The relationship between early Alzheimer’s Disease, Apolipoprotein E genotyping & Hippocampal MRI Volumes

Bernard Anthony Walsh

Thesis submission for the degree of Master of Philosophy (Clinical Epidemiology), University of Newcastle, Australia.

February 2014
Declarations:

Statement of Originality:

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

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**Statement of Collaboration:**

I hereby certify that the work embodied in this thesis has been done in collaboration with another researcher, Dr Stuart Slater, senior radiologist, Hunter Imaging Group, Newcastle Australia, whose input involved performing all cerebral MRI scanning and associated medial temporal lobe volume estimations conducted on the research subjects in this thesis.

Signed

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Bernard Anthony Walsh

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**Statement of Authorship:**

I hereby certify that the work embodied in this thesis contains published work of which I am a joint author. As part of thesis I provide the written statement, endorsed by my principal supervisor Professor Balakrishnan Nair, School of Medicine and Public Health, University of Newcastle, that I was the primary contributor and author of these publications.

Signed

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Bernard Anthony Walsh
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Associate Professor Patrick McElduff, senior statistician, University of Newcastle

All of the study participants who volunteered to be part of this research

Hunter New England Health Service
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List of Abbreviations:

Amnesic Mild Cognitive Impairment Syndrome  Amnesic MCI
ALlele FREquency Database (US Nat. Science Foundation)  ALFRED
Alzheimer’s Disease  AD
One Way Analysis of Variance  ANOVA
Amyloid Precursor Protein  APP
Apolipoprotein E  Apo E
Apolipoprotein E epsilon 2 allele  ApoE ε2
Apolipoprotein E epsilon 3 allele  ApoE ε3
Apolipoprotein E epsilon 4 allele  ApoE ε4
Apolipoprotein E2 protein isoform (coded by ApoE ε2 allele)  Apo E2
Apolipoprotein E2 protein isoform (coded by ApoE ε3 allele)  Apo E3
Apolipoprotein E2 protein isoform (coded by ApoE ε4 allele)  Apo E4
Cerebral Spinal Fluid  CSF
Clinical Dementia Rating scale  CDR
FluoroDeoxyGlucose  FDG
Medial Temporal Lobe (of the cerebrum)  MTL
Magnetic Resonance Imaging  MRI
Dementia of the Alzheimer’s Type  DAT
Mini Mental State Examination  MMSE
Positron Emission Tomography  PET
Pittsburgh Compound-B          PIB
Relative Ratio                RR
Single Photon Emission Computerised Tomography  SPECT
Tau family of intraneuronal proteins   Tau
Vascular Dementia              VasD
Abstract:
This thesis attempts to improve the clinical probability of correctly staging the degree of Alzheimer’s Disease (AD) neuropathology in an individual presenting with the syndrome of early Dementia, by exploring the relationship between the known subtypes of the cerebral protein Apolipoprotein E (ApoE) and the extent of AD neuropathology in such persons, using the surrogate of cerebral MRI volume loss within the Medial Temporal Lobe (MTL) regions as the maker of severity of AD neuropathology.

In this thesis Early Dementia is defined as “CDR 1.0” using the Clinical Dementia Rating scale (CDR), this being the classification in most common use by Dementia clinicians. The research subjects were drawn from CDR 1.0 persons residing within the community (as opposed to more serve dementia persons typically needing to reside within assisted care programs and who typically have more advanced neurodegeneration). These subjects are the most likely group where improved definition of the degree of neurodegeneration within an early AD individual would be of most clinical use.

No demonstrable relationship between the ApoE ε4 allele and increasing MTL volume loss in early AD was found in the research studies of this thesis and hence the presence of one or more ε4 alleles cannot be used by the clinician to estimate pathological disease load within an equivalent degree of cognitive impairment in early AD.

One peer reviewed medical literature publication and one published abstract resulted from this thesis work.
Chapter 1: Introduction.

1.1 Overview

Dementia is defined as a clinical syndrome of progressive cognitive and functional changes due to one or more of a wide variety of underlying degenerative neuropathologies (e.g. Alzheimer’s Disease, Diffuse Lewy Body Disease, cerebral ischemia, Tauopathies, TDP-43 proteinopathies) (1). As many of these underlying neuropathologies remain essentially post mortem diagnoses and can be very uncertain during life, the Dementia clinician must currently rely on estimating clinical probabilities of such from the medical history, physical examination and investigations to propose a specific neuropathology (such as AD) in any single, living individual with Dementia. Furthermore these clinical probabilities must currently also be used by the clinician to chose subsequent therapeutic treatments – which vary markedly between the underlying neuropathologies and their efficacy rapidly diminishes if prescribed for the wrong neuropathology. The above situation is inherently inexact and carries with it the recurrent potential for both wrong diagnosis and wrong treatment for the person with Dementia.

There is lack of consensus regarding the utility of ApoE genotyping in the clinical management and prognostication of individuals with AD, where the ApoE e4 allele is reported to have multiple negative outcomes. As a cumulative and progressive neurodegenerative process, therapeutic intervention in the earliest stages of AD offers the most potential for preventing or slowing progression of the disease. The Clinical Dementia Rating Scale (CDR) (2) is both a useful and common tool for classification of dementia severity and is used by many AD clinicians but lacks the ability to predict the
prognosis, rate of decline, response to drug therapy or the onset of behavioral and psychotic symptoms.

This thesis attempts to improve the clinical probability of correctly staging the degree of AD neuropathology in an individual presenting with the syndrome of early Dementia, by exploring the relationship of the known subtypes of the cerebral protein Apolipoprotein E in such situations, using the surrogate of cerebral MRI volume loss in the MTL regions as the maker of severity of AD as the scientific literature (see below) suggests that Apo E genotyping and MRI volumetric measures independently have the potential to be leading candidates in identifying the underlying progressive neurodegenerative process occurring in any particular individual with Dementia. However further epidemiological work is required to relate ApoE genotype to in vivo early neuropathological changes seen in AD, one of which is MTL atrophy and identifying the strength of relationship between these two measures forms the rationale for this thesis.

Although the degree of hippocampal MRI volume loss in AD is commonly accepted as a marker of disease severity it remains expensive, unavailable or not tolerated by many patients. Examining the potential of the ApoE e4 allele as a simple, readily available substitute to MRI to infer the extent of hippocampal shrinkage has significant appeal, especially in the early stages of the disease.

Chapter 1 covers the topics of:
• the current inherent in-vivo difficulties of diagnosing AD within the clinical syndrome of Dementia, and hence the use of “Dementia of the Alzheimer’s Type” in-vivo to reflect this uncertainty.

• the Australian demographic profile of AD and its clinical course, including the Clinical Dementia Rating scale (CDR) and its development.

• the amyloid hypothesis for AD histopathology development, and areas of regional cortical destruction in AD via the Braak Classification System.

• the weakness of the current clinical grading system for dementia to reflect both the degree of underlying neurodegeneration in AD and how such is an explanation of the variability of medication response and rate of decline in early AD.

• the scientific background of the Apolipoprotein E gene and its alleles.

• the over-representation of the ApoE ε4 in AD groups when compared to standard community groups.

• the scientific background of cerebral MRI imaging techniques as they relate to Dementias.

• the emergence of multiple biomarkers for AD including MRI MTL volume measures.

• MRI MTL volume measures and their temporal relationships to AD neurodegeneration.
• this thesis’ rationale of attempting to correlate the degree of underlying AD based neurodegeneration to the presence or absence of the ApoE ε4 allele.

• A literature review of the relevant published works on the relationship of ApoE genotyping with MRI MTL volume measures on AD.

• the need to generate two inter-related hypotheses for this thesis.

1.2 Study rationale & scientific background

Being the clinical state of progressive cognitive and functional changes, Dementia is due to one (or more) neuropathologies (e.g. AD, Diffuse Lewy Body Disease, cerebral ischemia, Tauopathies, TDP-43 proteinopathies) (1). When examining the AD subgroup, the medical paradigm most commonly used proposes both the clinical syndrome of “Dementia of the Alzheimer’s Type” as outlined in the current Diagnostic and Statistical Manual of Mental Disorders (3) for individuals during life and the histopathological diagnosis of AD that can currently only be made on post mortem examination of the cerebrum (4). It is widely recognized that Dementia of the Alzheimer’s Type is not exact for underlying AD pathology and that this clinical label can be erroneously applied to quite different underlying neurodegenerative disease states (5) with the ensuing possibility of wrong treatment, prognosis and complications that such incorrect diagnostic labeling poses. These inherent in-vivo difficulties of diagnosing AD during life have driven the identification of multiple biomarkers for individuals suspected of developing AD (6) – of which MRI imaging is prominent (see below) and also the pre AD concept of Amnesic MCI (see below).
AD is the most common cause of Dementia in the Australian population with 110,000 Australians having AD in the 2009 (7). It is a terminal disease, where in a “natural history” study of 126 persons with untreated DAT, mean age 77.6y, the median survival was 5.3 years from enrolment (range 0.2 – 7.2) and 9.3 years from symptom onset with shorter survival times if the MMSE<18, wandering and falling were present (RR 2.1) or behavioural problems occurred (RR 1.4) (8). Subsequent use of the anti-dementia Cholinesterase Inhibitor drug group have slowed the functional and cognitive deterioration in AD (9) although slowing of annual mortality rates is unclear with this drug group (10, 11). The effect of Apo E allele profile is difficult to quantify although the presence of an ApoE ε4 allele is likely to have a negative effect on AD mortality rates (12).

Mild Cognitive Impairment syndrome (MCI) (13) is conceptualized as a clinically intermediate but progressive phase between normal cognitive function and Dementia with the subgroup of Amnesic MCI converting to Dementia of the Alzheimer’s Type (DAT) at an annual rate between 10 and 14% per year, with almost all Amnesic MCI persons who hold the ApoE ε4 allele converting to DAT within 5 years (14).

The clinical course of DAT itself has relatively predictable phases although the presence and severity of individual symptoms vary widely with the initial phase often being a fluctuating, subtle psychological one of depression, anxiety and panic episodes followed within a 12 to 24 months by persistent and progressive memory impairment, often with parallel, progressive loss of expressive speech skills. Mild disease is dominated by a
phase of behavioral and psychiatric symptoms including aggression, resistiveness, paranoia and delusions. Motor dysfunction dominates the terminal phase of DAT with progressive apraxia, gait disturbance, falls and abnormal swallowing/eating (15). The current lack of a quantifiable biomarker that correlates well with all these clinical stages of DAT has meant that DAT staging is based on history taking by a physician trained in dementia care, who then applies the Clinical Dementia Rating Scale (CDR) (2). Although a numerical form of the CDR has subsequently been proposed (16), this has not gained wide acceptance as the original purpose of the scale was to make primary use of the clinical skills of senior Dementia physicians when grading Dementia severity. This most common method of using the original form of the CDR was therefore applied in this thesis.

First recognized over 100 years ago by Dr Alois Alzheimer (17), the accumulation within the cerebral matrix of the two neurodegenerative proteins species, Aβ amyloid and Tau, is the commonly accepted histopathological hallmark of AD where on light microscopy Aβ amyloid forms large extracellular plaques, while various tau proteins form smaller intraneuronal aggregates (18). Although peri-plaque inflammation and peri-vascular accumulation of Aβ amyloid have more recently been suggested as extra findings, the main histopathological addition to these Aβ amyloid “plaques” and Tau protein neurofibrillary “tangles” of AD in the last 30 years is accompanying widespread synaptic loss and its close correlation with symptom progression in AD (19). Although the Aβ amyloid deposition in AD is fairly uniform throughout the entire cerebral cortex, neuronal death (and concurrent gray matter atrophy) is much more localized in early AD
and progresses in a typical pattern with hippocampi and entorhinal cortices bilaterally being the first structures to undergo this atrophy. This neuropathological staging of AD on post mortem histopathology is based on a well defined pattern of spread of tau neurofibrillary intracellular tangles, beginning in these MTL structures of the entorhinal cortex and the CA2 area of the hippocampus, then spreading to the cortical association areas of the parietal lobes, and only involving the primary sensory, motor and visual cortices much later in the disease course (4). These limbic system structures of the hippocampus and entorhinal cortex - where the areas of interest in the hippocampus involve the dentate gyrus and CA2 area - flow onto the output memory pathways that form the fornix which itself relays back to the hippocampal formation via the mammillary body (20).

Whilst tau neurofibrillary tangles correlate well with synaptic loss and neuronal death in AD (21), the more generalized Aβ amyloid deposition within the cerebral matrix is considered to develop first (22), with soluble oligomers of Aβ amyloid (both Aβ\textsubscript{1-40} amyloid Aβ\textsubscript{1-42} amyloid fibrils) being the main neurotoxic species (23). Aβ\textsubscript{1-40} amyloid Aβ\textsubscript{1-42} amyloid fibrils are produced by α-secretase and γ-secretase mediated proteolytic metabolism of the Amyloid Precursor Protein (APP), a ubiquitous transmembrane protein within the cerebral cortex (24). This work, plus the identification of multiple single gene abnormalities in the coding of these secretase proteins in the rare autosomal dominant forms of younger onset AD (25), has lead to the proposed Amyloid Hypothesis of AD neuropathology. This hypothesis states that disordered metabolism of APP and subsequent production of neurotoxic Aβ\textsubscript{1-40} amyloid and Aβ\textsubscript{1-42} amyloid fibrils, produce
amyloid plaques possibly decades before clinical dementia emerges, and that these amyloid products (in their soluble oligomer form) subsequently lead to neuronal death where at least some of the intraneuronal tau tangles formed are part of this cell destruction (26). That diffuse amyloid plaques not associated with major amounts of tau neurofibrillary tangles are found at autopsy in a significant minority of cognitively normal deceased elderly without evidence of clinical dementia (27-29) adds further weight to the temporal sequence of this Amyloid hypothesis for AD.

Apo E is the major apolipoprotein species within the brain, and is involved in the catabolism of triglyceride-containing lipoproteins and the transport of these, plus fat soluble vitamins and cholesterol(30). It is a 34-kDa protein mediating the binding of lipoproteins to the Low Density Lipoprotein (LDL) receptor (mainly on neuronal membranes) and as such plays a key role in the mobilization of cholesterol and phospholipids during neuronal membrane remodeling associated with synaptic plasticity and neuronal repair following injury(31).

There are three major isoforms of the Apo E protein (Apo E2, Apo E3, Apo E4) encoded by three alleles (ApoE ε2, ApoE ε3, ApoE ε4) of the Apo E gene, located on chromosome 19. These protein isoforms differ from each other by single amino acid substitutions only. However these substitutions appear to greatly effect the ability of Apo E to bind to the Aβ amyloid protein produced in AD and to subsequently produce an insoluble complex interfering with the normal metabolism of Aβ amyloid in affected individuals. The Apo E gene is 299 amino acids long consisting of four exons and three
introns, totaling 3597 base pair (32) and Table 1 identifies the allele amino acid substitutions that determine the subsequent protein isoforms.

**Table 1. Structural differences of the three allelic forms of the Apolipoprotein E gene**

<table>
<thead>
<tr>
<th>Amino acid substitutions In ApoE species</th>
<th>ε2 allele</th>
<th>ε3 allele</th>
<th>ε4 allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position 112</td>
<td>Cysteine</td>
<td>Cysteine</td>
<td>Arginine</td>
</tr>
<tr>
<td>Position 158</td>
<td>Cysteine</td>
<td>Arginine</td>
<td>Arginine</td>
</tr>
</tbody>
</table>

From 1994 onwards, there has been an expanding literature on the subtyping of Apo E proteins and its relationship to both the pathology and diagnosis of AD so that currently this genotyping is considered the most prominent genetic influence in sporadic AD (33).

Of the Apo E protein subtypes, Apo E4 binds tightest to Aβ amyloid protein in the cerebral cortex, producing a combined molecule too large to pass through the cerebral endothelium and into the blood for metabolism – hence the build up of Aβ amyloid around the cerebral vasculature to produce the picture of “Aβ amyloid angiopathy” that has recently become a major histopathological finding of AD (34) (along with amyloid plaques and Tau tangles, plus widespread neuronal death). This affinity of ApoE ε4 to bind tightly to Aβ Amyloid(35) suggests the ApoE ε4 allele risk factor status for AD is primarily through its action on impeding Aβ amyloid metabolism(36, 37). This increased deposition of Aβ amyloid within the brain is reflected by reduced amyloid concentrations in the CSF (38).
Figure 1. Immunofluorescence staining of the combined ApoE4 - Aβ Amyloid protein complex within the cerebral cortex of Alzheimer’s Disease (1)

Positivity for one or more ε4 alleles is linked to decreased acetylcholine neurotransmitter levels, increased synaptic pruning and impaired response to drug therapy in AD. Plastic neuronal remodeling in AD is also impaired if an Apo ε4 allele is present (39). The increasing risk of AD pathology in the presence of Apo E4 also displays a gene dose effect (40) identified through individuals with the rarer homozygous form (41) and this persists in both relatives of AD persons and in cognitively normal persons possessing the Apo E4 allele (42).
There is a marked over representation of the ApoE E4 gene in AD patients – the prevalence rising from approximately 14% in general populations to 60% in the AD population, suggesting that heterozygous ApoE E4 carriers are between 3 and 4 times more likely to develop AD than non E4 individuals, and homozygous Apo E4 carriers are between 10 and 12 times more likely to develop AD (40). ApoE E4 gene frequencies across general Caucasian populations are currently 14.9% (British), 13.8% (Poles) and 18.1% (Swedes) (43). Also, there is a protective effect with ApoE E2 gene and its ApoE ε2 allele, where persons possessing this gene have a lower lifetime risk of developing AD, possibly due to this allele’s poor binding affinity with Aβ Amyloid (44).

There are currently five major biomarkers of AD ( PET Amyloid imaging, CSF Aβ42 levels, CSF Tau levels, FDG PET imaging and MRI MTL volume measurements), all being dynamic biomarkers based on the amyloid cascade hypothesis. In this hypothesis, Aβ amyloid is initially deposited as plaques within the extracellular space of the cerebral matrix in cognitively normal persons, followed soon by Tau protein associated synaptic injury and neuronal loss, and then emerging MTL atrophy. This MTL atrophy is thought to occur around the time that clinical cognitive impairment is first able to be identified as the pre-dementia Mild Cognitive Impairment Syndrome before the formal onset of clinical dementia (6).

Currently, AD biomarkers are divided into those identifying Aβ amyloid accumulation and those identifying neuronal destruction. Table 2 is a summary of the conceptual timed
relationships between various biomarkers and these progressive stages of the amyloid hypothesis.

**TABLE 2. The five major biomarkers of Alzheimer’s Disease and their relationship to the Amyloid hypothesis stages (adapted from Jack et al (6))**

<table>
<thead>
<tr>
<th></th>
<th>Initial Aβ amyloid deposition</th>
<th>Neuronal death and synaptic Injury from amyloid toxicity</th>
<th>Focal cerebral atrophy (MTL) from neuronal death</th>
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<tr>
<td>CSF Aβ levels</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PET Amyloid Imaging</td>
<td></td>
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</tr>
<tr>
<td>CSF Tau levels</td>
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<td>✓</td>
<td></td>
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<tr>
<td>Structural MRI Imaging</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>PET FDG Imaging*</td>
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<td></td>
<td>✓</td>
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</table>

PET FDG imaging identifies patterns of lobar hypometabolism, which is considered a marker of cerebral atrophy.

CSF = CerebroSpinal Fluid. PET = Positron Emission tomography. FDG = FluoroDeoxyGlucose

Low CSF levels of the Aβ amyloid derivative subprotein “Aβ42 amyloid” are a useful AD biomarker as they correlate with AD found on autopsy (45, 46), reflecting the concept of amyloid protein trapping within the cerebral matrix as part of AD neuropathology (see above section on Aβ amyloid angiopathy). Unfortunately this measure is not quantitative below a “rule-in” cut off level (47).
PET Amyloid Imaging uses several ligands that are relatively specific for Aβ amyloid, to identify the presence of cerebral amyloid plaques. The main current ligand being “PIB” Pittsburgh Compound-B (48) but due to its short half life affecting availability for more general diagnostic use, $^{18}$F labeled compounds (i.e Fluorine 18 ligands) are becoming more common in PET Amyloid Imaging due to their easier use in peripheral diagnostic centers (49, 50). PET Amyloid Imaging correlates extremely well with the presence of post mortem AD findings (27). PET Amyloid Imaging is not currently quantifiable below a “rule-in” cut off level (27).

Elevated CSF Tau levels (both phosphorylated and total levels) (51) correlate with neocortical neurofibrillary AD pathology (52) but is relatively nonspecific, being a marker of neuronal damage in nonAD pathologies as well(53), although there is early work suggesting a variation in CSF Tau levels depending on the extent of dementia is present and could therefore be used as a marker of severity of cognitive impairment, if not neuronal destruction (54).

FDG PET imaging is conceptually quite different to PET Amyloid imaging and instead identifies cerebral lobar hypometabolism, which is considered a marker of lobar cerebral atrophy, itself a hallmark of the macroscopic changes in AD (18). This hypometabolism is based on measuring neuronal glucose metabolism via FDG, which is believed to reflect synaptic activity and neuronal death (55). Decreased glucose metabolism in the temporal and parietal lobes on FDG PET imaging correlates with the lobar atrophy of AD subsequently confirmed on autopsy (56).
Medial Temporal Lobe (MTL) neural loss and associated atrophy is both an early AD neuropathological change and infers the relative degree of cortical destruction, especially in these early stages AD (4). This MTL atrophy is increasingly being able to be identified through MRI imaging technology (see below). Attempts to increase the specificity and sensitivity of in-vivo AD diagnosis by combining multiple biomarkers continue to develop (54, 57-59).

In Table 3, the author attempts to conceptualize that of these five AD biomarkers, only MRI measures of MTL atrophy currently provides quantitative measures of the degree of neuronal destruction in AD— a key reason behind the choice of MRI as the biomarker used in this thesis’ research.

TABLE 3. The five major biomarkers of Alzheimer’s Disease: Ability of each to correlate with the level of neuronal destruction in AD

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Not quantifiable below a “rule-in” level</th>
<th>Quantifiable below a “rule-in” level</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF Aβ levels</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>PET Amyloid Imaging</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>CSF Tau levels</td>
<td>✓</td>
<td>??</td>
</tr>
<tr>
<td>Structural MRI Imaging</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>PET FDG Imaging</td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>
Within AD diagnostic paradigms, MRI structural imaging has changed from being used to exclude non AD pathologies as a cause of cognitive decline (e.g. brain tumour) to now being used to “rule in” AD by measuring the volumes of the medial temporal lobe structures (i.e. hippocampus and entorhinal cortex) and identifying shrinkage of these cerebral cortex structures when compared to non AD persons (60-63). Higher atrophy rates of the entorhinal cortex than hippocampus in AD have been proposed (64) although the technical difficulties of reliably measuring the entorhinal structure remain formidable (65).

In the universally accepted pathological staging of AD post mortem i.e. the “Braak Staging” (18), Tau based neurofibrillary tangles and associated neuronal death are initially distributed in the medial temporal and the medial parietal lobes and produce focal atrophy of these structures (including the hippocampi). MRI structural imaging can provide volumetric measures of atrophy in these areas and the severity of hippocampal atrophy especially correlates with this Braak staging in AD persons at autopsy (66-68). MRI volumetric studies of focal cerebral atrophy in AD persons as correlate well tau neurofibrillary tangle distribution at subsequent autopsy (69). MRI measures of hippocampal volume loss over time in AD persons has been studied for both predictors and rates (70). Rate of this volume loss are significantly greater amongst ApoE E4 allele carriers and with increasing age, both in AD and in MCI Syndrome (70-73).

MRI brain imaging in AD is not limited to volume measures. Other, but presently only experimental, MRI diagnostic techniques in AD includes MRI diffusion imaging (where
white matter tracts patterns alter as the neuronal destruction of AD changes water diffusion across the cell membrane) (74), MRI perfusion imaging (to confirm the bilateral temporoparietal hypoperfusion patterns of AD well recognized previously in FDG PET or the older SPECT scanning) (75, 76), and functional MR imaging. Functional MR imaging is both performed in the resting state and also during task based demands and shows decreased activation in persons with AD (77). However all of these listed MRI diagnostic techniques remain experimental and have not entered routine clinical practice.

1.3 Aims
The aim of this thesis is to improve the clinical probability of more correctly staging the degree of Alzheimer’s Disease (AD) neuropathology within an individual presenting with the syndrome of Early Dementia, by exploring the relationship between the known subtypes of the cerebral protein Apolipoprotein E (ApoE) and the extent of AD neuropathology in such persons, using the surrogate of cerebral MRI volume loss within the cerebral Medial Temporal Lobe (MTL) regions as the maker of severity of AD neuropathology.

1.4 Research hypotheses – Major and Minor.
This thesis’ two hypotheses are interrelated in that before the relationship between ApoE subgroups and MRI findings in early clinic-based AD persons could be examined (i.e. the major study) it appeared prudent to confirm in this community-dwelling group that indeed there was the same over-representation of the ApoE ε4 allele that would be expected if indeed these subjects really did have AD.
Major Study (88 subjects) = Research Hypothesis (1)

Regarding the relationship between the ApoE $\varepsilon$ 4 allele and medial temporal lobe MRI volumes in early Alzheimer’s Disease:

In newly presenting AD patients in the Hunter region of Australia, the Apo E4 allele subgroup is associated with smaller hippocampal and/or entorhinal cortex volumes as measured by MRI volumetric measurements when compared to the non Apo E4 allele subgroup (hence inferring more extensive medical temporal lobe destruction at diagnosis in this E4 subgroup).

Minor Study (520 subjects) = Research Hypothesis (2)

Regarding local population estimates of Apolipoprotein E genotypes:

The incidence of the Apo E4 genotype subgroup in early AD patients presenting to an Australian community based Memory Clinic confirms an over-representation of the Apo E4 allele and protein in such community dwelling early AD persons compared to general population norms.
Chapter 2. Literature review

This section deals specifically with the published literature of MRI Medial Temporal Lobe volume measurements and their relationships to Apolipoprotein E e4 allele in AD only. “Section 1.2 scientific Background” examines the broader literature pertaining to this thesis.

Medline search using the MesH headings (plus article index cross referencing) of “Apolipoprotein E4 AND Alzheimer’s Disease AND Magnetic Resonance Imaging” noted 111 potential articles which were screened to identify 14 articles linking ApoE genotype and MRI changes. However five articles were rejected due to lack of apoE data and/or MRI measures of the MTL structures. So currently, ten studies attempt to examine a link between ApoE genotype and the structural MRI changes in AD persons. Table 3 (71-78).

Table 4. The ten studies examining links between Apolipoprotein E genotype and the structural Magnetic Resonance Imaging changes in Alzheimer’s Disease persons

<table>
<thead>
<tr>
<th>Citation</th>
<th>Baker (78)</th>
<th>Geroldi (79)</th>
<th>Geroldi (80)</th>
<th>Hashimoto (81)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study type</td>
<td>Observational, cross-sectional</td>
<td>Observational, cross-sectional</td>
<td>Observational, cross-sectional</td>
<td>Observational, cross-sectional</td>
</tr>
<tr>
<td>Population</td>
<td>25 AD, 24 VasD, 24 Lewy-body</td>
<td>28 AD (14 e4-, 9 e4+, 5 e4:E4)</td>
<td>28 AD (14 e4-, 9 e4+, 5 e4:e4)</td>
<td>138 AD (46 e4-, 46 e4+, 46e4:e4)</td>
</tr>
<tr>
<td>Comparator</td>
<td>Visual rating medial temporal lobe</td>
<td>Manual tracing of MRI volumes</td>
<td>“Asymmetry Index percentage” of MRI volumes hippocampus, amygdale, whole</td>
<td>MR...</td>
</tr>
<tr>
<td><em><strong>Outcome</strong></em></td>
<td>No association between ApoE &amp; MTL/WML</td>
<td>Smaller MTL volumes with increasing e4 allele number</td>
<td>Dose dependent effect of e4 allele on hippocampal volume asymmetry in AD group</td>
<td>Smaller volumes of hippocampus &amp; amygdala with increasing e4 allele number</td>
</tr>
<tr>
<td>----------------</td>
<td>---------------------------------------</td>
<td>-----------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td><em><strong>Variables adjusted for</strong></em></td>
<td>Age, Sex</td>
<td>Nil</td>
<td>Handiness (all AD were Right handed)</td>
<td>Age, sex, educational level, disease duration, cognitive severity</td>
</tr>
<tr>
<td><em><strong>Sample size</strong></em></td>
<td>71 total (24 AD)</td>
<td>58 total (28 AD)</td>
<td>58 total (28 AD)</td>
<td>138 AD</td>
</tr>
<tr>
<td><em><strong>STATS</strong></em></td>
<td>Analysis of variance (ANOVA) Mean volume scores Vs E4 allele status P&lt;0.05</td>
<td>Mean MRI Volumes Vs E4 allele groups were assessed with the t-test or analysis of variance (ANOVA) for independent samples</td>
<td>Linear regression model of volumes Vs 3 level allele variable (AD -/-, AD e4/-, AD e4/e4)</td>
<td>The relationship between the number of E4 alleles and the volume of each structure was tested using the Spearman rank correlation coefficients</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Citation</strong></th>
<th><strong>Jack (82)</strong></th>
<th><strong>Juottonen (83)</strong></th>
<th><strong>Lethovirta (84)</strong></th>
<th><strong>Mueller (85)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study type</strong></td>
<td>Observational, cross-sectional</td>
<td>Observational, cross-sectional</td>
<td>Observational, cross-sectional</td>
<td>Observational, cross-sectional</td>
</tr>
<tr>
<td><strong>Population</strong></td>
<td>49 AD 13 MCI 125 controls</td>
<td>27 AD 16 e4 positive 11 e4 negative 31 controls</td>
<td>26 AD 12 e4- 9 e4 + 5 e4:e4 16 controls</td>
<td>15 AD 5 e4 negative 10 e4 positive 66 controls</td>
</tr>
<tr>
<td><strong>Comparator</strong></td>
<td>MRI hippocampal</td>
<td>MRI Entorhinal cortex volumes</td>
<td>MRI volumes hippocampus,</td>
<td>MRI volumes, hippocampus</td>
</tr>
<tr>
<td>Outcome</td>
<td>No difference in hippocampal volumes across genotypes in AD (but analysis included MCI group)</td>
<td>Smaller Entorhinal cortex volume with e4 allele in AD (especially in females)</td>
<td>Smaller hippocampus &amp; amygdala volumes with increasing e4 allele number</td>
<td>Smaller CA3 and dentate gyrus (i.e. hippocampus subfields) volumes in e4 positive controls and AD</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Variables adjusted</td>
<td>Age, educational level</td>
<td>Age &amp; sex matched</td>
<td>Age, age of onset, disease severity, sex matched</td>
<td>Nil</td>
</tr>
<tr>
<td>Sample size</td>
<td>187 (49 AD)</td>
<td>58 (27 AD)</td>
<td>42 (26 AD)</td>
<td>88 (15 AD)</td>
</tr>
<tr>
<td>Statistical methods</td>
<td>Two sample tests for comparison between the two groups – Spearman rank correlations as the volumes were ordinal.</td>
<td>Multivariate analysis of variance (MANOVA) for comparisons between group means</td>
<td>Analysis of variance for independent sample (ANOVA) to detect differences in group means: volumes Vs apoE status</td>
<td>Mann-Whitney test Volumes Vs ApoE status</td>
</tr>
</tbody>
</table>

**Citation**

**Tanaka (86)**

<table>
<thead>
<tr>
<th>Study type</th>
<th>Observational, cross-sectional</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>34 AD - all institutionalized: 15 CDR 2.0 19 CDR 3.0 22 controls</td>
</tr>
<tr>
<td>Comparator</td>
<td>Whole brain &amp; ventricular volumes via CT, New temporal</td>
</tr>
<tr>
<td>Outcome</td>
<td>Lower ITI &amp; IMTI with e4, higher IMTI with e4</td>
</tr>
<tr>
<td>---------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Variables adjusted</td>
<td>Age, sex</td>
</tr>
<tr>
<td>Sample size</td>
<td>56 (34 AD)</td>
</tr>
<tr>
<td>Statistical Methods</td>
<td>Two way analysis of variance (ANOVA) Volumes Vs ApoE status</td>
</tr>
</tbody>
</table>

The number of AD persons recruited for each of these studies were all below 50 except for one study of 138 persons, with an average study size of only 41. The clinical severity level of AD (i.e. CDR 1.0, 2.0 or 3.0) either was not stated or trended towards the more severe stages, with significant recruitment of persons being from institutions. It appeared few persons were actually newly diagnosed, community-dwelling AD persons.

MRI volumetric techniques were ill defined in most studies, with the majority not clearing stating whether manual or computer generated measures were used. 4 of 9 studies did not distinguish between single and double ApoE e4 allele carriers. The 5 studies that did so, had distributions of homozygous ApoE e4 individuals approximately what would be expected from published community gene frequencies for apoE e4.

7 of the 9 studies confirmed a trend towards smaller hippocampal and/or amygdala volumes with the ApoE e4 allele and 3 out of 9 proposed a dose dependent effect where
homozygous for ApoE e4 allele persons had the smallest volumes. Most studies adjusted for the variables of age and sex.

3.1 Overview and study designs.

Both the major and minor study were cross sectional and observational in design, with the 88 subjects of the major study being a subgroup of the 520 persons of the minor study.

3.2 Ethics committee application

On the 30th October 2008 The Hunter New England Human Research Ethics Committee gave written approval for the author’s submitted protocol – see appendices – HNEHREC reference number No:08/10/15/5/05, NSW HREC reference No: 08/HNE/336. This Human Research Ethics Committee is constituted and operates in accordance with the National Health and Medical Research Council’s “National Statement on Ethical Conduct in Human Research (2007)(National Statement) and the CPMP/ICH Note for guidance on Good Clinical Practice.

3.3 Studies populations (minor and major studies)

The study population comprised community dwelling individuals attending three major outpatient memory clinics within the Newcastle region of NSW state, Australia

- The Geriatric Medicine & Cognitive Impairment Clinic, Charlestown
- The Memory Disorders Clinic, Rankin Park Hospital (HNEHS)
- The Geriatric Medicine Clinic, Wallsend Campus (HNEHS)
These clinics cover a large section of Alzheimer’s Disease (AD) presenting in the Newcastle geographical region. There were no financial costs to the patient or state health service for this study.

### 3.4 Sample size estimates (minor and major studies)

For the hypothesis on Apo E4 genotype frequencies in AD and using published data suggesting the gene frequency in the normal population of heterozygous E4 being 14% and homozygous being 2% (i.e. 16% allele prevalence), a study size of 200 would be needed to demonstrate an E4 allele frequency of 50% in the AD group with confidence intervals of +/- 7%. A total of 520 subjects subsequently were analyzed. For the hypothesis involving the E4 allele and MRI volumes in early stage AD, previous published studies have used up to 30 participants to suggest a positive correlation. A total of 88 subjects were subsequently recruited in this thesis’ study.

### 3.5 Patient selection, recruitment & consent plus data storage protocols (minor and major studies)

88 patients meeting the criteria for Mild Dementia using the Clinical Dementia Rating scale (CDR 1.0) and meeting the DSM IV criteria for probable Alzheimer’s Disease agreed to be entered into the major study, with the information and consent forms being provided (see appendices). The responder rate for these eighty-eight study subjects was 100%. At entry to the study, no subject meet the criteria for Temporal Lobe Epilepsy, Herpes Simplex Encephalitis or Temporal Lobe space occupying tumor – these being the three main disease states associated with temporal lobe swelling.
Retrospective examination of the above clinics’ consecutive patient medical records identified 520 patients with ApoE genotyping and meeting the DSM IV criteria for probable Alzheimer’s Disease, for the minor study. Data storage protocol dictates that all original data be kept in a unique locked storage cabinet within the author’s locked office located on the grounds of the John Hunter Hospital complex, at the Rankin Park Unit.

3.6 Outcome measures

Apo E genotyping was collected on all participants as single, nonfasting genetic blood test performed on peripheral blood lymphocytes.

The senior radiologist (Dr Stuart Slater) performed bilateral hippocampal volumetric studies on the MRI scans of the study patients ex gratia and has secured the special software program needed for this task. He was blinded to the patient’s memory clinic assessment results.

The author, a Senior Geriatrician within HNEHealth Service (Dr Bernard Walsh) performed the memory assessment and cognitive testing to confirm diagnosis of AD and classification of severity using the Clinical Dementia Rating Scale(2).

3.7 MRI image acquisition
All patients were imaged with 1.5T (Signa Excite General Electric Medical Systems, Milwaukee, WI. All volume measurements were derived from T1 weighted spoiled gradient-recalled echo sequence with 25 degree flip angle, 1.6mm slice thickness, 22x19cm field of view, TR 27, TE 9 and 320x224 matrix and 1.0 NWX. All measurements were made by manual tracing of the coronal sections of the hippocampus and the ERC and the volume measurements were calculated using the software provided by General Electric Medical Systems. The hippocampi were measured from the level of the mammillary body to the posterior margin of the quadrigeminal plate on the coronal images. The ERC measurements were made on a single slice at the level of the mammillary body.

3.8 Statistical analysis (Major Study)

The independent variables were:

1) ApoE4 status (nil, 1 positive allele, 2 positive alleles)

2) MRI volume of the right hippocampus

3) MRI volume of the left hippocampus

The measures of the Entorhinal cortices (ERC) were excluded from the study analysis due to the lack of technically reliable measures of this structure, as was noted in previous imaging work on this brain structure (64). The statistical software package used was STATA version 11 (copyright 2009 StataCorp LP, College Station, Texas, USA)

There were no significant differences in the distribution of sex and baseline cognitive scores between the groups defined by ApoE status.
As was consistent with previous literature, the expected difference in the age of onset of the homozygous E4 group compared to the single E4 and non E4 groups was demonstrated.

Assumptions of normality covering the spread of data were made for:

1) Age distribution of diseased cohort
2) Sex distribution of diseased cohort
3) Baseline Minimental and ClockFace cognitive test results

3.8 Statistical analysis (Minor Study)

Linear regression modelling was used with ApoE genotype as predictor (Double E4, Single E4, No E4) and age of onset as the outcome measure. Further linear regression modelling was performed with the ApoE genotype as predictor (Double E4, single E4, No E4) and mean MMSE at diagnosis as the outcome measure. Using the Chi Squared test for association ($x^2 = 20.5$, $df = 3$, $p > 0.01$).
Chapter 4. Results.

Chapter 4.a. Results (Major Study).

Table 5 provides the basic demographics of the 3 genotype groups. Participants were predominantly non-smokers and had mean MMSE scores between 22 and 24. There was a predominance of younger and female participants in the e4/e4 group. This non-normal distribution of age-of-onset across the three genotype groups is consistent with published literature that e4/e4 individuals have a 5 to 10 year earlier onset of Alzheimer’s compared to the other genotype groups (Graph 1).

One way ANOVA (analysis of variance) was applied to test for any difference in hippocampal volume (left then right cortex) across the three allele groups. No differences between the mean volume measurements across the individual genotype groups were demonstrated (Graph 2) (left hippocampal volumes: p>0.86, right hippocampal volume: p>0.68). Given the imbalance in age and gender between the 3 groups, we also demonstrated that this lack of association between genotype and hippocampal volume remained when adjusted for age and sex using linear regression (Table 6).
### Table 5: Clinical characteristics of study population (Eighty eight Clinical Dementia Rating 1.0 patients)

<table>
<thead>
<tr>
<th></th>
<th>No e4 (31)</th>
<th>Single e4 (46)</th>
<th>Double e4 (11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female (%)</td>
<td>14 (45.2%)</td>
<td>26 (56.5%)</td>
<td>10 (91%)</td>
</tr>
<tr>
<td>Age at Diagnosis (mean, years)</td>
<td>77.4</td>
<td>77.3</td>
<td>71.8</td>
</tr>
<tr>
<td>MMSE (-/30)</td>
<td>22.5</td>
<td>22.3</td>
<td>24.1</td>
</tr>
<tr>
<td>Non-Smoker (%)</td>
<td>29 (93.5%)</td>
<td>43 (93.5%)</td>
<td>11 (100%)</td>
</tr>
</tbody>
</table>

### Table 6: Multiple Linear regression of hippocampal volume

<table>
<thead>
<tr>
<th></th>
<th>Left hippocampal volume</th>
<th>Right Hippocampal volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>p-value</td>
</tr>
<tr>
<td>apoE (1 allele)</td>
<td>-0.05</td>
<td>0.60</td>
</tr>
<tr>
<td>apoE (2 alleles)</td>
<td>0.002</td>
<td>0.99</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>-0.02</td>
<td>0.001</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.13</td>
<td>0.13</td>
</tr>
</tbody>
</table>
Graph 1. Apolipoprotein E e4 count and Age-of-Diagnosis (nil, 1 and 2 e4 alleles) with the vertical axis being Density Estimations using age specific histograms, together with a Kernel Smoothing plotted line.
Graph 2: Left and Right Hippocampal volumes across ApoE e4 load (Nil,1,2)
Chapter 4.b. Results (Minor Study).

The demographics and cognitive tests results of the study group of 520 community dwelling CDR 1.0 subjects are detailed in Table 7, with 43.5% not possessing an ApoE e4 allele. The actual ApoE e4 allele frequency in this group of 520 persons is constructed in Table 8 and this allele frequency is then included in Table 9 where the comparative E4 frequency in study group of CDR 1.0 persons is compared to known, published general Caucasian populations data sets.

Table 7: Demographics and Results of study group of five hundred and twenty community dwelling Clinical Dementia Rating 1.0 subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Double E4</th>
<th>Single E4</th>
<th>No E4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (Total 520)</td>
<td>56 (10.8%)</td>
<td>238(45.8%)</td>
<td>226(43.5%)</td>
</tr>
<tr>
<td>Mean Age (y) At diagnosis</td>
<td>74.95(5.9)</td>
<td>78.42(6.6)</td>
<td>79.58(7.6)</td>
</tr>
<tr>
<td>Median Age (y)</td>
<td>64.45</td>
<td>77.45</td>
<td>67.7</td>
</tr>
<tr>
<td>Max. Age (y)</td>
<td>85.8</td>
<td>91.5</td>
<td>93.8</td>
</tr>
<tr>
<td>Min. Age (y)</td>
<td>57.5</td>
<td>52.7</td>
<td>54.2</td>
</tr>
<tr>
<td>Age Range (y)</td>
<td>28.3</td>
<td>38.8</td>
<td>39.6</td>
</tr>
<tr>
<td>Mean MMSE</td>
<td>21.03</td>
<td>21.46</td>
<td>21.18</td>
</tr>
<tr>
<td>at diagnosis (SD)</td>
<td>(4.3)</td>
<td>(3.29)</td>
<td>(3.84)</td>
</tr>
<tr>
<td>-----------------</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Median MMSE at diagnosis</td>
<td>19</td>
<td>22.5</td>
<td>25</td>
</tr>
<tr>
<td>Max. MMSE at diagnosis</td>
<td>30</td>
<td>29</td>
<td>28</td>
</tr>
<tr>
<td>Min. MMSE at diagnosis</td>
<td>12</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>% females</td>
<td>69.64%</td>
<td>57.14%</td>
<td>68%</td>
</tr>
<tr>
<td>% males</td>
<td>30.34%</td>
<td>42.86%</td>
<td>32%</td>
</tr>
<tr>
<td>F/M ratio</td>
<td>1.75</td>
<td>1.33</td>
<td>2.16</td>
</tr>
</tbody>
</table>

Table 8: Apolipoprotein E ε4 allele frequency in the study group of five hundred and twenty community dwelling Clinical Dementia Rating 1.0 subjects

<table>
<thead>
<tr>
<th>ApoE ε4 allele present</th>
<th>No ApoE ε4 allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>E4:E4 subjects</td>
<td>112 (i.e. 2 x 56 subjects)</td>
</tr>
<tr>
<td>E4:E3 or E4:E2 subjects</td>
<td>238</td>
</tr>
<tr>
<td>E3:E3 or E3:E2 subjects</td>
<td>0</td>
</tr>
<tr>
<td>Total allele count</td>
<td>350</td>
</tr>
<tr>
<td>Total allele frequency</td>
<td>33.7%</td>
</tr>
</tbody>
</table>
Table 9 – Comparative E4 frequency in study group of Clinical Dementia Rating 1.0 persons compared to known general Caucasian populations data

<table>
<thead>
<tr>
<th></th>
<th>ApoE ε4 allele Frequency</th>
<th>Sample size (alleles)</th>
<th>Sample size (subjects)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study group (CDR 1.0)</td>
<td>33.7%</td>
<td>1040</td>
<td>520</td>
</tr>
<tr>
<td>Poles *</td>
<td>13.8%</td>
<td>94</td>
<td>47</td>
</tr>
<tr>
<td>Swedes *</td>
<td>18.1%</td>
<td>182</td>
<td>91</td>
</tr>
<tr>
<td>British *</td>
<td>14.9%</td>
<td>316</td>
<td>158</td>
</tr>
</tbody>
</table>

*General populations data. Source: ALDRED. The ALlele FREquency Database (US National Science Foundation) (43)

The frequency of the ApoE4 allele in the study group of 520 Early AD persons (Table 8) is excessive when compared to known ApoE4 frequencies in nondemented, general populations of persons in multiple areas of the world possessing caucasian origins (Table 9).
Chapter 5. Discussion

Chapter 5.a. Discussion (Major Study).

In an attempt to improve clinical staging of the degree of AD neuropathology within the syndrome of Early Dementia, this study examined the relationship between the known subtypes of the cerebral protein ApoE and cerebral MRI volume loss within the cerebral MTL regions - as the maker of severity of AD neuropathology. This study relied on first demonstrating that the study group had a similar distribution of the three ApoE allele subgroups (e2, e3, e4) that would be reasonably expected in a group of AD persons – such being confirmed in the results of the Minor Study in this thesis (see below).

The study demonstrated no association between ApoE e4 status and hippocampal volumes in AD patients living in within the community and meeting the criteria for Early Dementia using the Clinical Dementia Rating Scale (i.e. CDR 1.0), so confirming that the presence of one or more e4 alleles cannot be used by the clinician to estimate pathological disease load within an equivalent degree of cognitive impairment in early AD rather than across the whole AD disease spectrum.

There were no significant differences in the distribution of gender and baseline cognitive scores between the groups defined by ApoE status. As is consistent with previous literature, the expected difference in the age of onset of the double e4 group compared to the single e4 and non e4 groups was demonstrated (40).
The strengths of this study are its unique community setting of early, well defined AD patients where the clinical utility of ApoE genotype would be of most benefit. The large sample size and consecutive recruitment of these community dwellers increases the study’s ability to reflect the true community AD ApoE e4 distribution. Also, all clinical assessments and disease severity staging were performed by a single, senior geriatrician applying standard AD grading (CDR) and diagnostic criteria (DSM IV-TR), with a single observer conducting all MRI volume measurements blinded to both ApoE genotype and clinical details.

As the results of this study rely on the published literature’s consensus that there is a correlation between AD pathological load and medial temporal lobe atrophy measured by MRI, it is appropriate to state that if this correlation was disproved in the future research, further studies where a different and as yet technically undefined measure of in vivo AD pathological load which is not reliant on MRI imaging, could be used to examine the possible association of the ApoE ε4 allele and early AD pathology, would then be required.
Chapter 5.b. Discussion (Minor Study).

In the study group of Early Dementia caucasian Australians (CDR 1.0) presenting to a community based Memory Disorders Clinic with a clinical diagnosis of AD, there was an over representation of the ApoE4 allele when compared to the frequencies of nondemented, caucasian population databases.

The increased ApoE4 frequency of 33.7% in the study group is compatible with what would be expected for AD subjects from the published literature (see chapter 1.2.) provides strong support that this study group has AD rather than other neurodegenerative disorders as a basis of their dementia.

Possession of the E4 allele did not lead to any lower MMSE at diagnosis in this CDR 1.0 study, suggesting that all of the group fell with the Early Dementia category and were appropriately homogenous in the level of cognitive impairment from their illness. As expected, the E4:E4 subgroup had a younger mean and median age of onset of their AD, with a smaller range of years in the group age of onset. This is consistent with the published literature (87).

6.1 Overview.

Two publications resulted from the research outlined in this thesis, reflecting the major and minor studies and their associated hypotheses.

6.2 Major Study publication


6.3 Minor Study publication

Apolipoprotein E genotype, age of onset and MMSE in mild Alzheimer's disease in caucasians within an Australian community based memory disorders clinic

Bernard Walsh

HNEHealth, New Lambton, NSW, Australia

520 (age range 52 to 94 years, 59.3% females) consecutive, community dwelling caucasians with mild Alzheimer's disease presenting to the Adult Cognitive Impairment Clinic, Newcastle, Australia in the years 2005, 2006 & 2007 were studied for ApoE genotype, age at diagnosis and MMSE. This clinic functions within a primary community setting with a defined catchment area and so appears more representative of AD persons and their community distribution than perhaps more tertiary institutions. Each subject meets the criteria for both "Probable Alzheimer's disease" via DSM IV and for "Mild Dementia" using the Clinical Dementia Rating Scale (CDR 1:0). The results demonstrated a similar excess of females across all subgroups. The ApoE4 allele frequency for the group was 56.4% comprising of 10.75% homozygous (E4:E4) and 45.65% heterozygous (single E4 allele). This is markedly in excess of the 13% frequency demonstrated in reference non-diseased populations. With increasing ApoE4 allele load, the study demonstrated a progressively younger mean age of onset (E4:E4 = 75y, Single E4 = 78.4y, NoE4 = 79.6y, E2 without E4 = 81.3y), a lower median MMSE score (E4:E4 = 19, Single E4 = 22.5, NoE4 = 25), and a smaller range of age of onset (E4:E4 = 28.3y, Single E4 = 38.8y, No E4 = 39.6y). When the subgroup of persons having an E2 allele (but no E4 allele) was compared to the E4:E4, single E4 and E3:E3 groupings, this E2 subgroup had the highest mean age of disease onset of any genotype.

Conclusion: Community living caucasians in Australia with Mild Alzheimer's Disease demonstrate a younger age of onset, a narrower range of age of onset and a lower mean MMSE at diagnosis with increasing ApoE4 allele load and this is consistent with the current literature on the interaction of ApoE4 and the amyloid protein of AD.
**ApoE genotype, age of onset and MMSE in mild Alzheimer’s Disease (CDR 1.0) Caucasians within an Australian community based, Memory Disorders Clinic**

**Method**

**Materials & Methods**: 520 (age range 52 to 94 years) consecutive community dwelling Caucasians presenting to a Community based Memory Disorders Clinic of Newcastle, Australia in the years 2005, 2006 & 2007 were examined for ApoE genotype, age at diagnosis and MMSE at diagnosis. ApoE genotype was identified via peripheral blood leukocytes and the diagnosis and MMSE at diagnosis. ApoE genotype was determined via polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis.

**Design**: Cross sectional, observational. The predictor is the ApoE genotype (as Double E4, Single E4, No E4 groups). The outcome measures are the age of onset and cognition (as measured by the Mini Mental State Examination) and age of onset as the outcome measure.

**Conclusion**

In the study group of mild Alzheimer’s Disease Caucasians (CDR 1.0) presenting to a community based Memory Disorders Clinic, the frequency of the ApoE4 allele in the group of mild AD persons (Table 2) is excessive when compared to ApoE4 frequencies in non-demented, general populations of persons in multiple areas of the world possessing Caucasian origins (Table 3). Using the Chi-squared test for association, χ² = 20.5, df = 3, p = 0.007. The frequency of the ApoE4 allele in the group of mild AD persons (Table 2) is excessive when compared to ApoE4 frequencies in non-demented, general populations of persons in multiple areas of the world possessing Caucasian origins (Table 3). Using the Chi-squared test for association, χ² = 20.5, df = 3, p = 0.007.

Further linear regression modeling was performed with the ApoE4 allele as predictor (Double E4, Single E4, No E4) and mean MMSE at diagnosis as the outcome measure.

**Results**

**Table 1: Study group results**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Double E4</th>
<th>Single E4</th>
<th>No E4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>56 (10.8%)</td>
<td>238 (45.8%)</td>
<td>226 (43.5%)</td>
</tr>
<tr>
<td>Age range (in years)</td>
<td>28.3</td>
<td>38.8</td>
<td>39.6</td>
</tr>
<tr>
<td>Mean Age</td>
<td>74.95 (5.9)</td>
<td>78.42 (6.6)</td>
<td>79.58 (7.6)</td>
</tr>
<tr>
<td>Median Age</td>
<td>64.45</td>
<td>77.46</td>
<td>67.7</td>
</tr>
<tr>
<td>Male %</td>
<td>85.8</td>
<td>91.5</td>
<td>93.2</td>
</tr>
<tr>
<td>Female %</td>
<td>14.2</td>
<td>8.5</td>
<td>6.8</td>
</tr>
<tr>
<td>Mean MMSE</td>
<td>21.03 (4.3)</td>
<td>21.46 (3.29)</td>
<td>21.18 (3.84)</td>
</tr>
</tbody>
</table>

**Table 2: ApoE allele frequency in study group**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Double E4</th>
<th>Single E4</th>
<th>No E4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele frequency</td>
<td>33.7%</td>
<td>66.3%</td>
<td>0</td>
</tr>
<tr>
<td>E4 allele count</td>
<td>172 (32.7%)</td>
<td>680 (129.8%)</td>
<td>0</td>
</tr>
<tr>
<td>E4 allele group</td>
<td>112 (21.2%)</td>
<td>580 (113.2%)</td>
<td>0</td>
</tr>
<tr>
<td>E4 = 0 group</td>
<td>104 (19.6%)</td>
<td>570 (110.8%)</td>
<td>0</td>
</tr>
<tr>
<td>E4 = 1 - E4 = 2 group</td>
<td>0</td>
<td>20 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>E4 = &gt; 2 group</td>
<td>0</td>
<td>20 (0.4%)</td>
<td>0</td>
</tr>
<tr>
<td>Male %</td>
<td>82</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>Female %</td>
<td>18</td>
<td>53</td>
<td>53</td>
</tr>
</tbody>
</table>

**Table 3 – Comparative E4 frequency versus known general populations (Chi-squared test)**

<table>
<thead>
<tr>
<th>E4 Allele</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study group</td>
<td>520 (CDR 1.0)</td>
</tr>
<tr>
<td>British</td>
<td>14.9%</td>
</tr>
<tr>
<td>Swedes</td>
<td>18.1%</td>
</tr>
<tr>
<td>Poles</td>
<td>13.8%</td>
</tr>
<tr>
<td>British</td>
<td>15.3%</td>
</tr>
</tbody>
</table>

**Dr Bernard Walsh FRACP**

University of Newcastle, Australia
7.1 Bibliography


43. ALFRED. ALelle FREquency Database. US National Science Foundation; alfred.med.yale.edu.


8. Appendices.

8.1 Structured UoN course work.

- Enrolment in “Molecular Epidemiology” semester 1, 2009, University of Newcastle (EPID6450) CCEB – completed.

- Enrolment in “Genetic Epidemiology” semester 2, 2009, University of Newcastle (EPID6450) CCEB – completed.

- STATA statistical software training course: UoN School of Medicine and Public Health, February 2011
20 November 2008

Dr Dr Bernard Walsh
Staff Specialist in Geriatric Medicine
Memory Disorders Clinic
1st Floor
Rankin Park Hospital

Dear Dr Walsh

Re: The relationship between Apolipoprotein E genotyping and hippocampal/entorhinal cortex MRI volumes in mild Alzheimer's Disease

HNEHREC reference number: 08/10/15/5.05
HREC reference number: 08/HNE/336
SSA reference number: 08/HNE/337

Thank you for submitting an application for authorisation of this project. I am pleased to inform you that authorisation has been granted for this study to take place at the following sites:

- Hunter New England Health

The following conditions apply to this research project. These are additional to those conditions imposed by the Human Research Ethics Committee that granted ethical approval:

1. Proposed amendments to the research protocol or conduct of the research which may affect the ethical acceptability of the project, and which are submitted to the lead HREC for review, are copied to the research governance officer;

2. Proposed amendments to the research protocol or conduct of the research which may affect the ongoing site acceptability of the project, are to be submitted to the research governance officer.

Yours faithfully

Lisa Woccen
Research Governance Officer
Hunter New England Health
Study Information Sheet

Cerebral MRI & ApoE genotyping Study

As part of the initial tests that confirmed your early Alzheimer’s Disease, a brain scan (cerebral MRI) and a blood test looking at some proteins in your blood (Apolipoprotein E genotyping) were performed. The results of these tests have helped Dr Walsh in working out your diagnosis and management plan, which has now been relayed to your local doctor.

Dr Walsh would like your permission to see if there are any links between these two tests you have already had by doing a short study. YOU DO NOT NEED ANY EXTRA TESTS IN THIS STUDY.

Using statistical methods Dr Walsh will analyse the results of these two tests over a large number of Memory Clinic patients, looking for patterns in the data. In the future, these patterns may help doctors with management of people who have memory loss. There is no individual feedback to participants planned for this study.

This study would not contain any information that might identify you and there would be no more tests for you to do. You would also be free to withdraw your consent at any time.

There would also be no extra time needed by you to be part of this study.

Declining to be part of this study would in no way alter the treatment you are receiving from the Memory Clinic.

Please take this sheet home, and at your next routine appointment in the this clinic, Dr Walsh will ask you if he can have your permission to use the results of your brain scan and blood test in his study.

If you have any complaints about this study or wish to talk to an independent officer, please contact the Dr Nicole Gerrand, Hunter New England Health Research Ethics and Governance Unit, Locked Bag No1, New Lambton, NSW 2305. (Phone 49214950)

Thank you for considering this request.
Dr Bernard Walsh (Chief Investigator)
### Table 10. Clinical Dementia Rating Scale (CDR).

<table>
<thead>
<tr>
<th>CDR 1</th>
<th>CDR 2</th>
<th>CDR 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mid dementia</strong></td>
<td><strong>Moderate dementia</strong></td>
<td><strong>Severe dementia</strong></td>
</tr>
<tr>
<td><strong>Memory</strong></td>
<td>Moderate memory loss, more marked for recent events</td>
<td>Highly marked memory loss, difficulty with familiar material, rapidly lost</td>
</tr>
<tr>
<td><strong>Orientation</strong></td>
<td>May have geographical disorientation</td>
<td>Usually disoriented in time, often in place</td>
</tr>
<tr>
<td><strong>Judgement &amp; problem solving</strong></td>
<td>Handling complex problems: social, judgment usually intact</td>
<td>Impaired judgment, easily distracted</td>
</tr>
<tr>
<td><strong>Community affairs</strong></td>
<td>Unable to engage in social activities, may still appear normal to casual inspection</td>
<td>Impaired judgment, easily distracted</td>
</tr>
<tr>
<td><strong>Home &amp; hobbies</strong></td>
<td>Mid but definite return at home, more complicated hobbies abandoned</td>
<td>Unable to perform everyday activities, interests poorly sustained</td>
</tr>
<tr>
<td><strong>Personal care</strong></td>
<td>Requires assistance in dressing, hygiene, eating, toileting</td>
<td>No significant function in own room</td>
</tr>
<tr>
<td><strong>Name:</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
8.5 Diagnostic and Statistical Manual of Mental Disorders Diagnostic criteria for

Dementia of the Alzheimer’s Type (DSM-IV-TR. 294x1)

A. The development of multiple cognitive deficits manifested by both
   1) memory impairment (impaired ability to learn new information or to recall previously learned information)
   2) one (or more) of the following cognitive disturbances:
      (a) aphasia (language disturbance)
      (b) apraxia (impaired ability to carry out motor activities despite intact motor function)
      (c) agnosia (failure to recognize or identify objects despite intact sensory function)
      (d) disturbance in executive functioning (i.e. planning, organizing, sequencing, abstracting)

B. The cognitive deficits in criteria A1 and A2 each cause significant impairment in social or occupational functioning and represent a significant decline from a previous level of functioning.

C. The course is characterized by gradual onset and continuing cognitive decline.

D. The cognitive deficits in criteria A1 and A2 are not due to any of the following:
   1) other central nervous system conditions that cause progressive deficits on memory and cognition (e.g. CVA, Parkinson’s Disease, Huntington’s disease, subdural hematoma, normal pressure hydrocephalus, brain tumor)
   2) systemic conditions that are known to cause dementia (e.g. hypothyroidism, vitamin B12 or folic acid deficiency, niacin deficiency, hypercalcaemia, neurosyphilis, HIV infection)
   3) substance –induced conditions

E. The deficits do not occur exclusively during the course of a delirium

F. The disturbance is not better accounted for by another Axis 1 disorder (e.g. major depressive Disorder, Schizophrenia)
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