). There were no differences across groups in the temporal WM.



**Figure 7. 3a. Bar graph showing mean GFAP immunoreactivity in [A] frontal and [B] temporal white matter in Control, PSND, PSD and AD, respectively. SPSS generated p values using ANOVA ( $p = 0.034$ ) in the frontal WM. post - hoc Tukey’s test was used to compare means of each group. \* $p < 0.05$ . Bars show  $\pm 2$  SEM.**



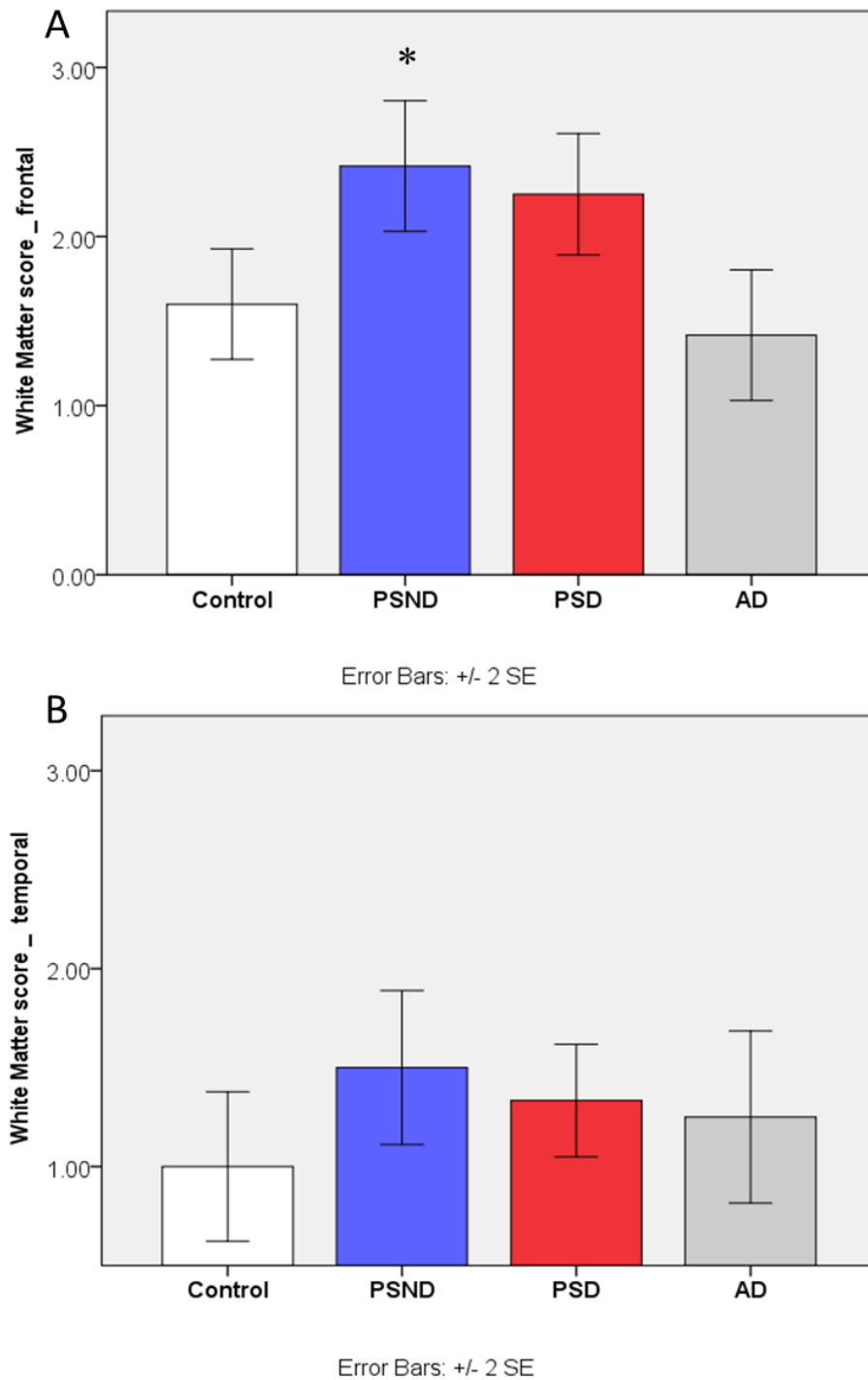
**Figure 7. 3b. Scatter plot showing relationship between astrocyte count per 0.5 mm<sup>2</sup> and GFAP immunoreactivity percentage area. \*p < 0.001.**

GFAP count per 0.5 mm<sup>2</sup> correlated positively with GFAP staining per unit area (Fig 7. 3b). A significant positive correlation was also found between frontal and temporal GFAP IR ( $r = 0.500$ ,  $p = 0.003$ ). GFAP immunoreactivity correlated positively with WM neuritic plaque ( $r = 0.398$ ,  $p = 0.013$ ). GFAP staining intensity measures correlated negatively with cognitive scores in both frontal [CAMCOG total ( $r = -0.624$ ,  $p = 0.010$ ) CAMCOG executive ( $r = -0.601$ ,  $p = 0.014$ ) and temporal WM ( $r = -0.616$ ,  $p = 0.011$ )].

### 7.3.2. Assessment of White Matter Severity

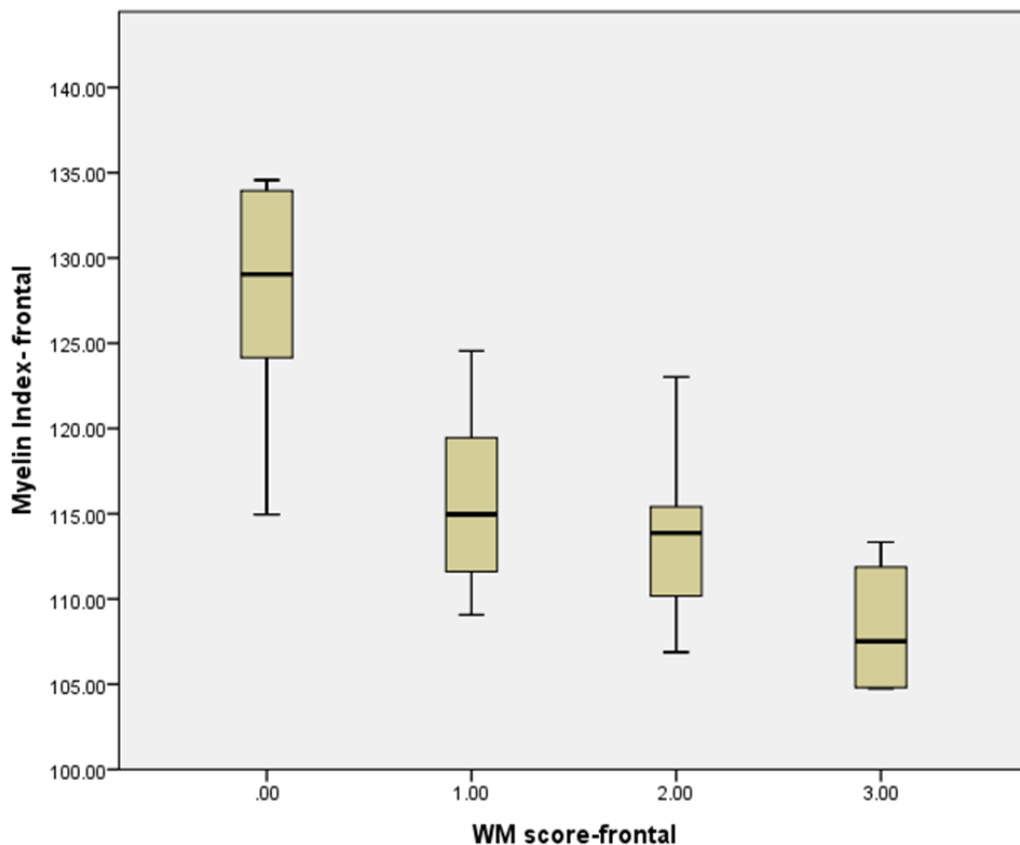
White matter severity score showed non - normal distribution (Shapiro – Wilk Test). Figures 7.4 shows the distribution of WM severity across disease groups in both frontal (a) and temporal (b) WM respectively, highlighting statistically significant lower WM scores in the AD and control groups in the frontal WM ( $p = 0.002$ , Kruska - Wallis

Test) but not in the temporal WM ( $p = 0.240$ , Kruska - Wallis Test).



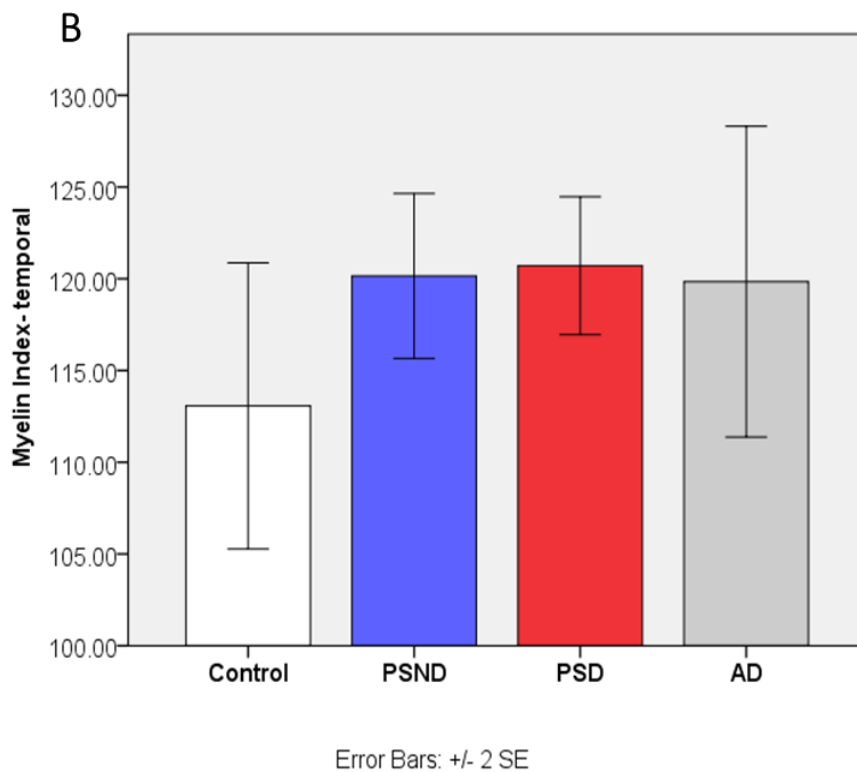
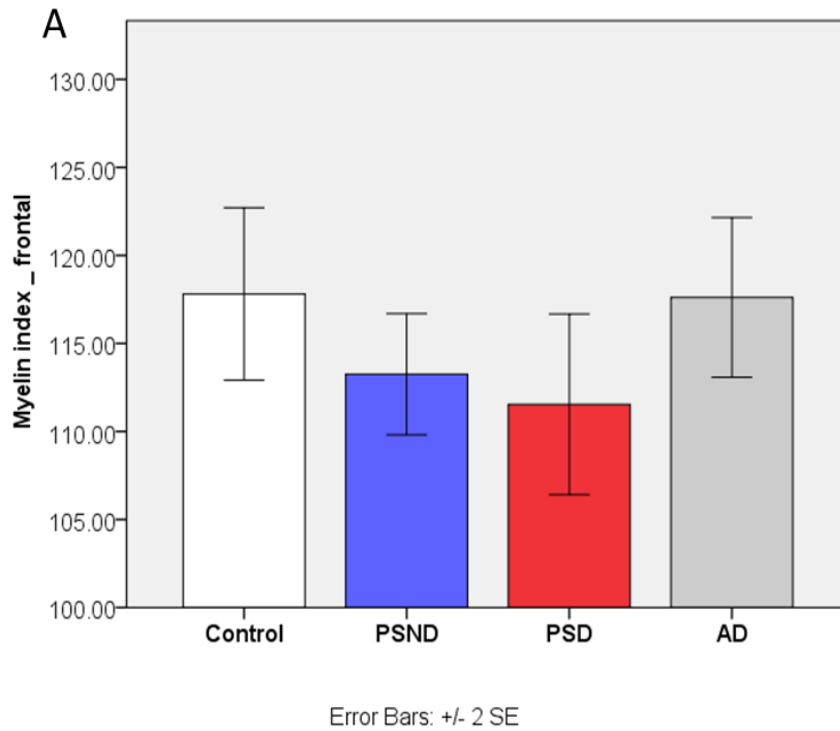
**Figure 7. 4. Bar graph shows white matter score in [A] frontal white matter and [B] temporal white matter in Control, PSND, PSD and AD groups. SPSS generated p values using Kruskal – Wallis Test ( $p = 0.002$ ) for the frontal WM. No significant difference across groups in temporal WM. Bars show  $\pm 2$  SEM \* Mann Whitney U test was used to compare means of each group. \* $p < 0.05$ .**

The WM severity score correlated positively with the vascular score ( $\rho = 0.674$ ,  $p < 0.001$ ) (Table 7.1) but inversely with the Myelin Index ( $\rho = - 0.750$ ,  $p < 0.001$ ); higher Myelin Index (MI) meaning better preserved myelin (Figure 7.5a) since MI in this context is a measure of *normal myelin*. Distribution of (MI) across the frontal and temporal WM is shown in Figure 7.5b. Myelin Index was relatively higher in the control and AD groups compared to PSND and PSD groups in the frontal WM, and the variation showed a trend towards significance ( $p = 0.069$ , Kruskal – Wallis Test). The difference in myelin index between controls and the pathological groups (AD, PSND, PSD) was surprisingly lower than expected in both frontal and temporal WM, possibly due to a significant amount of white matter disease in the control group.



**Figure 7.5a. Correlation between Myelin Index (MI) and WM severity rating** ( $\rho = - 0.745$ ,  $p < 0.001$ ). The figure shows an inverse relationship with higher WM severity scores corresponding to lower MI scores. The WM score is graded 0 = normal; 1 – mild; 2 – moderate and 3 = severe (Appendix 7.1)

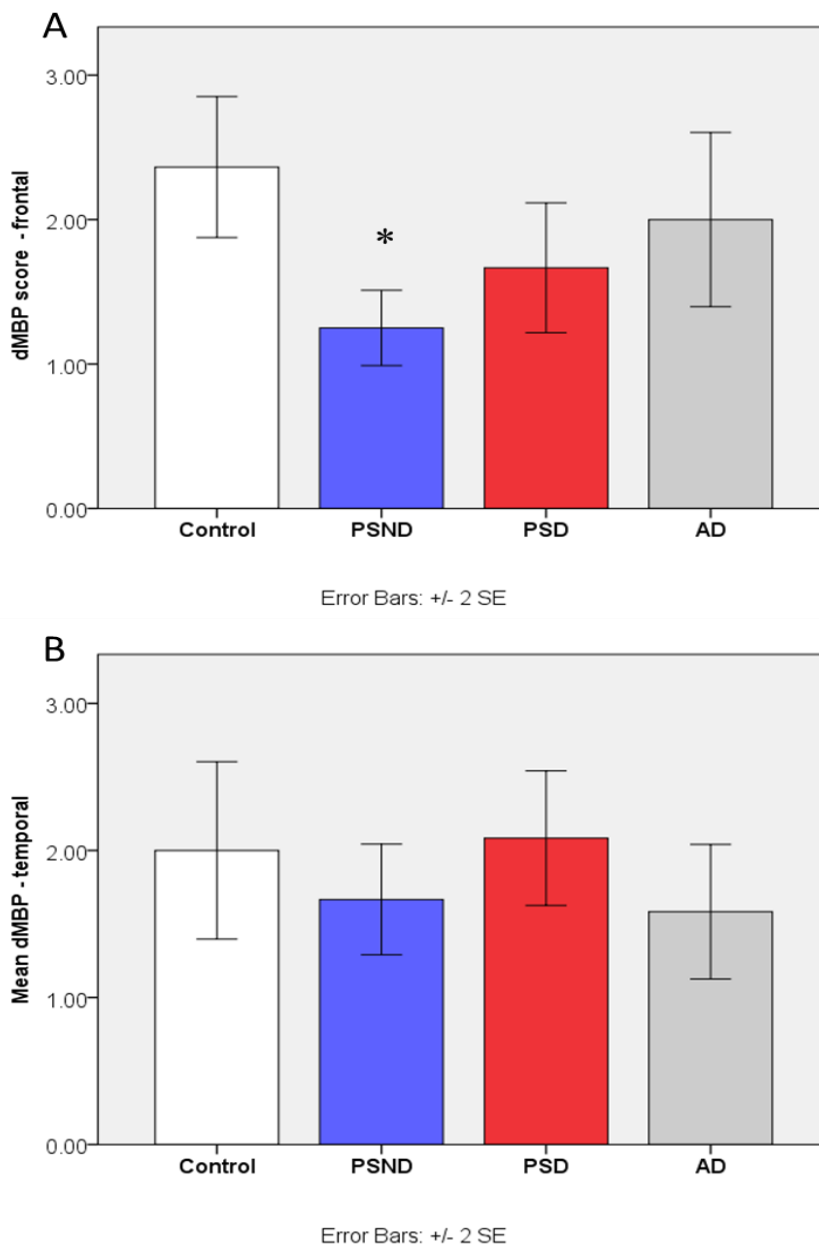




**Figure 7.5b.** Bar graph shows mean myelin index(MI) in [A] frontal white matter and [B] temporal white matter in Control, PSND, PSD and AD groups. SPSS generated p values using Kruskal – Wallis Test ( $p = 0.069$ ) for frontal WM. Bars show  $\pm 2$  SEM.

### 7.3.3. degraded Myelin Basic Protein (dMBP)

Degraded myelin basic protein assessed semi – quantitatively showed non – normal distribution and varied significantly across the groups in the frontal WM ( $p = 0.014$ , Kruska – Wallis Test). Immunoreactivity was significantly different between PSND and control ( $p = 0.003$ , Mann – Whitney U Test), but not different from PSD ( $p = 0.069$ ) and AD ( $p = 0.332$ , Mann – Whitney U Test) (Figure 7.6).

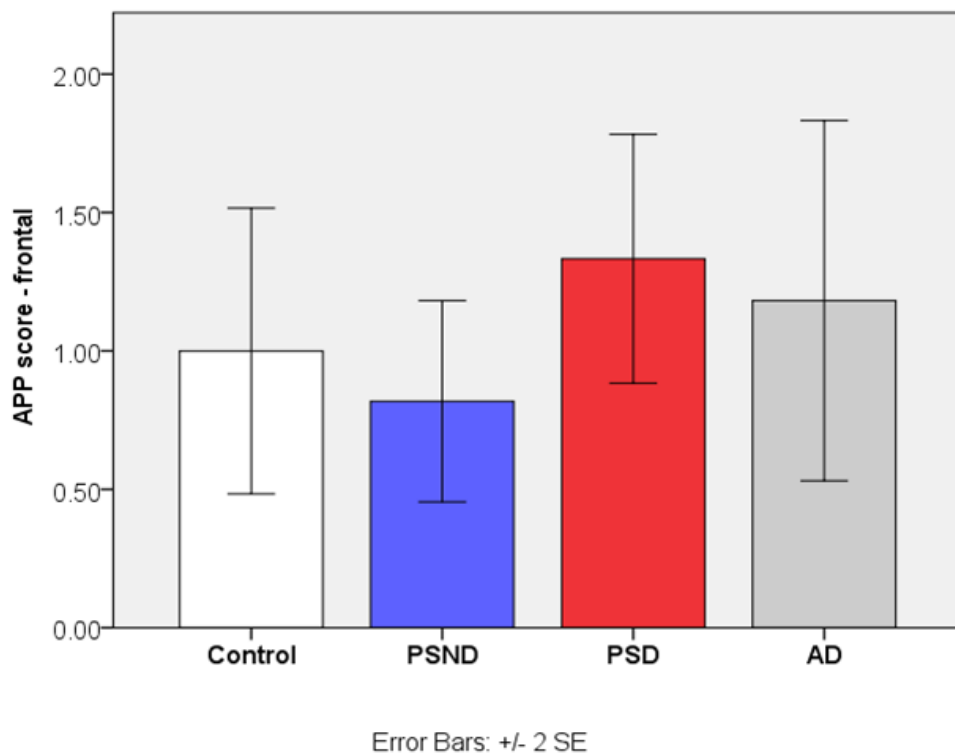


**Figure 7.6:** Bar graph shows mean dMBP score in [A] frontal and [B] temporal WM in Control, PSND, PSD and AD. SPSS generated p values using Kruskal – Wallis Test ( $p = 0.014$ ) for the frontal WM. Mann Whitney U test was used to compare means of each group. \* $p < 0.05$ . Bars show  $\pm 2$  SEM \*

However, no significant variations were detected across disease groups in the temporal WM ( $p = 0.367$ , Kruskal – Wallis Test) (Figure 7.6b). Degraded MBP showed significant positive correlation with the axonal markers - APP, microglial marker - CD 68 and myelin index (Table 7.2).

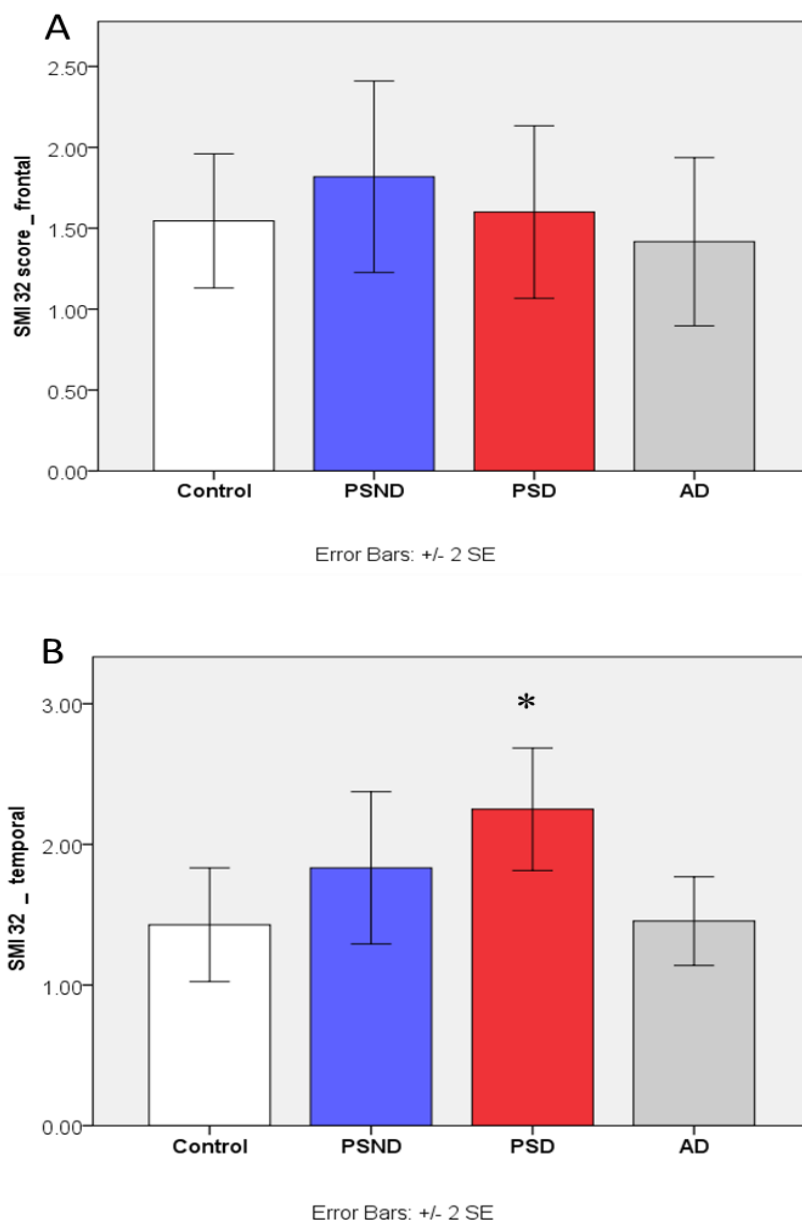
#### 7.3.4. Axonal damage

Axonal damage in both frontal and temporal MW was assessed with two different markers, APP and SMI 32. Although the distribution of APP immunoreactivity did not differ significantly across disease groups in both frontal and temporal WM ( $p > 0.05$ ), in the frontal WM APP IR showed an increase in the PSD group compared to the PSND and control group (Fig 7.8). There was no correlation with age, post – mortem delay or length of fixation period ( $p > 0.05$ ). However, APP demonstrated significant correlation with CD68 and dMBP (Table 7.2).



**Figure 7.7:** Bar graph shows mean APP score in the frontal white matter in AD, PSD, PSND and control groups. Bars show  $\pm 2$  SEM.

S MI – 32 demonstrated a significant variation in immunoreactivity in the temporal WM ( $p = 0.039$ , Kruska –Wallis Test) but not in the frontal WM. (Figure 7.8a and b). The IR was significantly higher in the PSD than in the control ( $p = 0.013$ , Mann Whitney U Test) and AD ( $p = 0.013$ , Mann Whitney U Test) groups, but not significantly different from the PSND group ( $p = 0.244$ , Mann Whitney U Test). However, there were no significant correlations with age, post –mortem delay or length of fixation period. There was also no correlation with cognitive or pathologic measures.



**Figure 7. 8:** Bar graph shows mean SMI32 score in [A] frontal and [B] temporal WM in Control, PSND, PSD and AD. SPSS generated p values using Kruskal – Wallis Test ( $p = 0. 039$ ) for temporal WM Bars show  $\pm 2$  SEM \* Mann Whitney U test was used to compare means of each group\* $p < 0.05$ .

### **7.3.5. Double Fluorescent confocal imaging**

Confocal images of dual staining of Glial Fibrillary Acidic Protein (GFAP) with Amyloid Precursor Protein (APP) in the frontal white matter show a predominant GFAP staining of astrocytes with higher immunostaining in AD and PSD compared to PSND and control group with weak APP staining in the background (Figure 7.9)

Spearman's rho		CD68	GFAP	WM score	Myelin Index	dMBP	APP	SMI32
<b>CD68</b>	rho	1.000	0.235	- 0.070	- 0.150	<b>0.378*</b>	<b>0.498**</b>	0.184
	p	.	.182	.658	.349	<b>.015</b>	<b>.001</b>	.249
	n	42	34	42	41	41	39	41
<b>GFAP</b>	rho	0.235	1.000	0.130	- 0.097	<b>0.393*</b>	<b>0.354*</b>	<b>0.311†</b>
	p	.182	.	.437	.573	<b>.015</b>	<b>.032</b>	<b>.061</b>
	n	34	39	38	36	38	37	37
<b>WM score</b>	rho	-0.070	0.130	1.000	- <b>0.387*</b>	0.208	<b>0.284†</b>	<b>0.272†</b>
	p	.658	.437	.	<b>.010</b>	.170	<b>.065</b>	<b>.074</b>
	n	42	38	46	43	45	43	44
<b>Myelin Index</b>	rho	- 0.150	- 0.097	- <b>0.387*</b>	1.000	- <b>0.344*</b>	- <b>0.294†</b>	- 0.200
	p	.349	.573	<b>.010</b>	.	<b>.024</b>	<b>.062</b>	.199
	n	41	36	43	44	43	41	43
<b>dMBP</b>	rho	<b>0.378*</b>	<b>0.393*</b>	0.208	- <b>0.344*</b>	1.000	<b>0.463**</b>	<b>0.276†</b>
	p	<b>.015</b>	<b>.015</b>	.170	<b>.024</b>	.	<b>.002</b>	<b>.070</b>
	n	41	38	45	43	46	43	44
<b>APP</b>	rho	<b>0.498**</b>	<b>0.354*</b>	<b>0.284†</b>	- <b>0.294†</b>	<b>0.463**</b>	1.000	<b>0.292†</b>
	p	<b>.001</b>	<b>.032</b>	<b>.065</b>	<b>.062</b>	<b>.002</b>	.	<b>.060</b>
	n	39	37	43	41	43	44	42
<b>SMI32</b>	rho	0.184	<b>0.311†</b>	<b>0.272†</b>	- 0.200	<b>0.276†</b>	<b>0.292†</b>	1.000
	p	.249	<b>.061</b>	<b>.074</b>	.199	<b>.070</b>	<b>.060</b>	.
	n	41	37	44	43	44	42	45

**Table 7 .2. Inter - markers correlation matrix .** Correlation matrix of markers of glial activation (CD68, GFAP). white matter severity (WM score and Myelin Index), demyelination (dMBP) and axonal damage (APP and SMI 32) . Statistical significance and trend designated by the following p values: \*\* p < 0.01; \*p < 0.05 †p < 0.1. Abbreviations: CD68 cluster of differentiation; GFAP, glial fibrillary acidic protein; dMBP, degraded myelin basic protein; SMI32, nonphosphorylated neurofilament H; APP, amyloid precursor protein; WM score, white matter severity score; MI , myelin index.

### **7.3.6. Correlational analysis**

Correlational analysis of all the markers together (Table 7.2) indicated that CD68 showed significant positive association with dMBP and APP while GFAP showed significant association with dMBP, APP and a trend with SMI32. Myelin Index was significantly associated with WM score and dMBP, and also showed a trend with APP while dMBP, in addition, showed significant association with APP and a trend with SMI32. APP and SMI32 both showed a trend of association with each other as well as with the markers of WM severity.

Table 7.3 shows the correlation analysis of cognitive scores with the two glial markers used in the study: CD 68 and GFAP. The table shows that both markers were significantly inversely associated with the measures of global cognitive functioning as well as measures of specific domains of memory and executive function. There were no significant association with the markers of demyelination, axonal damage and WM severity.

Spearman's rho		CD68	GFAP	MMSE	Memory-CAMCOG	Executive-CAMCOG	Total-CAMCOG
<b>CD68</b>	rho	1.000	0.077	- 0.404	- <b>0.516</b> *	- <b>0.651</b> **	- <b>0.562</b> *
	p	.	.672	.121	<b>.041</b>	<b>.006</b>	<b>.024</b>
	n	38	33	16	16	16	16
<b>GFAP</b>	rho	0.077	1.000	- <b>0.622</b> *	- 0.374	- <b>0.601</b> *	- <b>0.624</b> **
	p	.672	.	<b>.010</b>	.154	<b>.014</b>	<b>.010</b>
	n	33	41	16	16	16	16
<b>MMSE</b>	rho	- 0.404	- <b>0.622</b> *	1.000	<b>0.843</b> **	<b>0.784</b> **	<b>0.939</b> **
	p	.121	<b>.010</b>	.	<b>.000</b>	<b>.000</b>	<b>.000</b>
	n	16	16	20	20	20	20
<b>Memory-CAMCOG</b>	rho	- <b>0.516</b> *	- 0.374	<b>0.843</b> **	1.000	<b>0.896</b> **	<b>0.863</b> **
	p	<b>.041</b>	.154	<b>.000</b>	.	<b>.000</b>	<b>.000</b>
	n	16	16	20	20	20	20
<b>Executive-CAMCOG</b>	rho	- <b>0.651</b> **	- <b>0.601</b> *	<b>0.784</b> **	<b>0.896</b> **	1.000	<b>0.792</b> **
	p	<b>.006</b>	<b>.014</b>	<b>.000</b>	<b>.000</b>	.	<b>.000</b>
	n	16	16	20	20	20	20
<b>Total-CAMCOG</b>	rho	- <b>0.562</b> *	- <b>0.624</b> **	<b>0.939</b> **	<b>0.863</b> **	<b>0.792</b> **	1.000
	p	<b>.024</b>	<b>.010</b>	<b>.000</b>	<b>.000</b>	<b>.000</b>	.
	n	16	16	20	20	20	20

**Table 7.3. Correlation Matrix of markers of glial activation (CD68, GFAP) and cognitive scores.** Statistical significance and trend designated by the following p values: \*\* p < 0.01; \*p < 0.05 †p < 0.1. Abbreviations: CD68 cluster of differentiation; GFAP, glial fibrillary acidic protein; MMSE, mini mental state examination; CAMCOG, Cambridge Cognitive Examination.



## 7.4. Discussion

The objective of this chapter was to evaluate microglial and astrocytic activation, demyelination, axonal damage and severity of WM damage in post – stroke groups compared to AD subjects and normal controls. We hypothesized that markers of glial activation, demyelination and axonal damage would be differentially expressed in the WM of non – demented and demented post - stroke subjects compared with the control and AD groups.

We found significantly higher microglial activity in the temporal cortex of the PSD group compared to others, higher astrocytic activity in PSD and AD groups in the frontal cortex and higher WM severity in the post - stroke groups (PSND and PSD). We found no significant difference in the marker of acute axonal damage (APP) across groups whereas the marker of chronic axonal damage (SMI 32) showed differential expression in the temporal WM being significantly higher in the PSD group compared to others. Furthermore, there were significant positive associations of glial markers with the markers of demyelination and axonal damage, and inverse correlation with the metrics of global cognitive functioning, executive function and memory. Surprisingly, the control group despite being cognitively normal, demonstrated a relatively high level of WM pathology comparable to findings in the AD group.

Significantly higher expression of microglial (CD68) and astrocytic (GFAP) activation in PSD compared to PSND but largely similar myelin index and white matter severity score between PSD and PSND. Whereas APP expression was similar between PSND and PSD, SMI 32 expression was significantly higher in PSD than PSND. The expression of neuroinflammatory markers (CD68, GFAP), axonal markers (APP) in expression in AD was largely similar to PSD, while expression in controls was lower than in PSND.

Our finding of significantly increased astrocytic and microglial activity with associated worse cognitive performance particularly in the PSD group may suggest a higher degree of neuroinflammation with accentuated cerebral injury leading to faster progression of cognitive decline to dementia status. This is consistent with previous findings in different models of chronic cerebral hypoperfusion with cognitive deficits associated with blood brain barrier dysfunction and activated glial response, especially the

microglia (Sloane *et al.*, 1999; Wakita *et al.*, 2002; Shibata *et al.*, 2004; Whitehead *et al.*, 2007; Coltman *et al.*, 2011). Studies in man have also found microglial activation in the ageing WM (Fernando *et al.*, 2006; Simpson *et al.*, 2007a; Simpson *et al.*, 2007b)(Simpson *et al.*, 2007a, 2007b; Fernando *et al.*, 2006), AD (Englund, 1998; Sjobeck and Englund, 2003; Edison *et al.*, 2008) and even systemic inflammatory state such as sepsis (Lemstra *et al.*, 2007).

Glial cells particularly, microglia, are at the centre of neuroinflammation and thus play important roles in the pathogenesis WM damage (Pantoni and Garcia, 1995; Pantoni, 2010). They are also intricately involved in the aetiopathogenesis of stroke (Moskowitz *et al.*, 2010), AD and vascular cognitive impairment (Levine and Langa, 2011; Zotova *et al.*, 2011). In the healthy brain, microglia are involved in the process of pruning synapses and remodeling circuits and networks during brain development (Hughes, 2012) (Tremblay, 2011; Tremblay and Majewska, 2011) while in the adult brain microglia phagocytose apoptotic debris or breakdown products . In an attempt to clear debris from the brain, microglia also release pro - inflammatory mediators such as tumour necrosis factor, interleukins, nitric oxide which secondarily result in compromise of the blood brain barrier and damage to axons and their myelin sheaths (Sloane *et al.*, 1999; Edison *et al.*, 2011; Singh *et al.*, 2013).

Different neuroinflammatory phenotypes of microglia have been described in AD (Colton *et al.*, 2006). The M1 phenotype is associated with inflammatory cytokines that cause damage while the M2 phenotype is associated with wound repair (McCoy *et al.*, 2006). These have been found to influence the severity of pathology, natural history and therapeutic responsiveness in a cohort of early AD (Sudduth *et al.*, 2013). There is a distinct possibility that this phenotypic difference may have influenced the trajectory of cognition after stroke in our cohort resulting in higher neuroinflammation in the PSD group.

Myelin score was used as an index of ‘normality’ in this study. The significant negative correlation between the myelin index and the semi – quantitative WM score in our cohort suggests good concurrent validity of the two measures of white abnormality utilized in our study in agreement with previous findings (Sjobeck *et al.*, 2005; Ihara *et al.*, 2010b). Myelin index also showed significant correlation with the expression of

degraded myelin basic protein in agreement with previous findings (Akiguchi *et al.*, 2004; Yamamoto *et al.*, 2009; Ihara *et al.*, 2010a)

Axonal damage may be a consequence of ischemia – or immune – induced neuroinflammation or may occur secondary to cortical neurodegeneration (Medana and Esiri, 2003; Kalaria and Ihara, 2013). In agreement with previous reports, we found higher chronic axonal damage in the vascular than non – vascular category of subjects (Craggs *et al.*, 2013). Previous literature has shown worse axonal damage in frontal areas in comparison with temporal areas, but in our study we found relatively similar level of axonal damage in the two areas. The heterogeneity of our cohort and a distinct possibility of mixed vascular and degenerative aetiology of the axonal damage might offer an explanation for this. The temporal location of this degenerative process is in tandem with recent understanding that dissociative processes consisting of predominant frontal vascular and temporoparietal degenerative pathways might additively drive dementing processes (Kalaria and Ihara, 2013) (Haight *et al.*, 2013). Increased axonal damage in the temporal WM in the PSD group might thus have contributed to their worse performance in episodic memory compared to the PSND group.

We found a robust association between glial activation, degraded myelin and acute axonal injury (APP). This would support a central role for glial cells (microglia and astrocytes) indicative of neuroinflammation, and possible co-occurrence of demyelination and acute axonal damage in the pathogenesis of WM disease. While hypoxia from chronic hypoperfusion secondary to small vessel disease initiates the process of ischaemic demyelination, activated microglia are soon chemoattracted to the site to phagocytose the degraded myelin. The inflammatory mediators released by the microglia in the process of tissue repair might then worsen the demyelination and further damage the axons, especially depending on the phenotype of the glial cells (Sudduth *et al.*, 2013). The association between microglial activation and axonal damage is further corroborated by a recent finding of microglial nodules associated with degenerating axons in the WM of patients with early multiple sclerosis (Singh *et al.*, 2013). These findings are further substantiated by a recent DTI study in a cohort of subjects with small vessel disease where evidence was found for co-existing demyelination and axonal damage predicting executive dysfunction (Lawrence *et al.*, 2013).

Unexpectedly, we found a level of WM pathology in the control group comparable to that in the AD group. Although ageing alone is a significant risk factor for WM pathology (Gunning-Dixon *et al.*, 2009; Vernooij *et al.*, 2009) (Kennedy and Raz, 2009a; Vernooij *et al.*, 2009) the level found in this control group may have exceeded the threshold attributable to ageing alone. This begs the question of how ‘normal’ this group really was. Recent attention is being focused on the subject of ‘normal controls’ which are meant to be a reference group providing normative data that are used as a ‘reference range’ to a study population. At least one third of normal control samples may have moderate to severe WM hyperintensities (Zylberstein *et al.*, 2009) (DeBette and Markus, 2010; Aine *et al.*, 2013).

There are distinct possibilities that these subjects may have harboured, while alive, sub-clinical vascular disorders that did not produce overt symptoms and signs and thus attract specific diagnostic labels in the absence of significant clinical expression of disease. It is also possible that this WM pathology did not produce overt cognitive symptoms and deficits because of significant ‘cognitive reserve’ in the individuals that enabled them to maintain cognition in spite of significant brain pathology (Stern, 2009; Valenzuela *et al.*, 2012). However, it is also plausible that these individuals were not frequent hospital attenders during their life time and never really had rigorous diagnostic and cognitive profiling that could have picked abnormalities of clinical significance.

In all, findings in this study suggest that neuroinflammation indicated by glial activation, ischemia – induced demyelination and axonal damage are important cellular and tissue substrates of WM pathology and may contribute to the differential trajectory of cognition and speed of progress to dementia in post - stroke survivors. And, given that neuroimaging findings in the Nigerian African cohort shared a great degree of similarity with prior neuroimaging findings in the Newcastle cohort, it is intuitive to anticipate that similar neuropathologic findings will be unmasked in the Nigerian cohort in the process of time.

The limitations of the present study include: semi - quantitative assessment of dMBP and axonal markers may have been less sensitive than quantitative assessments in detecting significant differences across groups, and of correlations with other metrics of WM pathology. Furthermore, the size of the sample affected the statistical power to

detect significant changes. Hence, the current findings require external validation in independent bigger samples of a different cohort. In addition we acknowledge the potential influence of multiple pairwise comparisons and the impact on our conclusions

Future work to take this work forward will include: assay of blood brain barrier markers to examine their roles, assay for hypoxia - inducible factors (HIF) to determine if there is a differential regulation between PSD and PSND groups and determination of the neuroinflammatory phenotypes of the cases and groups.

## 7.5. Chapter Summary

Marker	Neuropathological Categories			
	Control	PSND	PSD	AD
GFAP	↔	↔	↑	↑
CD68	↔	↑	↑↑	↑
Myelin Index	↑	↔	↔	↑
WM severity	↔	↑	↑	↔
APP	↔	↔	↑	↔
SMI - 32	↔	↑	↑↑	↔

**Table 7.4. Summary of findings on markers of neuroinflammation, demyelination and axonal damage in frontal and temporal white matter**

**Annotation:** (↔ = little or no change; ↑ = increase; ↑↑ = moderate increase ;  
 ↓ = decrease)

### ▪ *Correlations*

- Inter - marker correlations were significant between :
  - CD68 and dMBP
  - CD68 and APP
  - GFAP and dMBP
  - GFAP and APP
  - WM score and Myelin Index
  - dMBP and Myelin Index
  - dMBP and APP
- Correlation between markers and cognitive scores
  - CD68 showed significant negative correlation with CAMCOG Total, Memory and Executive scores
  - GFAP showed significant negative correlation with MMSE score, CAMCOG Executive and Memory scores

## Chapter 8. General Discussion

### 8.1. Introduction

According to the Global Burden of Disease (GBD) Study 2010 report on the global and regional burden of stroke during the period 1990 – 2010, stroke remains the second leading cause of death and the leading cause of disability worldwide (Feigin *et al.*, 2013). The report further highlighted the absolute increase in the number of people with first stroke (16.9 million), stroke survivors (33 million), disability – adjusted years (DALYs) lost to stroke (102 million) and stroke – related deaths (5.9 million) in 2010 compared to previous years. This implies that the population of stroke survivors (33 million) is quite close to that of people living with dementia (36 million) (Prince *et al.*, 2013) and similar to that of people living with HIV/AIDS (34 million) (<http://www.niaid.nih.gov/topics/HIVAIDS/Understanding>). Nevertheless, there is disproportionate public inattention to stroke and other non – communicable disorders thus warranting their recent description as “*a public health emergency in slow motion*” by the Secretary General of the United Nations (Ban – Ki – Moon, 2011)([www.world-heart-federation.org](http://www.world-heart-federation.org) ). With the ageing of populations worldwide, more especially in the developing regions of the world and improvements in acute and rehabilitative stroke services, it is likely that the absolute number of stroke survivors will continue to rise. These individuals, their families and caregivers will necessarily have to contend with the long term consequences of stroke, especially cognitive impairment and dementia (Kalaria *et al.*, 2008; Pendlebury and Rothwell, 2009a; Ogunniyi and Akinyemi, 2010).

The cognitive consequences of stroke are of utmost concern to people affected by stroke. In a recent Scottish survey among stroke survivors, carers of stroke survivors and providers of stroke care services, the research question “*What are the best ways to improve cognition after stroke ?*” headed the list of top ten research priorities relating to life after stroke (Pollock *et al.*, 2012). Mitigating the cognitive sequelae of stroke requires a proper understanding of the burden and determinants as well as the mechanisms of causation in order to inform proper planning, policy formulation and development of new preventive and therapeutic interventions. This underscores the critical importance of this current study aimed at understanding the burden of post - stroke cognitive dysfunction, in a largely un – researched lower middle income sub –

Saharan African population as well as the mechanisms underlying brain injury producing cognitive impairment after stroke.

The primary aim of the project reported in this thesis was to establish a comparative cohort of Nigerian stroke survivors to investigate the profile and determinants of post – stroke vascular cognitive impairment (post – stroke VCI) and further explore the mechanisms of cerebral injury and cognitive impairment following stroke in post – mortem brains collected from the Newcastle cohort who had come to autopsy. In Chapter 3, we evaluated the frequency, pattern and determinants of post – stroke VCI in a cohort of Nigerian African stroke survivors while in Chapter 4, we examined the neuroimaging factors associated with post - stroke VCI in a sub – sample of the cohort. Recognizing that medial temporal atrophy also occurred in Nigerian African stroke survivors who developed cognitive impairment, in Chapter 5, we examined hippocampal Alzheimer pathology in post – mortem brain tissue of subjects with post – stroke dementia compared with non – demented post – stroke subjects, other dementias and ageing controls. In Chapter 6, we examined the synaptic integrity of hippocampal neurons in post – stroke dementia compared with non – demented post – stroke subjects, ageing controls and AD while in Chapter 7, we examined frontal and temporal white matter changes in the demented and non – demented post – stroke cohort compared with AD and normal ageing controls.

The main findings listed below are discussed in the light of extant literature in the sections that follow, and providing important implications for public health and future research.

- Three months after stroke, there was a **high frequency of VCI** of 48.3% in a cohort of Nigerian African stroke survivors (39.9% had vCIND and 8.4% had PSD). **Factors** associated with post – stroke VCI include **older age at baseline, female gender and lower educational attainment** while pre – stroke moderate – **heavy physical activity and daily fish intake were protective.**
- **Medial temporal lobe atrophy (MTLA)** was independently associated with post –stroke VCI at 3 months and also correlated significantly with cognitive performance and **white matter hyperintensities (WMHs)** in Nigerian African stroke survivors.

- Increased Alzheimer pathologic changes (especially  $\beta$  amyloid) occurred in post – stroke subjects relative to controls. However, amyloid and tau pathologies were differentially accumulated and showed weak association with ante – mortem cognitive performance suggesting that **non – AD** pathologic mechanisms may be primarily responsible for driving stroke – related cognitive impairment and differentiating PSND from PSD. We also found a possible influence of APOE  $\epsilon$ 4 allele on the differential deposition of amyloid and tau in sub – regions of the hippocampus and entorhinal cortex. In contrast, SorL1, a neuronal sorting protein, appeared to serve a protective role in hippocampal CA2 neuronal cells in response to hypoxia.
- **Significant reduction in the expression of synaptic markers:** vesicular glutamate transporter -1 (VGLUT - 1) and Drebrin in PSD and AD compared to control and PSND groups and positive correlation with cognitive performance scores.
- Significantly **higher microglial and astrocytic activation and axonal damage** in the temporal and frontal white matter of PSD and AD groups compared to PSND; significant correlation of glial markers immunoreactivity with cognitive performance scores and inter – correlations among markers of neuroinflammation, demyelination and axonal damage.

These findings, nonetheless, were subject to a few limitations:

- The size of the Nigerian African sample analyzed for this preliminary report is modest although still found some associations. A bigger sample would probably have generated more robust associations with improved generalizability.
- The neuroimaging sub – sample was also modest due to limitation of funds and MRI access, and this may also have influenced the statistical power of the study in the detection of significant associations and differences besides those that were reported.
- Rating of WMHs in the neuroimaging study were performed semi – quantitatively because of the nature of images produced by the limited magnet strength of the available MRI machine hence volumetric analysis could not be performed on the images. Volumetric analysis might have



yielded more significant associations than that found with the semi – quantitative assessment.

- Semi - quantitative assessment of axonal markers (APP and SMI 32) and demyelination marker (dMBP) might also have affected the robustness of their quantification and associations.

The CogFAST – Nigeria Study however is expected to be continued with the aim to undertake more rigorous evaluation of genetic and lifestyle risk factors. Subsequently, reports are expected to involve larger samples with longer survival data similar to the Newcastle CogFAST study (Allan *et al.*, 2011)). We also anticipate performing more brain MRI scans with stronger magnetic fields. This should facilitate more robust volumetric analysis and permit identification of novel associations.

## **8.2. Post stroke VCI in the CogFAST - Nigeria cohort.**

Results from the Nigerian African cohort reveal a relatively high burden of post – stroke VCI. The frequency of vCIND of 39.9 % at three months post – stroke obtained is comparable to findings from Sydney, Australia (39.4%) (Sachdev *et al.*, 2004a); Chongqing, China (37.1%) (Zhou *et al.*, 2005); Santiago, Chile (39.0%) (Delgado *et al.*, 2010) but lower than 49.9 % from Korea (Yu *et al.*, 2013), 55% from Lisbon, Portugal (Madureira *et al.*, 2001) and 54.8% from Singapore (Dong *et al.*, 2012).

In all these studies, subjects were assessed between 3 and 6 months after stroke. However, the frequency of vCIND was much higher in subjects assessed sooner after stroke: such as 63.5 % in a mixed ancestry cohort assessed two weeks after stroke in Durban, South Africa (Hoffmann, 2001) and 72.4 % in a cohort assessed at 1 month after stroke in Maastricht, Netherlands (Rasquin *et al.*, 2005a). The high frequency found in subjects assessed very early after stroke could reflect a true high frequency of early post - stroke vCIND (Pendlebury, 2009) or cases with incompletely resolved post – stroke delirium (Desmond DW, 1996)(Desmond et al, 1996, (Kalaria and Mukaetova-Ladinska, 2012), so that lower rates reported at 3 months and later would then suggest an improvement over time or resolution of acute post – stroke delirium (Desmond DW, 1996; Rasquin *et al.*, 2005b; Rasquin *et al.*, 2013). The rates were however lower in Newcastle, UK ranging from 24% (Stephens *et al.*, 2004) to 32% (Ballard *et al.*, 2002) as well as in a previous study from Hong Kong, China (21.8%) (Tang *et al.*, 2006). The

prevalence of cognitive impairment three months after stroke in the South London Stroke Register (SLSR) cohort using the MMSE is about 22% (Douiri *et al.*, 2013b).

Differences in populations, study designs, cut - off ages of the cohort, the cognitive assessment instrument and diagnostic criteria may offer explanations for the variations (Pendlebury and Rothwell, 2009a). Nonetheless, it is also plausible that lower rates in some studies may reflect true low prevalence as a result of inherent lower risk (genetic or epigenetic factors), protection from cognitive reserve (Stern, 2009) or low frequency of acute post - stroke complications as a result of good acute and restorative stroke services (Hachinski *et al.*, 2010; Norrving and Kissela, 2013) or some other yet unknown factors. On the other hand, high rates as seen in the current study may reflect a genetic predisposition of blacks to post - stroke cognitive impairment, unhealthy lifestyles or poor healthcare systems incapable of providing effective acute and post-discharge rehabilitative services to stroke survivors (Norrving and Kissela, 2013). It is also possible that cultural and religious biases against orthodox management of stroke as previously documented in a previous survey of Nigerian hospital workers (Akinyemi *et al.*, 2009) might have had an adverse effect on the uptake of appropriate stroke care with resultant impact on outcomes including cognitive functions.

Identification of stroke patients with vCIND at three months is of clinical significance. This stems from the fact it is possible to institute secondary prevention and long – term rehabilitative measures with the potential outcome of reversal of, or slowing further cognitive decline. In the Newcastle cohort, at least 50% of subjects experienced improvement in cognitive functioning at 15 – months follow up (Ballard *et al.*, 2003a) and aggregate cardiovascular risk factor load predicted long term outcome of progression to dementia and/or death (Allan *et al.*, 2011). Secondary prevention has been shown to improve long – term cognitive outcome in the survivor cohort of the South London Stroke Registry (SLSR)(Douiri *et al.*, 2013a) .

This also has implications for the recent 5<sup>th</sup> revision of the Diagnostic and Statistical Manual (DSM – V) which now has two categories of ‘Major’ and ‘Minor’ Neurocognitive Disorders (American Psychiatric Association, 2013). Subjects with vCIND fall into the category of ‘*Minor Neurocognitive Disorders*’, a specific diagnostic category that will be well captured in clinical trials and treatment guidelines. The multi – domain pattern of impairment identified in this cohort possibly underscores the

sensitivity and robustness of the NINDS – CSN VCI Harmonization Standards Neuropsychological Battery in detecting impairments in the multiple domains of executive function, memory, language and visuospatial/ visuoconstructive function (Hachinski *et al.*, 2006a) .

Older age, female gender and low educational attainment were significant risk factors while pre – stroke physical activity and fish intake were protective factors identified in the cohort. As populations age and life expectancy grows in the developing regions of the world, the burden of ageing – associated disorders including stroke and dementia have been projected to grow (Prince *et al.*, 2012; Feigin *et al.*, 2013; Prince *et al.*, 2013). This calls for planning, policy formulation and promotion of preventive interventions, especially in the LMIC regions that are now in epidemiologic transition (Yusuf *et al.*, 2001b; Yusuf *et al.*, 2001a).

Whereas age is a non – modifiable factor, educational attainment is a modifiable factor and surrogate of cognitive reserve (Satz *et al.*, 1993; Stern, 2009; Satz *et al.*, 2011) . Educational attainment has been found to protect against cognitive impairment in MCI (Mortamais *et al.*, 2013), AD (Stern, 2012) vascular dementia (Meng and D'Arcy, 2012), CADASIL (Zieren *et al.*, 2013), HIV neurocognitive disorder (Manly *et al.*, 2011) MS (Sumowski *et al.*, 2013b), Parkinson's disease (Armstrong *et al.*, 2012), traumatic brain injury (Sumowski *et al.*, 2013a) etc. Educational attainment together with occupational complexity and social engagement constitute the paradigm 'Cognitive Lifestyle' (Valenzuela and Sachdev, 2006; Valenzuela *et al.*, 2011; Valenzuela *et al.*, 2012) which has been associated with a reduced long – term risk of dementia (Valenzuela *et al.*, 2011) and found to be associated with neurotrophic changes in the prefrontal lobe consistent with a compensatory process (Valenzuela *et al.*, 2012).

In this current study, educational attainment significantly stratified stroke survivors into cognitive categories three months after stroke suggesting that education and related cognitive reserve might have provided some compensation against vascular brain injury to preserve cognitive functioning in the cognitively normal group compared to the impaired group. It is not clear, however, how effective cognitive stimulation therapy is in improving cognitive function if applied after stroke. In recent Cochrane reviews, cognitive stimulation training and cognitive rehabilitation have not provided sufficient

evidence of efficacy in randomized controlled trials in subjects with mild to moderate AD and vascular dementia (Woods *et al.*, 2012; Bahar-Fuchs *et al.*, 2013).

Physical activity promotes brain health by enhancing cerebral blood flow and the production of growth factors (Dishman *et al.*, 2006; Cotman and Berchtold, 2007; Cotman *et al.*, 2007). Our study found pre – stroke moderate - to - heavy physical exercise to be protective against cognitive impairment at three months after stroke. There are also suggestions that physical activity after stroke may impact positively on cognition (Quaney *et al.*, 2009), although a recent meta – analysis of randomized control trials of the effect of exercise training on cognitive function in older adults with mild cognitive impairment found limited evidence of beneficial effect of exercise (Gates *et al.*, 2013). In a recent study of physical activity, sedentary behavior and metabolic control among stroke survivors, reduced physical activity associated with poorer metabolic control was reported in a cohort of stroke survivors in the immediate post – stroke period up to 6 months (Moore *et al.*, 2013). There was however a positive impact of interventional exercise therapy on metabolic profile and cerebral blood flow in the same cohort over a period of twenty weeks (Moore, 2013).

The positive impact of dietary intake of fish on cardiovascular and brain health is supported by numerous evidence in literature (Takata *et al.*, 2013; Virtanen *et al.*, 2013) . Although, we found a positive association between pre – stroke fish intake and protection against post-stroke cognitive impairment in this study, it is needful to further characterize this protection by teasing out the specific species, quantities and modes of preparation. The influence of fruits and vegetable also require further exploration with a bigger sample of stroke patients.

Medial temporal lobe atrophy (MTLA) and white matter hyperintensities (WMHs) are important imaging biomarkers of cognitive decline in cognitive ageing, neurodegenerative and vascular dementias (Knopman, 2007; Mills *et al.*, 2007) The predictive role of MTLA in stratifying cognitive categories of stroke survivors and predicting cognitive decline, dementia and death had been shown in the Newcastle cohort (Firbank *et al.*, 2012). Although pre - stroke cognitive decline has been associated with pre – existing Alzheimer disease in some studies (Henon *et al.*, 1998) we found no correlation between pre – stroke cognitive decline and MTLA in the Nigerian cohort although the small sample size of the sub - cohort that was imaged may

be a factor. Nevertheless, WMHs showed significant correlation with measures of executive dysfunction and memory as previously documented in the Newcastle cohort (Burton *et al.*, 2003; Burton *et al.*, 2004) and by others (Jokinen *et al.*, 2005). More recent studies in other post - stroke survivor cohorts utilizing diffusion tensor imaging (DTI) in post – stroke cohorts have provided more detailed findings of changes in mean diffusivity and generalized fractional anisotropy particularly in frontal white matter (Jin Thong *et al.*, 2013; Lawrence *et al.*, 2013). These findings provided some mechanistic insight regarding the relative contributions of demyelination and axonal damage to the neurobiology of WM changes in post - stroke subjects. The combination of these two important neuroimaging findings (MTLA and WMHs) provided a conceptual framework for the subsequent laboratory studies we undertook on hippocampal AD pathology and on the role of glial activation, demyelination and axonal damage in frontal and temporal WM of representative samples of brain tissue from subjects in the Newcastle cohort who had come to autopsy in order to establish mechanistic differences between demented and non – demented post – stroke subjects.

### **8.3 Are there mechanistic differences between the non – demented (PSND) and demented (PSD) post – stroke laboratory cohort?**

An important objective of the project undertaken in this thesis was to explore mechanistic differences between the non – demented (PSND) and demented (PSD) post – stroke cohort who had come to autopsy. Given the significant findings of MTLA and correlative white matter changes from the neuroimaging studies in the Nigerian African cohort which were in tandem with previous reports from the Newcastle cohort, we investigated neurodegenerative hippocampal Alzheimer pathology and synaptic changes, as well as frontal and temporal white matter abnormalities in post - mortem brains collected from the Newcastle cohort.

Our results showed increased amyloid deposition in the post – stroke group following cerebral hypoxic stimulation (Lewis *et al.*, 2006; Okamoto *et al.*, 2012) but largely no significant differences were observed between PSND and PSD except for 4G8 (total amyloid) immunoreactivity in the subiculum and entorhinal cortex where higher immunoreactivity was observed in the PSND compared to the PSD group. Our results further revealed that this disparity was due to higher proportion of APOE  $\epsilon$ 4 allele in

the PSND group in keeping with its propensity to drive amyloid deposition, particularly A $\beta$  (42) (Schmechel *et al.*, 1993; Nagy *et al.*, 1995; Polvikoski *et al.*, 1995; Saito *et al.*, 2002). These results are in concordance with a recent study examining the genetic associations of vascular dementia subtypes in which an association was found between APOE- $\epsilon$ 4 allele and mixed dementia, stroke - related dementia and subcortical ischemic vascular dementia (SIVD) as well as higher A $\beta$  1 - 42 levels (Jones *et al.*, 2011). It is indeed intuitive to project that subjects in the PSND group with high amyloid burden might have progressed to develop mixed dementia (AD\_VaD) had they lived long enough. Among older subjects with MCI or AD, neurofibrillary tangles load tends to show better correlation with cognitive scores than amyloid plaques (Nelson *et al.*, 2009; Nelson *et al.*, 2012) while a clinico – pathologic study of a cohort of AD, DLB and VCI subjects showed that tangle load correlated significantly with MTLA rather than plaque or Lewy body pathology (Burton *et al.*, 2009). In this study however, AT8 immunoreactivity due to hyperphosphorylated tau was not significantly different between PSND and PSD even though it correlated significantly with memory score in the subiculum. Therefore, hippocampal AD – pathologic mechanisms do not seem to separate PSND and PSD. Neocortical AD pathology, other non – AD neurodegenerative mechanisms as well as other non - neurodegenerative mechanisms such as vascular and inflammatory/immune mechanisms require research attention.

Exploration of hippocampal synaptic markers yielded significant differences between PSND and PSD in the expression of vesicular glutamate transporter (VGLUT - 1) and drebrin. VGLUT -1 is a pre – synaptic marker involved in glutamatergic excitatory neurotransmission (Fremeau *et al.*, 2004a; Fremeau *et al.*, 2004b) while Drebrin is a marker of dendritic spine morphology and functioning on the post – synaptic density (Hayashi *et al.*, 1996; Sekino *et al.*, 2007). The expression of these two markers was significantly higher in PSND compared to PSD and showed significant positive correlation with cognitive performance in general cognitive functioning and memory domain. The higher expression of these two markers in the PSND group would suggest the presence of a stronger synaptic network and correlative higher cognitive functioning. Synaptic markers have been described as structural correlates of cognitive reserve (Bennett *et al.*, 2012; Boyle *et al.*, 2013a; Boyle *et al.*, 2013b) ; (Honer *et al.*, 2012) and reduced expression may be associated with dementia even in the oldest – old (Beeri *et al.*, 2012). Significant upregulation of pre – frontal lobe VGLUT -1 expression

has also been previously reported in non – demented post - stroke subjects (Kirvell *et al.*, 2010). Another complementary study within our research group has demonstrated preserved expression of Hu C/D, a marker of neuronal maintenance [Burke *et al.*, 2013 (unpublished observation)] in the CA1 and CA2 hippocampal sub – regions of control and non - demented stroke (PSND) subjects compared to demented (PSD) subjects. Using the techniques of stereology, it has also been demonstrated within this study cohort that PSND and control subjects have higher neuronal volume than PSD in the pyramidal neurons of CA2 and CA1 (Gemmell *et al.*, 2012). Putting all these together, we can surmise that the preservation of cognitive function in the PSND group depicted by the higher expression of synaptic markers may be related to higher neuronal volume and higher expression of neuronal maintenance markers (Gemmell *et al.*, 2012); Burke 2013 (unpublished data). Evidence is accruing on the microstructural correlates of cognitive reserve. In a 1988 pioneering study, Katzman and colleagues demonstrated that some individuals who remained cognitively intact despite significant AD pathology exhibited higher brain weight and greater number of neurons (Katzman *et al.*, 1988). In a neuropathologic arm of the Nun Study, participants who remained cognitively intact at the time of death showed widespread neuronal hypertrophy which was linked to greater linguistic ability in early life (Iacono *et al.*, 2009). More recently, results from the MRC Cognitive Function and Ageing Study (CFAS) have demonstrated an association between ‘*high cognitive lifestyle*’ (educational attainment, occupational complexity and social engagement) and increased cortical thickness, increased neuronal volume and density in the prefrontal lobe (Brodmann area 9) of a sub - cohort as a result of a compensatory neuronal processes (Valenzuela *et al.*, 2011; Valenzuela *et al.*, 2012).

Coupled with the finding of educational attainment as a determinant factor of cognitive impairment in the Nigerian cohort, our results from the post – mortem studies add to the growing body of knowledge on the strategic importance of cognitive reserve and its demographic, lifestyle and microstructural correlates in influencing the trajectory of cognitive function following brain injury, vascular in the current context.

To further establish mechanistic differences between the PSND and PSD groups, glial activation, demyelination, and axonal damage were explored in the frontal and temporal white matter of our cohort. The results revealed higher microglia and astrocytic activation and axonal damage in the PSD group compared with the PSND group with significant inter - correlations among markers, and of glial markers with cognitive

scores (inverse). This difference in inflammatory mechanisms may synergize with synaptic changes to differentiate between the PSND and PSD groups. Further studies will be necessary to unmask the basis of the neuroinflammatory differences whether this is due to phenotypic differences in inflammatory cells (Sudduth *et al.*, 2013) or it is related to systemic inflammatory processes (Arfanakis *et al.*, 2013) or post – stroke complications (Gottesman and Hills, 2010; Grube *et al.*, 2013; Wang *et al.*, 2013).

#### **8.4. Public Health Perspectives**

The critical role of early life education in the development of cognitive reserve has been well documented (Brayne *et al.*, 2010). For instance, although educational attainment was not documented for the CogFAST Newcastle cohort (Ballard *et al.*, 2002; Allan *et al.*, 2011), historical records indicate that most subjects in the Newcastle cohort, the oldest of whom was born in 1907 (personal communication) might have had a minimum of 9 years of early life education because of the promulgation of the ‘1918 Education Act’ <http://www.legislation.gov.uk/ukpga/Geo5/8-9/39> - accessed 07 - 11- 2013) which raised the school leaving age to 14 years, planned expansion of tertiary education and evolved plans for services to pupils with special needs. The median age of participants in the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS) [1991 – 2002] (Seidel *et al.*, 2009) which included participants from Newcastle was about 9 years (semi – interquartile range : 9 – 9 years). It is therefore conceivable that improved early life education might have contributed to cognitive reserve in the Newcastle cohort such that the cohort reported relatively lower rates of early post - stroke vascular cognitive impairment compared to other cohorts and even some younger cohorts such as the Nigerian cohort with a mean age of about 60 years. Apart from early life education, the importance of maintaining an active cognitive lifestyle (Valenzuela *et al.*, 2012) across the lifespan cannot be over emphasized. This will invariably include adopting a lifestyle of lifelong learning and engaging in cognitively stimulating leisure activities with robust social engagement. It is important, particularly in developing countries of the world, to develop effective public policies and laws to encourage early life education and lifelong learning in order to build



necessary '*mental capital*' for economic and social development, and at the same time build cognitive reserves to compensate for possible future brain injuries.

Adoption of healthy lifestyle including physical activity and healthy diets including the consumption of omega - 3 fatty acids are also of great public health importance. The impact of the healthcare system as well might also have been contributory. With the introduction of the National Health Service (NHS) in the United Kingdom in the late 40's (Shapiro, 2010), citizens including subjects in the Newcastle cohort had better healthcare access in their youth and mid – life. It is also possible that the Newcastle cohort may have enjoyed relatively good cardiovascular and brain health during their youth and middle – age. Historical records indicate that during the great depression and world wars of the 1930's and 1940's, food was rationed in the UK , access to meat was limited and families resorted to, and had to grow their own supply of vegetables in lieu. People also probably walked more because of scarcity of fuel. The obvious 'caloric restriction', increased intake of vegetables and likely increased physical activity at that time during early life and middle age of the Newcastle cohort coupled with the introduction of the NHS in the late 40's enhancing access to better healthcare might have synergized with improved early life education to provide them with good brain and cardiovascular health. The outcome of this may have been that the cognitive consequences of a stroke even after age of 75 years was ameliorated such that the proportion of the CogFAST – Newcastle cohort who had early vCIND was not more than 32 % at three months after stroke. Upon subsequent follow up, it is interesting to note that 50% of this cohort had improved cognitive function at 1 year follow up (Ballard *et al.*, 2003a) and over an average follow up of 5.8 years only 25% developed incident dementia (Allan *et al.*, 2011).

## **8.5. Conclusions**

The project described in this thesis was designed to establish a new cohort of Nigerian African stroke survivors to investigate the profile and determinants of vascular cognitive impairment after stroke and further explore the mechanisms underlying cognitive impairment after stroke in post – mortem brains from the Newcastle cohort who had come to autopsy.

Findings from this CogFAST Newcastle – Nigeria Study are contributing to the general fund of knowledge and advancing the field as follows:

- Chapter 3 was dedicated to the determination of profile and determinants of post – stroke VCI in the Nigerian African stroke survivors cohort. We have established a unique Nigerian African cohort of stroke survivors who will be studied well into the future. This is the first comprehensive, detailed and prospective African study. We have used a robust cognitive assessment battery to detect multi – domain cognitive impairment in the cohort that is well stratified into categories based on clinically useful criteria. The findings of the protective effect of pre – stroke daily fish intake and physical activity are unique and important in an African population that is currently in epidemiologic transition.
- Chapter 4 details the neuroimaging factors associated with post - stroke VCI in a sub – sample of the Nigerian cohort. This represents the first attempt to examine the neuroimaging substrates of post – stroke VCI among sub – Saharan Africans. Despite the modest sample size of this sub – cohort, robust correlations were established among neuroimaging, cognitive and clinical parameters apart from the important finding of MTLA and WMHs, of which MTLA demonstrated significant association with cognitive categories. These findings provided support for the vascular basis of neurodegeneration from an indigenous African population. And since similar findings were reported in the Newcastle cohort, we proceeded in subsequent chapters to examine in post – mortem brain tissue from the Newcastle cohort immuno - histochemical correlates of the MTLA and WMHs in appropriate brain regions.
- In Chapter 5, given the significant findings of MTLA and correlative WM changes from the previous chapter, we examined hippocampal Alzheimer pathology in post – mortem brains collected from the Newcastle cohort. We evaluated subjects with post - stroke dementia compared with non – demented post – stroke subjects, other dementias and ageing controls. The findings in this chapter demonstrate for the first time that non - AD pathologic mechanisms were more likely responsible for driving the dementing process after stroke.

Besides, this chapter also demonstrates, for the first time, that differential upregulation of SorL1 may occur in response to hypoxia as a protective mechanism for the hippocampal CA2 sub – region which is known to be resistant to common biological insults. Based on these findings, we sought to explore different synaptic markers to see if they could differentiate the non – demented from the demented post – stroke subjects.

- In Chapter 6, we explored hippocampal synaptic integrity in post – stroke dementia compared with non – demented post – stroke subjects, ageing controls and AD to ascertain if they could tease out the difference between demented and non – demented post – stroke subjects. Our results showed, for the first time, differential hippocampal expression of VGLUT – 1 in post-stroke subjects as well higher hippocampal expression of Drebrin in non – demented compared to demented post – stroke subjects.
- In Chapter 7, we explored white matter changes in the demented and non – demented post – stroke cohort compared with AD and normal ageing controls. We found higher expression of glial markers (CD68 and GFAP) and chronic axonal damage ( SMI -32) in the temporal white matter of demented post-stroke groups suggesting a higher level of neuroinflammation and axonal damage may differentiate non – demented from demented post – stroke subjects. Taken together, these findings provide complementary evidence from clinico – epidemiological and clinico – pathological studies regarding the profile, determinants and mechanisms of vascular cognitive impairment after stroke while opening up new vistas of further research.

## **8.6. Future Directions**

### *8.6.1. The CogFAST - Nigeria Study:*

Having established the initial studies, future efforts will be directed at:

- Extending the baseline recruitment and longitudinal follow up of the cohort. This will enable us study the natural history of post – stroke VCI in this unique population exposed to different genetic and lifestyle factors.
- Further neuroimaging of the cohort will be extremely useful in order to confirm previous findings and establish new associations of neuroimaging factors associated with post-stroke VCI. Use of MRI scanner with higher magnetic strength will facilitate volumetric analysis and correlation with clinical and neuropsychological findings.
- Exploration of genetic and biochemical factors e.g. omega-3 fatty acids and vitamins associated with post - stroke VCI in this unique population are anticipated.
- Future clinico - neuropathological studies of subjects extensively assessed in the clinic who come to post – mortem are anticipated.
- Randomized controlled clinical trials involving the subjects are anticipated e.g. studies assessing intervention to slow down the progression of cognitive impairment.

#### 8.6.2. *The CogFAST – Newcastle Study.*

- Exploration of non – AD neuropathologic mechanisms such as  $\alpha$  – synuclein and TDP – 43 may be useful in order to ascertain their possible contribution to cognitive decline in the subjects who had come to autopsy.
- It will be very useful as well to explore other brain regions such as the frontal, parietal and temporal regions in order to quantify the load of degenerative pathologies in these regions and correlate them with clinical and cognitive records.
- Further exploration of synaptic markers in other brain regions including the prefrontal and temporal region and correlate with available clinical and cognitive dataset.
- Further neurochemical evaluation of the frontal and temporal white matter to explore markers of glial activation, demyelination and axonal damage
- Determination of neuroinflammatory/immune phenotypes of the total cohort, blood brain barrier markers, blood inflammatory markers.

## References

- Aarsland, D., Sardahaee, F.S., Anderssen, S. and Ballard, C. (2010) 'Is physical activity a potential preventive factor for vascular dementia? A systematic review', *Aging Ment Health*, 14(4), pp. 386-95.
- Aho, K., Harmsen, P., Hatano, S., Marquardsen, J., Smirnov, V.E. and Strasser, T. (1980) 'Cerebrovascular disease in the community: results of a WHO collaborative study', *Bull World Health Organ*, 58(1), pp. 113-30.
- Aho, L., Jolkkonen, J. and Alafuzoff, I. (2006) 'Beta-amyloid aggregation in human brains with cerebrovascular lesions', *Stroke*, 37(12), pp. 2940-5.
- Ahtiluoto, S., Polvikoski, T., Peltonen, M., Solomon, A., Tuomilehto, J., Winblad, B., Sulkava, R. and Kivipelto, M. (2010) 'Diabetes, Alzheimer disease, and vascular dementia: a population-based neuropathologic study', *Neurology*, 75(13), pp. 1195-202.
- Aine, C.J., Sanfratello, L., Adair, J.C., Knoefel, J.E., Qualls, C., Lundy, S.L., Caprihan, A., Stone, D. and Stephen, J.M. (2013) 'Characterization of a normal control group: Are they healthy?', *Neuroimage*, 84c, pp. 796-809.
- Aizenstein, H.J., Nebes, R.D., Saxton, J.A., Price, J.C., Mathis, C.A., Tsopelas, N.D., Ziolkowski, S.K., James, J.A., Snitz, B.E., Houck, P.R., Bi, W., Cohen, A.D., Lopresti, B.J., DeKosky, S.T., Halligan, E.M. and Klunk, W.E. (2008) 'Frequent amyloid deposition without significant cognitive impairment among the elderly', *Arch Neurol*, 65(11), pp. 1509-17.
- Akiguchi, I., Tomimoto, H., Wakita, H., Kawamoto, Y., Matsuo, A., Ohnishi, K., Watanabe, T. and Budka, H. (2004) 'Topographical and cytopathological lesion analysis of the white matter in Binswanger's disease brains', *Acta Neuropathol*, 107(6), pp. 563-70.
- Akinyemi, R.O., Ogah, O.S., Ogundipe, R.F., Oyesola, O.A., Oyadoke, A.A., Ogunlana, M.O., Otubogun, F.M., Odeyinka, T.F., Alabi, B.S., Akinyemi, J.O., Osinfade, J.K. and Kalaria, R.N. (2009) 'Knowledge and perception of stroke amongst hospital workers in an African community', *Eur J Neurol*, 16(9), pp. 998-1003.
- Akinyemi, R.O., Okubadejo, N.N., Akinyemi, J.O., Owolabi, M.O., Owolabi, L.F. and Ogunniyi, A. (2008) 'Cognitive dysfunction in Nigerians with Parkinson's disease', *Mov Disord*, 23(10), pp. 1378-83.
- Albert, M.S., DeKosky, S.T., Dickson, D., Dubois, B., Feldman, H.H., Fox, N.C., Gamst, A., Holtzman, D.M., Jagust, W.J., Petersen, R.C., Snyder, P.J., Carrillo, M.C., Thies, B. and Phelps, C.H. (2011) 'The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease', *Alzheimers Dement*, 7(3), pp. 270-9.
- Alexopoulos, P., Guo, L.H., Tsolakidou, A., Kratzer, M., Grimmer, T., Westerteicher, C., Jiang, M., Bujo, H., Diehl-Schmid, J., Kurz, A. and Perneczky, R. (2012) 'Interrelations between CSF soluble AβetaPPbeta, amyloid-beta 1-42, SORL1, and tau levels in Alzheimer's disease', *J Alzheimers Dis*, 28(3), pp. 543-52.
- Allan, L.M., Rowan, E.N., Firkbank, M.J., Thomas, A.J., Parry, S.W., Polvikoski, T.M., O'Brien, J.T. and Kalaria, R.N. (2011) 'Long term incidence of dementia, predictors of mortality and pathological diagnosis in older stroke survivors', *Brain*, 134(Pt 12), pp. 3716-27.
- Almeida, O.P. and Almeida, S.A. (1999) 'Short versions of the geriatric depression scale: a study of their validity for the diagnosis of a major depressive episode according to ICD-10 and DSM-IV', *Int J Geriatr Psychiatry*, 14(10), pp. 858-65.
- Almkvist, O. and Winblad, B. (1999) 'Early diagnosis of Alzheimer dementia based on clinical and biological factors', *Eur Arch Psychiatry Clin Neurosci*, 249 Suppl 3, pp. 3-9.
- Alzheimer, A. (1907) 'Über eine eigenartige Erkrankung der Hirnrinde', *Allg Z Psychiatr Psych-Gerichtl Med*, 64, pp. 146-148.
- Amaral, D.G. (1993) 'Emerging principles of intrinsic hippocampal organization', *Curr Opin Neurobiol*, 3(2), pp. 225-9.
- American Psychiatric Association (1994) *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*.

- American Psychiatric Association (2013) *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)*.
- Andersen, G., Vestergaard, K., Riis, J.O. and Ingelman - Nielsen, M. (1996) 'Intellectual impairment in the first year following stroke, compared to an age matched population sample.', *Cerebrovasc Dis*, 6, pp. 363-69.
- Andersen, O.M., Reiche, J., Schmidt, V., Gotthardt, M., Spoelgen, R., Behlke, J., von Arnim, C.A., Breiderhoff, T., Jansen, P., Wu, X., Bales, K.R., Cappai, R., Masters, C.L., Gliemann, J., Mufson, E.J., Hyman, B.T., Paul, S.M., Nykjaer, A. and Willnow, T.E. (2005) 'Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein', *Proc Natl Acad Sci U S A*, 102(38), pp. 13461-6.
- Andresen, E.M., Malmgren, J.A., Carter, W.B. and Patrick, D.L. (1994) 'Screening for depression in well older adults: evaluation of a short form of the CES-D (Center for Epidemiologic Studies Depression Scale)', *Am J Prev Med*, 10(2), pp. 77-84.
- Ang, E.T., Wong, P.T., Moochhala, S. and Ng, Y.K. (2003) 'Neuroprotection associated with running: is it a result of increased endogenous neurotrophic factors?', *Neuroscience*, 118(2), pp. 335-45.
- Arai, H. (1996) 'Biological markers for the clinical diagnosis of Alzheimer's disease', *Tohoku J Exp Med*, 179(2), pp. 65-79.
- Arfanakis, K., Fleischman, D.A., Grisot, G., Barth, C.M., Varentsova, A., Morris, M.C., Barnes, L.L. and Bennett, D.A. (2013) 'Systemic inflammation in non-demented elderly human subjects: brain microstructure and cognition', *PLoS One*, 8(8), p. e73107.
- Armani, C., Landini, L., Jr. and Leone, A. (2009) 'Molecular and biochemical changes of the cardiovascular system due to smoking exposure', *Curr Pharm Des*, 15(10), pp. 1038-53.
- Armstrong, M.J., Naglie, G., Duff-Canning, S., Meaney, C., Gill, D., Eslinger, P.J., Zadikoff, C., Mapstone, M., Chou, K.L., Persad, C., Litvan, I., Mast, B.T., Fox, S., Tang-Wai, D.F. and Marras, C. (2012) 'Roles of Education and IQ in Cognitive Reserve in Parkinson's Disease-Mild Cognitive Impairment', *Dement Geriatr Cogn Dis Extra*, 2(1), pp. 343-52.
- Bahar-Fuchs, A., Clare, L. and Woods, B. (2013) 'Cognitive training and cognitive rehabilitation for mild to moderate Alzheimer's disease and vascular dementia', *Cochrane Database Syst Rev*, 6, p. Cd003260.
- Bailey, J.A. and Lahiri, D.K. (2006) 'Neuronal differentiation is accompanied by increased levels of SNAP-25 protein in fetal rat primary cortical neurons: implications in neuronal plasticity and Alzheimer's disease', *Ann N Y Acad Sci*, 1086, pp. 54-65.
- Baiyewu, O., Unverzagt, F.W., Lane, K.A., Gureje, O., Ogunniyi, A., Musick, B., Gao, S., Hall, K.S. and Hendrie, H.C. (2005) 'The Stick Design test: a new measure of visuoconstructional ability', *J Int Neuropsychol Soc*, 11(5), pp. 598-605.
- Ball, K., Berch, D.B., Helmers, K.F., Jobe, J.B., Leveck, M.D., Marsiske, M., Morris, J.N., Rebok, G.W., Smith, D.M., Tennstedt, S.L., Unverzagt, F.W. and Willis, S.L. (2002) 'Effects of cognitive training interventions with older adults: a randomized controlled trial', *JAMA*, 288(18), pp. 2271-81.
- Ballard, C., McKeith, I., O'Brien, J., Kalaria, R., Jaros, E., Ince, P. and Perry, R. (2000) 'Neuropathological substrates of dementia and depression in vascular dementia, with a particular focus on cases with small infarct volumes', *Dement Geriatr Cogn Disord*, 11(2), pp. 59-65.
- Ballard, C., Rowan, E., Stephens, S., Kalaria, R. and Kenny, R.A. (2003a) 'Prospective follow-up study between 3 and 15 months after stroke: improvements and decline in cognitive function among dementia-free stroke survivors >75 years of age', *Stroke*, 34(10), pp. 2440-4.
- Ballard, C., Stephens, S., Kenny, R., Kalaria, R., Tovee, M. and O'Brien, J. (2003b) 'Profile of neuropsychological deficits in older stroke survivors without dementia', *Dement Geriatr Cogn Disord*, 16(1), pp. 52-6.
- Ballard, C., Stephens, S., McLaren, A., Wesnes, K., Kenny, R.A., Burton, E., O'Brien, J. and Kalaria, R. (2002) 'Neuropsychological deficits in older stroke patients', *Ann N Y Acad Sci*, 977, pp. 179-82.

- Ballard, C.G., Burton, E.J., Barber, R., Stephens, S., Kenny, R.A., Kalaria, R.N. and O'Brien, J.T. (2004a) 'NINDS AIREN neuroimaging criteria do not distinguish stroke patients with and without dementia', *Neurology*, 63(6), pp. 983-8.
- Ballard, C.G., Morris, C.M., Rao, H., O'Brien, J.T., Barber, R., Stephens, S., Rowan, E., Gibson, A., Kalaria, R.N. and Kenny, R.A. (2004b) 'APOE epsilon4 and cognitive decline in older stroke patients with early cognitive impairment', *Neurology*, 63(8), pp. 1399-402.
- Bamford J, S.P., Dennis M, Burn J, Warlow C (1991) 'Classification and natural history of clinically identifiable subtypes of cerebral infarction', *Lancet* 337(8756), pp. 1521-1526.
- Barba, R., Martinez-Espinosa, S., Rodriguez-Garcia, E., Pondal, M., Vivancos, J. and Del Ser, T. (2000) 'Poststroke dementia : clinical features and risk factors', *Stroke*, 31(7), pp. 1494-501.
- Barber, R., Ghohkar, A., Scheltens, P., Ballard, C., McKeith, I.G. and O'Brien, J.T. (1999) 'Medial temporal lobe atrophy on MRI in dementia with Lewy bodies', *Neurology*, 52(6), pp. 1153-8.
- Barclay, L.L., Linden, C. and Murtagh, R. (1992) 'Medial temporal atrophy as a magnetic resonance imaging marker for Alzheimer's disease', *J Neuroimaging*, 2(3), pp. 131-5.
- Bast, T. and Feldon, J. (2003) 'Hippocampal modulation of sensorimotor processes', *Prog Neurobiol*, 70(4), pp. 319-45.
- Bast, T., Wilson, I.A., Witter, M.P. and Morris, R.G. (2009) 'From rapid place learning to behavioral performance: a key role for the intermediate hippocampus', *PLoS Biol*, 7(4), p. e1000089.
- Bastos-Leite, A.J., van der Flier, W.M., van Straaten, E.C., Staekenborg, S.S., Scheltens, P. and Barkhof, F. (2007) 'The contribution of medial temporal lobe atrophy and vascular pathology to cognitive impairment in vascular dementia', *Stroke*, 38(12), pp. 3182-5.
- Beeri, M.S., Haroutunian, V., Schmeidler, J., Sano, M., Fam, P., Kavanaugh, A., Barr, A.M., Honer, W.G. and Katsel, P. (2012) 'Synaptic protein deficits are associated with dementia irrespective of extreme old age', *Neurobiol Aging*, 33(6), pp. 1125.e1-8.
- Bejot, Y., Aboa-Eboule, C., Durier, J., Rouaud, O., Jacquin, A., Ponavoy, E., Richard, D., Moreau, T. and Giroud, M. (2011) 'Prevalence of early dementia after first-ever stroke: a 24-year population-based study', *Stroke*, 42(3), pp. 607-12.
- Bennett, D.A., Schneider, J.A., Arvanitakis, Z., Kelly, J.F., Aggarwal, N.T., Shah, R.C. and Wilson, R.S. (2006) 'Neuropathology of older persons without cognitive impairment from two community-based studies', *Neurology*, 66(12), pp. 1837-44.
- Bennett, D.A., Wilson, R.S., Boyle, P.A., Buchman, A.S. and Schneider, J.A. (2012) 'Relation of neuropathology to cognition in persons without cognitive impairment', *Ann Neurol*, 72(4), pp. 599-609.
- Bennett, S., Grant, M.M. and Aldred, S. (2009) 'Oxidative stress in vascular dementia and Alzheimer's disease: a common pathology', *J Alzheimers Dis*, 17(2), pp. 245-57.
- Benoit, S.C., Clegg, D.J., Seeley, R.J. and Woods, S.C. (2004) 'Insulin and leptin as adiposity signals', *Recent Prog Horm Res*, 59, pp. 267-85.
- Birks, J., McGuinness, B. and Craig, D. (2013) 'Rivastigmine for vascular cognitive impairment', *Cochrane Database Syst Rev*, 5, p. Cd004744.
- Black, S., Gao, F. and Bilbao, J. (2009) 'Understanding white matter disease: imaging-pathological correlations in vascular cognitive impairment', *Stroke*, 40(3 Suppl), pp. S48-52.
- Black, S., Roman, G.C., Geldmacher, D.S., Salloway, S., Hecker, J., Burns, A., Perdomo, C., Kumar, D. and Pratt, R. (2003) 'Efficacy and tolerability of donepezil in vascular dementia: positive results of a 24-week, multicenter, international, randomized, placebo-controlled clinical trial', *Stroke*, 34(10), pp. 2323-30.
- Blake, H., McKinney, M., Treece, K., Lee, E. and Lincoln, N.B. (2002) 'An evaluation of screening measures for cognitive impairment after stroke', *Age Ageing*, 31(6), pp. 451-6.
- Blennow, K., Bogdanovic, N., Alafuzoff, I., Ekman, R. and Davidsson, P. (1996) 'Synaptic pathology in Alzheimer's disease: relation to severity of dementia, but not to senile

- plaques, neurofibrillary tangles, or the ApoE4 allele', *J Neural Transm*, 103(5), pp. 603-18.
- Blessed, G., Black, S.E., Butler, T. and Kay, D.W. (1991) 'The diagnosis of dementia in the elderly. A comparison of CAMCOG (the cognitive section of CAMDEX), the AGE-CAT program, DSM-III, the Mini-Mental State Examination and some short rating scales', *Br J Psychiatry*, 159, pp. 193-8.
- Blessed, G., Tomlinson, B.E. and Roth, M. (1968) 'The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects', *Br J Psychiatry*, 114(512), pp. 797-811.
- Bohannon, A.D., Fillenbaum, G.G., Pieper, C.F., Hanlon, J.T. and Blazer, D.G. (2002) 'Relationship of race/ethnicity and blood pressure to change in cognitive function', *J Am Geriatr Soc*, 50(3), pp. 424-9.
- Bossuyt, P.M. and Reitsma, J.B. (2003) 'The STARD initiative', *Lancet*, 361(9351), p. 71.
- Boyle, P.A., Wilson, R.S., Yu, L., Barr, A.M., Honer, W.G., Schneider, J.A. and Bennett, D.A. (2013a) 'Much of late life cognitive decline is not due to common neurodegenerative pathologies', *Ann Neurol*, 74(3), pp. 478-89.
- Boyle, P.A., Yu, L., Wilson, R.S., Schneider, J.A. and Bennett, D.A. (2013b) 'Relation of neuropathology with cognitive decline among older persons without dementia', *Front Aging Neurosci*, 5, p. 50.
- Braak, H. and Braak, E. (1991) 'Neuropathological staging of Alzheimer-related changes', *Acta Neuropathol*, 82(4), pp. 239-59.
- Brands, A.M., Biessels, G.J., de Haan, E.H., Kappelle, L.J. and Kessels, R.P. (2005) 'The effects of type 1 diabetes on cognitive performance: a meta-analysis', *Diabetes Care*, 28(3), pp. 726-35.
- Brayne, C., Ince, P.G., Keage, H.A., McKeith, I.G., Matthews, F.E., Polvikoski, T. and Sulkava, R. (2010) 'Education, the brain and dementia: neuroprotection or compensation?', *Brain*, 133(Pt 8), pp. 2210-6.
- Bredesen, D.E., Rao, R.V. and Mehlen, P. (2006) 'Cell death in the nervous system', *Nature*, 443(7113), pp. 796-802.
- Bresciani, L., Rossi, R., Testa, C., Geroldi, C., Galluzzi, S., Laakso, M.P., Beltramello, A., Soininen, H. and Frisoni, G.B. (2005) 'Visual assessment of medial temporal atrophy on MR films in Alzheimer's disease: comparison with volumetry', *Aging Clin Exp Res*, 17(1), pp. 8-13.
- Brickman, A.M., Stern, Y. and Small, S.A. (2011) 'Hippocampal subregions differentially associate with standardized memory tests', *Hippocampus*, 21(9), pp. 923-8.
- Brun, A. and Englund, E. (1986) 'A white matter disorder in dementia of the Alzheimer type: a pathoanatomical study', *Ann Neurol*, 19(3), pp. 253-62.
- Bullock, R., Erkinjuntti, T. and Lilienfeld, S. (2004) 'Management of patients with Alzheimer's disease plus cerebrovascular disease: 12-month treatment with galantamine', *Dement Geriatr Cogn Disord*, 17(1-2), pp. 29-34.
- Burgess, N., Maguire, E.A. and O'Keefe, J. (2002) 'The human hippocampus and spatial and episodic memory', *Neuron*, 35(4), pp. 625-41.
- Burton, E., Ballard, C., Stephens, S., Kenny, R.A., Kalaria, R., Barber, R. and O'Brien, J. (2003) 'Hyperintensities and fronto-subcortical atrophy on MRI are substrates of mild cognitive deficits after stroke', *Dement Geriatr Cogn Disord*, 16(2), pp. 113-8.
- Burton, E.J., Barber, R., Mukaetova-Ladinska, E.B., Robson, J., Perry, R.H., Jaros, E., Kalaria, R.N. and O'Brien, J.T. (2009) 'Medial temporal lobe atrophy on MRI differentiates Alzheimer's disease from dementia with Lewy bodies and vascular cognitive impairment: a prospective study with pathological verification of diagnosis', *Brain*, 132(Pt 1), pp. 195-203.
- Burton, E.J., Kenny, R.A., O'Brien, J., Stephens, S., Bradbury, M., Rowan, E., Kalaria, R., Firbank, M., Wesnes, K. and Ballard, C. (2004) 'White matter hyperintensities are associated with impairment of memory, attention, and global cognitive performance in older stroke patients', *Stroke*, 35(6), pp. 1270-5.



- Buttner, A., Rohrmoser, K., Mall, G., Penning, R. and Weis, S. (2006) 'Widespread axonal damage in the brain of drug abusers as evidenced by accumulation of beta-amyloid precursor protein (beta-APP): an immunohistochemical investigation', *Addiction*, 101(9), pp. 1339-46.
- Cagnin, A., Brooks, D.J., Kennedy, A.M., Gunn, R.N., Myers, R., Turkheimer, F.E., Jones, T. and Banati, R.B. (2001) 'In-vivo measurement of activated microglia in dementia', *Lancet*, 358(9280), pp. 461-7.
- Callahan, L.M. and Coleman, P.D. (1995) 'Neurons bearing neurofibrillary tangles are responsible for selected synaptic deficits in Alzheimer's disease', *Neurobiol Aging*, 16(3), pp. 311-4.
- Campbell, M.C. and Tishkoff, S.A. (2010) 'The evolution of human genetic and phenotypic variation in Africa', *Curr Biol*, 20(4), pp. R166-73.
- Candelario-Jalil, E., Yang, Y. and Rosenberg, G.A. (2009) 'Diverse roles of matrix metalloproteinases and tissue inhibitors of metalloproteinases in neuroinflammation and cerebral ischemia', *Neuroscience*, 158(3), pp. 983-94.
- Caruana, D.A., Alexander, G.M. and Dudek, S.M. (2012) 'New insights into the regulation of synaptic plasticity from an unexpected place: hippocampal area CA2', *Learn Mem*, 19(9), pp. 391-400.
- Catani, M. and ffytche, D.H. (2005) 'The rises and falls of disconnection syndromes', *Brain*, 128(Pt 10), pp. 2224-39.
- Censori, B., Manara, O., Agostinis, C., Camerlingo, M., Casto, L., Galavotti, B., Partziguian, T., Servalli, M.C., Cesana, B., Belloni, G. and Mamoli, A. (1996) 'Dementia after first stroke', *Stroke*, 27(7), pp. 1205-10.
- CFAS), N.G.o.t.M.R.C.C.F.a.A.S.M. (2001) 'Pathological correlates of late-onset dementia in a multicentre, community-based population in England and Wales. ', *Lancet*, 357(9251), pp. 169-75.
- Chetelat, G., Villemagne, V.L., Pike, K.E., Baron, J.C., Bourgeat, P., Jones, G., Faux, N.G., Ellis, K.A., Salvado, O., Szoeki, C., Martins, R.N., Ames, D., Masters, C.L. and Rowe, C.C. (2010) 'Larger temporal volume in elderly with high versus low beta-amyloid deposition', *Brain*, 133(11), pp. 3349-58.
- Chowdhury, R., Stevens, S., Gorman, D., Pan, A., Warnakula, S., Chowdhury, S., Ward, H., Johnson, L., Crowe, F., Hu, F.B. and Franco, O.H. 'Association between fish consumption, long chain omega 3 fatty acids, and risk of cerebrovascular disease: systematic review and meta-analysis', *BMJ*, 345, p. e6698.
- Chowdhury, R., Stevens, S., Gorman, D., Pan, A., Warnakula, S., Chowdhury, S., Ward, H., Johnson, L., Crowe, F., Hu, F.B. and Franco, O.H. (2012) 'Association between fish consumption, long chain omega 3 fatty acids, and risk of cerebrovascular disease: systematic review and meta-analysis', *BMJ*, 345, p. e6698.
- Chui, H.C., Mack, W., Jackson, J.E., Mungas, D., Reed, B.R., Tinklenberg, J., Chang, F.L., Skinner, K., Tasaki, C. and Jagust, W.J. (2000) 'Clinical criteria for the diagnosis of vascular dementia: a multicenter study of comparability and interrater reliability', *Arch Neurol*, 57(2), pp. 191-6.
- Chui, H.C., Victoroff, J.I., Margolin, D., Jagust, W., Shankle, R. and Katzman, R. (1992) 'Criteria for the diagnosis of ischemic vascular dementia proposed by the State of California Alzheimer's Disease Diagnostic and Treatment Centers', *Neurology*, 42(3 Pt 1), pp. 473-80.
- Clare, R., King, V.G., Wrenfeldt, M. and Vinters, H.V. (2010) 'Synapse loss in dementias', *J Neurosci Res*, 88(10), pp. 2083-90.
- Colcombe, S.J., Erickson, K.I., Scalf, P.E., Kim, J.S., Prakash, R., McAuley, E., Elavsky, S., Marquez, D.X., Hu, L. and Kramer, A.F. (2006) 'Aerobic exercise training increases brain volume in aging humans', *J Gerontol A Biol Sci Med Sci*, 61(11), pp. 1166-70.
- Cole, W.A. (1869) *Physico - Medical Essay Concerning the Late Frequency of Apoplexies Together With a General Method of Their Prevention and Cure: In a Letter to a Physician*. Oxford, United Kingdom: The Theater.

- Coltman, R., Spain, A., Tsenkina, Y., Fowler, J.H., Smith, J., Scullion, G., Allerhand, M., Scott, F., Kalaria, R.N., Ihara, M., Daumas, S., Deary, I.J., Wood, E., McCulloch, J. and Horsburgh, K. (2011) 'Selective white matter pathology induces a specific impairment in spatial working memory', *Neurobiol Aging*, 32(12), pp. 2324.e7-12.
- Colton, C.A., Mott, R.T., Sharpe, H., Xu, Q., Van Nostrand, W.E. and Vitek, M.P. (2006) 'Expression profiles for macrophage alternative activation genes in AD and in mouse models of AD', *J Neuroinflammation*, 3, p. 27.
- Connelly, S.J., Mukaetova-Ladinska, E.B., Abdul-All, Z., Alves da Silva, J., Brayne, C., Honer, W.G. and Mann, D.M. (2011) 'Synaptic changes in frontotemporal lobar degeneration: correlation with MAPT haplotype and APOE genotype', *Neuropathol Appl Neurobiol*, 37(4), pp. 366-80.
- Connor, M.D., Walker, R., Modi, G. and Warlow, C.P. (2007) 'Burden of stroke in black populations in sub-Saharan Africa', *Lancet Neurol*, 6(3), pp. 269-78.
- Cordoliani-Mackowiak, M.A., Henon, H., Pruvo, J.P., Pasquier, F. and Leys, D. (2003) 'Poststroke dementia: influence of hippocampal atrophy', *Arch Neurol*, 60(4), pp. 585-90.
- Cotman, C.W. and Berchtold, N.C. (2007) 'Physical activity and the maintenance of cognition: learning from animal models', *Alzheimers Dement*, 3(2 Suppl), pp. S30-7.
- Cotman, C.W., Berchtold, N.C. and Christie, L.A. (2007) 'Exercise builds brain health: key roles of growth factor cascades and inflammation', *Trends Neurosci*, 30(9), pp. 464-72.
- Counts, S.E., He, B., Nadeem, M., Wu, J., Scheff, S.W. and Mufson, E.J. (2012) 'Hippocampal drebrin loss in mild cognitive impairment', *Neurodegener Dis*, 10(1-4), pp. 216-9.
- Counts, S.E., Nadeem, M., Lad, S.P., Wu, J. and Mufson, E.J. (2006) 'Differential expression of synaptic proteins in the frontal and temporal cortex of elderly subjects with mild cognitive impairment', *J Neuropathol Exp Neurol*, 65(6), pp. 592-601.
- Craggs, L. and Kalaria, R.N. (2010) 'Revisiting dietary antioxidants, neurodegeneration and dementia', *Neuroreport*.
- Craggs, L.J., Yamamoto, Y., Ihara, M., Fenwick, R., Burke, M., Oakley, A.E., Roeber, S., Duering, M., Kretschmar, H. and Kalaria, R.N. (2013) 'White matter pathology and disconnection in the frontal lobe in CADASIL', *Neuropathol Appl Neurobiol*.
- Craig, D. and Birks, J. (2005) 'Rivastigmine for vascular cognitive impairment', *Cochrane Database Syst Rev*, (2), p. Cd004744.
- Crichton, G.E., Bryan, J., Murphy, K.J. and Buckley, J. (2010) 'Review of dairy consumption and cognitive performance in adults: findings and methodological issues', *Dement Geriatr Cogn Disord*, 30(4), pp. 352-61.
- Crum, R.M., Anthony, J.C., Bassett, S.S. and Folstein, M.F. (1993) 'Population-based norms for the Mini-Mental State Examination by age and educational level', *Jama*, 269(18), pp. 2386-91.
- Cummings, J.L. (1994) 'Vascular subcortical dementias: clinical aspects', *Dementia*, 5(3-4), pp. 177-80.
- Danesi, M., Okubadejo, N. and Ojini, F. (2007) 'Prevalence of stroke in an urban, mixed-income community in Lagos, Nigeria', *Neuroepidemiology*, 28(4), pp. 216-23.
- Das, S., Paul, N., Hazra, A., Ghosal, M., Ray, B.K., Banerjee, T.K., Burman, P. and Das, S.K. (2012) 'Cognitive Dysfunction in Stroke Survivors: A Community-Based Prospective Study from Kolkata, India', *J Stroke Cerebrovasc Dis*.
- Datson, N.A., Meijer, L., Steenbergen, P.J., Morsink, M.C., van der Laan, S., Meijer, O.C. and de Kloet, E.R. (2004) 'Expression profiling in laser-microdissected hippocampal subregions in rat brain reveals large subregion-specific differences in expression', *Eur J Neurosci*, 20(10), pp. 2541-54.
- Davies, C.A., Mann, D.M., Sumpter, P.Q. and Yates, P.O. (1987) 'A quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with Alzheimer's disease', *J Neurol Sci*, 78(2), pp. 151-64.
- Dawe, R.J., Bennett, D.A., Schneider, J.A. and Arfanakis, K. (2011) 'Neuropathologic correlates of hippocampal atrophy in the elderly: a clinical, pathologic, postmortem MRI study', *PLoS One*, 6(10), p. e26286.

- de Koning, I., Dippel, D.W., van Kooten, F. and Koudstaal, P.J. (2000) 'A short screening instrument for poststroke dementia : the R-CAMCOG', *Stroke*, 31(7), pp. 1502-8.
- de Koning, I., van Kooten, F., Koudstaal, P.J. and Dippel, D.W. (2005) 'Diagnostic value of the Rotterdam-CAMCOG in post-stroke dementia', *J Neurol Neurosurg Psychiatry*, 76(2), pp. 263-5.
- de la Torre, J.C. (2002) 'Alzheimer disease as a vascular disorder: nosological evidence', *Stroke*, 33(4), pp. 1152-62.
- de la Torre, J.C. and Mussivand, T. (1993) 'Can disturbed brain microcirculation cause Alzheimer's disease?', *Neurol Res*, 15(3), pp. 146-53.
- de Leeuw, F.E., de Groot, J.C., Achten, E., Oudkerk, M., Ramos, L.M., Heijboer, R., Hofman, A., Jolles, J., van Gijn, J. and Breteler, M.M. (2001) 'Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study', *J Neurol Neurosurg Psychiatry*, 70(1), pp. 9-14.
- Deak, F., Schoch, S., Liu, X., Sudhof, T.C. and Kavalali, E.T. (2004) 'Synaptobrevin is essential for fast synaptic-vesicle endocytosis', *Nat Cell Biol*, 6(11), pp. 1102-8.
- Deane, R., Bell, R.D., Sagare, A. and Zlokovic, B.V. (2009) 'Clearance of amyloid-beta peptide across the blood-brain barrier: implication for therapies in Alzheimer's disease', *CNS Neurol Disord Drug Targets*, 8(1), pp. 16-30.
- Debette, S. and Markus, H.S. (2010) 'The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis', *BMJ*, 341, p. c3666.
- Debette, S., Seshadri, S., Beiser, A., Au, R., Himali, J.J., Palumbo, C., Wolf, P.A. and DeCarli, C. (2011) 'Midlife vascular risk factor exposure accelerates structural brain aging and cognitive decline', *Neurology*, 77(5), pp. 461-8.
- DeCarli, C., Grady, C.L., Clark, C.M., Katz, D.A., Brady, D.R., Murphy, D.G., Haxby, J.V., Salerno, J.A., Gillette, J.A., Gonzalez-Aviles, A. and Rapoport, S.I. (1996) 'Comparison of positron emission tomography, cognition, and brain volume in Alzheimer's disease with and without severe abnormalities of white matter', *J Neurol Neurosurg Psychiatry*, 60(2), pp. 158-67.
- DeCarli, C., Kawas, C., Morrison, J.H., Reuter-Lorenz, P.A., Sperling, R.A. and Wright, C.B. (2012) 'Session II: Mechanisms of age-related cognitive change and targets for intervention: neural circuits, networks, and plasticity', *J Gerontol A Biol Sci Med Sci*, 67(7), pp. 747-53.
- DeKosky, S.T. and Scheff, S.W. (1990) 'Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity', *Ann Neurol*, 27(5), pp. 457-64.
- Delgado, C., Donoso, A., Orellana, P., Vasquez, C., Diaz, V. and Behrens, M.I. (2010) 'Frequency and determinants of poststroke cognitive impairment at three and twelve months in Chile', *Dement Geriatr Cogn Disord*, 29(5), pp. 397-405.
- den Heijer, T., Vermeer, S.E., van Dijk, E.J., Prins, N.D., Koudstaal, P.J., Hofman, A. and Breteler, M.M. (2003) 'Type 2 diabetes and atrophy of medial temporal lobe structures on brain MRI', *Diabetologia*, 46(12), pp. 1604-10.
- Deramecourt, V., Slade, J.Y., Oakley, A.E., Perry, R.H., Ince, P.G., Maurage, C.A. and Kalaria, R.N. (2012) 'Staging and natural history of cerebrovascular pathology in dementia', *Neurology*, 78(14), pp. 1043-50.
- Derouesne, C. and Poirier, J. (1999) '[Cerebral lacunae: still under debate]', *Rev Neurol (Paris)*, 155(10), pp. 823-31.
- Desmond DW, M.J., Sano M, Stern Y (1996) 'Recovery of cognitive function after stroke.', *Stroke*, 27, pp. 1798 – 1803.
- Desmond, D.W., Moroney, J.T., Sano, M. and Stern, Y. (2002) 'Incidence of dementia after ischemic stroke: results of a longitudinal study', *Stroke*, 33(9), pp. 2254-60.
- Di Maio, V. (2008) 'Regulation of information passing by synaptic transmission: a short review', *Brain Res*, 1225, pp. 26-38.
- Ding, Y., Li, J., Luan, X., Ding, Y.H., Lai, Q., Rafols, J.A., Phillis, J.W., Clark, J.C. and Diaz, F.G. (2004) 'Exercise pre-conditioning reduces brain damage in ischemic rats that may

- be associated with regional angiogenesis and cellular overexpression of neurotrophin', *Neuroscience*, 124(3), pp. 583-91.
- Dishman, R.K., Berthoud, H.R., Booth, F.W., Cotman, C.W., Edgerton, V.R., Fleshner, M.R., Gandevia, S.C., Gomez-Pinilla, F., Greenwood, B.N., Hillman, C.H., Kramer, A.F., Levin, B.E., Moran, T.H., Russo-Neustadt, A.A., Salamone, J.D., Van Hoomissen, J.D., Wade, C.E., York, D.A. and Zigmond, M.J. (2006) 'Neurobiology of exercise', *Obesity (Silver Spring)*, 14(3), pp. 345-56.
- Dodson, S.E., Andersen, O.M., Karmali, V., Fritz, J.J., Cheng, D., Peng, J., Levey, A.I., Willnow, T.E. and Lah, J.J. (2008) 'Loss of LR11/SORLA enhances early pathology in a mouse model of amyloidosis: evidence for a proximal role in Alzheimer's disease', *J Neurosci*, 28(48), pp. 12877-86.
- Dolan, D., Troncoso, J., Resnick, S.M., Crain, B.J., Zonderman, A.B. and O'Brien, R.J. (2010) 'Age, Alzheimer's disease and dementia in the Baltimore Longitudinal Study of Ageing', *Brain*, 133(Pt 8), pp. 2225-31.
- Dong, Y., Venketasubramanian, N., Chan, B.P., Sharma, V.K., Slavin, M.J., Collinson, S.L., Sachdev, P., Chan, Y.H. and Chen, C.L. (2012) 'Brief screening tests during acute admission in patients with mild stroke are predictive of vascular cognitive impairment 3-6 months after stroke', *J Neurol Neurosurg Psychiatry*, 83(6), pp. 580-5.
- Donnan, G.A. (2008) 'The evidence to date does not seem to be enough to alter clinical practice and we wait with interest more definitive information', *Int J Stroke*, 3(2), p. 77.
- Dotchin, C.L., Akinyemi, R.O., Gray, W.K. and Walker, R.W. (2013) 'Geriatric medicine: services and training in Africa', *Age Ageing*, 42(1), pp. 124-8.
- Douiri, A., McKeivitt, C., Emmett, E.S., Rudd, A.G. and Wolfe, C.D. (2013a) 'Long-term effects of secondary prevention on cognitive function in stroke patients', *Circulation*, 128(12), pp. 1341-8.
- Douiri, A., Rudd, A.G. and Wolfe, C.D. (2013b) 'Prevalence of poststroke cognitive impairment: South London Stroke Register 1995-2010', *Stroke*, 44(1), pp. 138-45.
- Downes, E.C., Robson, J., Grailly, E., Abdel-All, Z., Xuereb, J., Brayne, C., Holland, A., Honer, W.G. and Mukaetova-Ladinska, E.B. (2008) 'Loss of synaptophysin and synaptosomal-associated protein 25-kDa (SNAP-25) in elderly Down syndrome individuals', *Neuropathol Appl Neurobiol*, 34(1), pp. 12-22.
- Driver, H.S. and Taylor, S.R. (2000) 'Exercise and sleep', *Sleep Med Rev*, 4(4), pp. 387-402.
- Dunn, A.L., Trivedi, M.H., Kampert, J.B., Clark, C.G. and Chambliss, H.O. (2005) 'Exercise treatment for depression: efficacy and dose response', *Am J Prev Med*, 28(1), pp. 1-8.
- Duron, E. and Hanon, O. (2008) 'Hypertension, cognitive decline and dementia', *Arch Cardiovasc Dis*, 101(3), pp. 181-9.
- Duvernoy, H. (2005) *The Human Hippocampus : Functional Anatomy, Vascularization and Serial Sections with MRI*. 3rd edn. Heidelberg: Springer.
- Edison, P., Archer, H.A., Gerhard, A., Hinz, R., Pavese, N., Turkheimer, F.E., Hammers, A., Tai, Y.F., Fox, N., Kennedy, A., Rossor, M. and Brooks, D.J. (2008) 'Microglia, amyloid, and cognition in Alzheimer's disease: An [11C](R)PK11195-PET and [11C]PIB-PET study', *Neurobiol Dis*, 32(3), pp. 412-9.
- Edison, P., Hinz, R. and Brooks, D.J. (2011) 'Technical aspects of amyloid imaging for Alzheimer's disease', *Alzheimers Res Ther*, 3(4), p. 25.
- Eichenbaum, H. (2001) 'The hippocampus and declarative memory: cognitive mechanisms and neural codes', *Behav Brain Res*, 127(1-2), pp. 199-207.
- Eichenbaum, H. (2004) 'Hippocampus: cognitive processes and neural representations that underlie declarative memory', *Neuron*, 44(1), pp. 109-20.
- Englund, E. (1998) 'Neuropathology of white matter changes in Alzheimer's disease and vascular dementia', *Dement Geriatr Cogn Disord*, 9 Suppl 1, pp. 6-12.
- Erkinjuntti, T. and Gauthier, S. (2009) 'The concept of vascular cognitive impairment', *Front Neurol Neurosci*, 24, pp. 79-85.
- Erkinjuntti, T. and Kalaria, R. (2006) 'Vascular Cognitive Impairment', in Growdon, J. and Rossor, M. (eds.) *The Dementias*. Elsevier.

- Erkinjuntti, T., Roman, G. and Gauthier, S. (2004) 'Treatment of vascular dementia--evidence from clinical trials with cholinesterase inhibitors', *J Neurol Sci*, 226(1-2), pp. 63-6.
- Erten-Lyons, D., Woltjer, R.L., Dodge, H., Nixon, R., Vorobik, R., Calvert, J.F., Leahy, M., Montine, T. and Kaye, J. (2009) 'Factors associated with resistance to dementia despite high Alzheimer disease pathology', *Neurology*, 72(4), pp. 354-60.
- Esiri, M.M., Nagy, Z., Smith, M.Z., Barnettson, L. and Smith, A.D. (1999) 'Cerebrovascular disease and threshold for dementia in the early stages of Alzheimer's disease', *Lancet*, 354(9182), pp. 919-20.
- Esiri, M.M., Wilcock, G.K. and Morris, J.H. (1997) 'Neuropathological assessment of the lesions of significance in vascular dementia', *J Neurol Neurosurg Psychiatry*, 63(6), pp. 749-53.
- Fanselow, M.S. and Dong, H.W. (2010) 'Are the dorsal and ventral hippocampus functionally distinct structures?', *Neuron*, 65(1), pp. 7-19.
- Farrag, A., Farwiz, H.M., Khedr, E.H., Mahfouz, R.M. and Omran, S.M. (1998) 'Prevalence of Alzheimer's disease and other dementing disorders: Assiut-Upper Egypt study', *Dement Geriatr Cogn Disord*, 9(6), pp. 323-8.
- Fassbender, K., Simons, M., Bergmann, C., Stroick, M., Lutjohann, D., Keller, P., Runz, H., Kuhl, S., Bertsch, T., von Bergmann, K., Hennerici, M., Beyreuther, K. and Hartmann, T. (2001) 'Simvastatin strongly reduces levels of Alzheimer's disease beta -amyloid peptides Abeta 42 and Abeta 40 in vitro and in vivo', *Proc Natl Acad Sci U S A*, 98(10), pp. 5856-61.
- Fatoye, F.O., Komolafe, M.A., Eegunranti, B.A., Adewuya, A.O., Mosaku, S.K. and Fatoye, G.K. (2007) 'Cognitive impairment and quality of life among stroke survivors in Nigeria', *Psychol Rep*, 100(3 Pt 1), pp. 876-82.
- Feigin, V.L., Forouzanfar, M.H., Krishnamurthi, R., Mensah, G.A., Connor, M., Bennett, D.A., Moran, A.E., Sacco, R.L., Anderson, L., Truelsen, T., O'Donnell, M., Venketasubramanian, N., Barker-Collo, S., Lawes, C.M.M., Wang, W., Yukito, S., Witt, E., Ezzati, M., Naghavi, M. and Murray, C. (2013) 'Global and regional burden of stroke during 1990 - 2010 : findings from the Global Burden of Disease Study 2010', *The Lancet*, S0140 -6736 (13) pp. 61953-4
- Fernando, M.S., O'Brien, J.T., Perry, R.H., English, P., Forster, G., McMeekin, W., Slade, J.Y., Golkhar, A., Matthews, F.E., Barber, R., Kalaria, R.N. and Ince, P.G. (2004) 'Comparison of the pathology of cerebral white matter with post-mortem magnetic resonance imaging (MRI) in the elderly brain', *Neuropathol Appl Neurobiol*, 30(4), pp. 385-95.
- Fernando, M.S., Simpson, J.E., Matthews, F., Brayne, C., Lewis, C.E., Barber, R., Kalaria, R.N., Forster, G., Esteves, F., Wharton, S.B., Shaw, P.J., O'Brien, J.T. and Ince, P.G. (2006) 'White matter lesions in an unselected cohort of the elderly: molecular pathology suggests origin from chronic hypoperfusion injury', *Stroke*, 37(6), pp. 1391-8.
- Ferrer, I. (1999) 'Neurons and their dendrites in frontotemporal dementia', *Dement Geriatr Cogn Disord*, 10 Suppl 1, pp. 55-60.
- Ferrer, I., Kaste, M. and Kalimo, H. (2008) 'Vascular diseases', in Love, S., Louis, D. and Ellison, D. (eds.) *Greenfield's Neuropathology*. 8th edn. Oxford: Oxford University Press, pp. 121 - 240.
- Firbank, M.J., Allan, L.M., Burton, E.J., Barber, R., O'Brien, J.T. and Kalaria, R.N. (2012) 'Neuroimaging predictors of death and dementia in a cohort of older stroke survivors', *J Neurol Neurosurg Psychiatry*, 83(3), pp. 263-7.
- Firbank, M.J., Burton, E.J., Barber, R., Stephens, S., Kenny, R.A., Ballard, C., Kalaria, R.N. and O'Brien, J.T. (2007) 'Medial temporal atrophy rather than white matter hyperintensities predict cognitive decline in stroke survivors', *Neurobiol Aging*, 28(11), pp. 1664-9.
- Firbank, M.J., He, J., Blamire, A.M., Singh, B., Danson, P., Kalaria, R.N. and O'Brien, J.T. (2011) 'Cerebral blood flow by arterial spin labeling in poststroke dementia', *Neurology*, 76(17), pp. 1478-84.

- Firbank, M.J., Minett, T. and O'Brien, J.T. (2003) 'Changes in DWI and MRS associated with white matter hyperintensities in elderly subjects', *Neurology*, 61(7), pp. 950-4.
- Fisher, C.M. (1968) 'The arterial lesions underlying lacunes', *Acta Neuropathol*, 12(1), pp. 1-15.
- Folstein, M.F., Folstein, S.E. and McHugh, P.R. (1975) "'Mini-mental state". A practical method for grading the cognitive state of patients for the clinician', *J Psychiatr Res*, 12(3), pp. 189-98.
- Fraser, A., Elliott, S.F. and Cohen, D. (2012) 'Stroke strategy: Six steps to achieve evidence-based care', *Health Serv J*, 122(6306), pp. 22-4.
- Freneau, R.T., Jr., Kam, K., Qureshi, T., Johnson, J., Copenhagen, D.R., Storm-Mathisen, J., Chaudhry, F.A., Nicoll, R.A. and Edwards, R.H. (2004a) 'Vesicular glutamate transporters 1 and 2 target to functionally distinct synaptic release sites', *Science*, 304(5678), pp. 1815-9.
- Freneau, R.T., Jr., Voglmaier, S., Seal, R.P. and Edwards, R.H. (2004b) 'VGLUTs define subsets of excitatory neurons and suggest novel roles for glutamate', *Trends Neurosci*, 27(2), pp. 98-103.
- Ganguli, M., Blacker, D., Blazer, D.G., Grant, I., Jeste, D.V., Paulsen, J.S., Petersen, R.C. and Sachdev, P.S. (2011) 'Classification of neurocognitive disorders in DSM-5: a work in progress', *Am J Geriatr Psychiatry*, 19(3), pp. 205-10.
- Gates, N., Fiatarone Singh, M.A., Sachdev, P.S. and Valenzuela, M. (2013) 'The Effect of Exercise Training on Cognitive Function in Older Adults with Mild Cognitive Impairment: A Meta-analysis of Randomized Controlled Trials', *Am J Geriatr Psychiatry*, 21(11), pp. 1086-97.
- Gdovinova, Z., Habalova, V. and Novosadova, Z. (2006) 'Polymorphism of apolipoprotein E in relation with Alzheimer and vascular dementia', *Cell Mol Neurobiol*, 26(7-8), pp. 1219-24.
- Geddes, J.W., Hess, E.J., Hart, R.A., Kesslak, J.P., Cotman, C.W. and Wilson, M.C. (1990a) 'Lesions of hippocampal circuitry define synaptosomal-associated protein-25 (SNAP-25) as a novel presynaptic marker', *Neuroscience*, 38(2), pp. 515-25.
- Geddes, J.W., Wilson, M.C., Miller, F.D. and Cotman, C.W. (1990b) 'Molecular markers of reactive plasticity', *Adv Exp Med Biol*, 268, pp. 425-32.
- Gemmell, E., Bosomworth, H., Allan, L., Hall, R., Khundakar, A., Oakley, A.E., Deramecourt, V., Polvikoski, T.M., O'Brien, J.T. and Kalara, R.N. (2012) 'Hippocampal neuronal atrophy and cognitive function in delayed poststroke and aging-related dementias', *Stroke*, 43(3), pp. 808-14.
- Geschwind, N. (1965a) 'Disconnexion syndromes in animals and man. I', *Brain*, 88(2), pp. 237-94.
- Geschwind, N. (1965b) 'Disconnexion syndromes in animals and man. II', *Brain*, 88(3), pp. 585-644.
- Girouard, H. and Iadecola, C. (2006) 'Neurovascular coupling in the normal brain and in hypertension, stroke, and Alzheimer disease', *J Appl Physiol*, 100(1), pp. 328-35.
- Girouard, H., Park, L., Anrather, J., Zhou, P. and Iadecola, C. (2007) 'Cerebrovascular nitrosative stress mediates neurovascular and endothelial dysfunction induced by angiotensin II', *Arterioscler Thromb Vasc Biol*, 27(2), pp. 303-9.
- Gold, G., Giannakopoulos, P., Montes-Paixao Junior, C., Herrmann, F.R., Mulligan, R., Michel, J.P. and Bouras, C. (1997) 'Sensitivity and specificity of newly proposed clinical criteria for possible vascular dementia', *Neurology*, 49(3), pp. 690-4.
- Goldman-Rakic, P.S., Selemon, L.D. and Schwartz, M.L. (1984) 'Dual pathways connecting the dorsolateral prefrontal cortex with the hippocampal formation and parahippocampal cortex in the rhesus monkey', *Neuroscience*, 12(3), pp. 719-43.
- Gorelick, P.B., Brody, J., Cohen, D., Freels, S., Levy, P., Dollear, W., Forman, H. and Harris, Y. (1993) 'Risk factors for dementia associated with multiple cerebral infarcts. A case-control analysis in predominantly African-American hospital-based patients', *Arch Neurol*, 50(7), pp. 714-20.
- Gorelick, P.B., Scuteri, A., Black, S.E., Decarli, C., Greenberg, S.M., Iadecola, C., Launer, L.J., Laurent, S., Lopez, O.L., Nyenhuis, D., Petersen, R.C., Schneider, J.A., Tzourio, C.,

- Arnett, D.K., Bennett, D.A., Chui, H.C., Higashida, R.T., Lindquist, R., Nilsson, P.M., Roman, G.C., Sellke, F.W. and Seshadri, S. (2011) 'Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association', *Stroke*, 42(9), pp. 2672-713.
- Gottesman, R., Hillis AE (2010) 'Predictors and assessment of cognitive dysfunction resulting from ischaemic stroke.', *Lancet Neurol*, 9, pp. 895-905.
- Greber, S., Lubec, G., Cairns, N. and Fountoulakis, M. (1999) 'Decreased levels of synaptosomal associated protein 25 in the brain of patients with Down syndrome and Alzheimer's disease', *Electrophoresis*, 20(4-5), pp. 928-34.
- Grube, M.M., Koennecke, H.C., Walter, G., Meisel, A., Sobesky, J., Nolte, C.H., Wellwood, I. and Heuschmann, P.U. (2013) 'Influence of acute complications on outcome 3 months after ischemic stroke', *PLoS One*, 8(9), p. e75719.
- Guerchet, M., Houinato, D., Paraiso, M.N., von Ahnen, N., Nubukpo, P., Otto, M., Clement, J.P., Preux, P.M. and Dartigues, J.F. (2009) 'Cognitive impairment and dementia in elderly people living in rural Benin, west Africa', *Dement Geriatr Cogn Disord*, 27(1), pp. 34-41.
- Guerchet, M., M'Belesso, P., Mouanga, A.M., Bandzouzi, B., Tabo, A., Houinato, D.S., Paraiso, M.N., Cowppli-Bony, P., Nubukpo, P., Aboyans, V., Clement, J.P., Dartigues, J.F. and Preux, P.M. (2010) 'Prevalence of dementia in elderly living in two cities of Central Africa: the EDAC survey', *Dement Geriatr Cogn Disord*, 30(3), pp. 261-8.
- Gunning-Dixon, F.M., Brickman, A.M., Cheng, J.C. and Alexopoulos, G.S. (2009) 'Aging of cerebral white matter: a review of MRI findings', *Int J Geriatr Psychiatry*, 24(2), pp. 109-17.
- Gureje, O., Unverzagt, F.W., Osuntokun, B.O., Hendrie, H.C., Baiyewu, O., Ogunniyi, A. and Hali, K.S. (1995) 'The CERAD Neuropsychological Test Battery: norms from a Yoruba-speaking Nigerian sample', *West Afr J Med*, 14(1), pp. 29-33.
- Gustafsen, C., Glerup, S., Pallesen, L.T., Olsen, D., Andersen, O.M., Nykjaer, A., Madsen, P. and Petersen, C.M. (2013) 'Sortilin and SorLA display distinct roles in processing and trafficking of amyloid precursor protein', *J Neurosci*, 33(1), pp. 64-71.
- Gyllys, K.H., Fein, J.A., Yang, F., Wiley, D.J., Miller, C.A. and Cole, G.M. (2004) 'Synaptic changes in Alzheimer's disease: increased amyloid-beta and gliosis in surviving terminals is accompanied by decreased PSD-95 fluorescence', *Am J Pathol*, 165(5), pp. 1809-17.
- Haag, M.D., Hofman, A., Koudstaal, P.J., Breteler, M.M. and Stricker, B.H. (2009) 'Duration of antihypertensive drug use and risk of dementia: A prospective cohort study', *Neurology*, 72(20), pp. 1727-34.
- Hachinski, V. (1983) 'Multi-infarct dementia', *Neurol Clin*, 1(1), pp. 27-36.
- Hachinski, V., Donnan, G.A., Gorelick, P.B., Hacke, W., Cramer, S.C., Kaste, M., Fisher, M., Brainin, M., Buchan, A.M., Lo, E.H., Skolnick, B.E., Furie, K.L., Hankey, G.J., Kivipelto, M., Morris, J., Rothwell, P.M., Sacco, R.L., Smith, S.C., Jr., Wang, Y., Bryer, A., Ford, G.A., Iadecola, C., Martins, S.C., Saver, J., Skvortsova, V., Bayley, M., Bednar, M.M., Duncan, P., Enney, L., Finklestein, S., Jones, T.A., Kalra, L., Kleim, J., Nitkin, R., Teasell, R., Weiller, C., Desai, B., Goldberg, M.P., Heiss, W.D., Saarelma, O., Schwamm, L.H., Shinohara, Y., Trivedi, B., Wahlgren, N., Wong, L.K., Hakim, A., Norrving, B., Prudhomme, S., Bornstein, N.M., Davis, S.M., Goldstein, L.B., Leys, D. and Tuomilehto, J. (2010) 'Stroke: working toward a prioritized world agenda', *Stroke*, 41(6), pp. 1084-99.
- Hachinski, V., Iadecola, C., Petersen, R.C., Breteler, M.M., Nyenhuis, D.L., Black, S.E., Powers, W.J., DeCarli, C., Merino, J.G., Kalaria, R.N., Vinters, H.V., Holtzman, D.M., Rosenberg, G.A., Dichgans, M., Marler, J.R. and Leblanc, G.G. (2006a) 'National Institute of Neurological Disorders and Stroke-Canadian Stroke Network vascular cognitive impairment harmonization standards', *Stroke*, 37(9), pp. 2220-41.
- Hachinski, V., Iadecola, C., Petersen, R.C., Breteler, M.M., Nyenhuis, D.L., Black, S.E., Powers, W.J., DeCarli, C., Merino, J.G., Kalaria, R.N., Vinters, H.V., Holtzman, D.M., Rosenberg, G.A., Wallin, A., Dichgans, M., Marler, J.R. and Leblanc, G.G. (2006b)

- 'National Institute of Neurological Disorders and Stroke-Canadian Stroke Network vascular cognitive impairment harmonization standards', *Stroke*, 37(9), pp. 2220-41.
- Hachinski, V., Oveisgharan, S., Romney, A.K. and Shankle, W.R. (2012) 'Optimizing the Hachinski Ischemic Scale', *Arch Neurol*, 69(2), pp. 169-75.
- Hachinski, V.C. and Bowler, J.V. (1993) 'Vascular dementia', *Neurology*, 43(10), pp. 2159-60; author reply 2160-1.
- Hachinski, V.C., Iliff, L.D., Zilhka, E., Du Boulay, G.H., McAllister, V.L., Marshall, J., Russell, R.W. and Symon, L. (1975) 'Cerebral blood flow in dementia', *Arch Neurol*, 32(9), pp. 632-7.
- Hachinski, V.C., Lassen, N.A. and Marshall, J. (1974) 'Multi-infarct dementia. A cause of mental deterioration in the elderly', *Lancet*, 2(7874), pp. 207-10.
- Haight, T.J., Landau, S.M., Carmichael, O., Schwarz, C., DeCarli, C. and Jagust, W.J. (2013) 'Dissociable effects of Alzheimer disease and white matter hyperintensities on brain metabolism', *JAMA Neurol*, 70(8), pp. 1039-45.
- Hall, K.S., Gao, S., Emsley, C.L., Ogunniyi, A.O., Morgan, O. and Hendrie, H.C. (2000) 'Community screening interview for dementia (CSI 'D'); performance in five disparate study sites', *Int J Geriatr Psychiatry*, 15(6), pp. 521-31.
- Hara, Y., Rapp, P.R. and Morrison, J.H. (2012) 'Neuronal and morphological bases of cognitive decline in aged rhesus monkeys', *Age (Dordr)*, 34(5), pp. 1051-73.
- Hardy, J. and Selkoe, D.J. (2002) 'The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics', *Science*, 297(5580), pp. 353-6.
- Hardy, J.A. and Higgins, G.A. (1992) 'Alzheimer's disease: the amyloid cascade hypothesis', *Science*, 256(5054), pp. 184-5.
- Harigaya, Y., Shoji, M., Shirao, T. and Hirai, S. (1996) 'Disappearance of actin-binding protein, drebrin, from hippocampal synapses in Alzheimer's disease', *J Neurosci Res*, 43(1), pp. 87-92.
- Hatanpaa, K., Isaacs, K.R., Shirao, T., Brady, D.R. and Rapoport, S.I. (1999) 'Loss of proteins regulating synaptic plasticity in normal aging of the human brain and in Alzheimer disease', *J Neuropathol Exp Neurol*, 58(6), pp. 637-43.
- Hayashi, K., Ishikawa, R., Ye, L.H., He, X.L., Takata, K., Kohama, K. and Shirao, T. (1996) 'Modulatory role of drebrin on the cytoskeleton within dendritic spines in the rat cerebral cortex', *J Neurosci*, 16(22), pp. 7161-70.
- Heinonen, O., Soininen, H., Sorvari, H., Kosunen, O., Paljarvi, L., Koivisto, E. and Riekkinen, P.J., Sr. (1995) 'Loss of synaptophysin-like immunoreactivity in the hippocampal formation is an early phenomenon in Alzheimer's disease', *Neuroscience*, 64(2), pp. 375-84.
- Hendrie, H.C. (2006) 'Lessons learned from international comparative crosscultural studies on dementia', *Am J Geriatr Psychiatry*, 14(6), pp. 480-8.
- Hendrie, H.C., Murrell, J., Gao, S., Unverzagt, F.W., Ogunniyi, A. and Hall, K.S. (2006) 'International studies in dementia with particular emphasis on populations of African origin', *Alzheimer Dis Assoc Disord*, 20(3 Suppl 2), pp. S42-6.
- Hendrie, H.C., Osuntokun, B.O., Hall, K.S., Ogunniyi, A.O., Hui, S.L., Unverzagt, F.W., Gureje, O., Rodenberg, C.A., Baiyewu, O. and Musick, B.S. (1995) 'Prevalence of Alzheimer's disease and dementia in two communities: Nigerian Africans and African Americans', *Am J Psychiatry*, 152(10), pp. 1485-92.
- Henon, H., Durieu, I., Guerouaou, D., Lebert, F., Pasquier, F. and Leys, D. (2001) 'Poststroke dementia: incidence and relationship to prestroke cognitive decline', *Neurology*, 57(7), pp. 1216-22.
- Henon, H., Pasquier, F., Durieu, I., Godefroy, O., Lucas, C., Lebert, F. and Leys, D. (1997) 'Preexisting dementia in stroke patients. Baseline frequency, associated factors, and outcome', *Stroke*, 28(12), pp. 2429-36.
- Henon, H., Pasquier, F., Durieu, I., Pruvo, J.P. and Leys, D. (1998) 'Medial temporal lobe atrophy in stroke patients: relation to pre-existing dementia', *J Neurol Neurosurg Psychiatry*, 65(5), pp. 641-7.



- Henon, H., Pasquier, F. and Leys, D. (2006) 'Poststroke dementia', *Cerebrovasc Dis*, 22(1), pp. 61-70.
- Hoffman, L.B., Schmeidler, J., Lesser, G.T., Beeri, M.S., Purohit, D.P., Grossman, H.T. and Haroutunian, V. (2009) 'Less Alzheimer disease neuropathology in medicated hypertensive than nonhypertensive persons', *Neurology*, 72(20), pp. 1720-6.
- Hoffmann, M. (2001) 'Higher cortical function deficits after stroke: an analysis of 1,000 patients from a dedicated cognitive stroke registry', *Neurorehabil Neural Repair*, 15(2), pp. 113-27.
- Hoffmann, M. (2013) 'The human frontal lobes and frontal network systems: an evolutionary, clinical, and treatment perspective', *ISRN Neurol*, 2013, p. 892459.
- Honer, W.G., Barr, A.M., Sawada, K., Thornton, A.E., Morris, M.C., Leurgans, S.E., Schneider, J.A. and Bennett, D.A. (2012) 'Cognitive reserve, presynaptic proteins and dementia in the elderly', *Transl Psychiatry*, 2, p. e114.
- Horsburgh, K., Reimer, M.M., Holland, P., Chen, G., Scullion, G. and Fowler, J.H. (2011) 'Axon-glia disruption: the link between vascular disease and Alzheimer's disease?', *Biochem Soc Trans*, 39(4), pp. 881-5.
- Hortobagyi, T., Wise, S., Hunt, N., Cary, N., Djurovic, V., Fegan-Earl, A., Shorrock, K., Rouse, D. and Al-Sarraj, S. (2007) 'Traumatic axonal damage in the brain can be detected using beta-APP immunohistochemistry within 35 min after head injury to human adults', *Neuropathol Appl Neurobiol*, 33(2), pp. 226-37.
- House, A., Dennis, M., Warlow, C., Hawton, K. and Molyneux, A. (1990) 'The relationship between intellectual impairment and mood disorder in the first year after stroke', *Psychol Med*, 20(4), pp. 805-14.
- Howard, G., Wagenknecht, L.E., Cai, J., Cooper, L., Kraut, M.A. and Toole, J.F. (1998) 'Cigarette smoking and other risk factors for silent cerebral infarction in the general population', *Stroke*, 29(5), pp. 913-7.
- Hsieh, H., Boehm, J., Sato, C., Iwatsubo, T., Tomita, T., Sisodia, S. and Malinow, R. (2006) 'AMPA removal underlies Aβ-induced synaptic depression and dendritic spine loss', *Neuron*, 52(5), pp. 831-43.
- Hughes, C.P., Berg, L., Danziger, W.L., Coben, L.A. and Martin, R.L. (1982) 'A new clinical scale for the staging of dementia', *Br J Psychiatry*, 140, pp. 566-72.
- Hughes, T.F., Borenstein, A.R., Schofield, E., Wu, Y. and Larson, E.B. (2009) 'Association between late-life body mass index and dementia: The Kame Project', *Neurology*, 72(20), pp. 1741-6.
- Hughes, V. (2012) 'Microglia: The constant gardeners', *Nature*, 485(7400), pp. 570-2.
- Hyman, B.T. and Trojanowski, J.Q. (1997) 'Consensus recommendations for the postmortem diagnosis of Alzheimer disease from the National Institute on Aging and the Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer disease', *J Neuropathol Exp Neurol*, 56(10), pp. 1095-7.
- Iacono, D., Markesbery, W.R., Gross, M., Pletnikova, O., Rudow, G., Zandi, P. and Troncoso, J.C. (2009) 'The Nun study: clinically silent AD, neuronal hypertrophy, and linguistic skills in early life', *Neurology*, 73(9), pp. 665-73.
- Iadecola, C. (2004) 'Neurovascular regulation in the normal brain and in Alzheimer's disease', *Nat Rev Neurosci*, 5(5), pp. 347-60.
- Iadecola, C. (2010) 'The overlap between neurodegenerative and vascular factors in the pathogenesis of dementia', *Acta Neuropathol*, 120(3), pp. 287-96.
- Iadecola, C. and Davisson, R.L. (2008) 'Hypertension and cerebrovascular dysfunction', *Cell Metab*, 7(6), pp. 476-84.
- Ihara, M. and Kalara, R.N. (2007) 'Amyloid-beta and synaptic activity in mice and men', *Neuroreport*, 18(12), pp. 1205-6.
- Ihara, M., Polvikoski, T.M., Hall, R., Slade, J.Y., Perry, R.H., Oakley, A.E., Englund, E., O'Brien, J.T., Ince, P.G. and Kalara, R.N. (2010a) 'Quantification of myelin loss in frontal lobe white matter in vascular dementia, Alzheimer's disease, and dementia with Lewy bodies', *Acta Neuropathol*, in press.

- Ihara, M., Polvikoski, T.M., Hall, R., Slade, J.Y., Perry, R.H., Oakley, A.E., Englund, E., O'Brien, J.T., Ince, P.G. and Kalaria, R.N. (2010b) 'Quantification of myelin loss in frontal lobe white matter in vascular dementia, Alzheimer's disease, and dementia with Lewy bodies', *Acta Neuropathol*, 119(5), pp. 579-89.
- Ihara, M. and Tomimoto, H. (2011) 'Lessons from a mouse model characterizing features of vascular cognitive impairment with white matter changes', *J Aging Res*, 2011, p. 978761.
- Ihle-Hansen, H., Thommessen, B., Fagerland, M.W., Wyller, T.B., Engedal, K., Oksengard, A.R., Stenset, V., Loken, K. and Fure, B. (2012) 'Impact of white matter lesions on cognition in stroke patients free from pre-stroke cognitive impairment: a one-year follow-up study', *Dement Geriatr Cogn Dis Extra*, 2(1), pp. 38-47.
- Insausti, R. and Amaral, D.G. (2008) 'Entorhinal cortex of the monkey: IV. Topographical and laminar organization of cortical afferents', *J Comp Neurol*, 509(6), pp. 608-41.
- Insausti, R., Amaral, D.G. and Cowan, W.M. (1987) 'The entorhinal cortex of the monkey: III. Subcortical afferents', *J Comp Neurol*, 264(3), pp. 396-408.
- Insausti, R., Annese, J., Amaral, D.G. and Squire, L.R. (2013) 'Human amnesia and the medial temporal lobe illuminated by neuropsychological and neurohistological findings for patient E.P.', *Proc Natl Acad Sci U S A*, 110(21), pp. E1953-62.
- Inzitari, D., Di Carlo, A., Pracucci, G., Lamassa, M., Vanni, P., Romanelli, M., Spolveri, S., Adriani, P., Meucci, I., Landini, G. and Ghetti, A. (1998) 'Incidence and determinants of poststroke dementia as defined by an informant interview method in a hospital-based stroke registry', *Stroke*, 29(10), pp. 2087-93.
- Ishibashi, K., Tomiyama, T., Nishitsuji, K., Hara, M. and Mori, H. (2006) 'Absence of synaptophysin near cortical neurons containing oligomer Abeta in Alzheimer's disease brain', *J Neurosci Res*, 84(3), pp. 632-6.
- Ishunina, T.A., Kamphorst, W. and Swaab, D.F. (2004) 'Metabolic alterations in the hypothalamus and basal forebrain in vascular dementia', *J Neuropathol Exp Neurol*, 63(12), pp. 1243-54.
- Ivan, C.S., Seshadri, S., Beiser, A., Au, R., Kase, C.S., Kelly-Hayes, M. and Wolf, P.A. (2004) 'Dementia after stroke: the Framingham Study', *Stroke*, 35(6), pp. 1264-8.
- Jaillard, A., Grand, S., Le Bas, J.F. and Hommel, M. (2010) 'Predicting cognitive dysfunctioning in nondemented patients early after stroke', *Cerebrovasc Dis*, 29(5), pp. 415-23.
- Jaracz, K. and Kozubski, W. (2003) 'Quality of life in stroke patients', *Acta Neurol Scand*, 107(5), pp. 324-9.
- Jellinger, K.A. (2008a) 'Morphologic diagnosis of "vascular dementia" - a critical update', *J Neurol Sci*, 270(1-2), pp. 1-12.
- Jellinger, K.A. (2008b) 'The pathology of "vascular dementia": a critical update', *J Alzheimers Dis*, 14(1), pp. 107-23.
- Jellinger, K.A. (2013) 'Pathology and pathogenesis of vascular cognitive impairment-a critical update', *Front Aging Neurosci*, 5, p. 17.
- Jin, C., Liu, X., Zhang, F., Wu, Y., Yuan, J., Zhu, J., Zhang, F., Wang, G. and Cheng, Z. (2013) 'An updated meta-analysis of the association between SORL1 variants and the risk for sporadic Alzheimer's disease', *J Alzheimers Dis*, 37(2), pp. 429-37.
- Jin Thong, J.Y., Du, J., Ratnarajah, N., Dong, Y., Soon, H.W., Saini, M., Tan, M.Z., Tuan Ta, A., Chen, C. and Qiu, A. (2013) 'Abnormalities of cortical thickness, subcortical shapes, and white matter integrity in subcortical vascular cognitive impairment', *Hum Brain Mapp*.
- Jokinen, H., Kalska, H., Mantyla, R., Ylikoski, R., Hietanen, M., Pohjasvaara, T., Kaste, M. and Erkinjuntti, T. (2005) 'White matter hyperintensities as a predictor of neuropsychological deficits post-stroke', *J Neurol Neurosurg Psychiatry*, 76(9), pp. 1229-33.
- Jokinen, H., Lipsanen, J., Schmidt, R., Fazekas, F., Gouw, A.A., van der Flier, W.M., Barkhof, F., Madureira, S., Verdelho, A., Ferro, J.M., Wallin, A., Pantoni, L., Inzitari, D. and

- Erkinjuntti, T. (2012) 'Brain atrophy accelerates cognitive decline in cerebral small vessel disease: the LADIS study', *Neurology*, 78(22), pp. 1785-92.
- Jones, E.L., Kalaria, R.N., Sharp, S.I., O'Brien, J.T., Francis, P.T. and Ballard, C.G. (2011) 'Genetic associations of autopsy-confirmed vascular dementia subtypes', *Dement Geriatr Cogn Disord*, 31(4), pp. 247-53.
- Kalaria, R. (2002a) 'Similarities between Alzheimer's disease and vascular dementia', *J Neurol Sci*, 203-204, pp. 29-34.
- Kalaria, R.N. (1996) 'Cerebral vessels in ageing and Alzheimer's disease', *Pharmacol Ther*, 72(3), pp. 193-214.
- Kalaria, R.N. (2000) 'The role of cerebral ischemia in Alzheimer's disease', *Neurobiol Aging*, 21(2), pp. 321-30.
- Kalaria, R.N. (2002b) 'Similarities between Alzheimer's disease and vascular dementia', *J Neurol Sci*, 203-204, pp. 29-34.
- Kalaria, R.N. (2009a) 'Linking cerebrovascular defense mechanisms in brain ageing and Alzheimer's disease', *Neurobiol Aging*, 30(9), pp. 1512-4.
- Kalaria, R.N. (2009b) 'Neurodegenerative disease: Diabetes, microvascular pathology and Alzheimer disease', *Nat Rev Neurol*, 5(6), pp. 305-6.
- Kalaria, R.N. (2010) 'Vascular basis for brain degeneration: faltering controls and risk factors for dementia', *Nutr Rev*, 68 Suppl 2, pp. S74-87.
- Kalaria, R.N. (2012a) 'Cerebrovascular disease and mechanisms of cognitive impairment: evidence from clinicopathological studies in humans', *Stroke*, 43(9), pp. 2526-34.
- Kalaria, R.N. (2012b) 'Risk factors and neurodegenerative mechanisms in stroke related dementia', *Panminerva Med*, 54(3), pp. 139-48.
- Kalaria, R.N., Akinyemi, R. and Ihara, M. (2012a) 'Does vascular pathology contribute to Alzheimer changes?', *J Neurol Sci*, 322(1-2), pp. 141-7.
- Kalaria, R.N. and Ballard, C. (1999) 'Overlap between pathology of Alzheimer disease and vascular dementia', *Alzheimer Dis Assoc Disord*, 13 Suppl 3, pp. S115-23.
- Kalaria, R.N., Bhatti, S.U., Lust, W.D. and Perry, G. (1993a) 'The amyloid precursor protein in ischemic brain injury and chronic hypoperfusion', *Ann N Y Acad Sci*, 695, pp. 190-3.
- Kalaria, R.N., Bhatti, S.U., Palatinsky, E.A., Pennington, D.H., Shelton, E.R., Chan, H.W., Perry, G. and Lust, W.D. (1993b) 'Accumulation of the beta amyloid precursor protein at sites of ischemic injury in rat brain', *Neuroreport*, 4(2), pp. 211-4.
- Kalaria, R.N. and Erkinjuntti, T. (2006) 'Small vessel disease and subcortical vascular dementia', *J Clin Neurol*, 2(1), pp. 1-11.
- Kalaria, R.N. and Ihara, M. (2013) 'Dementia: Vascular and neurodegenerative pathways-will they meet?', *Nat Rev Neurol*, 9(9), pp. 487-8.
- Kalaria, R.N., Kenny, R.A., Ballard, C.G., Perry, R., Ince, P. and Polvikoski, T. (2004) 'Towards defining the neuropathological substrates of vascular dementia', *J Neurol Sci*, 226(1-2), pp. 75-80.
- Kalaria, R.N., Maestre, G.E., Arizaga, R., Friedland, R.P., Galasko, D., Hall, K., Luchsinger, J.A., Ogunniyi, A., Perry, E.K., Potocnik, F., Prince, M., Stewart, R., Wimo, A., Zhang, Z.X. and Antuono, P. (2008) 'Alzheimer's disease and vascular dementia in developing countries: prevalence, management, and risk factors', *Lancet Neurol*, 7(9), pp. 812-26.
- Kalaria, R.N. and Mukaetova-Ladinska, E.B. (2012) 'Delirium, dementia and senility', *Brain*, 135(Pt 9), pp. 2582-4.
- Kalaria, R.N., Ogeng'o, J.A., Patel, N.B., Sayi, J.G., Kitinya, J.N., Chande, H.M., Matuja, W.B., Mtui, E.P., Kimani, J.K., Premkumar, D.R., Koss, E., Gatere, S. and Friedland, R.P. (1997) 'Evaluation of risk factors for Alzheimer's disease in elderly east Africans', *Brain Res Bull*, 44(5), pp. 573-7.
- Kalaria, R.N., Perry, R.H., O'Brien, J. and Jaros, E. (2012b) 'Atheromatous disease in small intracerebral vessels, microinfarcts and dementia', *Neuropathol Appl Neurobiol*, 38(5), pp. 505-8.
- Kalmijn, S., Feskens, E.J., Launer, L.J., Stijnen, T. and Kromhout, D. (1995) 'Glucose intolerance, hyperinsulinaemia and cognitive function in a general population of elderly men', *Diabetologia*, 38(9), pp. 1096-102.

- Kalmijn, S., Foley, D., White, L., Burchfiel, C.M., Curb, J.D., Petrovitch, H., Ross, G.W., Havlik, R.J. and Launer, L.J. (2000) 'Metabolic cardiovascular syndrome and risk of dementia in Japanese-American elderly men. The Honolulu-Asia aging study', *Arterioscler Thromb Vasc Biol*, 20(10), pp. 2255-60.
- Kandel, E.R. (2001) 'The molecular biology of memory storage: a dialogue between genes and synapses', *Science*, 294(5544), pp. 1030-8.
- Kasai, H., Matsuzaki, M., Noguchi, J., Yasumatsu, N. and Nakahara, H. (2003) 'Structure-stability-function relationships of dendritic spines', *Trends Neurosci*, 26(7), pp. 360-8.
- Kashani, A., Betancur, C., Giros, B., Hirsch, E. and El Mestikawy, S. (2007) 'Altered expression of vesicular glutamate transporters VGLUT1 and VGLUT2 in Parkinson disease', *Neurobiol Aging*, 28(4), pp. 568-78.
- Kashani, A., Lepicard, E., Poirel, O., Videau, C., David, J.P., Fallet-Bianco, C., Simon, A., Delacourte, A., Giros, B., Epelbaum, J., Betancur, C. and El Mestikawy, S. (2008) 'Loss of VGLUT1 and VGLUT2 in the prefrontal cortex is correlated with cognitive decline in Alzheimer disease', *Neurobiol Aging*, 29(11), pp. 1619-30.
- Katzman, R., Terry, R., DeTeresa, R., Brown, T., Davies, P., Fuld, P., Renbing, X. and Peck, A. (1988) 'Clinical, pathological, and neurochemical changes in dementia: a subgroup with preserved mental status and numerous neocortical plaques', *Ann Neurol*, 23(2), pp. 138-44.
- Keith, D. and El-Husseini, A. (2008) 'Excitation Control: Balancing PSD-95 Function at the Synapse', *Front Mol Neurosci*, 1, p. 4.
- Kennedy, K.M. and Raz, N. (2009a) 'Aging white matter and cognition: differential effects of regional variations in diffusion properties on memory, executive functions, and speed', *Neuropsychologia*, 47(3), pp. 916-27.
- Kennedy, K.M. and Raz, N. (2009b) 'Pattern of normal age-related regional differences in white matter microstructure is modified by vascular risk', *Brain Res*, 1297, pp. 41-56.
- Kennelly, S.P., Lawlor, B.A. and Kenny, R.A. (2009) 'Blood pressure and the risk for dementia: a double edged sword', *Ageing Res Rev*, 8(2), pp. 61-70.
- Keverne, J.S., Low, W.C., Ziabreva, I., Court, J.A., Oakley, A.E. and Kalaria, R.N. (2007) 'Cholinergic neuronal deficits in CADASIL', *Stroke*, 38(1), pp. 188-91.
- Khedr, E.M., Hamed, S.A., El-Shereef, H.K., Shawky, O.A., Mohamed, K.A., Awad, E.M., Ahmed, M.A., Shehata, G.A. and Eltahtawy, M.A. (2009) 'Cognitive impairment after cerebrovascular stroke: Relationship to vascular risk factors', *Neuropsychiatr Dis Treat*, 5, pp. 103-16.
- Kidd, P.M. (2008) 'Alzheimer's disease, amnesic mild cognitive impairment, and age-associated memory impairment: current understanding and progress toward integrative prevention', *Altern Med Rev*, 13(2), pp. 85-115.
- Kieft-de Jong, J.C., Chowdhury, R. and Franco, O.H. (2012) 'Fish intake or omega-3 fatty acids: greater than the sum of all parts?', *Eur J Epidemiol*, 27(12), pp. 891-4.
- Kim, A.S. and Johnston, S.C. (2013) 'Temporal and geographic trends in the global stroke epidemic', *Stroke*, 44(6 Suppl 1), pp. S123-5.
- Kirvell, S.L., Elliott, M.S., Kalaria, R.N., Hortobagyi, T., Ballard, C.G. and Francis, P.T. (2010) 'Vesicular glutamate transporter and cognition in stroke: a case-control autopsy study', *Neurology*, 75(20), pp. 1803-9.
- Kirvell, S.L., Esiri, M. and Francis, P.T. (2006) 'Down-regulation of vesicular glutamate transporters precedes cell loss and pathology in Alzheimer's disease', *Journal of Neurochemistry*, 98(3), pp. 939-950.
- Kitaguchi, H., Tomimoto, H., Ihara, M., Shibata, M., Uemura, K., Kalaria, R.N., Kihara, T., Asada-Utsugi, M., Kinoshita, A. and Takahashi, R. (2009) 'Chronic cerebral hypoperfusion accelerates amyloid beta deposition in APPSwInd transgenic mice', *Brain Res*, 1294, pp. 202-10.
- Kivipelto, M., Helkala, E.L., Laakso, M.P., Hanninen, T., Hallikainen, M., Alhainen, K., Soininen, H., Tuomilehto, J. and Nissinen, A. (2001) 'Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population based study', *BMJ*, 322(7300), pp. 1447-51.

- Kivipelto, M., Laakso, M.P., Tuomilehto, J., Nissinen, A. and Soininen, H. (2002) 'Hypertension and hypercholesterolaemia as risk factors for Alzheimer's disease: potential for pharmacological intervention', *CNS Drugs*, 16(7), pp. 435-44.
- Klann, E. and Dever, T.E. (2004) 'Biochemical mechanisms for translational regulation in synaptic plasticity', *Nat Rev Neurosci*, 5(12), pp. 931-42.
- Klimkiewicz, A., Dziedzic, T., Slowik, A. and Szczudlik, A. (2002) 'Incidence of pre- and poststroke dementia: cracow stroke registry', *Dement Geriatr Cogn Disord*, 14(3), pp. 137-40.
- Klunk, W.E., Engler, H., Nordberg, A., Wang, Y., Blomqvist, G., Holt, D.P., Bergstrom, M., Savitcheva, I., Huang, G.F., Estrada, S., Ausen, B., Debnath, M.L., Barletta, J., Price, J.C., Sandell, J., Lopresti, B.J., Wall, A., Koivisto, P., Antoni, G., Mathis, C.A. and Langstrom, B. (2004) 'Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B', *Ann Neurol*, 55(3), pp. 306-19.
- Knopman, D., Boland, L.L., Mosley, T., Howard, G., Liao, D., Szklo, M., McGovern, P. and Folsom, A.R. (2001) 'Cardiovascular risk factors and cognitive decline in middle-aged adults', *Neurology*, 56(1), pp. 42-8.
- Knopman, D.S. (2006) 'Dementia and cerebrovascular disease', *Mayo Clin Proc*, 81(2), pp. 223-30.
- Knopman, D.S. (2007) 'Cerebrovascular disease and dementia', *Br J Radiol*, 80 Spec No 2, pp. S121-7.
- Knopman, D.S. (2009) 'Hypertension and late-life dementia: A real link?', *Neurology*, 72(20), pp. 1716-7.
- Kojima, N. and Shirao, T. (2007) 'Synaptic dysfunction and disruption of postsynaptic drebrin-actin complex: A study of neurological disorders accompanied by cognitive deficits', *Neuroscience Research*, 58(1), pp. 1-5.
- Kokmen, E., Whisnant, J.P., O'Fallon, W.M., Chu, C.P. and Beard, C.M. (1996) 'Dementia after ischemic stroke: a population-based study in Rochester, Minnesota (1960-1984)', *Neurology*, 46(1), pp. 154-9.
- Kopf, D. and Frolich, L. (2009) 'Risk of incident Alzheimer's disease in diabetic patients: a systematic review of prospective trials', *J Alzheimers Dis*, 16(4), pp. 677-85.
- Krikorian, R., Nash, T.A., Shidler, M.D., Shukitt-Hale, B. and Joseph, J.A. (2010) 'Concord grape juice supplementation improves memory function in older adults with mild cognitive impairment', *Br J Nutr*, 103(5), pp. 730-4.
- Krikorian, R., Shidler, M.D., Nash, T.A., Kalt, W., Vinqvist-Tymchuk, M.R., Shukitt-Hale, B. and Joseph, J.A. 'Blueberry supplementation improves memory in older adults', *J Agric Food Chem*, 58(7), pp. 3996-4000.
- Kuusisto, J., Koivisto, K., Mykkanen, L., Helkala, E.L., Vanhanen, M., Hanninen, T., Kervinen, K., Kesaniemi, Y.A., Riekkinen, P.J. and Laakso, M. (1997) 'Association between features of the insulin resistance syndrome and Alzheimer's disease independently of apolipoprotein E4 phenotype: cross sectional population based study', *BMJ*, 315(7115), pp. 1045-9.
- Lace, G., Savva, G.M., Forster, G., de Silva, R., Brayne, C., Matthews, F.E., Barclay, J.J., Dakin, L., Ince, P.G. and Wharton, S.B. (2009) 'Hippocampal tau pathology is related to neuroanatomical connections: an ageing population-based study', *Brain*, 132(Pt 5), pp. 1324-34.
- Lancaster, G.A., Dodd, S. and Williamson, P.R. (2004) 'Design and analysis of pilot studies: recommendations for good practice', *J Eval Clin Pract*, 10(2), pp. 307-12.
- Lautenschlager, N.T., Cox, K.L., Flicker, L., Foster, J.K., van Bockxmeer, F.M., Xiao, J., Greenop, K.R. and Almeida, O.P. (2008) 'Effect of physical activity on cognitive function in older adults at risk for Alzheimer disease: a randomized trial', *JAMA*, 300(9), pp. 1027-37.
- Lavenex, P. and Banta Lavenex, P. (2013) 'Building hippocampal circuits to learn and remember: Insights into the development of human memory', *Behav Brain Res*, 254, pp. 8-21.

- Lawrence, A.J., Patel, B., Morris, R.G., MacKinnon, A.D., Rich, P.M., Barrick, T.R. and Markus, H.S. (2013) 'Mechanisms of cognitive impairment in cerebral small vessel disease: multimodal MRI results from the St George's cognition and neuroimaging in stroke (SCANS) study', *PLoS One*, 8(4), p. e61014.
- Lee, J.H., Kim, S.H., Kim, G.H., Seo, S.W., Park, H.K., Oh, S.J., Kim, J.S., Cheong, H.K. and Na, D.L. (2011a) 'Identification of pure subcortical vascular dementia using 11C-Pittsburgh compound B', *Neurology*, 77(1), pp. 18-25.
- Lee, M.H., Kim, H., Kim, S.S., Lee, T.H., Lim, B.V., Chang, H.K., Jang, M.H., Shin, M.C., Shin, M.S. and Kim, C.J. (2003) 'Treadmill exercise suppresses ischemia-induced increment in apoptosis and cell proliferation in hippocampal dentate gyrus of gerbils', *Life Sci*, 73(19), pp. 2455-65.
- Lee, S., Shafe, A.C. and Cowie, M.R. (2011b) 'UK stroke incidence, mortality and cardiovascular risk management 1999-2008: time-trend analysis from the General Practice Research Database', *BMJ Open*, 1(2), pp. 2011-000269.
- Lemstra, A.W., Groen in't Woud, J.C., Hoozemans, J.J., van Haastert, E.S., Rozemuller, A.J., Eikelenboom, P. and van Gool, W.A. (2007) 'Microglia activation in sepsis: a case-control study', *J Neuroinflammation*, 4, p. 4.
- Leon, A.C., Davis, L.L. and Kraemer, H.C. (2011) 'The role and interpretation of pilot studies in clinical research', *J Psychiatr Res*, 45(5), pp. 626-9.
- Leuba, G., Savioz, A., Vernay, A., Carnal, B., Kraftsik, R., Tardif, E., Riederer, I. and Riederer, B.M. (2008) 'Differential changes in synaptic proteins in the Alzheimer frontal cortex with marked increase in PSD-95 postsynaptic protein', *J Alzheimers Dis*, 15(1), pp. 139-51.
- Levine, D.A. and Langa, K.M. (2011) 'Vascular cognitive impairment: disease mechanisms and therapeutic implications', *Neurotherapeutics*, 8(3), pp. 361-73.
- Lewis, H., Beher, D., Cookson, N., Oakley, A., Piggott, M., Morris, C.M., Jaros, E., Perry, R., Ince, P., Kenny, R.A., Ballard, C.G., Shearman, M.S. and Kalaria, R.N. (2006) 'Quantification of Alzheimer pathology in ageing and dementia: age-related accumulation of amyloid-beta(42) peptide in vascular dementia', *Neuropathol Appl Neurobiol*, 32(2), pp. 103-18.
- Leys, D., Henon, H., Mackowiak-Cordoliani, M.A. and Pasquier, F. (2005) 'Poststroke dementia', *Lancet Neurol*, 4(11), pp. 752-9.
- Li, H. and Xin, X. (2013) 'Nitrogen dioxide (NO<sub>2</sub>) pollution as a potential risk factor for developing vascular dementia and its synaptic mechanisms', *Chemosphere*, 92(1), pp. 52-8.
- Lin, J.H., Lin, R.T., Tai, C.T., Hsieh, C.L., Hsiao, S.F. and Liu, C.K. (2003) 'Prediction of poststroke dementia', *Neurology*, 61(3), pp. 343-8.
- Lin, M.T. and Beal, M.F. (2006) 'Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases', *Nature*, 443(7113), pp. 787-95.
- Linden, T., Skoog, I., Fagerberg, B., Steen, B. and Blomstrand, C. (2004) 'Cognitive impairment and dementia 20 months after stroke', *Neuroepidemiology*, 23(1-2), pp. 45-52.
- Lindner, M., Fokuhl, J., Linsmeier, F., Trebst, C. and Stangel, M. (2009) 'Chronic toxic demyelination in the central nervous system leads to axonal damage despite remyelination', *Neurosci Lett*, 453(2), pp. 120-5.
- Liu, X., Li, L., Liu, F., Deng, S., Zhu, R., Li, Q. and He, Z. (2012) 'ApoE gene polymorphism and vascular dementia in Chinese population: a meta-analysis', *J Neural Transm*, 119(3), pp. 387-94.
- Looi, J.C. and Sachdev, P.S. (1999) 'Differentiation of vascular dementia from AD on neuropsychological tests', *Neurology*, 53(4), pp. 670-8.
- Lopes, M.A., Firbank, M.J., Widdington, M., Blamire, A.M., Kalaria, R.N. and O'Brien, J.T. (2012) 'Post-stroke dementia: the contribution of thalamus and basal ganglia changes', *Int Psychogeriatr*, 24(4), pp. 568-76.
- Lorento de No, R. (1934) 'Studies on the structure of the cerebral cortex II. Continuation of the study of the ammonic system.', *J. Psychol. Neurol*, 46, pp. 113 - 117.

- Love, S., Siew, L.K., Dawbarn, D., Wilcock, G.K., Ben-Shlomo, Y. and Allen, S.J. (2006) 'Premorbid effects of APOE on synaptic proteins in human temporal neocortex', *Neurobiol Aging*, 27(6), pp. 797-803.
- Lowery, K., Ballard, C., Rodgers, H., McLaren, A., O'Brien, J., Rowan, E. and Stephens, S. (2002) 'Cognitive decline in a prospectively studied group of stroke survivors, with a particular emphasis on the >75's', *Age Ageing*, 31 Suppl 3, pp. 24-7.
- Luchsinger, J.A. (2010) 'Diabetes, related conditions, and dementia', *J Neurol Sci*, 299(1-2), pp. 35-8.
- Luchsinger, J.A. and Gustafson, D.R. (2009) 'Adiposity, type 2 diabetes, and Alzheimer's disease', *J Alzheimers Dis*, 16(4), pp. 693-704.
- Madureira, S., Guerreiro, M. and Ferro, J.M. (2001) 'Dementia and cognitive impairment three months after stroke', *Eur J Neurol*, 8(6), pp. 621-7.
- Maguire, E.A., Gadian, D.G., Johnsrude, I.S., Good, C.D., Ashburner, J., Frackowiak, R.S. and Frith, C.D. (2000) 'Navigation-related structural change in the hippocampi of taxi drivers', *Proc Natl Acad Sci U S A*, 97(8), pp. 4398-403.
- Manly, J.J., Smith, C., Crystal, H.A., Richardson, J., Golub, E.T., Greenblatt, R., Robison, E., Martin, E.M. and Young, M. (2011) 'Relationship of ethnicity, age, education, and reading level to speed and executive function among HIV+ and HIV- women: the Women's Interagency HIV Study (WIHS) Neurocognitive Substudy', *J Clin Exp Neuropsychol*, 33(8), pp. 853-63.
- Marchant, N.L., Reed, B.R., DeCarli, C.S., Madison, C.M., Weiner, M.W., Chui, H.C. and Jagust, W.J. (2012) 'Cerebrovascular disease, beta-amyloid, and cognition in aging', *Neurobiol Aging*, 33(5), pp. 1006.e25-36.
- Marchant, N.L., Reed, B.R., Sanossian, N., Madison, C.M., Kriger, S., Dhada, R., Mack, W.J., DeCarli, C., Weiner, M.W., Mungas, D.M., Chui, H.C. and Jagust, W.J. (2013) 'The aging brain and cognition: contribution of vascular injury and abeta to mild cognitive dysfunction', *JAMA Neurol*, 70(4), pp. 488-95.
- Marques, F.Z., Markus, M.A. and Morris, B.J. (2009) 'Resveratrol: cellular actions of a potent natural chemical that confers a diversity of health benefits', *Int J Biochem Cell Biol*, 41(11), pp. 2125-8.
- Massaad, C.A., Amin, S.K., Hu, L., Mei, Y., Klann, E. and Pautler, R.G. (2010) 'Mitochondrial superoxide contributes to blood flow and axonal transport deficits in the Tg2576 mouse model of Alzheimer's disease', *PLoS One*, 5(5), p. e10561.
- Mbelesso, P., Tabo, A., Guerchet, M., Mouanga, A.M., Bandzouzi, B., Houinato, D., Paraiso, M.N., Cowppli-Bony, P., Aboyans, V., Nubukpo, P., Preux, P.M., Dartigues, J.F. and Clement, J.P. (2012) '[Epidemiology of dementia in elderly living in the 3rd borough of Bangui (Central African Republic)]', *Bull Soc Pathol Exot*, 105(5), pp. 388-95.
- McCoy, M.K., Martinez, T.N., Ruhn, K.A., Szymkowski, D.E., Smith, C.G., Botterman, B.R., Tansey, K.E. and Tansey, M.G. (2006) 'Blocking soluble tumor necrosis factor signaling with dominant-negative tumor necrosis factor inhibitor attenuates loss of dopaminergic neurons in models of Parkinson's disease', *J Neurosci*, 26(37), pp. 9365-75.
- McGuinness, B., Carson, R., Barrett, S.L., Craig, D. and Passmore, A.P. (2010a) 'Apolipoprotein epsilon4 and neuropsychological performance in Alzheimer's disease and vascular dementia', *Neurosci Lett*, 483(1), pp. 62-6.
- McGuinness, B., Craig, D., Bullock, R. and Passmore, P. (2009a) 'Statins for the prevention of dementia', *Cochrane Database Syst Rev*, (2), p. CD003160.
- McGuinness, B., O'Hare, J., Craig, D., Bullock, R., Malouf, R. and Passmore, P. (2010b) 'Statins for the treatment of dementia', *Cochrane Database Syst Rev*, (8), p. CD007514.
- McGuinness, B., Todd, S., Passmore, P. and Bullock, R. (2006) 'The effects of blood pressure lowering on development of cognitive impairment and dementia in patients without apparent prior cerebrovascular disease', *Cochrane Database Syst Rev*, (2), p. CD004034.

- McGuinness, B., Todd, S., Passmore, P. and Bullock, R. (2009b) 'Blood pressure lowering in patients without prior cerebrovascular disease for prevention of cognitive impairment and dementia', *Cochrane Database Syst Rev*, (4), p. CD004034.
- Medana, I.M. and Esiri, M.M. (2003) 'Axonal damage: a key predictor of outcome in human CNS diseases', *Brain*, 126(Pt 3), pp. 515-30.
- Meng, X. and D'Arcy, C. (2012) 'Education and dementia in the context of the cognitive reserve hypothesis: a systematic review with meta-analyses and qualitative analyses', *PLoS One*, 7(6), p. e38268.
- Miller, A.A., Drummond, G.R., Schmidt, H.H. and Sobey, C.G. (2005) 'NADPH oxidase activity and function are profoundly greater in cerebral versus systemic arteries', *Circ Res*, 97(10), pp. 1055-62.
- Mills, S., Cain, J., Purandare, N. and Jackson, A. (2007) 'Biomarkers of cerebrovascular disease in dementia', *Br J Radiol*, 80 Spec No 2, pp. S128-45.
- Minger, S.L., Honer, W.G., Esiri, M.M., McDonald, B., Keene, J., Nicoll, J.A., Carter, J., Hope, T. and Francis, P.T. (2001) 'Synaptic pathology in prefrontal cortex is present only with severe dementia in Alzheimer disease', *J Neuropathol Exp Neurol*, 60(10), pp. 929-36.
- Mirra, S.S., Heyman, A., McKeel, D., Sumi, S.M., Crain, B.J., Brownlee, L.M., Vogel, F.S., Hughes, J.P., van Belle, G. and Berg, L. (1991) 'The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease', *Neurology*, 41(4), pp. 479-86.
- Miyashita, A., Koike, A., Jun, G., Wang, L.S., Takahashi, S., Matsubara, E., Kawarabayashi, T., Shoji, M., Tomita, N., Arai, H., Asada, T., Harigaya, Y., Ikeda, M., Amari, M., Hanyu, H., Higuchi, S., Ikeuchi, T., Nishizawa, M., Suga, M., Kawase, Y., Akatsu, H., Kosaka, K., Yamamoto, T., Imagawa, M., Hamaguchi, T., Yamada, M., Moriaha, T., Takeda, M., Takao, T., Nakata, K., Fujisawa, Y., Sasaki, K., Watanabe, K., Nakashima, K., Urakami, K., Ooya, T., Takahashi, M., Yuzuriha, T., Serikawa, K., Yoshimoto, S., Nakagawa, R., Kim, J.W., Ki, C.S., Won, H.H., Na, D.L., Seo, S.W., Mook-Jung, I., St George-Hyslop, P., Mayeux, R., Haines, J.L., Pericak-Vance, M.A., Yoshida, M., Nishida, N., Tokunaga, K., Yamamoto, K., Tsuji, S., Kanazawa, I., Ihara, Y., Schellenberg, G.D., Farrer, L.A. and Kuwano, R. (2013) 'SORL1 is genetically associated with late-onset Alzheimer's disease in Japanese, Koreans and Caucasians', *PLoS One*, 8(4), p. e58618.
- Mok, V., Leung, E.Y., Chu, W., Chen, S., Wong, A., Xiong, Y., Lam, W., Ho, C.L. and Wong, K.S. (2010) 'Pittsburgh compound B binding in poststroke dementia', *J Neurol Sci*, 290(1-2), pp. 135-7.
- Mok, V., Wong, K.K., Xiong, Y., Wong, A., Schmidt, R., Chu, W., Hu, X., Leung, E.Y., Chen, S., Chen, Y., Tang, W.K., Chen, X., Ho, C.L., Wong, K.S. and Wong, S.T. (2011) 'Cortical and frontal atrophy are associated with cognitive impairment in age-related confluent white-matter lesion', *J Neurol Neurosurg Psychiatry*, 82(1), pp. 52-7.
- Montine, T.J., Phelps, C.H., Beach, T.G., Bigio, E.H., Cairns, N.J., Dickson, D.W., Duyckaerts, C., Frosch, M.P., Masliah, E., Mirra, S.S., Nelson, P.T., Schneider, J.A., Thal, D.R., Trojanowski, J.Q., Vinters, H.V. and Hyman, B.T. (2012) 'National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach', *Acta Neuropathol*, 123(1), pp. 1-11.
- Moore, S.A., Hallsworth, K., Plotz, T., Ford, G.A., Rochester, L. and Trenell, M.I. (2013) 'Physical activity, sedentary behaviour and metabolic control following stroke: a cross-sectional and longitudinal study', *PLoS One*, 8(1), p. e55263.
- Moorhouse, P. and Rockwood, K. (2008) 'Vascular cognitive impairment: current concepts and clinical developments', *Lancet Neurol*, 7(3), pp. 246-55.
- Mori, E., Hirono, N., Yamashita, H., Imamura, T., Ikejiri, Y., Ikeda, M., Kitagaki, H., Shimomura, T. and Yoneda, Y. (1997) 'Premorbid brain size as a determinant of reserve capacity against intellectual decline in Alzheimer's disease', *Am J Psychiatry*, 154(1), pp. 18-24.
- Mormino, E.C., Kluth, J.T., Madison, C.M., Rabinovici, G.D., Baker, S.L., Miller, B.L., Koeppe, R.A., Mathis, C.A., Weiner, M.W. and Jagust, W.J. (2009) 'Episodic memory



- loss is related to hippocampal-mediated beta-amyloid deposition in elderly subjects', *Brain*, 132(Pt 5), pp. 1310-23.
- Moroney, J.T., Bagiella, E., Desmond, D.W., Paik, M.C., Stern, Y. and Tatemichi, T.K. (1996) 'Risk factors for incident dementia after stroke. Role of hypoxic and ischemic disorders', *Stroke*, 27(8), pp. 1283-9.
- Morris, C.M., Ballard, C.G., Allan, L., Rowan, E., Stephens, S., Firbank, M., Ford, G.A., Kenny, R.A., O'Brien, J.T. and Kalaria, R.N. (2011) 'NOS3 gene rs1799983 polymorphism and incident dementia in elderly stroke survivors', *Neurobiol Aging*, 32(3), pp. 554 e1-6.
- Morris, M.C., Scherr, P.A., Hebert, L.E., Glynn, R.J., Bennett, D.A. and Evans, D.A. (2001) 'Association of incident Alzheimer disease and blood pressure measured from 13 years before to 2 years after diagnosis in a large community study', *Arch Neurol*, 58(10), pp. 1640-6.
- Morrison, J.H. and Baxter, M.G. (2012) 'The ageing cortical synapse: hallmarks and implications for cognitive decline', *Nat Rev Neurosci*, 13(4), pp. 240-50.
- Mortamais, M., Portet, F., Brickman, A.M., Provenzano, F.A., Muraskin, J., Akbaraly, T.N., Berr, C., Touchon, J., Bonafe, A., le Bars, E., Menjot de Champfleury, N., Maller, J.J., Meslin, C., Sabatier, R., Ritchie, K. and Artero, S. (2013) 'Education Modulates the Impact of White Matter Lesions on the Risk of Mild Cognitive Impairment and Dementia', *Am J Geriatr Psychiatry*.
- Moser, M.B. and Moser, E.I. (1998) 'Functional differentiation in the hippocampus', *Hippocampus*, 8(6), pp. 608-19.
- Moskowitz, M.A., Lo, E.H. and Iadecola, C. (2010) 'The science of stroke: mechanisms in search of treatments', *Neuron*, 67(2), pp. 181-98.
- Muangpaisan, W. and Brayne, C. (2010) 'Systematic review of statins for the prevention of vascular dementia or dementia', *Geriatr Gerontol Int*, 10(2), pp. 199-208.
- Mucke, L. and Selkoe, D.J. (2012) 'Neurotoxicity of amyloid beta-protein: synaptic and network dysfunction', *Cold Spring Harb Perspect Med*, 2(7), p. a006338.
- Mufson, E.J., Chen, E.Y., Cochran, E.J., Beckett, L.A., Bennett, D.A. and Kordower, J.H. (1999) 'Entorhinal cortex beta-amyloid load in individuals with mild cognitive impairment', *Exp Neurol*, 158(2), pp. 469-90.
- Mukaetova-Ladinska, E.B., Abdell-All, Z., Andrade, J., da Silva, J.A., Boksha, I., Burbaeva, G., Kalaria, R.J. and O'Brien, J.T. (2013a) 'Platelet Tau Protein as a Potential Peripheral Biomarker in Alzheimer's disease: An Explorative Study', *Curr Alzheimer Res*.
- Mukaetova-Ladinska, E.B., Andras, A., Milne, J., Abdel-All, Z., Borr, I., Jaros, E., Perry, R.H., Honer, W.G., Cleghorn, A., Doherty, J., McIntosh, G., Perry, E.K., Kalaria, R.N. and McKeith, I.G. (2013b) 'Synaptic proteins and choline acetyltransferase loss in visual cortex in dementia with Lewy bodies', *J Neuropathol Exp Neurol*, 72(1), pp. 53-60.
- Mukaetova-Ladinska, E.B., Xuereb, J.H., Garcia-Sierra, F., Hurt, J., Gertz, H.J., Hills, R., Brayne, C., Huppert, F.A., Paykel, E.S., McGee, M.A., Jakes, R., Honer, W.G., Harrington, C.R. and Wischik, C.M. (2009) 'Lewy body variant of Alzheimer's disease: selective neocortical loss of t-SNARE proteins and loss of MAP2 and alpha-synuclein in medial temporal lobe', *ScientificWorldJournal*, 9, pp. 1463-75.
- Murthy, S.B., Jawaid, A., Qureshi, S.U., Kalkonde, Y., Wilson, A.M., Johnson, M.L., Kunik, M.E. and Schulz, P.E. (2010) 'Does diabetes mellitus alter the onset and clinical course of vascular dementia?', *Behav Neurol*, 23(3), pp. 145-51.
- Nagy, Z., Esiri, M.M., Jobst, K.A., Johnston, C., Litchfield, S., Sim, E. and Smith, A.D. (1995) 'Influence of the apolipoprotein E genotype on amyloid deposition and neurofibrillary tangle formation in Alzheimer's disease', *Neuroscience*, 69(3), pp. 757-61.
- Nave, K.A. (2010) 'Myelination and support of axonal integrity by glia', *Nature*, 468(7321), pp. 244-52.
- Ndung'u, M., Hartig, W., Wegner, F., Mwenda, J.M., Low, R.W., Akinyemi, R.O. and Kalaria, R.N. (2012) 'Cerebral amyloid beta(42) deposits and microvascular pathology in ageing baboons', *Neuropathol Appl Neurobiol*, 38(5), pp. 487-99.

- Neeper, S.A., Gomez-Pinilla, F., Choi, J. and Cotman, C. (1995) 'Exercise and brain neurotrophins', *Nature*, 373(6510), p. 109.
- Neeper, S.A., Gomez-Pinilla, F., Choi, J. and Cotman, C.W. (1996) 'Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain', *Brain Res*, 726(1-2), pp. 49-56.
- Nelson, P.T., Alafuzoff, I., Bigio, E.H., Bouras, C., Braak, H., Cairns, N.J., Castellani, R.J., Crain, B.J., Davies, P., Del Tredici, K., Duyckaerts, C., Frosch, M.P., Haroutunian, V., Hof, P.R., Hulette, C.M., Hyman, B.T., Iwatsubo, T., Jellinger, K.A., Jicha, G.A., Kovari, E., Kukull, W.A., Leverenz, J.B., Love, S., Mackenzie, I.R., Mann, D.M., Masliah, E., McKee, A.C., Montine, T.J., Morris, J.C., Schneider, J.A., Sonnen, J.A., Thal, D.R., Trojanowski, J.Q., Troncoso, J.C., Wisniewski, T., Woltjer, R.L. and Beach, T.G. (2012) 'Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature', *J Neuropathol Exp Neurol*, 71(5), pp. 362-81.
- Nelson, P.T., Braak, H. and Markesbery, W.R. (2009) 'Neuropathology and cognitive impairment in Alzheimer disease: a complex but coherent relationship', *J Neuropathol Exp Neurol*, 68(1), pp. 1-14.
- Nelson, P.T., Smith, C.D., Abner, E.L., Wilfred, B.J., Wang, W.X., Neltner, J.H., Baker, M., Fardo, D.W., Kryscio, R.J., Scheff, S.W., Jicha, G.A., Jellinger, K.A., Van Eldik, L.J. and Schmitt, F.A. (2013) 'Hippocampal sclerosis of aging, a prevalent and high-morbidity brain disease', *Acta Neuropathol*, 126(2), pp. 161-77.
- Nieto, A., Montejo de Garcini, E. and Avila, J. (1989) 'Altered levels of microtubule proteins in brains of Alzheimer's disease patients', *Acta Neuropathol*, 78(1), pp. 47-51.
- Nishii, K., Nakaseko, C., Jiang, M., Shimizu, N., Takeuchi, M., Schneider, W.J. and Bujo, H. (2013) 'The soluble form of LR11 protein is a regulator of hypoxia-induced, urokinase-type plasminogen activator receptor (uPAR)-mediated adhesion of immature hematological cells', *J Biol Chem*, 288(17), pp. 11877-86.
- Nishio, K., Ihara, M., Yamasaki, N., Kalaria, R.N., Maki, T., Fujita, Y., Ito, H., Oishi, N., Fukuyama, H., Miyakawa, T., Takahashi, R. and Tomimoto, H. (2010) 'A mouse model characterizing features of vascular dementia with hippocampal atrophy', *Stroke*, 41(6), pp. 1278-84.
- Nordahl, C.W., Ranganath, C., Yonelinas, A.P., Decarli, C., Fletcher, E. and Jagust, W.J. (2006) 'White matter changes compromise prefrontal cortex function in healthy elderly individuals', *J Cogn Neurosci*, 18(3), pp. 418-29.
- Norrving, B. and Kissela, B. (2013) 'The global burden of stroke and need for a continuum of care', *Neurology*, 80(3 Suppl 2), pp. S5-12.
- Notkola, I.L., Sulkava, R., Pekkanen, J., Erkinjuntti, T., Ehnholm, C., Kivinen, P., Tuomilehto, J. and Nissinen, A. (1998) 'Serum total cholesterol, apolipoprotein E epsilon 4 allele, and Alzheimer's disease', *Neuroepidemiology*, 17(1), pp. 14-20.
- Nys, G.M., van Zandvoort, M.J., de Kort, P.L., Jansen, B.P., de Haan, E.H. and Kappelle, L.J. (2007) 'Cognitive disorders in acute stroke: prevalence and clinical determinants', *Cerebrovasc Dis*, 23(5-6), pp. 408-16.
- O'Brien, J.T., Erkinjuntti, T., Reisberg, B., Roman, G., Sawada, T., Pantoni, L., Bowler, J.V., Ballard, C., DeCarli, C., Gorelick, P.B., Rockwood, K., Burns, A., Gauthier, S. and DeKosky, S.T. (2003) 'Vascular cognitive impairment', *Lancet Neurol*, 2(2), pp. 89-98.
- O'Donnell, M., Xavier, D., Diener, C., Sacco, R., Lisheng, L., Zhang, H., Pias, P., Truelsen, T., Chin, S.L., Rangarajan, S., Devilliers, L., Damasceno, A., Mondo, C., Lanus, F., Avezum, A., Diaz, R., Varigos, J., Hankey, G., Teal, P., Kapral, M., Ryglewicz, D., Czlonkowska, A., Skowronska, M., Lopez-Jaramillo, P., Dans, T., Langhorne, P. and Yusuf, S. (2010a) 'Rationale and design of INTERSTROKE: a global case-control study of risk factors for stroke', *Neuroepidemiology*, 35(1), pp. 36-44.
- O'Donnell, M.J., Xavier, D., Liu, L., Zhang, H., Chin, S.L., Rao-Melacini, P., Rangarajan, S., Islam, S., Pais, P., McQueen, M.J., Mondo, C., Damasceno, A., Lopez-Jaramillo, P., Hankey, G.J., Dans, A.L., Yusuf, K., Truelsen, T., Diener, H.C., Sacco, R.L., Ryglewicz, D., Czlonkowska, A., Weimar, C., Wang, X. and Yusuf, S. (2010b) 'Risk

- factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the INTERSTROKE study): a case-control study', *Lancet*, 376(9735), pp. 112-23.
- O'Sullivan, M., Jones, D.K., Summers, P.E., Morris, R.G., Williams, S.C. and Markus, H.S. (2001) 'Evidence for cortical "disconnection" as a mechanism of age-related cognitive decline', *Neurology*, 57(4), pp. 632-8.
- O'Sullivan, M., Jouvent, E., Saemann, P.G., Mangin, J.F., Viswanathan, A., Gschwendtner, A., Bracoud, L., Pachai, C., Chabriat, H. and Dichgans, M. (2008) 'Measurement of brain atrophy in subcortical vascular disease: A comparison of different approaches and the impact of ischaemic lesions', *Neuroimage*.
- O'Sullivan, M., Ngo, E., Viswanathan, A., Jouvent, E., Gschwendtner, A., Saemann, P.G., Duering, M., Pachai, C., Bousser, M.G., Chabriat, H. and Dichgans, M. (2009) 'Hippocampal volume is an independent predictor of cognitive performance in CADASIL', *Neurobiol Aging*, 30(6), pp. 890-7.
- Offe, K., Dodson, S.E., Shoemaker, J.T., Fritz, J.J., Gearing, M., Levey, A.I. and Lah, J.J. (2006) 'The lipoprotein receptor LR11 regulates amyloid beta production and amyloid precursor protein traffic in endosomal compartments', *J Neurosci*, 26(5), pp. 1596-603.
- Ogata, J., Yamanishi, H. and Ishibashi-Ueda, H. (2011) 'Review: role of cerebral vessels in ischaemic injury of the brain', *Neuropathol Appl Neurobiol*, 37(1), pp. 40-55.
- Ogbole, G.I., Owolabi, M.O. and Yusuf, B.P. (2013) 'White matter changes on magnetic resonance imaging: a risk factor for stroke in an african population?', *Journal of Stroke & Cerebrovascular Diseases*, 22(7), pp. e227-33.
- Ogun, S.A., Ojini, F.I., Ogungbo, B., Kolapo, K.O. and Danesi, M.A. (2005) 'Stroke in south west Nigeria: a 10-year review', *Stroke*, 36(6), pp. 1120-2.
- Ogun, S.A., Oluwole, O., Fatade, B., Ogunseyinde, A.O., Ojini, F.I. and Odusote, K.A. (2002) 'Comparison of Siriraj Stroke Score and the WHO criteria in the clinical classification of stroke subtypes', *Afr J Med Med Sci*, 31(1), pp. 13-6.
- Ogunniyi, A. and Akinyemi, R.O. (2010) 'Dementia in Africa : A review', *Archives of Ibadan Medicine*, 10(1 & 2), pp. 40 - 46.
- Ogunniyi, A., Baiyewu, O., Gureje, O., Hall, K.S., Unverzagt, F., Siu, S.H., Gao, S., Farlow, M., Oluwole, O.S., Komolafe, O. and Hendrie, H.C. (2000) 'Epidemiology of dementia in Nigeria: results from the Indianapolis-Ibadan study', *Eur J Neurol*, 7(5), pp. 485-90.
- Okamoto, Y., Yamamoto, T., Kalaria, R.N., Senzaki, H., Maki, T., Hase, Y., Kitamura, A., Washida, K., Yamada, M., Ito, H., Tomimoto, H., Takahashi, R. and Ihara, M. (2012) 'Cerebral hypoperfusion accelerates cerebral amyloid angiopathy and promotes cortical microinfarcts', *Acta Neuropathol*, 123(3), pp. 381-94.
- Okonkwo, O.C., Xu, G., Dowling, N.M., Bendlin, B.B., Larue, A., Hermann, B.P., Kosciak, R., Jonaitis, E., Rowley, H.A., Carlsson, C.M., Asthana, S., Sager, M.A. and Johnson, S.C. (2012) 'Family history of Alzheimer disease predicts hippocampal atrophy in healthy middle-aged adults', *Neurology*, 78(22), pp. 1769-76.
- Oosterman, J.M., Oosterveld, S., Rikkert, M.G., Claassen, J.A. and Kessels, R.P. (2012) 'Medial temporal lobe atrophy relates to executive dysfunction in Alzheimer's disease', *Int Psychogeriatr*, 24(9), pp. 1474-82.
- Oosterman, J.M., Vogels, R.L., van Harten, B., Gouw, A.A., Scheltens, P., Poggesi, A., Weinstein, H.C. and Scherder, E.J. (2008) 'The role of white matter hyperintensities and medial temporal lobe atrophy in age-related executive dysfunctioning', *Brain & Cognition*, 68(2), pp. 128-33.
- Osuntokun, B.O., Hendrie, H.C., Ogunniyi, A.O., Hall, K.S., Lekwauwa, U.G., Brittain, H.M., Norton, J.A., Jr., Oyediran, A.B., Pillay, N. and Rodgers, D.D. (1992) 'Cross-cultural studies in Alzheimer's disease', *Ethn Dis*, 2(4), pp. 352-7.
- Owolabi, M.O. (2011) 'Taming the burgeoning stroke epidemic in Africa: stroke quadrangle to the rescue', *West Indian Med J*, 60(4), pp. 412-21.
- Owolabi, M.O. and Agunloye, A.M. (2013) 'Risk factors for stroke among patients with hypertension: a case-control study', *J Neurol Sci*, 325(1-2), pp. 51-6.
- Owolabi, M.O. and Ogunniyi, A. (2009) 'Profile of health-related quality of life in Nigerian stroke survivors', *Eur J Neurol*, 16(1), pp. 54-62.

- Owolabi, M.O. and Platz, T. (2008) 'Proposing the Stroke Levity Scale: a valid, reliable, simple, and time-saving measure of stroke severity', *Eur J Neurol*, 15(6), pp. 627-33.
- Pacher, P., Beckman, J.S. and Liaudet, L. (2007) 'Nitric oxide and peroxynitrite in health and disease', *Physiol Rev*, 87(1), pp. 315-424.
- Packard, C.J., Westendorp, R.G., Stott, D.J., Caslake, M.J., Murray, H.M., Shepherd, J., Blauw, G.J., Murphy, M.B., Bollen, E.L., Buckley, B.M., Cobbe, S.M., Ford, I., Gaw, A., Hyland, M., Jukema, J.W., Kamper, A.M., Macfarlane, P.W., Jolles, J., Perry, I.J., Sweeney, B.J. and Twomey, C. (2007) 'Association between apolipoprotein E4 and cognitive decline in elderly adults', *J Am Geriatr Soc*, 55(11), pp. 1777-85.
- Palop, J.J., Chin, J. and Mucke, L. (2006) 'A network dysfunction perspective on neurodegenerative diseases', *Nature*, 443(7113), pp. 768-73.
- Pantoni, L. (2010) 'Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges', *Lancet Neurol*, 9(7), pp. 689-701.
- Pantoni, L. and Garcia, J.H. (1995) 'The significance of cerebral white matter abnormalities 100 years after Binswanger's report. A review', *Stroke*, 26(7), pp. 1293-301.
- Panza, F., Solfrizzi, V., Colacicco, A.M., D'Introno, A., Capurso, C., Palasciano, R., Todarello, O., Capurso, S., Pellicani, V. and Capurso, A. (2006) 'Cerebrovascular disease in the elderly: lipoprotein metabolism and cognitive decline', *Aging Clin Exp Res*, 18(2), pp. 144-8.
- Paraiso, M.N., Guerchet, M., Saizonou, J., Cowppli-Bony, P., Mouanga, A.M., Nubukpo, P., Preux, P.M. and Houinato, D.S. (2011) 'Prevalence of dementia among elderly people living in Cotonou, an urban area of Benin (West Africa)', *Neuroepidemiology*, 36(4), pp. 245-51.
- Patel, M., Coshall, C., Rudd, A.G. and Wolfe, C.D. (2003) 'Natural history of cognitive impairment after stroke and factors associated with its recovery', *Clin Rehabil*, 17(2), pp. 158-66.
- Pendlebury, S.T. (2009) 'Stroke-related dementia: rates, risk factors and implications for future research', *Maturitas*, 64(3), pp. 165-71.
- Pendlebury, S.T., Mariz, J., Bull, L., Mehta, Z. and Rothwell, P.M. (2012) 'MoCA, ACE-R, and MMSE versus the National Institute of Neurological Disorders and Stroke-Canadian Stroke Network Vascular Cognitive Impairment Harmonization Standards Neuropsychological Battery after TIA and stroke', *Stroke*, 43(2), pp. 464-9.
- Pendlebury, S.T. and Rothwell, P.M. (2009a) 'Prevalence, incidence, and factors associated with pre-stroke and post-stroke dementia: a systematic review and meta-analysis', *Lancet Neurol*, 8(11), pp. 1006-18.
- Pendlebury, S.T. and Rothwell, P.M. (2009b) 'Prevalence, incidence, and factors associated with pre-stroke and post-stroke dementia: a systematic review and meta-analysis', *Lancet Neurol*, 8, pp. 1006-1018.
- Penzes, P. and Vanleeuwen, J.E. (2011) 'Impaired regulation of synaptic actin cytoskeleton in Alzheimer's disease', *Brain Res Rev*, 67(1-2), pp. 184-92.
- Perdahl, E., Adolfsson, R., Alafuzoff, I., Albert, K.A., Nestler, E.J., Greengard, P. and Winblad, B. (1984) 'Synapsin I (protein I) in different brain regions in senile dementia of Alzheimer type and in multi-infarct dementia', *J Neural Transm*, 60(2), pp. 133-41.
- Perez, L., Heim, L., Sherzai, A., Jaceldo-Siegl, K. and Sherzai, A. (2012) 'Nutrition and vascular dementia', *J Nutr Health Aging*, 16(4), pp. 319-24.
- Perry, R.H. and Oakley, A. (1993) *Newcastle Brain Map*. London: Wolfe.
- Petersen, R.C., Smith, G.E., Waring, S.C., Ivnik, R.J., Tangalos, E.G. and Kokmen, E. (1999) 'Mild cognitive impairment: clinical characterization and outcome', *Arch Neurol*, 56(3), pp. 303-8.
- Petrovitch, H., Ross, G.W., Steinhorn, S.C., Abbott, R.D., Markesbery, W., Davis, D., Nelson, J., Hardman, J., Masaki, K., Vogt, M.R., Launer, L. and White, L.R. (2005) 'AD lesions and infarcts in demented and non-demented Japanese-American men', *Ann Neurol*, 57(1), pp. 98-103.
- Petrovitch, H., White, L.R., Izmirilian, G., Ross, G.W., Havlik, R.J., Markesbery, W., Nelson, J., Davis, D.G., Hardman, J., Foley, D.J. and Launer, L.J. (2000) 'Midlife blood

- pressure and neuritic plaques, neurofibrillary tangles, and brain weight at death: the HAAS. Honolulu-Asia aging Study', *Neurobiol Aging*, 21(1), pp. 57-62.
- Poels, M.M., Ikram, M.A., van der Lugt, A., Hofman, A., Niessen, W.J., Krestin, G.P., Breteler, M.M. and Vernooij, M.W. (2012) 'Cerebral microbleeds are associated with worse cognitive function: the Rotterdam Scan Study', *Neurology*, 78(5), pp. 326-33.
- Pohjasvaara, T., Erkinjuntti, T., Vataja, R. and Kaste, M. (1997) 'Dementia three months after stroke. Baseline frequency and effect of different definitions of dementia in the Helsinki Stroke Aging Memory Study (SAM) cohort', *Stroke*, 28(4), pp. 785-92.
- Pohjasvaara, T., Mantyla, R., Aronen, H.J., Leskela, M., Salonen, O., Kaste, M. and Erkinjuntti, T. (1999) 'Clinical and radiological determinants of prestroke cognitive decline in a stroke cohort', *J Neurol Neurosurg Psychiatry*, 67(6), pp. 742-8.
- Pollock, A., St George, B., Fenton, M. and Firkins, L. (2012) 'Top ten research priorities relating to life after stroke', *Lancet Neurol*, 11(3), p. 209.
- Polvikoski, T., Sulkava, R., Haltia, M., Kainulainen, K., Vuorio, A., Verkkoniemi, A., Niinisto, L., Halonen, P. and Kontula, K. (1995) 'Apolipoprotein E, dementia, and cortical deposition of beta-amyloid protein', *N Engl J Med*, 333(19), pp. 1242-7.
- Prince, M., Acosta, D., Ferri, C.P., Guerra, M., Huang, Y., Llibre Rodriguez, J.J., Salas, A., Sosa, A.L., Williams, J.D., Dewey, M.E., Acosta, I., Jotheeswaran, A.T. and Liu, Z. (2012) 'Dementia incidence and mortality in middle-income countries, and associations with indicators of cognitive reserve: a 10/66 Dementia Research Group population-based cohort study', *Lancet*, 380(9836), pp. 50-8.
- Prince, M., Bryce, R., Albanese, E., Wimo, A., Ribeiro, W. and Ferri, C.P. (2013) 'The global prevalence of dementia: a systematic review and metaanalysis', *Alzheimers Dement*, 9(1), pp. 63-75.e2.
- Qiu, C., Zhang, Y., Bronge, L., Herlitz, A., Aspelin, P., Backman, L., Fratiglioni, L. and Wahlund, L.O. (2012) 'Medial temporal lobe is vulnerable to vascular risk factors in men: a population-based study', *Eur J Neurol*, 19(6), pp. 876-83.
- Quaney, B.M., Boyd, L.A., McDowd, J.M., Zahner, L.H., He, J., Mayo, M.S. and Macko, R.F. (2009) 'Aerobic exercise improves cognition and motor function poststroke', *Neurorehabil Neural Repair*, 23(9), pp. 879-85.
- Quayhagen, M.P., Quayhagen, M., Corbeil, R.R., Hendrix, R.C., Jackson, J.E., Snyder, L. and Bower, D. (2000) 'Coping with dementia: evaluation of four nonpharmacologic interventions', *Int Psychogeriatr*, 12(2), pp. 249-65.
- Querfurth, H.W. and LaFerla, F.M. (2010) 'Alzheimer's disease', *N Engl J Med*, 362(4), pp. 329-44.
- Rasquin, S., Lodder, J. and Verhey, F. (2005a) 'The association between psychiatric and cognitive symptoms after stroke: a prospective study', *Cerebrovasc Dis*, 19(5), pp. 309-16.
- Rasquin, S.M., Lodder, J. and Verhey, F.R. (2005b) 'Predictors of reversible mild cognitive impairment after stroke: a 2-year follow-up study', *J Neurol Sci*, 229-230, pp. 21-5.
- Rasquin, S.M., Verhey, F.R., Lousberg, R., Winkens, I. and Lodder, J. (2002) 'Vascular cognitive disorders: memory, mental speed and cognitive flexibility after stroke', *J Neurol Sci*, 203-204, pp. 115-9.
- Rasquin, S.M., Welter, J. and van Heugten, C.M. (2013) 'Course of cognitive functioning during stroke rehabilitation', *Neuropsychol Rehabil*.
- Reed, B.R., Mungas, D.M., Kramer, J.H., Ellis, W., Vinters, H.V., Zarow, C., Jagust, W.J. and Chui, H.C. (2007) 'Profiles of neuropsychological impairment in autopsy-defined Alzheimer's disease and cerebrovascular disease', *Brain*, 130(Pt 3), pp. 731-9.
- Reitz, C., Bos, M.J., Hofman, A., Koudstaal, P.J. and Breteler, M.M. (2008) 'Prestroke cognitive performance, incident stroke, and risk of dementia: the Rotterdam Study', *Stroke*, 39(1), pp. 36-41.
- Reitz, C., Tang, M.X., Luchsinger, J. and Mayeux, R. (2004) 'Relation of plasma lipids to Alzheimer disease and vascular dementia', *Arch Neurol*, 61(5), pp. 705-14.
- Reynolds, R.M., Strachan, M.W., Labad, J., Lee, A.J., Frier, B.M., Fowkes, F.G., Mitchell, R., Seckl, J.R., Deary, I.J., Walker, B.R. and Price, J.F. (2010) 'Morning cortisol levels and

- cognitive abilities in people with type 2 diabetes: the Edinburgh type 2 diabetes study', *Diabetes Care*, 33(4), pp. 714-20.
- Robinson, J.G., Ijioma, N. and Harris, W. (2010) 'Omega-3 fatty acids and cognitive function in women', *Womens Health (Lond Engl)*, 6(1), pp. 119-34.
- Rogaeva, E., Meng, Y., Lee, J.H., Gu, Y., Kawarai, T., Zou, F., Katayama, T., Baldwin, C.T., Cheng, R., Hasegawa, H., Chen, F., Shibata, N., Lunetta, K.L., Pardossi-Piquard, R., Bohm, C., Wakutani, Y., Cupples, L.A., Cuenco, K.T., Green, R.C., Pinessi, L., Rainero, I., Sorbi, S., Bruni, A., Duara, R., Friedland, R.P., Inzelberg, R., Hampe, W., Bujo, H., Song, Y.Q., Andersen, O.M., Willnow, T.E., Graff-Radford, N., Petersen, R.C., Dickson, D., Der, S.D., Fraser, P.E., Schmitt-Ulms, G., Younkin, S., Mayeux, R., Farrer, L.A. and St George-Hyslop, P. (2007) 'The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease', *Nat Genet*, 39(2), pp. 168-77.
- Rogers, I., Kerr, F., Martinez, P., Hardy, J., Lovestone, S. and Partridge, L. (2012) 'Ageing increases vulnerability to abeta42 toxicity in Drosophila', *PLoS One*, 7(7), p. e40569.
- Roman, G.C. (1999) 'A historical review of the concept of vascular dementia: lessons from the past for the future', *Alzheimer Dis Assoc Disord*, 13 Suppl 3, pp. S4-8.
- Roman, G.C. (2008) 'The Epidemiology of vascular dementia', *Handb Clin Neurol*, 89, pp. 639-58.
- Roman, G.C. and Kalaria, R.N. (2006) 'Vascular determinants of cholinergic deficits in Alzheimer disease and vascular dementia', *Neurobiol Aging*, 27(12), pp. 1769-85.
- Roman, G.C., Salloway, S., Black, S.E., Royall, D.R., Decarli, C., Weiner, M.W., Moline, M., Kumar, D., Schindler, R. and Posner, H. (2010) 'Randomized, placebo-controlled, clinical trial of donepezil in vascular dementia: differential effects by hippocampal size', *Stroke*, 41(6), pp. 1213-21.
- Roman, G.C., Tatemichi, T.K., Erkinjuntti, T., Cummings, J.L., Masdeu, J.C., Garcia, J.H., Amaducci, L., Orgogozo, J.M., Brun, A., Hofman, A. and et al. (1993) 'Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop', *Neurology*, 43(2), pp. 250-60.
- Rosenberg, G.A. (2009) 'Inflammation and white matter damage in vascular cognitive impairment', *Stroke*, 40(3 Suppl), pp. S20-3.
- Rosenberg, G.A., Sullivan, N. and Esiri, M.M. (2001) 'White matter damage is associated with matrix metalloproteinases in vascular dementia', *Stroke*, 32(5), pp. 1162-8.
- Rosene, D.L. and Van Hoesen, G.W. (1977) 'Hippocampal efferents reach widespread areas of cerebral cortex and amygdala in the rhesus monkey', *Science*, 198(4314), pp. 315-7.
- Roth, M., Tym, E., Mountjoy, C.Q., Huppert, F.A., Hendrie, H., Verma, S. and Goddard, R. (1986) 'CAMDEX. A standardised instrument for the diagnosis of mental disorder in the elderly with special reference to the early detection of dementia', *Br J Psychiatry*, 149, pp. 698-709.
- Rovio, S., Spulber, G., Nieminen, L.J., Niskanen, E., Winblad, B., Tuomilehto, J., Nissinen, A., Soininen, H. and Kivipelto, M. (2008) 'The effect of midlife physical activity on structural brain changes in the elderly', *Neurobiol Aging*.
- Rowan, E., Morris, C.M., Stephens, S., Ballard, C., Dickinson, H., Rao, H., Saxby, B.K., McLaren, A.T., Kalaria, R.N. and Kenny, R.A. (2005) 'Impact of hypertension and apolipoprotein E4 on poststroke cognition in subjects >75 years of age', *Stroke*, 36(9), pp. 1864-8.
- Rusanen, M., Kivipelto, M., Quesenberry, C.P., Jr., Zhou, J. and Whitmer, R.A. (2010) 'Heavy Smoking in Midlife and Long-term Risk of Alzheimer Disease and Vascular Dementia', *Arch Intern Med*, 171(4), pp. 333-9.
- Sacco, R.L., Kasner, S.E., Broderick, J.P., Caplan, L.R., Connors, J.J., Culebras, A., Elkind, M.S., George, M.G., Hamdan, A.D., Higashida, R.T., Hoh, B.L., Janis, L.S., Kase, C.S., Kleindorfer, D.O., Lee, J.M., Moseley, M.E., Peterson, E.D., Turan, T.N., Valderrama, A.L. and Vinters, H.V. (2013) 'An updated definition of stroke for the 21st century: a statement for healthcare professionals from the American Heart Association/American Stroke Association', *Stroke*, 44(7), pp. 2064-89.
- Sachdev, P. (1999) 'Vascular cognitive disorder', *Int J Geriatr Psychiatry*, 14(5), pp. 402-3.

- Sachdev, P. (2000) 'Is it time to retire the term "dementia"?', *J Neuropsychiatry Clin Neurosci*, 12(2), pp. 276-9.
- Sachdev, P.S., Brodaty, H., Valenzuela, M.J., Lorentz, L., Looi, J.C., Berman, K., Ross, A., Wen, W. and Zagami, A.S. (2006) 'Clinical determinants of dementia and mild cognitive impairment following ischaemic stroke: the Sydney Stroke Study', *Dement Geriatr Cogn Disord*, 21(5-6), pp. 275-83.
- Sachdev, P.S., Brodaty, H., Valenzuela, M.J., Lorentz, L., Looi, J.C., Wen, W. and Zagami, A.S. (2004a) 'The neuropsychological profile of vascular cognitive impairment in stroke and TIA patients', *Neurology*, 62(6), pp. 912-9.
- Sachdev, P.S., Brodaty, H., Valenzuela, M.J., Lorentz, L.M. and Koschera, A. (2004b) 'Progression of cognitive impairment in stroke patients', *Neurology*, 63(9), pp. 1618-23.
- Sachdev, P.S., Chen, X., Brodaty, H., Thompson, C., Altendorf, A. and Wen, W. (2009) 'The determinants and longitudinal course of post-stroke mild cognitive impairment', *J Int Neuropsychol Soc*, 15(6), pp. 915-23.
- Sachdev, P.S., Chen, X., Joscelyne, A., Wen, W., Altendorf, A. and Brodaty, H. (2007a) 'Hippocampal size and dementia in stroke patients: the Sydney stroke study', *J Neurol Sci*, 260(1-2), pp. 71-7.
- Sachdev, P.S., Chen, X., Joscelyne, A., Wen, W. and Brodaty, H. (2007b) 'Amygdala in stroke/transient ischemic attack patients and its relationship to cognitive impairment and psychopathology: the Sydney Stroke Study', *Am J Geriatr Psychiatry*, 15(6), pp. 487-96.
- Sagare, A., Deane, R., Bell, R.D., Johnson, B., Hamm, K., Pendu, R., Marky, A., Lenting, P.J., Wu, Z., Zarccone, T., Goate, A., Mayo, K., Perlmutter, D., Coma, M., Zhong, Z. and Zlokovic, B.V. (2007) 'Clearance of amyloid-beta by circulating lipoprotein receptors', *Nat Med*, 13(9), pp. 1029-31.
- Sager, K.L., Wu, J., Herskowitz, J.H., Mufson, E.J., Levey, A.I. and Lah, J.J. (2012) 'Neuronal LR11 expression does not differentiate between clinically-defined Alzheimer's disease and control brains', *PLoS One*, 7(8), p. e40527.
- Sager, K.L., Wu, J., Leurgans, S.E., Rees, H.D., Gearing, M., Mufson, E.J., Levey, A.I. and Lah, J.J. (2007) 'Neuronal LR11/sorLA expression is reduced in mild cognitive impairment', *Ann Neurol*, 62(6), pp. 640-7.
- Saito, Y., Suzuki, K., Nanba, E., Yamamoto, T., Ohno, K. and Murayama, S. (2002) 'Niemann-Pick type C disease: accelerated neurofibrillary tangle formation and amyloid beta deposition associated with apolipoprotein E epsilon 4 homozygosity', *Ann Neurol*, 52(3), pp. 351-5.
- Satz, P., Cole, M.A., Hardy, D.J. and Rassovsky, Y. (2011) 'Brain and cognitive reserve: mediator(s) and construct validity, a critique', *J Clin Exp Neuropsychol*, 33(1), pp. 121-30.
- Satz, P., Morgenstern, H., Miller, E.N., Selnes, O.A., McArthur, J.C., Cohen, B.A., Wesch, J., Becker, J.T., Jacobson, L., D'Elia, L.F. and et al. (1993) 'Low education as a possible risk factor for cognitive abnormalities in HIV-1: findings from the multicenter AIDS Cohort Study (MACS)', *J Acquir Immune Defic Syndr*, 6(5), pp. 503-11.
- Scarmeas, N., Luchsinger, J.A., Schupf, N., Brickman, A.M., Cosentino, S., Tang, M.X. and Stern, Y. (2009) 'Physical activity, diet, and risk of Alzheimer disease', *JAMA*, 302(6), pp. 627-37.
- Scheff, S.W., DeKosky, S.T. and Price, D.A. (1990) 'Quantitative assessment of cortical synaptic density in Alzheimer's disease', *Neurobiol Aging*, 11(1), pp. 29-37.
- Scheff, S.W. and Price, D.A. (2003) 'Synaptic pathology in Alzheimer's disease: a review of ultrastructural studies', *Neurobiol Aging*, 24(8), pp. 1029-46.
- Scheltens, P., Barkhof, F., Leys, D., Pruvo, J.P., Nauta, J.J., Vermersch, P., Steinling, M. and Valk, J. (1993) 'A semiquantitative rating scale for the assessment of signal hyperintensities on magnetic resonance imaging', *J Neurol Sci*, 114(1), pp. 7-12.
- Scheltens, P., Leys, D., Barkhof, F., Huglo, D., Weinstein, H.C., Vermersch, P., Kuiper, M., Steinling, M., Wolters, E.C. and Valk, J. (1992) 'Atrophy of medial temporal lobes on

- MRI in "probable" Alzheimer's disease and normal ageing: diagnostic value and neuropsychological correlates', *J Neurol Neurosurg Psychiatry*, 55(10), pp. 967-72.
- Scher, A.I., Xu, Y., Korf, E.S., Hartley, S.W., Witter, M.P., Scheltens, P., White, L.R., Thompson, P.M., Toga, A.W., Valentino, D.J. and Launer, L.J. (2011) 'Hippocampal morphometry in population-based incident Alzheimer's disease and vascular dementia: the HAAS', *J Neurol Neurosurg Psychiatry*, 82(4), pp. 373-6.
- Schmechel, D.E., Saunders, A.M., Strittmatter, W.J., Crain, B.J., Hulette, C.M., Joo, S.H., Pericak-Vance, M.A., Goldgaber, D. and Roses, A.D. (1993) 'Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease', *Proc Natl Acad Sci U S A*, 90(20), pp. 9649-53.
- Schneider, J.A., Wilson, R.S., Bienias, J.L., Evans, D.A. and Bennett, D.A. (2004) 'Cerebral infarctions and the likelihood of dementia from Alzheimer disease pathology', *Neurology*, 62(7), pp. 1148-55.
- Schoonenboom, N.S., Pijnenburg, Y.A., Mulder, C., Rosso, S.M., Van Elk, E.J., Van Kamp, G.J., Van Swieten, J.C. and Scheltens, P. (2004) 'Amyloid beta(1-42) and phosphorylated tau in CSF as markers for early-onset Alzheimer disease', *Neurology*, 62(9), pp. 1580-4.
- Seidel, D., Jagger, C., Brayne, C., Matthews, F.E. and Cfas, M. (2009) 'Recovery in instrumental activities of daily living (IADLs): findings from the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS)', *Age Ageing*, 38(6), pp. 663-8.
- Sekino, Y., Kojima, N. and Shirao, T. (2007) 'Role of actin cytoskeleton in dendritic spine morphogenesis', *Neurochemistry International*, 51(2-4), pp. 92-104.
- Selkoe, D.J. (2002) 'Alzheimer's disease is a synaptic failure', *Science*, 298(5594), pp. 789-91.
- Selkoe, D.J. (2008) 'Biochemistry and molecular biology of amyloid beta-protein and the mechanism of Alzheimer's disease', *Handb Clin Neurol*, 89, pp. 245-60.
- Seshadri, S. (2006) 'Elevated plasma homocysteine levels: risk factor or risk marker for the development of dementia and Alzheimer's disease?', *J Alzheimers Dis*, 9(4), pp. 393-8.
- Seshadri, S. and Wolf, P.A. (2007) 'Lifetime risk of stroke and dementia: current concepts, and estimates from the Framingham Study', *Lancet Neurol*, 6(12), pp. 1106-14.
- Seux, M.L., Thijs, L., Forette, F., Staessen, J.A., Birkenhager, W.H., Bulpitt, C.J., Girerd, X., Jaaskivi, M., Vanhanen, H., Kivinen, P., Yodfat, Y., Vanska, O., Antikainen, R., Laks, T., Webster, J.R., Hakamaki, T., Lehtomaki, E., Lilov, E., Grigorov, M., Janculova, K., Halonen, K., Kohonen-Jalonen, P., Kermowa, R., Nachev, C. and Tuomilehto, J. (1998) 'Correlates of cognitive status of old patients with isolated systolic hypertension: the Syst-Eur Vascular Dementia Project', *J Hypertens*, 16(7), pp. 963-9.
- Shapiro, J. (2010) 'The NHS: the story so far (1948-2010)', *Clin Med*, 10(4), pp. 336-8.
- Sharp, E.S. and Gatz, M. (2011) 'Relationship between education and dementia: an updated systematic review', *Alzheimer Dis Assoc Disord*, 25(4), pp. 289-304.
- Sharp, S.I., Aarsland, D., Day, S., Sonnesyn, H. and Ballard, C. (2011) 'Hypertension is a potential risk factor for vascular dementia: systematic review', *Int J Geriatr Psychiatry*.
- Sheng, M. and Hoogenraad, C.C. (2007) 'The postsynaptic architecture of excitatory synapses: a more quantitative view', *Annu Rev Biochem*, 76, pp. 823-47.
- Shepherd, J., Blauw, G.J., Murphy, M.B., Bollen, E.L., Buckley, B.M., Cobbe, S.M., Ford, I., Gaw, A., Hyland, M., Jukema, J.W., Kamper, A.M., Macfarlane, P.W., Meinders, A.E., Norrie, J., Packard, C.J., Perry, I.J., Stott, D.J., Sweeney, B.J., Twomey, C. and Westendorp, R.G. (2002) 'Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial', *Lancet*, 360(9346), pp. 1623-30.
- Shibata, M., Ohtani, R., Ihara, M. and Tomimoto, H. (2004) 'White matter lesions and glial activation in a novel mouse model of chronic cerebral hypoperfusion', *Stroke*, 35(11), pp. 2598-603.
- Shibata, M., Yamada, S., Kumar, S.R., Calero, M., Bading, J., Frangione, B., Holtzman, D.M., Miller, C.A., Strickland, D.K., Ghiso, J. and Zlokovic, B.V. (2000) 'Clearance of Alzheimer's amyloid-ss(1-40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier', *J Clin Invest*, 106(12), pp. 1489-99.



- Shibata, M., Yamasaki, N., Miyakawa, T., Kalaria, R.N., Fujita, Y., Ohtani, R., Ihara, M., Takahashi, R. and Tomimoto, H. (2007) 'Selective impairment of working memory in a mouse model of chronic cerebral hypoperfusion', *Stroke*, 38(10), pp. 2826-32.
- Shimohama, S., Kamiya, S., Taniguchi, T., Akagawa, K. and Kimura, J. (1997) 'Differential involvement of synaptic vesicle and presynaptic plasma membrane proteins in Alzheimer's disease', *Biochem Biophys Res Commun*, 236(2), pp. 239-42.
- Simpson, J.E., Fernando, M.S., Clark, L., Ince, P.G., Matthews, F., Forster, G., O'Brien, J.T., Barber, R., Kalaria, R.N., Brayne, C., Shaw, P.J., Lewis, C.E. and Wharton, S.B. (2007a) 'White matter lesions in an unselected cohort of the elderly: astrocytic, microglial and oligodendrocyte precursor cell responses', *Neuropathol Appl Neurobiol*, 33(4), pp. 410-9.
- Simpson, J.E., Hosny, O., Wharton, S.B., Heath, P.R., Holden, H., Fernando, M.S., Matthews, F., Forster, G., O'Brien, J.T., Barber, R., Kalaria, R.N., Brayne, C., Shaw, P.J., Lewis, C.E. and Ince, P.G. (2009) 'Microarray RNA expression analysis of cerebral white matter lesions reveals changes in multiple functional pathways', *Stroke*, 40(2), pp. 369-75.
- Simpson, J.E., Ince, P.G., Higham, C.E., Gelsthorpe, C.H., Fernando, M.S., Matthews, F., Forster, G., O'Brien, J.T., Barber, R., Kalaria, R.N., Brayne, C., Shaw, P.J., Stoeber, K., Williams, G.H., Lewis, C.E. and Wharton, S.B. (2007b) 'Microglial activation in white matter lesions and nonlesional white matter of ageing brains', *Neuropathol Appl Neurobiol*, 33(6), pp. 670-83.
- Singh, S., Metz, I., Amor, S., van der Valk, P., Stadelmann, C. and Bruck, W. (2013) 'Microglial nodules in early multiple sclerosis white matter are associated with degenerating axons', *Acta Neuropathol*, 125(4), pp. 595-608.
- Sjoberck, M. and Englund, E. (2003) 'Glial levels determine severity of white matter disease in Alzheimer's disease: a neuropathological study of glial changes', *Neuropathol Appl Neurobiol*, 29(2), pp. 159-69.
- Sjoberck, M., Haglund, M. and Englund, E. (2005) 'Decreasing myelin density reflected increasing white matter pathology in Alzheimer's disease--a neuropathological study', *Int J Geriatr Psychiatry*, 20(10), pp. 919-26.
- Skoog, I. (2000) 'Vascular aspects in Alzheimer's disease', *J Neural Transm Suppl*, 59, pp. 37-43.
- Skoog, I., Lernfelt, B., Landahl, S., Palmertz, B., Andreasson, L.A., Nilsson, L., Persson, G., Oden, A. and Svanborg, A. (1996) '15-year longitudinal study of blood pressure and dementia', *Lancet*, 347(9009), pp. 1141-5.
- Sloane, J.A., Hollander, W., Moss, M.B., Rosene, D.L. and Abraham, C.R. (1999) 'Increased microglial activation and protein nitration in white matter of the aging monkey', *Neurobiol Aging*, 20(4), pp. 395-405.
- Small, D.H. (1998) 'The Sixth International Conference on Alzheimer's disease, Amsterdam, The Netherlands, July 1998. The amyloid cascade hypothesis debate: emerging consensus on the role of A beta and amyloid in Alzheimer's disease', *Amyloid*, 5(4), pp. 301-4.
- Small, S.A. (2011) 'Neurobiological correlates of imaging', *Network*, 22(1-4), pp. 162-72.
- Small, S.A., Schobel, S.A., Buxton, R.B., Witter, M.P. and Barnes, C.A. (2011) 'A pathophysiological framework of hippocampal dysfunction in ageing and disease', *Nat Rev Neurosci*, 12(10), pp. 585-601.
- Smallwood, A., Oulhaj, A., Joachim, C., Christie, S., Sloan, C., Smith, A.D. and Esiri, M. (2012) 'Cerebral subcortical small vessel disease and its relation to cognition in elderly subjects: a pathological study in the Oxford Project to Investigate Memory and Ageing (OPTIMA) cohort', *Neuropathol Appl Neurobiol*, 38(4), pp. 337-43.
- Snowdon, D.A., Greiner, L.H., Mortimer, J.A., Riley, K.P., Greiner, P.A. and Markesbery, W.R. (1997) 'Brain infarction and the clinical expression of Alzheimer disease. The Nun Study', *JAMA*, 277(10), pp. 813-7.
- Sofi, F., Cesari, F., Abbate, R., Gensini, G.F. and Casini, A. (2008) 'Adherence to Mediterranean diet and health status: meta-analysis', *BMJ*, 337, p. a1344.

- Soininen, H.S. and Scheltens, P. (1998) 'Early diagnostic indices for the prevention of Alzheimer's disease', *Ann Med*, 30(6), pp. 553-9.
- Solomon, A., Kivipelto, M., Wolozin, B., Zhou, J. and Whitmer, R.A. (2009) 'Midlife serum cholesterol and increased risk of Alzheimer's and vascular dementia three decades later', *Dement Geriatr Cogn Disord*, 28(1), pp. 75-80.
- Sommer, W. (1880) 'Erkrankung des Ammon's horn als aetiologis ches moment der epilepsien', *Arch Psychiatr Nurs* 10, pp. 631 - 675.
- Squire, L.R., Stark, C.E. and Clark, R.E. (2004) 'The medial temporal lobe', *Annu Rev Neurosci*, 27, pp. 279-306.
- Srikanth, V.K., Anderson, J.F., Donnan, G.A., Saling, M.M., Didus, E., Alpitsis, R., Dewey, H.M., Macdonell, R.A. and Thrift, A.G. (2004) 'Progressive dementia after first-ever stroke: a community-based follow-up study', *Neurology*, 63(5), pp. 785-92.
- Staff, R.T., Murray, A.D., Deary, I.J. and Whalley, L.J. (2004) 'What provides cerebral reserve?', *Brain*, 127(Pt 5), pp. 1191-9.
- Starr, J.M., Whalley, L.J., Inch, S. and Shering, P.A. (1993) 'Blood pressure and cognitive function in healthy old people', *J Am Geriatr Soc*, 41(7), pp. 753-6.
- Stephens, S., Kenny, R.A., Rowan, E., Allan, L., Kalaria, R.N., Bradbury, M. and Ballard, C.G. (2004) 'Neuropsychological characteristics of mild vascular cognitive impairment and dementia after stroke', *Int J Geriatr Psychiatry*, 19(11), pp. 1053-7.
- Stern, R.A., Silva, S.G., Chaisson, N. and Evans, D.L. (1996) 'Influence of cognitive reserve on neuropsychological functioning in asymptomatic human immunodeficiency virus-1 infection', *Arch Neurol*, 53(2), pp. 148-53.
- Stern, Y. (2002) 'What is cognitive reserve? Theory and research application of the reserve concept', *J Int Neuropsychol Soc*, 8(3), pp. 448-60.
- Stern, Y. (2009) 'Cognitive reserve', *Neuropsychologia*, 47(10), pp. 2015-28.
- Stern, Y. (2012) 'Cognitive reserve in ageing and Alzheimer's disease', *Lancet Neurol*, 11(11), pp. 1006-12.
- Stern, Y. (2013) 'Cognitive Reserve: Implications for Assessment and Intervention', *Folia Phoniatr Logop*, 65(2), pp. 49-54.
- Stern, Y., Albert, S., Tang, M.X. and Tsai, W.Y. (1999) 'Rate of memory decline in AD is related to education and occupation: cognitive reserve?', *Neurology*, 53(9), pp. 1942-7.
- Strong, K., Mathers, C. and Bonita, R. (2007) 'Preventing stroke: saving lives around the world', *Lancet Neurol*, 6(2), pp. 182-7.
- Sudduth, T.L., Schmitt, F.A., Nelson, P.T. and Wilcock, D.M. (2013) 'Neuroinflammatory phenotype in early Alzheimer's disease', *Neurobiol Aging*, 34(4), pp. 1051-9.
- Sudhof, T.C. (2004) 'The synaptic vesicle cycle', *Annu Rev Neurosci*, 27, pp. 509-47.
- Sultana, R., Banks, W.A. and Butterfield, D.A. (2010) 'Decreased levels of PSD95 and two associated proteins and increased levels of BCl2 and caspase 3 in hippocampus from subjects with amnesic mild cognitive impairment: Insights into their potential roles for loss of synapses and memory, accumulation of Abeta, and neurodegeneration in a prodromal stage of Alzheimer's disease', *J Neurosci Res*, 88(3), pp. 469-77.
- Sumowski, J.F., Chiaravalloti, N., Krch, D., Paxton, J. and DeLuca, J. (2013a) 'Education Attenuates the Negative Impact of Traumatic Brain Injury on Cognitive Status', *Arch Phys Med Rehabil*.
- Sumowski, J.F., Rocca, M.A., Leavitt, V.M., Riccitelli, G., Comi, G., DeLuca, J. and Filippi, M. (2013b) 'Brain reserve and cognitive reserve in multiple sclerosis: what you've got and how you use it', *Neurology*, 80(24), pp. 2186-93.
- Suzuki, W.A. and Amaral, D.G. (2004) 'Functional neuroanatomy of the medial temporal lobe memory system', *Cortex*, 40(1), pp. 220-2.
- Swanwick, G.R., Coen, R.F., Lawlor, B.A., O'Mahony, D., Walsh, J.B. and Coakley, D. (1996) 'Utility of ischemic scores in the differential diagnosis of Alzheimer's disease and ischemic vascular dementia', *Int Psychogeriatr*, 8(3), pp. 413-24.
- Sze, C.I., Bi, H., Kleinschmidt-DeMasters, B.K., Filley, C.M. and Martin, L.J. (2000) 'Selective regional loss of exocytotic presynaptic vesicle proteins in Alzheimer's disease brains', *J Neurol Sci*, 175(2), pp. 81-90.

- Sze, C.I., Troncoso, J.C., Kawas, C., Mouton, P., Price, D.L. and Martin, L.J. (1997) 'Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with cognitive decline in Alzheimer disease', *J Neuropathol Exp Neurol*, 56(8), pp. 933-44.
- Takahashi, W., Takagi, S., Ide, M., Shohtsu, A. and Shinohara, Y. (2000) 'Reduced cerebral glucose metabolism in subjects with incidental hyperintensities on magnetic resonance imaging', *J Neurol Sci*, 176(1), pp. 21-7.
- Takata, Y., Zhang, X., Li, H., Gao, Y.T., Yang, G., Gao, J., Cai, H., Xiang, Y.B., Zheng, W. and Shu, X.O. (2013) 'Fish intake and risks of total and cause-specific mortality in 2 population-based cohort studies of 134,296 men and women', *Am J Epidemiol*, 178(1), pp. 46-57.
- Takeda, S., Sato, N., Takeuchi, D., Kurinami, H., Shinohara, M., Niisato, K., Kano, M., Ogihara, T., Rakugi, H. and Morishita, R. (2009) 'Angiotensin receptor blocker prevented beta-amyloid-induced cognitive impairment associated with recovery of neurovascular coupling', *Hypertension*, 54(6), pp. 1345-52.
- Tan, Z.S., Harris, W.S., Beiser, A.S., Au, R., Himali, J.J., Debette, S., Pikula, A., Decarli, C., Wolf, P.A., Vasan, R.S., Robins, S.J. and Seshadri, S. (2012) 'Red blood cell omega-3 fatty acid levels and markers of accelerated brain aging', *Neurology*, 78(9), pp. 658-64.
- Tan, Z.S., Seshadri, S., Beiser, A., Wilson, P.W., Kiel, D.P., Tocco, M., D'Agostino, R.B. and Wolf, P.A. (2003) 'Plasma total cholesterol level as a risk factor for Alzheimer disease: the Framingham Study', *Arch Intern Med*, 163(9), pp. 1053-7.
- Tang, W.K., Chan, S.S., Chiu, H.F., Ungvari, G.S., Wong, K.S., Kwok, T.C., Mok, V., Wong, K.T., Richards, P.S. and Ahuja, A.T. (2004) 'Frequency and determinants of poststroke dementia in Chinese', *Stroke*, 35(4), pp. 930-5.
- Tang, W.K., Chan, S.S., Chiu, H.F., Ungvari, G.S., Wong, K.S., Kwok, T.C., Mok, V., Wong, K.T., Richards, P.S. and Ahuja, A.T. (2006) 'Frequency and clinical determinants of poststroke cognitive impairment in nondemented stroke patients', *J Geriatr Psychiatry Neurol*, 19(2), pp. 65-71.
- Tatemichi, T.K., Desmond, D.W., Stern, Y., Paik, M., Sano, M. and Bagiella, E. (1994) 'Cognitive impairment after stroke: frequency, patterns, and relationship to functional abilities', *J Neurol Neurosurg Psychiatry*, 57(2), pp. 202-7.
- Tatemichi, T.K., Foulkes, M.A., Mohr, J.P., Hewitt, J.R., Hier, D.B., Price, T.R. and Wolf, P.A. (1990) 'Dementia in stroke survivors in the Stroke Data Bank cohort. Prevalence, incidence, risk factors, and computed tomographic findings', *Stroke*, 21(6), pp. 858-66.
- Tay, S.Y., Ampil, E.R., Chen, C.P. and Auchus, A.P. (2006) 'The relationship between homocysteine, cognition and stroke subtypes in acute stroke', *J Neurol Sci*, 250(1-2), pp. 58-61.
- Thabane, L., Ma, J., Chu, R., Cheng, J., Ismaila, A., Rios, L.P., Robson, R., Thabane, M., Giangregorio, L. and Goldsmith, C.H. (2010) 'A tutorial on pilot studies: the what, why and how', *BMC Med Res Methodol*, 10, p. 1.
- Thal, D.R., Capetillo-Zarate, E., Del Tredici, K. and Braak, H. (2006) 'The development of amyloid beta protein deposits in the aged brain', *Sci Aging Knowledge Environ*, 2006(6), p. re1.
- Thal, D.R., Ghebremedhin, E., Rub, U., Yamaguchi, H., Del Tredici, K. and Braak, H. (2002a) 'Two types of sporadic cerebral amyloid angiopathy', *J Neuropathol Exp Neurol*, 61(3), pp. 282-93.
- Thal, D.R., Holzer, M., Rub, U., Waldmann, G., Gunzel, S., Zedlick, D. and Schober, R. (2000) 'Alzheimer-related tau-pathology in the perforant path target zone and in the hippocampal stratum oriens and radiatum correlates with onset and degree of dementia', *Exp Neurol*, 163(1), pp. 98-110.
- Thal, D.R., Rub, U., Orantes, M. and Braak, H. (2002b) 'Phases of A beta-deposition in the human brain and its relevance for the development of AD', *Neurology*, 58(12), pp. 1791-800.
- Tham, W., Auchus, A.P., Thong, M., Goh, M.L., Chang, H.M., Wong, M.C. and Chen, C.P. (2002) 'Progression of cognitive impairment after stroke: one year results from a longitudinal study of Singaporean stroke patients', *J Neurol Sci*, 203-204, pp. 49-52.

- Tomlinson, B.E., Blessed, G. and Roth, M. (1968) 'Observations on the brains of non-demented old people', *J Neurol Sci*, 7(2), pp. 331-56.
- Tomlinson, B.E., Blessed, G. and Roth, M. (1970) 'Observations on the brains of demented old people', *J Neurol Sci*, 11(3), pp. 205-42.
- Tong, L., Shen, H., Perreau, V.M., Balazs, R. and Cotman, C.W. (2001) 'Effects of exercise on gene-expression profile in the rat hippocampus', *Neurobiol Dis*, 8(6), pp. 1046-56.
- Tremblay, M.E. (2011) 'The role of microglia at synapses in the healthy CNS: novel insights from recent imaging studies', *Neuron Glia Biol*, 7(1), pp. 67-76.
- Tremblay, M.E. and Majewska, A.K. (2011) 'A role for microglia in synaptic plasticity?', *Commun Integr Biol*, 4(2), pp. 220-2.
- Truelsen, T., Heuschmann, P.U., Bonita, R., Arjundas, G., Dalal, P., Damasceno, A., Nagaraja, D., Ogunniyi, A., Oveisgharan, S., Radhakrishnan, K., Skvortsova, V.I. and Stakhovskaya, V. (2007) 'Standard method for developing stroke registers in low-income and middle-income countries: experiences from a feasibility study of a stepwise approach to stroke surveillance (STEPS Stroke)', *Lancet Neurol*, 6(2), pp. 134-9.
- Tyas, S.L., White, L.R., Petrovitch, H., Webster Ross, G., Foley, D.J., Heimovitz, H.K. and Launer, L.J. (2003) 'Mid-life smoking and late-life dementia: the Honolulu-Asia Aging Study', *Neurobiol Aging*, 24(4), pp. 589-96.
- Unverzagt, F.W., Morgan, O.S., Thesiger, C.H., Eldemire, D.A., Luseko, J., Pokuri, S., Hui, S.L., Hall, K.S. and Hendrie, H.C. (1999) 'Clinical utility of CERAD neuropsychological battery in elderly Jamaicans', *J Int Neuropsychol Soc*, 5(3), pp. 255-9.
- Valenzuela, M., Brayne, C., Sachdev, P., Wilcock, G. and Matthews, F. (2011) 'Cognitive lifestyle and long-term risk of dementia and survival after diagnosis in a multicenter population-based cohort', *Am J Epidemiol*, 173(9), pp. 1004-12.
- Valenzuela, M.J., Matthews, F.E., Brayne, C., Ince, P., Halliday, G., Kril, J.J., Dalton, M.A., Richardson, K., Forster, G. and Sachdev, P.S. (2012) 'Multiple biological pathways link cognitive lifestyle to protection from dementia', *Biol Psychiatry*, 71(9), pp. 783-91.
- Valenzuela, M.J. and Sachdev, P. (2006) 'Brain reserve and cognitive decline: a non-parametric systematic review', *Psychol Med*, 36(8), pp. 1065-73.
- van der Hel, W.S., Verlinde, S.A., Meijer, D.H., de Wit, M., Rensen, M.G., van Gassen, K.L., van Rijen, P.C., van Veelen, C.W. and de Graan, P.N. (2009) 'Hippocampal distribution of vesicular glutamate transporter 1 in patients with temporal lobe epilepsy', *Epilepsia*, 50(7), pp. 1717-28.
- van Gelder, B.M., Tijhuis, M., Kalmijn, S. and Kromhout, D. (2007) 'Fish consumption, n-3 fatty acids, and subsequent 5-y cognitive decline in elderly men: the Zutphen Elderly Study', *Am J Clin Nutr*, 85(4), pp. 1142-7.
- van Gelder, B.M., Tijhuis, M.A., Kalmijn, S., Giampaoli, S., Nissinen, A. and Kromhout, D. (2004) 'Physical activity in relation to cognitive decline in elderly men: the FINE Study', *Neurology*, 63(12), pp. 2316-21.
- van Oijen, M., de Jong, F.J., Wittman, J.C., Hofman, A., Koudstaal, P.J. and Breteler, M.M. (2007) 'Atherosclerosis and risk for dementia', *Ann Neurol*, 61(5), pp. 403-10.
- van Spronsen, M. and Hoogenraad, C.C. (2010) 'Synapse pathology in psychiatric and neurologic disease', *Curr Neurol Neurosci Rep*, 10(3), pp. 207-14.
- Velliquette, R.A., O'Connor, T. and Vassar, R. (2005) 'Energy inhibition elevates beta-secretase levels and activity and is potentially amyloidogenic in APP transgenic mice: possible early events in Alzheimer's disease pathogenesis', *J Neurosci*, 25(47), pp. 10874-83.
- Verghese, J., Lipton, R.B., Hall, C.B., Kuslansky, G. and Katz, M.J. (2003) 'Low blood pressure and the risk of dementia in very old individuals', *Neurology*, 61(12), pp. 1667-72.
- Vernooij, M.W., Ikram, M.A., Vrooman, H.A., Wielopolski, P.A., Krestin, G.P., Hofman, A., Niessen, W.J., Van der Lugt, A. and Breteler, M.M. (2009) 'White matter microstructural integrity and cognitive function in a general elderly population', *Arch Gen Psychiatry*, 66(5), pp. 545-53.

- Vinters, H.V., Ellis, W.G., Zarow, C., Zaias, B.W., Jagust, W.J., Mack, W.J. and Chui, H.C. (2000) 'Neuropathologic substrates of ischemic vascular dementia', *J Neuropathol Exp Neurol*, 59(11), pp. 931-45.
- Virtanen, J.K., Siscovick, D.S., Lemaitre, R.N., Longstreth, W.T., Spiegelman, D., Rimm, E.B., King, I.B. and Mozaffarian, D. (2013) 'Circulating omega-3 polyunsaturated fatty acids and subclinical brain abnormalities on MRI in older adults: the cardiovascular health study', *J Am Heart Assoc*, 2(5), p. e000305.
- Viswanathan, A., Rocca, W.A. and Tzourio, C. (2009) 'Vascular risk factors and dementia: how to move forward?', *Neurology*, 72(4), pp. 368-74.
- Wahlund, L.O., Julin, P., Johansson, S.E. and Scheltens, P. (2000) 'Visual rating and volumetry of the medial temporal lobe on magnetic resonance imaging in dementia: a comparative study', *J Neurol Neurosurg Psychiatry*, 69(5), pp. 630-5.
- Wakita, H., Tomimoto, H., Akiguchi, I., Matsuo, A., Lin, J.X., Ihara, M. and McGeer, P.L. (2002) 'Axonal damage and demyelination in the white matter after chronic cerebral hypoperfusion in the rat', *Brain Res*, 924(1), pp. 63-70.
- Waldstein, S.R., Lefkowitz, D.M., Siegel, E.L., Rosenberger, W.F., Spencer, R.J., Tankard, C.F., Manukyan, Z., Gerber, E.J. and Katzel, L. (2010) 'Reduced cerebral blood flow in older men with higher levels of blood pressure', *J Hypertens*, 28(5), pp. 993-8.
- Walker, R., Whiting, D., Unwin, N., Mugusi, F., Swai, M., Aris, E., Jusabani, A., Kabadi, G., Gray, W.K., Lewanga, M. and Alberti, G. (2010) 'Stroke incidence in rural and urban Tanzania: a prospective, community-based study', *Lancet Neurol*, 9(8), pp. 786-92.
- Wang, L.Y., Larson, E.B., Sonnen, J.A., Shofer, J.B., McCormick, W., Bowen, J.D., Montine, T.J. and Li, G. (2009) 'Blood pressure and brain injury in older adults: findings from a community-based autopsy study', *J Am Geriatr Soc*, 57(11), pp. 1975-81.
- Wang, P.L., Zhao, X.Q., Du, W.L., Wang, A.X., Ji, R.J., Yang, Z.H., Wang, C.X., Liu, L.P., Wang, Y.L., Ju, Y. and Wang, Y.J. (2013) 'In-hospital medical complications associated with patient dependency after acute ischemic stroke: data from the China National Stroke Registry', *Chin Med J (Engl)*, 126(7), pp. 1236-41.
- Wang, T.J., Massaro, J.M., Levy, D., Vasani, R.S., Wolf, P.A., D'Agostino, R.B., Larson, M.G., Kannel, W.B. and Benjamin, E.J. (2003) 'A risk score for predicting stroke or death in individuals with new-onset atrial fibrillation in the community: the Framingham Heart Study', *Jama*, 290(8), pp. 1049-56.
- Wen, H.M., Mok, V.C., Fan, Y.H., Lam, W.W., Tang, W.K., Wong, A., Huang, R.X. and Wong, K.S. (2004) 'Effect of white matter changes on cognitive impairment in patients with lacunar infarcts', *Stroke*, 35(8), pp. 1826-30.
- Wesnes, K. (2002) 'Assessing cognitive function in clinical trials: latest developments and future directions', *Drug Discov Today*, 7(1), pp. 29-35.
- Wetterling, T., Kanitz, R.D. and Borgis, K.J. (1996) 'Comparison of different diagnostic criteria for vascular dementia (ADDTC, DSM-IV, ICD-10, NINDS-AIREN)', *Stroke*, 27(1), pp. 30-6.
- White, L., Petrovitch, H., Hardman, J., Nelson, J., Davis, D.G., Ross, G.W., Masaki, K., Launer, L. and Markesbery, W.R. (2002) 'Cerebrovascular pathology and dementia in autopsied Honolulu-Asia Aging Study participants', *Ann N Y Acad Sci*, 977, pp. 9-23.
- Whitehead, S.N., Cheng, G., Hachinski, V.C. and Cechetto, D.F. (2007) 'Progressive increase in infarct size, neuroinflammation, and cognitive deficits in the presence of high levels of amyloid', *Stroke*, 38(12), pp. 3245-50.
- Whitehead, S.N., Hachinski, V.C. and Cechetto, D.F. (2005) 'Interaction between a rat model of cerebral ischemia and beta-amyloid toxicity: inflammatory responses', *Stroke*, 36(1), pp. 107-12.
- Whitmer, R.A., Gunderson, E.P., Barrett-Connor, E., Quesenberry, C.P., Jr. and Yaffe, K. (2005) 'Obesity in middle age and future risk of dementia: a 27 year longitudinal population based study', *BMJ*, 330(7504), p. 1360.
- Whitmer, R.A., Gunderson, E.P., Quesenberry, C.P., Jr., Zhou, J. and Yaffe, K. (2007) 'Body mass index in midlife and risk of Alzheimer disease and vascular dementia', *Curr Alzheimer Res*, 4(2), pp. 103-9.

- Wilkinson, P.R., Wolfe, C.D., Warburton, F.G., Rudd, A.G., Howard, R.S., Ross-Russell, R.W. and Beech, R.R. (1997) 'A long-term follow-up of stroke patients', *Stroke*, 28(3), pp. 507-12.
- Wilson, R.S., Boyle, P.A., Yu, L., Barnes, L.L., Schneider, J.A. and Bennett, D.A. (2013a) 'Life-span cognitive activity, neuropathologic burden, and cognitive aging', *Neurology*, 81(4), pp. 314-21.
- Wilson, R.S., Nag, S., Boyle, P.A., Hizek, L.P., Yu, L., Buchman, A.S., Schneider, J.A. and Bennett, D.A. (2013b) 'Neural reserve, neuronal density in the locus ceruleus, and cognitive decline', *Neurology*, 80(13), pp. 1202-8.
- Wong, A., Xiong, Y.Y., Wang, D., Lin, S., Chu, W.W., Kwan, P.W., Nyenhuis, D., Black, S.E., Wong, K.S. and Mok, V. (2013) 'The NINDS-Canadian stroke network vascular cognitive impairment neuropsychology protocols in Chinese', *J Neurol Neurosurg Psychiatry*, 84(5), pp. 499-504.
- Woods, B., Aguirre, E., Spector, A.E. and Orrell, M. (2012) 'Cognitive stimulation to improve cognitive functioning in people with dementia', *Cochrane Database Syst Rev*, 2, p. Cd005562.
- World Health Organization (1993) *The ICD-10 Classification of Mental and Behavioural Disorders*.
- World Health Organization (2010) 'WHO Guidelines Approved by the Guidelines Review Committee', in *mhGAP Intervention Guide for Mental, Neurological and Substance Use Disorders in Non-Specialized Health Settings: Mental Health Gap Action Programme (mhGAP)*. Geneva: World Health Organization
- World Health Organization.
- World Health Organization, M.P.P.I. (1988) 'The World Health Organization MONICA Project (Monitoring Trends and Determinants in Cardiovascular Disease): a major international collaboration', *J Clin Epidemiol*, 41, pp. 105-141.
- Yamada, M., Ihara, M., Okamoto, Y., Maki, T., Washida, K., Kitamura, A., Hase, Y., Ito, H., Takao, K., Miyakawa, T., Kalaria, R.N., Tomimoto, H. and Takahashi, R. (2011) 'The influence of chronic cerebral hypoperfusion on cognitive function and amyloid beta metabolism in APP overexpressing mice', *PLoS One*, 6(1), p. e16567.
- Yamaji, S., Ishii, K., Sasaki, M., Imamura, T., Kitagaki, H., Sakamoto, S. and Mori, E. (1997) 'Changes in cerebral blood flow and oxygen metabolism related to magnetic resonance imaging white matter hyperintensities in Alzheimer's disease', *J Nucl Med*, 38(9), pp. 1471-4.
- Yamamoto, Y., Ihara, M., Tham, C., Low, R.W., Slade, J.Y., Moss, T., Oakley, A.E., Polvikoski, T. and Kalaria, R.N. (2009) 'Neuropathological correlates of temporal pole white matter hyperintensities in CADASIL', *Stroke*, 40(6), pp. 2004-11.
- Young, V.G., Halliday, G.M. and Kril, J.J. (2008) 'Neuropathologic correlates of white matter hyperintensities', *Neurology*, 71(11), pp. 804-11.
- Yu, K.H., Cho, S.J., Oh, M.S., Jung, S., Lee, J.H., Shin, J.H., Koh, I.S., Cha, J.K., Park, J.M., Bae, H.J., Kang, Y. and Lee, B.C. (2013) 'Cognitive impairment evaluated with Vascular Cognitive Impairment Harmonization Standards in a multicenter prospective stroke cohort in Korea', *Stroke*, 44(3), pp. 786-8.
- Yuste, R. and Bonhoeffer, T. (2001) 'Morphological changes in dendritic spines associated with long-term synaptic plasticity', *Annu Rev Neurosci*, 24, pp. 1071-89.
- Yusuf, S., Reddy, S., Ounpuu, S. and Anand, S. (2001a) 'Global burden of cardiovascular diseases: part I: general considerations, the epidemiologic transition, risk factors, and impact of urbanization', *Circulation*, 104(22), pp. 2746-53.
- Yusuf, S., Reddy, S., Ounpuu, S. and Anand, S. (2001b) 'Global burden of cardiovascular diseases: Part II: variations in cardiovascular disease by specific ethnic groups and geographic regions and prevention strategies', *Circulation*, 104(23), pp. 2855-64.
- Zandi, P.P., Anthony, J.C., Khachaturian, A.S., Stone, S.V., Gustafson, D., Tschanz, J.T., Norton, M.C., Welsh-Bohmer, K.A. and Breitner, J.C. (2004) 'Reduced risk of Alzheimer disease in users of antioxidant vitamin supplements: the Cache County Study', *Arch Neurol*, 61(1), pp. 82-8.

- Zhan, S.S., Beyreuther, K. and Schmitt, H.P. (1993a) 'Quantitative assessment of the synaptophysin immuno-reactivity of the cortical neuropil in various neurodegenerative disorders with dementia', *Dementia*, 4(2), pp. 66-74.
- Zhan, S.S., Beyreuther, K. and Schmitt, H.P. (1993b) 'Vascular dementia in Spatz-Lindenberg's disease (SLD): cortical synaptophysin immunoreactivity as compared with dementia of Alzheimer type and non-demented controls', *Acta Neuropathol*, 86(3), pp. 259-64.
- Zhan, S.S., Beyreuther, K. and Schmitt, H.P. (1994) 'Synaptophysin immunoreactivity of the cortical neuropil in vascular dementia of Binswanger type compared with the dementia of Alzheimer type and nondemented controls', *Dementia*, 5(2), pp. 79-87.
- Zhang, X., Zhou, K., Wang, R., Cui, J., Lipton, S.A., Liao, F.F., Xu, H. and Zhang, Y.W. (2007) 'Hypoxia-inducible factor 1alpha (HIF-1alpha)-mediated hypoxia increases BACE1 expression and beta-amyloid generation', *J Biol Chem*, 282(15), pp. 10873-80.
- Zhao, X., Lein, E.S., He, A., Smith, S.C., Aston, C. and Gage, F.H. (2001) 'Transcriptional profiling reveals strict boundaries between hippocampal subregions', *J Comp Neurol*, 441(3), pp. 187-96.
- Zhou, D.H., Wang, J.Y., Li, J., Deng, J., Gao, C. and Chen, M. (2005) 'Frequency and risk factors of vascular cognitive impairment three months after ischemic stroke in china: the Chongqing stroke study', *Neuroepidemiology*, 24(1-2), pp. 87-95.
- Zieren, N., Duering, M., Peters, N., Reyes, S., Jouvent, E., Herve, D., Gschwendtner, A., Mewald, Y., Opherk, C., Chabriat, H. and Dichgans, M. (2013) 'Education modifies the relation of vascular pathology to cognitive function: cognitive reserve in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy', *Neurobiol Aging*, 34(2), pp. 400-7.
- Zotova, E., Holmes, C., Johnston, D., Neal, J.W., Nicoll, J.A. and Boche, D. (2011) 'Microglial alterations in human Alzheimer's disease following Abeta42 immunization', *Neuropathol Appl Neurobiol*, 37(5), pp. 513-24.
- Zuccala, G., Onder, G., Marzetti, E., Monaco, M.R., Cesari, M., Cocchi, A., Carbonin, P. and Bernabei, R. (2005) 'Use of angiotensin-converting enzyme inhibitors and variations in cognitive performance among patients with heart failure', *Eur Heart J*, 26(3), pp. 226-33.
- Zylberstein, D.E., Lissner, L., Bjorkelund, C., Mehlig, K., Thelle, D.S., Gustafson, D., Ostling, S., Waern, M., Guo, X. and Skoog, I. (2009) 'Midlife homocysteine and late-life dementia in women. A prospective population study', *Neurobiol Aging*.

# Appendix