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Exploring Early Signs of Structural Brain Changes in Mid-Age Apolipoprotein E epsilon-4 Carriers

by

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I hereby declare that this thesis has not been and will not be, submitted in whole or in part to another University for the award of any other degree.

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Abstract

Background: Neuronal degeneration, a key feature of Alzheimer's disease (AD), marks a crucial preclinical phase, where a person has no apparent cognitive symptoms but show some pathophysiological changes. Several studies have explored the time course of these early changes using Cerebrospinal Fluid measures and Ionizing Imaging scans. But these methods are quite invasive, and new non-invasive approaches such as blood biomarkers and magnetic resonance imaging (MRI) offer a new pathway towards early investigations. We ask whether early pathophysiological changes are present in mid-age healthy people carrying the Apolipoprotein E epsilon-4 (APOE4) genotype, the biggest risk factor for late onset, non-familial AD.

Objectives: To assess whether APOE4 status is associated with subtle brain changes in mid age healthy adults and whether such changes, if any, are related to fine deficits in cognition or to inflammatory markers.

Methods: The study comprised three phases: **Phase1** Recruitment and genotyping of mid-age adults (42-59), subsequently pseudo-randomly selected to participate in the study. **Phase2** comprised a blood sample, for biomarker analysis and kidney function to confirm eligibility for the final phase, and a memory task. **Phase3** comprised a 70-minute brain scan on 3T scanner, including structural acquisitions and gadolinium-based dynamic contrast-enhanced MRI.

Results: Although non-significant at the conventional statistical level, subtle differences in blood-brain barrier permeability, inflammatory biomarkers and brain structure were identified in the composite profiles between healthy mid-age APOE4 carriers and non-carriers, matched on age, education and gender.

Discussion: This study demonstrated a trend towards change emerging from mid-age, with quantitative but not qualitative differences observable on a number of the measures. Results from this study have potential for impact on early diagnosis of AD and will facilitate development of early interventions to change the trajectory of decline.

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List of Abbreviations

FDG	^{18}F -fluoro-2-deoxyglucose (FDG)
AF	Acceleration factor
AD	Alzheimers disease
A β	Amyloid- β
APOE ϵ 4	Apolipoprotein E epsilon-4
AxD	Axial Diffusivity
PET	Positron Emission Tomography
BET	Brain Extraction Tool
BBB	Blood-brain barrier
BSMS RGEC	Brighton and Sussex Medical School Research Governance and Ethics Committee
CNS	Central nervous system
CMB	Cerebral Micro-Bleeds
CISC	Clinical Imaging Science Centre
CV	Coefficient of Variation
CT	Computed Tomography
CNR	Contrast to Noise Ratio
CRP	C-Reactive Protein
CSF	Cerebral Spinal Fluid
CypA	Cyclophilin A
MMP-9	Matrix metalloproteinase-9
DMN	Default-mode network
DTI	Diffusion tensor imaging
DWI	Diffusion Weighted Imaging
DCE-MRI	Dynamic contrast enhanced MRI scan of the brain
TE	Time of Echo
eGFR	Estimated glomerular filtration rate
EES	Extravascular-extracellular space
qMRI	Quantitative Magnetic Resonance Imaging

FA	Flip Angle
FH	Family history
FOV	Field of view
FL	Frontal Lobe
FA	Fractional Anisotropy
fMRI	functional magnetic resonance imaging
Gd	Gadolinium (Gadoterate meglumine)
GRAPPA	Generalized Auto Calibrating Partial Parallel Acquisition
GM	Grey matter
HTA	Human Tissue Act
iPAT	Integrated Parallel Imaging Technique
IFN γ	Interferon gamma
IL6	Interleukin-6
f_{iso}	Isotropic diffusion fraction
KSU	King Saud University
T_1	Longitudinal relaxation time
LRP1	Low-density lipoprotein receptor-related protein 1
MRI	Magnetic resonance imaging
MPRAGE	Magnetization Prepared Rapid Acquisition Gradient Echo
MTsat	Magnetization transfer saturation
WMH	Matter hyper-intensities
MCI	Mild Cognitively Impaired
MD	Mean Diffusivity
MTL	Medial-temporal lobe
MSD	Meso Scale Discovery
MB	Micro-bleeds
MTR	Magnetisation Transfer Ratio
MPM	Multi-Parametric Mapping
MS	Multiple Sclerosis
NDI	Neurite Density Index

NODDI	Neurite Orientation Dispersion and Density Imaging
NfL	Neurofilament-Light
NCI	Non-Cognitively Impaired
ODI	Orientation Dispersion Index
PH	Peak Heights
PP	Peak Positions
Qalb	Plasma albumin ratio
PM	Prospective Memory questions
PRMQ	Prospective-Retrospective Memory Questionnaire
RD	Radial Diffusivity
RF	Radiofrequency Field
TR	Repetition Time
RM	Retrospective Memory questions
ROI	Region of Interest
RSUH	Royal Sussex University Hospital
SACB	Saudi Arabian Cultural Bureau in the United Kingdom
SMC	Smooth muscle cells
SDOM	Standard Deviation of the Mean
MNI	Standard Montreal Neurological Institute
SOP	Standard Operating Procedure
SPM	Statistical Parametric Mapping
SLF	Superior Longitudinal Fasciculus
SWI	Susceptibility-Weight Imaging
TIV	Total Intracranial Volume
T2*	Transverse relaxation rate
TNF α	Tumor Necrosis Factor alpha
vAD	Vascular Alzheimer's Disease
vMCI	Vascular MCI
VBM	Voxel-Based Morphometry
WBC	White Blood Cells

WHO	World Health Organization
WM	White Matter
WMAcc	White Matter Anterior cingulate cortex

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Chapter 1: Introduction

1.1 Dementia

Dementia is a chronic syndrome usually thought to be associated with old age in which the cognitive function of an individual deteriorates i.e. the ability to perform everyday tasks, memory loss, impaired thinking as well as changes in behaviour (<https://www.who.int/news-room/fact-sheets/detail/dementia>). The World Health Organization (WHO) recorded about 50 million people diagnosed with dementia worldwide and about 10 million are diagnosed every year. As of 2019, dementia is ranked the 7th cause of death worldwide (<https://www.who.int/news-room/fact-sheets/detail/dementia>). While dementia is a component of many forms of neurodegenerative disease, including Alzheimer's disease (AD), vascular dementia, mixed dementia, dementia with Lewy bodies, frontotemporal dementia and Parkinson disease the most common cause of dementia is AD, accounting for about 60-70% of dementia, as shown in Figure 1.1.

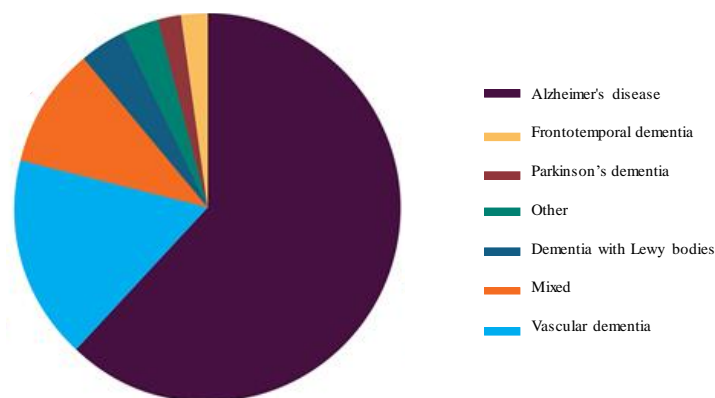


Figure 1.1 Pie chart showing percentage of different forms and causes of dementia. (Modified and sourced from <https://www.alzheimersresearchuk.org/kids/teens/what-is-dementia/causes-of-dementia/>).

1.2 Alzheimer's Disease (AD)

AD is often identified as a disease of old age and is usually diagnosed at the age of 65 or over (Figure 1.2) (Gatz et al., 2006). AD is defined as a subtle, slow, progressive and degenerative disease of the neurons (Bondi et al., 2017). Symptoms of AD include progressive cognitive decline in memory, language, behaviour and executive function, which in turn cause the

inability to perform daily life activities and becoming dependent on a caregiver (Weller et al., 2018).

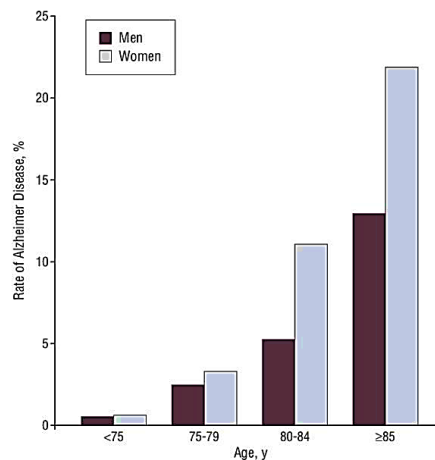


Figure 1.2 Rate of AD as a function of age of detection (Sourced from Gatz et al., 2006).

As neuronal degeneration is the main feature of Alzheimer's disease, the pathophysiological changes behind such defect need to be investigated at an early stage to help in prevention prior to the onset of the disease and development of innovative, therapeutic pathways to prevent, arrest or retard these pathological changes. The challenge of finding a cure for this neurodegenerative disease has attracted many researchers to focus on improving early detection, which could provide the opportunity for early intervention to improve cognitive health in older age, and slow down the progression of this disease.

1.3 AD Brain Morphology

Recent studies have suggested that the neuropathology of the disease, begin years before clinical diagnosis is made i.e. 10-20 years before the start of dementia (Sperling et al., 2014). The pathologies suggested in the literature that appear before the first stage of dementia include amyloid- β ($A\beta$) deposition and the development of tau tangles (Ewers et al., 2011; Sperling et al., 2014). $A\beta$ deposition and tau tangles have been shown to be present in cognitively healthy older adults, which suggests that AD pathology begin to progress years before clinical diagnosis is made (Leal et al., 2018). Further information about $A\beta$ deposition and tau tangles are explained in chapter 6.

1.3.1 Neurovascular Brain Morphology in AD

While blood vessels across the whole body have the same anatomical structure and function, the blood vessels in the brain are naturally formed with a strong structural framework to enable protection of the very delicate brain organ against neurotoxic blood-derived substances that

may lead to damage to the neuronal structure and therefore neurodegenerative disease including AD. To promote a healthy brain, the blood-brain barrier (BBB) is tightly bonded to prevent toxins and pathogens from entering the brain including blood derived materials (Keaney and Campbell, 2015). In AD, the BBB is found to be dysfunctional, allowing leakage of neurotoxic material into the brain tissue, which in turn leads to neuronal damage and accumulation of neuro-toxins (Nelson et al., 2016; Zenaro et al., 2017).

There has been a rising interest in AD research to investigate BBB permeability prior to the onset of disease. A leaky BBB is thought to be one of the first stages leading to neuronal damage and contributing to cognitive decline in neurodegenerative disease (Montagne et al., 2015; Nelson et al., 2016; Sweeney et al., 2018). Figure 1.3 illustrates the factors that might contribute to pathologies that can give rise to changes in brain structure (morphology) over time.

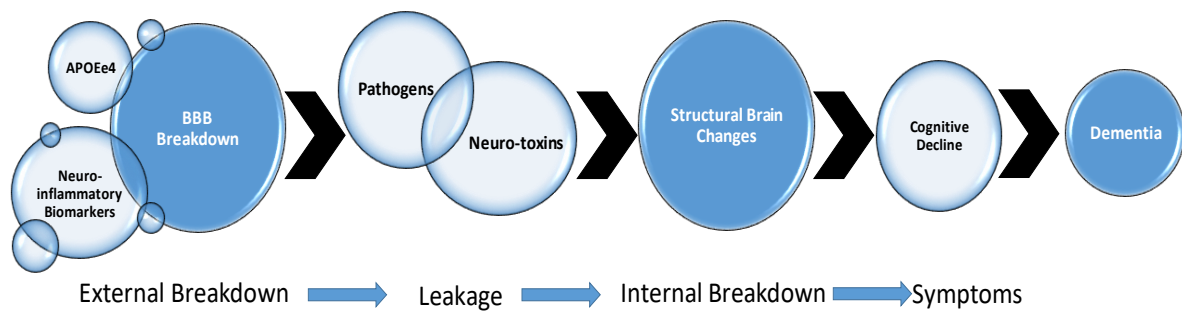


Figure 1.3 Simple illustration showing factors that might contribute to pathologies that can give rise to changes in brain structure (morphology) over time. (APOEε4: Apolipoprotein E epsilon4: AD strongest known genetic risk factor).

1.3.2 Structural Brain Morphology in AD

Some early macro-structural brain changes such as lobar specific and whole brain atrophy together with cognitive decline have been identified in the literature to be linked to Aβ deposition (Storandt et al., 2009). Similarly, micro-structural brain changes have been a rising interest in research and have shown to be detected during early stages of AD. These include white matter (WM) tract disruption cerebral micro-bleeds (CMB) and white matter hyperintensities (WMH), which also may be detected earlier in people who are at higher risk of developing late-onset sporadic AD (Poels et al. 2010; Apostolova et al., 2012; Poliakova et al., 2016; Cavedo et al., 2017).

1.4 AD Risk Factors

AD onset is likely the result of a cascade of different factors. Cardiovascular disease is one of the most important of those risk factors and has been a focus in understanding the onset of the disease and prevention pathways (Vijayan and Reddy, 2016). Cardiovascular risk factors in mid-age that have been linked to the development of AD later on in life include hypertension, high cholesterol, diabetes, heart disease and vascular pathologies (Kivipelto et al., 2001; Vijayan and Reddy, 2016). Because AD is a non reversible disease, identifying and understanding AD risk factors at a very early age and working towards preventive paths prior to the development of any structural or functional changes in the brain is a greatly important route for researchers to focus on (Edwards III et al., 2019).

1.5 AD Strongest Genetic Risk Factor (APOEε4)

Apolipoprotein (APOE) is a protein associated with movement of cholesterol. The gene encoding for APOE is implicated in cardiovascular and neurovascular diseases, but is independently considered to be the biggest genetic risk factor for late onset AD (Liu et al., 2013). APOE is a gene that has three allelic variants in humans (epsilon2, epsilon3 and epsilon4; henceforth e2, e3, e4). The most common APOE allele in populations worldwide is e3, accounting for 65-85%, followed by e4 accounting for 15-25% of the population, while e2 is far less common and may even be absent in some ethnic groups (Mahley et al., 1988; Corbo et al., 1999; Huebbe & Rimbach, 2017; Hubacek et al, 2021).

Important to note first is the terminology of APOE (the gene) and its polymorphism, and the way these terms are used in this thesis. The various alleles are usually represented by the Greek letter epsilon (ϵ), so that the different isoforms have the corresponding notations APOE ϵ 2, ϵ 3, ϵ 4 alleles (in the gene), while the respective corresponding proteins are usually named ApoE2, E3, E4. For the purpose of this thesis, I will be using APA notation for the alleles, so they are written in this thesis in the following format APOE e2, e3, e4.

The influence of APOEε4 genotype on AD is of great importance in all ethnic groups worldwide, but some physiological variations among the African ethnic groups were

recognized (Beydoun et al., 2021; Teruel et al., 2011; Farrer et al., 1997). The global distribution of APOE ϵ 4 is not random (as shown in Figure 1.4) and is found to be lower in southern regions of Europe and Asia compared to Northern regions, for example, less than 10%

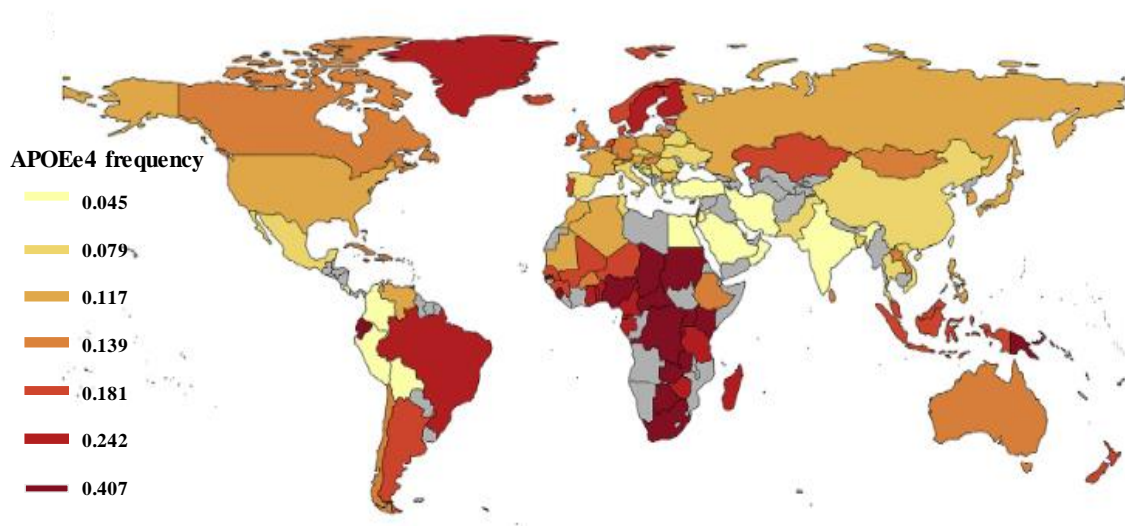


Figure 1.4 Global APOE ϵ 4 distribution demonstrated on World map. (Modified and sourced from Belloy et al., 2019).

of the population in South China are carriers of APOE ϵ 4. Indigenous people of Australia and central Africa have a higher percentage of APOE ϵ 4 carriers, ranging between 26-40% of the population (Farrer et al., 1997; Corbo et al., 1999; Egert et al., 2012). Therefore, taking the ethnic background into consideration is important when conducting a study in relation to the ApoE4 gene.

APOE ϵ 4 allele has been recognized as a risk factor for developing cardiovascular disease and has been notably recognized as the strongest genetic risk factor for developing AD and may influence the rate of cognitive decline most significantly at the early stages of AD (Davignon et al. 1988; Cosentino et al., 2008). Mortality rates due to cardiovascular disease or cognitive decline is significantly higher in APOE ϵ 4 compared to non-APOE ϵ 4 carriers (Ewbank, 2004). Although Figure 1.5 illustrates the APOE frequency in AD is higher in carriers of at least one ϵ 4 allele, Davidson et al (2007) suggests that the presence of two ϵ 4 alleles (ϵ 4/ ϵ 4) are at higher risk of developing early onset AD than the presence of one ϵ 4 allele (ϵ 4/ ϵ 2), (ϵ 4/ ϵ 3)) (Davidson et al., 2007; Yang et al., 2016). While, it has been agreed that APOE ϵ 4 has a strong influence on the onset of the disease, how it contributes to the onset and early progression of

AD remains debatable (Sun et al., 2016). There are several factors that have been studied to demonstrate how APOE ϵ 4 influences the development of AD.

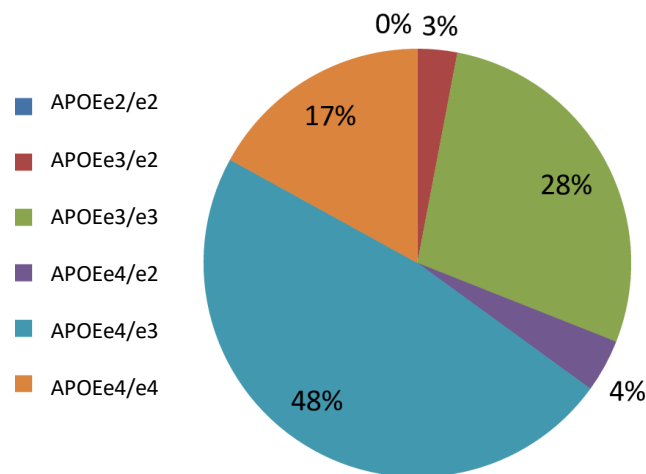


Figure 1.5 Pie chart of APOE distribution in AD patients. (Modified and sourced from Yang et al., 2016).

Studies suggest the presence of APOE ϵ 4 genotype accelerates the process of aging (Cacciaglia et al., 2018) independent of its additional relationship to AD onset. The ϵ 4 allele of the APOE gene has effects on multiple aspects of the AD chain such as demyelination in healthy mid-age individuals thought to contribute to reduced cognitive performance compared with non APOE ϵ 4 carriers, (Bartzokis et al., 2007), lobar micro-bleeds (MB) in APOE ϵ 4 carriers causing the neurophysiological function to worsen (Caselli et al., 2009), cortical thinning in regions of the hippocampus also contributing to memory decline in APOE ϵ 4 carriers when compared to non-carriers (Donix et al., 2010), and long term memory decline in mid-age in APOE ϵ 4 carriers (Taylor et al. 2011).

Furthermore, studies have shown that the ApoE4 gene is a risk factor for developing vascular disease which, could promote AD via blood-brain barrier (BBB) dysfunction (Nelson et al., 2015; Montagne et al., 2015, 2016, 2017, 2018, 2020, 2021). Therefore, the APOE ϵ 4 variant of this gene has attracted attention as the single most important known genetic risk factor for late onset, non-familial AD.

1.5.1 APOE ϵ 4 and Age

Studies propose that AD pathology (such as amyloid- β accumulation and hippocampal dysfunction) starts a decade or two prior to the detection of symptoms and diagnosis of neurological degeneration (Sperling et al., 2014). Evidence suggest that the APOE ϵ 4 gene has

a different impact on cognition across the lifespan (Han and Bondi, 2008), therefore, age is an important factor in understanding the role and contribution of APOEε4 to AD. Although APOEε4 is associated with an increased occurrence of age-related cognitive impairment (Deary et al., 2002), unexpectedly, studies show it could be associated with advantages in cognition in younger population, where younger APOEε4 carriers showed better progress in higher education than non APOEε4s and higher IQs during their 20s (Mondadori et al., 2007; Rusted et al., 2013; Evans et al., 2013; Dowell et al., 2013; Taylor et al., 2017). Even in healthy mid-age adults, the APOEε4 variant was not associated with significant cognitive change (Cacciaglia et al., 2019), but may be the point of transition towards poorer performance, being associated with steeper age-related decline in cognitive ability (Caselli et al., 2009; Cacciaglia et al., 2018; Mishra et al., 2018).

1.5.2 APOEε4 and Gender

Studies suggest that there is a gender effect in the impact of the APOE gene. Women were at a higher risk of developing AD compared to men, as well as developing AD related pathologies (Corder et al., 2004; Calderón-Garcidueñas & de la Monte, 2017), and some studies have suggested that females carrying one ε4 allele have greater risk for developing AD compared to APOEε3 carriers (Payami et al., 1996; Farrer et al., 1997; Bretsky et al., 1999).

Several neuroimaging studies demonstrated structural and functional differences in female APOEε4 carriers in comparison to male carriers of the same allele. In a study on AD patients, there was a decrease in the entorhinal cortex volume in female APOEε4 carriers compared to males (Juottonen et al., 1998). Another study on healthy aging individuals showed a decrease in functional connectivity in the default-mode network (DMN) of female APOEε4 carriers (Damoiseaux et al., 2012). This is particularly interesting because the DMN is functionally linked with the hippocampus, which is one of the first regions of the brain to show pathology related to AD (Braak and Braak, 1991). Furthermore, there was a significant correlation between functional connectivity and white matter (WM) integrity of the cingulum tract connecting those regions, where female APOEε4 carriers showed a decrease in WM integrity which suggests female APOEε4 carriers are at an increased risk of AD (Verena et al., 2014).

1.5.3 APOEε4 and Family History

Family history (FH) of AD (APOEε4 carriers), is a recognized risk factor for the development of sporadic AD (Ten Kate et al., 2016; Donix et al., 2012; Huang et al., 2004). In a study that

focused on studying grey matter (GM) volume on cognitively healthy mid-aged individuals by looking at differences between APOE status and first-degree family history, they identified a reduction in GM volume in the striatum of APOEε4+ compared to APOEε4-, a reduction in GM volume in the right precuneus of participants with FH compared to participants without FH, while maternal and paternal FH demonstrated similar atrophy patterns (Ten Kate et al., 2016). This study concluded that both APOEε4 carriers and family history are associated with regional GM atrophy in cognitively healthy mid-age participants. Ten Kate et al (2016) and Huang et al (2004), both suggested that the presence of APOEε4 together with family history increased the risk of developing AD later in life compared to non APOEε4 carriers.

1.6 APOEε4 and Cognition

APOEε4 genotype has been identified to impact cognitive status differently in different age groups. As mentioned earlier APOEε4 contributes to stronger cognitive performance in young age (Rusted et al., 2013; Dowell et al., 2016), but has been associated with over activity in the hippocampus and other regions, which may contribute to deteriorating cognition as people age, effectively accelerating the aging effect (Evans et al., 2014; Dowell et al., 2016). Additionally, Deary et al., 2002 suggests that APOEε4 has an age dependent negative effect on cognition throughout the life span of carriers independent of risk of developing AD (Deary et al., 2002). Hestad et al also confirmed that cognitive decline was related to APOEε4 genotype, where memory difficulties were higher in APOEε4 carriers compared to APOEε3s and APOEε2s in healthy elderly, mild cognitive impaired (MCI) and AD cohorts, and memory loss was even higher in carriers of the double ε4 allele (ε4/ε4) compared to one ε4 allele (Hestad et al., 2021). Therefore, it is important to address both the age where APOEε4 starts to build a negative effect on cognition and the physiological changes APOEε4 contributes to in the human brain.

1.7 Brain Pathology in APOEε4 (structural and physiological)

APOEε4 status has been linked to brain pathologies such as Amyloid-β (Aβ) accumulation and hippocampal atrophy as well as cognitive decline from a pre-symptomatic stage (Filippini et al., 2009; Ewers et al., 2011; Bateman et al., 2012; Reinvang et al., 2013; Shi et al., 2019). However, a Mayo Clinic study of aging has shown that an increase in Aβ deposition and APOEε4 has no significant influence on cognition in mid-age (Mielke et al., 2016). Briefly below we introduce some of the structural changes happening in APOEε4 carriers who are at

higher risk in developing late onset AD, while further more detailed backgrounds are described in relevant chapters in the thesis.

1.7.1 Hippocampal Atrophy

Although several MRI studies have recognized hippocampal atrophy in MCI and AD patients, the extent to which hippocampal volume is reduced in healthy carriers of the genetic risk for AD (APOEε4) is still controversial (de Leon et al., 1996; Rusinek et al., 2003; Apostolova et al., 2006; Devanand et al., 2007). Two studies on cognitively healthy older age participants showed significant hippocampal and amygdalar atrophy in APOEε4 carriers compared to APOEε3s (Honea et al., 2009; den Heijer et al., 2002). Another study on healthy older women documented that the presence of a single ε4 allele is linked to an increase in the rate of hippocampal atrophy (Cohen et al., 2001). In contrast, Ten Kate et al (2016) study on healthy mid-aged individuals found that hippocampal atrophy was associated with APOEε4 gene and family history (Ten Kate et al., 2016).

1.7.2 Cortical Thinning

Cortical thinning is a structural biomarker evident in MCI and AD patients (Thompson et al., 2007; Mishra et al., 2018). Correspondingly, older adult APOEε4 carriers showed a significant increase in cortical thinning in the subiculum and entorhinal cortex as well as average cortical thinning across all medial temporal lobe sub-regions combined, suggesting APOEε4 is a risk factor for developing AD (Donix et al., 2010). Moreover, localized to the hippocampal sub-regions, cortical thinning was also observed in mid age (45-55) APOEε4 carriers (Burggren et al., 2008). In contrast, Dowell et al reported higher cortical thickness in mid-age APOEε4 carriers compared to the APOEε3 peers (Dowell et al., 2016).

1.7.3 Grey Matter (GM) Atrophy

A study on healthy mid-age population showed an increase in regional GM atrophy in APOEε4 carriers compared to non-carriers as well as differential effects on brain regions usually affected in AD (Striatum GM atrophy) (Klunk et al., 2007; Liu et al., 2010; ten Kate et al., 2016). However, GM atrophy was not detected in the right superior frontal gyrus in APOEε4 carriers (Honea et al., 2009; ten Kate et al., 2016).

1.7.4 Hippocampal Activity

In a study on neurologically healthy mid to old age participants, APOEε4 carriers had an increase in both the magnitude and extent of brain activation (during memory and recall of

words tasks) during functional magnetic resonance imaging (fMRI). The increased activation was seen in regions related to memory such as the hippocampus as well as throughout the brain compared to non APOEε4s (Bookheimer et al., 2000). However, in another fMRI study, where hippocampal activity had been observed in patients with MCI, the presence of the APOEε4 did not contribute independently to hippocampal dysfunction and memory decline (Tran et al., 2017).

1.7.5 Medial-temporal Lobe (MTL) Activation

A study on cognitively normal older APOEε4 carriers showed less task-associated activation in the medial-temporal lobe (MTL) compared to non-carriers, but no increase in specific regions in task-associated activations in APOEε4s (Borghesani et al., 2008). In another study, increase in MTL activation while performing a task was found in APOEε4 carriers but not in non-carriers; these were older age cognitively healthy participants (Han et al., 2007).

1.7.6 White Matter Hyperintensities (WMHs) and White Matter (WM) Structure

Studies suggest that WMHs could be a biomarker for cerebral small vessel disease and BBB dysfunction (Wardlaw et al., 2015). In a study APOEε4 carriers (ε4/ε4) had significantly increased rate of WMH accumulation (22.5% per year) than (ε4/ε3) (10.0% per year) and (ε3/ε3) (6.6% per year) suggesting that APOEε4 is a major factor in WMHs (Sudre et al., 2017). WM structure has been identified in neuro-imaging studies to be disrupted in APOEε4 carriers (Heise et al., 2011; Westlye et al., 2012).

1.7.7 Blood-Brain Barrier

BBB is a crucial anatomical structure for the health and wellbeing of the tissues of the central nervous system (CNS). Having a leaky BBB causes neurotoxins to accumulate in the brain that will eventually cause neuronal death which, leads to neurodegenerative disease. Several studies on mice and post-mortem have shown BBB leakage in APOEε4 carriers. Accumulation of neuron poisoning substances such as hemosiderin, fibrin and thrombin (serum proteins) in the brain of APOEε4 mice have been reported and the rate of neuronal damage increased with age (Grammas, 2011). Other studies by Zlokovic et al (2011) and Bell et al on transgenic mice carrying the human APOEε4 showed BBB breakdown promoting the accumulation of toxic serum proteins in the brain inducing neuronal degeneration (Zlokovic et al., 2011; Bell et al., 2012). On the other hand, a study on a large cohort (n=292) of cognitively normal older Swedish participants (≥60-year-old) showed no significant difference in BBB permeability

measured by cerebral-spinal fluid (CSF)/plasma albumin ratio (Qalb) between APOEε4 and non-carriers (Janelidze et al., 2017).

1.8 APOEε4 and Blood Biomarkers

There has been a rising interest in research to recognize blood inflammatory biomarkers that contribute to AD and correlating these with the presence of APOEε4 genotype. Some of the blood biomarkers that differ between APOEε4 carriers and non-carriers that have been linked with AD or age-related cognitive decline are below. More background on blood biomarkers are presented in chapter 6.

1.8.1 C-Reactive Protein (CRP)

Higher than normal CRP often signals that there is inflammation somewhere in the body. The higher the CRP level in the blood, the greater the likelihood of acute disease or injury, including cardiovascular disease such as stroke and heart attack (Nehring et al., 2021). CRP is produced by the liver as a response to acute disease to indicate inflammation and thought to have a negative effect when produced excessively in certain cases causing more damage to the tissue involved by over activation of inflammatory cytokines (Cleland and Eranki, 2020). Higher levels of CRP correlated with cognitive decline in menopausal APOEε4+ compared to APOEε4- (Bojar et al., 2016). Moreover, a longitudinal study has linked chronically high CRP to an increased risk of AD in APOEε4 carriers only (Tao et al., 2018). However, several studies have shown lower CRP levels in APOEε4 carrier compared to APOEε3s, and associated the high CRP levels in non APOEε4s with cognitive decline and risk of dementia (Marz et al., 2004; Austin et al., 2004; Kahri et al., 2006; Haan et al., 2008). These studies were on Latino, German, Japanese and Finnish cohorts. A study on a Korean population has also found lower CRP in APOEε4 but higher white blood cells (WBC) suggesting that APOEε4 influences CRP production through a non-inflammatory response (Yun. et al., 2015). Therefore, more research is required to determine how inflammation may damage the brain and the neurovascular system and possibly contribute to AD, through understanding the risk factors such the APOEε4 and how it influences the bodies anti-inflammatory and inflammatory response.

1.8.2 Pro-Inflammatory Cytokines

Interleukin-6 (IL6), tumor necrosis factor alpha (TNFα) and Interferon gamma (IFNγ) have an inflammatory response associated with the progression of AD (Fillit et al., 1991; Blasko et al.,

1999; Silva et al., 2021). An association between increased levels of AD pathology (total tau) and levels of all inflammatory cytokines were established in APOEε4 carriers but not in APOEε3s in post-mortem community-based cohort, suggesting APOEε4 increases total tau through facilitating pro-inflammatory response (Friedberg et al., 2020). Further research is needed to evaluate the level of serum cytokines on mid-age APOEε4 carriers.

1.9 Hypothesis and Aim of this Study

The aim of this study was to develop methods to investigate the possible early detection of subtle brain changes in people at a higher risk of developing late-onset, non-familial AD later in life (APOEε4). The information obtained will increase our knowledge of the potential neuropathological effects of the APOEε4 gene in mid-age individuals who carry the additional risk factor. Specifically, we aim to assess whether APOEε4 can lead to subtle brain changes (BBB leakage, cerebral MBs, volumetric atrophy, structural brain changes and increase in inflammatory markers) in mid-age and whether such changes, if any, are related to fine deficits in cognition. This may provide the opportunity for early intervention to maintain and improve individuals' cognitive health as they age.

Chapter 2: Methodology

2.1 Ethics

The Brighton and Sussex Medical School Research Governance and Ethics Committee (BSMS RGEC) assessed the application of this project initially titled Blood-brain barrier permeability in mid-age APOEε4 carriers, which has been changed at a later stage to Exploring early signs of structural brain changes in mid-age Apolipoprotein E epsilon-4 carriers. The application is available in Appendix A. RGEC granted this project ethical and research governance approval to proceed starting November 26th 2018. The ethics Certificate of Approval is provided in Appendix B. An ethics application amendment was approved March 26th 2019, provided in Appendix C. The Minor amendment included extension of participants age as well as adding to the inclusion criterion “No current self-reported serious physical and/or mental illness”. The ethics Certificate of Approval for the amendment is provided in Appendix D.

2.2 Study Protocol/ Design

Data were collected while keeping the APOE status anonymized at all times. A qualitative protocol development tool version 1, 13 October 2017, research reference number (IRAS number: ER/NA391/2) was created to help design this study, shown in Appendix E. The study was divided into three phases; phase 1: genotyping, phase 2: blood test and cognitive task and phase 3: dynamic contrast enhanced MRI scan of the brain (DCE-MRI). During all three phases of the study, it was made clear to potential participants that they have no obligation to take part in the study and had the right to withdraw at any stage without any given reason; also that data would be shredded and destroyed upon the request of the withdrawing participant, but that this was not possible once the data has been published. This was clearly explained verbally as well as in both the information sheet and consent forms. The information sheet was provided to potential participants electronically via email during recruitment and a paper copy was provided upon request during their first visit. Participants signed three consent forms, where one was provided at the beginning of each phase during their visit. A documentation of undertaking tasks in the study in accordance with the data management and data protection policies was available for every participant at each phase. Participant information sheets, consent forms and documentation of undertaking tasks in the study are provided in Appendix F, G and H.

2.2.1 Participant information sheet and consent

Care was taken to ensure that recruitment was free from undue influence and recruitment material made no diagnostic nor therapeutic promise. Only healthy participants that met the inclusion but none of the exclusion criteria were invited to take part in the study. The information sheet was sent to the participants electronically and a paper copy was provided during their visit upon request. Volunteers were given two weeks to consider their participation. Plenty of opportunity was given to discuss the study and ask any questions before giving formal consent. Each phase had its unique consent form pertaining to the specific part of the study. Participants were asked to read each statement on the consent forms carefully, and print their initials next to each statement. They were then asked to sign on the back of the page including the date. Consents were then stored in a Human Tissue Act (HTA) folder. The HTA folders holding participant consents for each phase were stored at two different locations: phase 1 consent forms were stored at School of Psychology Human Psychopharmacology Lab (Pevensey 2) under HTA license, whereas phase 2 and 3 consent forms were stored at Trafford Labs on campus.

2.2.2 Compensation

Phase 1 (genotyping): the visit lasted approximately 5 minutes and participants received 5GBP Costa voucher. Phase 2 (blood test and 15minute cognitive task): this visit lasted approximately 30 minutes and participants received 7GBP and were offered a cup of tea or coffee at the end of the visit. Phase 3 (DCE-MRI): this last visit took about 90 minutes and participants received 60 GBP and offered water, a cup of tea or coffee at the end of this visit. This reimbursement was approved by the BSMS RGEC, based on the participant age group and level of investigation at each phase. In a couple of cases, where participants had cancelled their MRI visit, a repeat of the blood work was required. For each extra visit the participants made they were compensated again. All participants received a picture of their brain a few days after the scan and were also compensated for travel expenses and parking charges upon their request.

2.2.3 Location

Phase 1 took place at Human Psychopharmacology Lab (Pevensey 2). Phase 2 and 3 took place at the Clinical Imaging Science Centre (CISC), both locations are at Sussex University campus. Processing and Analysis of blood samples took place at Royal Sussex County Hospital and serum samples were processed and analysed at Affinity Biomarker Labs in London (Office G11, Imperial College London White City Campus, 80 Wood Ln, London W12 0BZ, United Kingdom).

2.2.4 Participants (sample size)

A target number was set to recruit 20 APOEε4 carriers (ε4+) and 20 APOEε3 carriers (ε4-) for this study. Sample size was computed using nQuery power calculator (<https://www.statsols.com/nquery>) by inputting mean and standard deviation values from two studies with similar imaging approaches to this project. First both studies, nQuery produced a sample size of 4 in each group, which would have a 95% power to detect a difference in means of 0.02 assuming that the common SD is 0.002 using a two-group t-test with a 0.1% two-sided significance level. Comparator studies measured BBB permeability using DCE-MRI in normal appearing white and grey matter, comparing multiple sclerosis (MS) patients with healthy controls (Cramer et al., 2014; Montagne et al. 2016). Both these studies had a sample size of 18-24 per group. Furthermore, a published study on a middle age cohort evaluated structural brain changes using neurite orientation dispersion and density imaging (NODDI) parameters between APOEε4+ and APOEε4- groups with 20 participants per group (Dowell et al., 2016). For this reason, we concluded a sample size of 20 per group was well powered for the primary measure analyses for this project.

2.2.5 Participants (recruitment)

Since the ε4 allele of the APOE gene is present in only ~15-25% of the population (Heffernan et al., 2016), the target population was achieved through a) new recruitment and genotyping (see below) and (b) approaches to volunteers on a database with ~200 previously genotyped mid-age volunteers from a previous project at Sussex university (ER/JENNYR/1); all volunteers had previously consented to be approached for further research studies. This database was supplemented by ~150 volunteers recruited under this study. All recruitment methods were approved by the BSMS RGEC as explained earlier.

Advertisement for the study was accomplished by 1. posting the approved advert around Sussex University and Brighton University campuses, 2. emailing several Sussex University Schools staff through the schools' faculty mailing lists and 3. placing the advert on the Sussex University website, at local schools, libraries and public meeting places. (Copy of the advertising material is in Appendix I).

2.2.6 Participants (inclusion/ exclusion criteria)

Participants' inclusion criteria for this study were: ages between 42-59, generally healthy and willing to participate in an MRI study if selected. Volunteers were excluded if: 1. They were people of African descent, which is due to the differences in physiological consequences of APOE gene (Farrer et al., 1997). 2. They had a current serious physical and/or mental illness

(self-reported). 3. They had any MRI contraindications such as: implantable devices (e.g. non-MRI compliant cardiac pacemaker), metal fragment lodged in the eyes or body, large or dark tattoos on the head or neck, pregnancy or claustrophobia. 4. They had any of the contrast media contraindications including: asthma, history of renal disease/kidney problems, allergies/sensitivity to contrast media. Participants who fell into the inclusion but not the exclusion criteria were contacted to take part in phase 2 of the study. A sample of the study eligibility questionnaire is provided in Appendix J.

2.2.7 Participants (eligibility)

During phase 2, participants underwent a blood test, to collect blood samples to measure inflammatory markers as well as to confirm participant eligibility to take part in phase 3 (DCE-MRI). Participants were considered eligible for phase 3 when the blood test results showed normal kidney function indicated by an estimated glomerular filtration rate (eGFR) >60 and were confirmed eligible by a physician from BSMS. The importance of a normal kidney function in a contrast enhanced imaging study is to make sure the participants' kidneys are able to excrete all contrast out of their system within a short period of time after injection. Participants were scheduled for the imaging phase within 2 weeks of their blood test and if phase 3 scheduling was delayed (more than 2 weeks) for any reason, the participant had to repeat the blood test to receive a recent kidney function result.

2.3 Study Materials

2.3.1 Phase 1 (Genotyping)

For each participant, an Isohelix swab with 5ml tube (DNA buccal swab- DNA isolation kit) from Cell Project Ltd Harrietsham Kent was used (Figure 2.1). Participants were compensated 5GBP Costa Coffee voucher at the end of this visit.



Figure 2.1 Isohelix SK-1S/MS-01 Buccal Swab with 5ml tube and a cap. Image from (<https://www.bocascientific.com/isohelix-tswab-buccal-swab-with-5ml-collection-tube-pack-of-100-p-2255.html>).

2.3.2 Phase 2 (Blood Test & Cognitive Task)

For the blood Test: Four BD vacutainer blood collection tubes were used per participant including 4ml (lavender cap), 5ml (gold/ gold top), 3.5ml (light blue cap) and 10ml (red cap EDTA tube), as shown in Figure 2.2 b. One BD vacutainer Safety-Lock blood collection set 21 G, 3/4" green, 7" (17cm) tubing, and a safety re-shielding cover, Luer™ adapter and a holder were used for the phlebotomy, shown in Figure 2.2 c. Blood sample in the 4ml lavender cap



Figure 2.3 Materials used for phase 2 blood sample collection. Tray with a. two Cryogenic vials, b. four vacutainers and c. one BD blood collecting set and one holder.

was taken by the researcher to Trafford labs immediately after the participant's visit with two 1.8 ml empty cryogenic vials, shown in Figure 2.2 c. for centrifuging and serum collection. A centrifuge was used in Trafford lab to separate plasma from serum. A Finn pipette of 1000 µL with disposable pipette tip

was used to transfer the serum from the EDTA tube to the cryogenic vials (Figure 2.3). Vials were stored in code order starting with NAS001 onwards in a special blue vial holder with a lid and in a zip log bag placed in a metal box with study number and researcher name printed on the exterior. These were stored in a shelf inside a -80° HTA freezer at Trafford Labs.

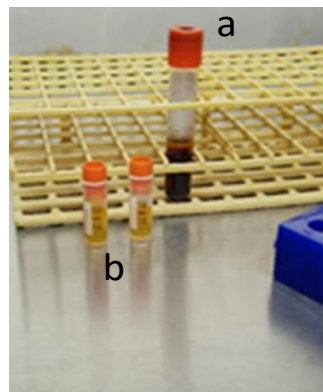


Figure 2.2 Phase 2 Serum sample collection: a. one 10ml red cap EDTA tube containing blood plasma and b. two 1.8ml Cryogenic Vials containing blood serum.

For the cognitive tests: two paper-based tasks were provided as well as an IBM-compatible computer with a 14-inch screen for two computer-based tasks. Complete tasks procedures and design are explained in section 2.4.2. At the end of this visit, participants were compensated 7GBP.

2.3.3 Phase 3 (DCE-MRI scan)

A Siemens PRISMA 3T MRI was used at CISC. An MRI safety questionnaire was completed and signed by a radiographer (Appendix K). Also, participants weight was measured prior to

entering the scanning room to calculate the contrast agent dosage. Cannulation equipment were prepared by experienced radiographers. This phase involved acquisition of structural brain images followed by contrast imaging to assess blood-brain barrier integrity. For this, participants were injected with Gadolinium contrast agent (Dotarem) followed by normal saline, this is to enable flushing of the lines to guarantee full dosage of the contrast is received by the participant. These are injected by a portable power injector controlled by the radiographers in the control room (Figure 2.4). Dotram is a paramagnetic macrocyclic ionic contrast agent used for MRI brain imaging intravenously. A leaflet containing information about Gadolinium (Dotarem) and its safety was sent to participants via email during the recruitment phase and is provided in (Appendix L). Three tissue mimicking phantom tubes were placed and secured with tape on the head coil before the start of the scan (Figure 2.5). These were used to correct for signal loss and are explained with more details in Chapter 5. Participants were given earplugs and headphones to help reduce the noise from



Figure 2.4 Phase 3 contrast injection equipment and material: a. MRI contrast power injector holding two syringes (each for contrast and normal saline) and b. Gadolinium (Dotarem) contrast vial. Images from a. (https://www.alibaba.com/product-detail/MRI-Zenith-C60-Dual-head-automatic_62578409413.html) & b. (<https://med.mahidol.ac.th/aimc/en/km/30jul2019-1517-0>).

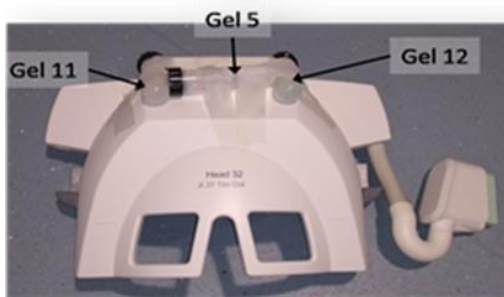


Figure 2.5 A 32 channel head coil and the three gel phantoms mimicking brain tissue (Gel 5, 11, 12).

the scanner and to enable communication between radiographer and participant in between scans. A screen displaying a BBC documentary video was available upon request to help soothe the length of the scan for the participant. A compensation of 60GBP cash and an adverse event management card was provided at the end of the session, which provided details of the contrast agent administered to the participant

and the dosage given in case of any adverse reaction after the participant left the building (provided in Appendix M).

2.4 Study Procedure

At the beginning of each phase, the researcher greeted the participant and thanked them for their interest in taking part in this study. The researcher asked the participant if they have read the information sheet and if they wanted a hard copy. Both the participant and researcher signed and dated the consent form and the researcher filled out the documentation of undertaking task. At the end of each phase the participant was thanked again for their participation and compensated for their visit.

2.4.1 Phase 1 (Genotyping)

This phase comprised 8 brief steps: 1. The participants were asked if they were NPO (had nothing per mouth) in the last hour. 2. Participants were instructed to rinse their mouth with water using a disposable cup. 3. The researcher explained the swabbing process while making sure not to touch the swab at any time, by displaying the swab, pointing to the cotton bud, and explaining that they need to rub the cotton bud firmly on the inside of one cheek for 30 seconds, then the other cheek for 30 seconds, while timing them. 4. After swabbing, they were asked to break the stick at a small indent while placing the swab inside the tube then securing it with a lid (Instructions for use of Buccal Swab provided in Appendix N) 5. The tube was labelled on both sides with a code (using a permanent marker). 6. The participant was thanked for their participation and compensated for their time. 7. The swabs were then stored in a -20° HTA freezer and consent form saved in the HTA folder, stored and locked in a secure HTA cabinet at the School of Psychology Human Psychopharmacology Lab (Pevensey 2). 8. The participant information, swab location and code were then documented in a secured HTA data base (item tracker).

School of Psychology at Sussex University has a standard operating procedure (SOP) for human genotyping-cheek swab (Copy of the SOP is provided in Appendix O). Care was taken to ensure participants genotype was confidential and not disclosed to the researcher at any time. Samples were batch sent to Biosearch Technologies LGC Hoddessdon UK with full compliance with HTA procedures and a Material Transfer Agreement in place (provided in Appendix P). The results were sent back to a third party in the school to ensure anonymization.

A triangulated procedure involving two anonymized codes per sample and a third party (another member of faculty) ensured that neither the testing researcher nor the volunteer is provided with genotype information. The third party selected from the returned genotyping procedure a subset of codes representing potential volunteers from all required genotypes (unlinked). In this instance, as in previous studies, we compared APOEε4 carriers (ε4/ε4 and

e4/e3) with the population norm, APOEε3 carriers (ε3/ε3). To avoid adding heterogeneity to the non-APOEε4 group, only APOEε3 were considered and the APOEε2 participants were excluded. The lead researcher translated these codes back to names, which were provided in batches of 20, with approximately 12 APOEε3 and 8 APOEε4 in each batch. All procedures for acquisition, storage and processing of samples for genotyping complied with the HTA license held by the University (Appendix Q). After all image processing was completed, final code breaking was completed on the anonymized data spreadsheet to allow statistical comparisons between genotype groups.

About 100 volunteer codes were returned to be contacted by the researcher to take part in phase 2 of the study. Over 55% were either ineligible due to newly developed health issues, did not reply to the invitation, contact information had changed or did not want to take part in this study. The remaining participants happily took part and were happy to take part in future research in this area.

2.4.2 Phase 2 (Blood Test & Cognitive Task)

The participant was invited to phase 2 via email after they have been randomly selected by a third party based on their APOE status. This phase comprised two parts, a blood sample collection and cognitive tasks. The blood sample collected enough blood (plasma and serum) to measure inflammatory markers as well as to measure the participant's kidney function. The kidney function measure, indicated by the eGFR results, determined if the participant is eligible for phase 3.

After the participant arrived to CISC, read and signed the consent form, he/she was taken to the phlebotomy room and the on-call physician asked the participant if they were happy for their blood to be taken, and collected four tubes of blood, labelled by the researcher with codes specific for this phase (NABxx). A request form supplied by the Royal Sussex University Hospital (RSUH) pathology laboratory was completed, with samples to be sent, including participant code, age, gender and the date. Three blood tubes were placed and secured into a bag attached to the request form which was then transported to the onsite university clinic, where a courier transported all blood tubes and samples to RSUH pathology laboratory to be analyzed. The sample results were then sent back to CISC in a confidential envelope a week to two weeks later. eGFR results were also available via phone 24 hours later, in order to confirm eligibility for phase 3. The remaining 10ml red cap EDTA tube was taken by the researcher to Trafford center labs for serum extraction after the cognitive task was completed. For serum

extraction, the EDTA tube was placed in a centrifuge at a speed of 2500 spins for 13 minutes. This was the standard protocol for all samples in this study, although in a couple of cases, spin time was increased to reach optimum separation. Once the plasma and serum were separated, under a fume hood, the serum was extracted from the tube via Finn pipettors and placed into cryogenic vials. The vials were labeled (NASxx) and stored in a -80° HTA freezer. The consent form was then stored in the HTA folder in Trafford Centre. The serum vials were stored at Trafford center until the data collection for the whole study was completed. Serum vials were then transported to Affinity Biomarker Labs in London, for serum analyses with full compliance with HTA procedures and a Material Transfer Agreement in place (as shown in Appendix O). Serum analysis panels and kits were purchased from Meso Scale Discovery (MSD), Maryland, USA. Individual standard procedure used for each inflammatory biomarker analysed by Affinity Biomarker Labs for this study are provided in Chapter 6.

After the blood collection, the participant was asked to go to a study room at CISC for the second part of this phase. This part required participants to perform 15-20-minute cognitive tasks. These were memory and executive function tasks which were selected on the basis that they were sensitive markers of early cognitive change associated with dementia pathology, especially with the presence of the APOEε4 gene (Deary et al., 2002; Albert et al., 2014).

The four tasks were always completed in the same order and comprised:

1. FAS Verbal Fluency Task was developed by Borkowski, Benton, and Spreen in 1967. Verbal fluency is an executive function task where words are retrieved from semantic memory (Lezak et al., 2004). The words to be produced start with the letters F, A and S as these letters have been shown to produce variable performance (Barry, Bates, & Labouvie, 2008). For this task, the participant was given full and clear instructions by the researcher: “I would like you to verbally say as many words as possible starting with a specific letter within one minute”. “The words must not be names of people such as Sarah, places such as Spain or brands such as Samsung”. “The words must not be the same word with different endings such as play, playing, played or sing, sang, singing”. The researcher said a letter, turned the stopwatch on and wrote the words as the participant said them. At a later time, after all data for all participants have been collected, the researcher reviewed and recorded the total of correctly recalled words, as well as total error (number of unrelated errors, semantic errors and phonemic errors).

2. Episodic Memory word Task: This is a working memory task that measures immediate verbal recall (Rusted and Warburton, 1989). In this task, both a computer and paper were

required. A PowerPoint was presented to the participant on a laptop screen. The researcher asked the participant to read the instructions on the slide carefully “you will see words come up on the screen one at a time. Please try to remember as many of the words as you can for later recall”. Each slide had a 2-second interval where one slide presented a word and followed with a blank slide for a total of 20 words. The last slide had the following instruction “On the sheet provided, please write down all the words that you can remember from the list”. The participant was given time to recall and write as many words from the list in any order. Total correct recalled words from the list were recorded as well as total error (number of unrelated errors, semantic errors and phonemic errors).

3. Prospective-Retrospective Memory Questionnaire (PRMQ): This is a paper-based questionnaire which includes a total of 16 questions (8 prospective memory questions (PM) and 8 retrospective memory questions (RM)) and the participant indicates to which extent this applies to him/her (Smith, Della Sala, Logie & Maylor, 2000). This task helps identify the type and frequency of memory mistakes in normal day life, where higher scores indicate more memory failures. A copy of the PRMQ is provided in (Appendix R). Scoring for the PRMQ task was straightforward, where the total PM scores indicated by (PM short term +PM long term) and total RM scores indicated by (RM short term +RM long term) and the total PRMQ score (PM+RM) were calculated.

4. Processing Speed and Prospective Memory Card Sorting Task: This task evaluates speed of processing, as well as the retrieval and implementation of a given prospective memory instruction (Rusted et al., 2009). For this task we used an E-prime software, and the key board number 1 and 2 keys had been labelled with a red HEART and a black SPADE. The tasks were divided into a baseline and a PM deck. where the first part (baseline) had 52 cards, and measured speed of processing of the ‘sort’ task, while the second part (PM) had 104 cards, and measured processing speed plus prospective memory. The card face was displayed on the screen for 750ms each time and the back of the card was displayed in between card faces for 1000ms, the card flipping was automatic. Instructions were presented to the participants on the screen as follows: “You will be presented with a set of cards, and you will have to sort them into HEARTs and SPADEs”, “you press the key with heart when you see a heart and press a spade when you see a spade. You will ignore the DIAMONDS and CLUBs”. “You must respond as quickly as possible.” Then the participant was asked “Did you understand the instructions?”, “Can you repeat the instructions to me please?”. Then the participant was told “The first part is a short practice followed by the card sorting task. Let me know when you are

ready.” After the first part of card sorting was completed, the participant was given a working memory task for distraction: The Backward Digit Span, where the participant verbally counted numbers backwards from a given three-digit number for one minute (example: 350) (Wechsler, 1981).

The participant was asked to look at the screen again to read the given instruction for the next and last part of the task: “You will be given another set of playing cards: you will continue with the same instructions given previously, but this time you will ALSO press the spacebar when you see a 7 card regardless of the suit”. This task recorded reaction time and number of correct/error cards. The participant was then compensated and thanked again for their visit.

2.4.3 Phase 3 (DCE-MRI)

Participants with normal eGFR results were confirmed eligible by a BSMS physician and were contacted to take part in the scanning phase. Each participant was given a unique CISC code. All codes for each phase were linked on a separate file. Prior to the scan, the participant was interviewed by a radiographer, asked to answer an MRI safety questionnaire and sign a consent form for CISC, in addition to the specific consent form for phase 3 of this study. The participant’s renal function result and date were checked again by the radiographer to ensure safety of Dotarem contrast injection. Weight of the participant was taken and documented to calculate the amount of contrast to be injected. The participant was asked to take off any metal on the body (jewellery, belts, hair clips, etc.) and put all belonging in a locker with a key kept in the control room. The participant was invited to the scanning room by the radiographer, asked to take off shoes for comfort and to lay on bed with a cushion under knees for comfort. A blanket was provided to keep them warm during the scan, ear plugs and head phones to reduce noise from the scanner and to enable communication with the radiographer from the control room and a buzzer button held by the participant in case they needed to stop the scan for any reason. The radiographer secured a cannula into the participants’ arm prior to the scan and connected to the power injector.

During this time the researcher secured the tissue mimicking phantoms on the head coil. The head coil was then placed over the participants’ head and the TV turned on to entertain the participant during the scan. The scanning time was about 70 minutes long, and the contrast was injected towards the last 15 minutes of the scan. Details of scanning protocol and acquisition is explained in Chapter 3.

After the scan, the cannula was removed by the radiographer and the participant was assisted while leaving the scanning room. The researcher provided the key to the locker and checks that the participant is feeling well. Participant was asked to stay in the waiting room for at least 10 minutes before leaving, offered a drink and instructed to drink lots of fluids to flush out the contrast. The participant was given an adverse event card, was thanked for taking part in this study and compensated for their time. Imaging data were saved on a storage space at CISC and were pre-processed and analyzed by the researcher after completion of data collection for phase 3.

2.5 Challenges and Limitations

Middle-aged people are usually at a very busy stage in life, considering family time and working hours, they have limited time to spare. As this study required a considerable amount of time, commitment which was sometimes difficult, and therefore recruitment was particularly challenging. Another important factor to note regarding the target age range, was that many people have developed chronic illnesses by mid-age, whether mental and/or physical which require medication, therefore, had to be excluded from this study. Nevertheless, the data collected from the target population set for this study reached the estimated sample size required with equal group sizes.

An additional challenge was the length of the scan during phase 3 which was sometimes not welcomed by participants, especially if they suffered from back problems. Although we expected the injection of a contrast agent would be an impediment for participants, luckily only a couple of eligible candidates rejected taking part due to the fear from contrast side effects.

Because the e4 variant of the APOE is only present in ~15-25% of the population, it required recruitment of over 150 new volunteers to supplement another 200 genotyped volunteers to be able to reach an equal number of participants per group (APOEe3 and APOEe4). Therefore, this was the most challenging phase for this study, especially given the challenges stated earlier with the target age group.

During phase 2, some limitations were faced with the collection of one serum vial rather than two for two participants. These were due to problems encountered with the centrifuging process, where the plasma clotted sooner than usual and only a small amount of pure serum was collected. Fortunately, this amount was adequate for serum analysis. Another limitation was with RSUH pathology laboratory, where in one case at the very beginning of phase 2, they discarded a complete sample of 3 blood tubes due to insufficient identifications labelled on the

tubes and consent form. This was made clear to the researcher after the loss of this sample, where two identification information must be labelled for every volunteer. In this case, the participant was re-contacted and asked to revisit the centre for more blood withdrawal and compensated for their visit again. One other participant had to delay phase 3 after completing phase 2 due to an unexpected scheduling for knee surgery, this resulted in a repeated blood test after recovery and within two weeks prior to phase 3. The participant was also compensated for the second visit.

Phase 3 was challenging as well for several reasons. The scanning slot was booked by the researcher in advance and could only be cancelled with no financial obligation 48 hours prior to the scanning date. The scanning slot booked for each participant was 90 minutes. Luckily, only one participant cancelled on the day of the scan, while all other participant invited to phase 3 attended their booked sessions. In a few cases, participants attended late, therefore scanning time was extended over the 90minute slot and caused conflicting situation with other booked researchers. On one occasion, after 10 minutes from the start of the scan, one participant felt unsure and panicked so pressed the button and asked to stop the scan and to leave the scanning room. Therefore, no phase 3 data were collected for this participant. The major limitation in this phase was the low image quality in some cases due to participants moving during the scan. It was very challenging for participants to stay still for 70 minutes, thus comforting the participant with knee pillow and a BBC documentary was beneficial to an extent.

2.6 Funding

This study was funded by King Saud University (KSU), Riyadh, through the Saudi Arabian Cultural Bureau in the United Kingdom (SACB), London. The funding covered phase 1, 2 and 3 equipment and material, 3690 minutes of scanning time, compensations and the transfer and processing of DNA and serum samples.

Chapter 3: Overview of Structural MRI Techniques and Imaging Protocol Development

3.1 Introduction

Certainly one of the greatest medical advancements in history is the development of imaging modalities to aid in the investigation and diagnosis of disease and injury of the human body and specifically for this study, the human brain. Understanding the anatomical properties of the brain and localization of physical defects are critical for diagnostic and research purposes. For this reason, structural brain imaging has been used for many years as a method of investigation, which enables clear visualization of all different brain structures and identification of abnormalities such as blood clots, haemorrhages, tumours or changes in size or homogeneity. Structural brain imaging has been used for the diagnosis and investigation of brain diseases, including Alzheimer's disease (AD) for almost a century (Hirsch et al., 2015).

For many years, neuroscientists and medical physicists have worked together on the improvement of image quality and sensitivity for the achievement of accurate detection of pathological or physiological changes at its earliest stages. Moreover, the safety of the imaging techniques used for investigation are of great importance, therefore, the development of less/non-invasive imaging modalities was essential. Although, the advancement of neuroimaging modalities to date is extraordinary, each has advantages and disadvantages when it comes to AD investigations (Table 3.1).

Table 3.1 Comparison of structural neuroimaging techniques for AD studies including advantages and disadvantages of each technique.

Structural Imaging technique	Advantages	Disadvantages	usage for AD studies
CT ^a +	<ul style="list-style-type: none"> -Mostly available. -Fast acquisition time and image processing time. -Useful for visualization of brain structure including skull and brain abnormalities. -extensively used in brain injury. 	<ul style="list-style-type: none"> -Exposure to ionizing radiation (x-ray). -Lower spatial resolution compared to MRI. 	<ul style="list-style-type: none"> -Detection of whole brain atrophy.
PET ^b +++	<ul style="list-style-type: none"> -Less sensitive to motion artefacts compared to other techniques. -Useful in identifying glucose metabolic changes in AD. 	<ul style="list-style-type: none"> -Low availability in medical centres. -Highly invasive with injection of radioactive tracers. -exposure to ionizing radiation (gamma rays). -Lower spatial resolution compared to other techniques. -Expensive and complex procedure. 	<ul style="list-style-type: none"> - Useful for differentiating between AD and MCI. - BBB^c permeability measures (Qalb^d), especially in vAD^e.

PET ^b +++		-exposure to ionizing radiation (gamma rays). -Lower spatial resolution compared to other techniques. -Expensive and complex procedure.	
qMR ^f and DCE-MRI ^g ++	-Commonly available (1.5T but not always 3T scanner). -Clear visualization of different brain tissues (grey matter and white matter track). -useful for Identification of brain abnormalities, homogeneity of brain tissue, localization of volume changes, tissue connectivity and vascular changes. -High spatial resolution. -less/non-invasive	-Noisy. -uncomfortable especially for claustrophobic people. -Long image acquisition and image processing time. -Complex procedure. -Cannot be used in the presence of metallic implants such as pacemakers.	-Identification of ROI's with volumetric changes. -Localising micro bleeds. -Useful for longitudinal studies and repetitive scans for disease progression. -BBB permeability measures (Patlak model) ^h

(Hirsch et al., 2015; Ortiz-Terán et al., 2011).

+ / ++ / +++ cost of imaging modalities where, + is lower cost and +++ is higher cost

^a Computed Tomography

^b Positron Emission Tomography

^c Blood-Brain Barrier

^d CSF/Plasma albumin ratio

^e Vascular Alzheimer's Disease

^f Quantitative Magnetic resonance imaging

^g Dynamic contrast enhanced MRI

^h A pharmacokinetic analysis model to measure the concentration of Gadolinium in the extravascular-extracellular space (EES) (Patlak et al. 1983)

Structural brain imaging using higher tesla MRI scanners such 3T scanners, has been shown to produce high resolution brain images. This is very useful for the early identification of brain defects, making this a valuable technique for the investigation of subtle brain changes in a disease-free population but at a higher risk of developing AD at a later age. Additionally, structural MRI is non-invasive and free from ionizing radiation, making this a safe neuroimaging technique for both patients and research participants.

Developing an accurate and precise neuroimaging protocol that evaluates structural differences between healthy and diseased groups and detects very low BBB permeability at its earliest stages is of high importance in early detection and prevention studies. The aim of this chapter was to:

1. provide an overview of structural brain MRI acquisitions used for AD investigations and image analysis.
2. Develop a BBB imaging protocol that acquires subtle changes in permeability with high accuracy in a healthy population at mid-age (Pilot Study)
3. To evaluate the developed protocol on the actual main project and to provide some of the limitation encountered from the BBB protocol in the main study.

3.2 Overview of Structural Brain MRI

Structural imaging of the brain in AD studies, includes a couple of techniques, where each can provide valuable information of the brain. These include, quantitative MR Imaging (qMR) and dynamic contrast enhanced MRI (DCE MRI).

3.2.1 Quantitative MR Imaging (qMR)

Quantitative MRI of the brain is an imaging technique used to quantify structures to enable estimation of normal appearance, level of change or severity of the brain (Rosenkrantz et al., 2015). For many years, qMR has been used extensively for the study and understanding of the complex, normal appearing brain tissues and regions, as well as for the investigation of underlying changes in disease or injury cases (<http://www.rsna.org/QIBA.aspx>). Due to their high sensitivity, qMR techniques used in neuroimaging had developed greatly and this advancement in technology has enabled not only early detection of AD but also the investigation of early structural brain changes prior to the diagnosis of disease (Pierpaoli,

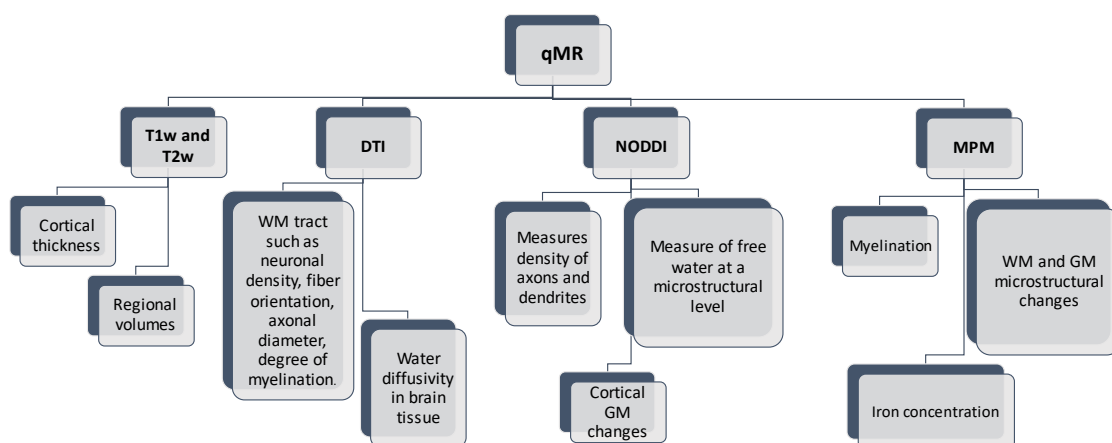


Figure 3.1 Simple diagram showing qMR imaging acquisitions and the type of data each can acquire for AD studies. (qMR)= quantitative magnetic resonance techniques. (T1w and T2w)=T1 weighted and T2 weighted structural imaging. (DTI)= diffusion tensor imaging. (NODDI)= neurite orientation dispersion and density imaging. (MPM)= multi-parametric mapping. (WM)= white matter. (GM)= grey matter.

2010). Some of the important qMR techniques include; T1 weighted (T1w) and T2 weighted (T2w) imaging, diffusion tensor imaging (DTI), neurite orientation dispersion and density imaging (NODDI) and multi-parametric mapping (MPM). An overview of the data that can be acquired from each of these qMR techniques for AD studies are shown in figure 3.1.

3.2.1.1 T1-weighted (T1w) and T2-weighted (T2w) imaging

Regional volumes and cortical thickness are important imaging indicators for normal and abnormal appearing brain in AD studies. As GM and WM are closely adjacent to each other, it is very important to be able to differentiate the two tissues in imaging studies to study volumetric changes in specific region of interest. Because T1w and T2w imaging have high spatial¹ resolution as well as high contrast between GM and WM, they are greatly used for volumetric studies (Chandra et al., 2019). T1w images are produced by short echo time(TE)² and short repetition time (TR)³, which are very useful in gadolinium (Gd) enhanced studies that assess vascular structures and BBB integrity (<https://radiopaedia.org/articles/mri-sequences-overview>). Gd appears very bright in T1w images due to changes in signal intensity by shortening T1. On the other hand, T2w images are produced by longer TE and TR, which are useful in detecting hyper-intensity lesions and plaque.

3.2.1.2 Diffusion Weighted Imaging (DWI)

DWI is an MR imaging technique that measures random motion of water particles (Brownian motion) within the tissues (Baliyan et al., 2016). DWI is quantified by a diffusion coefficient map and is used to evaluate disease progression (Baliyan et al., 2016). The pixels collected in

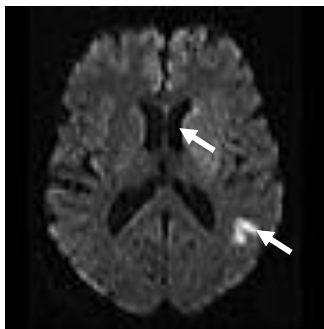


Figure 3.2 Diffusion weighted imaging of the brain of a patient showing CSF in ventricles in black and ischaemic stroke in white (image sourced and modified from Hobohm et al., 2014).

DWI can greatly identify diffusivity by the greyscale colour of the voxels, for example CSF in the ventricles will look dark due to high diffusivity and regions with ischemic stroke will appear white due to low diffusivity as shown in Figure 3.2 (Holdsworth et al., 2008). Because most cell structure has limited permeability, diffusion is often restricted to a specific direction (called anisotropic diffusion), in fiber for example, WM diffusion is higher than and perpendicular to fiber, this allows for evaluation of structural dysfunction (Holdsworth et al., 2008). Therefore, DWI allows for acquiring many volumes with

¹ Spatial resolution in MRI is defined as the ability to separate two adjacent structures through the selection of very small pixel sizes usually <1mm (Reeth et al., 2012).

² TE is the amount of time between the delivery of the pulse and the echo signal received.

³ TR is the amount of time between magnetic pulse sequences applied to the slice.

DW directions distributed across several different directions. The advancement in DWI, allows for different diffusion models to be applied to the imaging data to explain the diffusion weighting patterns that are observed (i.e. the diffusion of water molecules in the neuronal tissue) and in this scenario the diffusion-weighted signals can be measured as diffusion-tensor (DT) (Basser et al., 1994b; Bammer et al., 2009). Several microstructural diffusion properties can be measured from DT models in multi diffusion directions (Quantitative MRI of the Brain: Principles of Physical Measurement, 1st Edition, 2018, CRC Press, Pages 28, ISBN 9781315363578).

3.2.1.3 Diffusion Tensor Imaging (DTI)

DTI is a model of diffusion in the brain, which measures how the water diffuses in brain tissue. The DT model is used in DTI which produces parameters that quantify the average diffusivity and requires a minimum of 6 different diffusion directions. These parameters are: **Fractional Anisotropy (FA)**, as the MRI scanner detects direction of water diffusion, axons tend to direct water parallel to WM. FA can go from 0 to 1. Isotropic diffusion of water molecules is FA=0 such as in CSF, while diffusion purely along a single direction (anisotropic diffusion) is FA=0.7-0.9 such as in Fiber bundles. In other words, FA is a parameter that quantifies the susceptibility of water molecules to diffuse in a particular direction; it is higher in ordered microstructural tissues such as WM and is often used as a marker for WM integrity (Quantitative MRI of the Brain: Principles of Physical Measurement, 1st Edition, 2018, CRC Press, Pages 28, ISBN 9781315363578). Loss of WM integrity is expected to result in reduced FA. **Mean Diffusivity (MD)** is a parameter that measures the direction of diffusion of water molecules in neuronal tissue (Alexander et al., 2007). It simply describes how easily the water molecules diffuse. Higher MD means the water moves more freely, possibly due to structural breakdown. **Axial Diffusivity (AxD)** This is the diffusion coefficient in the preferred direction of diffusion only (e.g. parallel to the WM fiber tract) i.e. it is the measure of anisotropy and clinically it is used as a measure of axonal degeneration (Alexander et al., 2007). Higher AxD is thought to reflect damage to the axons. **Radial Diffusivity (RD)** is the diffusion coefficient perpendicular to the preferred direction of diffusion and has been used as an indicator of WM pathology (Stebbins and Murphy 2009). Increase in RD is linked to myelin abnormalities.

DTI is considered a sensitive imaging technique for neuropathology via the combination of all DT parameters, however, it has many limitations including low signal to noise ratio (SNR) which may be overcome by reducing resolution or increasing scanning time, and in the case of long scanning time, motion artefacts will increase, partial volume averaging between tissue

and regions of fiber crossing (Ranzenberger and Snyder, 2020). Low specificity is another major limitation in DTI, where it is nonspecific to individual tissue microstructures (Alexander et al., 2007).

3.2.1.4 Neurite Orientation Dispersion and Density Imaging (NODDI)

NODDI is a more advanced model that requires more DW images than DTI, where NODDI needs two shells (i.e. 2 b-values), however the DTI model will also work with these data too. NODDI was developed to overcome the limitation of DTI where NODDI is more sensitive to subtle microstructural change (Timmers et al., 2016). NODDI models DW signals by combining three tissue compartments: neurites, extra-neurites, and cerebro-spinal fluid (CSF) (Zhang et al., 2012; Palacios et al., 2020), each with different patterns of diffusion, and enables in vivo estimation of the following parameters: **neurite density index (NDI)** is a quantitative measure of the density of axons and dendrites in the brain. NDI has been shown to be a reliable scanning parameter in detection of age-related microstructural changes in the brain due to its high sensitivity compared to DTI measures (Genc et al., 2017). Lower cortical NDI indicates disruption to the cortical microstructure (Parker et al., 2017). **Orientation dispersion index (ODI)** which is the degree of different neurite orientations/ alignments. If many different orientations are detected in a location, this will result in a high ODI, whereas if the neurite directions are more consistent at that location, then ODI will be lower. Higher ODI in WM indicates axonal ineffectiveness, while lower ODI in GM indicates damage to dendrites. ODI has been shown to be highly sensitive to age-related cortical GM changes, where ODI is lower in GM regions indicating GM microstructural disruption that increases with age (Nazeri et al., 2015). **Isotropic diffusion fraction (f_{iso})** which represents the fraction of free water within the tissue at a microstructural level which diffuses isotropically (Zhang et al., 2011). Increase in free water concentration (f_{iso}) indicates microstructural damage (Raghavan et al., 2021).

Although NODDI model has an advantage of both higher sensitivity and high specificity for microstructural pathology than DTI, NODDI requires a more complex and extensive amount of time for image processing compared to DTI, where NODDI may require up to 25 hours to calculate parameters, DTI requires about 10 seconds (Zhang et al., 2012). From here, combining both models with multiple parameters for increased sensitivity and specificity at microstructural level will produce information that may complement one another and confirm accuracy of results especially in subtle diffusivity measures (Kamiya et al., 2020).

3.2.1.5 Multi-Parametric Mapping (MPM)

MPM is a quantitative imaging technique that can provide measurable quantities mainly for comparison between groups, imaging centres or different time points via combining multiple parameters to produce images of the whole brain in a 20-minute time frame (Weiskopf et al., 2013). MPM image acquisition uses multiple echo gradient with different flip angles (5° , 15° , 25°) to allow quantification of these parameters; magnetization transfer saturation (MTsat), proton density (PD), longitudinal relaxation rate (T1), and transverse relaxation rate (T2*). More information about the clinical implications of each parameter is found in chapter 4. In general, MPM provides valuable information with high sensitivity and high resolution about the microstructures of WM and GM and in particular, iron concentration and myelin (Weiskopf et al., 2013; Carey et al., 2018; Polzehl & Tabelow 2019; Cooper et al., 2020).

3.2.2 Dynamic contrast enhanced MRI (DCE-MRI)

DCE-MRI is a dynamic T1w acquisition of the circulation of contrast medium across a time frame and typically, it is an in vivo study of tissue vascularity (Nielsen et al., 2012). Detection and evaluation of blood vessel permeability in the human brain is of great importance in studying progression and development of neurodegenerative disease such as AD. As the blood-brain barrier (BBB) is a critical area of investigation due to its delicate properties and important functions in protecting the brain tissue from any toxins and pathogens (Keaney and Campbell, 2015), it was very important for neuroscientists to develop an imaging technique that enables clear and safe visualisation of the BBB and in vivo. Therefore, in recent years DCE-MRI has been developed and used extensively for vascular studies including BBB permeability (Tofts and Kermode, 1991; Larsson et al., 2008,2009; Tofts, 2010; Armetage et al., 2011; Cramer et al., 2014, 2019; Heye et al., 2014,2016; Barnes et al., 2015; Thrippleton et al., 2019; Verhagen et al., 2020). Although BBB disruption is part of normal aging, it has been found to be detectable at a pre-clinical stage of AD, prior to the development of any cognitive symptoms (Medina et al., 2016; Verheggen et al., 2020). Consequently, the development of BBB imaging protocol through a selection of parameters sensitive to subtle change in permeability and leakage is of great interest.

3.2.2.1 DCE-MRI Modelling

Neuroscientists have investigated BBB permeability for several years, and have implemented different methods to quantify BBB permeability, including DCE-MRI, CSF/serum albumin quotient (Qalb) and BBB leakage measurements in positron emission tomography (PET), each method comes with a set of strengths and limitations shown in table 3.2. Signal changes in

DCE-MRI images can be modelled for BBB permeability by a number of different approaches with differing complexities. e.g. two popular approaches are Tofts model which is a 3-parameter fit and Patlak model which is a 2-parameter fit. Patlak model is most appropriate for subtle leakage (e.g. in healthy individuals) because it doesn't need to consider back flux (Gadolinium (Gd)) going back into the blood vessels) and so only has 2 parameters to fit.

Table 3.2 Summary of quantitative BBB permeability techniques for AD investigations.

Quantitative BBB permeability methods in AD studies	Advantages	Disadvantages	What it measures for AD studies
CSF/serum albumin quotient (Qalb) ^a	-Indicator for vascular AD (vAD)	-Highly invasive (lumbar puncture) -Cannot Localize BBB leakage -Unreliable (May produce false positive results of Qalb or underestimate BBB leakage) ^a -Long procedure time	-quantification of Qalb as a measure of BBB permeability
Diminished glucose transport (FDG-PET) ^b	-Can detect very early BBB malfunction before evidence of cognitive decline -useful in pre-clinical stages and early AD -Short procedure time	-Highly invasive with injection of radioactive tracers (ionizing radiation) -FDG does not metabolize easily and is eliminated from the brain very slowly	-Evaluates cerebral blood flow via FDG brain uptake (estimates BBB function) -indicating stage of AD
T1 or T2*-weighted with contrast ^c	-short scanning time	-can only detect high leakage rates - Non quantitative method	-Pixel intensity changes due to contrast leakage
DCE-MRI Patlak model ^d	-Sensitive to subtle BBB changes -High accuracy -Localization of BBB leakage -fast acquisition time -Quantitative analysis ^f	-Low image quality due to long scanning time -image brightness due to contrast ^e -Complex -motion artefacts	-Estimates small leakage (K_{trans}) -Measures fractional plasma volume in the selected region (V_p)

^a (Janelidze et al.,2017; Raja et al., 2018; Sweeney et al.,2018).

^b ¹⁸F-fluoro-2-deoxyglucose (FDG) uptake in positron emission tomography (Sweeney et al., 2018)

^c (Taheri et al.,2011)

^d (Tofts 1991 Tofts and Kermode, 1991; Larsson et al., 2008,2009; Tofts, 2010; Armetage et al., 2011; Cramer et al., 2014, 2019; Heye et al., 2014,2016; Barnes et al., 2015; Thrippleton, 2019; Verheggen et al., 2020)

^e Boxerman et al., 1995

^f Bowden and Barrett, 2011

3.2.2.2 Patlak Modelling

Patlak model is a fitting model used in DCE-MRI acquisition to measure and localize BBB leakage via extraction of several parameters including K_{trans} and V_p . K_{trans} is the metric used to quantify permeability of the BBB, and used to evaluate permeability differences between groups. V_p is the fractional plasma volume in the selected region of interest (Tofts et al., 1999; Tofts, 2010; Barnes et al., 2015; Montagne et al., 2015,2020; Thrippleton, 2019). A Patlak modelling diagram illustrating parameters generated by the Patlak model is shown in figure 3.2.

While several factors influence DCE-MRI acquisition protocol, including image analysis, acquisition time and temporal resolution⁴. Introducing the Patlak model with high quality parameters was suggested for subtle leakage rates (Cramer and Larsson, 2014). Lower permeability measures indicated by lower K_{trans} values are evidence of subtle disorders as in early AD (Barnes et al., 2015; Cramer and Larsson, 2014). In addition to all the strengths Patlak model has to offer, it was advantageous over other models by the simplicity of only a few parameters which reduces the chances of error in overfitting as in complex models (Raja et al., 2018).

Therefore, because DCE-MRI using Patlak model is advantageous over other techniques especially the high sensitivity in assessing subtle BBB changes and accurate

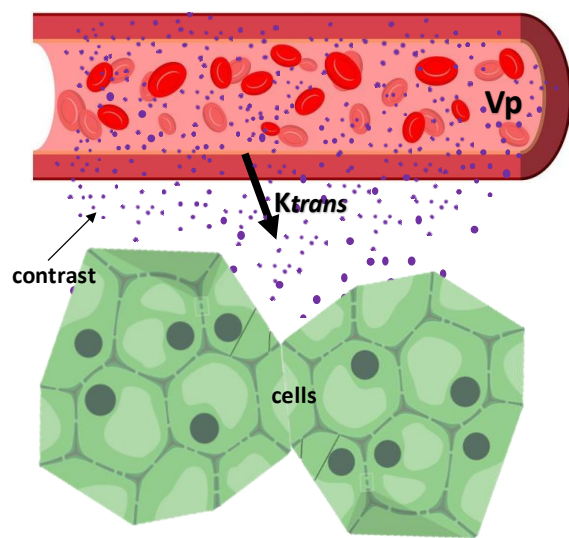


Figure 3.3 Diagram showing Patlak model with quantitative parameters: volume transfer constant (K_{trans}), extravascular-extracellular space (EES) and blood-plasma volume (V_p). The red tube like figure is a sliced section of a blood vessel, the red elipsical shapes are blood cells, the purple clusters are contrast media, and the green figures are brain cells (Blood vessel image from: <https://www.colourbox.com/vector/bloody-artery-blockage-of-blood-vessels-sick-artery-with-leukocytes-blood-cells-in-human-vein-close-up-healthy-and-painful-human-artery-vector-16867749>).

localisation of the region mostly effected, DCE-MRI (Patlak model) has been used extensively in recent BBB investigation and selected as the most accurate and less invasive technique compared to other modalities (Tofts et al., 1999; Tofts, 2010; Cramer and Larsson, 2014;

⁴ Temporal resolution in neuroimaging is defined as the time frame for a single dynamic process (<https://radiopaedia.org/articles/temporal-resolution>).

Barnes et al., 2015; Montagne et al., 2015; Van de Haar et al., 2016,2017; Verheggen et al. 2020, Thrippleton, 2019; 2020; Montagne et al., 2020; Li et al., 2021).

Acquiring high spatial resolution in BBB permeability measures is thought to be very important for the detection of subtle BBB leakage, therefore a lower temporal resolution is favoured (Barnes et al., 2016). Another major influencing factor for high quality BBB leakage measures is the contrast concentration curve which is generated to enable accurate calculation of leakage rate; this requires collecting pre-contrast data prior to the bolus injection of contrast as shown in Figure 3.4. Studies have shown that increased pre-contrast scanning time will result in an improved contrast to noise ratio (CNR) which results in greater sensitivity to permeability measurements (Barnes et al., 2016). It was also found that increased total acquisition time will increase the sensitivity of the measures in subtle-leakage investigations. The longer the scanning time after contrast injection will allow for enough time for the contrast to leak into the extravascular-extracellular space (EES), thus, more data are acquired for accurate K_{trans} measures (Barnes et al., 2016; van de Haar et al., 2017).

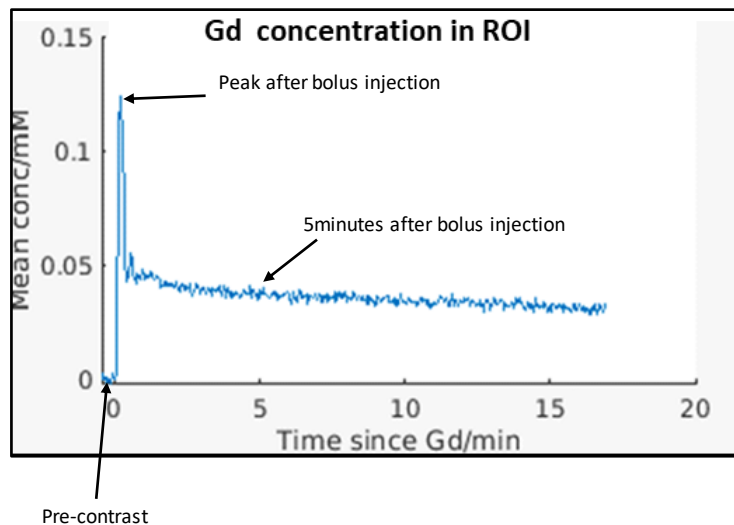


Figure 3.4 A contrast concentration curve for one ROI showing pre and post contrast signals. The longer the acquisition time, the more data-points are acquired which provide better precision in fitted parameters, therefore, more accurate measurements.

3.2.3 General Imaging Analysis Techniques

Most image analysis techniques are applicable to most structural imaging techniques such as NODDI, MPM, DTII and DCE-MRI. Here is a review of the general imaging analysis techniques and steps applied in the main study.

3.2.3.1 Image Co-registration

Because brain sizes and shapes are different from one person to another, voxels from each part of the brain need to correspond to the same anatomical region for all participants in group analysis. Images were coregistered using SPM12-coregistration (https://rdrr.io/cran/spm12r/man/spm12_coregister.html). This was achieved by first a process called affine transformation to align all images from the same person, then warping images to standard Montreal Neurological Institute (MNI) parameter space template as shown in Figure 3.5. Warping images to a parameter space is also called normalisation which, is the process of mapping different people's brains into a common space, to perform regional/ pixel-wise comparisons between people or groups of people. This template is agreed and used across

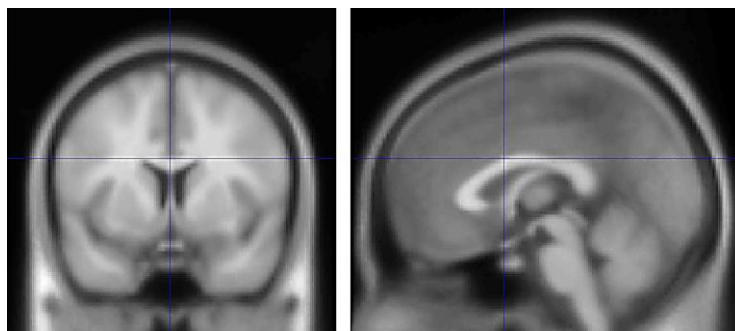


Figure 3.5 An example of MNI152 brain template. 152 is the number of healthy adult brains that were averaged to create this template that is commonly used across research centres. (https://andysbrainbook.readthedocs.io/en/latest/SPM/SPM_Short_Course/SPM_04_Preprocessing/03_SPM_Coregistration.html).

scanning centres because it has standard dimensions and coordinates (standardized space), where the participants brain images will be realigned with standard space (https://andysbrainbook.readthedocs.io/en/latest/SPM/SPM_Short_Course/SPM_04_Preprocessing/03_SPM_Coregistration.html). This process of normalization increases accuracy of coregistration.

3.2.3.2 Image Segmentation

Freesurfer image analysis suite (<http://surfer.nmr.mgh.harvard.edu>) is usually used for image reconstruction and volumetric segmentation from T1w MPAGE images. It also involves motion correction and exclusion of non-brain regions such as eyes and skull by hybrid watershed/surface deformation procedure (Dowell et al., 2016).

3.2.3.3 Image Masking

Images obtained contain a large amount of unwanted voxels from around the head including air. To reduce image analysis time, these unwanted voxels can be removed simply by masking the regions of interest or masking only brain pixels. The masked brain image will have voxel values of 1, and anything other than brain will have a voxel value of 0 as shown in Figure 3.6. Similar to the idea of tracing an image and cutting the traced pattern out. Therefore, only brain data is analysed while anything non-brain is ignored (https://andysbrainbook.readthedocs.io/en/latest/AFNI/AFNI_Short_Course/AFNI_Preprocessing/06_AFNI_Masking_Scaling.html?highlight=image%20masking). This method of masking was applied to all regions of interest (ROIs), such as, WM regions, GM regions, CSF filled regions.

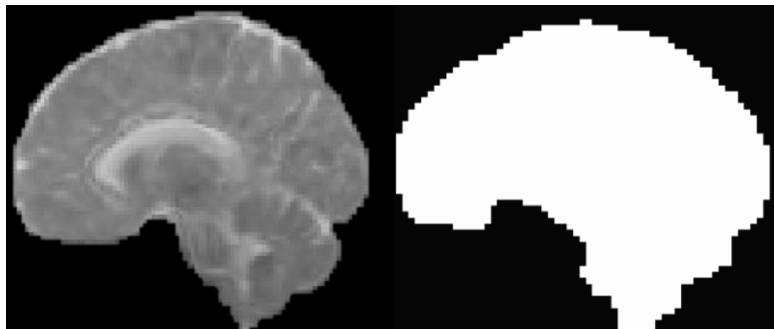


Figure 3.6 An example of image masking, where the original brain image is on the left and the masked brain is on the right.

3.2.3.4 Region of Interest Analysis

After image masking, the mask model was fitted to each participants' brain and group analysis can be performed, whether it is for the whole brain or specific ROIs. The ROI analysis produced estimated mean pixel values for each volume and will identify differences across groups. FSLeyes (<https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLeyes>) was used to visualize results for each participant. ROI analysis provides a more accurate estimation of mean values and group differences than whole brain group differences. ROI analysis is achieved by using SPM12 (<https://www.fil.ion.ucl.ac.uk/spm>) extension and computational anatomy toolbox - CAT12 (<http://www.neuro.uni-jena.de/cat/>) in Matlab versions R2007a (7.4) (MathWorks, <https://uk.mathworks.com/>).

3.2.3.5 Cluster wise analysis

SPM12 (<https://www.fil.ion.ucl.ac.uk/spm>) extension was used to identify clusters (pixels) differences between groups as shown in Figure 3.7. DTI and NODDI parameter outputs were entered in SPM, where 2 sample t-test was performed and clusters where pixel values are different between groups were highlighted and therefore clusters with higher or lower values between groups are identified.

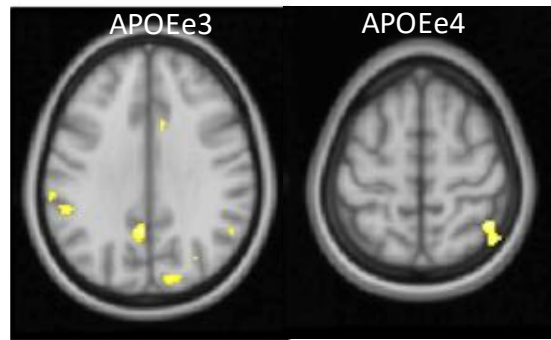


Figure 3.7 Clusters (pixels) of NDI differences between two APOE groups, where APOE ϵ 3 group shows clusters of increased NDI which indicates better neuronal density in this group.

3.2.3.6 Histogram Analysis

Histogram analysis has been widely used to analyse MR parameters when evaluating subtle microstructural changes in the whole brain. Histogram is the frequency of each pixel intensity in the image of the chosen parameter (Vadmal et al., 2020). Histograms are more and more used in diffusion parameters that measure global changes in the brain such as DTI parameters and do not require ROI generating (Paul Tofts and Gerard Davies, 2003, Histograms: Volumetric Analysis, 2003, Quantitative MRI of the Brain (581-610) John Wiley & Sons, Ltd, ISBN: 0-470-84721-2). The only disadvantages of histogram analysis are that the localization of changes are not identified, and are not enabled in the presence of brain atrophy.

In simplified steps, histogram analysis requires brain segmentation, smoothing of images, normalization to correct for brain volume differences and that is achieved by dividing each histogram pixel count by the sum of pixel count for each individual brain and then multiplying by 100 (Paul Tofts and Gerard Davies, 2003, Histograms: Volumetric Analysis, 2003, Quantitative MRI of the Brain (581-610) John Wiley & Sons, Ltd, ISBN: 0-470-84721-2). In this stage, normalized histogram data is available for statistical analysis.

Two measures were extracted from the histogram data, peak height and peak position as shown in Figure 3.8, these are calculated via excel and extracted to SPM to be statistically analysed.

Whole brain, WM or GM were compared between groups. Peak height represents homogeneity of whole brain, where lower peak height indicates more heterogeneity potentially due to subtle diffuse effects; peak position reveals changes in the actual diffusivity value, where higher diffusivity values (higher peak position) indicates breakdown at a microstructural level.

3.3 Optimization of BBB Acquisition

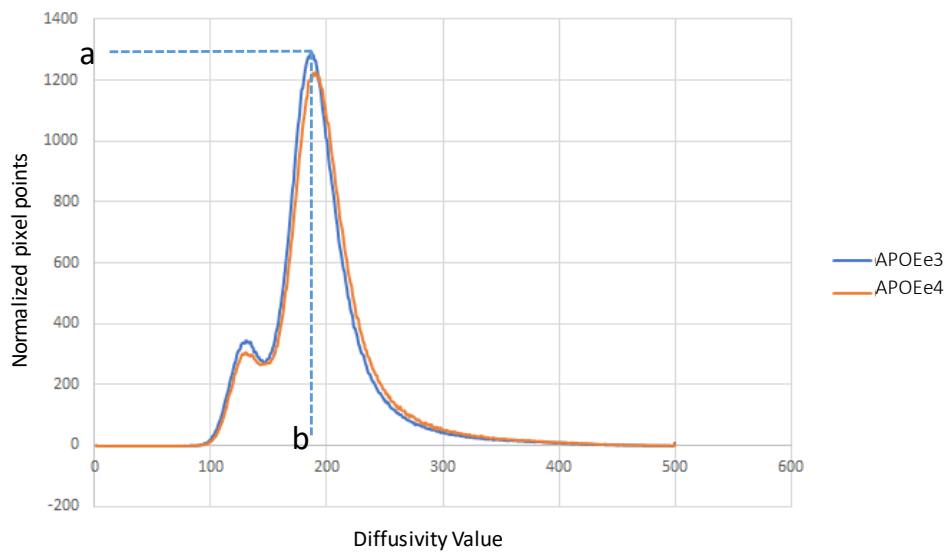


Figure 3.8 Histogram analysis of GM axial diffusivity between two APOE groups, APOEε3 (blue) and APOEε4 (red), (a) corresponds to peak height and (b) corresponds to peak position.

Two of the best BBB scanning approaches using DCE-MERI were developed by Tofts and Larsson. Both methodologies needed to be evaluated here to determine the best approach for this project. Tofts has presented a quantitative measure of BBB permeability after a bolus injection of (Gd-DTPA) MR contrast and measured the Gd leakage at different flip angles (Tofts et al., 2001). While Larsson's group estimated measurements of brain perfusion, blood volume, and BBB permeability, using T₁w imaging on 3T scanner while introducing a Generalized auto calibrating partial parallel acquisition (GRAPPA), which is a parallel imaging technique that reduces acquisition time. Higher acceleration factor (AF) gives shorter acquisition times (Cramer and Larsson, 2014).

The aims of this pilot study were:

1. To develop the best combination of qMR imaging techniques that will acquire high quality imaging data with high sensitivity to subtle changes.

2. To compare two BBB scanning approaches and identify how much variability there is in the data over the course of the BBB acquisition, therefore deciding on the optimal approach for subtle BBB measures.

3. To overcome scanner instability issues during the scan, and the best solution to reduce motion artefacts, whether it was positioning or image processing solutions.

The BBB acquisition did not include the administration of contrast (Gd) during the pilot study, and for that reason, pixel values were expected to be consistent throughout the acquisition run. Any variability in pixel values would most likely be as a result of movement, artefact, or scanner instability. The difference in method applied in the pilot study from flip angles, acceleration factors and acquisition time, most likely will cause the difference in pixel values and we will use the variation in the signal as the metric to assess which method is the best. The optimal imaging protocol developed here was implemented in the main research project with the addition of contrast injection.

3.3.1 Methods

3.3.1.1 Ethical approval

The pilot study titled “Development of new MRI sequence” gained ethical approval from Brighton and Sussex Medical School Research Governance and Ethics Committee (BSMS RGEC). RGEC granted this project ethical and research governance approval in August 2015 (Version 5). The ethics certificate of approval and amendment approval are provided in Appendix S.

3.3.1.2 Participants

Eleven healthy participants with different ages were invited for the pilot study through word of mouth advertisement. Participants were excluded if pregnant, claustrophobic, had metallic implants or tattoo around the neck or head. All participants were given enough time to read the information sheet, and then were invited to CISC at Sussex university campus and asked to read and sign the consent form (information sheet and consent form are provided in Appendix T and U). Participants were compensated for their visit with £5 Costa voucher and a picture of their brain. Participant instructions and scanning procedure were described in chapter 2.

3.3.1.3 Imaging Acquisition

All imaging data were acquired on a Siemens 3Tesla MRI scanner and 32-channel phased-array receive-only, head coil. Several scanning parameters were used in the pilot study and

were divided into protocol 1 (Tofts approach) and protocol 2 (Larsson approach). Table 3.3 presents the scanning parameter used for both BBB protocols.

Table 3.3 Scanning parameter used for protocol 1(Tofts approach) and protocol 2 (Larsson approach).

Sequence	Imaging Plane	TR ^a (ms)	TE ^b (ms)	FOV ^c (mm ³)	Matrix	GRAPPA ^d Factor	Flip angle (°)	Acquisition time (min)	Number of volumes	Number of slices	Slice thickness (mm)
Protocol 1											
3D GRE	Sagittal	30	5.93	240	96x96	2	°5	5	1	56	3
	Sagittal	30	5.93	240	96x96	2	°15	5	1	56	3
	Sagittal	30	5.93	240	96x96	2	°25	5	1	56	3
Protocol 2											
T1 vibe	Sagittal	2.56	0.86	280	224x168	0/3	°15	5	144	30	5
T1 vibe	Sagittal	2.56	0.86	280	224x168	0/3	°15	5	144	30	5
T1 vibe	Sagittal	2.56	0.86	280	224x168	0/3	°15	5	144	30	5

^aRepetition Time, which is the amount of time between magnetic pulse sequences applied to the slice.

^bTime of Echo, which is the amount of time between the delivery of the pulse and the echo signal received.

^cdependant on pixel size and slice thickness.

^dGeneralized auto calibrating partial parallel acquisition, which is a parallel imaging technique which is considered an acceleration technique that speeds up the sequence.

For **protocol 1**, slow gradient echo imaging was acquired, generating 56 images in an acquisition time of 5 minutes. Repeated the same acquisition in 3 flip angles °5, °15 and °25. The choice of flip angle is critical for determining both signal intensity as well as image contrast. Higher flip angles produce higher sensitivity but lower contrast. For example, °5 flip angle is too low to identify Gd leakage, it will increase the contrast but reduce the sensitivity. Therefore, identifying the best optimal flip angle is crucial. We collected filtered and unfiltered images to compare image quality by adding a post-processing (B1) filter to the reconstructed image. B1 is a radiofrequency field (RF) used to improve quantitative MRI measures (Vaidya et al, 2016) explained in Figure 3.9, and a B1 filter is thought to enhance image contrast while reducing noise, by reducing any variation in signals due to movement (Placidi et al., 2003). An integrated parallel imaging technique (iPAT) was added to this acquisition, which is an acceleration factor (AF) that helps in improving spatial resolution while shortening acquisition time. Also called GRAPPA (Generalized auto calibrating partial parallel acquisition). AF0 was used in all 3 flip angle acquisitions and images were generated in a 5minute scanning time with a total of 100 images generated.

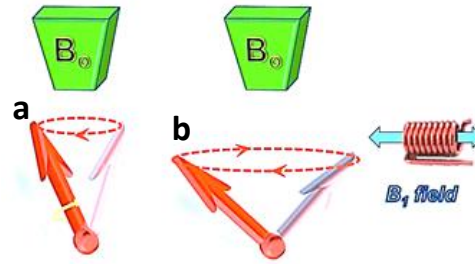


Figure 3.9 Illustration of the difference between B_1 and B_0 . B_1 is a RF produced by an RF coil and B_0 is the main magnetic field. The net magnetisation in B_0 (a) rotates in a small angle, while net magnetisation with the application of B_1 rotates at a larger angle (b). sourced from (<http://mriquestions.com/how-does-b1-tip-m.html>).

For **protocol 2**, fast T1w VIBE imaging was acquired, where VIBE (a volumetric interpolated brain examination sequence) is a gradient-echo MR sequence that can shorten acquisition time. T1w VIBE is a relatively a new technique and BBB leakage is measured during this acquisition (Tofts 2001). This sequence generated a total of 5100 images in 5-minute acquisition time. The purpose of the fast T1w VIBE was to decrease acquisition time without degrading image quality. Initially AF0 was used resulting in blurry images, but then the acceleration factor was increased to AF3 to increase resolution. 1. Three sets of T1w VIBE (AF0) imaging were acquired each for 5 minutes for a total 15minute scan. 2. Three sets of T1w VIBE (AF3) imaging were acquired each for 5 minutes for a total 15minute scan.

3.3.1.4 Image Processing

For **protocol 1**, we followed the same algorithms that Tofts used in his image processing (Tofts et al., 2001). Images were coregistered using two pre-processing software's for comparison, FSL (<https://fsl.fmrib.ox.ac.uk/fsl/fslwiki>) and a statistical parametric mapping (SPM) software (<https://www.fil.ion.ucl.ac.uk/spm/software/download/>). Image co-registration realigns the images to a common image space, thereby eliminating the spatial offset of pixels and allowing a pixel-wise analysis of the data. Three ROI's were then selected manually including frontal lobe, WM and hippocampus to compare signal intensities across different regions of the brain. This was repeated for all three flip angles 5° , 15° and 25° , filtered and unfiltered images. Image processing for Tofts approach took about 4 hours per participant to complete.

For **protocol 2**, we followed the same algorithms as Larsson et al., 2014 used in their image processing (Cramer and Larsson, 2014). By first separating filtered and unfiltered images, then manually drawing ROI's of different regions of the brain (frontal lobe, WM and hippocampus)

on MPAGE images. MPAGE was used for selecting ROIs due to its high contrast images, which improves accuracy of manual ROI drawing. This was not the case with protocol 1 where MPAGE was not acquired, and image quality was reduced. Images were then coregistered on the high resolution MPR space, and repeated for both AF0 and AF3. Image processing for Larsson's approach took about 24 hours per participant to complete.

For model fitting, BBB studio (Version: 2.1.0-dirty-master (bbblar) fitting using GSL tools) was used. Which is a program that applies a fitting algorithm to time series of GRE datasets. Modelling of the signal intensities as they increase over time with BBB leakage generates a patlak plot and uses linear regression to extract two major parameters; Gd concentration (k_{trans}) and blood plasma volume fraction in tissue (v_p). The Patlak model equation below calculates these two parameters:

$$C_t(t) = v_p c_p(t) + k_{trans} \int_0^t c_p(t') dt'$$

Where $c_p(t)$ is the Gd concentration in blood plasma (Thrippleton et al., 2019).

3.3.2 Results

3.3.2.1 Optimization for Protocol 1 (Tofts Method)

Tofts method was optimised where image quality, high sensitivity measures and high accuracy were major requirements. The unfiltered images showed brightness near the edge of the brain, but darker in the middle. The filtered images seemed to correct occurrence by reducing the edges, and boosting the deeper brain regions. Low FA (5°) increased variability but decreased sensitivity, making it too low to be able to identify subtle Gd leakage. In table 3.4 Mean pixel

Table 3.4 Evaluation of the Tofts method, comparing filtered and unfiltered data for FA5°, FA15° and FA25°.

		HIPP			WM			FL		
		FA5°	FA15°	FA25°	FA5°	FA15°	FA25°	FA5°	FA15°	FA25°
Ave % diff from mean	Filtered	0.51	1.12	0.88	0.60	0.55	0.51	0.41	0.68	0.36
	Unfiltered	0.54	0.80	0.88	0.65	0.63	0.63	0.68	0.59	0.51
SDOM	Filtered	0.42	1.05	0.79	0.42	0.64	0.69	0.36	0.89	0.74
	Unfiltered	0.32	0.62	0.47	0.41	0.86	0.77	0.45	0.91	0.81

Average SDOM and the average % difference from the mean across all subjects for the ROIs in Tofts method, comparing filtered and unfiltered data for FA5°, FA15° and FA25°.

HIPP= Hippocampus

WM=White Matter

FL= Frontal Lobe

FA= Flip Angle

SDOM= Standard Deviation of Mean

values, standard deviation of the mean (SDOM⁵) and coefficient of variation (CV), for each volume were calculated to quantify for variability in the measures and showing stability of signals. These were plotted for comparison including FA, filtered and unfiltered data. The data were compared within the Tofts method, because we used two analysing software's to compare variability between measures. The software's are statistical parametric mapping -SPM12 (<https://www.fil.ion.ucl.ac.uk/spm>) extension and FSL toolboxes from the FMRIB Software Library v5.0 (Created by the Analysis Group, functional MRI of the brain (FMRB), Oxford, UK). The program producing low variability between participants is the best approach. Figure 3.10 shows bar graphs comparing means of frontal lobe measures in Tofts approach using SPM and FSL tools on both filtered and unfiltered images, where SPM shows less variability in data points compared to FSL.

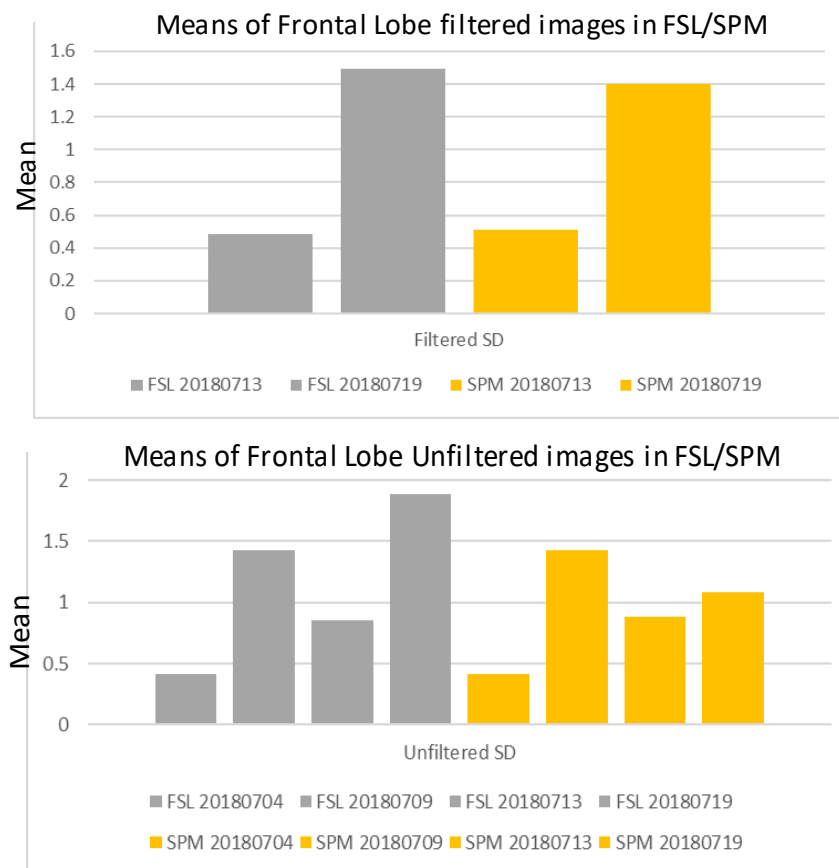


Figure 3.10 Bar graphs illustrating variability between SPM and FSL tools in measuring mean values of the frontal lobe in both filtered and unfiltered images.

⁵ SDOM takes into account the number of data points.

3.3.2.2 Optimization for Protocol 2 (Larsson Method)

Similar to Tofts method, filtered images reduced the edges while boosting the deeper brain regions. Table 3.5 presents a comparison within the Larsson method between filtered and unfiltered image and between AF0 and AF3, where mean pixel values, SDOM and CV, for each volume were calculated to quantify variability in the measures and showing stability of signals.

Table 3.5 Evaluation of the Larsson method, comparing filtered and unfiltered data for AF0 and AF3.

		HIPP		WM		FL	
		AF0	AF3	AF0	AF3	AF0	AF3
Ave % diff from mean	Filtered	2.06	1.79	1.31	0.94	1.36	1.02
	Unfiltered	1.94	1.59	1.3	0.93	1.49	1.14
SDOM	Filtered	0.26	0.16	0.27	0.18	0.35	0.23
	Unfiltered	0.17	0.11	0.21	0.15	0.33	0.23

Average SDOM and the average % difference from the mean across all subjects for the ROIs in Larsson method, comparing filtered and unfiltered data for AF0 and AF3.

HIPP= Hippocampus

WM=White Matter

FL= Frontal Lobe

FA= Flip Angle

SDOM= Standard Deviation of Mean

3.3.2.3 Comparison of Protocol 1 and Protocol 2 (Tofts and Larsson Method)

The grand average SDOMs across all three ROI's were calculated and compared between both Tofts and Larsson approaches shown in table 3.6 and Figure 3.11.

Table 3.6 Comparison of the Tofts /Larsson techniques.

	TOFTS			LARSSON	
	FA5	FA15	FA25	AF0	AF3
Unfiltered	0.39	0.80	0.68	0.23	0.17
filtered	0.40	0.86	0.73	0.30	0.19

The grand average standard deviation of mean across all ROI's comparing the Tofts /Larsson techniques and filtered /unfiltered images for both techniques, the three flip angles for Tofts method (5°, 15°, 25°) and the acceleration factors for Larsson method (0, 3).

FA= Flip Angle (5 °, 15°, 25 °) for Tofts technique

AF= Acceleration Factor (GRAPPA 0 and 3) for Larsson technique

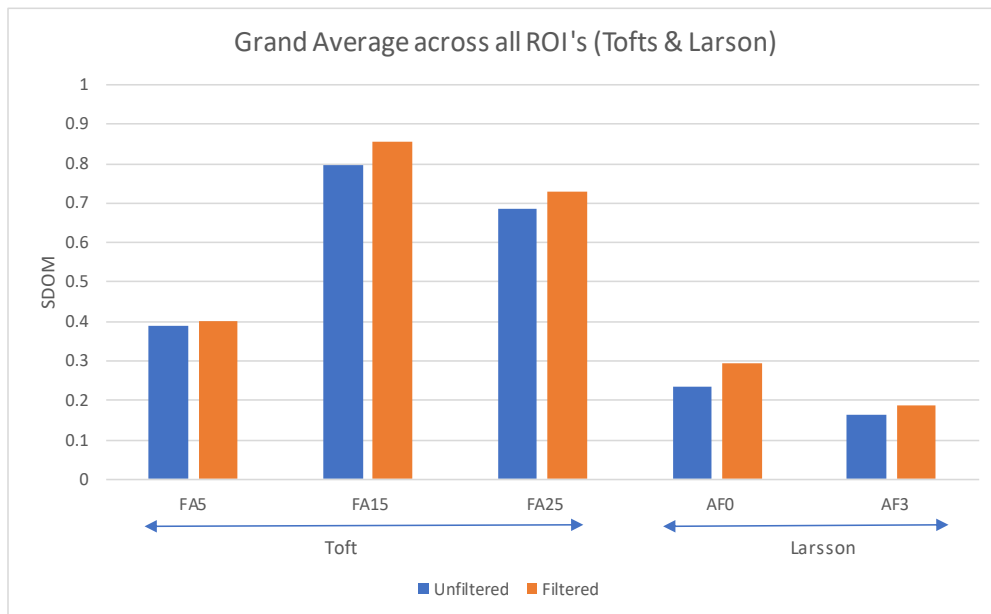


Figure 3.11 Bar graph showing grand average standard deviation of mean across all ROI's in Tofts and Larsson techniques and filter status. Where FA represents flip angle in Tofts, AF represents acceleration factor in Larsson and SDOM is the standard deviation of mean.

3.3.3 Conclusion

The main goal of this pilot study was to determine the optimal imaging approach to quantify subtle BBB leakage by assessing and comparing two of the best BBB scanning protocols using SDOM as the metric of evaluation of number of data points acquired. Table 3.7 summarises the differences between Tofts and Larsson's approaches in quantifying subtle BBB leakage. For the image processing software of the Tofts method, SPM was the approach decided on due to the big variability in data points when using FSL. B1 filter was selected due to its ability to improve any variation on signals due to movement. T1w VIBE was selected for the main study with a FA °15 due to its optimal results with high sensitivity together with GRAPPA (AF3) to reduce acquisition time. Therefore, we concluded protocol 2 (Larsson's method) for subtle BBB leakage measures was for our primary study.

Table 3.7 Summary of the strengths and weaknesses of both Tofts and Larsson's BBB imaging methods.

BBB permeability Methods in DCE-MRI	Advantages	Disadvantages
Tofts	Thin Slices High Resolution Shorter processing time ~4hr	Long acquisition time Motion artefacts Less data collected
Larsson	Short Acquisition Time More data collected Clarity in drawing ROI's on MPR Less motion artefacts	Thick Slices Blurry images Longer processing time ~24 hr

3.4 Evaluation of the BBB Protocol on the Main Project and Some of the Limitations

BBB data were collected for 36 healthy mid-age participants (42-59) divided into two groups; APOEε4 (n=17) and APOEε3 (n=19). The methodology, imaging acquisition and results for this part of the study is explained in chapter 5. The aim of the study was to compare BBB permeability status between APOEε4 and APOEε3 carriers, specifically, to identify increased BBB leakage if any, in the APOEε4 group.

In this section, I present an explanation of the image analysis used to quantify BBB permeability in key regions and the challenges faced during the image processing stage and quality of imaging data collected.

For BBB ROI analysis, we used in-house software called BBB Studio written in Matlab version R2007a (7.4) (MathWorks, <https://uk.mathworks.com/>) to perform BBB fitting; this enabled us to preselect a ROI for each participant individually then perform a Patlak modelling to extract K_{trans} and v_p values in each ROI. The graph represents signal intensities in the ROI, in which some ROIs are composed of ~5% blood vessels and the general trend is to see a drop in signal intensity. This smooth change in signal intensity is a reflection of the blood Gd concentration dropping as it is being cleared by the kidneys (Figure 3.12.a), but in many cases here, we identified unexpected and unstable changes in signal intensities precisely towards the middle or end of the BBB acquisition (Figure 3.12.b).

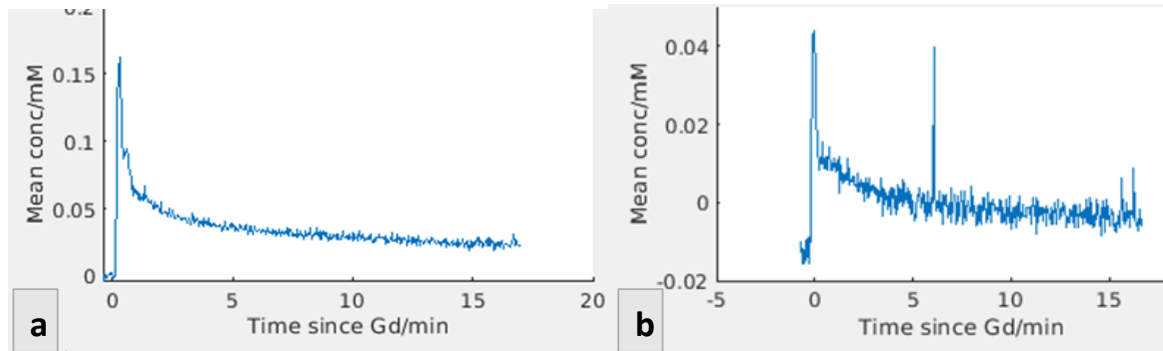


Figure 3.12 Graphs showing Gd concentration in ROI, (a) smooth change in signal intensity as the Gd leaks into the tissue, and (b) unstable changes in signal intensities as the Gd leaks into the tissue.

BBB studio also produced a p-value (P) (Shown in Figure 3.13) which indicates the goodness of the fit and is ideally $P < 0.001$, which in several cases here was high in the range of $P = 0.598897$, $P = 0.145539$. Furthermore, in several ROIs and participants, negative K_{trans} values were produced. Another observation was, after warping MNI-space ROIs to individual participant brains, different ROI sizes were produced based on the Patlak model fitting, where the ROIs were enlarged. Although they were in the correct location in the brain, this was problematic because unwanted pixels from outside the selected ROIs were included. This is mainly due to:

1. the nature of the human brain, where there is a substantial variability between individuals' anatomical appearance at an imaging level.
2. the very small anatomical structures of the ROIs selected for this study. During segmentations and masking, this process included unwanted pixels from outside the region of interest such as eyes or produced a strange line up the cerebellum in a few cases (Figure 3.14).

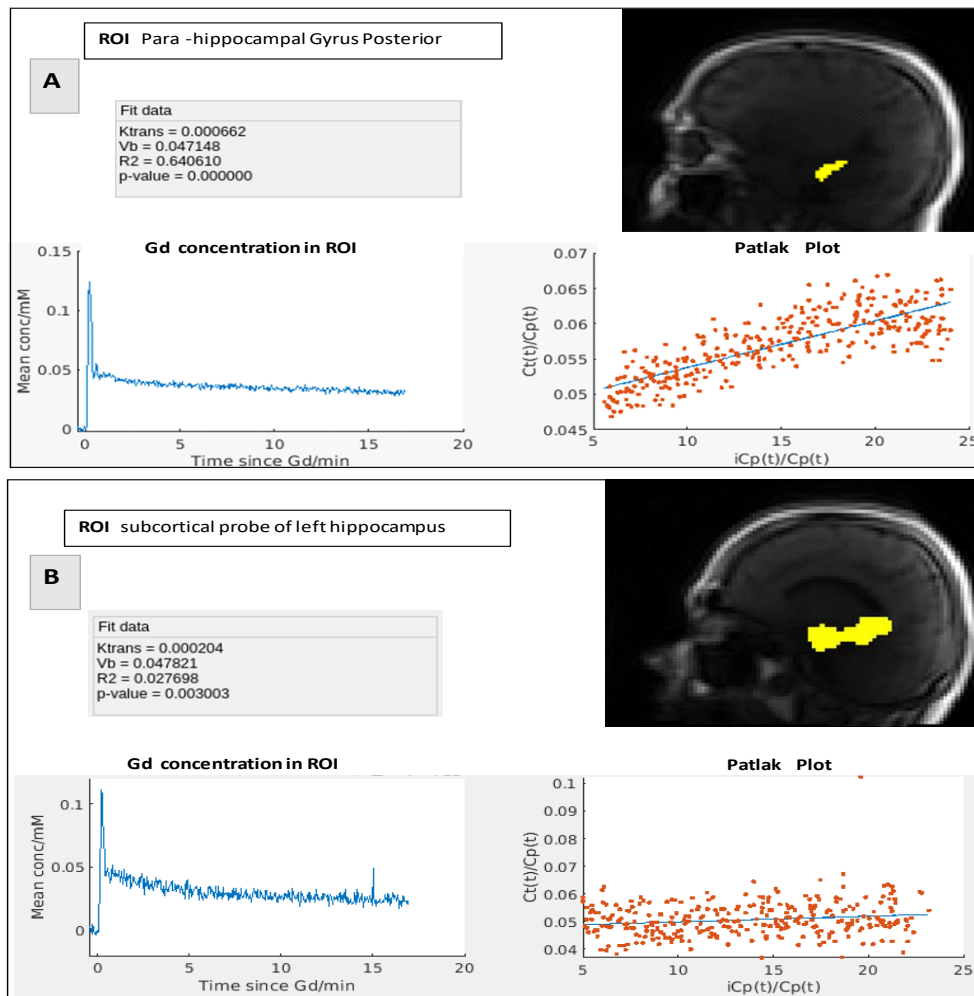


Figure 3.14 BBB fitting program (BBB studio) for a participant showing in (A) the ROI (Para-hippocampal gyrus posterior), with Ktrans value in the region of interest and the Patlak plot representing the Gd uptake in that particular region and where there is Gd leakage we expect an increase (the blue line slopes up) as shown in this example. (B) Another ROI (subcortical probe of left hippocampus) for different participant shows the Patlak plot with a flat slope (blue line), which means there is no Gd leakage. The graphs on the left represent the Gd concentration in ROI, where the spike at the beginning of the ROI signal graph is when the Gd first enters the tissue and the signals smoothly reduce with time as shown in A. The instability of the signals in B and the spike at 15 minutes may be attributed to motion artefacts.



Figure 3.13 T1w VIBE sequence sagittal view after applying model fitting producing a white line.

To overcome the segmentation issues, we experimented with different threshold values in SPM12. Initially, the threshold value used was 0.7 which produced a black line, then we reduced the threshold value to overcome the black line but the lower threshold was becoming too permissive and letting through non brain (eye and skull). A segmentation software FSL-BET2 (Brain Extraction Tool) was then used which incorrectly removed large parts of the brain in several volunteers. Then we tried FSL-BET as a variation to FSL-BET2 command which had a couple of useful options including a robust brain centre estimation and an eye & optic nerve voxel clean-up which BET2 can sometimes leave behind. In this segmentation experiment, we found that FSL-BET2 seemed to chop off brain values while the FSL-BET version did not (Figure 3.15).

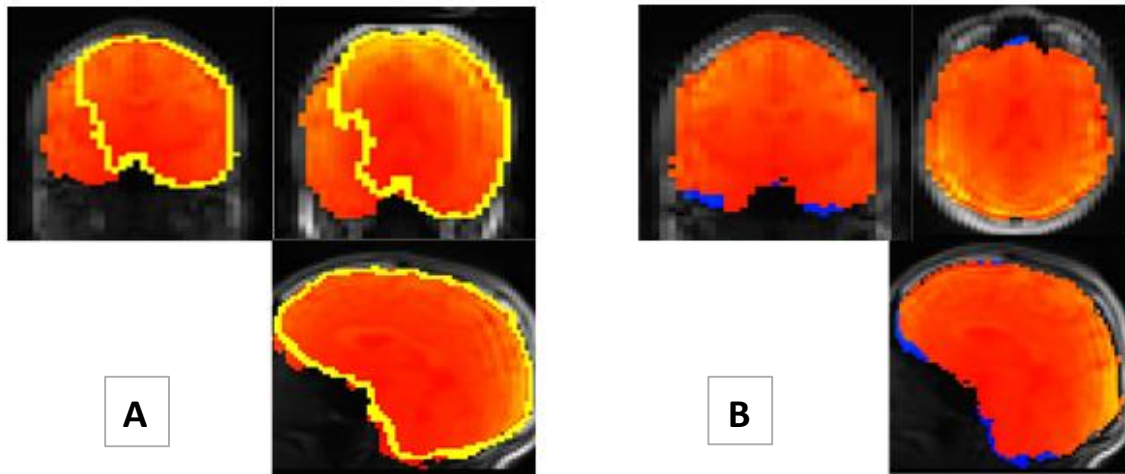


Figure 3.15 Brain images showing the difference between bet2 and bet overlay. (A) Bet2 overlay in yellow over a pre-Gd non-distorted brain in red representing bet2 failure to fit over the whole brain. (B) Brain images showing bet overlay in red over a pre-Gd non-distorted brain in Blue.

In order to extract the *ktrans* values from native space ROIs, first we had to exclude the non-fitting pixels by applying a mask that describes where the Patlak model worked, and masking out pixels that did not fit, creating a mask for where *ktrans* was less than zero, by applying a threshold and reducing the number of bins. Because we were trying to detect very low *ktrans* values, histogram analysis was used to increase the sensitivity in the identification of subtle diffusion differences. Histograms of the mean *ktrans* for each ROI were produced, histograms were smoothed using FWHM of 8, where *ktrans* value was in the range (0 - 0.008 min⁻¹) to overcome noise, and then normalized to correct for brain volume differences between participants. Peak heights and peak positions for each ROI of each volunteer were calculated, where peak height represents the number of pixels under the histogram, and peak position

corresponds to the k_{trans} value that is most common in the ROI. In healthy BBB, the histograms would present a very well-defined peak, whereas, increase in BBB permeability is identified by either a shift in peak position or a depressed peak height at the same peak position as in normal BBB (Tofts et al., 2003). However, peak heights are usually depressed with a broader appearance due to histogram normalization.

This chapter focused on an in-depth technical description of imaging acquisition and parameters used in investigation of microstructural changes, a pilot study that aimed on evaluating the most optimal DCE-MRI protocol for quantification of subtle BBB leakage and the image analysis and challenges faced during the implementation of BBB permeability approach in the main study. The structural imaging study (qMRI) And BBB permeability study (DCE-MRI) used in the main project are explained in details in both chapter 4 and 5.

Chapter 4: Structural Changes in Mid-Age APOEε4 Carriers

Neuroimaging studies have shown a variation between cognitively healthy APOEε4 carriers and non-carriers in both structural and functional brain imaging (Donohue et al., 2017). One of the powerful non-invasive techniques of investigation is the use of Magnetic resonance imaging (MRI). This chapter describes an in-depth exploration of subtle structural changes that are found in AD/APOEε4 carriers and may start to show in mid-age APOEε4 carriers with essentially normal-appearing brain tissue.

4.1 Overview of Structural Changes Found in APOEε4 Carriers

4.1.1 Volumetric Atrophy (Regional and Whole Brain)

Several MRI studies have recognized hippocampal atrophy in MCI and AD patients (de Leon et al., 1996; Rusinek et al., 2003; Apostolova et al., 2006; Devanand et al., 2007), but the extent to which the hippocampal volume is reduced in healthy carriers of the genetic risk factor for AD, APOEε4, is still controversial. A study on MCI (n=276) and AD (n=129) patients between the ages of 55 and 75 showed significant hippocampal and amygdalar atrophy in APOEε4 carriers compared to non-carriers (Tang et al., 2015). Other studies also observed greater hippocampal atrophy in APOEε4 carriers in non-demented older age participants (Honea et al., 2009; den Heijer et al., 2002). Furthermore, a study on healthy older women (mean age 55 ± 6 years), non-APOEε4 carriers (n=9) and APOEε4 carriers (n=16), observed over two years, showed that the presence of a single ε4 allele is linked to an increase in the rate of hippocampal atrophy (Cohen et al., 2001). In a large cohort study involving 533 participants, healthy mid-age APOEε4 carriers (age 45-75) showed a decrease in hippocampal volume in comparison to non APOEε4 (Cacciaglia et al., 2018). A longitudinal study on cognitively healthy people from mid-age onwards explored the rate of volumetric change in subcortical region and in particular the hippocampus and amygdala as well as the age where the changes start to show (Mishra et al., 2018). Mishra et al., 2018, found that the rate of hippocampal and amygdalar atrophy increased in APOEε4 carriers at mid-age when compared to non-APOEε4 carriers, and the start of volumetric changes in these regions start earlier at the age 57 and 66 years respectively. In contrast, a study on a small sample of mid-age APOEε4 (n=17) and non APOEε4 (n=20) carriers (age 45-55) showed a significant increase in WM volume in APOEε4 carriers in left anterior cingulate and Para-hippocampal cortex (Dowell et al., 2016). In the same sample, no hippocampal volumetric difference was reported between genotype but there was a positive link described in non-APOEε4 carriers only where increased neurite orientation-dispersion

(ODI) linked to better recall performance, and increased hippocampal volume linked to better recognition performance (Evans et al., 2020). Rusted et al., 2013, suggested young APOEε4 carriers are cognitively advantaged compared to non APOEε4 carriers when, investigating a large healthy cohort (n=98), age range between 18-30 years, i.e. young APOEε4 carriers scored higher in academic performance and cognitive tests compared to young non-APOEε4 carriers (Rusted et al., 2013). A recent study observed no direct effect of APOEε4 on structural MRI measures from 20-50 years of age, but rather, APOEε4 carriers (n=50) had a protective effect with aging compared to non APOEε4 carriers (n=107), where the volumes of cingulate and temporal cortex regions were preserved up to the age of 50 and executive function performance was maintained in younger APOEε4 carriers (Taylor et al., 2017). These findings suggest that the presence of APOEε4 has an advantageous effect in youth, which may be lost with age, and this could indicate the beginning of the turning point is later in life or possibly suggests a mid-age transition where structural differences may begin to be detected in very specific regions and may anticipate poorer cognitive performance in the presence of APOEε4 in mid-age.

In early AD, neurodegeneration is observed in regions related to cognitive impairment and is detected in structural imaging. Cortical thinning is one of the major structural changes that is detected in AD (Bobinski et al., 1999, Thompson et al., 2008; Morra et al., 2009). Older cognitively normal and AD patients who are carriers of the APOEε4 allele showed a significant increase in cortical thinning in the subiculum and entorhinal cortex as well as increased average cortical thinning across all medial temporal lobe sub-regions combined (Li et al., 2017), therefore acknowledging APOEε4 as a major genetic risk factor for developing AD. Studies on healthy middle-aged APOEε4 carriers have been limited, however. One study found significantly increased cortical thinning in the entorhinal cortex and the subiculum in carriers of the APOEε4 allele as compared to non-carriers in mid-age with mean age 61 (Donix et al., 2010). A recent study also revealed differences in several cortical regions between healthy carriers and non-carriers of APOEε4 in mid-age (Mishra et al., 2018), where cortical thinning of para-hippocampal cortex and insula appeared to be accelerated in mid-age of APOEε4 carriers and was observed as early as the age of 51 and 47 years respectively but volumetric changes of the entorhinal cortex started to accelerate at the age of 71 onwards. Further studies on healthy middle-aged people showed an increase in grey matter (GM) atrophy in APOEε4 carriers compared to non-carriers specifically in regions of the brain that are usually affected in AD including the hippocampus, frontal lobe and temporal lobe (Klunk et al., 2007; Liu et al., 2010; Ten Kate et al., 2016; Cacciaglia et al., 2018, 2019). Older MCI patients (mean age 75)

who progressed to mild AD and were carriers of the APOEε4 genotype had increased whole brain atrophy associated with greater decline in cognitive function compared to non-carriers (Okonkwo et al., 2010). In a population of healthy mid-age individuals (mean age 56.8 years), the rate of whole brain volume loss was also significantly increased in APOEε4 carriers (Chen et al., 2007).

In summary, these studies on cognitively healthy mid-aged individuals have shown that the rate and percentage of volumetric loss in whole brain and several regions of the brain, including regions of the hippocampus, para-hippocampal cortex, insula, amygdala, cingulate, temporal cortex, subiculum and entorhinal cortex, are influenced negatively by the presence of APOEε4 gene, where volume loss is increased in APOEε4 carriers, and these volumetric changes begin to be detectable in imaging acquisitions, somewhere in mid-age and as early as 50 years.

4.1.2 White Matter Hyperintensities (WMHs) and Microbleeds (MBs)

White matter hyperintensities (WMH) are brain lesions found within cerebral white matter that are usually detected as bright regions in MRI brain scans (Figure 4.1) and are more common in AD and MCI patients than in normal aging (Holland et al., 2008). WMHs are found to be associated with whole brain atrophy and cortical thinning (Aribisala et al., 2013) and are linked to axonal loss and demyelination (Gouw et al., 2010). WMHs are also linked to cognitive decline in elderly and increases with age (Wang et al., 2018; Debette et al., 2010). Studies suggest that WMHs could be a biomarker for cerebral small vessel disease and blood-brain barrier dysfunction (Wardlaw et al., 2015). APOEε4 has been shown to be a genetic risk factor for the accumulation of WMHs in AD and in non-AD individuals while being significantly linked to cognitive decline (Mirza et al., 2019; Wang et al., 2018; Sudre et al., 2017). Indeed, relative to non-carriers, WMHs are shown to accumulate more in healthy elderly APOEε4 carriers (≤ 75 years) (Lyall et al., 2019; Sudre et al., 2017, Godin et al., 2009) and in healthy mid-age APOEε4 carriers (≥ 45 years) (Salvadó et al., 2019; Rojas et al., 2021). Therefore,

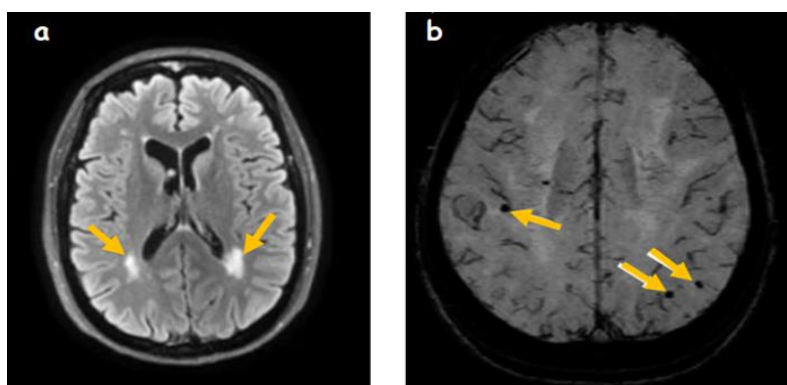


Figure 4.1 Illustration of white matter hyperintensities (WMH) and lobar cerebral microbleeds (MB). a. T2 FLAIR image showing WMH (Rojas et al., 2017). b. Susceptibility weighted

WMHs in mid-age APOEε4 carriers could be a strong neuroimaging marker for accelerated neurodegeneration and disease later in life.

Cerebral microbleeds (MBs), also called microhaemorrhages are small accumulations of blood haemorrhages in the brain due to ruptured small blood vessels which are usually visible as black spots in susceptibility-weight imaging (SWI) MRI scans (Figure 4.1). Cerebral MBs are common observations in populations at high risk of cardiovascular disease and the rate of occurrence increases with age (>40 years) (Daugherty et al., 2017; Poels et al., 2010). Cerebral MBs are also found in cognitively impaired individuals and interestingly, MB are found in cortical regions, specifically in the frontal-temporal lobe which is a region impaired early in AD (Poliakova et al., 2016; Van Veluw et al., 2016; Norden et al., 2011). Also, the level of cognitive decline increased with increase in number of MBs (Qiu et al., 2010). The number of MBs detected in early AD patients is higher than that found in age-matched non-AD patients (Poliakova et al., 2016; Brundel et al., 2012; van der Flier et al., 2012) and 92% of MBs in AD patients were detected in lobar specific regions rather than in deep brain regions (Brundel et al., 2012).

Studies have also shown that MBs have a pathological significance in high-risk population for AD, where MBs were common in APOEε4 carriers and were detected from as early as mid-age (45-60), but were localised in strictly lobar regions (Poels et al., 2010). Additionally, a recent study suggested APOEε4 carriers are at a higher cerebrovascular vulnerability compared to non-carrier and carriers of ε4 homozygote are even at a higher risk as APOEε4 carriers developed more MB and WMH than non-carriers which increased with age (Ingala et al., 2020). Figure 4.2 shows a prevalence of how APOEε4 increases the risk of MBs and how MBs are increased with age in relation to APOE status. In contrast, MBs detected in deep regions were associated with WMHs but not with APOEε4 gene (Martinez-Ramirez et al., 2014). These

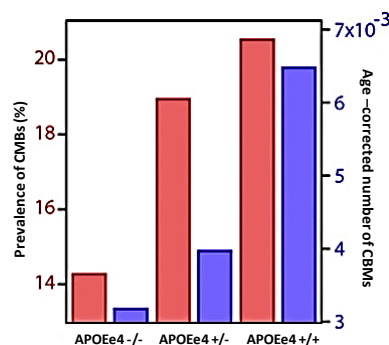


Figure 4.1 Frequency of CMBs in APOEε4 genotype (blue) and CMBs with age-corrected number of CMBs (red) (Modified and sourced from Ingala., 2020).

studies suggest that WMHs and MBs may be proposed as biomarkers for early structural findings that may be detected in APOEε4 carriers and these biomarkers could be evident from as early as mid-age.

4.1.3 White Matter Structural Disruption

White Matter (WM) microstructure has been shown to be disrupted in early AD, even at pre-symptomatic stage (Kantarci et al., 2017). Understanding the factors influencing such changes in WM is essential. Several studies observed WM tract disruption in healthy elderly APOEε4 carriers using Diffusion Tensor Imaging (DTI) to explore axonal structure changes, these studies showed:

1. A decrease in fractional anisotropy (FA), a parameter that quantifies the tendency of water molecules to diffuse in a certain direction; it is higher in ordered microstructural tissues such as WM and is often used as a marker for WM integrity⁶.
2. An increase in radial diffusivity (RD), a parameter that indicates myelin dysfunction, in regions of the WM such as the Corpus Callosum, cingulum, in the inferior fronto-occipital and longitudinal fasciculus.
3. An increase in mean diffusivity (MD), indicating higher free water diffusion likely due to microstructural or cellular breakdown, in the genu, right internal capsule, superior longitudinal fasciculus and corona radiata (Honea et al., 2009; Zhang et al., 2015; Cavado et al., 2017; Williams et al., 2019).

Studies on cognitively healthy mid-age population (45-74 years), comparing WM integrity between APOEε4+ and APOEε4- expressed WM tract disruption in regions affected by AD (Operto et al., 2018; Slattery et al., 2017). In contrast, Dowell et al (2016), showed greater WM volume in the left anterior cingulate of healthy mid-age (45-55) APOEε4 carriers than in non-APOEε4 carriers. These differences may relate to the different age-ranges and confirm that carriers of the APOEε4 genotype start to show structural brain changes in healthy mid-age, but make it clear that our understanding of the impact of the APOEε4 genotype on WM integrity in AD in its early stages remains limited.

Understanding from where and how APOEε4 may be implicated in the trajectory of pathological changes leading to AD is crucial in tackling this neurological disease, especially

⁶ Lower FA and Higher MD, AxD and RD

when AD diagnosis is often delayed until there are observable changes in cognitive performance. As structural imaging studies on middle age APOEε4 population are often contradictory, it is critical to examine in more detail the trajectory of the physiological changes that start years prior to cognitive decline, and which is thought to be during mid-age.

In this chapter we examine structural differences between healthy mid-age APOEε4 and APOEε3 carriers in regions identified in the literature that are most relevant to AD, associating with neurological and cognitive defects and that have also shown genetic differences in healthy elderly individuals. We developed a set of high sensitivity imaging techniques in our MRI protocol which are novel for this specific age range to be able to detect possible early subtle differences between genotypes in a healthy population. In particular, we included measures of whole brain volume, regional volumes, WMHs, MB and WM integrity (using specific quantitative MR techniques) in the same population of mid-age volunteers. We also collected additional measures allowing correlations of structural differences with blood inflammatory markers. We selected recognised biomarkers that influence the prognosis of AD, including NFL, ferritin, fibrinogen and total Tau. A set of cognitive measures were also collected to explore links to subtle structural measures of the brain that may be occurring as early as mid-age, and that may increase risk for later pathological outcomes. These additional measures for exploring the data are reported in subsequent chapters.

4.2 Hypotheses

We predicted, through the application of the novel combination of structural brain imaging parameters on a cognitively healthy mid-age cohort who are considered high-risk AD population (APOEε4 carriers):

1. Volumetric differences in regions of the brain between APOEε4 and APOEε3 carriers in mid-age, with APOEε4 carriers showing reduced whole brain volume.
2. An increase in WMHs and MBs in APOEε4s relative to age-matched APOEε3 carriers.
3. Differences in diffusivity measures which are strong indicators of WM integrity, between APOEε4 and APOEε3 carriers, where we predict Lower FA and Higher MD, AxD and RD in APOEε4 carriers.

4.3 Methods

4.3.1 Participants and genotyping

Forty-one healthy mid-age participants (age, 52 ± 4 years; range, 42-59 years; 33 females, 8 males) participated in the study. One participant from the initial eligible 41 was claustrophobic and withdrew from phase 3 prior to the start of the scanning session, while part of the structural data collected for two of the participants were corrupted during data transfer. Details about the recruitment process inclusion and exclusion criteria, ethical approval and consent forms are presented in chapter 2. All genotype information was withheld from both researchers and participants through a triangulated recruitment procedure (described in Methods chapter 2) and was added to the anonymised imaging data only after all pre-processing had been completed.

4.4 Structural MRI protocol

All imaging data were acquired on a Siemens 3 Tesla MRI scanner and 32-channel phased-array receive-only, head coil. The series of multiple scanning sequences were designed for the purpose of detection of microstructural differences from different brain microstructural tissues with high sensitivity and reliability detection properties. Throughout the scan, participants were instructed to keep their head still and limit other body part movement as much as possible. Total acquisition time was about 70 minutes including the BBB acquisition described in the following chapter. Several quantitative magnetic resonance (qMR) techniques have been used as biomarkers for changes or differences in the brain. The qMR parameters used in this study are:

4.4.1Voxel-based morphometry (VBM)

VBM is a relatively easy automated technique developed a little over a decade ago, and has been used extensively since then to assess volumetric structural changes in brain tissue (Ashburner and Friston, 2000). Basically, VBM uses T1 weighted (T1w) imaging to collect volumes of the brain tissue then statistically analysing voxels to estimate differences in brain tissue volume across study groups (Whitwell, 2009). T1-weighted Magnetization Prepared Rapid Acquisition Gradient Echo (T1w MPRAGE) and T2-weighted SPACE (T2w SPACE) anatomical imaging sequence is used to produce regional volumetric and cortical thickness measurements which have high spatial resolution (each pixel represents $< 1\text{mm}$) and high contrast between WM and GM. The images are used to divide the brain into regions, which allows for measurement of volume of a particular region or to determine the thickness of the cortex. Volumetric measurements are widely used to diagnose and study disease progression

in AD (Chandra et al., 2019). Here we used the following sequences and metrics: For T1 MPRAGE, a matrix= 320x320, 224 slices and voxel size= 0.8x0.8x0.8, and for T2 SPACE, echo time (TE)=534ms, voxel size= 0.9x0.9x0.9, matrix= 256x256 and 192 slices. The same metrics were repeated with reversed polarity acquisition (for eddy current correction) and DWI low b-value acquisition 2minutes 30 seconds with diffusion directions=30 and b-value=800s/mm².

4.4.2 Diffusion tensor imaging (DTI)

DTI is considered a sensitive qMR technique which provides a measure of structural connectivity giving a sense of where there are directions in the flow of the fiber tracts specifically in WM. (Ortiz-Terán et al., 2011). This is an important technique used in AD research to enable assessment of neuronal connectivity via evaluation of neuronal density, fiber orientation, axonal diameter and level of myelination (Soares et al., 2013). Therefore, DTI has been applied as a method of structural imaging to assess both diffusion of water molecules in WM microstructure and WM tracts, making it a valuable technique in assessing microstructural changes (WM integrity) in the brain. Studies have also identified a relationship between DTI parameters and cognitive function in healthy mid-age cohorts (Dowell et al., 2013; Operto et al., 2018). Here we used DWI high b-value acquisition, acquisition time 4minutes 30seconds, repetition time (TR)= 3600ms, TE=80ms, MB factor=2, field of view (FOV)=240x240mm. 60 slices were acquired, diffusion directions=64 and b-value=2600.

Four parameters were extracted from DTI techniques including Fractional Anisotropy (FA), Mean Diffusivity (MD), Axial Diffusivity (AxD) and Radial Diffusivity (RD), explained in more detail in chapter 3. In AD, FA is found to be lower in AD compared to non-AD individuals due to microstructural damage, whereas MD, AxD and RD are found to be higher in several region including the hippocampus, these results in DTI parametric measures significantly correlated with cognitive decline indicating WM integrity disruption in AD (Mayo et al., 2018). In healthy older APOEε4 carriers, FA was found to be lower compared APOEε4- in WM of the left hippocampal gyrus which correlated with cognitive decline evaluation suggesting a genetic effect resulting in the loss of WM integrity with age (Honea et al., 2009). In healthy mid-age APOEε4 carriers, MD, RD and AxD were found to be higher indicating increase in WM diffusivity, and also suggesting demyelination in regions found to be effected in AD (Operto et al., 2018).

4.4.3 Neurite orientation dispersion and density imaging (NODDI)

NODDI is qMR imaging technique used in recent years, developed as a diffusion weighted imaging (DWI) model to overcome DTI limitations (differences between NODDI and DTI were explained in chapter 3). NODDI assists in the investigation of age related changes in the brain and pathological progression in neurodegenerative diseases including AD. Studies have showed NODDI is more sensitive to subtle differences than DTI in early investigations (Timmers et al., 2016). Therefore, a combination of both acquisitions maybe a useful method for early investigation.

NODDI comprises three parameters, Neurite Density Index (NDI), Orientation Dispersion Index (ODI) and Isotropic Diffusion Fraction (f_{iso}) which provide valuable measures of free water at microstructural levels, neurite density and cortical GM changes (Zhang et al., 2011). Further details about each parameter are found in chapter 3. Cortical NDI and ODI measures were significantly lower in young onset AD compared to healthy age matched controls indicating disruption to the cortical microstructure (Parker et al., 2018). Moreover, ODI has been shown to be reduced in the neocortex with age and suggested to have an age related effect which indicates disruption in the GM microstructure that increases with age (Nazeri et al., 2015). Raghavan et al., 2021, identified differences in NODDI measures between cognitively healthy, MCI and dementia patients in a cohort with mean age (68.3 ± 13.1) years, where f_{iso} was increased in dementia and MCI groups represents an increase in free water concentration which indicates disruption at a microstructural level (Raghavan et al., 2021). In young onset AD, NDI was lower in APOEε4 carriers compared to non APOEε4 carriers which also correlated with cognitive decline indicating APOEε4 contributes to WM neurodegeneration in AD (Slattery et al., 2017). On a healthy mid-age cohort (45-55) ODI was greater in APOEε4-group compared to APOEε4+ in the hippocampus and was linked to better cognition, suggesting APOEε4 carriers start to show GM microstructural breakdown from mid-age which may in turn lead to cognitive decline and AD later in life (Evans et al., 2020). Further NODDI studies are required to understand the mechanism of structural change at a microstructural level on healthy carriers of the APOEε4 gene especially in healthy mid-age. The same DWI acquisition was used for both DTI and NODDI data.

4.4.4 Multi-Parametric Mapping (MPM)

MPM is used in several qMRI studies to provide measurable quantities that are useful in comparisons especially across scanning sites and different time points (Weiskopf et al, 2013). MPM is highly sensitive to measuring the microstructural composition of brain tissue typically

axons, myelin and iron concentrations (Polzehl & Tabelow 2019). **Magnetization transfer saturation (MTsat)** quantifies the magnetisation transfer from MR-visible water molecules to myelin, where loss of myelin (demyelination) nearby is represented by lower MTsat. Therefore, MTsat has been a reliable method for myelin measurements where it provides a semi-quantitative measure of myelin concentration (Hagiwara et al., 2017, 2018). **Longitudinal relaxation time (T₁)** is a useful parameter for information about water content (increase in water results in a longer T₁), myelin content (increase in myelin results in a lower T₁) and iron content (increase in iron results in a lower T₁) (<https://radiopaedia.org/articles/t1-relaxation-time>). We used a flip angle °28 for MTw, °5 for PDw and °7 for MTw, where PDw is proton density weighted, which is related to the amount of hydrogen protons rather than the magnetic characteristics of hydrogen and MTw is magnetisation transfer weighted. T1w, PDw and T1w used 8 gradient echoes at TE= 2.46,4.95,7.43,9.89,12.35,14.81,17.27,19.73ms. MTw used a TR=4.2ms and was achieved by applying an off-resonance pulse prior to the excitation. **Transverse relaxation rate (T₂^{*})** is a measure of the rate-of-loss of observable magnetization. T₂^{*} is sensitive to changes in the local microstructural environment, e.g., the presence of iron disrupts the magnetic field to accelerate this rate, therefore, T₂^{*} was shown to be higher in AD patients compared to healthy controls, representing higher iron concentration in specific regions (Damulina et al., 2020).

4.4.5 Susceptibility Weighted Imaging (SWI)

SWI is an MRI technique which is sensitive to blood derived products such as iron, as well as calcification which cause distortion to the local magnetic field (<https://radiopaedia.org/articles/susceptibility-weighted-imaging-1>). SWI has been widely used for the detection of MB (Kaaouana et al., 2017). The SWI sequence applied in this study was for 4 minute 3 seconds, echo time 10ms, repetition time 20ms, with a 15° flip angle, FOV= 240mm, 2mm slice thickness, 88 slices were acquired in a sagittal plane and matrix (320x320).

4.5 Image Analysis

4.5.1 Voxel-Based Morphometry (VBM)

VBM was achieved by using a statistical parametric mapping - SPM12 (<https://www.fil.ion.ucl.ac.uk/spm>) extension, computational anatomy toolbox - CAT12 (<http://www.neuro.uni-jena.de/cat/>) in Matlab versions R2007a (7.4) (MathWorks, <https://uk.mathworks.com/>) . GM and WM volumes and cortical thickness were segmented from the T1w MPRAGE and T2w SPACE anatomical images using a segmentation model. Total intracranial volume (TIV) was calculated to account for differences in head size across

participants. Imaging data were then smoothed with 8mm Gaussian kernel, and masked with a threshold value of 0.1. Volumetric group data (ROI volumes divided by TIV) were then compared statistically using a one tailed t-test (family-wise uncorrected; $p < 0.001$; with TIV entered as a covariate).

4.5.2 Diffusion Tensor Imaging (DTI)

DTI data were pre-processed and analysed using FSL tool-boxes from the FMRIB Software Library v5.0 (Created by the Analysis Group, functional MRI of the brain (FMRB), Oxford, UK) (Jenkinson et al., 2012). Using ANTs (<http://picsl.upenn.edu/software/ants/>), B0 (non-DW) images were normalized to the standard Montreal Neurological Institute (MNI) parameter space template. This warping process was applied to all DTI parameters including FA, MD, RD and AxD maps. Each DTI parameter was analysed across the whole brain to localise regions of difference between genotypes. All parameters went through spatial smoothing of (8x8x8mm), which increases signal to noise ratio and therefore increases significance of results where cluster threshold was set at $p < 0.001$ (uncorrected) for all parameters.

Histogram analysis has become widely used to identify subtle changes or disease in a large area due to its sensitivity and requiring no ROI to be identified (Paul Tofts and Gerard Davies, 2003, Histograms: Volumetric Analysis, 2003, Quantitative MRI of the Brain (581-610) John Wiley & Sons, Ltd, ISBN: 0-470-84721-2), More detail about histogram analysis for qMRI parameters is available in chapter 3. Histogram analysis was used on the DTI data and, to overcome the noise in the histograms, gaussian smoothing was applied using FWHM⁷ of 0.024 mm²/s for FA maps and FWHM of 0.02 x 10⁻³ mm²/s for MD, AxD and RD. The DTI maps were then normalized to account for brain volume differences between participants as explained in chapter 3. Peak heights and peak positions of both whole brain and WM were compared for both groups. Lower peak height and higher peak position are indicators of increased diffusivity likely due microstructural damage.

4.5.3 Neurite orientation dispersion and density imaging (NODDI)

Global brain analysis was performed for group comparison of each of the NODDI parameters NDI, ODI and f_{iso} . Similar to DTI, FSL v5.0 was used for pre-processing and ANTs was used for images to be warped to the MNI parameter space template. Eddy correction was used to exclude non-brain tissue and to correct for motion. NODDI volumes were normalised to account for differences in brain size, and regions of increased NDI, ODI and f_{iso} between

⁷ Full width half maximum.

groups were warped to MNI space Using ANTs (<http://picsl.upenn.edu/software/ants>). Furthermore, using SPM to identify clusters of pixels in the brain that had differences between groups and the Harvard-Oxford cortical and subcortical structural atlas, five ROI's were selected following the methodology used by (Adluru et al., 2014 and Dowell et al., 2016). The means of ROI's were selected for genotype comparison, and were the right/left (hippocampus, WM anterior cingulate cortex (WMAcc), Superior longitudinal fasciculus (SLF)) and anterior/posterior parahippocampal gyrus. NDI, ODI and f_{iso} for the 8 ROI's were statistically compared using a two-tailed t-test (equal variance assumed) in SPSS version 26. Significance level was set at $p=0.006$ after adjusting for multiple comparisons using Bonferroni correction for all regions.

4.5.4 Magnetization transfer saturation (MTsat) analysis

SPM12 (<https://www.fil.ion.ucl.ac.uk/spm>) was used to normalize to MNI space and analyse the MTsat data. Pixel-wise analysis approach was used for voxel wise analysis. MT maps were segmented into GM, WM and CSF using MNI space template (Balbastre et al., 2021). Whole brain statistical comparison between genotypes of MTsat volumes were completed to identify clusters of myelin concentration.

4.5.5 Susceptibility Weighted Imaging (SWI)

One neuroimaging trained neurologist conducted the image analysis while blinded to participant information via quantifying the number of MB lesions using a standard microbleed anatomic rating scale (MARS) (Brundel et al., 2012; Poliakova et al., 2016).

4.6 Results

4.6.1 Voxel-based morphometry (VBM)

The study showed no significant differences between groups in GM volume, WM volume, CSF and cortical thickness (Table 4.1). Although not significant at the conventional 5% level, the VBM analysis of the high-resolution structural images showed some localised differences

Table 4.1 Brain volume ratio (expressed as mean \pm standard deviation) for the APOE ϵ 4 and APOE ϵ 3 groups, including the p -value from the two-tailed t-test.

	ϵ 4 (n=20)APOE	APOE ϵ 3 (n=20)	p
GM volume ratio	0.44 \pm 0.02	0.45 \pm 0.02	0.18
WM volume ratio	0.35 \pm 0.02	0.35 \pm 0.02	0.31
CSF volume ratio	0.21 \pm 0.03	0.19 \pm 0.03	0.17
Cortical thickness	2.68 \pm 0.82	2.57 \pm 0.79	0.17

GM=grey matter; WM= white matter; CSF=cerebrospinal fluid.

between the APOEε4 and APOEε3 groups, where APOEε4 carriers had greater WM volume than the APOEε3 group in the Right superior temporal gyrus, reduced WM volume in the Right sub-gyral frontal lobe, left medial temporal gyrus and lower GM volume in the left superior frontal gyrus. These differences are shown in Figure 4.3.

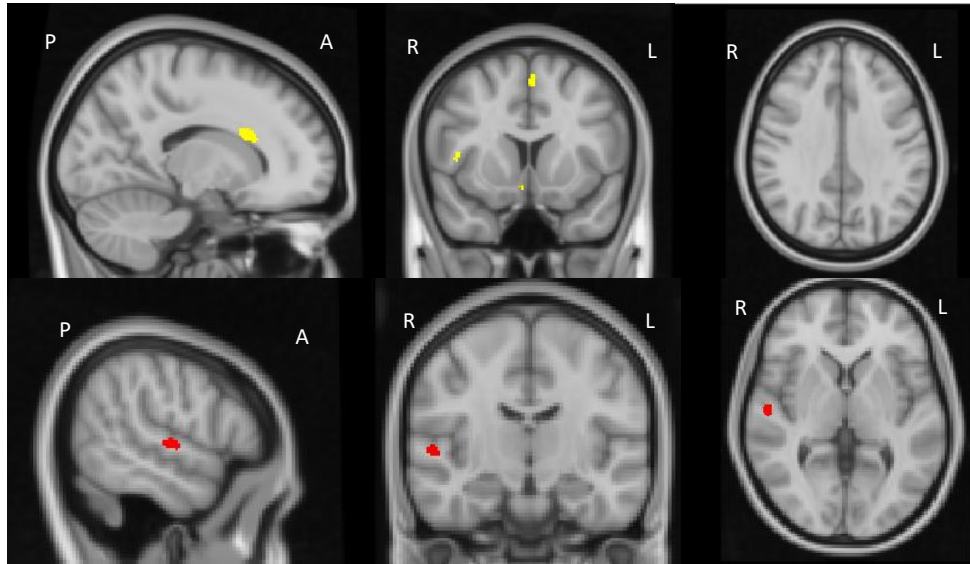


Figure 4.2 GM and WM volumes in APOEε3 and APOEε4 carriers. MRI slices showing clusters of greater GM and WM volumes in APOEε3 carriers (in yellow) and greater volumes in APOEε4 (in red). The cluster threshold was set at $T=3.3$ and $p<0.001$ family wise uncorrected. A, anterior; P, posterior; R, right; L, left.

4.6.2 Diffusion Tensor Imaging (DTI)

SPM clusters of higher FA in APOEε3s as compared to APOEε4s were observed in the right hemisphere grey matter, specifically in postcentral gyrus of the parietal lobe and inferior frontal gyrus of the frontal lobe shown in (Figure 4.4). No clusters were observed in the APOEε4 group. Clusters of greater MD in APOEε4s relative to APOEε3s were detected in several

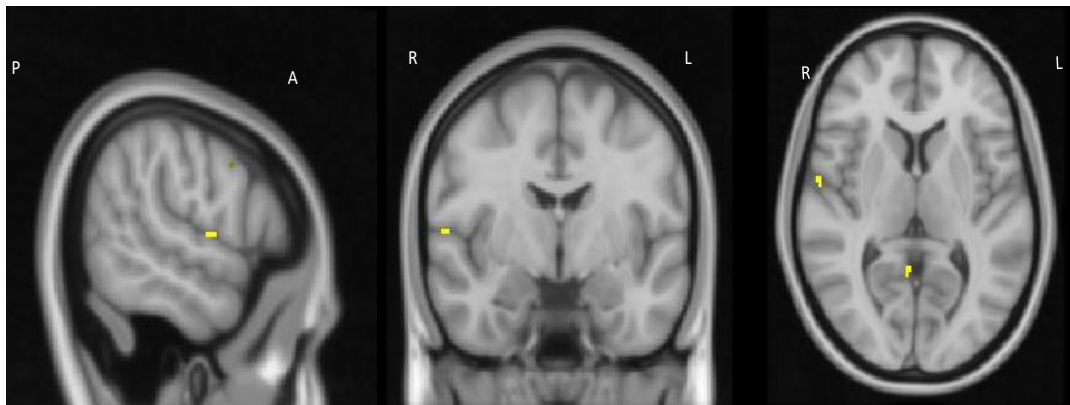


Figure 4.4 Clusters of greater FA (in yellow) for APOEε3s. The cluster threshold was $p<0.001$ uncorrected. A, anterior; P, posterior; R, right; L, left.

regions including sub-gyrus of the parietal lobe of both right and left hemispheres and temporal gyrus of the right hemisphere, inferior frontal gyrus of right hemisphere and lingual gyrus of occipital lobe in left hemisphere (Figure 4.5 (a)). The APOEε3 group did not show any clusters.

Greater AxD was observed in APOEε4 group relative to APOEε3 in the parietal lobe of both hemispheres, cingulate gyrus of limbic lobe and fusiform gyrus of temporal lobe in the left hemisphere, as well as; the sub-lobar insula, precentral gyrus of frontal lobe, middle-temporal gyrus of the right hemisphere, and right anterior cerebellum (Figure 4.5 (b)). Finally, higher RD was also observed in APOEε4s compared to APOEε3s in the following regions: sub gyrus of the parietal lobe in the left hemisphere, insula and inferior gyrus of parietal lobe, middle temporal gyrus, precentral and postcentral gyrus of frontal lobe in the right hemisphere; and the anterior lobe of both left and right cerebellum (Figure 4.5 (c)).

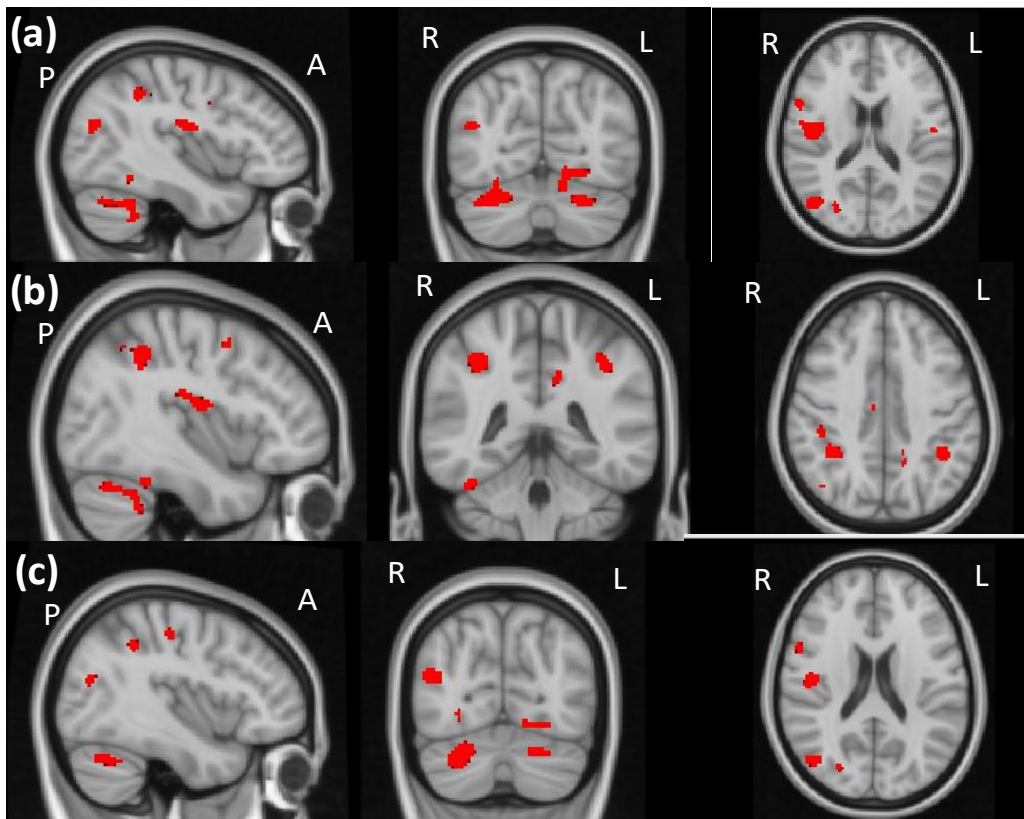


Figure 4.5 MD clusters in APOEε4 and APOEε3 carriers (a) Clusters of greater MD in APOEε4 carriers (shown in red). The cluster threshold was $p < 0.001$ uncorrected. A, anterior; P, posterior; R, right; L, left. (b) Clusters of greater AxD in APOEε4 carriers (shown in red). The cluster threshold was $p < 0.001$ uncorrected. A, anterior; P, posterior; R, right; L, left. (c) Clusters of greater RD in APOEε4 carriers (shown in red). The cluster threshold was $p < 0.001$ uncorrected. A, anterior; P, posterior; R, right; L, left.

In addition to the DTI pixel wise analysis, a supplementary histogram analysis was completed to achieve more sensitive information regarding brain tissue microstructure regardless of ROIs.

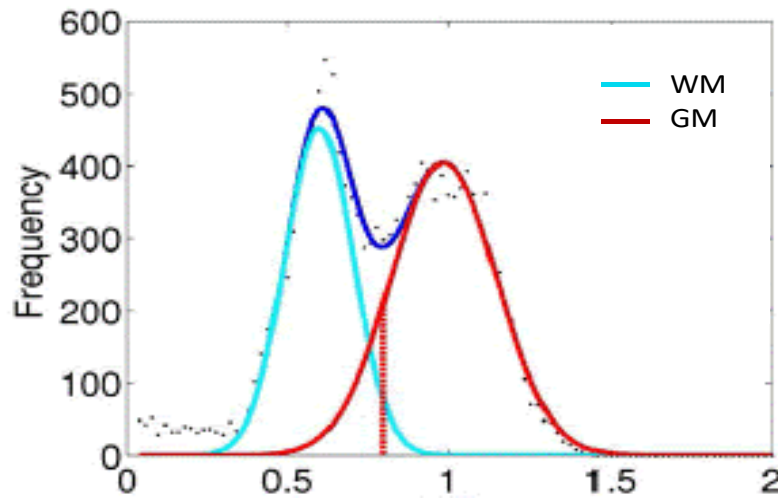


Figure 4.3 Partial volume effect in WM histogram. Generating GM histogram resulted in a double peak histogram with WM histogram which produces a partial volume effect (Sourced from Grinberg et al., 2017).

DTI maps (FA, MD, RD and AxD) of whole brain and WM were generated. GM histograms were not generated due to issues with the grey matter masks which included WM peaks together with the GM peak⁸, which is known as partial volume effect as shown Figure 4.6. To compare between groups, peak height and peak position, shown in table 4.2, were calculated for each DTI parameter of the whole brain and white matter. Histograms of whole brain in the four diffusivity measures used in the histogram analysis are shown in Figure 4.7. Means of the diffusivity histogram measures obtained in APOEε3 and APOEε4 participants were compared using a one-tailed *t* test. Statistical significance was set at $p < 0.013$ following Bonferroni correction for multiple comparisons. None of the measures showed a significant difference between groups but rather an underlying trend of group difference was observed in peak height of the whole brain for mean MD ($p = 0.02$) and AxD ($p = 0.02$) as well as peak position of whole brain for axial diffusivity ($p = 0.01$). All differences in whole brain were in the direction of higher peaks in the APOEε3 carriers except for FA, where higher peak was observed in the APOEε4 group. No trend of group differences in white matter measures were observed.

⁸ Attempts to erode the GM mask to remove the partial volume effect resulted in a GM ROI that was too small to provide useful histograms (too few voxels) and this will cause the histograms to be too noisy. Therefore, GM histograms for the DTI measures were not obtained.

Table 4.2 APOEε3 and APOEε4 carriers group means of Histogram peak height and peak position of diffusivity parameters for each of the whole brain and white matter.

	Whole Brain			White Matter		
	APOEε3(20)	APOEε4(18)	<i>p</i>	APOEε3(20)	APOEε4(18)	<i>p</i>
Peak Height						
FA	0.813	0.873	0.025	0.58	0.58	0.47
MD	1.66	1.496	0.015	3.79	3.84	0.30
AxD	1.697	1.617	0.017	1.46	1.46	0.47
RD	1.12	1.035	0.046	2.30	2.31	0.20
Peak Position						
FA	0.112	0.109	0.11	0.424	0.417	0.15
MD	0.507	0.512	0.37	0.488	0.495	0.11
AxD	0.717	0.726	0.014	0.694	0.701	0.1
RD	0.487	0.499	0.316	0.362	0.371	0.18

Where FA is Fractional Anisotropy, MD is Mean Diffusivity, AxD is Axial Diffusivity and RD is Radial Diffusivity. Peak height diffusivity measures and peak position of FA are in units of mm^2/s and the remaining diffusivity measures on peak positions (MD, AxD, RD) are in units of $\times 10^{-3} \text{mm}^2/\text{s}$.

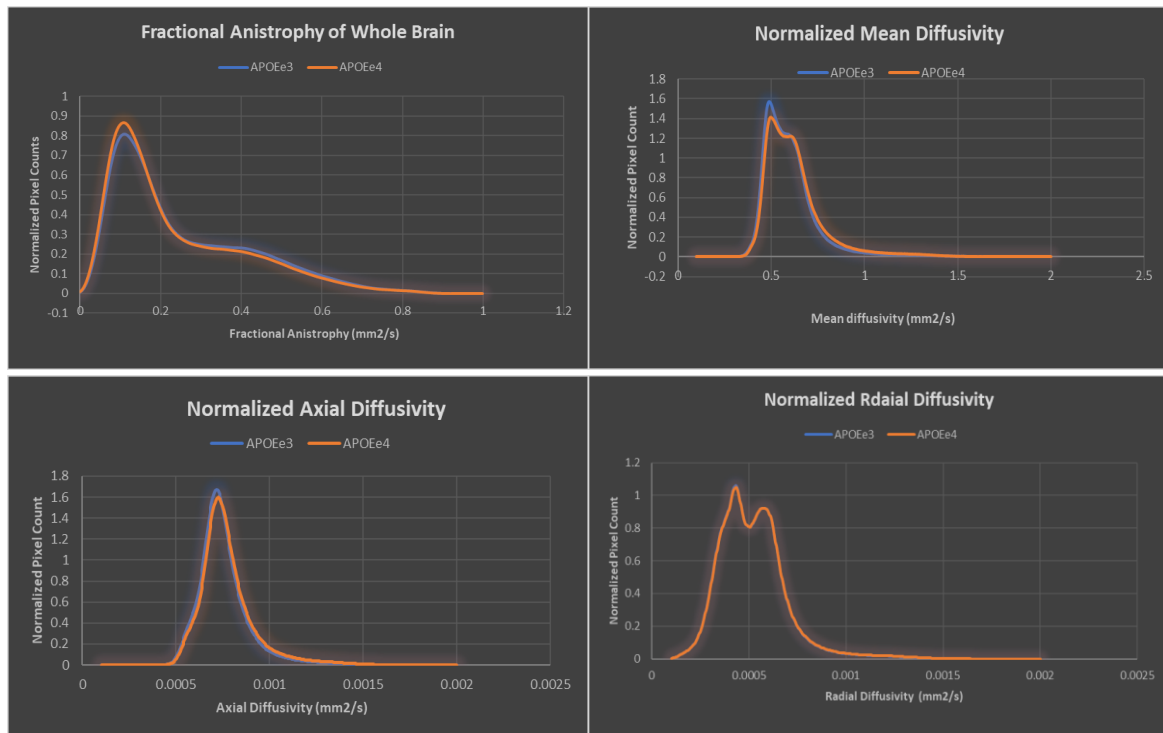


Figure 4.4 Histograms of whole brain diffusivity measures comparing APOEε3 (blue) and APOEε4 (red) groups. The higher peak representing white matter and lower peak representing grey matter. Peak height is the corresponding value on the y-axis and the peak position is the value on the x-axis (diffusivity value ($\times 10^{-3} \text{mm}^2/\text{s}$)).

4.6.3 Neurite Orientation Dispersion and Density Imaging (NODDI)

Although non-significant, NDI was lower in the APOEε4 group than APOEε3 in the left hemisphere of the superior frontal gyrus, middle temporal gyrus and parietal lobe, as well as the superior temporal gyrus of both the right and left cerebrum (Figure 4.8. yellow clusters),

while NDI was lower in APOEε3 in the inferior parietal lobe of the right hemisphere only (Figure 4.8.). Relative to APOEε3 carriers, f_{iso} was slightly higher in APOEε4s in the sub-lobar, extra nuclear and the sub gyrus, parietal lobe of the right hemisphere as well as the anterior lobe of the right cerebellum and posterior lobe of the left cerebellum (Figure 4.9). Finally, relative to APOEε3 carriers, ODI was observed to be lower in APOEε4 carriers in the superior frontal gyrus of the left hemisphere and inferior temporal gyrus, cingulate gyrus of limbic lobe, superior and middle frontal gyrus of the right hemisphere (Figure 4.10), while ODI was higher in APOE4s relative to APOEε3s only in the extra nuclear, Sub-lobar, Intra hemispheric region (Figure 4.10). No statistically significant differences after Bonforroni correction between genotypes was shown for ROI analysis, as shown in table 4.3 and bar graphs in Figure 4.11.

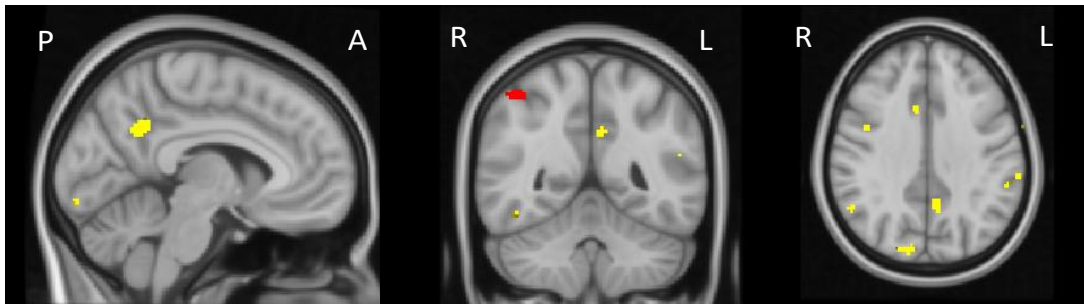


Figure 4.8 Clusters showing regions of greater NDI shown in yellow in APOEε3 group while APOEε4 groups greater cluster is shown in red. The cluster threshold was $p < 0.001$ uncorrected. A, anterior; P, posterior; R, right; L, left.

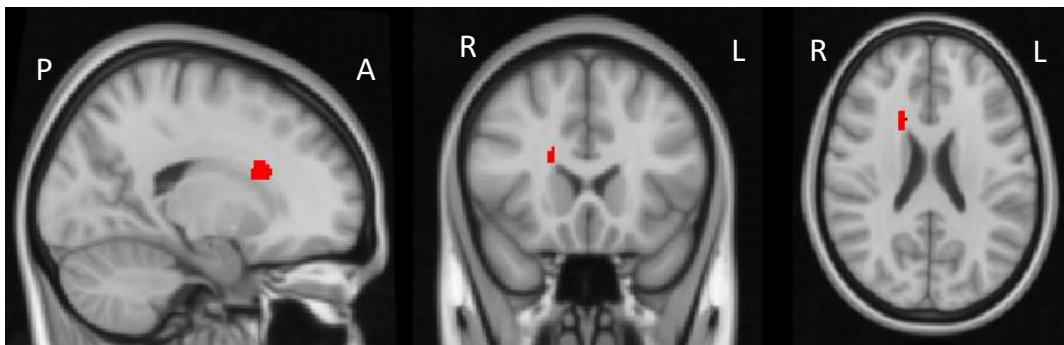


Figure 4.5 Clusters showing regions of greater f_{iso} shown in red in APOEε4 groups. The cluster threshold was $p < 0.001$ uncorrected. A, anterior; P, posterior; R, right; L, left.

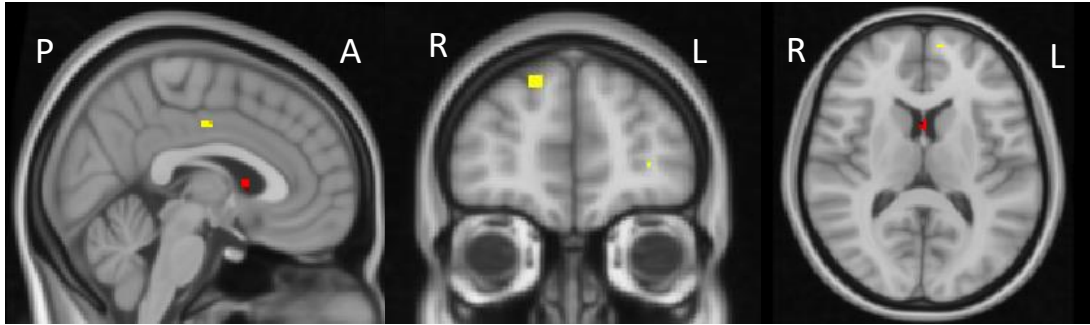


Figure 4.10 Clusters showing regions of greater ODI shown in yellow for APOEε3 carriers and in red for APOEε4 carriers. The cluster threshold was $p < 0.001$ uncorrected. A, anterior; P, posterior; R, right; L, left.



Figure 4.6 Bar chart showing differences between APOEε3 and APOEε4 carriers in NODDI parameters of different ROI's. A. NDI differences, B. ODI differences, C. Fiso differences.

Table 4.3 Mean (Standard deviation) and significance level of NODDI parameters for each ROI for APOEε3 and APOEε4 carriers.

	NDI			ODI			Fiso		
	APOEε3(20)	APOEε4(18)	p	APOEε3(20)	APOEε4(18)	p	APOEε3(20)	APOEε4(18)	p
Left Hippocampus	0.44	0.44	0.64	0.49	0.48	0.24	0.13	0.13	0.70
Right Hippocampus	0.43	0.43	0.47	0.48	0.48	0.89	0.14	0.14	0.85
Left Superior longitudinal fasciculus	0.57	0.55	0.08	0.30	0.30	0.68	0.09	0.09	0.47
Right Superior longitudinal fasciculus	0.58	0.57	0.20	0.30	0.29	0.24	0.09	0.09	0.94
Left WM ACC	0.45	0.45	0.46	0.38	0.38	0.54	0.09	0.10	0.25
Right WM ACC	0.45	0.43	0.03	0.40	0.40	0.65	0.12	0.13	0.63
Para-hippocampal Gyrus Anterior	0.55	0.54	0.40	0.48	0.48	0.75	0.23	0.24	0.49
Para-hippocampal Gyrus Posterior	0.48	0.48	0.40	0.49	0.50	0.29	0.16	0.17	0.43

Significant ($p < 0.006$) after Bonferroni correction

4.6.4 Magnetisation Transfer (MTsat)

No significant differences were observed between APOEε4 and APOEε3 groups for MTsat. However, localised cluster differences were observed between groups where the APOEε3 group showed higher MTsat in the Right sub-lobar lateral ventricular wall, GM of Right sub-lobar caudate head and the GM of the anterior cingulate of the limbic lobe (Figure 4.12).

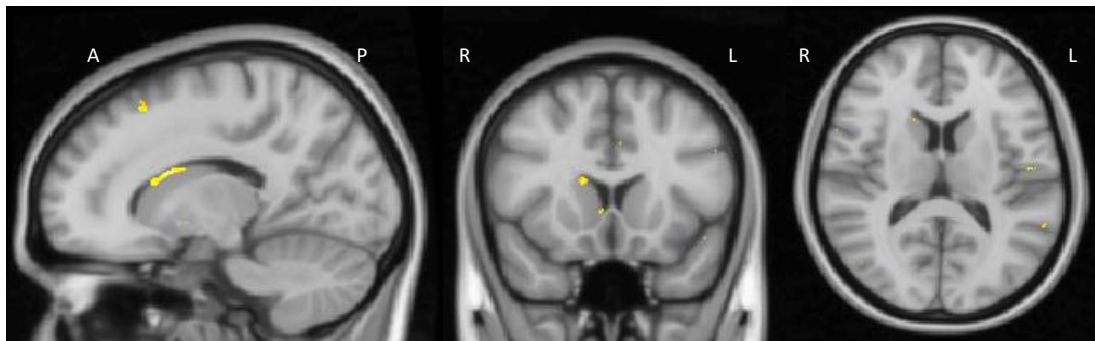


Figure 4.7 Clusters of greater MTsat shown in yellow for APOEε3 carriers. The cluster threshold was $p < 0.001$ uncorrected. A, anterior; P, posterior; R, right; L, left.

4.6.5 Susceptibility Weighted Imaging (SWI)

From visualized quantification of number MB lesions, no MB were detected in any of the participants in our cohort.

4.7 Discussion

In this study a series of structural imaging acquisitions was developed using a high performance 3T scanner to investigate subtle structural differences between APOEε4 and APOEε3 carriers in a disease free, symptom free mid-aged cohort. The series of imaging techniques employed here including diffusivity measures (DTI and NODDI), magnetization transfer and multiple

parametric mapping provide a variety of structural information with high sensitivity for detection of subtle changes or differences. Although non-significant at the conventional 5% level, subtle trends were detected between genotypes in several localized regions.

Although conflicting with some of the literature (Williams et al., 2019; Operto et al., 2018; Cavedo et al., 2017), higher WM volume was found in the temporal lobe of APOEε4 carriers. This is consistent with the findings of a previous study from our research group (Dowell et al., 2016) in a different mid-age sample, and was interpreted by the authors as evidence for a possible neurogenic effect as a result of regional over-engagement in APOEε4 carriers (i.e. the brain working harder) from a younger age. WM volume was found to be lower in regions of the frontal lobe of APOEε4 relative to the APOEε3 carriers, and GM volume was lower in the temporal lobe. Both of these findings are consistent with the literature (Operto et al., 2018; Cacciaglia et al., 2018,2019), indicating that there are subtle differences between APOEε4s and APOEε3s visible with high quality structural imaging from mid-age.

In this study we combine two *in vivo* diffusion models to investigate brain microstructure. Regions of lower FA and higher MD were found in people with AD, and interpreted as evidence for WM tract disruption (Stebbins et al., 2009). The measure of AxD is thought to be sensitive to axonal health and RD is more related to myelin conditions. In AD, AxD and RD are both found to be increased (Acosta-Cabronero et al., 2012).

In the current study, at cluster threshold $p < 0.001$ uncorrected for all DTI measures, lower FA and higher MD was found in APOEε4 carriers, which may indicate the starting point of fiber tract disruption. We also found regions of slightly higher AxD and RD in APOEε4 carriers, which may indicate the very early stages of axonal damage and demyelination. Previously, however, our group has reported higher AxD, in the absence of changes in RD and MD, in healthy young APOEε4 carriers (Dowell et al., 2013).

To identify subtle diffuse differences between genotypes that effect a large area of the brain and may not be detected in small regions or cluster analysis, we performed histogram analysis on the DTI parameters, (Paul Tofts and Gerard Davies, 2003, Histograms: Volumetric Analysis, 2003, Quantitative MRI of the Brain (581-610) John Wiley & Sons, Ltd, ISBN: 0-470-84721-2). After applying multiple comparison correction $p = 0.01$, none of the measures showed a significant difference between groups but rather an underlying trend of group difference was observed in peak height of the whole brain for MD $p = 0.02$ and AxD $p = 0.02$ as well as peak position of whole brain for AxD $p = 0.01$. Lower peak height and higher peak

position was found in the APOEε4 group in MD and AxD measures, meaning more variability which is possibly due to subtle diffuse damage. This may be interpreted as the start point for loss of WM integrity and axonal damage.

Neurite density index (NDI) is a measure of neurite volume indicative of WM neurodegeneration which is found to be reduced in young onset AD and in APOEε4 carriers / AD and correlated with cognitive decline (Parker et al., 2017; Slattery et al., 2017). Here, at cluster threshold $p < 0.001$ uncorrected for all NODDI measures, NDI was found to be slightly lower in APOEε4s in localised areas of the brain, which may indicate a beginning of change towards axonal and dendritic damage starting to emerge in mid-age APOEε4 carriers. Fraction of water in the isotropic compartment (f_{iso}), is found to be increased in dementia and MCI patients indicating increase in free water concentration and damage at a microstructural level (Raghavan et al., 2021). In this current study, f_{iso} was found to be higher in healthy mid-age APOEε4 carriers, in the sub-lobar, extra nuclear and the sub gyrus, parietal lobe of the right hemisphere as well as the anterior lobe of the right cerebellum and posterior lobe of the left cerebellum which may indicate subtle negative changes in axonal structures. Variability in neurite orientation is estimated by orientation dispersion index (ODI) and studies have shown that ODI is reduced with normal aging (Nazeri et al., 2015), was found even lower in young onset AD (Parker et al., 2017), and also lower in healthy mid-age APOEε4 carriers which correlated with lower cognitive performance (Evans et al., 2020). Here we found ODI to be lower in APOEε4 carriers relative to their APOEε3 counterparts in regions identified in early onset of AD indicating GM microstructural breakdown might start to develop in APOEε4 carriers at mid-age. These regions included WM of right inferior temporal gyrus, left superior frontal gyrus, right middle frontal gyrus and GM of the right superior frontal gyrus and right cingulate gyrus of limbic lobe, which are implicated in early changes in AD (Parker et al., 2018; Xiuwei Fu et al., 2020).

To add to the series of imaging acquisitions we included MTsat imaging which is a marker for myelin concentration, where lower MTsat indicates demyelination (Hagiwara et al., 2018). No significant differences between groups were found in our cohort of healthy mid-age volunteers at cluster threshold $p < 0.001$ uncorrected, but subtle reduction in myelin was found in APOEε4 carriers in localised regions compared to APOEε3 carriers. These regions included; Right lateral ventricular wall, GM of right caudate head and right anterior cingulate of limbic lobe. These differences may be suggestive of better myelination in those specific regions in the APOEε3 group, but also could be considered an artefact at the regions very near to the

ventricles (an edge effect because CSF in ventricles has no MT value, while surrounding tissues have high MT values). MT ratio (MTR) has been shown to be reduced in GM of AD, but not in the same regions identified in our study (Seiler et al., 2014; Colonna et al., 2021). MTR is a technique used to quantify myelin concentration but its acquisition time is very long with complicated processing (Saccenti et al., 2020) and MTsat is a technique used to improve MTR with higher contrast, which may be the reason for different regional results in this study (Wallaert et al., 2017).

On the other hand, lesions of cerebral microbleeds (CMB) and white matter hyperintensities (WMH) were not detected in any of the genotype groups, which suggests CMB and WMH, that are found to be increased in AD and in older APOEε4 carriers (Brundel et al., 2012; van der Flier et al., 2012; Poliakova et al., 2016; Ingala et al., 2020), do not develop at mid-age but rather later on with age in APOEε4 carriers.

Concluding comments and summary

Here we experimented with a combination of structural imaging techniques to help identify subtle differences between the brain structures of healthy mid-age APOEε4 and APOEε3 carriers. We also aimed to investigate whether the differences identified in the literature associated with later life AD pathology are emerging at mid-age in people with a higher risk for AD pathology in later age. We did indeed identify some subtle changes in the APOEε4 group relative to their APOEε3 peers. While these did not reach the conventional 5% level of statistical significance, and therefore could not be considered just yet to identify any significant structural differences between genotypes at this age range, interestingly we think that together they demonstrate a trend towards change starting to emerge. This may implicate, some detrimental change in APOEε4 structure starting to emerge, which may be identified in a longitudinal study on the same cohort.

A major limitation of this study is the small sample size. The precise specifications in the recruitment criteria made it very challenging to find volunteers in mid-age with time to spare for the study, with no underlying health conditions and willing to receive a contrast agent during the lengthy MRI scanning session.

Previous qMR studies on APOE have focused on specific techniques, for example, either NODDI or DTI but not a full range of qMR techniques together. On the positive side, this is the first study to report on full range of subtle structural markers completed on the same group of volunteers, using 3T imaging. In this study we also focused on the likely age where structural changes related to AD may be detectable but prior to the development of any cognitive deficit, with a narrow age range 42-59. Previous work has reported data over a much wider age range, whereas here we focused on pinpointing a more limited and specific age range that could show small changes in the brain structure between the two genotypes.

Chapter 5: BBB Breakdown in Mid-Age APOEε4 Carriers

5.1 Introduction to BBB breakdown in mid-age APOEε4 carriers

Early biomarkers contributing to AD are of importance for reaching a complete understanding of how and when this neurodegenerative disease starts for preventive and therapeutic purposes. As described in previous chapters, this study has investigated blood and structural differences between higher risk APOEε4 carriers and their APOEε3 counterparts, and early biomarkers indicative of potential risk have been identified. This chapter focuses on blood-brain barrier breakdown which is a major structural biomarker that has been described as potentially where neurodegenerative disease begins.

One of the natural anatomical structures are the vessel walls separating blood from tissues of the central nervous system (CNS). The blood vessel walls of the CNS are different from the vessel walls in the rest of the body as shown in Figure 5.1, due to the sensitivity and delicacy of the brain tissue which cannot tolerate any leakage of neuro-toxic substances into the neuronal tissue. The vessel wall of the CNS is called the blood-brain barrier (BBB) and is structured in a way where the cells are very tightly bonded to perform their main function, which is to prevent toxins and pathogens from entering the neuronal tissues including blood derived materials (Keaney and Campbell, 2015). This is essential for the brain to perform its normal functions.

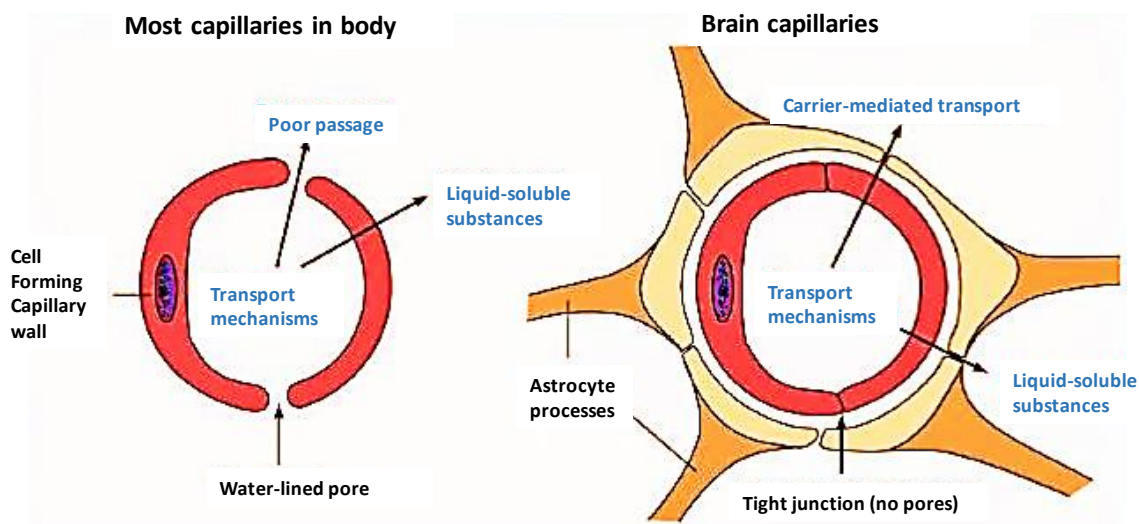


Figure 5.1 Difference between brain capillaries (BBB) and capillaries in the rest of the body, demonstrating the tight capillary wall junctions in the BBB (<https://kevinbinz.files.wordpress.com/2016/01/neuroendocrine-bbb.png>).

As the BBB carries out a major role in maintaining the health and wellbeing of the CNS, it is unsurprising that in AD the BBB is found to be damaged leading to increase in vascular permeability and neuro-toxin accumulation, and in turn, contributing to cognitive decline and neuronal damage (Erickson and Banks, 2013; Montagne et al., 2015; Nelson et al., 2016; Zenaro et al., 2017). It is thought that BBB breakdown is one of the first steps leading to neuronal injury resulting in preclinical neurodegenerative disease including AD (Nelson et al., 2016; Sweeney et al., 2018). This was concluded because A β toxins are thought to play an important role in AD pathogenesis (Teunissen et al., 2018), and are found to be accumulated early in brains of AD patients even before memory decline (Leal et al., 2018) due to cerebrovascular damage including BBB leakage. Consequently, BBB leakage leads to blood derived neurotoxins entering the brain tissue, such as leakage of fibrinogen in the brain tissue which is thought to bind with A β (A β -Fibrinogen), and therefore, contributing to A β accumulation, leading to neurodegeneration (Cortes-Canteli et al., 2012; Oijen et al., 2005).

Through the years, several methods have been used to investigate and quantify BBB leakage in vivo. One of these methods has been extensively used in recent years in BBB investigations, namely dynamic contrast enhanced MRI (DCE-MRI), (Tofts and Kermode, 1991; Larsson et al., 2008,2009; Tofts, 2010; Cramer et al., 2014, 2019; Heye et al., 2014,2016; Barnes et al., 2015; Thrippleton, 2019; Raja et al., 2019; Verheggen et al., 2020). DCE-MRI has been used together with the Patlak fitting model to measure BBB leakage as well as localization of the leakage (Tofts et al., 1999; Tofts, 2010; Barnes et al., 2015; Montagen et al., 2015,2020; Thrippleton, 2019). The Patlak model is able to quantify several parameters including K_{trans} and V_p , where K_{trans} is the metric used to estimate the permeability of the BBB and V_p is the fractional plasma volume in the selected region (Tofts, 2010). More details about the different BBB permeability measurements and modalities are found in chapter 3 including DCE-MRI (Patlak model), CSF/serum albumin quotient (Qalb) and BBB leakage measurements in PET.

To continue our review on BBB permeability, vascular-structural changes such as the breakdown of the BBB have been shown to be increased with age, both in people with AD and also in healthy older individuals, which indicates BBB disruption is part of normal ageing (Verheggen et al., 2020). BBB leakage was also detectable prior to the development of apparent cognitive decline at a preclinical stage in AD (Medina et al., 2016). Understanding the timing of when the BBB breakdown occurs is critical for prevention and feasible treatments. To date, most BBB studies have been on murine models (Bell et al., 2012; Daneman et al., 2010) or human post-mortem individuals (Sengillo et al., 2013, Toledo et al., 2013), but in a seminal

study, Montagne et al. (2015) reported findings from a cohort of non-cognitively impaired (NCI) and mild cognitively impaired (MCI) participants of a wide age-range (23-91), where they were divided into young NCI, older NCI and MCI groups. This study explored two indices of BBB integrity including in vivo *Ktrans* BBB measurements and CSF/plasma albumin ratio (Qalb). Their findings indicated age-related changes in NCI participants that suggest BBB breakdown is part of normal aging which increases, but is not qualitatively distinct, in MCI patients. The loss of BBB integrity was found to be increased in the hippocampus, which is a region identified as showing earliest and most extensive damage in AD post-mortem cases (Sengillo et al., 2013) and may suggest the target region of early BBB breakdown in AD. A more recent study on vascular MCI (vMCI) participants found that BBB leakage increased in vMCI patients compared to healthy age-matched controls and cognitive decline was significantly increased, correlating with the rate of BBB leakage in vMCI patients (Li et al., 2021).

The mechanism contributing to BBB breakdown which is thought to lead to AD pathology has been the focus of most recent studies. Because APOEε4 is the most dominant genetic risk factor leading to late onset AD and has been found to contribute to cognitive decline (Moreno-Grau et al., 2017; El Haj et al., 2016), it was important to many researchers to investigate the relationship between the presence of APOEε4 and BBB integrity. A study revealed that the presence of APOEε4 genotype contributes to BBB breakdown in cognitively healthy carriers but the BBB disruption is further increased in MCI patients (Montagne et al., 2020). Montagne et al., 2020 study was performed on 245 participants over the age of 45 divided in four groups: 130 healthy APOEε3, 76 healthy APOEε4, 14 MCI APOEε3 and 25 MCI APOEε4 with mean age 69.9, 67.3, 73.8 and 69.4, respectively. BBB permeability was measured using several modalities including MRI, PET, lumbar puncture and blood test. They found the BBB breakdown increased in healthy APOEε4 carriers as indicated by *Ktrans* levels, and these correlated with increase in two inflammatory markers; Cyclophilin A (CypA) and matrix metalloproteinase-9 (MMP-9). The CypA-MMP-9 pathway is a brain pro-inflammatory pathway in pericytes⁹ of the BBB endothelial walls (Bell et al., 2014)) (further details about these blood biomarkers are found in chapter 6). Furthermore, BBB breakdown was found to be higher in APOEε4/MCI individuals compared to APOEε4/healthy, and the increased BBB permeability was identified in the hippocampal and medial temporal lobe regions (Montagne

⁹ Pericytes are cells along vessel walls which are extremely important in CNS vessels as they have a major role in maintaining the BBB integrity. Pericytes degeneration is found in AD (Sengillo et al., 2013).

et al., 2020). The Montagne et al. (2020) study agrees with a human post-mortem brain tissue study in AD APOEε4 carriers which suggested that BBB breakdown is initiated due to pericyte degeneration, and that the pericyte degeneration was due to the accumulation of the pro-inflammatory cytokines CypA and MMP-9 in the pericytes and endothelial cells (Halliday et al., 2016). However, not all studies have reported a link to APOE. A study on a large cohort of elderly AD patients (n=75) and controls (n=65) (76yrs \pm 7) found that BBB damage was associated with diabetes but not with APOE status (Janelidze et al., 2017). This contradictory finding may be attributed to the method used to measure BBB integrity (CSF/Plasma albumin ratio (Qalb)), because Qalb is not able to localize BBB leakage and cannot differentiate between blood leakage in brain from elsewhere in the CNS making this method less informative for BBB permeability investigations (Raja et al., 2018). Qalb is also an invasive method (via lumbar puncture) whereas other methods measuring BBB leakage use a less invasive approach such as DCE-MRI scans with a *K_{trans}* parameter.

Table 5.1 reviews details of recent BBB permeability human studies discussed in this chapter and the methods used to quantify BBB leakage rate. From the literature to date, we can conclude that BBB breakdown leads to neurodegenerative disease including AD, BBB permeability is increased in MCI and is more prominent in carriers of the APOEε4 gene. The age at which BBB leakage begins is of great importance. A DCE-MRI study involving people in the early stages of AD (age range: 59-85) found that BBB dysfunction was significantly increased in several regions of white and grey matter in patients in their early stages of AD compared to healthy age-matched controls, and that cognitive decline increased significantly with increase in the rate of BBB leakage (van de Haar et al., 2016, 2017). In two recent studies on cognitively healthy volunteers, BBB breakdown was found to increase with age, where BBB leakage is increased in older adults compared to mid-age (Verheggen et al., 2020) and increased in middle aged rats compared to young (Bors et al., 2018) suggesting, as reported by Montagne et al. (2016), that BBB dysfunction is part of normal aging.

So far, BBB breakdown has been investigated in post-mortem, AD patients, transgenic mice, APOEε4 carriers with AD, healthy older individuals who are either APOEε4 or non-APOEε4 carriers, and in middle age rats. Only one recent study investigated BBB permeability in

Table 5.1 Review of human BBB leakage *in vivo* measurements and cohorts

Paper	Cohort type	Cohort age	Measurement method
Li et al., 2021	Vascular MCI	71.04 ± 8.99	Patlak model ^a
Montagne et al., 2020	APOEε4/APOEε3 NCI and MCI ≥45		Patlak model/ PET ^b /Qalb ^c
Verheggen et al., 2020	Healthy	47-91	Patlak model
Van de Haar et al., 2016, 2017	Early AD	59-85	Patlak model
Janelidze et al., 2017	clinical form of dementia	>60	Qalb
Montagne et al., 2015	NCI & MCI	23-91	Patlak model/ Qalb

^a A pharmacokinetic analysis model to measure the concentration of Gd in the extravascular-extracellular space (EES) (Patlak et al. 1983).

^b *Positron Emission Tomography*.

^c Albumin quotient (CSF/plasma albumin ratio) measured via lumbar puncture.

*Research papers cited in this chapter on rat models or post-mortem were not included in this table.

cognitively unimpaired APOEε4 carriers above 45 years of age, but with an exceptionally wide age range, which compared differences between groups but did not look for differences specifically in a narrow mid-age range. In recent years, the focus of most BBB studies has been on the mechanism of how APOEε4 has a negative influence on BBB breakdown rather than the age at which BBB dysfunction begins. The timeline of when and where these physiological changes occur that lead to increased risk for neurodegenerative disease is of extreme importance, for prevention and therapeutic purposes.

In this chapter, we examine BBB permeability differences between healthy middle age APOEε4 and APOEε3 carriers in brain regions identified in the literature that are most relevant to AD, associating with cognitive deficits, and that have also shown sensitivity to genetic differences in healthy elderly individuals. No previous studies have explored BBB integrity in mid-age healthy adults who differ only in their APOE genotype. We developed a high sensitivity 3T MR imaging protocol to enable detection of early subtle BBB permeability differences. In the same individuals, we also collected blood inflammatory markers which have been identified as biomarkers for the dysfunction of BBB in AD, including MMP2, MMP9, LRP1 and CypA. These blood inflammatory markers were measured and analysed, and are reported in chapter 6. In this study we fitted the Patlak model to the DCE-MRI data to determine K_{trans} , a parameter extensively applied to assess BBB integrity (K_{trans}) which is a quantitative measure of BBB leakage and has been shown to be ideal for detecting group differences (Montagne et al., 2015, 2018, 2020; Zhang et al., 2017; van de Haar et al., 2016;

Taheri et al., 2011). The Patlak model has been shown to estimate very low vascular permeability measures (K_{trans}) with high accuracy, therefore, ideal for detection of subtle BBB leakages (Barnes et al., 2015; Cramer and Larsson, 2014).

The aim of this chapter was to determine whether, compared to their APOEε3 counterparts, increased BBB permeability is present in healthy middle aged APOEε4 carriers. We hypothesised that the APOEε4 group may show subtle increase in permeability measures in key regions which may indicate that the BBB tight junction has begun to become dysfunctional at this age in carriers of the APOEε4 gene.

5.2 Methods

5.2.1 Participants

Forty-one healthy mid-age participants (mean age 52 (42-59) years; 33 females, 8 males) participated in the study. One participant from the initial eligible 41 was claustrophobic and withdrew from phase 3 (imaging phase) prior to the start of the scanning session, while two other participants withdrew from the scanner session prior to the collection of the BBB data due to: 1. anxiety about the MR contrast injection (Gd) and 2. fatigue from the length of the scan (70 minutes). Details about recruitment process inclusion and exclusion criteria, ethical approval and consent forms are presented in the methods chapter 2. All genotype information was withheld from both researchers and participants through a triangulated recruitment procedure (described in chapter 2) and was added to the anonymised imaging data only after all pre-processing had been completed.

5.2.2 DCE-MRI protocol

BBB imaging data were acquired on a Siemens 3 Tesla MRI scanner using a 32-channel phased-array receive-only, head coil. The BBB scanning protocol was developed after modelling of the DCE signal in a pilot study (described in chapter 3). The DCE sequence acquired in this study included 3 identical T1 weighted 3D VIBE sequences with TR=2.56ms, TE=0.86ms, flip angle=15°, GRAPPA with parallel imaging factor=2, acquired matrix =96(base), 75%(phase), 78%(slice), field of view= 240x240x180 mm³, reconstructed into 36 slices of 5.0mm slice thickness. The DCE acquisition comprised 432 repeated volumes, each with an acquisition time of 2.4 seconds resulting in a total acquisition time of approximately 17 minutes. All slices collected in this sequence were in pure sagittal orientation. Ten T1w VIBE volumes were collected prior to the infusion of contrast agent to provide a baseline image

intensity measure. Gadoterate meglumine (Dotarem) was administered remotely using an automated injector with speed of 3ml/s, followed by a saline flush at the same rate. A single dose of the contrast agent Dotarem was injected based on participant weight (0.05mmol/kg body weight) by an MR radiographer, where the participants' weight was measured immediately before the scan. Throughout the scan participants were instructed to keep their head still and limit other body part movement as much as possible. After the scan, participants were given clear instructions by the radiographer to keep drinking fluids throughout the day to flush out all the contrast from the body. Although it was unlikely, but following the safety protocol by CISC, participants were given a telephone number to contact in case they felt a side effect from the injected contrast medium.

5.2.3 Imaging Acquisition

Prior to the beginning of the scan, 3 phantoms were placed precisely above and outside the coil, to avoid heating from the participant which may result in changes to the T1 and signal intensities. Signal drift during DCE-MR acquisition has a major effect on the estimation of diffusion parameter. Therefore, the phantoms were used to mimic different brain tissues to identify signal drift, by allowing to estimate and compensate for signal loss due to the instability of the DCE-MRI data prior to image analysis (Vos et al., 2016). The B1 map and T1 map were acquired as part of the multi-parametric mapping (MPM) acquisition, obtained in the same scanning session. B1 map was introduced during the scan which is used to correct for unwanted bright and dark regions in parts of the brain, thus reducing any variation in signals due to movement (Sacolick et al., 2010) (further details are found in chapter 3).

5.2.4 Image Analysis

B1 maps, T1 maps and the DCE-MRI volumes were all co-registered to a common participant image space, using SPM12. Regions-of-interest (ROIs) were selected from the MNI¹⁰-space atlas and co-registered to participant space for region-specific statistical analysis. In-house software was used to determine K_{trans} and V_p in a pixel wise manner by fitting the DCE-MRI data, T1 map and B1 map, using the Patlak model. These parameters from the Patlak model are further described in chapter 3. Smoothing (Gaussian kernel 4-mm isotropic) was applied to the T1 maps and DCE-MRI data to improve the robustness of the fit.

¹⁰ MNI-space: Montreal Neurological Institute (MNI) is a template which defines a brain that is more representative of the population (<https://brainmap.org>).

On the basis of previous literature, six ROIs were selected for the BBB analysis in both the right and left hemispheres including; hippocampus, White Matter Anterior cingulate cortex (WM Acc), anterior cortex and subcortical probe of the hippocampus, as well as, anterior and posterior Para-hippocampal Gyrus (Figure 5.2). The ROIs are located in the frontal and temporal lobes (cortical and subcortical regions) including different tissue types (GM & WM), thus were selected to cover a variety of brain regions found to be affected with different pathophysiological changes in the early clinical stages of AD (Bobinski et al., 1999, Smith, 2002; Thompson et al., 2007; Morra et al., 2009; Kantarci et al., 2017) and also in healthy middle aged APOEε4 carriers (Klunk et al., 2007; Liu et al., 2010; Donix et al., 2010; Ten Kate et al., 2016; Slattery et al., 2017; Operto et al., 2018; Mishra et al., 2018; Cacciaglia et al., 2018, 2019).

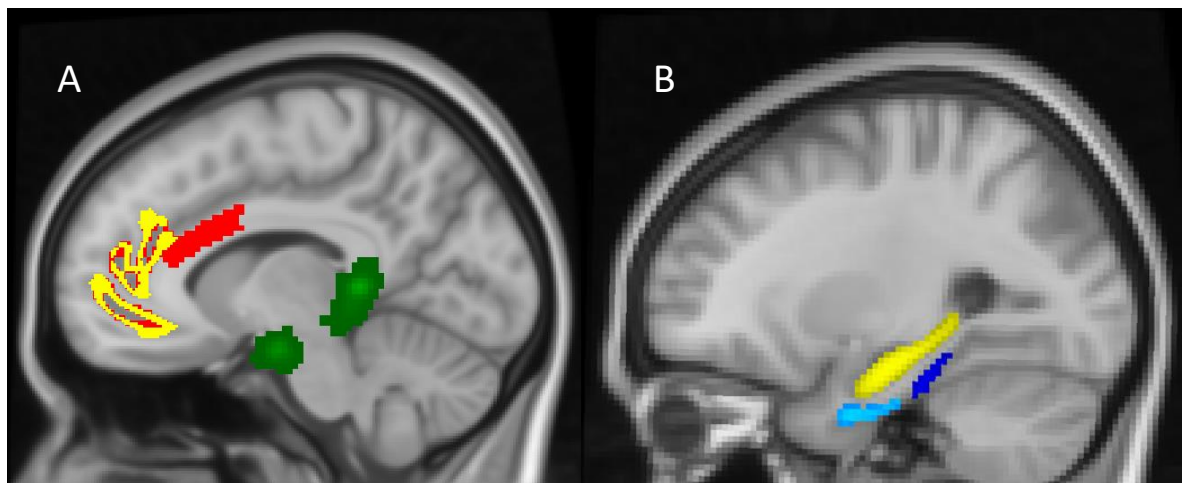


Figure 5.2 Sagittal view of the left hemisphere showing the ROIs selected in this study. An example image of the ROIs selected in this study showing a sagittal view of the left hemisphere. Image A Shows Left Anterior cortex in (yellow), Left WM Acc in (red) and subcortical probe of left hippocampus in (green). Image B shows left hippocampus in (yellow), para-hippocampal gyrus posterior (dark blue) and para-hippocampal gyrus Anterior (light blue).

A ROI BBB histogram analysis was performed on all participants by implementing three key steps: 1. Transforming (warping) MNI-space ROIs to each participant image-space 2. Applying the native-space¹¹ ROIs to each participant 3. Applying Patlak model fitting (linear fit) of the ROI data in order to extract K_{trans} and V_p values. To adjust for the resultant differences in size of the sampled areas of the ROIs, total number of pixels in each ROI were added as covariates in one-way Analysis of Covariance (SPSS version 26), so that the differences in the estimated parameters between APOEε3 and APOEε4 groups in the selected ROIs could be statistically analysed. In this study we focused on one research question to compare two parameters

¹¹ Native-space: Reconstructs the structural *MR* images to extract the localization data for each ROI.

between two groups in several anatomical regions. These two parameters were generated by the histograms and are the peak heights and peak positions. Peak height indicates the variation in the *Ktrans* value and is sensitive to subtle diffusion changes in the ROI, while peak position corresponds to the *Ktrans* value that is most common in the ROI. In healthy BBB, the histograms would present a very well-defined peak, whereas, increase in BBB permeability is identified by either a shift in peak position or a reduced peak height (Tofts & Davies, 2003). Peak height is reduced in normalized histograms, because they are broader, which is driven by increased variance in the *Ktrans* value which may possibly be due to damage. This study generated two comparisons across 10 different ROIs, and since this study has a single hypothesis being tested, statistical significance was retained at $P < 0.05$ (following Bors et al., 2018; Verheggen et al. 2020; Man Li et al., 2021).

5.3 Results

A total of 38 healthy middle-aged (45-59) participants provided data for this part in this study and as described previously, were classified into two groups (APOEε4 and APOEε3 carriers). BBB histograms for each ROI were generated and an example is shown in Figure 5.3. *Ktrans* Means and significance values for regional BBB analyses are shown in table 5.2. Higher BBB leakage, quantified by higher *Ktrans* value, represented by peak position indicates more permeability and potential damage. All ROIs showed no significant difference in BBB leakage between groups although in 8 out of the possible 10 comparisons, the difference was in the direction of higher leakage in APOE4 carriers, where a clear distribution difference between groups is observed when using (GraphPad Prism 9.1.1), (Figure 5.4). These directional differences were identified in the subcortical regions but not the cortex.

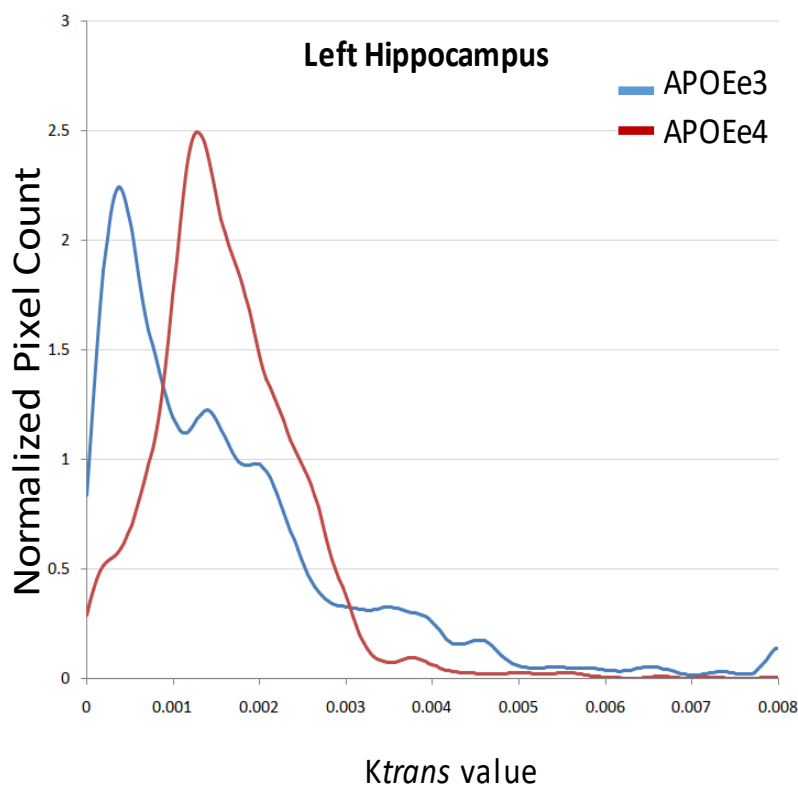


Figure 5.3 Normalized BBB histograms of the left hippocampus. the APOEε3 group shown in blue and APOEε4 group in red. (excel 2016).

Table 5. 2 Mean (Standard deviation) and significance level of BBB Ktrans Histogram peak height and peak position for each ROI for APOEε3 and APOEε4 carriers

	APOEε3(19)	APOEε4(17)	<i>p</i>	APOEε3(19)	APOEε4(17)	<i>p</i>
	Peak Height			Peak Position		
Left Hippocampus	4.66(1.8)	4.41(1.5)	0.704	1.21(1.2)	1.35(0.7)	0.791
Right Hippocampus	3.65(2.7)	4.06(4.4)	0.741	1.16(0.9)	1.29(0.9)	0.738
Left WM ACC	4.00(2.3)	3.43(1.7)	0.535	1.00(0.6)	1.02(0.6)	0.789
Right WM ACC	3.98(1.8)	4.16(2.1)	0.468	0.77(0.9)	0.78(0.6)	0.629
Left Anterior Cortex	9.95(9.9)	7.41(3.1)	0.442	0.78(0.6)	0.68(0.6)	0.302
Right Anterior Cortex	4.37(1.6)	4.20(2.2)	0.922	0.52(0.5)	0.56(0.4)	0.901
Para-hippocampal Gyrus Anterior	2.43(2.0)	2.11(2.0)	0.648	1.90(1.7)	1.85(1.3)	0.951
Para-hippocampal Gyrus Posterior	2.78(1.3)	3.20(1.4)	0.427	1.44(1.1)	1.72(1.3)	0.251
Subcortical probe Left hippocampus	3.44(1.5)	3.39(1.9)	0.974	1.16(1.0)	1.29(0.8)	0.646
Subcortical probe Right hippocampus	3.55(1.6)	3.56(1.1)	0.842	1.18(9.6)	1.29(0.9)	0.389

Peak position is in unit (x10⁻³min⁻¹)

Significant (*p*<.05)

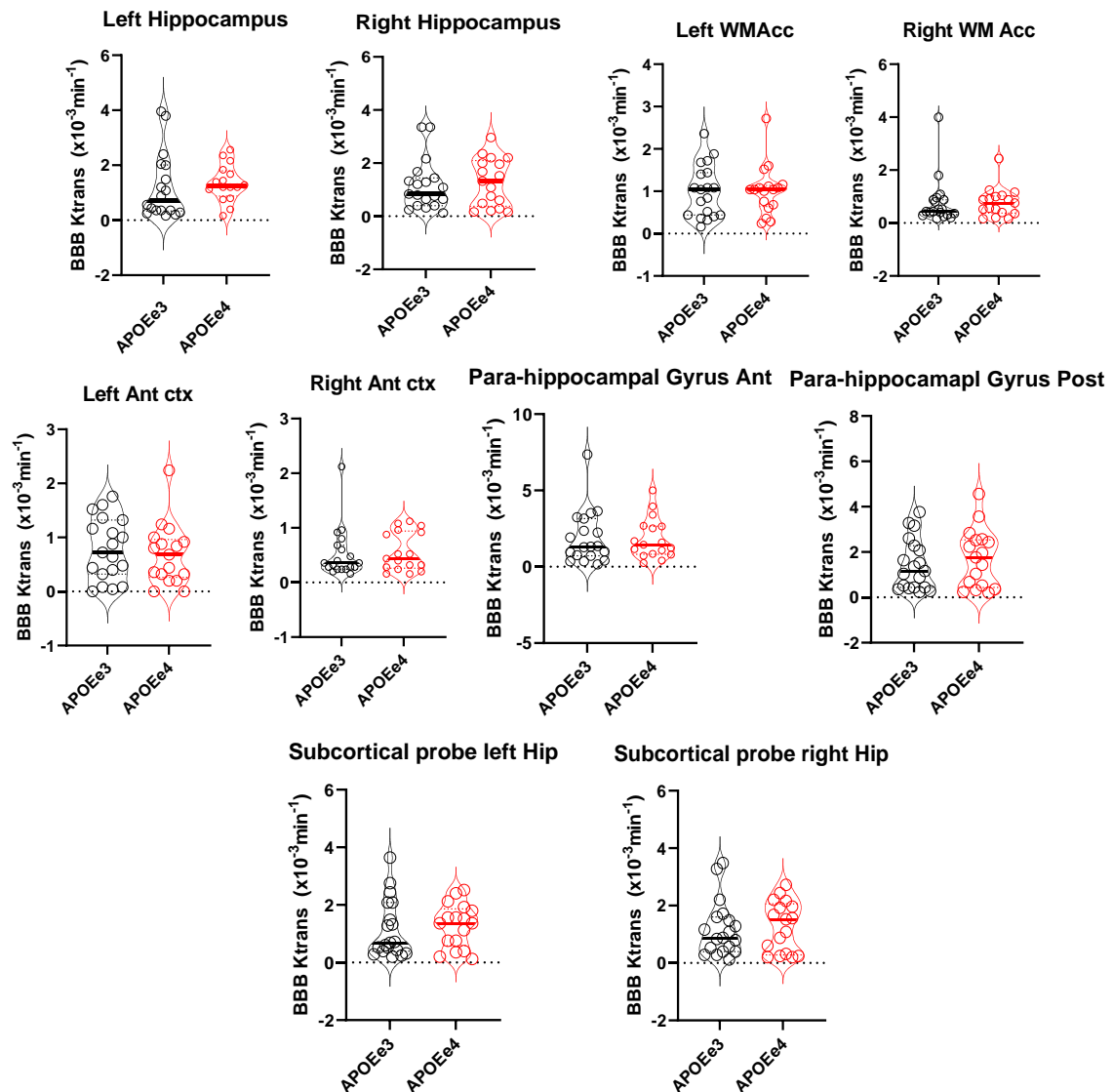


Figure 5.4 Violin plots showing regional BBB Ktrans in 10 brain regions in APOEε4 (red, n=17) and APOEε3 (black, n=19) carriers. Median shown as continuous line. Significance by ANCOVA for mean difference. Regions: left hippocampus, right hippocampus, left white matter anterior cingulate cortex, right white matter anterior cingulate cortex, left anterior cortex, right anterior cortex, anterior para-hippocampal gyrus, posterior para-hippocampal gyrus, left hippocampus subcortical probe and right hippocampus subcortical probe. (GraphPad Prism 9.1.1)

5.4 Discussion

BBB breakdown is thought to be one of the primary sources where many neurological diseases begin, including AD. BBB dysfunction has been found to be prominent in APOEε4 carriers in early onset AD (van de Haar et al., 2016, 2017). Since neuro-physiological changes between APOEε4 and APOEε3 carriers have been reported at mid-age prior to the detection of cognitive changes (Dowell et al., 2016; Ten Kate et al., 2016; Cacciaglia et al., 2018,2019; Mishra et al.,

2018; Rojas et al., 2018; Operto et al., 2018; Lyall et al., 2019; Salvadó et al., 2019), it was important to investigate BBB at this age range. To our knowledge, no human studies have previously investigated BBB permeability exclusively in healthy middle-aged APOEε4 carriers. The aim of this study, was to investigate BBB integrity in ROIs known to be affected early in AD and we hypothesised that subtle BBB leakage would be observed in healthy middle-aged carriers of the APOEε4 compared to age-matched APOEε3 carriers, since APOEε4 is an established risk factor for AD. We developed a BBB imaging acquisition using a high-performance MRI scanner to investigate subtle BBB permeability differences between APOEε4 and APOEε3 carriers in a disease free, symptom free middle-aged cohort. We collected quantitative measurements of contrast (Gd) leakage across the BBB at regions identified in the literature to be dysfunctional during early AD.

In this study we found no significant difference in the estimated BBB leakage between groups in any of the regions included in this study, but there was a strong trend in the data distribution of the *Ktrans* histogram analysis mapping a directional BBB leakage towards the APOEε4 carriers compared to their APOEε3 counterparts. The regions showing subtle difference are in subcortical regions, including the hippocampus. BBB breakdown in the hippocampus has been identified in several studies to be affected as part of an aging effect (Verheggen et al., 2020), increased with MCI (Wag et al., 2006), and the hippocampus has been recognised to be the first affected region in AD (Sengillo et al., 2013) and in APOEε4/MCI individuals (Montagne et al., 2015, 2020).

Visual representation of the violin plots reported in the present study may suggest that breakdown of the BBB tight junctions gradually and subtly begins in APOEε4 carriers at mid-age in the hippocampal region before it progresses to other regions, even in healthy individuals showing no cognitive changes. Indeed, previous AD research has suggested that microvascular pathology (identified as BBB breakdown) resulting in neuronal dysfunction, begins prior to the development of any cognitive defects (Medina et al., 2016) at a time as early as mid-age (Bors et al., 2020). In addition, the BBB breakdown in the hippocampus is an early indicator of AD pathology (Sengillo et al., 2013; Raven et al., 2013), and the BBB is more dysfunctional in older adult carriers of the APOEε4 gene than in non-carriers (Zipser et al., 2007; Montagne et al., 2015, 2020). Notably, *Ktrans* values reported here for the mid-age sample were comparable to the range of the Montagne et al (2020) *Ktrans* values for their older healthy controls, but the present sample had a much larger variability in *Ktrans* scores which, resulted in the

consistently-observed directional differences between APOE4 carriers and non-carriers failing to reach statistical significance. The BBB integrity, measured by *Ktrans* scores may be influenced by the gender of participants recruited in this study, who are mostly females and may be at a pre- or perimenopausal age, where estrogen plays a protective role to the brain (Maggioli et al., 2015). Nevertheless, observing the very early signs of a consistent pattern of BBB disruption occurring in the cognitively healthy mid-age individuals recruited to the present study ~~may be~~ is consistent with other BBB studies of age-related decline.

A major strength of this study was the focus on the time of life where BBB breakdown may be detectable through imaging, but prior to the development of any cognitive changes, with a narrow age range 42-59. In comparison to previous work which has reported data over a much wider and older age range, here we focus on pinpointing a more limited and specific age range that could show subtle, early BBB changes between the two genotypes in specific brain regions.

Nevertheless, there were several limitations in this present study that must be acknowledged. First, the sample size was small, but comparable with many other DCE-MRI studies. Given the precise specifications in the recruitment criteria, it was very challenging to find volunteers in mid-age with time to spare for the study, who had no underlying health conditions and were accepting the intravenous injection of an MR contrast agent and a considerably long MRI scan. Second, despite the pilot work that determined the protocol decisions, the quality of imaging data acquired in DCE-MRI to measure subtle *Ktrans* differences was disappointing. The images included a considerable amount of movement artefacts, and the quality of data collected were substantially reduced; this was likely primarily due to the length of the scanning time (Figure 5.5). Consequently, several ROIs in some participants gave negative *Ktrans* values in the Patlak plot, and the measures in these parameters indicated failure to fit. Although images can be realigned by co-registration, the image artefact and erroneous signal intensities remain.

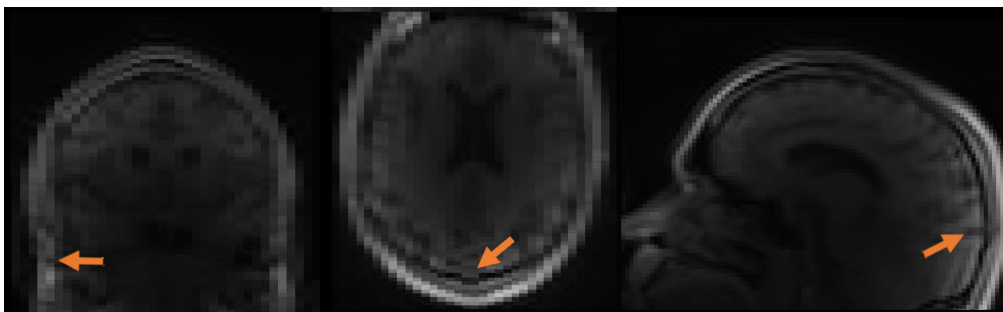


Figure 5.5 MRI image of a participant brain Coronal, axial and sagittal planes, showing motion artefacts due to movement during the scan.

Furthermore, while MR contrast agent (Gd) influences T1 weighted images, with increasing signal intensity enhancing the sensitivity of measures (Ibrahim et al., 2020), during bolus injection, Gd is highly concentrated in the vessels, and the increased brightness results in image artefact across a substantial region of the image (Boxerman et al., 1995). This problem was not identified in the pilot stage since that work concentrated on scanner stability and no contrast agent was used. Third, another major limitation was the loss of some data during the Patlak model fitting, which was due to the differences in ROI sizes between individuals, and subtle head movement during the scan, invalidating some of the B1 maps and resulting in failure of the model fitting. Finally, the disturbing noise signals limiting accurate parameter measurements maybe attributed to the small anatomical structure of the selected ROIs; this resulted in improper model fitting and loss of some important pixels. This last limitation may be addressed in future studies by selecting larger regions rather than small ones; for example, the whole para-hippocampal gyrus could have been sampled rather than dividing into anterior and posterior para-hippocampal gyrus.

In conclusion, using the less invasive DCE-MRI approach, the increasingly preferred methodology for BBB leakage measurements (Raja et al., 2018; Verheggen et al., 2020), we completed the first BBB permeability study on cognitively healthy, middle-aged APOEε4 and APOEε3 carriers. Our data suggests that the subcortical regions including the hippocampus may be the first region to develop changes in the BBB in APOEε4 carriers detectable using DCE-MR imaging as early as in the mid-forties, and prior to the development of any cognitive changes. A larger sample size and a longitudinal follow up on the same cohort would be recommended to further analyse the development of the subtle differences identified between the groups in the present work.

Chapter 6: Inflammatory Markers in Mid-Age APOEε4 Carriers

6.1 Introduction to Inflammatory Markers in relation to APOEε4

Apolipoprotein E4 has been shown to be a major risk factor for developing Alzheimer's disease (AD), and one mechanism is through neurovascular dysfunction (Bell et al., 2012). The pathological process by which the APOEε4 gene affects the neurovascular structures and/or pathways of the brain remains unclear, but there is evidence that neuro-inflammatory factors that impact neurovascular function are present during early stages of the disease (Mishra et al., 2018). Several key proteins were found to be adversely influenced by the APOEε4 such as cyclophilin A (CypA), Matrix metalloproteinase-9 (MMP9), Low-density lipoprotein receptor-related protein 1 (LRP1) and C-reactive protein (CRP), resulting in vascular dysfunction and therefore leakage of blood-derived material into the brain tissue causing neuronal toxicity (Bell et al., 2012; Mishra et al., 2018). Fibrinogen, another protein found to be accumulated in brains of AD patients due to vascular disruption (Cortes-Canteli et al., 2012), and pro-inflammatory cytokines are found to be elevated in serum of AD patients and are thought to be early inflammatory biomarkers for AD (Cojocaru et al., 2011, Wu et al., 2015). In addition, recognised inflammatory markers that have a role in the pathological progress of AD due to neuronal damage have been identified. It is of interest that the APOE gene appears to play an important role in the accumulation of amyloid beta 42 (Aβ42), Total Tau and Neurofilament-Light (NfL) (Bettcher et al., 2018; Qingyi Ma et al., 2018). While more research has been carried out on the former two proteins, NfL is likely to be a significant biomarker that may benefit from further assessment. Exploring the concentration of all of the identified early inflammatory markers that contribute to blood-brain barrier (BBB) dysfunction and combining the tests in a single study on healthy mid-aged individuals carrying the AD genetic risk factor APOEε4 may expose more associations that may help in understanding the mechanism behind early BBB breakdown.

6.2 Serum Neurofilament Light (NfL)

Neurofilaments (Nf) are proteins which largely exist in axons. They play an important role in maintaining axonal structure including shape, size and quality as well as axonal growth and the speed of electrical impulses through the axon (Yuan et al., 2012). Neurofilaments are of three subtypes, Nf High (NfH), Nf Medium (NfM) and Nf Light (NfL). NfL is the subtype most used as an early inflammatory biomarker for neurodegenerative disease including Alzheimer's

disease. NfL concentrations in cerebral spinal fluid (CSF) have been shown to be useful in measuring severity of neurodegenerative diseases (Scherling et al., 2014; Rohrer et al., 2016; Lin et al., 2018). Recently, several studies have measured NfL in serum with promising results providing a method of NfL measure that is both less invasive and easily accessible when compared to CSF measures (Lin et al., 2018; Preische et al., 2019; Rohrer et al., 2016). Plasma NfL measures are found to be biomarkers associated with cognitive decline in AD, where plasma NfL increases, cognitive measures decline in preclinical phases of this disease (Lin et al., 2018; Hu et al., 2019). In a further study, serum NfL concentration was associated with frontal lobe atrophy, as well as cortical thinning and cognitive changes in preclinical AD (Rohrer et al., 2016; Preiche et al., 2019).

Although a recent study found NfL to be associated with cognitive decline but not influenced by the presence of the APOEε4 genotype, where NfL concentration was measured in CSF of older participants aged (69.3 ± 8.3) (Bos et al., 2019). Plasma NfL has been found to be associated with cognitive decline in APOEε4 carriers aged (76 ± 5) (He et al., 2020). As serum NfL measures have been used for the detection of neurodegenerative disease including AD (Rohrer et al., 2016; Weston et al., 2017; Preiche et al., 2019), but have not yet been studied on APOEε4 carriers. Serum NfL measures in healthy, non-clinical mid-aged, cohort and its relation to the APOEε4 gene is a novel approach.

In this study we investigate whether NfL level is increased in cognitively healthy mid-age APOEε4 carriers, where none of the samples are at a pre-symptomatic stage. We hypothesised:

1. that serum NfL concentration would be elevated in APOEε4 carriers compared to APOEε3 carriers.
2. This elevation would correlate with subtle structural pathologies in our regions of interest in the brain, namely the frontal lobe and whole brain atrophy, as well as cortical thinning.

6.3 CyPA, MMP-9 & LRP1

Several studies suggest that BBB disruption should be considered a biomarker for early stages of AD (Montagne et al., 2015; Van de Haar et al., 2016; Bors et al., 2018; Sweeny et al., 2018). Capillary pericytes of the brain are responsible for protecting the integrity of the BBB. Recent studies on AD provided evidence that elevated levels of the soluble platelet-derived growth factor receptor β (sPDGFR β) in CSF correlates positively with local BBB leakage independent of the levels of A β 42 and total tau levels in early AD (Miners et al., 2019; Montagne et al.,

2015; Nation et al., 2019). CSF sPDGFR β also correlated with serum sPDGFR β (Miners et al., 2019) which should be investigated in mid-age individuals carrying the APOE ϵ 4 genotype in future studies. Therefore, pericyte dysfunction can contribute to BBB breakdown, causing leakage of blood derived material into the brain tissue that can be neurotoxic, leading to neuronal damage and cognitive decline (Bell et al., 2010). Moreover, studies have shown that the ϵ 4 variant of the APOE gene has a negative impact on capillary pericytes leading to BBB breakdown and impaired neuro-vascularity (Bell et al., 2014). Figure 6.1 shows the pericytes responsible for maintaining brain capillary integrity, and how the pathway is dysfunctional in the presence of the APOE ϵ 4 gene.

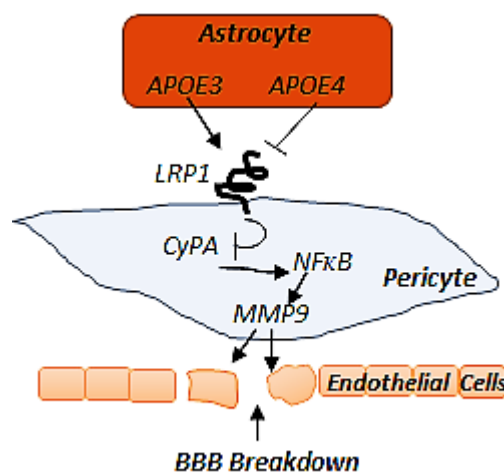


Figure 6.1 APOE ϵ 4 astrocyte leading to BBB breakdown due to activation of brain pro-inflammatory proteins pathway in pericytes (modified from Bell et al., 2014).

Cyclophilin A (CyPA) is an intracellular protein that belongs to the immunophilin family and is responsible for protein folding and trafficking (Nigro et al., 2013). CyPA is secreted extracellularly, stimulating pro-inflammatory signals in both endothelial cells and smooth muscle cells (SMC) in response to infection or oxidative stress contributing to vascular disease pathologies and neurodegenerative disease (Jin et al., 2000). Furthermore, white blood cells (WBC) responsible for detecting and fighting infections (Leukocytes, Monocytes and Lymphocytes) are chemically stimulated by secreted CyPA (Sherry et al., 1992). To further understand this, we explored the effect of the APOE alleles on WBC through CyPA pathway.

Matrix metalloproteinase-9 (MMP-9) is a binding protein that has been shown to play an important role in AD pathology. Studies on AD post-mortem human tissue and in transgenic mice had shown MMP-9 levels to be increased in hippocampal and cerebral cortex capillary walls (Mizoguchi et al., 2009, Bruno et al., 2009). Moreover, plasma MMP-9 levels were also

elevated in AD patients, which may contribute to BBB permeability (Lorenzl et al., 2008). This was explained by increased concentration of both CyPA and MMP-9 in pericytes leading to loss of BBB integrity (Bell et al., 2012).

Studies on post-mortem human tissue, transgenic mouse models and human CSF have demonstrated a negative influence of APOEε4 on vascular health. They suggest the presence of APOEε4 disrupts the normal pericyte pathways via activation of pro-inflammatory pathways (CyPA- NFκB- MMP-9) leading to loss of the tight junctions in pericytes, as well as loss of pericytes themselves which, results in BBB breakdown, neurotoxicity due to leakage of serum molecules into the brain tissue and loss of synapses (Bell et al., 2012, Halliday et al., 2016, 2013). All of these changes are typical of the pathogenic process that finally leads to AD.

Another marker of BBB integrity is Low-density lipoprotein receptor-related protein 1 (LRP1) where some studies observed an increase in plasma LRP1 levels in mild cognitively impaired (MCI) and AD patients (Sagare et al., 2011, Shinohara et al., 2017). LRP1 has been shown to be an important mediator for the clearance of extracellular Aβ from the brain across the BBB (Van Gool et al., 2019) via a regulatory pathway where LRP1 controls CyPA synthesis. This pathway is influenced by the APOE status, where APOEε3 has a tight bond with LRP1 in pericytes while APOEε4 loses its connection with LRP1 leading to uncontrolled CyPA expression in pericytes (Bell et al., 2012). As a result, Aβ accumulation is facilitated in APOEε4 carriers while Aβ clearance is facilitated in APOEε3 carriers (Shinohara et al., 2017; Qungyi Ma et al., 2018). An illustration of APOE role in the binding of Aβ to LRP1 and its role in active transport of Aβ out of cells is shown in figure 4.2.

Evaluating the levels of CyPA, MMP-9 & LRP1 proteins in non-pathological mid-aged carriers of the APOEε4 and compared to age-matched APOEε3 carriers is greatly important. As this study focusses on exploring non-invasive diagnostic methods for early detection of physiological changes that differentiate individuals who may in later life develop AD, we measured these proteins in serum. We hypothesised that each of the measured pro-inflammatory proteins would be elevated in healthy mid-age APOEε4 carriers compared to APOEε3 carriers.

6.4 Serum Total Tau & Aβ42

Many studies associated AD with as toxic build-up of Tau and Aβ proteins in the brain. Tau is an axonal specific protein which, if abnormally phosphorylated, accumulates causing

neurofibrillary tangles, which are thought to be causal in neuronal cell death and cognitive decline in AD.

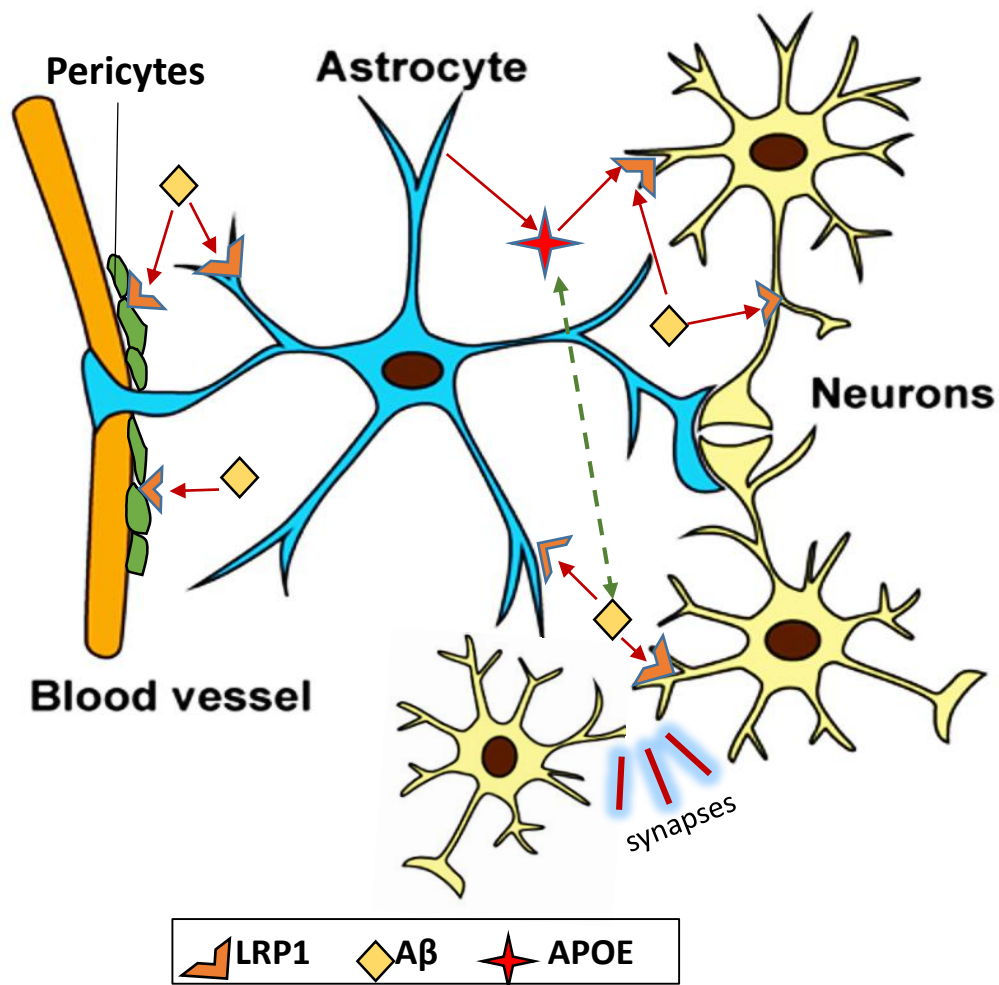


Figure 6.2 An illustration of APOE role in the binding of Aβ to LRP1 and its role in active transport of Aβ out of cells. Aβ production in neurons might be influenced by LRP1, where Aβ is secreted into the brain extracellular space. This will result in Aβ uptake through LRP1 pathway in neurons, astrocytes, pericytes and other cells which, may result in Aβ to be accumulated in cells inhibiting cellular toxicity. As APOE is produced and secreted from astrocytes in the brain, they interact with Aβ to influence LRP1- Aβ pathway or even bind with LRP1 rather than Aβ. (modified from Shinohara et al., 2017 and Harada et al., 2016).

Tau has been investigated mostly in CSF and CSF Tau level has been shown to be increased in AD patients and in MCI (Andreasen et al., 1998; Hampel et al., 2004). Using ligands that bind to Tau, brain imaging studies showed that APOEε4 was also associated with increased tau-PET uptake in the entorhinal cortex and hippocampus (Therriault et al., 2019). Recently, plasma Tau levels have been shown to be increased in the plasma of AD and MCI patients and this increase was associated with brain atrophy, specifically the hippocampal and ventricular

volumes (Mattson et al., 2016). Although plasma Tau levels have been clearly associated with AD in older age (Neergaard et al., 2018; Shekhar et al., 2016), they may also be age related and a normal part of the aging process, as levels are significantly increased in healthy elderly compared to healthy middle aged (Chiu et al., 2017).

Amyloid beta 42 (A β 42) is a second key protein associated with AD pathology, with build-up in brain tissue causing plaques and accumulation in the walls of blood vessels of the brain resulting in plaques bulid up, aiding in the obstruction of the BBB pathway (Hampel and Blennow, 2004). It has been suggested that A β 42 reduction in CSF starts at least two decades before the onset of symptoms of the disease (Bateman et al., 2012; Sperling et al., 2014). Moreover, APOE ϵ 4 carriers had increased accumulation in brain tissue of A β 42 in cognitively healthy mid-age adults, correlating with subtle differences in brain structure (Filippini et al., 2009; Reinvang et al., 2013). Traditionally, Amyloid beta 42 had been studied as CSF A β 42 or PET A β 42 (Fagan et al., 2006; Storandt et al., 2009; Cruchaga et al., 2012) where these two measures negatively correlate in preclinical AD, because increased retention of A β 42 in brain tissue associates with decreased levels in CSF. In the last few years, however, less invasive methods of investigating A β 42 were assessed. A β 42 has been measured in plasma and serum showing a positive correlation between plasma/CSF A β 42 and between serum/CSF A β 42, where A β 42 in CSF, plasma and serum are all decreased in AD patients compared to healthy controls, but these measures were not related to APOE status and were all on pathological or elderly cohort (Mehta et al., 2000; Emadi et al., 2016; Eke et al., 2020). Because CSF A β 42 was found to be declined in AD and associated with cognitive decline at a pre-symptomatic stage (Bateman et al., 2012), it is important to explore the potential to measure A β 42 in a minimally invasive way such as in serum.

To the extent of my knowledge, no study has been published that assesses Tau and A β 42 in serum in a healthy mid-aged population in relation to their APOE ϵ 4 status. Therefore, this study is novel being the first to measure serum Tau and serum A β 42 in a healthy mid-age cohort carrying the APOE ϵ 4 genotype and comparing to APOE ϵ 3 carriers. We predicted:

1. serum Tau would show a subtle increase in APOE ϵ 4 carriers compared to APOE ϵ 3 carriers.
2. serum A β 42 would show a subtle decrease in concentration in APOE ϵ 4 carriers compared to APOE ϵ 3 carriers.

6.5 Fibrinogen

Fibrinogen is a blood clotting protein made in the liver which is found in high concentration in the brain tissue of AD patients due to BBB leakage (Cortes-Canteli et al., 2012). Plasma Fibrinogen levels were associated with cognitive decline in MCI individuals and with increased risk of AD (Xu et al., 2008, Oijen et al., 2005).

Increased Fibrinogen levels were also linked to the presence of the APOEε4 genotype, where in a post-mortem study, increased Fibrinogen accumulation was observed in AD/APOEε4 carriers compared to AD/APOEε3 carriers, suggesting Fibrinogen is an important measure in AD pathology (Hultman et al., 2013).

In this study we also measured Fibrinogen levels in plasma of healthy mid-aged individuals based on their APOE status. We hypothesised that Fibrinogen levels in APOEε4 carriers would be higher than in APOEε3 carriers indicating a genotype difference at a pre-clinical phase.

6.6 CRP and Cytokines (IFNγ, IL-6, TNF)

C-reactive protein (CRP) is a protein produced in the liver in response to a rise in pro-inflammatory cytokines such as interleukin-6 (IL6). Normal CRP levels for healthy adults rarely go over 3 mg/L but in the incidence of an injury or underlying health issue such as cardiovascular disease or obesity, CRP levels rise to above 100 mg/L, indicating inflammation (Nehring et al., 2020). Reduction in total brain volume was associated with increased CRP levels in a non-demented cohort of adults (mean age was 60) (Jefferson et al., 2007). Elevated CRP levels paracellularly have also been linked to BBB permeability in transgenic mouse model (Hsueh et al., 2012). Furthermore, CRP concentrations were higher in APOEε4 carriers in a sample of post-menopausal females, and this rise in CRP level correlated with cognitive decline (Bojar et al., 2016). Also, APOEε4 carriers with CRP levels 8mg/L or over developed AD in a quicker pace compared with APOEε4 carriers with lower CRP levels (Tao et al., 2018). Conversely, several studies indicated that CRP concentration was reduced in APOEε4 carriers compared to APOEε3 carriers. For example, in a large Latino cohort (mean age 81), CRP was detected to be decreased in APOEε4 carriers compared to APOEε3 carriers and the elevated CRP level in APOEε3 carriers was linked to increased risk of dementia and cognitive decline (Hann et al., 2008). Similar results were found in German, Japanese and Finnish populations (Marz et al., 2004; Austin et al., 2004; Kahri et al., 2006).

Although Interleukin-6 (IL6) functions as an anti-inflammatory cytokine, it is also considered a pro-inflammatory cytokine and thought to play a role in the development of AD (Silva et al.,

2021). IL6 was found to be increased in AD patients when measured in both serum and plasma indicating this measure may be an early biomarker for AD (Cojocaru et al., 2011, Wu et al., 2015; Silva et al., 2021). Additionally, Tumour necrosis factor alpha (TNF α) is also a pro-inflammatory cytokine which is increased in serum of AD patients when compared to normal controls (Fillit et al., 1991) and was also associated with brain atrophy (Jefferson et al., 2007), though this marker was not related to the APOE genotype (Infante et al., 2002). Interferon gamma (IFN γ) is a third cytokine together with TNF α that is thought to promote the production of A β 42 (Blasko et al., 1999).

Similar to our approach, most CRP & cytokine studies measured these inflammatory biomarkers in serum. To date, no papers have explored the emergence of changes in this comprehensive set of potential biomarkers in mid-age individuals carrying the APOEe4 genotype and therefore at risk for late onset AD. We hypothesised that serum CRP and pro-inflammatory cytokines (IL6, TNF α and IFN γ) are elevated in APOEe4 carriers at mid-age.

In this chapter, we present the data from our sample of cognitively healthy mid-age volunteers, testing for potential differences in inflammatory markers between APOEe4 and APOEe3 carriers, and assessing the state of inflammation while correlating these biomarkers with proteins that may contribute to BBB dysfunction and in turn lead to tau and A β 42 accumulation.

6.7 Methods

6.7.1 Participants

Forty healthy mid-age participants (mean age 52 (42-59) years; 33 females, 8 males) participated in the study. The APOEe3 group consisted of e3/e3 phenotype, and the APOEe4 group included e4/e3 and e4/e4 phenotypes. All participants self-reported being physically and cognitively healthy with no chronic illness. Recruitment details and methodology for blood sample collection are presented in detail in Chapter 2.

6.8 Analysis and Results

Blood samples were analysed at Sussex Royal Hospital in Brighton and serum samples were processed and analysed at Affinity Biomarker Labs in London and some of the assays used were distributed by Meso Scale Discovery, a division of Meso Scale Diagnostics, LLC. 1601 Research Blvd, Rockville, MD 20850 USA. Participants' demographics and means (standard deviation) of all biomarkers are presented in table 6.1.

Table 6. 1 Participants demographics and biomarkers mean (SD).

	APOEε4 (21)	APOEε3 (19)
Age (years)	52±5	52±5
Gender (Male/Female)	5/15	3/18
CRP mg/L	1.0 (1.4)	1.4 (1.5)
Total tau ng/L	80.85 (135.8)	69.8 (103.5)
Aβ42 ng/L	20.5 (24.6)	25.1 (39.1)
TNFα ng/L	2.9 (0.47)	3.0 (0.7)
CypA ug/L	20.3 (29.9)	16.4 (22.6)
NfL ng/L	264.5 (577.7)	203.4 (482.3)
MMP-2 µg/L	81.1 (8.7)	79.2 (8.5)
MMP-9 µg/L	141.4 (62.3)	139.5 (55.4)
Fibrinogen	3.32 (0.57)	3.24 (0.61)

Age expressed as mean with ±5 years (SD).

6.8.1 Serum Neurofilament Light (NfL)

NfL Immunoassay was used, catalogue Number 70-7001, distributed by Uman Diagnostics AB, Tvistevägen 48C, 907 36 Umea, Sweden. Samples were analysed at Affinity Biomarker Labs in London. Throughout the study, where sample results came back with limit of detection (LOD), the LOD value was calculated and substituted similarly to previous studies (Croghan and Egeghy, 2003). NfL Sample results less than 33ng/L were reported as <33ng/L (APOEε4 (12) APOEε3 (7)) and the calculated LOD= 23.3 ng/L. NfL data were not normally distributed, and so was subjected to log transformation before analysis. Both raw and log-transformed data are shown in Figure 6.3. Comparing Serum NfL ng/L levels between the two groups (APOEε3 and APOEε4), we observed that there is a greater variability in NfL within the APOEε4 group. Since the notches in the boxplot overlap, we can conclude with 95% confidence, that the true medians do not differ. This was confirmed by a non-significant t-test between groups.

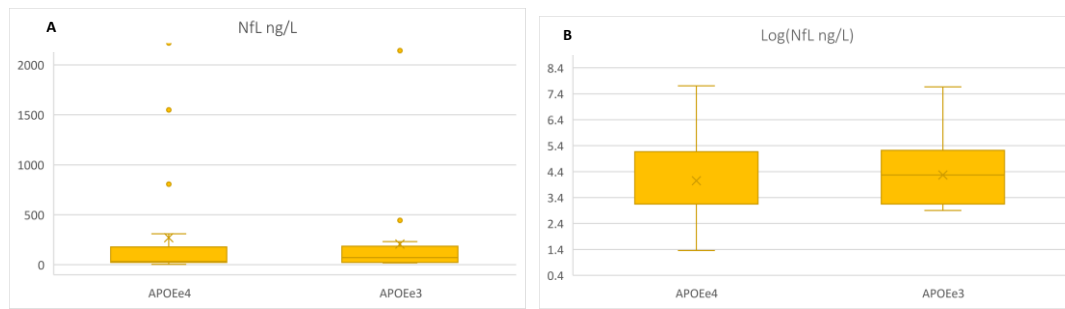


Figure 6.3 Boxplot showing NfL ng/L levels in APOE ϵ 4 (n=21) and APOE ϵ 3 (n=19) groups. A. Raw NfL data and B. Log-transformed NfL data.

6.8.2 LRP1, CyPA and MMP-9

ELISA Kit, catalogue number abx251415, distributed by Abbexa Ltd, Innovation Centre, Cambridge Science Park, Cambridge, CB4 0EY, UK, was used to measure LRP1 ng/L in serum. Sample results less than 0.098 ng/mL were reported as <0.098ng/mL and in this study 39/40 of the samples were measured at <0.098ng/mL irrespective of the genotype. Unfortunately, no analysis of LRP1 was enabled due to the insensitivity of the measures.

For serum CyPA analysis, we used Biovendor Cyclophilin A ELISA kit, catalogue number RD191329200R, distributed by Oxford Biosystems Ltd, 115 Olympic Avenue, Milton Park, Oxfordshire, OX14 4SA. Results less than 0.28 μ g/L are reported as <0.28 μ g/L (not recorded for any of the samples), but an upper limit of detection >75 μ g/L occurred for only four participants and analysed at 75 μ g/L (APOE ϵ 4 (3), APOE ϵ 3(1)).

Serum MMP-9 was measured using Human MMP-3 plex custom assay kit, catalogue number K15034C-1 distributed by Meso Scale Discovery. The lower limit of detection range of the MMP-9 assay was 61.0-102.6ng/L, however, 100% of the samples were detected within the reference range (169-705 μ g/L).

We compared serum CyPA and MMP-9 levels between the two APOE groups. Both sets of data were not normally distributed, and so were subjected to log transformation before analysis. Both raw and log-transformed data are shown (Figure 6.4). We observed that there was a greater variability in CyPA within the APOE ϵ 3 group, but no significant difference between groups was identified in either serum CyPA and MMP-9, confirmed with independent t-tests. In an approach to further explore subtle differences between genotype, we checked for a correlation between CyPA secretion and white blood cell (WBC) levels of lymphocytes and monocytes, as well as Leukocytes. Significance level was Bonferroni corrected for multiple correlations, $p < 0.02$. For both genotypes (Figure 6.5) the analysis showed no significant

correlations on any measure. However, it should be noted that all three measures tended towards negative correlations with CyPA in APOEε4 carriers.

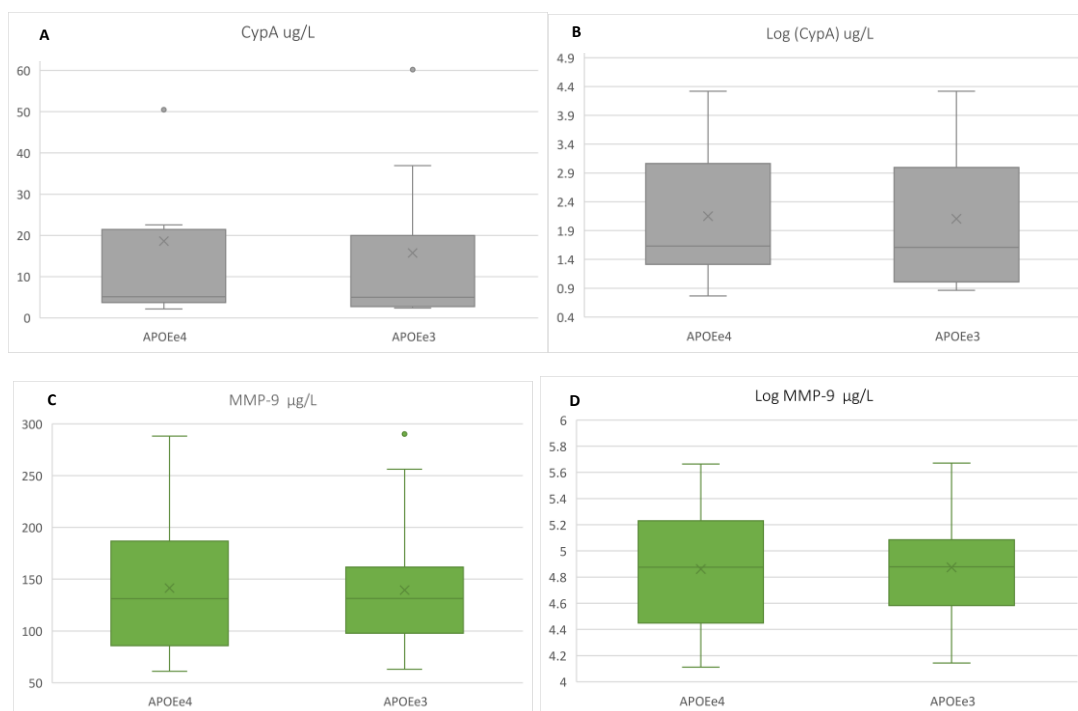


Figure 6.4 Boxplot of concentration of CyPA ug/L (A) and MMP-9 μg/L (C) in APOEε4 (n=21) and APOEε3 (n=19) groups. While B and D Are Log Transformed of CyPA and MMP-9.

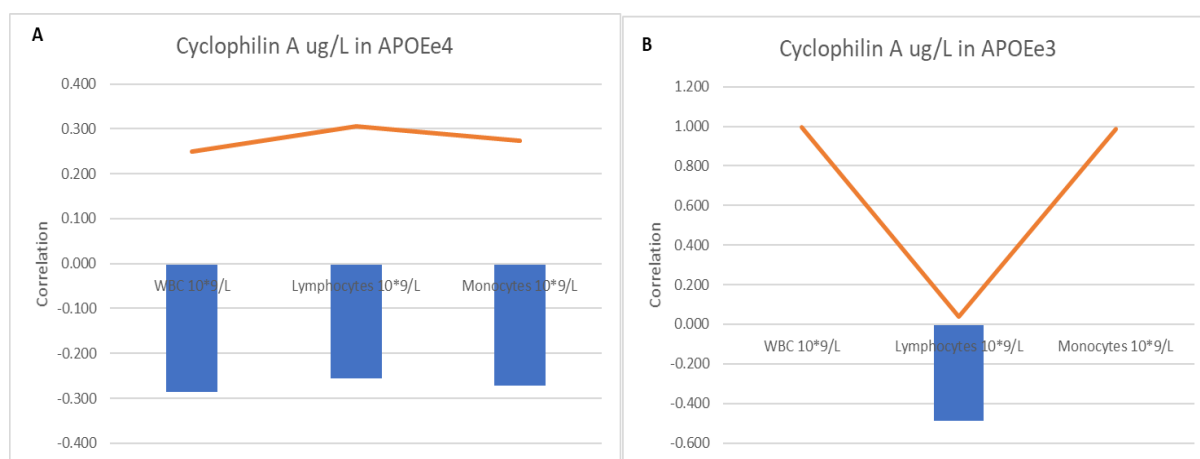


Figure 6.5 Within group correlation between CyPA and three measures (Leukocytes, Lymphocytes & Monocytes) for A) in APOEε4 group (n=21) and B) in APOEε3 group (n=19). Significance level was Bonferroni corrected for multiple correlations and set at $p < 0.017$.

6.8.3. Serum Total Tau & Aβ42

Serum Tau and Aβ42 were measured using MSD V Plex total tau protein assay and MSD V Plex Aβ42 Peptide (Aβ42) distributed by Meso Scale Discovery and samples were analysed at Affinity Biomarker Labs in London. Serum Tau results less than 63.45 ng/L were recorded as

<63.45 ng/L (APOEε4 (16), APOEε3(14)) and the calculated LOD= 44.87 ng/L. Both total Tau and Aβ42 resulted in non-normal distribution of scores, so the data were log transformed, and both raw and transformed data are presented below. The Box Plots in Figure 6.6 shows there was no significant difference between the two genotypes in either Tau ng/L nor Aβ42 ng/L. This was confirmed with an independent t-tests between genotypes. These results are strong indicators that BBB inflammatory biomarkers were not influenced by total tau and Aβ42

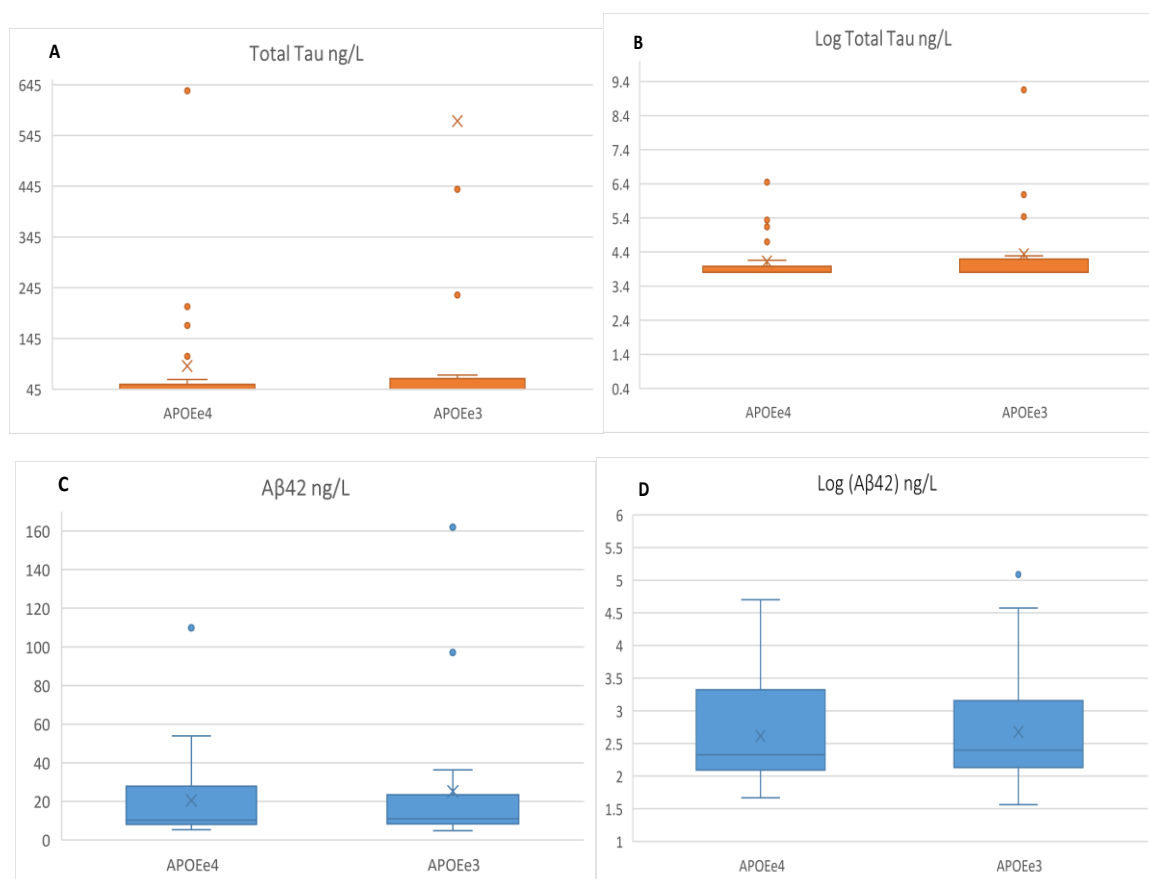


Figure 6.6 Boxplot of Tau ng/L (A) & Aβ42 ng/L (C) levels and Log Transformed plot of Tau (B) & Aβ42 at this stage of life (mid-age).

6.8.4. Fibrinogen

Plasma samples were collected on site at the clinical imaging science centre (CISC), Sussex University, Brighton UK, and were analysed within 8 hours of collection by Frontier Pathology, Brighton and Sussex University Hospital Trust and Surrey and Sussex Hospital Trust. Reference range for normal fibrinogen levels in adults is 2-4 g/L. Figure 6.7 shows a box plot of the fibrinogen g/L in APOEε4 (19) and APOEε3 (19) carriers. Two Fibrinogen sample results were not returned from Frontier Pathology Labs. Non-normal distribution of the data were corrected by log transform; both raw data and transformed data are shown below.

There was no significant difference identified in fibrinogen levels between the two groups, confirmed by independent t-test.

6.8.5. CRP and Cytokines

Serum CRP was centrifuged and analysed within 8 hours of collection by Frontier Pathology.

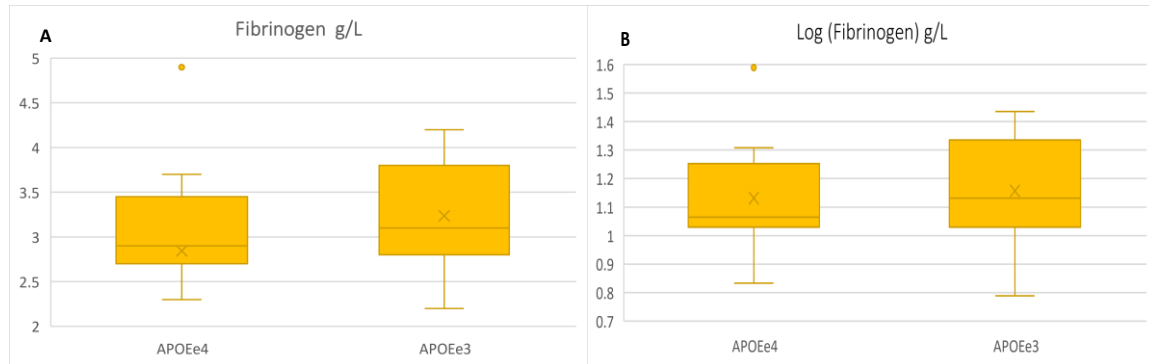


Figure 6.7 Boxplot: A) showing Fibrinogen level in APOEε4 (n=19) and APOEε3 (n=19) groups and B) Log Transformed plot of Fibrinogen.

Serum CRP results less than 1mg/L were recorded as <1mg/L (APOEε4 (14), APOEε3 (10)). Cytokines (IL6 ng/L, TNFα ng/L and IFNγ ng/L) serum samples were analysed at Affinity Biomarker Labs in London, UK using Human V plex custom Pro-inflammatory panel 1 assay kit (IL-6, TNF, IFNγ), catalogue number K151A0H-1 distributed by Meso Scale Discovery. LOD for IFNγ was recorded at <1.75ng/L (APOEε4 (1), APOEε3 (3)) and LOD was calculated to 1.24. Because 24 of the CRP samples collected were below reference range <1mg/L irrespective of their genotype, the CRP measured could not be analysed.

Statistical analysis of the pro-inflammatory biomarkers showed no significant differences between the APOE groups (APOEε3 and APOEε4) for all three measures IL6, TNF & IFNγ, shown in Figure 6.8. Due to non-normal distribution of the data, log transform was used on the data sets and transformed data are shown below.

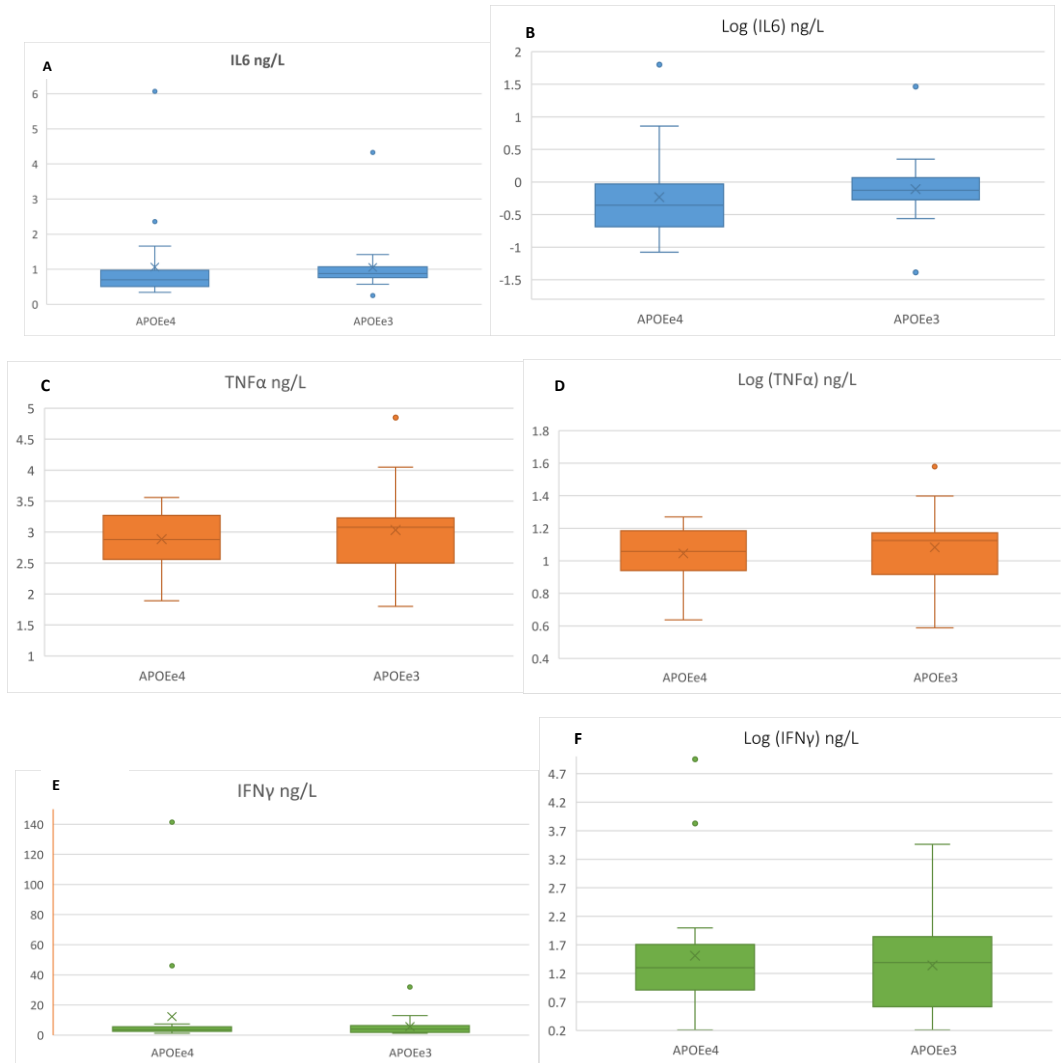


Figure 6.8 Box and Log Transformed plots showing IL6 ng/L, TNFα ng/L & IFNγ ng/L levels in APOEε4 (21) and APOEε3 (19) groups. Box plots are shown in A, C, E and Log transformed plots are shown in B, D, F.

6.8.6. Correlations between biomarkers

As reported above, between group t-test comparison on all variables with significance at $p < 0.05$, found no significant difference between APOEε4 and APOEε3 groups. However, we did observe a greater variability for CyPA in the APOEε3 group and NfL in the APOEε4 group, suggesting potential subtle differences between groups. Exploratory correlations were performed between the biomarkers, therefore, for each genotype separately, to see whether there was any evidence for differential activation patterns between genotypes. Within group correlation showed several significant correlations in APOEε4 carriers that were not observed in APOEε3 carriers with significance at ($p < 0.05$). In APOEε4 carriers' significant correlation

of biomarker levels were found between IL6 and IFN γ (0), A β 42 and total Tau (0.004), NfL and Total Tau (0) and between IL-6 and MMP-9 (0.006). We performed a Bonferroni correction ($p < 0.006$) and the significant relationship between the same measures was still observed (Figure 6.9). The only correlation observed within the APOE ϵ 3 group was between A β 42 and NfL (0) (Figure 6.10).

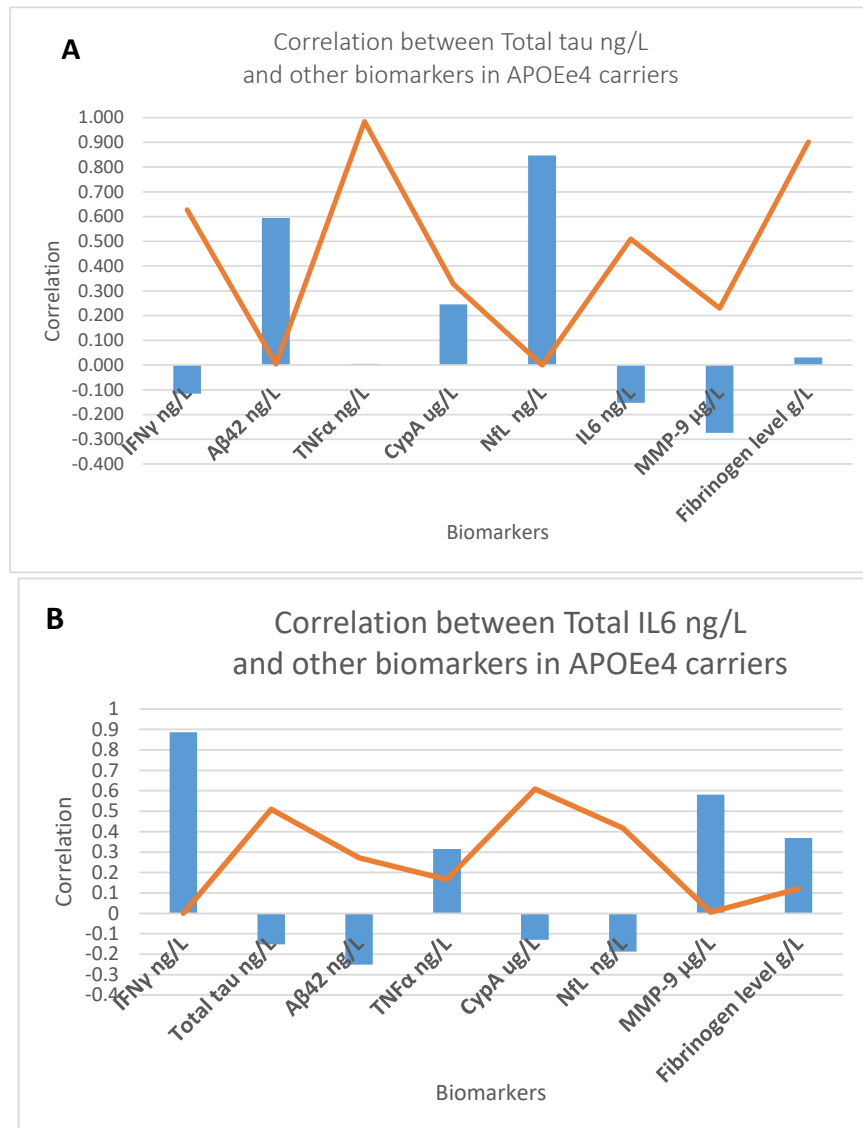


Figure 6.9 Correlations within the APOE ϵ 4 group, showing the relationship between 8 different biomarkers and Total Tau in A and the relationship between IL6 and the 8 biomarkers in B. Significance level set at 0.0 after Bonferroni correction. The orange line represents the significance values for each biomarker. Total Tau/ A β 42 correlate significantly (0.004), Total Tau/NfL (0), IL-6/IFN γ (0) and IL-6/MMP-9 (0.006).

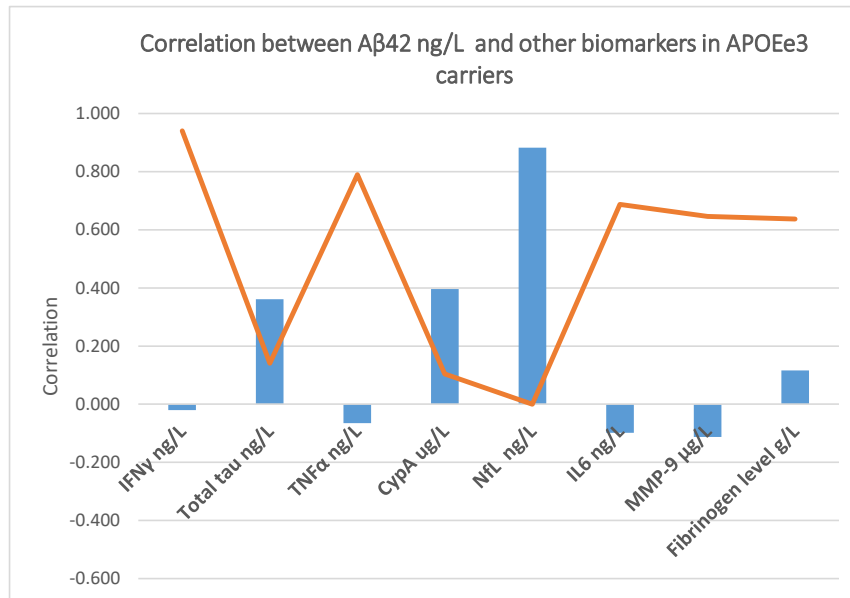


Figure 6.10 Correlation within the APOE ϵ 3 group, showing the relationship between A β 42 and all 8 biomarkers. Significance level set at 0.006 after Bonferroni correction. The orange line represents the significance values for each biomarker. The A β 42/NfL correlate significantly (0).

6.9 Discussion

Many biomarkers have been identified in CSF which are useful for diagnostic purposes, but this method of investigation is highly invasive requiring a lumbar puncture to test a sample of CSF. Therefore, it was crucial to try to explore different, less invasive and cheaper methods to acquire these biomarkers, such as through serum and plasma samples, which may aid in the early diagnostic and prevention process in the future. The aim of this study was to explore whether blood biomarkers investigation in mid-aged carriers of the AD risk gene, APOE ϵ 4, could reveal evidence of subtle changes happening that indicate a preclinical stage, and whether these biomarker changes may influence the integrity of the BBB.

In this chapter we discussed the different influences some inflammatory biomarkers have on the progression and development of AD and how they may or may not be related to an individual's APOE status. This chapter explored measured serum levels of several of biomarkers in healthy mid-aged individuals divided into two groups, APOE ϵ 4 carriers and APOE ϵ 3 carriers. We did not identify any significant differences in levels of the biomarkers between the two groups, although we did observe a greater variability for CyPA levels within the APOE ϵ 3 group and greater variability of NfL level within the APOE ϵ 4 group.

Further analyses, however, revealed that, within the APOE ϵ 4 group, several correlations were observed (namely, IL-6/IFN γ , IL-6/MMP-9, Total Tau/A β 42, Total Tau/NfL). These

correlations were not observed within the APOEε3 group. However, in the APOEε3 group one correlation was identified (between Aβ42/NfL). Although we had a small sample size in this study, high significance was detected within group analysis even after adjusting for multiple comparisons. NfL and Aβ42 have been found to correlate with total Tau levels in AD patients (Jin et al., 2019; Lewczuk et al., 2004), supporting the possibility in our finding, where NfL and Aβ42 both correlate with total Tau in the AD-known genetic risk factor APOEε4 but not in APOEε3, may have a clinical significance. In concert with the other correlations of inflammatory markers, seen only in APOEε4s, these findings may suggest that subtle differences in mobilizing these markers might be happening at mid-age in APOEε4 carriers. This may be taken as a further indication of dysregulation of a key biomarker.

WBC (Leukocytes, monocytes, lymphocytes) are naturally elevated in the presence of an infection or an inflammation and increased CyPA secretion supports WBC production. Therefore, CyPA and WBC are positively correlated in healthy individuals (Fisheries et al. 2017). However, in our study although we did not identify differences between genotypes in the level of CyPA and WBC, we must note that WBC measures tended towards negative correlations with CyPA in the APOEε4 group but not in APOEε3.

It is acknowledged that this study has several limitations that need to be recognised. The small sample limits generalisation as does the possible insensitivity of the measures in serum samples. Furthermore, the non-correlation between genotypes at this stage i.e. mid-age, could also be an indicator that serum inflammatory biomarkers do not differentiate the genotypes at this stage, but rather these changes happen rapidly at a later stage i.e. after the age of 60. Nevertheless, it is important to confirm that we did detect stronger correlations between biomarkers within the APOEε4 group in a healthy mid-aged cohort, which agrees with some of the literature which reported that APOEε4 has an influence on inflammatory markers that contribute to the onset of AD. These findings need to be further confirmed in larger groups of healthy mid-age individuals.

The necessity to tackle AD at its earliest stages has been increasingly acknowledged in the past few years. In general, this can be tackled by early prevention and intervention, and these are only obtainable by more understanding of the early biological changes that occur in the human body prior to the development of cognition decline. Here, we recognise AD's strongest known genetic risk factor (APOEε4) and investigate its effect on mid-age individuals who have no apparent symptoms of cognitive decline, and comparing them to the population norm APOEε3.

This study has served to illuminate some underexplored areas in this pathway, and we are able to answer some specific research questions from this study.

Chapter 7: General Discussion and Conclusion

7.1 Are there any detectable structural brain differences between APOEε3 and APOEε4 carriers at mid-age?

High quality neuroimaging techniques have advanced extensively in the past few decades with increased sensitivity measures and accuracy in exploring the different brain tissues and vascular structures. Here we experimented with a combination of structural imaging techniques of the brain, based on previous similar studies that investigated AD patients and healthy APOEε4 carriers at different age groups (Zhang et al., 2011; Soares et al., 2013; Timmers et al., 2016; Dowell et al., 2016; Cooper et al., 2020; Evans et al., 2020), while focusing on a specific target group (mid-age/ disease free/ APOEε4 carriers). We calculated regional brain volumes and cortical thickness through T1-w and T2-w imaging, measured WM integrity through evaluation of myelination, neuronal density, fiber orientation and axonal diameter through DTI and NODDI techniques, as well as WM and GM microstructures and iron concentration through MPM.

Although not significant, subtle structural differences were identified between APOEε3 and APOEε4 carriers at mid-age that complement the literature of genotype effect on the microstructures of the brain early on in life (Dowell et al 2016; Operto et al., 2018; Cacciaglia et al., 2018,2019).

7.1.1 Macro-structural Brain changes

Macro-structural brain changes defined as regional or whole brain volumetric changes were found, where APOEε4 carriers showed slightly greater WM volumes in the temporal lobe, similarly to the earlier published findings from our research group (Dowell et al., 2016), which may be interpreted as possible neurogenic effect as a result of regional over-engagement in APOEε4 carrier with a positive effect in cognition from young age that may continue on during the mid-age range identified in our study (45-59). On the other hand, WM volume was found to be lower in the frontal lobe, while GM volume was lower in the temporal lobe in APOEε4s compared to age matched APOEε3 carriers. These results are in line with similar recent research which found GM volume to be reduced in healthy APOEε4 carriers after the age of 55 (Mishra et al., 2018; Cacciaglia et al., 2018, 2019). Moreover, frontal lobe WM volume atrophy was also found in healthy elderly (age 65-75) APOEε4 carriers (Santos et al., 2017).

On the contrary, whole brain volume differences between genotypes were not detected in our mid-age cohort, but this outcome might be due to our small sample size. Collectively, these differences between genotypes indicate subtle structural disruption starting to emerge at mid-age and appear consistent with previous studies which identified similar differences in mid-age (Operto et al., 2018; Cacciaglia et al., 2018,2019).

7.1.2 Microstructural Brain Changes

Microstructural differences between genotypes were identified in this mid-age sample, where DTI parameters showed regions of slightly higher MD, RD, AxD and lower FA in APOEε4 carriers. Perhaps these may be interpreted as subtle disruption in structural connectivity in mid-age, where lower FA and higher MD are indicators of WM tract disruption as identified in AD patients and APOEε4 carriers (Cavedo et al., 2017; Zhang et al., 201; Williams et al., 2019). Similarly, higher RD and AxD may be inferred as possible axonal damage and demyelination which are identified in AD (Slattery et al., 2017; Operto et al., 2018). Whereas Dowell et al reported higher AxD only in younger APOEε4 carriers, which was interpreted by the authors as greater efficiency and improved information transmission in young individuals, by mid-age this study showed there are more consistent negative changes emerging across all parameters, which may be interpreted as indicators of a starting point for loss of WM integrity and axonal damage (Dowell et al 2013).

Likewise, NODDI parameters also expressed genotype differences, where NDI and ODI were found to be slightly lower in APOEε4 carriers, while fiso was slightly higher. These findings are similar to a recent study on diffusivity measures which were found in early onset AD patients, where first symptoms onset at age <65 years (Parker et al., 2018). These results in APOEε4 carriers indicate that:

1. neurite volume was reduced.
2. free water was increased.
3. neurite orientation dispersion was lower in WM of right inferior temporal gyrus, left superior frontal gyrus, right middle frontal gyrus and GM of the right superior frontal gyrus and right cingulate gyrus of limbic lobe. These regions were implicated in early changes in AD (Parker et al., 2018; Fu et al., 2019).

Interestingly, MTsat which is one of MPM parameters, showed subtle decrease in APOEε4 carriers indicative of myelin reduction in the right lateral ventricular wall, GM of right caudate

head and right anterior cingulate of the limbic lobe. However, these regions of demyelination were not identified in the literature and maybe attributed to the differences in parameter measures or artefacts.

We speculate that these findings together imply that there is a trend towards change starting to emerge in mid-age and that APOEε4 carriers may have a detrimental effect on brain microstructures at this stage of life. To our knowledge, this is the first study to report a full range of subtle structural markers completed on narrow mid-age group of volunteers, using 3T imaging.

7.2 Does BBB integrity disruption start at mid-age in carriers of the APOEε4?

BBB breakdown is a crucial biomarker that is known to lead to AD and has been identified in early onset AD who are carriers of the APOEε4 gene (van de Haar et al., 2016, 2017). In this study, we investigated BBB permeability in APOEε4 carriers and compared them to age matched APOEε3 carriers, by collecting quantitative measurements of contrast (Gd) leakage across the BBB at regions identified in the literature to be dysfunctional during early AD. Subcortical regions including the hippocampus showed a trend towards higher leakage in APOEε4 carriers. This is consistent with several studies that have identified BBB breakdown in the hippocampus to be the first region affected as part of aging, in AD and in APOEε4/MCI carriers (Sengillo et al., 2013; Montagne et al., 2015, 2020; Verheggen et al., 2020).

Even though non-significant, our results are consistent with the idea that breakdown of the BBB tight junction may gradually and subtly begin in APOEε4 carriers at mid-age in the hippocampal region before it progresses to other regions and that these changes can be detected even in healthy individuals showing no cognitive changes. The data trends are consistent with other findings (Medina et al., 2016; Bors et al., 2020) but were insufficiently powered to demonstrate this at a statistically significant level. Medina et al (2016) investigated vascular integrity on a large late-onset AD cohort mean age 73.4 (SD7.3) and suggested that early vascular dysfunction is associated with early cognitive decline. Bors et al (2020) was an animal study, and found an age-related BBB breakdown when comparing between cognitively unimpaired young and mid-age rats (Medina et al., 2016; Bors et al., 2020).

To our knowledge, no human studies have previously investigated BBB integrity exclusively in cognitively healthy mid-aged APOEε4 carriers. Our observation of the very early signs of

BBB disruption occurring in the cognitively healthy mid-age individuals may be considered a remarkable and important finding.

data does not support previous findings but it also not negating them and the trends are consistent with other findings but insufficiently powered to demonstrate this.

7.3 Do blood biomarkers reveal evidence of subtle changes happening in mid-age carriers of the APOEε4?

In this study, we focused on less invasive and cheaper methods to acquire neuro-inflammatory biomarkers through serum and plasma samples. We collected blood samples from healthy mid-age volunteers and comparing between APOEε4 and APOEε3 carriers. We analysed NfL, CyPA, MMP-9, LRP1, total tau, Aβ42, CRP and cytokines (IFNγ, IL-6, TNF) in serum, and fibrinogen and WBC in plasma. In general, we did not identify any significant differences in biomarker concentrations between the two groups, although we did observe a greater variability for CyPA levels within the APOEε3 group with a subtle increase in APOEε4s and greater variability and elevation of NfL levels within the APOEε4 group. These subtle fluctuations identified within the biomarker measures are not inconsistent with, but are clearly less marked than the biomarker changes reported in the very recent work from Montagne et al (2021) which found that APOEε4 accelerates BBB breakdown in older mice and that CyPA levels are a significant correlate of those changes (Montagne et al., 2021).

The present findings also are in line with previous studies which detected elevated CyPA and NfL in AD and in APOEε4. CyPA has been found to be higher in AD postmortem APOEε4 carrier compared to age match APOEε3s (Halliday et al., 2016) and levels of CSF CyPA were elevated in APOEε4 carriers (age 40-60) and correlated with BBB breakdown, suggesting an age-dependent BBB dysfunction in APOEε4 carrier before cognitive decline is detectable (Halliday et al., 2013). Lin et al investigated plasma NfL and found it to be elevated in AD patients compared to healthy age matched individuals, mean age 77.3 (SD 5.1) and suggested it could be a biomarker for cognitive decline in AD (Lin et al., 2018), while Hu et al found plasma NfL to be elevated in pre-clinical AD, mean age 72.76 (SD6.80), suggesting plasma NfL as an early biomarker of AD (Hu et al., 2019). Overall, our results demonstrate associations that are consistent with emerging vulnerability for neuro-biomarkers of BBB integrity in the presence of APOEε4 gene. The findings from the present human study may be interpreted as demonstrating early vulnerability in pro-inflammatory markers for

neurodegenerative disease, starting to be stimulated at mid-age in APOEε4 carriers, changes which may in turn promote BBB disruption and therefore Aβ accumulation.

7.3.1 How do these markers correlate with changes in BBB integrity?

Within group correlations showed significant correlation between IL-6/IFNγ, IL-6/MMP-9, NfL/total tau and between Aβ42/total tau in APOEε4 but not APOEε3. The latter two correlations are in accordance with recent findings reported in AD patients, where serum NfL has been found to correlate well with CSF NfL and more importantly here with AD biomarker (total tau) making it a very useful and accessible diagnostic biomarker for AD (Jin et al., 2019). Similarly, on a small sample Risacher et al found plasma Aβ42 to correlate well with CSF Aβ42 in healthy and AD participants, at the same time plasma Aβ42 correlated with CSF total tau (Risacher et al., 2019). Additionally, Shi et al concluded that plasma Aβ42, total tau and NfL are useful biomarkers that correlate well with volumetric brain atrophy and cognitive decline and are strong indicators for early AD (Shi et al., 2019). Therefore, our results with significant correlation between serum NfL/total tau and between Aβ42/total tau may implicate a clinical significance for early AD biomarkers happening at mid-age in carriers of AD-known genetic risk factor (APOEε4). It is important to note here, that measures of NfL, total tau and Aβ42 in serum of healthy, non-clinical mid-aged, cohort and its relation to the APOEε4 gene is a novel approach.

Despite the non-significant correlations between CyPA and WBCs, it should be noted that leukocytes tended towards a negative correlation with CyPA in APOEε4 carriers but not in APOEε3s. Because WBCs are chemically stimulated by CyPA and found to be positively correlated in healthy individuals (Fisheries et al. 2017; Dawar et al., 2017), we anticipate the negative correlation between CyPA and WBCs is indicative of an inflammatory response starting to emerge in mid-age APOEε4 carriers.

Although between group differences were not identified in our mid-age sample, this could simply mean that serum inflammatory biomarkers do not differentiate the genotypes at this stage, but rather that these changes happen rapidly at a later stage i.e., after the age of 60. However, it is important to acknowledge that we did detect stronger correlations between biomarkers within the APOEε4 group in this cognitively healthy population. A longitudinal follow up on the same group would be recommended to further analyse the temporal development of the subtle differences identified between the groups in the present work. Furthermore, the directional trend toward change happening in mid-age APOEε4 carriers

identified in this study needs to be further confirmed in larger groups of cognitively healthy mid-age individuals.

7.4 Strengths and Limitations

While other studies have tended to involve samples which cover a larger age range, this study focused on measurement of a wide range of biological markers including high quality imaging in a specific age range (45-59), with focus on a disease-free cohort to identify changes that may be occurring early on life in carriers of AD genetic risk factor (APOEε4). We combine multiple structural imaging modalities together with DCE-MRI on a 3T scanner to increase sensitivities of the measures and enable detection of subtle change. The high-quality imaging was combined with a range of neuro-inflammatory marker investigations, to identify subtle changes in serum and plasma of the same cohort.

The recruitment of mid-aged volunteers was challenging due to life obligations of this age range and busy schedules. Although comparable to other structural imaging and DCE-MRI studies, an acknowledged limitation is the small sample size which limits the generalization of results. One major factor that was not taken into account, was the fact that 80% of participants were females at or near menopause regardless of their genotype, and the hormonal changes happening at this stage could influence the results, since estrogen has a protective role on brain tissue at pre-menopausal age. Moreover, all health conditions were self reported and were taken into account that they are healthy, with no underlying health conditions based on the honesty and understanding of the participant which may not have been a reliable way of health evaluation.

Imagings' major limitation was the length of scanning time which was both an obstacle for some participants due to discomfort, and substantially reduced the quality of images acquired due to participants' movement throughout the scanning session resulting in image artefacts. Additionally, artefacts across substantial regions of the images were due to increased brightness from the high concentration of Gd after bolus injection. Moreover, differences in ROI sizes, the selection of small anatomical structures for the ROI's and subtle head movement during the scan resulted in failure of the Patlak model fitting causing loss of some data.

One of the imaging limitations was related to the selection of small anatomical regions of interest, which may be addressed in future studies by selecting larger regions rather; for example, the whole para-hippocampal gyrus could have been sampled rather than dividing into anterior and posterior para-hippocampal gyrus. Similarly, artefact maybe addressed in future

work by shortening scanning time. Additionally, despite providing high quality and sensitivity serum analysis kits, some of the kits had lower sensitivity than expected, therefore very low measures were not detected.

7.5 Recommendation for Future Research

My novel approach in methods of investigation of early noninvasive techniques and combining both blood biomarkers with sensitive and extensive imaging modalities, together with a focus on a specific population of cognitively healthy carriers of AD strongest genetic risk factor (APOEε4) has shaped further research questions to identify and endorse these findings. Some of the research questions that may be addressed in future work are:

- Do Aβ₄₂, total tau and NfL correlate with volumetric brain changes in APOEε4 carriers at mid-age?
- Does serum Aβ₄₂ provide as good an index as plasma Aβ₄₂ as an AD biomarker?
- Do APOEε4 carriers develop higher CypA and WBCs as they age, and can this be usefully tracked?
- Is BBB breakdown accelerated in mid-age APOEε4 carriers and how do these changes relate to serum CyPA?
- Do NODDI measures of structural change in cognitively healthy mid-age APOEε4 carriers correlate with inflammatory blood biomarkers?
- Are brain volumetric and microstructural changes correlated to cognitive change and blood inflammatory markers in APOEε4 mid-age?

Furthermore, a longitudinal study on the same cohort employing the same methodology is of great importance, perhaps to signify some of the results that were probably not detectable yet at our selected mid-age range but rather a few years later to confirm if BBB is breaking down at mid-age in cognitively healthy APOEε4 carriers and to correlate inflammatory biomarkers with the volumetric and microstructural brain changes as well as cognitive change.

7.6 General Conclusion

This study was presented for the advancement of knowledge and was designed for the development of reliable and accessible modalities for early investigation and innovation in detection and recognition of physiological changes that may occur early, on a population carrying an AD risk factor (APOEε4 gene), specifically at mid-age prior to the recognition of cognitive decline. The findings reported here indicate both how hard it is technically to identify

clear patterns of early change but also show promise for the value of careful and sensitive measures which might begin to map the temporal pattern of change and ultimately provide new directions for developing novel therapeutic inhibitors for inflammatory diseases.

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Tracer kinetic modelling for DCE-MRI quantification of subtle blood-brain barrier permeability—

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Appendices

Appendix A: Ethics

Ethical Review Application (ER/NA391/2) Nourah Alruwais	
Project Title	Blood Brain Barrier Permeability in mid age APOE e4 carriers (COPY)
Status	Approved
Department	Psychology
Email	N.Alruwais@sussex.ac.uk
Phone No.	
Applicant Status	PG (Research)
Project Start Date	04-Dec-2017
Project End Date	07-Oct-2020
External Funding in place	Yes
External Collaborators	No
Funder/Project Title	
Name of Funder	Saudi Cultural Bureau
Is this an IRP project?	No

Ethical Review Application ER/NA391/2 (continued)	
Project Description	<p>Alzheimer's disease (AD) affects about 850,000 people in the UK and is usually diagnosed after symptoms have developed at an older age. Several studies suggest that neuropathology of the disease (amyloid deposition and later tau tangles) begins years before clinical diagnosis is made. Some early structural brain changes such as lobar specific atrophy and ventricle enlargement may be identified in the early stages of AD and it is possible that other structural changes such as blood brain barrier abnormalities, microhemorrhages and haemosiderin deposits may be detected earlier in people who develop late-onset sporadic AD.</p> <p>Research has also shown a relationship between the presence of Apolipoprotein (APOE) genotype and AD. The APOE gene has effects on neural function. The e4 variant of this gene has attracted attention as the single most important known genetic risk factor for late onset, non-familial AD. Even in healthy adults, the e4 variant has been associated with steeper age-related decline in cognitive ability.</p> <p>The aim of this study is to develop methods to investigate the possible early detection of subtle brain changes in people at a higher risk of developing late onset, non-familial AD later in life. The information obtained will increase our knowledge of the potential neuropathological effects of the APOE4 gene and may provide the opportunity for early intervention to maintain and improve individuals' cognitive health as they age.</p> <p>People with AD have been shown to have increased blood leakage into the brain as a result of increased blood-brain barrier (BBB) permeability. This begs the question whether increased BBB permeability is present in healthy e4 carriers, who carry the risk for late onset, sporadic AD but do not currently (and may never) manifest the symptoms. We plan to investigate this by recruiting healthy volunteers into two groups: those that carry the e4 allele and those that do not. We will then be carrying out structural brain imaging using a gadolinium-based MRI contrast agent to help us identify and quantify subtle leakage of blood across the BBB.</p> <p>The use of contrast agent in this project is essential to identify the movement of blood into brain tissue. The gadolinium chelates constitute the largest group of MR contrast media and are considered to be extremely safe when administered within standard dosage guidelines.</p>

Roles (ER/NA391/2)					
Please list all investigators in this project's research team. If you specify role "Other" for any investigator please provide further details in the "Comments" field.					
Role	Name	Email	Phone	Institution	Comments
Student	NOURAH ALRUWAIS	N.Alruwais@sussex.ac.uk		School of Psychology, Sussex University	PhD student (cv attached)
Supervisor	Dr Nicholas Dowell	N.G.Dowell@bsms.ac.uk		CISC, Sussex University	Lecturer in Imaging Physics
Supervisor	Prof Jennifer Rusted	j.rusted@sussex.ac.uk		School of Psychology, Sussex University	Professor of Experimental Psychology
Supervisor	Dr Naji Tabet	n.tabet@bsms.ac.uk		Brighton & Sussex Medical School	Reader in Old Age Psychiatry (MD)

Ethical Review Form (BSMS version) (ER/NA391/2)	
Question	Response
>> Checklist	
C1. Will your study involve participants who are particularly vulnerable or unable to give informed consent or in a dependent position (e.g. children under 16, individuals with learning difficulties, mental health problems, people in care facilities or over-researched groups)?	No
C2. Will participants be required to take part in the study without their consent or knowledge at the time (e.g. covert observation of people in non-public places), and/or will deception of any sort be used?	No
C3. Will it be possible to link identities or information back to individual participants in any way? Will it be impossible to ensure that identities or information cannot be linked back to individual participants in any way (including after anonymisation?)	No
C4. Might the study induce psychological stress or anxiety, or produce humiliation or cause harm or negative consequences beyond the risks encountered in the everyday life of the participants?	Yes
C5. Will the study involve discussion of sensitive topics (e.g. sexual activity, drug use, ethnicity, political behaviour, potentially illegal activities)?	No
C6. Will any drugs, placebos or other substances (such as food substances or vitamins) be administered as part of this study or physiological interventions or processes outside of standard practice and will any invasive or potentially harmful procedures of any kind will be used?	Yes

C7. Will your project involve working with any substances and / or equipment which may be considered hazardous, e.g. such as radioactive materials?	Yes
C8. Will financial inducements (other than reasonable expenses, compensation for time or a lottery / draw ticket) be offered to participants?	No
C9. Will the research involve storage and/or analysis of human biological tissue ?	Yes
C9a. If you answered Yes to question C9, will this be carried out under University of Sussex HTA license?	Yes
C10. Is there a possibility that your investigations might uncover unexpected and possibly clinically relevant findings?	Yes
C11. Will the study include groups where permission is normally required for access to its members, for example groups based in the community, traditional communities or school pupils?	No
>> Section 1. Methodology, Data Collection and Analysis (Please provide full details)	

1.1. What is the principal research question/objective? Please clearly state the hypothesis to be tested. Please put this in language comprehensible to a lay person.	To assess whether APOE4 status can lead to subtle brain changes in mid age and whether such changes, if any, are related to fine deficits in cognition.
1.2. What research method(s) do you plan to use; e.g. interview, questionnaire/self-completion questionnaire, field observation, audio/audio-visual recording?	<p>Following consent participant will be contacted by telephone to screen for food allergies, asthma, any known allergies to contrast media and history of renal disease. Participants deemed eligible will continue with the study.</p> <p>Part 1: Genotyping is completed through a cheek swab acquired in a 5-minute procedure (details included in document attached). All procedures for genotyping have been approved in previous studies (JRSK0709) (ER/SLE27/1). A triangulated procedure involving two anonymized codes per sample and a third party (another member of faculty) ensures that neither the testing researcher nor the volunteer is provided with genotype information. The third party selects from the returned genotyping procedure a subset of codes representing potential volunteers from all required genotypes (unlinked). In this instance, as in previous studies, we will compare e4 carriers with the population norm, double e3 carriers. The lead researcher translates these codes back to names, which are provided to the researcher running the volunteers. All procedures for acquisition, storage and processing of samples for genotyping comply with the HTA license held by the university. Final code breaking is completed on the anonymized data spreadsheet only and after preprocessing of imaging data.</p> <p>Part 2: Participants randomly selected to represent required genotype groups will be invited to CISC for a blood sample to check for (normal renal function, full blood count and inflammatory markers), and basic cognitive assessments, including episodic and a prospective memory tasks, which may take up to 30 minutes to complete.</p> <p>Part 3: Participants with normal renal function will then be asked to visit CISC one more time. They will complete an MRI safety questionnaire and be screened by a radiographer before entering the scanner. The 3rd visit will last 90 minutes.</p>

1.3. How many people do you envisage will participate, who are they (e.g. age and gender) and how will they be selected?	We will invite previous volunteers to take part in this study who have been genotyped under (ER/JENNYR/1) and have previously indicated their willingness to be approached for further studies. We will also recruit about 100 healthy mid aged (45-55) people to be genotyped to supplement this volunteer database. Since the e4 allele is present in only ~15% of the population, we need to genotype this number of people to achieve our target number of 20 e4 carriers (e4+) and 20 non-e4 carriers (e4-) for the study. After genotyping, we will randomly select 20 individuals in each group to continue in the study. Personal details and genotype information are stored separately at all times (details below).
1.4. Please state the rationale for the number of participants to be recruited (please note that it is unethical to recruit either more, or less, participants than required to adequately power a study).	The number of participants were chosen based on similar studies of blood brain barrier permeability using gadolinium as the contrast agent where the number of participants ranged between 18-24 per group (Cramer et al 2014 & Montagne et al. 2016), as well as, e4 studies that showed subtle brain changes with 20 participants per group (Dowell et al. 2011).
1.5. What are the inclusion/exclusion criteria?	<p>INCLUSION CRITERIA:</p> <ol style="list-style-type: none"> 1. Age 45-55 2. Generally Healthy 3. Willingness to participate in MRI study if selected <p>EXCLUSION CRITERIA:</p> <ol style="list-style-type: none"> 1. People of Asian & African descent (due to different allelic ratios and physiological outcomes in these groups) 2. Current physical and/or mental illness 3. MRI contraindications: <ol style="list-style-type: none"> a. Implantable devices (e.g. cardiac pacemaker) b. Metal fragments lodged in the eyes or body c. Large or dark tattoos on the head or neck d. Pregnancy e. Claustrophobia 4. Contrast Media contraindications: <ol style="list-style-type: none"> a. Asthma b. History of renal disease/kidney problems (Self-reported) c. Allergies/Sensitivity to contrast media e. Claustrophobia
1.6. Where will the project be carried out e.g. public place, in researcher's office, in private office at an organisation? Please list all research locations to be used.	Phase1: Human Psychopharmacology Laboratory (Pevensy 2); Phase2 & 3 at CISC.
1.7. How will the results be analysed and by whom?	<p>The results will be analysed by Nourah Al Ruwais and supervisory team (Tabet, Dowell, Rusted). Some analyses of the anonymised datasets will be delegated to supervised undergraduate and masters students where this would facilitate data processing time.</p> <p>Confidentiality and Data Protection:</p> <p>Separate consent forms are used for the genotyping and the experimental sessions (All three are attached).</p> <p>Separate storage of participants information and data.</p> <p>Triangulation of genotype information.</p>

>> Section 2. Informed Consent and Recruitment	
2.1. How will participants be approached and recruited? What specific mechanisms will be used e.g. social media, university circulation lists, intranets or any other external websites?	Volunteers will be recruited through posted advertisement at the University of Sussex and University of Brighton and also on local community websites (e.g. Brighton Gumtree). Further, emails will also be sent to people who have previously recorded an interest in taking part in research at the University of Sussex. A passive recruitment strategy will be adopted- i.e. the first contact between participant and researcher will be made by potential participants themselves. This will ensure that participants do not feel coerced into taking part. A copy of the advertising material is attached in this application.
2.2. Please describe the process you will use to ensure your participants are freely giving fully informed consent to participate. In most instances this will include the provision of an Information Sheet and will require a Consent Form unless it is a purely self-completion questionnaire based study or there is justification for not doing so. (Please provide details if this is the case).	Care has been taken to ensure that recruitment is free from undue influence and recruitment material makes no diagnostic nor therapeutic promise. Only healthy participants that meet the inclusion but non of the exclusion criteria will be recruited to this study. The information sheet will be provided to them electronically, and volunteers will be given 2 weeks to consider their participation. Plenty of opportunity will be given to discuss the study and ask questions (either by phone or email) before giving formal consent to participants. During consenting, participants will also have an opportunity to ask further questions. A copy of the participant information sheet and consent forms for the three parts of the study are attached in this application.
2.3. Who will be taking informed consent? What training or experience have they received to do so?	Informed consents will be taken by principal researcher Nourah Al Ruwais (medical imaging technologist (CV attached)).
2.4. Participants should have the right to withdraw from the research at any time. Participants should also be able to withdraw their data if it is linked to them and should be told when this will no longer be possible (e.g. once it has been included in the final report). Please describe the exact arrangements for withdrawal from participation and withdrawal of data for your study.	At all stages of the study it will be made clear to potential participants that they have no obligation to take part in the study and have the right to withdraw at any stage without any given reason. Data will be shredded and destroyed upon the request of the withdrawing participant. However, this will not be possible once the data has been published, although, all data will be anonymized and cannot be traced to an individual. This is clearly explained in the information sheet and consent forms.
2.5. Does the study involve participants who are particularly vulnerable, or unable to give informed consent, or in a dependent position (e.g. children (under 18), people with learning difficulties, over-researched groups or people in care facilities, including prisons) ?	No.
2.6. Will a chaperone be required to be present during interviews? If so, please describe the chaperone arrangements.	No.
2.7. Is Disclosure and Barring Service (DBS) clearance necessary for this project?	No
2.8. Will participants be asked to take part in the study without their consent or knowledge at the time (e.g. covert observation of people) or will deception of any sort be involved? Please refer to the British Psychological Society Code of Ethics and Conduct for further information.	No.
2.9. Will participants be compensated for their time or be reimbursed for expenses? If so, how much?	The first visit will last approximately 10 minutes and participants will receive a coffee voucher for this visit. The second visit will last about 35 minutes and will receive 7GBP. The third & last visit will last about 90 minutes and participants will receive 60GBP at the end of this visit. This was decided upon based on the participant age group and level of investigation at each stage, and is in keeping with reimbursement rates in previous studies..

2.10. Could the study induce psychological stress or anxiety, or produce humiliation, or cause harm or negative consequences beyond the risks encountered in normal life?	Yes. Participants may experience anxiety due to venipuncture and the imaging study. Adverse reaction due to the injection of contrast media may occur, although very rare (explained later in detail)
2.11. Are alcoholic drinks, drugs, placebos or other substances (such as food substances or vitamins) to be administered to the study participants?	Yes. Contrast Media (injection).
2.12. If the research involves physical intervention (e.g. imaging) have you considered the possibility that your investigations might uncover unexpected and possibly clinically relevant findings? How will this be managed?	Very rarely brain imaging may reveal unexpected abnormalities. Should this occur, images will be sent to a consultant neuroradiologist to be reviewed. Should any incidental findings be found the participant and his/her GP will be informed and provided with a copy of the neuroradiologist report. This procedure follows standard CISC protocol. If renal blood analysis shows unexpected findings, the participant and GP will be notified. This is explained in the information sheet.
2.13. Can you think of anything else that might be potentially harmful to participants in this research?	Saliva samples will be collected in this study. This is not potentially harmful but might be considered invasive. Venipuncture: Taking a blood Sample is associated with mild discomfort. To minimize this, blood taking will be performed by a member of the onsite clinical team, whom are highly experienced in blood taking (CISC radiography team). MRI scanning: MRI scanning is safe. However, some participants may feel claustrophobic when in the scanner. To minimize this, all potential participants will be screened for history of claustrophobia. Contrast Media: Gadolinium based contrast agents have also been associated with side effects. The most common side effects (which occur in 1 in 100 people) include pain around the injection site, nausea, vomiting, itching, rash, headache and abnormal skin sensations. In people with poor kidney function they can also cause severe thickening of the skin and other tissues. However, this has never been reported in people who have normal kidney function. We will check every participant's kidney function at the beginning of the study and they will only be able to receive the contrast agent and continue in the study if this is normal (Dr Tabet will check the results of participant's kidney function to confirm eligibility). In some rare cases (fewer than 1 in 10,000 people) gadolinium based contrast agents can cause serious allergic or non-allergic reactions. The staff at the imaging center that will administer the contrast agent are experienced and trained in dealing with any acute side effects that may arise. A trained clinician will also be present within the imaging centre during all imaging.
2.14. Will you inform participants of the results of the research? Please give details of how you will inform participants or justify if not doing so.	Yes. Participants will receive a short executive summary of the findings, via email, and copies of all manuscripts resulting from this research upon individual request.
>> Section 3. Data Protection, Confidentiality and Records Management	
3.1. Does the project require access to personal records?	No
3.2. How will you ensure that the processing of personal information and personal identifiable information related to the study will be in full compliance with the Data Protection Act 1998 (DPA)?	All data held on computers will be anonymized and stored under a participant recruitment number. Participants initials will not be used, nor will dates of birth to minimize the risk of participant identification from the study recruitment code. All data will be stored in accordance with Data Protection Act. All researchers involved in this study are aware of the importance of confidentiality and confidentiality clauses exist in all research staff contracts.

3.3. If you are processing any personal information outside of the European Economic Area (EEA) you must explain how compliance with the DPA will be ensured.	No.
3.4. Will you take steps to ensure the confidentiality of personal information?	Yes
3.5. Please provide details of anonymisation procedures and physical and technical security measures here.	Participants with APOE e4+, or double e3 genotype will be selected randomly from their pool. A triangulated procedure involving two anonymized codes per sample and a third party (another member of faculty) ensures that neither the testing researcher nor the volunteer is provided with genotype information. This is in keeping with the Guidelines for the American College of Medical Genetics (see appendix A attached: Non-Disclosure document). The third party selects from the returned genotyping procedure a subset of codes representing potential volunteers from all required genotypes (unlinked). The lead researcher translates these codes back to names, which are provided to the researcher running the volunteers. All procedures for acquisition, storage and processing of samples for genotyping comply with the HTA license held by the university. Final code breaking is completed on the anonymized data spreadsheet only. Security measures: all study data will be stored in locked filing cabinets and password-protected PCs.
3.6. Will all personal information related to this study be retained and shared in a form that is fully anonymised?	Yes
3.7. If you answered "no" to the above question, you must ensure that these arrangements are detailed in the Information Sheet and that participant consent will be in place. If relevant, please outline arrangements here.	
3.8. Will the Principal Investigator take full responsibility during the study, for ensuring appropriate storage and security of information (including research data, consent forms and administrative records) and, where appropriate, will the necessary arrangements be made in order to process copyright material lawfully?	Yes
3.9. If you answered "no" to the above question, please give further details.	
3.10. Who will have access to personal information relating to this study?	Nourah Al Ruwais Prof Jennifer Rusted Dr Nicholas Dowell Dr Naji Tabet
3.11. Data management responsibilities after the study: State how long study information including research data, consent forms and administrative records will be retained, in what format(s) and where the information will be kept. Please see the University's Research Data Management Policy.	7 years as paper and electronic records. Secure filing cabinets within CISC or the School of Psychology.
>> Section 4. Researcher(s) Safety and Wellbeing	

4.1. Does the project involve working with any substances and/or equipment which may be considered hazardous? (Please refer to the University's Control of Hazardous Substances Policy).	Yes
4.2. If yes, briefly state the substance(s) or equipment and briefly describe measures you will take to ensure the researchers safety and wellbeing.	<p>Venipuncture: Blood taking is performed by a member of the onsite clinical team, whom are highly experienced.</p> <p>Contrast Media: Gadolinium based contrast agents are non radioactive substances and have no harm to the radiographer administering it.</p> <p>all CISC staff are trained in Standard operating procedures for adverse events</p>
4.3. Could the nature or subject of the research potentially have an emotionally disturbing impact on the researcher(s)?	No
4.4. If yes, briefly describe what measures will be taken to help the researcher(s) to manage this.	
4.5. Could the nature or subject of the research potentially expose the researcher(s) to threats of physical violence and/or verbal abuse?	No
4.6. If yes, briefly describe what measures will be taken to mitigate this.	
4.7. Does the research involve any fieldwork - Overseas or in the UK?	No
4.8. If yes, where will the fieldwork take place? If the fieldwork will take place overseas please ensure that an OTSSRA form has been completed and the University of Sussex Insurance Manager has been consulted (insurance@sussex.ac.uk).	
4.9. Will any researchers be in a lone working situation?	No
4.10. If yes, briefly describe what measures will be taken to mitigate this.	
4.11. Can you think of anything else that might be potentially harmful to the researcher(s) in this research?	
>> Section 5. Other Ethical Clearances, Gatekeepers and Permissions	
>> Section 5. Other Ethical Clearances, Gatekeepers and Permissions	
5.1. Are any other ethical clearances or gatekeeper permissions required for access to participants or the research sites?	No
5.2. If yes, please give further details including the name and address of the organisation. If other ethical approval has already been received please attach evidence of approval, otherwise you will need to supply it when ready.	
5.3. If the research involves storing and/or analysing human tissue or any human material will this be carried out under the University's HTA license?	Yes
5.4. Please also consider whether there are other ethical issues you should be covering here. Please also make reference to the professional code of conduct you intend to follow in your research.	-
>> Section 6. Conflicts of interest	
6.1. Do any researchers have any financial interests in this research or its outcomes or any relevant affiliations?	No
6.2. If yes, please give further details.	

Appendix B: Ethics Certificate of Approval



BSMS Research Governance Ethics Committee

Certificate of Approval	
Reference Number	ERA/NA391/2/1
Title Of Project	Blood Brain Barrier Permeability in mid age APOE e4 carriers (COPY) (AMENDMENT)
Principal Investigator (PI):	Nourah Alruwais
Student	Nourah Alruwais
Collaborators	Professor Jennifer Rusted Dr Naji Tabet Dr Nicholas Dowell
Date Of Approval	26-Nov-2018
Approval Expiry Date	07-Oct-2020
RGEC Chair	Caroline Brooks
Name of Authorised Signatory	Professor Kevin Davies
Date	26-Nov-2018

The Brighton and Sussex Medical School Research Governance and Ethics Committee (RGEC) has assessed your application and granted Ethical and Research Governance Approval to proceed with the above named project.

Approval is granted on the following basis:

Duration of Approval

Approval covers the period stated above. Research must commence within 12 months of the certificate start date; any delay beyond 12 months and this certificate of approval will lapse necessitating renewed review of the project.

Project Amendments

Any substantial changes or minor amendments to the project following issue of the certificate of approval should be submitted to the Research Governance and Ethics Committee for review and authorisation prior to implementation. Please submit your application for an amendment to the Committee (via rgec@bsms.ac.uk) using the Request for an Amendment Form.

Reporting Adverse and Unexpected Events

Any incidents occurring during the project's lifespan presenting ethical and safety implications must be reported immediately to the Chair of the Research Governance and Ethics Committee. In the event of an adverse (undesirable and unintended) and unexpected event occurring during the project, research must be stopped immediately and events reported to the Chair of the Research Governance and Ethics Committee within 24 hours of its occurrence.

Monitoring

The Medical School has a duty to ensure all its research is conducted in accordance with the University of Sussex's Code of Practice for Research and Research Governance and Ethical Review Framework. In order to ensure compliance auditing may be undertaken annually and /or periodic monitoring of a percentage of approved research studies. If your project is selected you will be given 4 weeks' notice to prepare all study documentation for inspection.

Notification of End of Study

Please notify the Research Governance and Ethics Committee once the study has completed. It is also your responsibility to inform the Committee in the event of early termination of the project or if the work is not completed.

Appendix C: Ethics Amendment

Amendment made to the ERA/NA391/2/2 application Include:

Age range:

First version: 45-55

Second version: 42-59.

In the exclusion Criteria:

First version: Current physical and/or mental illness

Second version: Current serious physical and/or mental illness

In the participant information sheet:

First version:

Why have I invited to participate?

You have been invited to take part as you fit the age criteria for our study. We are looking for 40 volunteers aged between 45 and 55 years who are generally healthy. You should not currently be taking any regular medications, be a non-smoker and have no previous or current mental or physical illness, particularly problems with kidney function, asthma or other allergies. If you take health supplements that include creatine this can affect the results of your kidney function test, so you may not be able to take part in this study. If you have experienced been claustrophobia in the past, then you may want to reconsider your participation due to the tunnel-like MRI machine.

Second Version:

Why have I invited to participate?

You have been invited to take part as you fit the age criteria for our study. We are looking for 40 volunteers aged between 42 and 59 years who are generally healthy. You should be a non-smoker and have no current serious mental or physical illness, particularly problems with kidney function, asthma or other allergies. If you take health supplements that include certain this can affect the results of your kidney function test, so you may not be able to take part in this study. If you have experienced claustrophobia in the past, then you may want to reconsider your participation due to the tunnel-like MRI machine.

Appendix D: Ethics Certificate of Approval (Amendment)



BSMS Research Governance Ethics Committee

Certificate of Approval	
Reference Number	ER/NA391/3
Title Of Project	Blood Brain Barrier Permeability in mid age APOE e4 carriers (COPY)
Principal Investigator (PI):	Nourah Alruwais
Student	Nourah Alruwais
Collaborators	
Date Of Approval	26-Mar-2019
Approval Expiry Date	07-Oct-2020
RGEC Chair	Caroline Brooks
Name of Authorised Signatory	Professor Kevin Davies
Date	26-Mar-2019

The Brighton and Sussex Medical School Research Governance and Ethics Committee (RGEC) has assessed your application and granted Ethical and Research Governance Approval to proceed with the above named project.

Approval is granted on the following basis:

Amendment to extend the age range of participants and to include the phrase: No current "serious" physical or mental illness.

Duration of Approval

Approval covers the period stated above. Research must commence within 12 months of the certificate start date; any delay beyond 12 months and this certificate of approval will lapse necessitating renewed review of the project.

Project Amendments

Any substantial changes or minor amendments to the project following issue of the certificate of approval should be submitted to the Research Governance and Ethics Committee for review and authorisation prior to implementation. Please submit your application for an amendment to the Committee (via rgec@bsms.ac.uk) using the Request for an Amendment Form.

Reporting Adverse and Unexpected Events

Any incidents occurring during the project's lifespan presenting ethical and safety implications must be reported immediately to the Chair of the Research Governance and Ethics Committee. In the event of an adverse (undesirable and unintended) and unexpected event occurring during the project, research must be stopped immediately and events reported to the Chair of the Research Governance and Ethics Committee within 24 hours of its occurrence.

Monitoring

The Medical School has a duty to ensure all its research is conducted in accordance with the University of Sussex's Code of Practice for Research and Research Governance and Ethical Review Framework. In order to ensure compliance auditing may be undertaken annually and /or periodic monitoring of a percentage of approved research studies. If your project is selected you will be given 4 weeks' notice to prepare all study documentation for inspection.

Notification of End of Study

Please notify the Research Governance and Ethics Committee once the study has completed. It is also your responsibility to inform the Committee in the event of early termination of the project or if the work is not completed.



Qualitative Protocol Development Tool

Research protocol forms an essential part of a research project. It is a full description of the research study and will act as a 'manual' for members of the research team to ensure adherence to the methods outlined. As the study gets underway, it can then be used to monitor the study's progress and evaluate its outcomes.

The protocol should go into as much detail about the research project as possible, to enable the review bodies to fully understand your study.

The use of this collated consensus guidance and template is not mandatory. The guidance and template are published as standards to encourage and enable responsible research.

The document will:

- Support researchers developing protocols where the sponsor does not already use a template
- Support sponsors wishing to develop template protocols in line with national guidance
- Support sponsors to review their existing protocol template to ensure that it is in line with national guidance.

A protocol which contains all the elements that review bodies consider is less likely to be delayed during the review process because there will be less likelihood that the review body will require clarification from the applicant.

We would appreciate self-declaration of how you've used this template so we are able to measure its uptake.

Please indicate the compatibility of this template with any existing templates you already use by stating one of the following on the front of each submitted protocol:

- **This protocol has regard for the HRA guidance and order of content; OR**
- **This protocol has regard for the HRA guidance; OR**
- **This protocol does not have regard to the HRA guidance and order of content**

AIM OF THE STUDY

- ✓ Develop methods to investigate the possible early detection of subtle brain changes
- ✓ Verify these structural brain changes
- ✓ To assess whether APOE4 status can lead to subtle brain changes in mid age and whether such changes, if any, are related to fine deficits in cognition

SHORT STUDY TITLE

BBB permeability in mid age APOE e4 carriers

PROTOCOL VERSION NUMBER AND DATE

Version 1.2 26 March 2019

RESEARCH REFERENCE NUMBERS

IRAS Number:

ER/NA391/2/2

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the study in compliance with the approved protocol and will adhere to the principles outlined in the Declaration of Helsinki, the Sponsor's SOPs, and other regulatory requirement.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:

Signature:

Date:

...../...../.....

.....

Name (please print):

.....

Position:

.....

Chief Investigator:

Signature:

Date:

.....Nourah.....

..25../..3../2019

Name: (please print):

.....NOURAH ALRUWAIS.....

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KEY STUDY CONTACTS

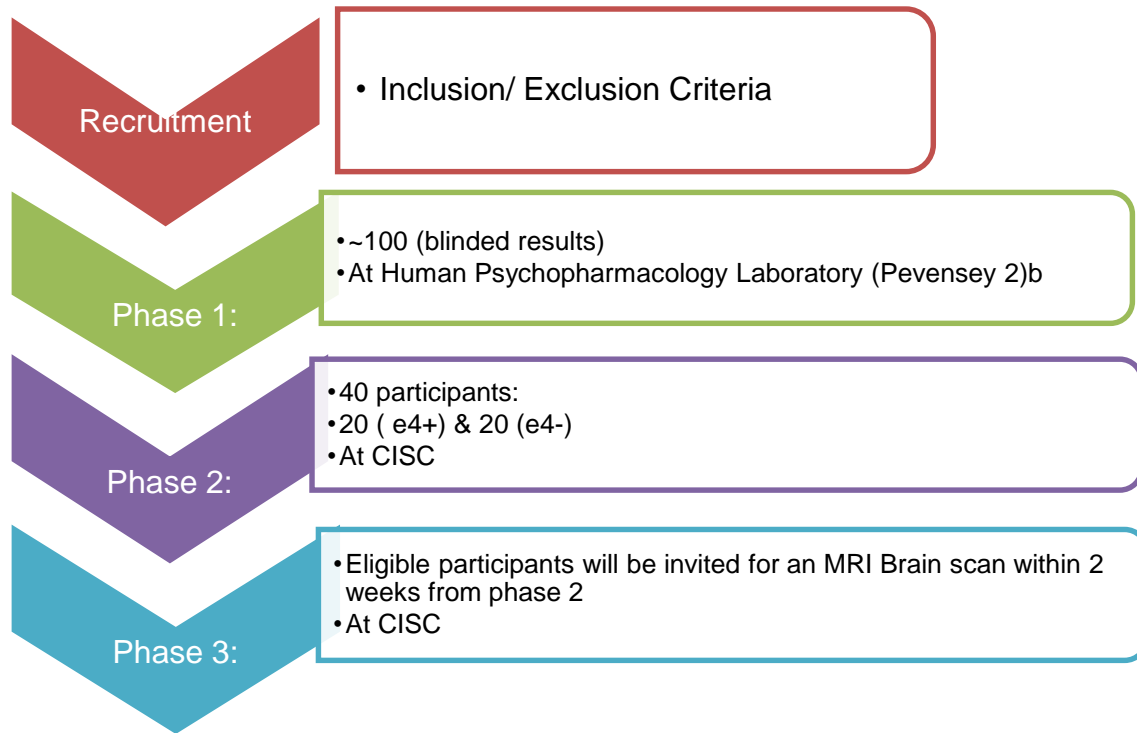
Insert full details of the key study contacts including the following

Chief Investigator	Nourah Al Ruwais, N.Alruwais@sussex.ac.uk
Study Co-ordinator	Prof. Jennifer Rusted, j.rusted@sussex.ac.uk , +44 1273 678325
Institute	School of Psychology, University of Sussex
Funder(s)	Saudi Cultural Bureau
Key Protocol Contributors	Prof. Jennifer Rusted, j.rusted@sussex.ac.uk , Dr Naji Tabet, N.Tabet@bsms.ac.uk Dr Nicholas Dowell, N.G.Dowell@bsms.ac.uk
Committees	Research Governance and Ethics Committee (RGEC) , : c.e.brooks@bsms.ac.uk , Tel: 01273 641470

STUDY SUMMARY

Study Title	Blood Brain Barrier Permeability in mid age APOE e4 carriers
Internal ref. no. (or short title)	BBB IN MID AGE
Study Design	Phase 1: Genotyping Phase 2: Cognitive Assessment & blood sample Phase3: Brain Imaging (MRI)
Study Participants	Healthy mid age adults
Planned Size of Sample (if applicable)	Initial ~100 for genotyping 20 APOE +e4 & 20 APOE -e4
Follow up duration (if applicable)	
Planned Study Period	2 years
Research Question/Aim(s)	<ul style="list-style-type: none"> ✓ Develop methods to investigate the possible early detection of subtle brain changes. ✓ Verify these structural brain changes. ✓ To assess whether APOE4 status can lead to subtle brain changes in mid age and whether such changes, if any, are related to fine deficits in cognition.

STUDY FLOW CHART



FUNDING AND SUPPORT IN KIND

FUNDER(S) (Names and contact details of ALL organizations providing funding and/or support in kind for this study)	FINANCIAL AND NON FINANCIALSUPPORT GIVEN
Saudi Cultural Bureau	Saudi Cultural Bureau
University of Sussex (School of Psychology)	University of Sussex (School of Psychology)
	CISC, BSMS

STUDY PROTOCOL

BLOOD BRAIN BARRIER PERMEABILITY IN MID AGE APOE4 CARRIERS

1 BACKGROUND

Alzheimer's disease (AD) affects about 850,000 people in the UK and is usually diagnosed after symptoms have developed at an older age. Several studies suggest that neuropathology of the disease (amyloid deposition and later tau tangles) begins years before clinical diagnosis is made. Some early structural brain changes such as lobar specific atrophy and ventricle enlargement may be identified in the early stages of AD and it is possible that other structural changes such as blood brain barrier abnormalities, microhemorrhages and haemosiderin deposits may be detected earlier in people who develop late-onset sporadic AD.

Research has also shown a relationship between the presence of Apolipoprotein (APOE) genotype and AD. The APOE gene has effects on neural function. The e4 variant of this gene has attracted attention as the single most important known genetic risk factor for late onset, non-familial AD. Even in healthy adults, the e4 variant has been associated with steeper age-related decline in cognitive ability.

The aim of this study is to develop methods to investigate the possible early detection of subtle brain changes in people at a higher risk of developing late onset, non-familial AD later in life. The information obtained will increase our knowledge of the potential neuropathological effects of the APOE4 gene and may provide the opportunity for early intervention to maintain and improve individuals' cognitive health as they age.

People with AD have been shown to have increased blood leakage into the brain as a result of increased blood-brain barrier (BBB) permeability. This begs the question whether increased BBB permeability is present in healthy e4 carriers, who carry the risk for late onset, sporadic AD but do not currently (and may never) manifest the symptoms. We plan to investigate this by recruiting healthy volunteers into two groups: those that carry the e4 allele and those that do not. We will then be carrying out structural brain imaging using a gadolinium-based MRI contrast agent to help us identify and quantify subtle leakage of blood across the BBB.

The use of contrast agent in this project is essential to identify the movement of blood into brain tissue. The gadolinium chelates constitute the largest group of MR contrast media and are considered to be extremely safe when administered within standard dosage guidelines.

1.1 Objectives

To assess whether APOE4 status can lead to subtle brain changes in mid age and whether such changes, if any, are related to fine deficits in cognition.

1.2 Outcome

Subtle structural Brain changes (BBB leakage, cerebral microhaemorrhages, Hippocampus atrophy) are identified in healthy mid-age APOEε4 carriers.

2 STUDY SETTING

Phase1 (Genotyping): Human Psychopharmacology Laboratory (Pevensey 2)
 Phase2 (Cognitive assessment & blood sample): CISC
 Phase3 (MRI Brain Scan): CISC

2.1 Participants Compensation for their time

Phase 1 (Genotyping): Coffee voucher
 Phase 2 (Cognitive assessment & blood sample): £7
 Phase 3 (MRI Brain Scan): £60

3 SAMPLE AND RECRUITMENT

3.1 Eligibility Criteria

Healthy mid age participants with no known kidney abnormalities (healthy renal function).

3.1.1 Inclusion criteria

1. Age 42-59
2. Generally Healthy
3. Willingness to participate in MRI study if selected

3.1.2 Exclusion criteria

1. People of African descent (due to differences in physiological consequences of APOE)
2. Current significant physical and/or mental illness as judged by Dr Tabet.
3. MRI contraindications: a. Implantable devices b. Metal fragments lodged in the eyes or body c. Large or dark tattoos on the head or neck d. Pregnancy e. Claustrophobia
4. Contrast Media contraindications: a. Asthma b. History of renal disease/kidney problems Self-reported and documented by eGFR < 60 c. Allergies/Sensitivity to contrast media including gadolinium agents d. pregnancy e. breastfeeding f. severe liver disease or history of liver transplantation g. current use of psychoactive substances

3.2 Sampling

We will invite previous volunteers to take part in this study who have been genotyped under (ER/JENNYR/1) and have previously indicated their willingness to be approached for further studies. We will also recruit about 100 healthy mid aged (42-59) people to be genotyped to supplement this volunteer database. Since the ε4 allele is present in only ~15% of the population, we need to genotype this number of people to achieve our target

number of 20 e4 carriers (e4+) and 20 non-e4 carriers (e4-) for the study. After genotyping, we will randomly select 20 individuals in each group to continue in the study. Personal details and genotype information are stored separately at all times.

3.2.1 Size of sample

The number of participants were chosen based on similar studies of BBB permeability using gadolinium as the contrast agent where the number of participants ranged between 18-24 per group (Cramer et al 2014 & Montagne et al. 2016), as well as, e4 studies that showed subtle brain changes with 20 participants per group (Dowell et al. 2012).

3.2.2 Sampling technique

Phase 1: A triangulated procedure involving two anonymized codes per sample and a third party (another member of faculty) ensures that neither the testing researcher nor the volunteer is provided with genotype information. The third party selects from the returned genotyping procedure a subset of codes representing potential volunteers from all required genotypes (unlinked). In this instance, as in previous studies, we will compare e4 carriers with the population norm, double e3 carriers. The lead researcher translates these codes back to names, which are provided to the researcher running the volunteers. All procedures for acquisition, storage and processing of samples for genotyping comply with the HTA license held by the university. Final code breaking is completed on the anonymized data spreadsheet only and after pre-processing of imaging data.

Phase 2: Participants randomly selected to represent required genotype groups will be invited to CISC for a blood sample to check for (normal renal function, full blood count and inflammatory markers), and a basic cognitive assessment, including episodic and a prospective memory tasks, which may take up to 30 minutes to complete.

Phase 3: Participants with normal renal function will then be asked to visit CISC one more time. They will complete an MRI safety questionnaire and be screened by a radiographer before entering the scanner. The third visit will last about 90 minutes.

3.3 Recruitment

Volunteers will be recruited through posted advertisement at the University of Sussex and University of Brighton and also on local community websites (e.g. Brighton Gumtree). Further, emails will also be sent to people who have previously recorded an interest in taking part in research at the university of Sussex. A passive recruitment strategy will be adopted- i.e. the first contact between participant and researcher will be made by potential participants themselves. This will insure that participants do not feel coerced into taking part.

We will invite previous volunteers to take part in this study who have been genotyped under (ER/JENNYR/1) and have previously indicated their willingness to be approached for further studies. We will also recruit about 100 healthy mid aged (42-59) people to be genotyped to supplement this volunteer database. Since the e4 allele is present in only ~15% of the population, we need to genotype this number of people to achieve our target number of 20 e4 carriers (e4+) and 20 non-e4 carriers (e4-) for the study. After genotyping, we will randomly select 20 individuals in each group to continue in the study. Personal details and genotype information are stored separately at all times.

3.3.1 The rational for the number of participants recruited

The number of participants were chosen based on similar studies of blood brain barrier permeability using gadolinium as the contrast agent where the number of participants ranged between 18-24 per group (Cramer et al 2014 & Montagne et al. 2016), as well as, E4 studies that showed subtle brain changes with 20 participants per group (Dowell et al. 2011).

3.3.2 Consent

Care has been taken to ensure that recruitment is free from undue influence and recruitment material makes no diagnostic nor therapeutic promise. Only healthy participants that meet the inclusion but none of the exclusion criteria will be recruited to this study. The information sheet will be provided to them electronically, and volunteers will be given 2 weeks to consider their participation. Plenty of opportunity will be given to discuss the study and ask questions (either by phone or email) before giving formal consent to participation.

Informed consents will then be given prior the start of each phase by principal researcher Nourah Al Ruwais (Medical Imaging Technologist).

At all stages of the study it will be made clear to potential participants that they have no obligation to take part in the study and have the right to withdraw at any stage without any given reason. Data will be shredded and destroyed upon the request of the withdrawing participant. However, this will not be possible once the data has been published. This is clearly explained in the information sheet and consent forms.

4.1 Assessment and management of risk

Saliva samples will be collected in this study. This is not potentially harmful but might be considered invasive.

Venepuncture: Taking a blood Sample is associated with mild discomfort. To minimize this, blood taking will be performed by a member of the onsite clinical team, whom are highly experienced in blood taking (CISC radiography team).

MRI scanning: MRI scanning is safe. However, some participants may feel claustrophobic when in the scanner. To minimize this, all potential participants will be screened for history of claustrophobia.

Contrast Media: Gadolinium based contrast agents have also been associated with side effects. The most common side effects (which occur in 1 in 100 people) include pain around the injection site, nausea, vomiting, itching, rash, headache and abnormal skin sensations. In people with poor kidney function they can also cause severe thickening of the skin and other tissues. However, this has never been reported in people who have normal kidney function. We will check every participant's kidney function at the beginning of the study and they will only be able to receive the contrast agent and continue in the study if this is normal (Dr Tabet will check the results of participants Kidney function to confirm eligibility). In some rare cases (fewer than 1 in 10,000 people) gadolinium based contrast agents can cause serious allergic or non-allergic reactions. The staff at the imaging centre that will administer the contrast agent are experienced and trained in dealing with any acute side effects that may arise. A trained clinician will also be present within the imaging centre during all imaging.

Very rarely brain imaging may reveal unexpected abnormalities. Should this occur, images will be sent to a consultant neuroradiologist to be reviewed. Should any incidental findings be found the participant and his/her GP will be informed and provided with a copy of the

neuroradiologist report. This procedure follows standard CISC protocol. If renal blood analysis shows unexpected findings, the participant and GP will be notified.

4.2 Data protection and patient confidentiality

All data held on computers will be anonymized and stored under a participant recruitment number. Participants initials will not be used, nor will dates of birth to minimize the risk of participant identification from the study recruitment code. All data will be stored in accordance with Data Protection Act. All researchers involved in this study are aware of the importance of confidentiality and confidentiality clauses exist in all research staff contracts.

Participants with APOE e4+, or double e3 genotype will be selected randomly from their pool. A triangulated procedure involving two anonymized codes per sample and a third party (another member of faculty) ensures that neither the testing researcher nor the volunteer is provided with genotype information. The third party selects from the returned genotyping procedure a subset of codes representing potential volunteers from all required genotypes (unlinked). The lead researcher translates these codes back to names, which are provided to the researcher running the volunteers. All procedures for acquisition, storage and processing of samples for genotyping comply with the HTA license held by the university. Final code breaking is completed on the anonymized data spreadsheet only. Security measures: all study data will be stored in locked filing cabinets and password-protected PCs.

4.3 Access to personal information relating to this study

Prof Jennifer Rusted

Dr Nick Dowell

Dr Naji Tabet

Nourah Al Ruwais

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6. APPENDICIES

6.1 Appendix 1- Required documentation

- ✓ Participant Information Sheet
- ✓ Consent Phase 1
- ✓ Consent Phase 2
- ✓ Consent Phase 3
- ✓ Gadolinium Information Sheet (Contrast)
- ✓ Buccal Swab Information Sheet (for genotyping)

11.2 Appendix 2 – Schedule of Procedures (Example)

Procedures						
	Screeni ng	Phase 1	Phase 2	Phase 3	Data Analysi s	Image Analysi s
Information sheet	x					
Eligibility questioner	x					
Informed consent 1		x				
Informed consent 2			x			
Informed consent 3				x		
Genotyping		x			x	
Cognitive Assessment			x		x	
Blood Sample			x		x	
MRI screening	x			x		
Imaging				x	x	x
Adverse Event Management card				x		
Risk Assessment Pro-Forma				x		

Appendix F: Participant Information Sheet



PARTICIPANT INVITATION AND INFORMATION SHEET

Study title

Investigating Blood Brain Barrier permeability in mid age carriers of APOE genotype

Invitation paragraph

We would like to invite you to take part in a research study investigating blood brain barrier permeability in mid aged APOE e4 carriers.

Before you decide it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and please feel free to discuss your participation with friends and family if you wish. Ask us if there is anything that is not clear or if you would like more information.

Also please be aware that this is not intended to identify individual differences, nor is it intended to offer or suggest treatment.

What is the purpose of the study?

Alzheimer's disease (AD) affects about 850,000 people in the UK and is usually diagnosed after symptoms have developed at an older age. Several studies suggest that the progression of the disease begins years before clinical diagnosis is made. Early structural brain changes can be identified as signs leading to the progression of AD.

Although APOE genotyping is not used for clinical diagnosis, research has shown a relationship between the presence of apolipoprotein (APOE) genotype and AD in general. APOE gene has effects on neural function. The e4 variant of this gene has attracted attention as the single most important gene for risk of late onset, sporadic AD. Even in healthy adults, the e4 variant has been associated with steeper age-related decline in cognitive ability.

Therefore, **the aim of this study is to develop methods for early detection of brain changes associated with carrying the e4 gene. In the longer term, this knowledge may improve our understanding of who will and who will not go on to develop late onset AD. This will provide the opportunity for early intervention to improve patient's cognitive health in older age.**

We plan to investigate this by first identifying people from each of the main APOE genotypes and then by imaging the brain using an MRI contrast agent, Gadolinium, to enhance the quality of the scans that we are doing and to expose subtle changes that may be taking place.

Why have I been invited to participate?

You have been invited to take part as you fit the age criteria for our study. We are looking for 40 volunteers aged between 42 and 59 years who are generally healthy.

You should be a non-smoker and have no current serious mental or physical illness, particularly problems with kidney function, asthma or other allergies. If you take health supplements that include certain this can affect the results of your kidney function test, so you may not be able to take part in this study. If you have experienced claustrophobia in the past, then you may want to reconsider your participation due to the tunnel-like MRI machine.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form to keep. If you decide to take part you are still free to withdraw at any time, without needing to give a reason.

What will happen to me if I take part?

The study involves 3 phases:

Phase 1 (Genotyping): takes less than 10 minutes; it involves a cheek swab (a small cotton bud you rub on the inside cheek of your mouth) which will be analysed to determine your APOE genotype). We randomly select a number of e4 carriers and non-e4 carriers from phase 1 to complete phase 2.

You will be given a coffee voucher as a compensation for taking part in phase 1 which is located in The Human Psychopharmacology Laboratory (Pevensey 2), Sussex University.

Note: Your genotype will be kept confidential and will NOT be available to you nor to the researcher. Please consider carefully whether you wish to take part in this study on this basis, and feel free to contact anyone from the research team to answer any questions that may arise at any stage during the study.

Phase 2 (Blood sample and Cognitive Test): This involves you coming to the Clinical Imaging Sciences Centre (CISC) on the Falmer campus of the University of Sussex. When you arrive one of our team members will meet you at the reception desk. We will initially ask you a few basic health questions and then explain everything again. You will have another opportunity to ask any further questions. If you are happy to continue with the study you will be asked to fill in a consent form. You will then be invited to take a cognitive test on a computer in a private room. This will only take 15-30 minutes of your time and involves you answering some questions and completing a few short computerised tasks of mental agility. We will then take a blood sample. This will be sent to a laboratory to ensure that you have healthy kidney function and to also measure inflammatory markers and check your

full blood count. If you do not meet the healthy kidney function required for using gadolinium your participation in this research study will come to an end and you will be reimbursed £7 for your time.

Phase 3 (Imaging): A small cannula will be administered into your arm to allow us to give you the MRI contrast agent gadolinium. The cannula will remain until the end of the scan. You will then be taken through to the MRI scanner where you will be asked to remove any metal items that you may be wearing e.g. rings, piercings, watch, necklace and glasses and if needed given a pair of MRI safe glasses to wear. The MRI scanner is very noisy so we'll also give you earplugs and headphones to wear. We will ask you to lie still in the scanner while we take some images of your brain. Then you will be injected with the MRI contrast agent gadolinium and the scanner will continue for 70 minutes. During this scan we will ask you lay still and relax so we can get some clear images of your brain. You will be compensated £60 for your participation.

Phase 2&3 will take place at the Clinical Imaging Science Centre (CISC) on the Falmer campus of the University of Sussex.

What are the possible disadvantages and risks of taking part?

Gadolinium based contrast agents may have some side effects. The most common side effects (which occur in 1 in 100 people) include pain around the injection site, nausea, vomiting, itching, rash, headache and abnormal skin sensations. In people with poor kidney function they can also cause severe thickening of the skin and other tissues. However, this has never been reported in people who have normal kidney function. We will check your kidney function during phase 2 of the study and you will only be able to receive the contrast agent and continue in the study if this is normal. In some rare cases (fewer than 1 in 10,000 people) gadolinium based contrast agents can cause serious allergic or non-allergic reactions. The staff at the imaging centre that will administer the contrast agent are experienced and trained in dealing with any acute side effects that may arise.

If you experience any delayed side effects, you are advised to visit your GP or nearest A&E department telling them that you have recently had a gadolinium based contrast agent (Dotarem). We have also attached some additional information about gadolinium contrast agents for you to read.

MRI is a safe procedure that has been used for many years without any particular problems being found. It does not involve x-rays or other forms of harmful radiation. Some participants may experience dizziness or a slight tingling feeling (due to the fast changes of magnetic fields that occur during an MRI scan) or claustrophobia due to being inside of the scanner. While you are in the scanner you will have a button that you can press and you will also be able to communicate over an intercom, so if you should feel uncomfortable in the scanner you can alert the radiographer and the scan can be stopped.

What are the possible benefits of taking part?

Whilst participating in this research may not benefit you directly, you will be contributing towards the advancement of science and can generate benefits for generations to come. You will also receive £67 and a voucher on completion of the study to compensate you for your time taken to participate.

Will my information in this study be kept confidential?

Yes. We want to emphasise that all results obtained will be strictly confidential and will only be used for medical research purposes. All the information about your participation in this study will be secured against any unauthorised access. Although the overall results will be published in medical journals, no individual subjects will be identifiable from this. Confidential information regarding identity of participants will be kept secure for 7 years, and you may be contacted for follow-up studies related to this project in the future upon your approval.

Your GP will not routinely be notified that you have taken part in this study. However, if your kidney function tests or your scans show any unusual features that need to be followed up we will inform your GP and they will contact you.

What should I do if I want to take part?

You can contact Nourah Al Ruwais by email: N.Alruwais@sussex.ac.uk

What will happen to the results of the research study?

The results, along with all other information collected from you in the course of this research will be kept strictly confidential. The results will be statistically analysed and findings subsequently published in peer-reviewed journals. You will not be identified in any publication. You may request a copy of any publication resulting from this work that can be obtained by giving us your email address.

Forms with your name and contact details on them will all be stored in lockable cabinets in the CISC imaging centre or School of Psychology for the duration of the study. They will then be destroyed unless you have indicated that you would like to remain on our resources to be contacted for further studies. All other information about you that leaves the department will have your name and contact details removed so that you cannot be identified from it.

Who is organising and funding the research?

This research is conducted by a PhD student at the University of Sussex jointly supervised by senior faculty at BSMS and School of Psychology, and is funded by an international scholarship awarding committee.

Who has approved this study?

All medical research is looked at and approved by independent members of a Research Ethics Committee, to protect your interests. This study has been reviewed and given a favourable opinion by the Brighton & Sussex Medical School and Research Ethics Committees.

Contact for Further Information

If you have any questions before or during the study, please contact the following at any time:

Nourah Al Ruwais, PhD research student, School of Psychology, University of Sussex, Email: N.Alruwais@sussex.ac.uk

Prof. Jennifer Rusted, Professor of Experimental Psychology, School of Psychology, Sussex University
Email: j.rusted@sussex.ac.uk

Dr. Nicholas Dowell, Lecturer in Imaging Physics, Clinical Imaging Sciences Center, University of Sussex, Email: N.G.Dowell@bsms.ac.uk

Dr. Naji Tabet, Reader & Honorary Consultant, Centre for Dementia Studies, Brighton & Sussex Medical School, Email: n.tabet@bsms.ac.uk

University of Sussex has insurance in place to cover its legal liabilities in respect of this study.

Thank you

Many thanks for reading this. We hope you feel able to take part in our study, which will help us understand more about early structural brain changes in people at risk of developing Alzheimer's Disease at a later age.

Date

26/3/2019.

Appendix G: Consent Forms



Nourah Al Ruwais
Clinical Imaging Sciences Centre,
School of Psychology,
University of Sussex,
Brighton, BN1 9QG

CONSENT FORM (Phase 1: Genotype) – Confidential

Project Title: **Blood Brain Permeability in mid age APOE-e4 carriers**

Project ID: (ER/NA39/2)

Name of principal investigator: Nourah Al Ruwais

Please initial box

- 1 I confirm that I have read and understood the information sheet applicable to this study and have had the opportunity to ask questions. ☐
- 2 I confirm that I have had sufficient time to consider whether or not I want to be included in the study. ☐
- 3 I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my legal rights being affected. ☐
- 4 I understand that any information I give is completely confidential and will not be stored in such a way that it can be traced back to me. I agree that the data that I provide will be anonymised and stored for further analysis. ☐
- 5 I understand that I am being asked to provide saliva samples in this research and that these samples will be stored until the end of the research project. ☐
- 6 I understand that I will provide a saliva sample that will be genotyped for APOE ONLY. I understand that at the end of the study, or if I withdraw from the study, the sample will be destroyed by incineration. If I withdraw from the study, all my data will also be destroyed if I wish. However, this is not possible if the results have been published. ☐
- 7 I understand that I might be contacted to take part in phase 2 of this study (selection for phase 2 is random). I understand that I am under no obligation to take part in phase 2 if contacted. I understand that I might be contacted to participate in phase 3 of the study if eligible. I understand that I might also be invited to take part in other related studies (also with no obligation). I give permission to be contacted again. ☐
- 8 I understand that my genotype is confidential. I understand that neither I nor the researcher will have access to the results of my genotype. ☐
- 9 I agree to take part in the above study ☐



CONSENT FORM (Phase 1: Genotype) – Confidential

Project Title: **Blood Brain Permeability in mid age APOE-e4 carriers**

Project ID: (ER/NA39/2)

Name of participant

Date

Signature

Name of person taking consent

Date

Signature

Nourah AlRuweis

N.Alruwais@sussex.ac.uk

Researcher

Email**Comments or concerns during the study**

If you have any comments or concerns you may discuss these with the investigator. If you wish to go further and complain about any aspect of the way that you have been approached or treated during the course of the study, you should write or get in touch with Nourah Al Ruweis N.Alruwais@sussex.ac.uk, if she cannot resolve the issue you can also contact Prof Jennifer Rusted, primary supervisor at School of Psychology. Please quote the project ID number at the top of this consent form.



Nourah Al Ruwais
Clinical Imaging Sciences Centre,
School of Psychology,
University of Sussex,
Brighton, BN1 9QG

CONSENT FORM (Phase 2: Blood Sample/ Cognitive Task) – Confidential

Project Title: Blood Brain Permeability in mid age APOE-e4 carriers

Project ID: (ER/NA39/2)

Please initial box

Name of principal investigator: Nourah AlRuwais

1. I confirm that I have read and understood the information sheet applicable to this study and have had the opportunity to ask questions.

☐

2. I confirm that I have had sufficient time to consider whether or not I want to be included in the study.

☐

3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my legal rights being affected.

☐

4. I understand that any information I give is completely confidential and will not be stored in such a way that it can be traced back to me. I agree that the data that I provide will be anonymised and stored for further analysis.

☐

5. I understand that I am being asked to give a blood sample in this research and that this sample will be used to analyse my Kidney function in order to proceed to phase 3. I understand that the sample will also be analysed for biomarkers.

☐

6 I understand that after 12 months of the study, or if I withdraw from the study, the blood sample will be destroyed by incineration. If I withdraw from the study, all my data will also be destroyed if I wish. However, this is not possible if the results have been published.

☐

7 I understand that I might be contacted to participate in phase 3 of the study if eligible and I am under no obligation to take part in phase 3 if contacted. I understand that I might also be invited to take part in other related studies (also with no obligation). I give permission

☐

to be contacted again.

8 I understand that I am asked to take part in some short cognitive tasks and that individual results will not be available for these tasks.

☐

9 I understand that if there is a non-normal outcome from the Kidney test it will be reported back to me and to my GP.

☐

10 I understand that this study is not intended to identify individual differences or illnesses,

☐



Nourah Al Ruwais
 Clinical Imaging Sciences Centre,
 School of Psychology,
 University of Sussex,
 Brighton, BN1 9QG

CONSENT FORM (Phase 2: Blood Sample/ Cognitive Task) – Confidential

Project Title: **Blood Brain Permeability in mid age APOE-e4 carriers**
 Project ID: (ER/NA39/2)

Name of participant	Date	Signature
_____	_____	_____
Name of person taking consent	Date	Signature
_____	_____	_____
Nourah AlRuwais		N.Alruwais@sussex.ac.uk
Researcher		Email

Comments or concerns during the study

If you have any comments or concerns, you may discuss these with the investigator. If you wish to go further and complain about any aspect of the way that you have been approached or treated during the course of the study, you should write or get in touch with Nourah Al Ruwais N.Alruwais@sussex.ac.uk, if she cannot resolve the issue you can also contact Prof Jennifer Rusted, primary supervisor at School of Psychology. Please quote the project ID number at the top of this consent form.



Nourah Al Ruwais
Clinical Imaging Sciences Centre,
School of Psychology,
University of Sussex,
Brighton, BN1 9QG

CONSENT FORM (Phase 3: Brain Scan) – Confidential

Project Title: Blood Brain Permeability in mid age APOE-e4 carriers

Project ID: (ER/NA39/2)

Name of principal investigator: Nourah Al Ruwais

Please initial box

- 1 I confirm that I have read and understood the information sheet applicable to this study and have had the opportunity to ask questions.

☐

- 2 I confirm that I have had sufficient time to consider whether or not I want to be included in the study.

☐

- 3 I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my legal rights being affected.

☐

- 4 I agree to allow you to contact my GP should any abnormality be found on my brain scan.

☐

- 5 I understand that any information I give is completely confidential and will not be stored in such a way that it can be traced back to me. I agree that the data that I provide will be anonymised and stored for further analysis.

☐

- 6 I agree to receive Gadolinium contrast agent and I have been given information on the possible side effects

- 7 I understand that this study is not intended to identify individual differences or illnesses, nor is it intended to offer or suggest treatment.

☐

- 8 I agree to take part in the above study

☐



Nourah Al Ruwais
 Clinical Imaging Sciences Centre,
 School of Psychology,
 University of Sussex,
 Brighton, BN1 9QG

CONSENT FORM (Phase 3: Brain Scan) – Confidential

Project Title: **Blood Brain Permeability in mid age APOE-e4 carriers**
 Project ID: (ER/NA39/2)

Name of participant	Date	Signature
 _____	 _____	 _____
Name of person taking consent	Date	Signature
 _____	 _____	 _____
Nourah AlRuweis		N.Alruweis@sussex.ac.uk
_____ Researcher		_____ Email

Comments or concerns during the study

If you have any comments or concerns, you may discuss these with the investigator. If you wish to go further and complain about any aspect of the way that you have been approached or treated during the course of the study, you should write or get in touch with Nourah Al Ruwais N.Alruweis@sussex.ac.uk, if she cannot resolve the issue you can also contact Prof Jennifer Rusted, primary supervisor at School of Psychology. Please quote the project ID number at the top of this consent form.

Appendix H: Documents of Undertaking Tasks



Documentation of undertaking tasks in the study in accordance with the data management and data protection policies

Study Title: BBB permeability in mid-age APOE e4 carriers

Phase1 (Genotyping)

Participant Code: _____

Session Date: _____

Session Time: _____

	Name	Comment	Demographics	
Consenting			Age	
Explaining procedure			Gender	
Swab Taken			Height	
Swab Code			Weight	
HTA Registration			Highest qualification	
Location			Ethnic	
Disposal of material				
Participant Compensation		Coffee Voucher	Email (to contact for phase 2)	

Documentation of undertaking tasks in the study in accordance with the data management and data protection policies

Study Title: BBB permeability in mid-age APOE e4 carriers

Phase2/ Part 1 (Cognitive Assessment)

Participant Code: _____

Session Date: _____

Session Time: _____

		Duration	Demographics	
Assessment Code			Age	
Consenting			Gender	
Information sheet			Height	
A. Prospective memory task			Weight	
B. Memory word list			Highest qualification	
C. PRMQ			Ethnic	
D. Verbal Fluency			Compensation	7 GBP
Task Order			Email (to contact for phase 2)	

Phase2 / Part 2 (Blood Sample)

Participant Code: _____

Session Date: _____

Session Time: _____

	Name	Location	Time	No. of tubes	Results/ Received date
Consenting					
Blood Code					
Venipuncture					
Disposal of sharps and material					
Delivery of samples to local GP surgery					
Courier to BSUH					
Centrifuge (Freeze -80)					
EGFR %					
Eligible for phase 3/ Approved by Dr Naji Tabet					
Participant invited to phase 3			Date		
CRP					
CBC					
RBC					
WBC					
Hemoglobin					
Ferritin					
Cytosine					
LRP1					

Documentation of undertaking tasks in the study in accordance with the data management and data protection policies

Study Title: BBB permeability in mid-age APOE e4 carriers

Phase3 (MRI Imaging)

Participant Code: _____

Session Date: _____ **Session Time:** _____

	Name	Comment
Consenting		
MRI Screening questioner		
Info. Sheet		
Injecting contrast		
Adverse Event management card		
Scan Time		
Session completed	Yes / No	
Participant compensation		60 GBP

45-55 Year old
VOLUNTEERS WANTED
To take part in research



We are conducting a study to investigate early structural brain changes in mid-aged individuals. The study involves 3 phases:

Phase 1 is to determine eligibility and takes less than 10 minutes; it involves a buccal swab (a small cotton bud you rub on the inside cheek of your mouth) which will be analysed to provide us with information about your genotype.

Phase 2 involves a memory and attention task and a small blood sample to ensure you have healthy kidney function

Phase 3 will be at the Clinical Imaging Sciences Centre (CISC) at Sussex University and it will involve a brain scan.

You will be compensated for your time and participation at the End of each phase!

na391@sussex.ac.uk

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Appendix J: Study Eligibility Questionnaire

**Study Eligibility Questioner Prior to invitation to take part in phase 2**

1. Is there any likelihood no matter how small that you may be pregnant?
(If participant answers yes or unsure, a pregnancy test will then be taken prior to the imaging session).
2. Is your age between 45-55?
3. Do you have any current clinical or mental illness?
4. Do you have Asian or African links in your family history?
5. Do you have any implantable devices?
6. Do you have any metal fragments lodged in eye or body?
7. For females: Do you IUD? What kind? MIRENA, LILETTA, LIPPY LOOP are 3T MRI SAFE
8. Do you have any large or dark tattoos on the head or neck?
9. Are you claustrophobic?
10. Are you Asthmatic?
11. Do you have a history of renal/kidney problems?
12. Have you ever been allergic to contrast media?
13. Do you consider yourself generally healthy?

Appendix K: MRI Safety Questioner

Clinical Imaging Sciences Centre

CISC MRI Safety Questionnaire

Name	Date of Birth dd.mm.yyyy	Weight (kg)	Contact Number	Office Use Only CISC Number
Address		Height	Name & Address of GP	

****The MRI scanner uses a powerful magnetic field - we need to ensure that you are safe to enter the scanning room and don't have metal attached to you that can cause artifacts or heating effects.****

Before arriving for your scan:

REMOVE the following: ALL body piercings & loose metal objects including jewellery, mobile phones, watches, keys, coins, hairclips/grips/extensions, hearing aids, belts, removable metal dental work

Please do not wear make-up (particularly mascara) or you may need to remove it.

**If necessary, change into the scrubs provided.*

Do you have / ever had any of the following? If yes please include details and dates:			
	No	Yes	Details / Dates
Cardiac pacemaker/defibrillator?			
Heart surgery / valve replacement?			
Stents in any blood vessels?			
Head surgery including that to the eyes or ears (e.g. clips / coils / shunts / cochlear implants)?			
Surgery in the past 6 weeks? Provide details.			
Any other surgery (e.g. pins / plates / screws in any bones / joint replacements)?			
Camera capsule endoscopy (PillCam) within the past 2 weeks or Bravo pH monitoring procedure?			
Neurological stimulator or any other implanted electronic medical device (e.g. insulin pump)?			
Implanted contraceptive IUS/IUD (e.g. Mirena or copper coil)? Please provide make / model.			
Epilepsy?			
Skin patches? (e.g. HRT, nicotine, pain relief, contraceptive)			
Tattoos or permanent eye makeup?			
(If yes, where? Read & Sign overleaf)			
Have you EVER had metal fragments in your eyes or under your skin (e.g. from a car accident / shrapnel / welding / grinding / metal sheet worker)?			
Have you had a previous MRI scan? If yes, please indicate at CISC or elsewhere.			
Have you removed ALL loose metal from your person (see list above) as well as ALL 'smart' devices ?			
Is there any chance you could be pregnant?			

I confirm that I have answered and understood the above questions and that the information I have provided is correct to the best of my knowledge:

Patient / Participant Signature	Date:	Radiographer Signature	Date:
		Research Only: Latest Negative LFT (must be within 7 days) Date: Text Result Checked by (Initials):	

Consent for MRI Scanning of research volunteer with a tattoo

I confirm that I have had the risks associated with having an MRI scan with a tattoo explained to me by the researcher.

I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

 Name of Participant

 Date

 Signature

 Researcher

 Date

 Signature

 Name of Person taking consent (if different from the researcher)

 Date

 Signature

Appendix L: Gadolinium (Dotarem) Contrast



Gadolinium (Dotarem) contrast injection

This leaflet contains information on gadolinium (also known by its brand name Dotarem®), which is a contrast (dye) used during MRI scans.

If you have any further questions, please speak to Nourah Al Ruwais

N.Alruwais@sussex.ac.uk or Dr Nicholas Dowell N.G.Dowell@bsms.ac.uk .

What is gadolinium? Gadolinium (Dotarem®) is a clear colorless fluid that is used to make the images clearer during an MRI scan and help with diagnosis.

How is it given? Gadolinium is injected into one of your veins via a needle or a cannula (a soft, hollow, plastic tube) during your scan. The cannula is inserted through your skin into a vein using a needle.

Once the cannula is in place, the needle is removed leaving a small thin plastic tube in the blood vessel. This should be comfortable and will only be in place until your scan is finished.

Are there any side effects? Gadolinium is not suitable for everybody; therefore, a blood sample is taken to check your kidney function. Gadolinium should not be used in patients with reduced kidney function, or hepatorenal syndrome (a condition involving reduced function of the liver and kidneys) since it causes nephrogenic systemic fibrosis in patients with severely reduced kidney function.

It is important that you tell the research team before your scan if you have any history of kidney problems. Also, please let us know if you have had a previous allergic reaction to a contrast agent.

Gadolinium may cause side effects in some people but these are usually mild and short lasting.

Some of the more common side effects include injection site pain, nausea, vomiting, itching, rash, headache and paresthesia (abnormal skin sensation, such as prickling, burning or tingling). If you have any concerns about side effects, please speak to Nourah Al Ruwais N.Alruwais@sussex.ac.uk or Dr Nicholas Dowell

N.G.Dowell@bsms.ac.uk both available at CISC. Occasionally the injection may leak out from the vein to the tissues under the skin – this is known as extravasation. If this has happened, you will experience a stinging sensation where the contrast has gone into the tissue and it can be painful.

Allergic reactions to gadolinium contrast agents are uncommon but do occur.

Most occur during the injection or within the first hour following administration; however, some can occur up to several days after.



Serious allergic reactions have been rarely reported. **Please seek immediate medical attention** if you have any of the following:

Swelling of face, mouth, hands, feet or throat
Coughing/wheezing/sneezing
Difficulty in breathing or swallowing
Eye irritation
Fainting
Rash/hives/itchy red skin

(visit your GP or nearest A&E department telling them that you have recently had a gadolinium based contrast agent (Dotarem)).

NHS 111

Offers medical help and advice from fully trained advisers supported by experienced nurses and paramedics. Available over the phone 24 hours a day.
t: 111

NHS Choices

Provides online information and guidance on all aspects of health and healthcare, to help you make choices about your health.

w: www.nhs.uk

Appendix M: Adverse Event Management



Standard Operating Procedure:

Adverse Event Management A5 Card (Per Study)

NEW

- i. Submit with the RGEC application the Standard Operating Procedure for management of adverse events.
- ii. The SOP should provide details of the procedure to be followed in the case of an adverse reaction:
 - a. immediately following ingestion of the drug
 - b. after volunteers leave the laboratory.
- iii. Include as part of the Standard Operating Procedure, an A5 card for participants to present at A&E in the case of an adverse reaction, which includes PI contact details.
- iv. The card provides at a glance the name of the drug taken, its dose and time of ingestion as well as researchers contact details.



Today I participated in a research study at the University of Sussex. As part of the study:

I have/may have been given:(name of drug) **Dotarem**

The dose of (drug name): _(dose)_____

It was administered at: (time and date of ingestion)

Name/Contact details of persons who administered it:

(name of researcher)_____

PI Contact details: _____ (telephone number)_____

Appendix N: Instructions for use of Buccal Swab for phase 1



Instructions for use of Isohelix SK-1S/MS-01 Buccal Swabs with Isohelix Dri-Capsules

SK-1S, MS-01, SGC-3, SGC-50



Intended for the retrieval of buccal cells. Single use only.
Store at room temperature. Use only if swab wrapper remains intact.
Note: The fresh silica gel is coloured orange and turns green when moisture is absorbed.
In the event that the capsule is coloured green on removal from the foil pack, this indicates that the capsule has already been exposed to moisture and is not suitable for use.

<p>Take your DNA sample at least one hour after eating, drinking or cleaning your teeth. For best results, rinse mouth with water immediately prior to sampling.</p>	
<p>1</p>	<p>Pull open the package from one end.</p>
<p>2</p>	<p>Remove the swab from the tube, taking care not to touch the white swab head with your fingers.</p>
<p>3</p>	<p>Insert the swab into your mouth and rub firmly against the inside of your cheek or underneath lower or upper lip. For standard DNA collection rub for 1 minute and in all cases rub for a minimum of 20 seconds. Important – use reasonable, firm and solid pressure</p>
<p>4</p>	<p>Place the swab back into the tube. Do not touch the swab head with your fingers.</p>
<p>5</p>	<p>Place your thumbnail in the small groove set in the handle, then snap the handle in two by bending to one side. Let the swab head fall into the tube.</p>
<p>6</p>	<p>Remove the silica gel capsule from the foil wrapper and place in the tube so that the capsule sits on top of the swab shaft. Seal the tube securely with the cap provided.</p>

For Research Use only

Isohelix is a division of Cell Projects
For swab or DNA isolation queries email: info@isohelix.com www.isohelix.com
Molecular Biology Solutions www.cellprojects.com

Version August 2015

Appendix O: Standard Operating Procedure (Genotyping)

Standard Operating Procedure – human genotyping (cheek swab)

Prior to volunteer visit

- ☐ Screen participant for eligibility (depending on study requirements)
- ☐ Ensure participant has seen and read participant information sheet (PIS)
- ☐ Confirm time and date of appointment
- ☐ Give directions to Psychopharmacology lab if internal
- ☐ Give directions for parking and to Psychopharmacology lab if external, including map and Pay By Phone info
- ☐ Ensure you tell the participant how to gain entrance to the lab
- ☐ Remind participant not to eat or drink anything (other than water), brush teeth or have chewing gum 1 hour prior to appointment

At volunteer visit: consenting

- ☐ Greet volunteer, thank them for coming
- ☐ Give them PIS and consent form; explain that “this was the consent form that we sent by email, but you’re welcome to re-read if you want. Now is a good time to ask any questions you may have prior to signing the consent”
 - If consenting as “registration for database” session, explain that the first phase of all our studies is the same, and involves a cheek swab for genotyping and them consenting for us to securely hold that information and to invite them for studies in the future. Reiterate that they of course don’t have to participate in any study we invite them to; they can decide on a study-by-study basis
- ☐ Ask them to read through the consent form and put their **initials** in the box if they are happy with each statement
- ☐ Ensure they sign the back

At volunteer visit: swab

- ☐ Double check that they haven’t had food or drink (other than water), brushed teeth or had chewing gum in the last hour
- ☐ Get them to rinse mouth “as much as possible, basically trying to get your mouth as clean as possible without using a toothbrush”

- ☐ Explain swabbing: Show swab. Point to cotton bud, and explain that you'd like them to "rub the cotton bud firmly on the inside of one cheek for 30 seconds, then the other cheek for 30 seconds – I'll time you [demonstrate with finger against outside of own cheek]. Try not to touch the cotton bud anywhere but the inside of your cheek".
- ☐ After swabbing, explain how they break the stick: "there is a small indent here [point] – you need to pull the stick so the indent is level with the top of the tube. Then, trap the stick in place with your finger and snap the stick. If you don't trap it in place, the end will fly across the room!"
- ☐ Ask them to put the stick back in the cup they used.
- ☐ If they really can't snap the stick, cut with scissors – disinfect using wipe BEFORE AND AFTER.

At volunteer visit: information

- ☐ Reiterate that "neither you nor I will ever know your genotype. No one person will ever have your genotype and your name; one person will have your genotype and an ID, another an ID and your name. The reason for this is because at this point in time, there is no value to knowing your *APOE* genotype. We currently can't give any specific advice to those at higher genetic risk of AD, and in fact some of the research we're doing is trying to work out what we **can** tell $\epsilon 4$ carriers to reduce their risk of poor cognitive aging throughout their life – but currently, the advice is the same for people of all genotypes! Exercise, eat healthily, live a healthy lifestyle, keep your mind active"
- ☐ State that "we also take great care to invite people to studies in such a way that you **can't possibly infer your *APOE* genotype** from whether you are invited to participate in a study or not. We run a number of studies at any one time, and we try to get a good balance of things such as genotype, gender, and age for our studies – when we do this, we invite people from our subject pool using a statistically derived sampling procedure so that there is **no way** for you to be able to infer your *APOE* genotype".
- ☐ Check whether they need parking/transport payment. Get them to fill in a form if so (including address, phone, **signature**)
- ☐ Thank them again for coming. Tell them to let any friends know who might be interested.

After volunteer visit

- ☐ Label sample twice, writing in opposite directions
- ☐ Dispose of cup and stick in appropriate bin
- ☐ Put sample in HTA freezer and consent in HTA folder
- ☐ Add sample to itemtracker within 48 hours (working days)



Appendix P: Material Transfer Agreement



This is a controlled document
Any printed versions of this document will be classed as uncontrolled

Human Tissue Act
SOP – Transport of Human Tissue

SOP Reference:	SOP/HTA/05
Version Number V 4.0	Date: 17/07/2017
Effective Date: 30/07/2017	Review by: 30/7/2018

Reviewed: Dr Georgios Giamas	Signature 	Date 11/09/2017
Designation: Designated Individual School of Life Sciences Reviewed: Dr Robert Fowler Designation: Persons Designate School of Life Sciences		11/09/2017
Authorised By: UoS HTA Coordination group	Signatures not sought as minimal changes	

Version	Date	Reason for Change
2.0	30/01/2013	Text change to ensure SOP numbering correct within document Amendment to delivery procedure
3.0	28/7/2014	To reflect merger of BSMS and SoLS practices for UoS
4.0	17/07/2017	Change to reflect the update to DI

1.0 Purpose

This standard operating procedure details the transport of samples.



2.0 Introduction

The integrity of human tissue samples must be protected at all times, necessitating special packaging and direct transport. There are regulations covering the transportation of hazardous materials by Road, Rail, Air and Sea and these regulations must be followed at all times when transporting human tissues/samples.

3.0 Procedure to be followed for transport using road/rail/air services

All samples collected from hospitals or clinics require appropriate secure transportation and packaging must comply with the regulations mentioned above and must be accompanied at all times. Passenger aircraft may allow material to travel in the hold as luggage but this must be cleared by the individual airline.

Packaging

The total packaging must include:

1. A watertight, leakproof primary receptacle
2. Watertight, leakproof secondary packaging

Both of which must be able to maintain their integrity at the temperature of transport paying special attention to packages that require shipment on dry ice (-79oC, -109oF)

3. Outer packaging of sufficient strength for its capacity, mass and intended use

For transport at ambient temperature the primary receptacle should be plastic, metal or glass. If screw caps are used they should be reinforced with adhesive tape to ensure a leakproof seal.

For transport in dry ice, the dry ice should be placed around the secondary packaging and the outer packaging must allow the release of carbon dioxide gas to avoid build up of gas and potential rupturing of packaging or explosion. (Dry ice sublimates 5-10lbs every 24 hours.)

There is clear guidance in document:

SPG 37 – **Guidance for Transporting dangerous material** see web pages:

<http://www.sussex.ac.uk/lifesci/internal/servicesandsupport/healthandsafety/schoolpolicies/procedures>

Shipping of samples to outside establishments must be arranged via the appropriate PD and a reputable courier (eg FedEx, DHL, World Courier) must be used.

Sample returns

In the case where material is undelivered and returned to sender the integrity of the samples must be checked before either resending or stored. If, for example, the sample has thawed and the integrity compromised, the material must be destroyed as outlined in the SOP/HTA/12 which covers disposal of human tissue.

In the case of returned material due to non-delivery an adverse event report will need to be completed, see SOP/HTA/9 and 9a



Labelling and paperwork

- Paperwork, including a contents list, covering letter, materials transfer documents and service level agreement etc., should be placed in waterproof packaging and placed between the secondary packaging and the outer packaging.
- Labels on the primary and secondary packaging should be waterproof and, where handwritten, should be in permanent ink. Labels on the outer packaging must be durable, legible and clearly visible. They should contain the delivery address and the senders' details.
- Hazard labels should be fixed according to guidance document SPG-37
- The PD must ensure that the appropriate Human tissue database is updated accordingly

Delivery

Records of delivery of human material to or from anyone working under research licence numbers 12119 (Life Sci) and 12561 (BSMS) must be kept. A record book for each site must be kept where the details of each shipment are documented. These details should include contact names, journey start point, destination, and AirWayBill information. A record of these details should be made in the log book on arrival and staff should have prior knowledge of expected shipments (in and out) so that material can be promptly collected and stored.

There should never be an instance where human material is in transit and unaccounted for.

If the package appears to be leaking or damaged, it should only be opened in a biological safety cabinet by personnel who are trained in spill clean-up procedures and are wearing appropriate personal protective equipment. The person for whom the parcel is intended should be notified immediately.

4.0 Transport around campus

- Around campus
 - If material is being carried around campus on foot, the carriage of dangerous goods regulations do not apply, but transportation around the campus should follow the guidance in section 3.
- For transportation within a building (i.e. from freezer to bench)
 - Material should be transported in appropriate containers. These should have secure, tight fitting lids (ideally fasten-able) and made of smooth, impervious material such as plastic or metal which would retain liquid in the event of a spillage and can be easily disinfected and cleaned.
 - Material should not be carried in hands, open trays, pockets or loose in plastic bags.
 - If the specimen container is a tube, ensure it is tightly capped and placed in a rack to maintain an upright position.



5.0 Training

All persons undertaking any role in the transport chain should be properly trained to carry out their responsibilities to the required standards. They must appreciate the risks involved and have an understanding of the relevant regulations.

Appendix Q: Storage and Processing of Samples for Genotyping



Code of Practice for the Acquisition, Storage and Use of Human Biological Material for Research.	
Version Number V 6.0	Date: 17/07/2017
Effective Date: 30/07/2017	Review by: 30/07/2018

Reviewed: Dr Georgios Giamas Designation: Designated Individual School of Life Sciences Reviewed: Dr Robert Fowler Designation: Persons Designate School of Life Sciences	Signature 	Date 11/09/2017 11/09/2017
Approved by: HTA Governance Committee	Signatures not sought as minimal changes	

Version	Date	Reason for Change
V2.0	28/8/2012	Change to License details and named PDs. Inclusion of addition glossary terms
V3.0	20/2/2013	Change to scope of CoP Minor corrections as requested by HTA inspector
V.4.0	15/7/2015	Change to reflect the update license to University wide
V.5.0	19/2/2016	Change to reflect the update to DI
V6.0	17/07/2017	Change to reflect the update to DI

Summary of Document

This document sets out the Code of Practice for the acquisition, storage and use of human biological material for research purposes, with particular reference to activities relating to the Human Tissue Act research licence 12119 and defines the responsibilities and accountabilities in relation to such activities.

Distribution

The authorised Code of Practice will be distributed to the licence holder, Persons Designate on the HTA licence and cascaded to other staff as appropriate.



This Code of Practice together with associated Standard Operating Procedures, will also be posted on the School of Life Sciences public website and made available to all research active staff.

Review

The Code of Practice will be reviewed annually or more frequently if deemed necessary by the HTA Governance Committee.

Implementation

The Designated Individual (DI) on HTA research licence 12119 is responsible for ensuring that this code of practice is implemented across all related sites. The DI will, with the approval of the HTA Governance Committee, delegate the task of implementing this code of practice to the Persons Designate.

1. Introduction

The purpose of this Code of Practice is to ensure that all staff undertaking research using human specimens do so within the framework of the Human Tissue Authority (HTA) Licence and know and comply with relevant sections of the Human Tissue Act (2004), HTA licensing conditions, Codes of Practice and related policies.

The University has a duty of care to any person who has consented to the use and storage of their biological specimens for research to ensure that the material is treated with respect and is handled in accordance with the terms of the consent given and the appropriate legislation.

2. Definitions

Adverse Incident: HTA definition: Any event that:

- caused harm or had the potential to cause harm to staff, patients or visitors;
- any event that led to or had the potential to lead to a breach of security of the premises and the contents contained therein;
- any event that caused harm or had the potential to cause harm to stored human tissue (including loss);
- any other event that gives rise to an internal inquiry.
- any breach of the HT Act or the Codes of Practice

Anonymisation: samples or data have had any identifying information removed, such that it is not possible for the researcher using them to identify the individual to whom they relate. They may be:

- Linked anonymised – Specimens are fully anonymous to the people who use them but remain coded such that an appropriately authorised person could link them back to the person from whom they came.
- Unlinked anonymised -the link between the specimen and the person from whom it came has been irreversibly broken.

Appropriate consent: consent as defined by the Human Tissue Act and the guidance from the Human Tissue Authority in Code of Practice A: Consent.

Designated Individual: Person under whose supervision the licensed activity is authorised to be carried out.

Donated material/tissue: human biological material that has been removed with appropriate consent as set out in section 1 of the Act.

HTA: Human Tissue Authority, the independent body set up under the Human Tissue Act (2004) to regulate the removal, storage, use and disposal of human bodies, organs and tissue for a number of "Scheduled Purposes".

HTA Governance Committee: is responsible for developing policies and procedures and to ensure operational practise is compliant with the Human Tissue Act, HTA codes of practice and licensing requirements.



Human Biological Material: For the purposes of this policy Human Biological Material shall refer to material that has come from a human body and consists of or includes human cells (the definition of 'relevant material' used in the Human Tissue Act (2004) section 53).

Human Tissue Act (2004) ["The Act"]: legislative framework covering the uses to which human biological samples can be put. Material not covered by the Act include hair and nail of a living person, gametes and embryos, which are separately regulated by the Human Fertilisation and Embryology Act (1990), established cell lines, human material created outside the human body or rendered acellular by processing (eg serum, plasma, cell free supernatants, DNA).

HTA Licence - Research: Held by the University - licence number 12119

Persons designated: Named person to whom the licence extends and who provides advice, direction and guidance to those at the site.

Principal Investigator: The principal investigators are responsible for all aspects of their research activity including the acquisition, storage, use and disposal of human biological material. They must be familiar with all current requirements and procedures relating to research and the use of human biological material as defined in this Code of Practice

REC: Research Ethics Committee (REC) established under and operating to the standards set out in the governance arrangements issued by the UK Health Departments; or an ethics committee recognised by United Kingdom Ethics Committee Authority (UKECA), to review clinical trials of investigational medicinal products under the Medicines for Human Use (Clinical Trials) Regulations 2004. A University ethics committee is not, for the purpose of the consent exception considered to be a recognised research ethics committee.

Researcher/research staff: All staff involved in research must comply with all relevant SOP's, policies and standards of good practice to ensure compliance with the law, HTA licensing conditions, codes of practice and the Research Governance Framework

SOP: Standard Operating Procedure is a written document or instruction detailing all steps and activities of a process or procedure so that there is uniformity of a specific function

Surplus Human Biological Material: refers to material that has been removed from a patient for the primary purpose of diagnosis, treatment or a specific research project and is no longer required for that purpose.

3. Scope

The scope of this Code of Practice applies to the collection, storage, use and management of all specimens of human biological material for research purposes collected since 1st September 2006. It also applies to the storage, use and management of all specimens of human biological material for research purposes collected before 1st September 2006

It is the responsibility of all staff working with human material at this HTA licence site to know and comply with the requirement of the licences, the law and this policy.

4. Governance

The HTA Governance Committee will develop policy in relation to the Act, HTA licence conditions and codes of practice to ensure that anyone working under licence 12119 can comply with current legislation and guidance. The HTA Governance Committee is accountable to the University HTA Coordination Group.

HTA Governance Committee

- Georgios Giamas (Designated Individual and PD – Life Sciences)
- Kim Bowman (PD – BSMS)
- Jenny Rusted (PD – Psychology)
- Heather Fawcett (PD – GDSC)
- Lisa Woodbine (PD – GDSC)
- Robert Fowler (PD – Life Sciences)



5. Research Approvals

Research using human biological material cannot be carried out unless the project has been approved by an appropriate Research Ethics Committee (as defined by HT Act 2004, Statutory Instrument 2006 No 1260 Section 1 (2)).

6. Consent

Human biological material can only be acquired, stored and used for research if appropriate consent has been obtained. (For exceptions see: 7. Use of surplus diagnostic specimens). The giving of consent must be a positive act, the absence of refusal is not evidence of consent.

Anyone removing, storing or using material in circumstances for which the HT Act requires consent must be satisfied that consent is in place.

It is the principal investigator's responsibility to ensure that appropriate consent procedures are in place as well as Service Level Agreements in the case of a third party taking consent.

If under the NHS, a person's agreement or refusal to consent to the donation, storage or use of tissue for purposes under the Act must not affect the investigation or treatment that s/he receives. If the person being approached is a member of the University staff or student body their agreement or refusal must not affect their management or supervision whilst at the University.

Deceased patients

Consent is required for the removal, use and storage of relevant material from the deceased for ALL scheduled purposes listed in HT Act Schedule 1 (parts 1 and 2).

Explicit consent must be obtained separate from the consent for post mortem examination to remove organs or tissue from the deceased for the primary purpose of research.

7. Acquisition of Human Biological Material

It is the responsibility of those collecting/acquiring human biological material for research purposes to know and follow the local procedures.

Use of surplus diagnostic specimens

Specimens surplus to diagnosis and held in diagnostic/teaching archives can be used for ethically approved research with the donor's consent; unless it was stored prior to implementation of the HTA Act on 1 September 2006, in which case consent is not required, as it is regarded as an "existing holding".

Diagnostic tissue can only be released for research under the following circumstances:

- When the patient has given consent for use of their tissue in research (the preferable scenario);

Where tissue that has not been consented for research (other than existing holdings) can only be released if it is from a living person, and

- the researcher is not in possession, and not likely to come into possession of information that identifies the person from whom it has come;
AND
- where the material is released by a research tissue bank with generic ethical approval from a REC for research within the terms of the approval
OR
- it is to be used for a specific research project approved by a REC.



Use of DNA/RNA from human biological material

It is an offence to have any bodily material (i.e. material which has come from a human body and which consists of or contains human cells) with intent to analyse the DNA in it without qualifying consent, subject to certain exceptions. This section applies to any type of analysis of DNA as defined in genetic research.

8. Storage of Human Biological Material

Material acquired for a specific project

All material collected will be entered and tracked onto the research database until its disposal and any specific requirements detailed at the time of consent. If, during the time of the project, material that had been obtained with project specific consent is needed to be stored for unspecified future use, consent will need to be re-obtained.

Material acquired for unspecified future use

Surplus human biological material can only be stored for the primary purpose of future unspecified research (i.e. in a research tissue bank) under the terms of an HTA licence. Human biological material can only be stored for future unspecified research purposes if there is consent from the donor to do so.

Specimen tracking

A record within the research database must be made of all human biological material collected for the purpose of research. The exception to this is where samples are made acellular or transferred to another recipient within 5 working days. The database will contain information on consent, ethics approval, storage location and fate in order that an audit trail is maintained. Transfer of samples to other institutions must also be recorded on the research database.

Custodianship

The legal responsibility for the use and management of human biological material for research purposes lies with the Designated Individual on the HTA Research Licence.

As the corporate Licence Holder, the University of Sussex will have the formal responsibility for custodianship of the sample and has a responsibility to the donor under the terms of the consent to fulfil the donor's intention.

The University of Sussex and the Designated Individual will delegate the day-to-day management and responsibility for storage, tracking, safe-guarding donors' interest, control of use, disposal and transfer of the donated material to the local principal investigators of the ethically approved research project or tissue bank for which the sample was acquired.

9. Transfer of material

Human biological material can only be transferred to another organisation for a specific ethically approved research project and under the terms of the consent.

An agreement has been drawn up by the Contracts & Intellectual Property office and all human biological material that leaves the University of Sussex HTA licence sites must do so in accordance with the current SOP; being appropriately anonymised where practicable unless consent for a specific research project has been given, in which instance an identifier for the samples agreed between the University of Sussex and the receiving institution must be used. The transfer of the material must be logged on the appropriate laboratory information management system.

10. Disposal

The Human Tissue Authority states that as best practice all human tissue should be disposed of by incineration and separate from other clinical waste.



All human biological material will be disposed of as human tissue waste as detailed in the relevant SOP, and in line with the consent given. Detail of when and why the material is disposed of must be recorded in the research database.

11. Confidentiality

Confidentiality of donor/patient information is a high priority and every effort must be expended to ensure it. The University position on confidentiality and research governance is covered in documents within links below:

<http://www.sussex.ac.uk/res/documents/code.pdf>

http://www.sussex.ac.uk/res/documents/research_governance_code_of_practice.pdf

12. Adverse events

All adverse incidents involving the collection, transport, storage, use or disposal of human biological material for research purposes must be reported in accordance with the SOP for Adverse Incident Reporting.

Adverse incidents involving human biological material at any Sussex University sites must be reported and investigated through the incident reporting system and be notified to the DI so that they can feed into the School of Life Sciences and Psychology Management Committees.

All adverse events will be logged and any required changes to policies, risk assessments and standard operating procedures will be instituted by DI in consultation with the PDs.

13. Risk management systems

All activities relating to the acquisition, storage, use, transportation and disposal of human biological material requires risk assessment. This should be performed and recorded according to the University policies and procedures. The main criterion for risk assessment will be the integrity of the human biological material and all points made in the risk assessments will be clearly reflected in the standard operating procedures which will be recorded in the same manner as the risk assessments. These assessments will be reviewed every two years.

14. Complaints

During the consent process all donors will be made aware of our complaints procedure. A complaints form will be readily available and will be displayed on the School web pages. Complaints will be submitted to the DI who will respond after consultation with PDs and PIs. The complaint and its subsequent action will be reported to the relevant committee. Any required changes in policy, risk assessment or standard operating procedure will be instituted by the DI in consultation with the PDs.

15. Training

Basic induction into the use of human biological material for research and human tissue governance is included in Safety Induction which is provided to all new staff and post graduate students, will be provided by the PD or the DI at the commencement of any new employment contract.

16. Premises

Premises used for the storage and use of human tissue will be secure, out of hours security as well as general premises maintenance will be the responsibility of the University of Sussex. All areas required for the discharge of activities relating to the use of human tissue will be risk assessed. General health and safety issues will be the responsibility of the University of Sussex.



17. Equipment

Research and technical staff will be supplied with the appropriate equipment, including personal protective equipment in order to minimise the risk of contamination and to avoid compromising the integrity of human tissue. All equipment will be regularly (annual or bi-annual) calibrated, validated and serviced. Records of this will be securely stored.

18. Receipt and distribution of local and national alerts

National Alerts from the HTA will be received and subsequently circulated to staff and students working with human tissue by the DI.

More general alerts regarding issues such as local power loss or fire/flood will be provided by the Estates or Health and Safety Offices to the DI who will circulate this information to staff and students working with human tissue.

19. Contingency plans

Every School that uses human tissue must have contingency plans in place that take into consideration the need to protect human biological material from damage or loss.

Freezer failure

All ultra low temperature freezers are alarmed and connected to automatic diallers where the appropriate person is informed. In the case of a failure, samples will be immediately moved to alternative freezers which have been identified in existing local emergency plans, according to the relevant standard operating procedure.

Equipment failure

In this case, alternative similar equipment for the procedure should be found. In the case of biological safety cabinet failure, the procedure should be delayed until an appropriate Cat II cabinet is located.

Site failure

In the case of catastrophic site failure, the relevant University of Sussex policies on work continuation will be put into practice.

20. Monitoring and auditing

We are required to comply with the HTA licensing conditions and Codes of Practice in order to operate within the law and maintain the confidence of the public and patients. The HTA has a duty to inspect licensed premises and activities on a regular basis to ensure this is being done. Regular internal auditing and monitoring of consent, tissue tracking, storage, incident reporting and tissue disposal will be led by the HTA Governance Committee.

Appendix R: Storage and Processing of Samples for Genotyping

REMEMBERING TO DO THINGS

Prospective-Retrospective Memory Questionnaire as described in:

Smith, G., Della Sala, S., Logie, R.H. & Maylor, E.A. (2000). Prospective and Retrospective Memory in Normal Aging and Dementia: A Questionnaire Study. *Memory*, 8, 311-321.

In order to understand why people make memory mistakes, we need to find out about the kinds of mistakes people make, and how often they are made in normal everyday life. We would like you to tell us how often these kind of things happen to you. Please indicate by ticking the appropriate box.

Please make sure you answer all of the questions on both sides of the sheet even if they don't seem entirely applicable to your situation.

Please provide the following details about yourself.	Age _____	Male/Female _____
How many year of formal education have you had?	_____	
Have you suffered from brain or head injury resulting in hospitalisation (Y/N)	_____	
Please give brief details _____		

Please answer all of the questions as accurately as possible.

	Very Often	Quite Often	Sometimes	Rarely	Never
Do you decide to do something in a few minutes' time and then forget to do it?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Do you fail to recognise a place you have visited before?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Do you fail to do something you were supposed to do a few minutes later even though it's there in front of you, like take a pill or turn off the kettle?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

	Very Often	Quite Often	Sometimes	Rarely	Never
Do you forget something that you were told a few minutes before?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Do you forget appointments if you are not prompted by someone else or by a reminder such as a calendar or diary?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Do you fail to recognise a character in a radio or television show from scene to scene?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Do you forget to buy something you planned to buy, like a birthday card, even when you see the shop?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Do you fail to recall things that have happened to you in the last few days?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Do you repeat the same story to the same person on different occasions?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Do you intend to take something with you, before leaving a room or going out, but minutes later leave it behind, even though it's there in front of you?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Do you mislay something that you have just put down, like a magazine or glasses?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Do you fail to mention or give something to a visitor that you were asked to pass on?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Do you look at something without realising you have seen it moments before?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
If you tried to contact a friend or relative who was out, would you forget to try again later?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Do you forget what you watched on television the previous day?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Do you forget to tell someone something you had meant to mention a few minutes ago?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Appendix S: Ethics Certificate of Approval for “Development of new MRI sequence” and Amendment Approval.

R&D OFFICE

Research Director Prof Kevin Davies
R&D Manager: Scott Harfield
Research Governance Officer: Linda Rudden

E-mail: scott.harfield@bsuh.nhs.uk
Linda.rudden@bsuh.nhs.uk

Tel: 01273 696955 ext 3538 / 7497



Research & Development Directorate
Royal Sussex County Hospital
Clinical Investigation & Research Unit
Eastern Road
Brighton
BN2 5BE

26/11/2009

Professor Paul Tofts
Brighton & Sussex Medical School
University of Sussex
Falmer
Brighton
BN1 9PS

Dear Professor Paul Tofts

Full Study Title: Quantitative MR in Healthy Volunteers
R&D Ref No. : 09/156/TOF

I am writing to inform you that you have Research Governance approval to proceed with the above named project. This letter acknowledges that you have all the necessary internal and external regulatory approvals. The sites covered by this approval include:

- University of Sussex

Conditions of Approval

The approval covers the period stated in the Research Ethics Committee (REC) application and will be extended in line with any amendments agreed by the REC. Research must commence within 12 months of the issue date of this letter. Any delay beyond this may require a new review of the project resources.

Please note this approval does not apply to the earlier request to include MRI examinations of the eye. A separate application and information sheet should be submitted for this project, as the intervention requires the administration of an anaesthetic.

Amendments

Project amendment details dated after the issue of this approval letter should be emailed to the R&D Office for formal approval.

ICH-GCP Monitoring

The Medical School has a duty to ensure that all research is conducted in accordance with the Research Governance Framework and to ICH-GCP standards. The R&D Department will take responsibility for the ongoing monitoring of the study and reporting of any adverse events. In order to ensure compliance the department undertakes random audits. If your project is selected you will be given 4 weeks notice to prepare all documentation for inspection.

I wish you luck with your project and would be grateful if you could inform me when the project is complete or due to be closed on this site.

BSMS Research Governance & Ethics Committee (RGEC)

Chair: Professor Kevin Davies
 Deputy Chair: Professor Bobbie Farsides
 Secretary: Miss Caroline Brooks
 Tel: 01273 872855 c.e.brooks@bsms.ac.uk
 Applications and general enquiries: rgec@bsms.ac.uk



28/08/2015

Brighton and Sussex Medical School
 Medical Teaching Building
 University of Sussex
 Falmer
 Brighton
 BN1 9PX

Professor Mara Cercignani
 Clinical Imaging Sciences Centre (CISC)
 Brighton and Sussex Medical School
 University of Sussex, Falmer
 Brighton
 BN1 9RR

Dear Professor Cercignani

Full Study Title: Quantitative MR in Healthy Volunteers
R&D Ref No. : 09/156/TOF
Amendment No. : 3

I am writing to inform you that you have BSMS Research Governance and Ethics Committee (RGEC) approval to proceed with the above named project. This letter acknowledges that you have submitted evidence of all the necessary internal and external regulatory approvals in relation to this amendment. This approval also acknowledges all previous amendments.

The documents reviewed for this approval were:

Document	Version	Date
Request for Amendment Form – request for the following:	N/A	N/A
<ul style="list-style-type: none"> INCREASE IN SCANNING SESSION TIME: Currently the information sheet informs participants that the scan will last no longer than 60 minutes. However, we would like to increase the maximum total scan time to 90 minutes. This will require a change to the information sheet. It should be noted that this is a development project and the vast majority of scans will continue to take less than 1 hour. If the scan session lasts longer than an hour we will offer the participants a break. RESEARCH TEAM CHANGES: Dr Marica Cutajar is no longer involved in physics scanning at CISC and has been removed. Dr Samira Bouyagoub has joined the CISC physics team and has been added to this project. 		
Information Sheet	5	19/08/15
Consent Form	5	19/08/15

Conditions of Approval

The approval covers the period stated in the Research Governance and Ethics Committee (RGEC) application and will be extended in line with any amendments agreed by the RGEC. Research must commence within 12 months of the issue date of this letter. Any delay beyond this may require a new review of the project resources.

Amendments

Further project amendment details dated after the issue of this approval letter should be emailed to the Research Governance and Ethics Committee (RGEC) for formal approval.

Monitoring

The Medical School has a duty to ensure that all research is conducted in accordance with the University's Research Governance Code of Practice. In order to ensure compliance the department undertakes random

audits. If your project is selected for audit you will be given 4 weeks notice to prepare all documentation for inspection.

It is your responsibility to inform me in the event of early termination of the project or if you fail to complete the work.

I wish you luck with your project.

Yours sincerely

A handwritten signature in dark ink, appearing to read 'Kevin Davies', with a long horizontal flourish extending to the right.

Professor Kevin Davies
Chair of the BSMS Research Governance and Ethics Committee

Appendix T: Participant Information Sheet for “Development of new MRI sequence” pilot study.



Development of New MRI Sequences

Dear Participant

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

1. What is the purpose of the study?

The purpose is to develop and assess the performance of quantitative MRI techniques implemented on our scanner in healthy people. It is important that the quantities we measure on the scanner are accurate and reproducible and, in order to test this and develop data analysis methods, we need to perform our imaging techniques on a group of healthy volunteers.

2. Why have I been chosen?

You have been chosen because you have expressed an interest in helping out with our research project.

3. Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

4. What will happen to me if I take part?

You will undergo a MRI scan that lasts no longer than 90 minutes.

5. What do I have to do?

You will need to attend the Clinical Imaging Sciences Centre at the University of Sussex. Here you will be asked to fill in a safety questionnaire to confirm your suitability for the scan. You will then be required to lie in the MRI scanner for up to 90 minutes. Normally, you will be asked to relax and do nothing, although, on occasion, you may be asked to perform simple tasks, such as looking at an image or listening to a sound and asked to respond via a button box. You will be notified in advance if any tasks are planned. If your scan lasts longer than 60 minutes you will be given the opportunity to come out of the scanner for a break. Remember you can request a break or leave the study at any time.

6. What are the side effects of any treatment received when taking part?

The technique has been used for over 20 years in medicine and every year approximately 10 million people are scanned worldwide. There are no known side effects and MRI causes no pain or damage.

7. What are the other possible disadvantages and risks of taking part?

There are no disadvantages unless you are afraid of small spaces or loud noises. However occasionally unexpected findings are revealed by imaging. In this instance we will advise you to contact your GP who can arrange for further tests. It is important to note that the images are not being obtained for diagnosis. Therefore, you should not volunteer for the study as an alternative to seeking medical attention.

8. What are the possible benefits of taking part?

There are no immediate benefits in taking part, however you will be helping the development and testing of new MRI methods that will benefit many clinical projects.

9. What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. Please contact the researchers in the first instance (contact details in section 11) 10. Will my taking part in the study be kept confidential? Yes. All the information about your participation in this study will be kept confidential. The details are included in Part 2. 11.

Contact Details:

Professor Mara Cercignani, CISC, Brighton and Sussex Medical School, Brighton, BN1 9RR (m.cercignani@bsms.ac.uk). Tel: 01273 877879 Dr Nicholas Dowell, CISC, Brighton and Sussex Medical School, Brighton, BN1 9RR (n.g.dowell@bsms.ac.uk). Tel: 01273 876770 Dr Samira Bouyagoub, CISC, Brighton and Sussex Medical School, Brighton, BN1 9RR (s.bouyagoub@bsms.ac.uk). Tel: 01273 876783 Ludovico Minati, CISC, Brighton and Sussex Medical School, Brighton, BN1 9RR (lminati@ieee.org). Tel: 01273 678182

Part 2

1. What can I expect in the MRI scanner?

The MRI examination is performed in a special room that houses the MR system or “scanner”. The scanner consists of a circular magnetic tunnel which contains the radio coils. During your scan you will lie on a padded bed, which will move slowly into the scanner.

In preparation for the MRI examination, you will be asked to wear headphones or earplugs to protect your hearing as the scanner produces loud noises. These loud noises are normal and should not worry you. A number of scans will be taken with a pause in between so do not be alarmed if the scanner goes quiet. The most important thing is to relax and try to keep still. It is not dangerous if you move, but the resulting pictures may be blurred. Some minor movement of your body is possible between the scans. The radiographer will be able to hear and see you throughout the session and you will be provided



with a call button to alert them if you have any concerns. The whole session will take no longer than 90 minutes, but you will be offered a break after 60 minutes. You can request a break in scanning (or withdraw) at any time.

2. Complaints

If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions (contact details are in part 1, section 11).

3. Harm

In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against the Brighton and Sussex Medical School, but you may have to pay your legal costs.

4. Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. All MRI images are anonymized and do not contain participant names.

5. What will happen to my MR Images?

The results gained during this experiment will be confidential and your scans will be stored in an anonymous form. The results will be used to assess our improvements, and the accuracy and reproducibility of these scans.

6. What will happen to the results of the research study?

The results of the research study will be written up and published in a scientific journal.

7. Who is organising and funding the research?

The research is funded by CISC and BSMS.

8. Who has reviewed the study?

This study was given a favourable ethical opinion by the Research Governance Ethics Committee: Research & Development Directorate, Royal Sussex County Hospital, Clinical Investigation & Research Unit, Eastern Road, Brighton, BN2 5BE.

Thank you for taking the time to read this information sheet.

Appendix U: Consent Form for “Development of new MRI sequence” pilot study.

**CONSENT FORM****Title of Project: Development of New MRI Sequences****Name of Researchers:**

Prof Mara Cercignani
Dr Nicholas Dowell
Dr Samira Bouyagoub
Ludovico Minati, CEng CPhys CSci MIPeM

Please initial
box

I confirm that I have read and understand the information sheet dated August 2015 (Version 5) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

☐

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

☐

I agree that my images may be used for educational purposes, technical development and related publications on the understanding that they will be fully anonymized before being used in such a way.

☐

I agree to my GP being informed of my participation in the study, if necessary.

☐

I agree to take part in the above study.

☐

Name of Participant_____
Date_____
Signature_____
Name of Person taking consent
(if different from researcher)_____
Date_____
Signature_____
Researcher_____
Date_____
Signature

When completed, 1 for participant; 1 for researcher site file.

Development of New MRI Sequences
 Version 5
 August 2015

