EXPLORATORY LONGITUDINAL STUDIES ON CEREBRAL SMALL VESSEL DISEASE (CSVD) IN APPARENTLY ASYMPTOMATIC INDIVIDUALS WITH NEUROPSYCHOLOGICAL, NEUROIMAGING AND MICROPARTICLES PROFILING

BY

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KAJIAN PENEROKAAN TERHADAP PENYAKIT PEMBULUH DARAH KECIL BAGI INDIVIDU ASIMPTOMATIK MELALUI PROFIL NEUROPSIKOLOGI, NEUROPENGIMEJAN, DAN MIKROPARTIKEL.

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Tesis diserahkan bagi memenuhi sebahagian keperluan bagi ijazah

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Dedication

This work is dedicated to:

My mom, Kamariah, who brought me the world of love

My dad, Che Mohd Nassir, who brought me the world of words

My siblings, Nasrul, Nazlin, Nazdlia, Nasran and Nazatul, for bringing me the world

My nieces, nephews and other family members for bringing me joy

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LIST OF SYMBOLS AND ABBREVIATIONS

°C	degree Celsius
ACD	Citric Acid-Dextrose
AD	Axial Diffusivity
ADC	Apparent Diffusion Coefficients
AFM	Atomic Force Microscopy
ANOVA	Analysis of Variance
APC	Allophycocyanin
AR	Arithmetic
ATP	Adenosine Triphosphate
B_o	External Magnetic Field
B_1	Electromagnetic wave
BB	Annexin-V Binding Buffer
BD	Block Design
BET	Brain Extraction Tool
BOLD	Blood Oxygenated Level Dependent
CA	Cancellation
Ca^{2+}	Calcium
CC	Corpus Callosum
CD	Cluster Differentiation
COD	Coding
CI	Confidence Interval
CMBs	Cerebral Microbleeds
CO	Comprehension
Conc.	Concentration
COW	Circle of Willis
CSF	Cerebrospinal Fluid
CSVD	Cerebral Small Vessel Disease
CT	Computed Tomography
CTA	Computed Tomography-Angiography
DEC	Directionally Encoded Colour
df	Degree of Freedom

dH_2O	distilled Water
DICOM	Digital Imaging and Communications in Medicine (DICOM) format
DLS	Dynamic Light Scattering
dMRI	Diffusion Magnetic Resonance Imaging
DNA	Deoxyribose-Nucleic Acid
DS	Digit Span
DSC	Dynamic Susceptibility Contrast
DSCL	Deep Subcortical Lesion
DTI	Diffusion Tensor Imaging
DWI	Diffusion Weighted Imaging
DWM	Deep White Matter
ECC	Eddy Current Correction
EDTA	Ethylene Diamine Tetra Acetic Acid
ELISA	Enzyme Linked Immunosorbent Assay
EMP	Endothelial Cells Derived Microparticles
ER	Endoplasmic Reticulum
f^2	Effect size
FA	Fractional Anisotropy
FCM	Flow Cytometry
FDT	FSL Diffusion Toolbox
FITC	Fluorescein Isothiocyanate
FLAIR	Fluid Attenuated Inversion Recovery
fMRI	Functional Magnetic Resonance Imaging
FOV	Field of View
FRGS	Fundamental Research Grant Scheme
FSC	Forward Scatter
FSIQ	Full-Scale IQ
FSL	Functional Magnetic Resonance Imaging of The Brain Software Library
FW	Figure Weights
g	Gravity
GAI	General Ability Index

GLA	Carboxy-Glutamic Acid
H^+	Hydrogen atom
H_2O	Water
HRB	Halstead-Reitan Battery
HTML	Hypertext Mark-Up Language
HUSM	Hospital Universiti Sains Malaysia
ICU	Intensive Care Unit
IN	Information
JEPeM-USM	Jawatankuasa Etika Penyelidikan Manusia Universiti Sains Malaysia
JHU	John Hopkins University
JPEG	Joint Photographic Experts Group
kDa	kilo Dalton
KRK	Klinik Rawatan Keluarga
LACR	Left Anterior Corona Radiata
LDL	Low Density Lipoprotein
LEC	Left External Capsule
LGA	Lesion Growth Algorithm
LECAM	Leukocytes-Endothelial Cell Adhesions Molecules
LMP	Leukocytes Derived Microparticles
LN	Letter-Number Sequencing
LSCR	Left Superior Corona Radiata
LSLF	Left Superior Longitudinal Fasciculus
LST	Lesion Segmentation Tools
LV	Lesion Volume
MA	Mature Adults
MATLAB	Matrix Laboratory
MCA	Middle Cerebral Arteries
MCI	Mild Cognitive Impairment
MD	Mean Diffusivity
MHz	Mega Hertz
ml	Millilitre
mm	Millimetre

MMSE	Mini-Mental State Examination
MPM	Monocytes Microparticles
MPs	Microparticles
MtR	Matrix Reasoning
MR	Magnetic Resonance
MRA	Magnetic Resonance Angiography
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
msec	Milliseconds
NAWM	Normal Appearing White Matter
NIfTI	Neuroimaging Informatics Technology Initiative, (Nifti)
nm	Nanometre
NMP	Neutrophils Microparticles
NMR	Nuclear Magnetic Resonance
NO	Nitric Oxide
NOL	Number of Lesion
NOT	Number of Tracts
OA	Older Adults
PC	Phosphatidylcholine
PCm	Picture Completion
PCT	Perfusion Computed Tomography
PDMP	Platelet Derived Microparticles
PE	Phosphatidylethanolamine
PcE	Phycoerythrin
PerCP	Peridinin Chlorophyll Protein
PET	Positron Emission Tomography
PF	Platelet Free
PFP	Platelet Free Plasma
PLIC	The Posterior Limb of The Internal Capsule
POI	Perceptual Organization Index
PPACK	D-Phenylalanyl-L-Propyl-L-Arginine Chloro-Methyl Ketone
PPP	Platelet Poor Plasma
PRI	Perceptual Reasoning

PS	Phosphatidylserine
Pst	Polystyrene
PSI	Processing Speed Index
PVL	Periventricular Lesion
PVWM	Periventricular White Matter
PWI	Perfusion Weighted Imaging
QRISK2	Cardiovascular Risk Prediction Score
RACR	Right Anterior Corona Radiata
RBC	Red Blood Cells
rCBF	relative Cerebral Blood Flow
rCBV	relative Cerebral Blood Volume
RD	Radial Diffusivity
RF	Radiofrequency
RGB	Red, Green, and Blue
RICM	Reflection Interference Contrast Microscopy
RMP	Red Blood Cells Derived Microparticles
ROCK 1	'Rho-Associated' Coiled-Coil Containing Protein Kinase 1
ROI	Region of Interest
RSCR	Right Superior Corona Radiata
RSLF	Right Superior Longitudinal Fasciculus
SBI	Silent Brain Infarcts
SD	Standard Deviation
Sig.	Significant Different
SI	Similarities
SPM	Statistical Parametric Mapping Version 12
SPSS	Statistical Package for The Social Sciences
SS	Symbol Search
SSC	Side Scatter
STRIVE	Standards for Reporting and Imaging of Small Vessel Disease
SVD	Small Vessel Disease
T	Tesla
T_0	Baseline
T_{12}	12-months (One-year) Follow-Up

T1-relaxation	Spin-Lattice Relaxation
T2-relaxation	Spin-Spin Relaxation
TE	Echo Time
TF	Tissue Factor
TFPI	Tissue factor Pathway Inhibitor
TI	Inversion Time
TL	Tracts Lengths
TNF	Tumour Necrosis Factor
TR	Repetition Time
TV	Tracts Volume
VC	Vocabulary
VCI	Verbal Comprehension Index
VMWare v12	Virtual Machine Workstation
VP	Visual Puzzles
ω_o	Larmor Frequency
WAIS	Wechsler Adult Intelligence Scale
WB	Whole Brain
WBC	White Blood Cells
WISC	Wechsler Intelligence Scale for Children
WMHs	White Matter Hyperintensities
WMI	Working Memory Index
WMS	Wechsler Memory Scale
X^2	Chi-Square
γ	Gyromagnetic Ratio
YA	Young Adults
µl	Microliter
µm	Micro Metre
µM	Micro Molar

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ABSTRAK

Secara lazimnya penyakit pembuluh darah kecil otak (CSVD) yang kelihatan asimptomatik ('senyap') dilihat sebagai hiperintensiti jirim putih (WMHs) pada Pengimejan Resonans Magnetik (MRI). Kajian ini bertujuan untuk meninjau penanda pengganti baru untuk penilaian integriti jirim putih dalam CSVD di kalangan individu yang tidak bersimptom melalui profil neuropsikologi, neuropengimejan dan micropartikel (MPs). Subjek yang direkrut dengan skor ramalan risiko kardiovaskular yang rendah kepada sederhana seperti yang ditakrifkan oleh QRISK2 menjalani imbasan otak MRI, diikuti oleh penilaian neuropsikologi yang mengukur daya pemikiran persepsi (PRI), memori kerja (WMI) dan kelajuan pemprosesan (PSI) menggunakan Weschler Adults Intelligence Scale (WAIS -IV). Sebelum ujian 'flow cytometry' untuk mengira kadar MPs, 18 ml darah (puasa) telah diambil. Prosedur yang sama dijalankan selepas 12 bulan. Pada peringkat awal, 48 orang yang asimptomatik telah direkrut (purata umur: 38.81 ± 10.9); 24 orang dewasa muda, YA (purata umur 29.7 ± 4.33) dan 24 orang dewasa tua, MA (purata umur: 47.92 ± 7.01). Lima belas (29.4%) subjek mempunyai WMHs (WMH^{+}) telah dikesan, lima daripdanya adalah YA, dan 10 adalah MA. Walau bagaimanapun, selepas 12 bulan, hanya 40 (purata umur: 39.35 ± 11.31) dapat menyertai; lapan (20%) subjek tidak kembali menyertai kajian, empat belas (35%) daripada subjek adalah WMH^{+} dan 26

(65%) adalah WMH^-. Umur mempengaruhi kehadiran WMHs, dimana subjek WMH^+ mempunyai risiko CSVD yang lebih tinggi. WMH^+ subjek menunjukkan purata peratusan QRISK2 dan MPs yang lebih tinggi terutamanya MPs platlet (CD41a dan CD62P). Dari segi integriti jirim putih, skor QRISK2 meningkat apabila integriti berkurang (berdasarkan anisotropi fraksional, FA), terutamanya jirim putih seperti superior corona radiata (SCR) dan superior longitudinal fasciculus (SLF). Integrity jirim putih juga berkurang dengan peningkatan umur. Prestasi PRI dan WMI berbeza antara subjek, tetapi PSI secara konsisten lebih rendah dalam kalangan subjek WMH^+. Tidak ada perubahan temporal yang signifikan terhadap prestasi kognitif dan memori dari kedua-dua kumpulan. FA untuk kesemua jirim putih berkolerasi secara positif dengan profil neuropsikologi. Integriti jirim putih seperti SLF kiri dan ACR kiri adalah berkolerasi dengan MPs darah merah (CD235a) ($r= -0.313$, $p=0.030$) dan MPs platlet CD62P ($r=0.289$, $p= 0.047$). Kesimpulannya, walaupun dengan saiz sampel yang terhad dan tempoh susulan yang singkat, kajian ini telah membuktikan hubungan antara ramalan risiko kardio-serebrovaskular oleh QRISK2, prestasi indeks neuropsikologi, integriti jirim putih/WMHs dari MRI dan profil MPs sebagai penanda yang berpotensi untuk CSVD dalam individu-individu yang sihat dan asimtomatik.

EXPLORATORY LONGITUDINAL STUDIES ON CEREBRAL SMALL VESSEL DISEASE (CSVD) IN APPARENTLY ASYMPTOMATIC INDIVIDUALS WITH NEUROPSYCHOLOGICAL, NEUROIMAGING AND MICROPARTICLES PROFILING

ABSTRACT

The prevalence of cerebral small vessel disease (CSVD) for asymptomatic ('silent') manifestation is typically obtained from incidental findings of white matter hyperintensities (WMHs) on magnetic resonance imaging (MRI). This study aimed to explore novel surrogate markers for the assessment of white matter integrity in CSVD among apparently asymptomatic individuals with neuropsychological, neuroimaging and microparticles (MPs) profiling. The recruited subjects with low to moderate cardiovascular risk prediction score as defined by QRISK2 underwent MRI brain scan, followed by neuropsychological indices of Weschler Adults Intelligence Scale (WAIS-IV) that measured their perceptual reasoning (PRI), working memory (WMI) and processing speed (PSI). Prior to flow cytometry MPs subpopulations enumerations, 18 ml of fasting blood was collected. Similar procedures were carried out at baseline (T_0) and at 12 months follow-up (T_{12}). At baseline, 48 asymptomatic individuals were recruited (mean age: 38.81±10.9); 24 were young adults, YA (mean age 29.7±4.33) and 24 mature adults, MA (mean age: 47.92±7.01). Fifteen (29.4%) had WMHs (WMH^+) detected, of who five were YA, and 10 were MA. However, after one-year, 40 (mean age: 39.35±11.31) were able to participate; eight (20%) subjects were lost to follow up fourteen (35%) subjects were WMH^+ and 26 (65%) were WMH^-

. Age influenced the presence of WMHs and WMH^+ subject as having higher risk of CSVD. WMH^+ subjects showed higher mean percentage of QRISK2 and MPs especially platelet derived MPs (CD41a and CD62P). In term of white matter integrity, QRISK2 score increased with reduced integrity (measured by fractional anisotropy, FA) of white matter tracts of superior corona radiata (SCR) and superior longitudinal fasciculus (SLF). White matter integrity was also reduced with advancing age. The PRI and WMI performance varied between subjects, but PSI consistently lower in WMH+ subjects. There were no significant temporal changes on cognitive and memories performances from both groups. FA of all tracts consistently and positively correlated with neuropsychological profiles. The integrity of white matter tracts such as left SLF and left anterior corona radiata were correlated with red blood cell MPs (CD235a) ($r= -0.313$, $p=0.030$) and CD62P ($r=0.289$, $p= 0.047$), respectively. Although with a relatively small sample size and one-year follow-up period, this study had established the correlations between cardio-cerebrovascular risk prediction by QRISK, neuropsychological indices performance, WMHs/white matter integrity from diffusion MRI and MPs profiling as potential surrogate markers for CSVD in apparently asymptomatic individuals.

CHAPTER 1

INTRODUCTION

1.1 Background of The Study

Cerebral small vessel disease (CSVD) is a spectrum of histopathological, clinical and imaging abnormalities linked to the pathology of microvasculature of small penetrating arteries and arterioles in brain irrigating subcortical structures (Lammie, 2005; Pantoni, 2010). In general, it results in a brain parenchyma injury invariably associated with distal leptomeningeal and intracerebral vessel pathology that affects deep cerebral white matter integrity (Ogata *et al.*, 2014). CSVD is characterised by arteriosclerosis and/or micro-atheromatosis of cerebral arteries of small calibre (50-500 μm) caused by various pathologies (Lammie, 2005). Current data suggest that CSVD is one of the most prevalent neurological disorders in the ageing society of the developed world (Hachinski, 2008; Thompson & Hakim, 2009). The prevalence of CSVD's seemingly asymptomatic manifestation such as silent brain infarcts (SBI) or white matter hyperintensities (WMHs) is recognised to be higher with advancing age (Gunda *et al.*, 2012).

Generally, the microvasculature health of the small vessels is maintained by normal functioning endothelial cells that lines the vessel wall. However, the formation of microparticles (MPs) have been reported to contribute to the disorganisation of the

proper function of endothelium layers. For example, Martinez *et al*., 2011, have shown that endothelial dysfunction caused by MPs can induce vascular inflammation that potentially leads to a prothrombotic state in arteriolo- and atherosclerosis. Besides, the dysfunction is also demonstrated by the shedding of endothelial MPs that express platelet-endothelial cell adhesion molecule-1 (i.e. CD31) that has been implicated to feature in ischaemic stroke subtypes (Grammas *et al*., 2011). Aside from endothelial dysfunction, Schreiber *et al*., 2013, argued about another common pathomechanism of CSVD which is related to the disorganisations of the arterial segmental walls and luminal narrowing. These arose due to accumulations of MPs alongside with cholesterol crystals that caused arteriolosclerosis which may result in hypoperfusion that accompanied infarcts and WMHs (Ogata *et al*., 2011; Schreiber *et al*., 2013).

The ischaemic consequences of several manifestation of CSVD such as WMHs, lacunar strokes, cerebral microbleeds, enlarged perivascular spaces, and small subcortical infarcts can be detected using magnetic resonance imaging (MRI) (Lambert *et al*., 2015; Sorond *et al*., 2015; Yakushiji *et al*., 2016; Yakushiji *et al*., 2018). WMHs are commonly recognized as small 'lacunes' (Latin: for lake) in ageing brain or bright areas of small non-cavitated high signal intensities on fluid attenuated inversion recovery (FLAIR) and T2-weighted MRI sequences. The lesion will increase with age because they evolve over a few months to years (Ovbiagele & Saver, 2006; Valdés Hernández *et al*., 2015; Wharton *et al*., 2015).

Although the association between age and WMHs prevalence has been consistently shown across different studies, the correlation of WMHs with other cardiovascular risk factors and clinical proforma remain elusive. Since WMHs is common even in healthy individuals, risk prediction assessment would be beneficial. In addition to as well as a part of the risk assessment, essential clinical algorithms have become useful in the prevention and management of cardiovascular disease (CVD), including cerebrovascular events (Kanjilal *et al*., 2008). Thus, a tool such as QRISK2 has been developed to assess the cardiovascular risk including cerebrovascular disease such as CSVD. QRISK2 is an online based risk prediction of cardio-cerebrovascular disease (Collins & Altman, 2012).

Moreover, in healthy individuals, executive dysfunction seems to correspond with WMHs (O'Brien *et al*., 2002). The overall severity of WMHs is known to link with the reduction in speed of information processing and executive performance in patients with subcortical ischemic vascular dementia (Mungas *et al*., 2001). In contrast, such global cognitive deficits are not seen in those with additional heterogenous vascular dementia (Cohen *et al*. 2002). Also, a significant relationship between WMHs severity and processing speed in older adults (65-80 years of age) has been reported (Nebes *et al*., 2006). Multiple assessments tools have been developed to assess changes in cognitive performance in diseases and healthy individuals. To date, the Weschler Adults Intelligence Scale (WAIS) is the most widely used and enables assessing cognitive and memories function in terms of the perceptual reasoning index (PRI), the processing speed index (PSI) and also the working memory index (WMI) (Weschler *et al*., 2008).

To date, WMHs are characterised and rated in terms of volume, location, size and number of lesions. Hence, various visual rating scales have been developed in order to assess WMHs, including the Fazekas scale (Fazekas *et al*., 1987), which provides an overall impression of WMHs presence in the entire brain. Apart from that, the current advancement in neuroimaging technology has enabled various specific algorithmic techniques and tools to be developed which automatically detect the presence of WMHs in terms of number, volume and location. Such tools include lesion segmentation tool (LST) which provide more concise delineation of WMHs (Schmidt *et al*., 2012).

Finally, although the conventional MRI or even MRI based diffusion-weighted imaging (DWI) offers detailed insights about brain anatomy and pathology, it still remains limited in mapping cerebral white matter, (Wang & Melhem, 2008) and more importantly, in correlating clinical parameters including cognition that has often been only weak to moderate (Patel & Markus, 2011). Therefore, better correlations and reliable lesion surrogates are necessary to improve the assessment of white matter architecture and also connectivity, such as white matter tractography using diffusion based magnetic resonance imaging (dMRI) (Lee *et al.*, 2015; Nitkunan *et al.*, 2008). Diffusion tensor imaging (DTI) is a specific signal modelling technique under diffusion MRI (dMRI). DTI analyses provides important information regarding white matter integrity and health in terms of diffusion indices. Such indices include fractional anisotropy (FA), mean diffusivity (MD), radial and axial diffusivity (RD and AD). Changes in these parameters give an insight regarding the characteristics of specific white matter tracts in relation to certain abnormalities including WMHs.

1.2 Problem Statement and Study Rationale

'Silent' or 'asymptomatic' manifestation of CSVD is frequently detected an as incidental finding from brain imaging and is often used as a prognostic marker following a first symptomatic CSVD presentation (van Norden *et al*., 2011). On MRI, the microchanges in white matter are seen as WMHs that represent discrete lacunar infarcts and/or more diffuse areas of white matter lesion (Smith, 2010). Such changes as seen on a conventional MRI are of limited correlations with clinical parameters including cognition, ranging from weak to moderate (Patel & Markus, 2011). Therefore, more concise correlations and reliable lesion surrogates are needed to improve the assessment of white matter structure, including white matter tractography. Advancement in MRI techniques offer potential new information on disease pathomechanisms as well as surrogate markers of disease onset, progression and new therapy evaluations using newer MR-DTI modalities. The use of DTI offers better insight into white matter integrity in healthy and asymptomatic individuals, hence can serve as a plausible indicator of CSVD onset and progression, and to correlate with the manifestation of CSVD that would otherwise elude conventional MRI (Huynh *et al*., 2008; Rost *et al*., 2010).

In addition, and as part of the risk assessment, essential clinical algorithms have become useful in the prevention and management of CVD, including cerebrovascular events (Kanjilal *et al*., 2008). Hence, a tool such as QRISK2 has been developed to assess the cardiovascular risk as well as cerebrovascular disease such as CSVD. QRISK2 is an online based risk prediction of cardio-cerebrovascular disease (Collins

& Altman, 2012). However, correlation with MR-DTI findings in white matter changes with QRISK2 as well as the performance on neurocognitive assessments among healthy and asymptomatic individuals is still insufficient. Moreover, variation in MPs and its subpopulation counts in various diseases indicate their diagnostic importance, particularly in vascular pathologies. However, most of the previous MPs studies are skewed towards diseases populations and do not focus on healthy subjects with prevalence of WMHs. Hence, novel correlations of MPs and its subpopulation counts with an imaging modality, QRISK2 and neuropsychology profiles is need and can serve as potential surrogate markers for the onset and progression of the CSVD spectrum.

1.3 Objectives of The Study

1.3.1 General objective

To explore novel surrogate markers for the assessment of white matter integrity in cerebral small vessel disease (CSVD) among apparently healthy asymptomatic individuals with neuropsychological, neuroimaging and microparticles profiling.

1.3.2 Specific objectives

1. To determine the proportion of white matter hyperintensities (WMHs) among the study subjects
2. To compare and correlate the QRISK2 cardiovascular risk prediction with longitudinal changes in white matter integrity among subjects with and without the WMHs.
3. To compare and correlate the neuropsychological profiles with longitudinal changes in white matter integrity among subjects with and without the WMHs.
4. To compare and correlate the microparticles (MPs) and its subpopulations' profiles with longitudinal changes in white matter integrity among subjects with and without WMHs.
5. To determine novel multimodal correlation of QRISK2, MPs subpopulations and neuropsychological profiles with neuroimaging parameters.

1.3.3 Research Questions

1. Does QRISK2 cardio-cerebrovascular risk prediction differ with age and is it able to predict the integrity and health of white matter tracts?
2. Is there any relationship between QRISK2 cardio-cerebrovascular risk prediction with the prevalence of white matter hyperintensities (WMHs)?
3. Does QRISK2 relate to the neuropsychological performance among healthy and asymptomatic individuals?
4. Do the neuropsychological profiles among subjects with and without prevalence of WMHs significantly differ?
5. Is the prevalence of WMHs affected by age?
6. Does neuropsychological performance relate with white matter tracts' integrity?
7. Is there any correlation between QRISK2 and the level of circulating microparticles?
8. Does MPs and its subpopulations correlate with the number and volume of WMHs lesions in healthy and symptomatic subjects?
9. Does the level of circulating MPs influence a subject's performance in cognitive and memories functions?
10. Do changes in white matter integrity among healthy and asymptomatic subjects have to do with the level of circulating MPs?

1.3.4 Research hypothesis

1. There is a significant correlation between QRISK2 with age and changes in white matter integrity.
2. There is no significant relationship between cardiovascular risk prediction, level of circulating MPs with a subject's neuropsychology performance.
3. There is a significant association between the level of circulating MPs and QRISK2
4. The level of certain MPs subpopulations can influence specific white matter tracts' integrity.
5. There is a significant relationship between white matter tracts' integrity and QRISK2.
6. There is a significant correlation between white matter integrity and a subject's neuropsychology performance
7. This study hypothesised that imaging changes of CSVD over time as delineated by DTI studies may serve as surrogate markers of CSVD together with potential novel correlations between a panel of MPs subpopulations, QRISK2 and neuropsychological profiles among healthy and asymptomatic individuals.

CHAPTER 2

LITERATURE REVIEW

2.1 Literature Search Strategy

The search strategies for relevant literature employed the use of search engines and specific string of keywords within several electronic databases. The databases used included Google Scholar, Science Direct, PubMed, Wiley Online Library, and ISI Web of Knowledge. Only articles published in English were selected. The literature search approach was divided into several parts. The first part included cerebral small vessel disease and silent stroke, using keywords: “cerebral small vessel disease AND silent stroke”. The seconds part concerned microparticles, using keywords: “circulating microparticles and strokes”, “cell-derived microparticles AND subpopulations”. The third focused upon cardiovascular risk in relation with cerebral small vessel disease and also cognitive function using keywords: “cardiovascular risk AND cerebral small vessel disease AND cognitive function”, “ischemic stroke AND cognitive”. Finally, further search focused upon supplementary research completion.

Part 1: Cerebral Small Vessel Disease (CSVD)

2.2 Cerebral White Matter

White matter is the term used to reflect glistening "white" fatty myelin sheath derived from oligodendrocytes that encircle many axons (Figure 2.1). Neuronal axons can be unmyelinated, lightly myelinated or heavily myelinated. Proportion of myelinated fibres (groups of axons) increases following phylogenetic, embryologic and developmental growth. The conduction capacity of axons differs according to unmyelinated slow-conducting and myelinated fast conducting (Wang *et al*., 2008).

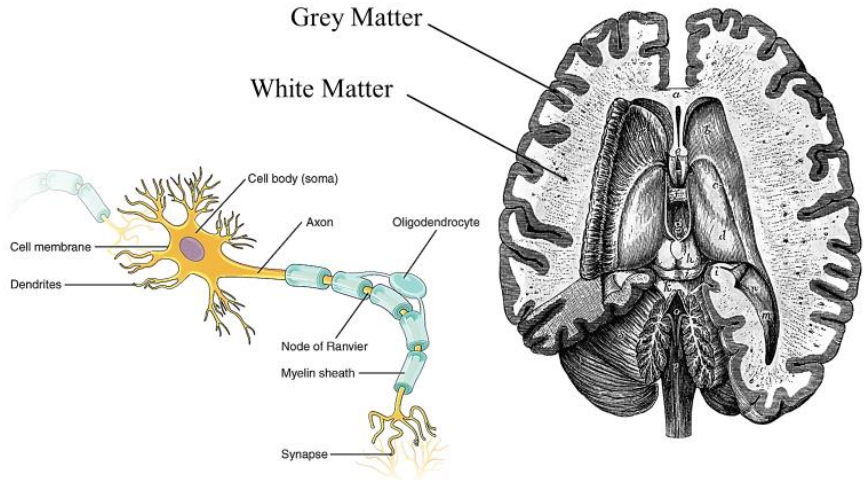

Figure 2.1: Schematic illustration of cerebral white matter. Left: diagrammatic representation of a neuron in which axon wrapped with oligodendrocytes forming myelin sheaths. Right: cerebral hemisphere, noted the different in white matter and grey matter. (image source for cerebral hemisphere adapted from: Baker & Haris, 1892, retrieved from http://etc.usf.edu/clipart/8100/8166/human_brain_8166.htm).

There are three major cerebral white matter fibres: commissural, projection and association. The commissural fibres, connects the two cerebral hemispheres across the midline, hence regarded as bi-hemispheric fibres (Nieuwenhuys *et al*., 2008) (Figure 2.2). There are two types of commissural fibres: homotopic and heterotopic fibres. The former connects corresponding region of two hemisphere. The latter connects one cortical area with non-corresponding areas of contralateral hemisphere. Examples of commissural fibres includes corpus callosum, anterior/posterior commissure and also hippocampal commissure (Standring *et al*., 2008).

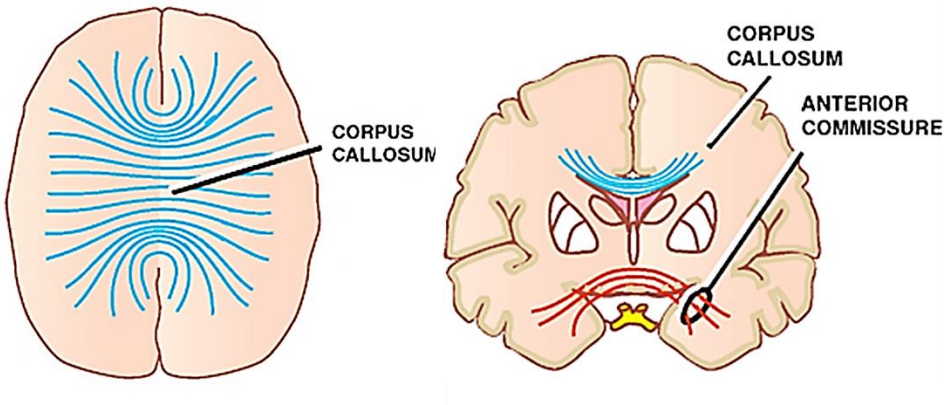

Figure 2.2: Schematic illustration of major types of commissural fibres (image source: https://neupsykey.com/cerebral-cortex-3/. Retrieved on 14th March 2018)

In contrast, the projection fibres, interconnect and transmit impulses from cerebral cortex to spinal cord, brain stem and subcortical regions (Figure 2.3). Thus, frequently regarded as uni-hemispheric fibres. Major example of projection fibres includes corticospinal tracts (CST), thalamic radiations, optic radiations, and also fornix (Nieuwenhuys *et al*., 2008). Tracts are defined as groups of axons that have

similar corresponding functions. Radiations are defined as white matter fibres that fanned out together from or towards one target (Standring *et al*., 2008).

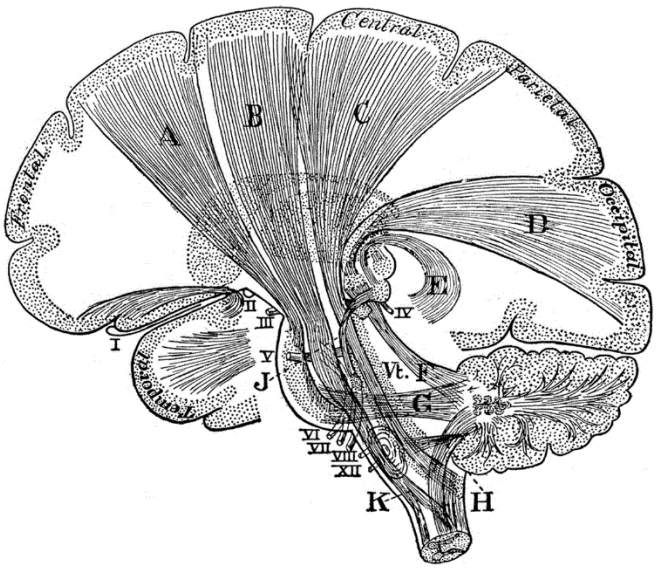

Figure 2.3: Schematic illustration of major projections fibres (A-H) which interconnect cerebral cortex with other regions such as midbrain, subcortical regions and also spinal cord (Image source adapted from: Newell & Ernst, 1917, retrieved from http://etc.usf.edu/clipart/ 44000/44026/44026_cerebrum.htm on 14th March 2018)

The next major white matter fibre is the association fibres which also known as uni-hemispheric fibres. These fibres interconnect multiple cerebral region in one particular hemisphere (Figure 2.4). Short U-fibres is the most prominent that interconnect adjacent gyri or long tracts that span the hemisphere (i.e. cingulum bundles, superior/inferior longitudinal fasciculus, arcuate fasciculus and also extreme capsule). Fasciculus is the term used for microscopically determinable fibres' groups (Nieuwenhuys *et al*., 2008). Whilst, capsules are sheet of fibres that curved and partially surround the grey matter (Standring *et al*., 2008).

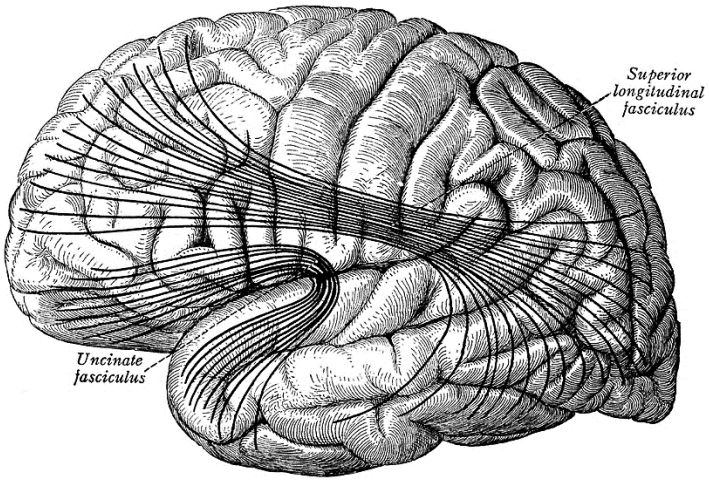

Figure 2.4: Schematic illustration of major association fibres, noted the superior longitudinal fasciculus and uncinate fasciculus (Image source adapted from: Sobatta, 1907; retrieved from http://etc.usf.edu/clipart/36300/36301/brain_36301_lg.gif, on 14th March 2018).

Therefore, cerebral white matter consists of fibres pathways that transmit information in the form of impulses through link of interconnected axons from one cerebral cortical areas to another including subcortical areas that form neural circuits (Schmahmann *et al*., 2008). From total brain volume, 60% are represented by circuits of white matter tracts.

2.3 Cerebral Small Vessels (Blood Supply)

The deep white matter, receives blood supply from the penetrating small vessel. The inner layer of these vessels is generally covered by endothelium monolayer with total area of 350m^2 (in human) (Pries *et al.*, 2000). The microvasculature health of the small vessel is maintained by normal functioning endothelial cells (Figure 2.5).

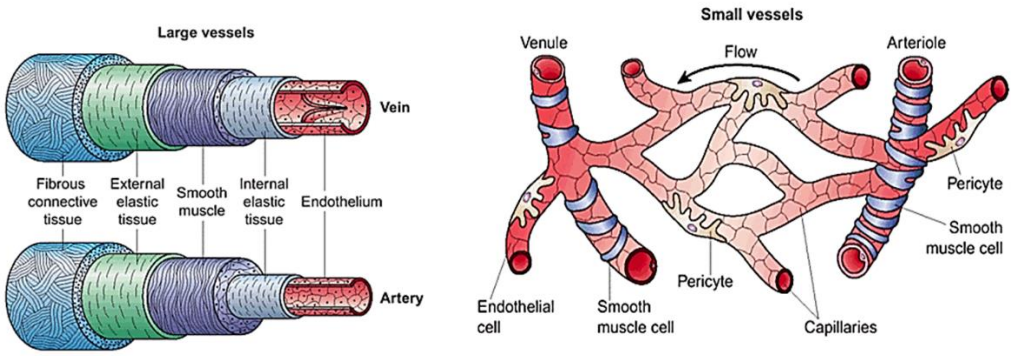

Figure 2.5: Schematic illustration of blood vessels. Left: larger vessels (vein and artery), with multiple layer covering the vessels; Right: representation of small vessel, noted that it is surrounded by endothelial cells monolayer and associated with pericytes (image source adapted from: Cleaver & Melton, 2003).

Current accepted definition of small vessels refers to all the vascular structures (small arteries, arterioles, capillaries, venules, and small veins) that are located in the brain parenchyma or in the subarachnoid space (Pantoni & Gorelick, 2014). Terminal arterioles including pial and medullary arterioles (4-5 cm long) are formed from cortical arteries that perpendicularly penetrate white matter (Martorella *et al.*, 2012) (Figure 2.6). Branches of short cortical arteries (3-4 mm) also supply juxta-cortical white matter (U-fibres). Multiple anastomoses were established by cortical arteries

provide the cerebral cortex with rich arteriolar network. As the arteries penetrated deep into white matter they establish few more capillary anastomoses alongside pial arterioles forming relatively independent arteriolar metabolic units (Martorella *et al*., 2012) (Figure 2.6). Apart from that, shorter deep sub-ependymal arteries that arise from choroid arteries also supply white matter. Moreover, basal ganglia are penetrated by another terminal branch of sub-ependymal arteries including thalamic and lenticulostriate perforating arteries originating from the larger vessel, middle cerebral artery (MCA). These penetrating arterioles are hardly anastomosed with each other thus parenchyma located at the adjacent of them are less vascularized. This is in contrast to superficial penetrating arteries which are more vascularized.

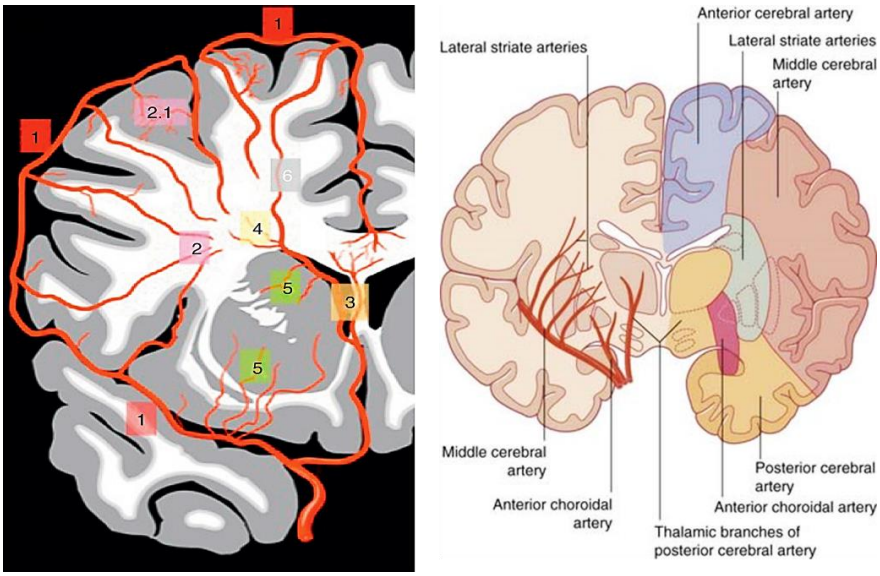

Figure 2.6: Schematic illustration of cerebral vasculature. Right: Different branches of cerebral arteries and their territories that supply cerebral white matter. Left: penetrating arteries and arterioles into deep white matter, from the image (1) represent cortical arteries (2) pial arterioles that supply deep white matter (2.1) short branches (3) anterior choroidal arteries that branch into sub-ependymal arteries (4) arterioles of sub-ependymal (5) MCA branches into thalamic and lenticulostriate perforating arteries that supply basal ganglia (adapted from: Martorella *et al*., 2012).

2.4 CSVD: Definition and Pathophysiology

One of the predominant types of strokes that resulted from occlusion (ischaemia) of small blood vessel deep within the brain is ischaemic stroke (Rouhl *et al.,* 2009; Smith, 2017). In ischemic or lacunar stroke, around up to 30% thought to be due to CSVD (Heye *et al.,* 2015; Patel & Markus, 2011; Rouhl *et al.,* 2009).

It is generally accepted that small vessel disease (SVD) is a term used to describe the pathologic consequences of SVD on the brain parenchyma rather than the underlying diseases of the vessels (Wardlaw *et al*., 2013). Thus, the term CSVD is preferred to describe a brain injury that resides in poorly collateralized subcortical grey and deep white matter due to several of vasculo-pathologic processes that affect and cause occlusion to the perforating cerebral capillaries and arteries (mostly branches of MCA) that penetrate and supply the brain subcortical region (Benjamin *et al.*, 2016; Hinman *et al.*, 2015; Novakovic, 2010) (Figure 2.7).

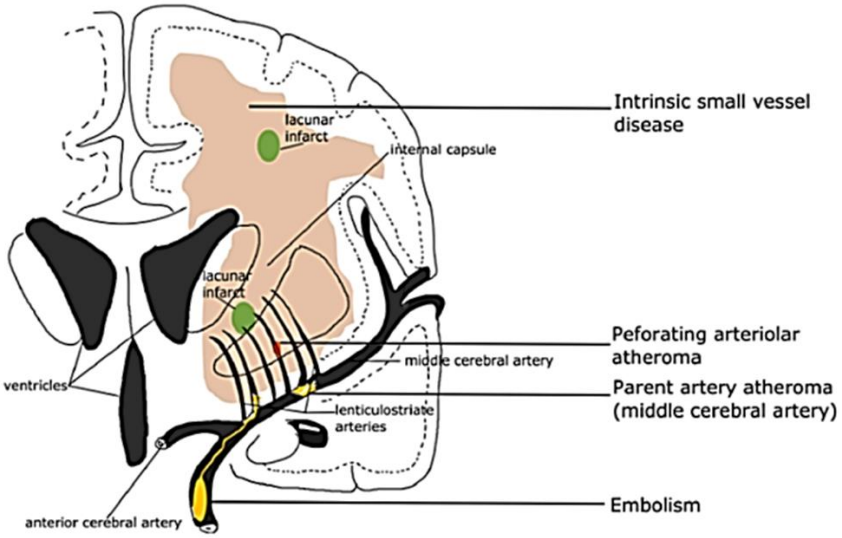

Figure 2.7: Illustration of general aetiologies of CSVD. The picture shows branches of MCA that penetrates subcortical region of white matter and also grey matter. Embolus or thrombus may accumulate and cause occlusion (atheroma) upon parent MCA and penetrating arteriolar. The occlusion of perforating arteriolar can cause ischemia and eventually lacunar infarct may have formed. The core infarcts might affect surrounding tissue (penumbra). Diffused disruption of blood brain barrier following intrinsic CSVD also happened at arteriolar level indicating that certain plasma component invasion and deposited in vessel wall (adapted from: Shi & Wardlaw, 2016).

Therefore, in general the term CSVD is used to describe a complex and heterogeneous disorder to indicate brain parenchymal injury associated with distal leptomeningeal and intracerebral vessel pathology (Ogata *et al.*, 2014; de Bresser *et al*., 2018) through clinical, radiological, or pathological phenomena with various aetiologies that affect the pathologies of small arteries, arterioles, venules, and capillaries of the brain (Sorond *et al.*, 2015; Yakushiji *et al*., 2018).

2.5 White Matter Hyperintensities (WMHs)

The ischaemic consequences of several manifestation of CSVD such as WMHs, lacunar strokes, cerebral microbleeds, enlarged perivascular spaces, and small subcortical infarcts can be detected using MRI (Lambert *et al.*, 2015; Sorond *et al.*, 2015; Yakushiji *et al*., 2016; Yakushiji *et al*., 2018). WMHs are commonly recognized as small '*lacunes*' (Latin: for lake) in ageing brain or bright areas of small non-cavitated high signal intensity on FLAIR and T2-weighted MRI parameters. The lesion will increase with age because they evolve over a few months to years (Ovbiagele & Saver, 2006; Valdés Hernández *et al.*, 2015; Wharton *et al.*, 2015). Recognising the expanding CVSD manifestations from MRI imaging (symptomatic and asymptomatic), Wardlaw and colleagues proposed what is known as Standards for Reporting and Imaging of Small Vessel Disease (STRIVE) for the methods of visual identification and classification CSVD spectrum (Wardlaw *et al*., 2013) (Figure 2.8).

In addition, WMHs are also regarded as an ischaemic white matter demyelination and can manifest as symptomatic or silent (asymptomatic) brain parenchyma lesion. Interestingly, this so-called 'silent' manifestation of CSVD is frequently reported as incidental finding from brain imaging of individuals who never experienced any symptom of stroke more frequently among the elderly (Valdés Hernández *et al.*, 2015). It is also been proposed as a prognostic marker following a first symptomatic CSVD presentation, for instance acute lacunar stroke (van Norden *et al.*, 2011).

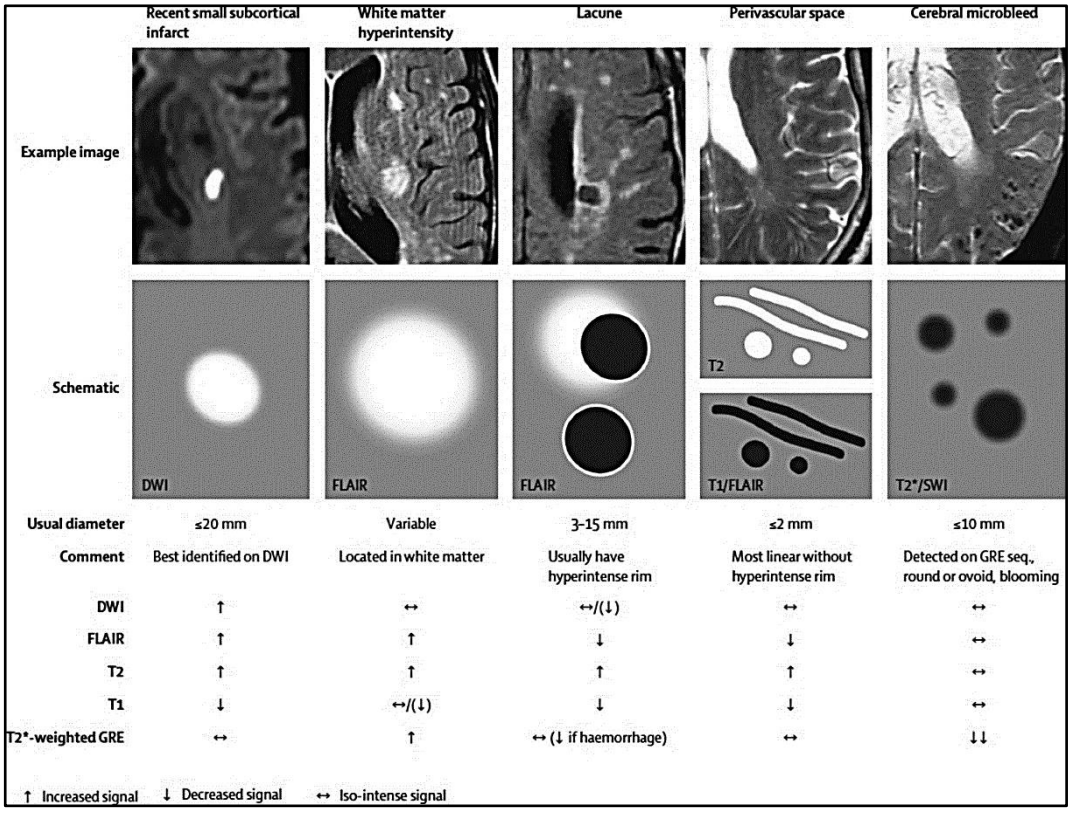

Figure 2.8: STRIVE method of identification and classification of CSVD spectrum. Top: example of MRI images; Middle: schematic representation; Bottom: characteristic of changes related to CSVD (adapted from: Wardlaw *et al*., 2013).

2.6 Aetiology and Consequences of WMHs

About 95% of asymptomatic or SBI are lacunar infarcts (as seen as WMHs on MRI imaging) and are arguably more prevalent than symptomatic ones. The two major contributors to the progression of SBI include age and hypertension (Norrving, 2015). The key difference between SBI and symptomatic lacunar infarcts are their location and size. This is because both SBI and symptomatic lacunar infarcts have similar pathological appearance (Bailey *et al*., 2012). For example, most asymptomatic SBI are located within white matter periventricular space (periventricular lesion, PVL) and centrum semiovale (deep subcortical lesions, DSCL) (Kaiser *et al.*, 2014; Wharton *et*

al., 2015), whereas, in symptomatic lacunar ischaemic stroke mostly affects the sensory and motor tracts (Valdés Hernández *et al.*, 2015).

However, the underlying pathomechanism of SBI or WMHs remains contentious, largely as a result of growing insights from histopathological, epidemiological, and physiological studies that contribute important information about it. In addition, healthy white matter is more myelinated than white matter of Alzheimer's patients (Bartzokis *et al*., 2003) and has a high content of long chain fatty acids and less water content (by 12%) compared with grey matter. Previous study reported that WMHs are consistently related to age, hypertension and other cardiovascular risk factors (Prabakharan *et al*., 2012). Therefore, individual with extensive WMHs are at high risk for future stroke, i.e. WMHs serving as prognostic marker. Nevertheless, it is estimated that SBI or WMHs occur around 30% of healthy subjects over 60 years of age, and with a linear prevalence increment with age (de Leeuw *et al*., 2001).

Reduced cerebral blood flow and focal neurologic signs are related to brain atrophy and have been found to correlate with number and volume of WMHs (Bahrani *et al*., 2017). Furthermore, WMHs are also associated with cognitive impairment, with the notion that a certain threshold must be achieved before this becomes clinically apparent (Debette & Markus, 2010). Alarmingly, WMHs have also been linked precursor of developing neuropsychiatric disorder such as schizophrenia (Berlow *et al*., 2010).

Part 2: Cerebrovascular Disease Risk and Risk Prediction

2.7 Risk Factors for Cerebrovascular Disease

In 1960, Framingham Heart Study was the first to recognised the non-modifiable and modifiable risk factors for cerebrovascular disease in the population setting (Mahmood *et al*., 2014). Understanding of such factors has helped facilitate the management and prevention of cardiovascular morbidities and mortality. Age, gender, race, ethnicity and heredity have been recognized as common non-modifiable risk factors for the onset and progression of stroke. Although these factors cannot be modified, they help identify those whose potentially at high risk, hence, enabling strategic management of modifiable risk factors such as smoking and cholesterol level.

In addition, age is the strongest determinant risk factor for stroke. It has been reported that, the risk for stroke for an individual is doubled at 10 successive years after 55 years of age, for both male and female (Smajlovic, 2015; Ovbiagele & Nguyen-Huynh, 2011). Furthermore, the incidence of stroke is 1.25 folds higher in male compared to female, as females tend to live longer than males (Morovic *et al*., 2012).

Stroke incidence and mortality are particularly varied among different race. Previous studies reported that, the incidence of stroke is double in black patients compared to white patients. For example, the incidence of stroke among black patient

is more than 2 folds higher than white, whilst Hispanic patients are 1.6 folds higher than white patients (Howard, 2013). Higher incidence of stroke also has been reported in Asians such as Chinese and Japanese (Ueshima *et al*., 2008).

Moreover, the incidence and prevalence of stroke also elevated in individual with family history of stroke. The reasons for this are a genetic tendency for stroke, a genetic determination of other stroke risk factors, and a common familial exposure to environment or lifestyle risk. Previous studies suggested that, an increase risk for male whose mothers died from stroke and female who had a family history of stroke (Seshadari *et al*., 2010). In the Framingham Study and offspring analysis revealed that both paternal and maternal history of stroke conferred an increased risk of stroke (Romero & Wolf, 2013).

2.8 Cardio-cerebrovascular Risk Prediction Assessment by QRISK2

Currently, an online calculator has been established to predict the risk for cardio-cerebrovascular disease within 10 years; such a tool includes QRISK 2 (Collins & Altman, 2010). QRISK2 is an online based risk prediction of cardio-cerebrovascular disease (http//:www.QRISK.org/index.php) (University of Nottingham and EMIS). It was developed in the United Kingdom (UK) based on information compiled between 1993 and 2010 that contained the data for over 10 million patients registered with 550 general practices in the UK. Hence, it is globally used and is annually updated.

QRISK2 is the updated version of QRISK1, whereby a new multivariable risk score that comprises all the risk factors in QRISK1 has been factored in, including self-assigned ethnicity and conditions associated with cardio-cerebrovascular risk including type 2 diabetes mellitus, hypertension, rheumatoid arthritis, renal disease and atrial fibrillation (Hippisley-Cox *et al*., 2008). Interactions between age and body mass index, systolic blood pressure, family history, smoking status, treated hypertension, diagnosed type 2 diabetes mellitus, and atrial fibrillation are also considered in QRISK2 (Table 2.1).