Does Apolipoprotein E modify the association of cerebral infarcts with Alzheimer's disease?

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners. I understand that my thesis may be made electronically available to the public.

Abstract

Background: Dementia is a disease known to cause chronic deterioration of intellectual functions severe enough to interfere with the ability to perform activities of daily living. Alzheimer's disease (AD) is the most frequent cause of dementia and is expected to have a substantial impact on the health care system as the Canadian population ages. Current therapies are ineffective at halting disease progression; thus, investigations examining risk factors for AD have become a popular avenue of research. A relationship between cerebrovascular disease and the risk of AD has been established, but the underlying mechanisms on how these morbidities are related remain unclear. The apolipoprotein E gene (ApoE) influences the development of AD with the apolipoprotein E-e4 allele (ApoE-e4) conferring increased risk. The underlying mechanism by which the ApoE-e4 allele influences AD is unclear. Since the ApoE-e4 allele is related to both AD and stroke, the impact of cerebral infarcts on AD may vary by ApoE-e4 allele status. **Objective:** The objective of this study was to assess if ApoE-e4 allele status modified the relationship between cerebral infarcts and AD. **Methods:** Secondary data from the Nun Study, a longitudinal clinico-pathologic study of aging representing 678 female participants 75+ years were used for this investigation. AD was diagnosed using criteria for clinical dementia and AD pathology. Dementia was diagnosed using standard criteria, including the Consortium to Establish a Registry for Alzheimer's Disease battery of neuropsychological tests and performances on activities of daily living. AD pathology was diagnosed using a modified version of the National Institute on Aging and Reagan Institute criteria. Infarcts were identified during gross neuropathologic assessment at autopsy.

Logistic regression was used to assess the relationship between AD and the presence, location, and size of cerebral infarcts. Regression models were then stratified by ApoE-e4 allele status to determine if this variable was a significant effect modifier. The relationship of ApoE-e4 allele status with AD, as well as presence of cerebral infarcts, was also explored. All regression models were adjusted for age at death, educational level, and, when appropriate, ApoE-e4 allele status. A sensitivity analysis using different definitions for the outcome AD was performed and showed that varying criteria for AD pathology did not change study results; however, the use of clinical dementia (regardless of pathology) as an outcome did produce significantly different results. Thus, the research questions were repeated using clinical dementia as an outcome.

Results: The presence of cerebral infarcts was not significantly associated with AD; this relationship did not change when location and size of infarcts were examined. ApoE-e4 was significantly associated with an increased risk of AD. ApoE-e4 was not associated with presence of cerebral infarcts. ApoE-e4 did not modify the relationship between presence of cerebral infarcts and AD. When the outcome clinical dementia was investigated, presence of cerebral infarcts significantly increased the risk of dementia. This relationship remained when location and size of infarcts were analyzed. ApoE-e4 allele status slightly modified the relationship between presence of cerebral infarcts and dementia. **Conclusions:** The findings from this study suggest that individuals with severe AD pathology are unlikely to be affected by cerebral infarcts. Future studies should focus on examining levels of severity of AD pathology in relation to cerebral infarcts. Cerebral infarcts appear to have an impact on dementia and this relationship was found to slightly vary by ApoE-e4 status. Future studies are recommended to examine how ApoE interacts with a variety of age-related risk factors to increase the risk of AD.

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AD	Alzheimer's disease
ADLs	Activities of Daily Living
ApoE	Apolipoprotein E
ApoE-e4	Apolipoprotein E-e4 allele
CDR	Clinical Dementia Rating
CERAD	The Consortium to Establish a Registry for Alzheimer's Disease
CI	Confidence Interval
DSM-IV	The American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, Fourth edition
MMSE	Mini-Mental State Exam
NIA-RI	The National Institute for Aging, Ronald and Nancy Reagan Institute of Alzheimer's Disease
NIA-RI working group	The National Institute of Aging, and Reagan Institute working group on diagnostic criteria for the neuropathological assessment of Alzheimer's disease
NINCDS-ADRDA	The National Institute of Neurological Disorders and Stroke-Alzheimer Disease and Related Disorders Association
NMDA	N-Methyl-D-aspartic acid
OR	Odds ratio
SD	Standard deviation

1.0 Introduction

According to the United Nations, population aging is one of the most significant demographic processes shaping the world today (United Nations Economic and Social Council, 2001). Population aging in Canada is accelerating as the oldest baby boomers begin turning 65 years old in 2011 (Statistics Canada, 2007). The generation of baby boomers, those born between 1946 and 1965, is the largest cohort in Canada and comprises approximately 30 percent of the population (Statistics Canada, 2007). In 2007, one in seven Canadians was 65 or older (Statistics Canada, 2007); furthermore, individuals 80 years or older constituted one of the fastest growing segments among all age groups (McPherson, 2004; Statistics Canada, 2007).

Population aging is a result of the demographic transition, which represents a shift from high fertility and mortality rates to low fertility and mortality rates (Omran, 1971). Delayed first pregnancies and the use of oral contraceptives have contributed to the declining fertility rate, which has dropped to 1.5 children per woman in Canada (McPherson, 2004). Life expectancy has increased because of improved sanitation, immunization, and the development of curative therapies (Omran, 1971). In the early 1900s, the life expectancy rate was approximately 40 years for Canadians (McPherson, 2004). In 2006, the rates stand at 82 years for women and 77 years for men, respectively (Statistics Canada, 2007).

The epidemiologic transition parallels the demographic transition and reflects changes in causes of death: in developed countries the major causes of mortality are currently due to age-related chronic diseases (Fries, 2005; Omran, 1971; see review by Tyas & Gutmanis, 2008). Chronic diseases are the largest cause of mortality in the world (Yach, Hawkes, Gould, & Hofman, 2004). These diseases are heavily age-dependent and are expected to increase in prevalence and severity as the population continues to grow old (Fries, 2005; Yach et al., 2004).

Numerous social and biological risk factors are known to increase the incidence or accelerate the progression of various chronic diseases (Yach et al., 2004). Thus, contrary to the original medical model of disease, a single risk factor is not generally sufficient to cause one chronic disease (Yach et al., 2004). Chronic diseases are detected once a symptom threshold is met: the time at which they become clinically obvious (Fries, 2005). However, by the time there is clear clinical disability, the disease process is significantly advanced, and interventions are likely to be suboptimal. Therefore, a current strategy to impede the development of chronic diseases is to apply primary prevention efforts by identifying risk factors associated with the disease.

Dementia is a disease known to cause chronic deterioration of intellectual functions severe enough to interfere with the ability to perform activities of daily living (Lindsay & Anderson, 2003). Dementing disorders significantly reduce the quality and length of life, impose a severe burden on families and caregivers, and are an enormous drain on the economy (Markesbery, 1998b). In only a few years, the worldwide societal costs of dementia have increased from $315 billion in 2005 to $422 billion in 2009 (US dollars) in direct health care and indirect personal costs (Wimo, Winblad, & Jonsson, 2010). There are over 60 different causes for dementia; however, investigations over the last several decades have determined that Alzheimer's disease (AD) is the most frequent cause (Lindsay & Anderson, 2003; Markesbery, 1998b).

According to the Rising Tide report (Alzheimer's Society of Canada, 2010), approximately 500,000 Canadians are currently suffering from AD or a related dementia. AD is the most significant cause of disability among Canadians 65 years or older. As life expectancy increases and the baby boom generation ages, the impact that AD will have on health services and society is of increasing concern to policy makers (Li et al., 2010; Lindsay et al., 2002).

Treatments for AD have the ability to mitigate symptoms associated with the decline in cognitive and functional performance, but are not currently effective at halting or reversing the disease (Alzheimer's Association, 2010; Alzheimer's Society of Canada, 2010). Although other major causes of mortality, such as heart disease, have been decreasing with innovative therapies, deaths due to AD have been rising dramatically (Alzheimer's Association, 2010). The Rising Tide report (Alzheimer's Society of Canada, 2010) claims that if dementia onset was delayed by approximately 2 years, the incidence of AD would decrease by 18 percent and a large reduction in economic costs would result.

Because of the aging population and lack of effective treatments for AD, risk factors for AD have become an increasingly critical area of research (Alzheimer's Association, 2010; Lindsay & Anderson, 2003). Research on risk factors has direct relevance to the primary prevention of AD (Patterson, Feightner, Garcia, & MacKnight, 2007). Risk factors for AD can be categorized as either immutable or potentially modifiable. Genetics and increasing age are examples of irreversible factors that can increase the risk of developing AD (Alzheimer's Association, 2010). Cardiovascular health and educational level are examples of modifiable factors that are related to the risk of developing AD (Patterson et al., 2007). Fries (2005) suggested that modifiable risk factors are the key to his compression of morbidity model, which, if successful, has direct effects on mortality. Modifying risk factors may decrease the slope of disease progression, which in turn causes clinical symptoms to be delayed or less severe and mortality to be delayed or prevented (Fries, 2005).

Cardiovascular health is known to affect AD onset and progression. Neuroimaging and postmortem evaluation of the brain has indicated that at least one-third of AD individuals have some degree of vascular pathology (Jellinger & Attems, 2003; Lee, Olichney, Hansen,

Hofstetter, & Thal, 2000; Snowdon et al., 1997; see review by Staessen, Richart, & Birkenhager, 2007). Specifically, cerebral infarcts, which are the pathological evidence of stroke in the brain, are an established factor that increases the risk of developing clinical symptoms of AD seen during life, such as memory impairment. In Canada, strokes are prevalent and are the third leading cause of death (Dai et al., 2009). Although cerebral infarcts are clearly implicated in the development of vascular dementia, the exact mechanisms by which cerebral infarcts influence the development of AD remains unknown.

The Apolipoprotein E (ApoE) gene has been repeatedly demonstrated to be strongly associated with an increased risk of developing AD (Seripa et al., 2009). The e4 allele of the ApoE gene remains the most established AD genetic susceptibility factor (Yip et al., 2005). Carriers of the ApoE-e4 allele who have a history of stroke are five times more likely than ApoE-e4 carriers without a history of stroke to develop AD (Johnston, Nazar-Stewart, Kelsey, Kamboh, & Ganguli, 2000). Little is known, however, about the role of the ApoE-e4 allele on cerebrovascular disease and cerebral infarcts (Schneider et al., 2005).

The purpose of this study is to examine if ApoE-e4 allele status influences the strength of the relationship between cerebral infarcts and AD using data from the Nun Study, a longitudinal clinico-pathological study on aging. Data on cerebral infarcts and ApoE-e4 allele status were analyzed to determine if these factors independently increased the risk of developing AD. Subsequently, ApoE-e4 allele status was analyzed as an effect modifier of the relationship between cerebral infarcts and AD.

If the impact of cerebral infarcts on AD is modified by ApoE-e4 allele status, our understanding of how cerebral infarcts contribute to the etiological process of AD will be expanded. In addition, individuals that have risk factors for stroke can be identified as high-risk

for the development of AD and targeted for primary and secondary prevention efforts. Since risk factors for stroke are modifiable, vascular therapies may be able to delay the onset of AD for these high-risk individuals or delay disease progression in individuals already diagnosed with AD.

2.0 Literature Review

2.1 Alzheimer's Disease

AD is a progressive degenerative disorder of the brain causing impairments in memory and a variety of additional cognitive disabilities (Khachaturian, 1985; see review by Tyas & Gutmanis, 2008). AD is a major form of dementia and is not a normal part of aging (Alzheimer's Society of Canada, 2010). As AD progresses through three symptomatic stages (mild, moderate, and severe), cognitive impairments become significant enough to interfere with an individual's ability to perform Activities of Daily Living (ADLs) (see review by Tyas & Gutmanis, 2008). Each stage has implications for care and management of the disease. Individuals with mild AD symptoms may be able to continue living independently, whereas individuals with moderate to severe symptoms may require assistance or supervision. The rate of decline in AD is highly variable, but AD is ultimately fatal, with death usually occurring within seven to ten years of diagnosis (Alzheimer's Society of Canada, 2010; see review by Citron, 2010). The body is weakened by inactivity and muscle wasting, and a decline in the body's immune functions allows for possible bacterial and viral infections (Alzheimer's Society of Canada, 2010).

The vast majority of AD cases represent the sporadic form, also known as late-onset AD; less than 10 percent of the AD population experiences familial AD, also known as early-onset AD (Alzheimer's Society of Canada, 2010). Incidence of late-onset AD increases as life expectancy increases; thus, late-onset AD is of increasing concern because of the current aging population (Khachaturian, 1985). In 2008, there was one new case of late-onset AD or an age-related dementia every five minutes, which translates to approximately 103,000 new cases per year (Alzheimer's Society of Canada, 2010). The estimated incidence is expected to increase to

one new case every two minutes by 2038, which translates to approximately 257,000 new cases per year (Alzheimer's Society of Canada, 2010).

In 1991, the Canadian Study on Health and Aging estimated that dementia occurred in eight percent of the Canadian population over the age of 65, and almost two-thirds of these dementia cases were due to AD (Lindsay, Sykes, McDowell, Verreault, & Laurin, 2004). Equal numbers of AD cases resided within the community and institutions; however, more severe cases tended to reside in institutions (Lindsay et al., 2004). In the Rising Tide Report (Alzheimer's Society of Canada, 2010), it is estimated that approximately 500,000 Canadians suffer from AD or a related dementia and this rate is expected to increase 2.5 times for individuals over the age of 65 by the year 2038 (Alzheimer's Society of Canada, 2010). Due to the increasing incidence and prevalence of AD, the economic burden of dementia is expected to double every decade from 15 billion in 2008 to 153 billion by 2038 (Alzheimer's Society of Canada, 2010). Therefore, research into prevention, treatment, and care for AD is crucial.

2.1.1 Etiology

Alzheimer was one of the first scientists to report on an AD case over a century ago (see review by Mott & Hulette, 2005). Unfortunately, an established cause for AD still remains unknown (de la Torre, 2011; Khachaturian, 1985). In Alzheimer's original report, he found amyloid plaques and neurofibrillary tangles in the cerebral cortex of his patient (see reviews by Mott & Hulette, 2005; Price et al., 1998). These key neuropathologic features of AD remain and represent the two major hypotheses for the pathogenesis of AD (see review by Tyas & Gutmanis, 2008).

Senile plaques can be divided into two types: neuritic and diffuse. Neuritic plaques are spherical extracellular deposits containing a central core of amyloid material, whereas diffuse

plaques are a collection of extracellular peptides that lack a dense core (see review by Mott & Hulette, 2005). Neuritic plaques are associated with a glial response (see review by Mott & Hulette, 2005). Hypotheses suggest that microglia attempt to remove amyloid deposits and are in a constant state of proinflammatory activation that ultimately leads to chronic inflammation and neurodegeneration (see review by Mott & Hulette, 2005). The role of diffuse plaques in the pathophysiology of AD remains uncertain (see review by Mott & Hulette, 2005). Many studies claim both types of senile plaques should be quantified during diagnosis (Geddes et al., 1997), while others disagree and suggest quantifying only neuritic plaques (Powers, 1997). Occasionally, distinguishing neuritic plaques from diffuse plaques can be difficult, especially in older adults (Halliday et al., 2002; Haroutunian et al., 2008). The overall hypothesis for senile plaques in the pathogenesis of AD involves aggregation and deposition of beta-amyloid (Pimplikar, 2009; see review by Price et al., 1998). The hypothesis suggests a mutation in the amyloid precursor protein or environmental factors, both of which can alter amyloid precursor protein metabolism (see review by Price et al., 1998). Altered metabolism creates excess toxic amyloid deposition and leads to plaques that are identified in AD (see review by Price et al., 1998). Amyloid plaques have been hypothesized to attack cholinergic neurotransmission, which, if damaged, has been shown to have direct effects on cognition (Francis, Palmer, Snape, & Wilcock, 1999). Amyloid can also be found deposited on the walls of blood vessels of the central nervous system, known as cerebral amyloid angiopathy, which is common in individuals with AD (Dickson, 1998). Sparse senile plaques can be found in cognitively intact individuals, but they are densely present in individuals with AD, especially within the neocortex (Bancher, Paulus, Paukner, & Jellinger, 1997; Heyman et al., 1998; see review by Mott & Hulette, 2005). Since senile plaques are not always correlated with a decline in cognition, researchers have

speculated that amyloid plaques could be a result of the disease rather than a cause (Maccioni, Farías, Morales, & Navarrete, 2010; Pimplikar, 2009).

Neurofibrillary tangles are insoluble fibrillary intracellular deposits found within neurons (see review by Mott & Hulette, 2005). These tangles are made up of a protein labeled tau, which normally forms microtubules within nerve cells (see review by Price et al., 1998). Microtubules help transport nutrients and other important substances from one part of the nerve cell to another (see review by Price et al., 1998). Hypotheses for AD predict that the tau protein becomes hyperphosphorylated, causing the microtubule system to weaken, which, in turn, creates helical filaments (see reviews by Castellani et al., 2007; Mott & Hulette, 2005). The aggregation of these filaments forms tangles within the neurons and, ultimately, disrupts cell transportation and communication (see review by Castellani et al., 2007). Oxidative stress has also been reported to be associated with tangle production (Good, Werner, Hsu, Olanow, & Perl, 1996). Neurofibrillary tangles can be seen in low numbers in the cognitively intact, in aged individuals, and in individuals with other neurodegenerative diseases (see review by Mott & Hulette, 2005). In AD, however, neurofibrillary tangles become more densely distributed within neurons found in critical areas of the brain, such as the hippocampus (Bancher et al., 1997; Heyman et al., 1998; see review by Mott & Hulette, 2005).

The cholinergic hypothesis has also been suggested to be a part of the pathogenesis of AD. This hypothesis proposes that cholinergic neurons degenerate and become ineffective at neurotransmission, both of which contribute to the deterioration of cognitive function (Craig, Hong, & McDonald, 2011; Francis et al., 1999). Many therapeutic strategies for AD aim to increase cholinergic neuronal transmission by decreasing the breakdown of the cholinergic neurotransmitter, acetylcholine (Francis et al., 1999). These medications may improve deficits in

cognition temporarily, but have not been shown to have any effects of the progression of AD pathology (Craig et al., 2011; Francis et al., 1999). Other theories about AD etiology include excitotoxicity of the N-Methyl-D-aspartic acid (NMDA) receptors (Hynd, Scott, & Dodd, 2004) and oxidative damage to neurons (Good et al., 1996). However, many researchers are starting to branch out from these independent theories of damage and examine how they all possibly work together, in addition with AD risk factors such as the ApoE-e4 allele, to influence AD pathology and cognitive impairment (Craig et al., 2011).

Vascular hypotheses have also been suggested as causal factors for the development of AD. In the past, vascular dysfunction resulting in dementia has been typically diagnosed as vascular dementia (see review by Altman & Rutledge, 2010). However, several common vascular risk factors have been shown to be associated with an increased risk of AD in old age (see review by Pinkston, Alekseeva, & Gonzalez Toledo, 2009; Roher et al., 2011). Atherosclerosis, stroke, and hypercholesterolemia all result in vascular pathologies, creating an inefficient blood supply to the brain (Roher et al., 2011). Recently, the impact cerebrovascular disease has on AD pathogenesis has been recognized and chronic hypoperfusion in AD likely contributes towards neuronal and synaptic damage (Roher et al., 2011).

2.1.2 Detection and Diagnosis

Diagnosing AD is a clinico-pathologic process; the clinical diagnosis is presumptive and requires post-mortem neuropathological examination for definitive confirmation (Khachaturian, 1985; Lim et al., 1999; Markesbery, 1998b). Overall, the correlation between clinical and pathological outcomes of AD is not well understood, especially within older populations (Khachaturian, 1985; Silver, Newell, Brady, Hedley-White, & Perls, 2002).

A universal definition for what constitutes normal aging does not exist; therefore, establishing concrete values for what constitutes normal cognitive aging versus abnormal cognitive aging is very difficult (Khachaturian, 1985). Early signs of AD, such as forgetfulness, may be misinterpreted as a part of normal aging rather than as a part of the disease process (Khachaturian, 1985; see review by Tyas & Gutmanis, 2008). During the later stages of AD, symptoms can be mistaken for other types of dementias or mental diseases until post-mortem analysis can be done (Khachaturian, 1985). Clinical symptoms of AD can include memory loss, difficulty performing familiar tasks, disorientation to time and place, changes in mood or behavior, and loss of initiative (see review by Tyas & Gutmanis, 2008).

Although the gold standard for diagnosing AD involves a post-mortem neuropathologic examination, a diagnosis of AD during life using clinical and neuropsychological evaluations are important (Markesbery, 1998b). These evaluations can establish the degree of disability and are crucial to set the course of therapy and assistance (McKhann et al., 1984). In addition, clinical information will be able to be compared to post-mortem information, once available. There are three main sets of criteria used when clinically diagnosing AD: the National Institute of Neurological Disorders and Stroke-Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984); the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (Morris et al., 1989); and the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, Fourth edition (DSM-IV) (American Psychiatric Association, 1994).

The NINCDS-ADRDA criterion defines AD as having deficits in two or more areas of cognition that impair ADLs (McKhann et al., 1984). This criterion classifies the diagnosis of AD during life as either possible or probable. The CERAD clinical criterion defines AD as

having any area of cognitive deficit that impacts ADLs (Morris et al., 1989). This criterion classifies the diagnosis of AD during life as either possible or probable. The DSM-IV criterion defines clinical AD as of cognitive impairment within two or more domains; however, memory has to be one of the areas affected (American Psychiatric Association, 1994) . This criterion classifies the diagnosis of AD as having dementia of the Alzheimer's type, either with or without behavioural disturbances. Each criterion requires that a complete medical history is obtained and a general clinical evaluation is performed. Through varying methods, each criterion aims to exclude other health issues, such as Parkinson's disease, that may lead to cognitive impairment, but also assesses issues typical of AD, such as gradual memory loss. Common tools are used during the neuropsychological evaluations to examine various dimensions of cognition, such as short-term recall, abstract thinking, apraxia, agnosia, and verbal fluency (see review by Tyas & Gutmanis, 2008). Currently, there is no single test or established biological marker for AD, leaving the diagnostic evaluation a lengthy process (see review by Tyas & Gutmanis, 2008). Regular follow-ups are recommended after diagnosis so that disease progression can be monitored and care adjusted accordingly (see review by Tyas & Gutmanis, 2008).

AD pathology is presumed to be progressing in the brain years before the onset of clinical symptoms. The gold standard for AD diagnosis is based on both clinical and pathological assessment. Some inconsistencies between clinical and pathological characteristics, however, do exist. Studies that have used both clinical and pathological information for AD diagnosis have found that the brains of some cognitively intact older adults contained sufficient numbers of senile plaques and neurofibrillary tangles to meet at least one set of neuropathologic guidelines for the diagnosis of AD. The ability to resist the clinical expression of AD has been termed asymptomatic AD (Tyas, Snowdon, Desrosiers, Riley, & Markesbery, 2009). In addition, a small

portion of individuals who do not meet neuropatholologic criteria for AD showed clinical symptoms of dementia during life (Snowdon et al., 1997). These dementia symptoms represent non-AD dementias, such Lewy body dementia, vascular dementia, or Parkinson's disease. Due to the inconsistencies between clinical and pathological outcomes, research has yet to prove if senile plaques and neurofibrillary tangles are markers of disease progression or instead are involved in another unknown way (see review by Castellani et al., 2007). Despite the inconsistencies, the majority of diagnostic criteria still use neurofibrillary tangle distribution and/or senile plaque density as the major components for AD progression. Initially, a macroscopic neuropathologic evaluation is performed, followed by a comprehensive microscopic evaluation that typically examines specific areas of the brain, including the middle frontal gyrus, inferior parital lobule, occipital cortex, and hippocampus (lateral geniculate nucleus, entorhinal cortex, and mid brain) (see review by Mott & Hulette, 2005). The entorhinal cortex, hippocampus, and amygdale are the sites of some of the most severe neuropathologic changes in AD (Markesbery, 1998b).

Numerous guidelines are presently used for the neuropathological diagnosis of AD (Jellinger, 2009). The Khachaturian criterion was developed in 1985 as a minimum microscopic diagnosis of AD and was the first attempt to standardize a neuropathologic diagnosis (Khachaturian, 1985). The criterion consisted of quantitative assessments of senile plaques in numerous brain regions; these assessments were then integrated with patient age for a final diagnosis (Khachaturian, 1985; Hyman, 1998). The Khachaturian criterion had vague recommendations for sampling and staining techniques; it was later proven to be of limited use (Bancher et al., 1997; see review by Mott & Hulette, 2005). Despite its limitations, the

Khachaturian criterion laid the groundwork for neuropathological diagnostics developed years later.

There are three common neuropathological assessments for AD that are recognized by current neuropathologists: Braak staging (Braak & Braak, 1991), the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria (Mirra et al., 1991), and the National Institute for Aging, Ronald and Nancy Reagan Institute of Alzheimer's Disease (NIA-RI) criteria (The National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease [NIA-RI working group], 1997). Each type of neuropathological assessment examines different pathological features in various regions of the brain and requires specific stains and techniques. Braak staging was developed as a neuropathological method to differentiate periods of AD progression and specifically examines neurofibrillary tangle distribution in different regions of the brain (Braak & Braak, 1991). The method originally had six stages of disease progression (0 plus I-VI) irrespective of age or clinical diagnosis although stages are often condensed (I-II, III-IV, V-VI) (Braak & Braak, 1991). The CERAD diagnostic criterion is a three-step semi-quantitative process that incorporates neuritic plaque density (level A, B, and C), age, and clinical diagnosis into levels of AD diagnosis: none, possible, probable, and definite (Mirra et al., 1991). CERAD also examines additional pathological features in the brain to rule out other diseases possibly contributing to impaired cognition (Mirra et al., 1991). The NIA-RI criterion combines the former criteria by examining neuritic plaque density, through the CERAD definition, and by also examining neurofibrillary tangle distribution through Braak staging. The NIA-RI criterion assumes that neuritic plaque densities and neurofibrillary tangle distributions are ideally correlated with each other (NIA-RI working group, 1997), but recognizes this may not always be the case. For

example, not every individual with a high neurofibrillary tangle distribution, (Braak score V-VI) also has a high neuritic plaque density (CERAD level C). Unfortunately, the criterion does not specify how to classify individuals that do not fit into the predefined categories, possibly leaving a high proportion of unclassified cases (NIA-RI working group, 1997). The likelihood that dementia during life was due to AD is classified according to three categories: low, intermediate, and high (NIA-RI working group, 1997).

2.1.3 Treatment and Care

Treatment options are dependent on the individual because the clinical symptoms of AD can be highly variable (Alzheimer's Society of Canada, 2010). Clinical symptoms progress from gradual episodic memory problems to global declines in cognitive function, leaving the individual fully dependent for care (see review by Citron, 2010). Life expectancy is significantly decreased as death is estimated to occur within seven to ten years of AD diagnosis (see review by Citron, 2010).

Once a diagnosis is made, acetylcholinesterase inhibitors are typically prescribed for mild or moderate AD (Alzheimer's Society of Canada, 2010; Citron, 2010). These medications aim to slow the rate of acetylcholine breakdown and ultimately cholinergic neuronal death (see review by Citron, 2010). Acetylcholinesterase inhibitors have the ability to mitigate symptoms associated with decline in memory, language, and attention, as well as delay functional impairments (Alzheimer's Society of Canada, 2010). Unfortunately, acetylcholinesterase inhibitors are not proven to stop or reverse the decline in AD (Alzheimer's Society of Canada, 2010). Memantine is another possible medication prescribed for moderate to severe AD (see review by Citron, 2010). Memantine is an NMDA receptor antagonist and is only proven to moderately decrease clinical deterioration temporarily (see review by Citron, 2010). Other types

of medications may also be introduced during moderate to severe AD to manage behavioural and/or psychological symptoms of dementia, such as mood disorders or agitation (Alzheimer's Society of Canada, 2010; see review by Citron, 2010). Even though current medications are not successful at halting the disease, several drugs are in development or under research with hopes to modify disease progression, such as beta-amyloid inhibitors, anti-inflammatories, and serotonin antagonists (see review by Sabbagh, 2009).

In addition to medication, education and support services are highly recommended for the patients and their family or caregivers (Alzheimer's Society of Canada, 2010). Individuals with mild AD are often recommended to get involved in community organizations and to take advantage of organizations offering support within the home (Alzheimer's Society of Canada, 2010). Once an individual has transitioned into moderate or severe AD, assisted living or long-term care may be required. Support for caregivers is also recommended to provide information on their loved one's changing need for care. During the final stages of disease, palliative care is necessary for minimizing pain and providing comfort (Alzheimer's Society of Canada, 2010).

2.1.4 Risk Factors

Autosomal dominant conditions, such as mutations in presenilin and amyloid precursor protein genes, lead to the development of familial AD (see review by Bookheimer & Burggren, 2009). These genetic disorders produce abnormal mechanisms for amyloid production resulting in the formation of amyloid plaques (see review by Bookheimer & Burggren, 2009). Risk factors that increase the likelihood of developing sporadic AD have also been established and are often divided into three categories: biologically determined, lifestyle, and co-morbid conditions. Risk factors that fit into these categories will be discussed below, for more information on additional risk factors, see reviews by Graves (2004) and Lindsay (2002).

2.1.4.1 Age

The most established biologically determined risk factor for sporadic AD is age. The likelihood of developing AD has been estimated to double every five years after the age of 65 (Jorm, Korten, & Henderson, 1987). However, this exponential risk has been hypothesized to reach a plateau in adults 85 years or older (von Strauss, Viitanen, De Ronchi, Winblad, & Fratiglioni, 1999). Studies examining individuals over the age of 90 are few because attaining a large enough study population to provide reliable estimates is difficult. Since age and AD are strongly associated, researchers have speculated that AD may be a result of multiple risk factors over a long latency period; thus, AD is a life course disease (von Strauss et al., 1999). Until effective prevention and treatment strategies are developed, the presence of AD will continue to rise given the aging population.

2.1.4.2 Genetics

Another established biological factor that increases the risk of developing AD is the ApoE gene. (For more details, see section 2.4.) This genetic factor has three common alleles: ApoE-e2, ApoE-e3, and ApoE-e4. There is a dose-response displayed within these alleles as individuals who are homozygous for the ApoE-e4 allele have an increased age-specific risk for developing AD compared to any other combination of ApoE alleles (Meyer et al., 1998) . ApoE-e4 carriers have also been shown to display a higher density of amyloid plaques compared to other ApoE allele carriers. However, the ApoE-e4 allele is not necessary or sufficient to cause AD. Individuals without the ApoE-e4 allele develop AD, and some individuals with the ApoE-e4 allele never develop AD, despite living to old age (Meyer et al., 1998). Since complex gene-environment interactions are likely involved in the development of AD, Graves (2004) recommends assessing possible environmental factors within their genetic context. Due to the

dose-response relationship, the ApoE allele status of the individual may be an effect modifier for of the development of AD depending on the presence of risk factors such as diabetes, hypertension, or obesity.

2.1.4.3 Gender

Gender represents a potential biologically determined risk factor for sporadic AD. Several studies have suggested that women are at a higher risk of developing AD compared to men, whereas other studies refute the increased risk (Janicki & Schupf, 2010; Markesbery, 1998b). The prevalence of AD in an older population appears higher in women; however, this may be due to the fact that women generally have a longer life expectancy and perhaps longer disease duration compared to men (Dal Forno et al., 2002). Longitudinal studies that have accounted for duration of the disease have had mixed results for differential incidence rates in men and women (Janicki & Schupf, 2010). Loss of estrogen in the aging female brain has been suggested to lead to an increased risk of developing AD; however, results are inconsistent. Difficulty measuring hormone levels in older adults is claimed to be the reason for inconsistent results; thus, research on estrogen and AD is still warranted (Janicki & Schupf, 2010). The relationship between gender and increased likelihood of developing AD remains unclear.

2.1.4.4 Lifestyle Factors

The most important lifestyle risk factor associated with AD is level of educational attainment (Stern et al., 1994). Hypotheses suggest a highly educated individual may be able to tolerate the disease process and remain asymptomatic longer compared to a less educated individual (Stern et al., 1994). The process of education may increase neuronal reserve by promoting synaptic growth in the brain. Advanced neuronal reserve may be protective, as it creates a higher threshold for damage before cognitive consequences of AD become clinically

present (Katzman, 1993; Stern et al., 1994). However, low educational level may be related to other factors that are also known to increase the risk of developing AD, such as poor nutrition or environmental exposures (Markesbery, 1998b).

Other lifestyle risk factors include head injury and tobacco use, both of which have been found to have harmful effects that span over several decades (Graves, 2004; Mortimer, French, Hutton, & Schuman, 1985; Tyas et al., 2003). Head injuries resulting in a loss of consciousness can be due to many lifestyle choices, such as helmet use or recreational activities. In addition to being a risk factor for developing AD, head injury has also been associated with earlier onset of AD (Graves, 2004). Tobacco use has a dose-response relationship with AD, where the amount smoked is associated with the risk of AD and AD-related neuropathologies, even when stratified by ApoE-e4 allele status (Tyas et al., 2003). However, many studies fail to find an association in heavy smokers, likely because of a survival bias (Tyas et al., 2003). Fortunately, head injury and tobacco use are preventable and/or modifiable through behaviour change, and public health strategies have lowered the prevalence of these risk factors.

2.1.4.5 Vascular Factors

Co-morbid conditions, such as diabetes and vascular diseases, are risk factors that have been shown to increase the likelihood of developing AD. Type 2 diabetes mellitus is positively associated with incident AD (Peila, Rodriguez, Launer, & Honolulu-Asia Aging Study, 2002). Individuals who are both diabetic and carriers of the ApoE-e4 allele have an even greater risk of developing AD and AD-related pathologies than individuals without either diabetes or an ApoE-e4 allele (Peila et al., 2002).Cardiovascular diseases are of great concern for increasing the risk of developing AD because of their high prevalence in the population. Neuroimaging and postmortem evaluation of the brain has indicated that at least one-third of AD individuals have

some degree of vascular pathology (Jellinger & Attems, 2003; Lee et al., 2000; Snowdon et al., 1997; see review by Staessen et al., 2007). The brain consumes large quantities of nutrients that are supplied through cerebral arteries; thus, the brain and heart are closely connected (see review by de la Torre, 2006). Since a healthy brain is dependent on a healthy blood supply, understanding the role of the vascular system in AD is becoming increasingly important (see review by de la Torre, 2006). Numerous studies have indicated that damaged vasculature in the brain may contribute to the clinical and pathological manifestations of AD (Roher et al., 2011). Several vascular risk factors have been found to be associated with cognitive decline and a greater risk of developing AD (Duron et al., 2009; Hanon et al., 2006; see review by Pinkston et al., 2009). Stroke, diabetes mellitus, metabolic syndrome, hypertension, atherosclerosis, thrombosis, and hyperlipedemia are examples of vascular insults and are all highly prevalent in Western society (see Figure 1) (see review by de la Torre, 2006; Duron et al., 2009; Regan et al., 2006). Over time, these factors cause damage to the cerebral blood vessels and major arteries, and lead to vascular pathologies, such as cerebral infarcts, white matter lesions, and cerebral amyloid angiopathy (Jellinger & Attems, 2003). Due to poor blood flow, neurons can become damaged and are not able to function or metabolize oxygen (see review by de la Torre, 2006; Jellinger & Attems, 2003; see review by McDonald, Craig, & Hong, 2010). Depending on the particular artery affected in the brain and the duration of the blockage, neuronal cell death can be local or global (see reviews by de la Torre, 2006; McDonald et al., 2010). Neurons in the hippocampus are particularly sensitive to vascular damage, suggesting that vascular pathologies found specifically within this area could lead to cognitive deficits similar to those observed in AD (see review by McDonald et al., 2010). Since vascular risk factors are modifiable through

lifestyle or medical interventions, their identification may be a new and effective approach to delay the onset of AD, or better yet, to create new preventative and therapeutic strategies.

Previous research from the Nun Study found that among participants who met neuropathologic criteria for AD, the risk of dementia was greater if cerebral infarcts were present when compared to participants without any infarcts (Snowdon et al., 1997).

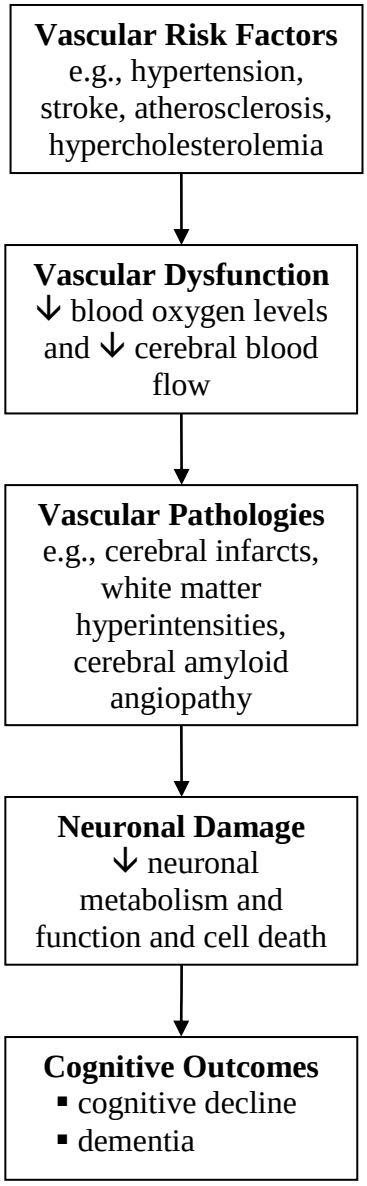

Figure 1. The Vascular Hypothesis for Dementia

2.2 Cerebral Infarcts

In Canada, 50,000 strokes occur annually and are the third leading cause of death (Dai et al., 2009). The risk of stroke doubles every 10 years after the age of 55 (Dai et al., 2009). The vast majority of strokes represent the ischemic type, which is a blockage of blood flow in the brain (see review by de la Torre, 2006). The minority of stroke cases, approximately 15 percent, experiences a more serious hemorrhagic type, which is abrupt leakage of blood in the brain (see review by de la Torre, 2006). Thus, most investigations typically examine only ischemic stroke. Cerebral infarcts are the pathological sign of stroke in the brain. Whether ischemic or hemorrhagic, cerebral infarcts have been shown to significantly reduce the likelihood of healthy aging and are an established factor that increases the risk of developing AD (see review by de la Torre, 2006; Norrving, 2008; see review by Staessen et al., 2007; Tyas, Snowdon, Desrosiers, Riley, & Markesbery, 2007).

Cerebral infarcts can be visualized through an autopsy analysis or structural magnetic resonance imaging (MRI) (see review by Chui, 2005). Occlusion of a major cerebral artery can result in a large infarct. Occlusion of a small or medium-sized arteriole can result in a lacunar infarct, which is a round lesion approximately 1.5 centimeters or less in diameter (Markesbery, 1998a). Not all cavitations, known as lacunes, in the deep white matter represent lacunar infarcts (see review by Chui, 2005). During autopsy analysis, the distinction between lacunes and lacunar infarcts may be clear to some, but consistency of their identification across all neuropathologists is questioned. Occlusion of small arteries or capillaries can result in microscopic or watershed infarcts, which usually cannot be detected by the unaided human eye (see reviews by Chui, 2005; Miklossy, 2003).

Not all individuals with cerebral infarcts at autopsy have a clinical history of stroke during life (see review by de la Torre, 2006; Honig, Kukull, & Mayeux, 2005). Strokes that do

not clinically present with symptoms during life are known as silent strokes (Honig et al., 2005). Silent strokes appear to have the same vascular risk factors as clinically symptomatic strokes and can range in size representing large or lacunar infarcts (Leary & Saver, 2003). The incidence of silent strokes rises sharply with increasing age, and the prevalence of one silent stroke triples the incidence of another silent stroke occurring (Vermeer et al., 2007). In the general population, silent strokes have been shown to be five times more prevalent than clinical strokes (Leary & Saver, 2003). Studies that examine the effects of clinical strokes on the risk of developing AD may not be accounting for the presence of silent strokes, which are most accurately detected though autopsy evaluation (Leary & Saver, 2003).

2.2.1 Cerebral Infarcts and Alzheimer's Disease Pathology

Previous research from the Nun Study reported that cerebral infarcts increased the likelihood of dementia in both those who met and didn't meet the neuropathologic criteria for AD when compared to individuals without any infarcts (Snowdon et al., 1997). Several studies have reported that AD pathology is not associated with the presence of infarcts and that cerebral infarcts do not increase AD pathology (Schneider, Wilson, Bienias, Evans, & Bennett, 2004; Snowdon et al., 1997; Troncoso et al., 2008). However, these results tend to be reported in autopsy-based studies that make temporality between infarcts and AD pathology difficult to establish. Cerebral infarcts and AD pathology have been tested to see if they increase the likelihood of dementia synergistically; results have varied (Schneider et al., 2004; Schneider, Boyle, Arvanitakis, Bienias, & Bennett, 2007; Troncoso et al., 2008). Significant interactions could depend on characteristics of the cerebral infarct (e.g., volume, location, number, and size).

Although the relationship between cerebral infarcts and AD pathology remains unclear, they both are related to dementia. Cerebral infarcts appear to have the most impact on cognition

in the early stages of dementia, when perhaps AD pathology is mild (Schneider et al., 2007). During the later stages of dementia, the severity of AD pathology is correlated with severe memory impairment (Fleischman et al., 2005). Cerebral infarcts, along with their cognitive consequences, could be related to the pathogenesis of AD pathology. If related, vascular damage could act as an early warning sign for AD and targeted vascular therapies could be used to potentially slow the process of AD pathology.

2.2.2 Effects of Cerebral Infarcts in Alzheimer's Disease

In addition to increasing the risk of developing AD, cerebral infarcts have been found to contribute to poorer global cognitive functioning in AD participants as measured by the Mini-Mental State Exam (MMSE), the Clinical Dementia Rating (CDR), and the Global Deterioration Scale (GDS) (Heyman et al., 1998; Schneider et al., 2004; Schneider et al., 2007; Snowdon et al., 1997; Song, Kim, Kim, Eah, & Lee, 2007). However, a small number of studies have reported that cerebral infarcts have no effect on global cognitive functioning (Lee et al., 2000; Zekry et al., 2003). These studies, however, had small sample sizes and were from clinic-based populations. Small sample sizes and clinically based populations are factors that limit sample variation, are not generalizable to the population of interest, and do not represent the full spectrum of AD cases. As a result, the statistical power to observe an association between cerebral infarcts and poor global cognitive functioning in these studies was limited.

Cerebral infarcts contribute to decline within specific cognitive domains, such as visuospatial skills, perceptual speed, episodic memory, semantic memory, and working memory (Heyman et al., 1998; Schneider et al., 2004; Schneider et al., 2007; Snowdon et al., 1997; Song et al., 2007). The structure in the brain that is highly responsible for the learning and memory domains is the limbic system, which is composed of the hippocampal formation and the

amygdala. Many researchers have hypothesized that the strength of the relationship between the presence of cerebral infarcts and cognitive decline is highly dependent on lesion size and, most importantly, location (Kovari et al., 2007; Zekry et al., 2002).

Large cerebral infarcts have a statistically significant impact on cognitive scores (Schneider et al., 2004; Schneider et al., 2007). Lacunar infarcts are studied less and their independent impact on cognition in older populations is not clear (Benisty et al., 2009). Quite often investigations group both lacunar and large infarcts together to represent one macroscopic infarct variable; however, teasing out the impact each size of infarct may have on cognition is important when understanding the etiologic process of dementia. The role of microscopic infarcts, which are not able to be seen with the unaided human eye, on cognition or towards the risk of developing dementia has had varying results (Lee et al., 2000; Schneider et al., 2004; Schneider et al., 2007; White et al., 2002). The effect of microscopic cerebral infarcts on cognition depends on their number and location in the brain (White et al., 2002).

Not surprisingly, individuals with multiple cerebral infarcts have been found to have poorer cognitive function compared to the cognitive functioning in individuals with only one cerebral infarct (Schneider et al., 2004; Schneider et al., 2007). However, prior research from the Nun Study has shown that just one or two lacunar infarcts, found in strategic locations in the brain, can have a significant impact on cognitive performance (Snowdon et al., 1997). Based on autopsy results of older adults or AD populations, common locations of infarcts in the brain are the basal ganglia, deep white matter and limbic areas (Schneider et al., 2007; Snowdon et al., 1997; Zekry et al., 2003). Prior research on the Nun Study sample has reported that the prevalence of dementia was higher when lacunar infarcts were located in the basal ganglia, deep white matter or thalamus (Snowdon et al., 1997).

2.3 Apolipoprotein E

ApoE, found mostly in the liver and brain, is a major protein that binds to lipids and transports them through various systems in the body (see reviews by Bu, 2009; Kim, Basak, & Holtzman, 2009). In the brain, astrocytes are the major cell type that produce ApoE lipoprotein particles, which deliver cholesterol and other essential lipids to neurons in the central nervous system (see review by Kim et al., 2009). Cholesterol, a crucial macromolecule, is used for composing membranes and myelin sheaths and is vital for synaptic integrity and neuronal function (see reviews by Bu, 2009; Kim et al., 2009). ApoE transports cholesterol though low-density-like-lipoprotein particles and binds to low density lipoprotein receptors. Low density lipoprotein receptors are the main receptors for cholesterol homeostasis (see review by Bu, 2009). Studies performed in vitro suggest that cholesterol released from ApoE lipoprotein particles is essential for synaptogenesis and the maintenance or repair of synaptic connections in the adult brain (see reviews by Bu, 2009; Kim et al., 2009).

The ApoE gene has several single nucleotide polymorphisms that change the coding sequence and result in the three common isoforms: ApoE-e2, ApoE-e3, and ApoE-e4 (see review by Kim et al., 2009). Although the ApoE alleles differ by only one amino acid, each has a unique structure and function (see review by Kim et al., 2009). The e3 allele is the most common and e2 the least common allele in the general population (see review by Bu, 2009). The three polymorphic alleles give rise to six possible genotypes: e2/e2, e2/e3, e2/e4, e3/e3, e3/e4, and e4/e4.

2.3.1 Apolipoprotein E and Alzheimer's Disease

The ApoE gene has been established as a risk factor for the development of sporadic AD (Seripa et al., 2009). Specifically, the e4 allele of the ApoE gene remains the most established

AD genetic susceptibility factor (Yip et al., 2005). In the general population, the e4 allele frequency is approximately 20 percent, with the e3/e4 genotype being most common and the e4/e4 genotype least common among all e4 genotypes (see reviews by Ashford, 2004; Bu, 2009). However, in the AD population, the ApoE-e4 allele frequency has an estimated prevalence of 60%; with the e3/e4 being most common and the e2/e4 least common among all e4 genotypes (see review by Ashford, 2004). In the AD population, the ApoE-e4 allele displays a gene-dose effect: individuals homozygous for the gene have the most risk for AD onset compared to heterozygous individuals (see review by Ashford, 2004; Dal Forno et al., 2002). The gene-dose effect has also been shown in global cognitive decline scores: individuals homozygous for the e4 allele experience a steeper decline in test scores compared to individuals heterozygous for the e4 allele (Shadlen et al., 2005). The prevalence of AD cases was estimated to decrease by half if the ApoE-e4 allele did not exist (see review by Ashford, 2004).

Individuals with the ApoE-e4 allele have been found to have an earlier age of AD onset compared to individuals without an ApoE-e4 allele (see review by Kim et al., 2009). The ApoE-e4 allele appears to exert its maximal effect on AD risk before the age of 70 because the effect of the allele was lost in older populations (see review by Ashford, 2004). ApoE-e4 is hypothesized to increase the formation of amyloid plaques and neurofibrillary tangles (Huang, 2006; Mortimer, Snowdon, & Markesbery, 2009); however, this theory has yet to be established. Since the exact mechanisms of ApoE-e4 remain unknown, further investigation into how ApoE-e4 influences the risk, onset, and progression of AD is required (Seripa et al., 2009; Shadlen et al., 2005).

2.3.2 Apolipoprotein E and Cerebral Infarcts

ApoE-e4 carriers with a history of stroke are five times more likely than ApoE-e4 carriers without a history of stroke to have AD (Johnston et al., 2000). The ApoE-e4 allele is related to indicators of vascular disease, such as cholesterol, but is not considered a major risk factor for vascular diseases (Schneider et al., 2005). Little is known about the relationship between ApoE-e4 and cerebrovascular disease (Schneider et al., 2005), specifically cerebral infarcts. Two possible ways ApoE-e4 allele status and cerebral infarcts may work together as risk factors to increase the likelihood of developing AD are through mediation or moderation.

Figure 2 is a modified version of the mediating hypothesis by Baron and Kenny (1986). As depicted in Figure 2, the independent or predictor variable is always antecedent to the mediating variable. Since ApoE-e4 allele status is genetically determined (antecedent), cerebral infarcts would have to act as the mediating variable. Given recent findings on epigenetic drift, some may argue that the ApoE gene should not always be considered an antecedent variable. ApoE has displayed effects of age-related methylation and thus its genetic impact within AD may not be the same across the lifespan (Wang, Oelze, & Schumacher, 2008). Although several studies have been performed, however, very few have been able to link ApoE genetic drift to risk of AD, and thus the ApoE-e4 allele was assumed antecedent for this study.

There are two causal paths feeding into the outcome variable AD: the direct impact of ApoE-e4 allele status (Path *c*) and the direct impact of cerebral infarcts (Path *b*). The path from ApoE-e4 allele status to cerebral infarcts (Path *a*) represents the mediation pathway. To function as a mediator, according to Baron and Kenny (1986), the relationship between ApoE and cerebral infarcts must meet the following conditions:

> . . . (a) variations in levels of the independent variable significantly account for variations in the presumed mediator (i.e., Path *a*), (b) variations in the mediator significantly account for variations in the dependent variable (i.e., Path *b*), and (c) when Paths *a* and *b* are controlled, a previously significant relation between the independent and dependent variables is no longer significant, with the strongest demonstration of mediation occurring when Path *c* is zero. (p. 1176)

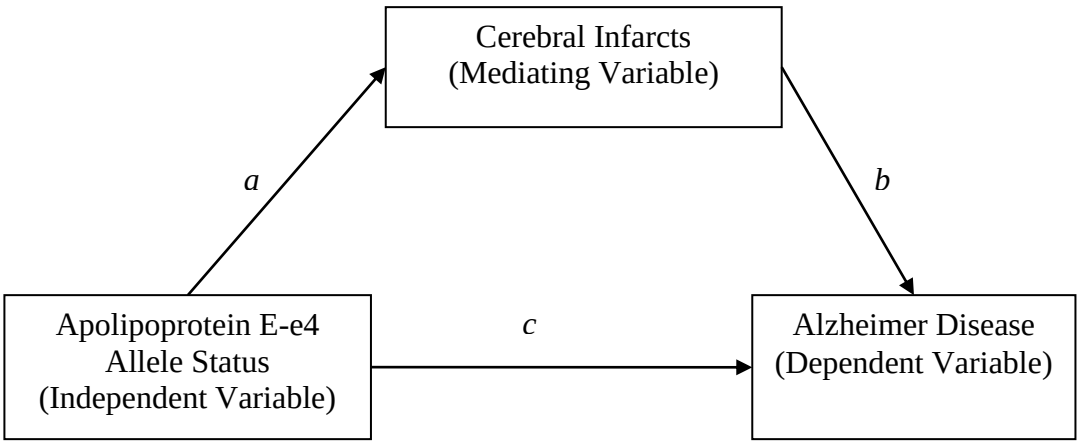

Figure 2. The Modified Mediation Hypothesis

According to conditions (a) and (b) of the mediating hypothesis, cerebral infarcts and ApoE-e4 allele status should be associated regardless of which risk factor is representing the mediating variable (Baron & Kenny, 1986). However, the ApoE genotype has not been found to be associated with the presence of cerebral infarcts and vice versa (Frikke-Schmidt, Nordestgaard, Thudium, Moes Gronholdt, & Tybjaerg-Hansen, 2001; Snowdon et al., 1997; Troncoso et al., 2008; Yip et al., 2005; Zhu et al., 2000). The only correlation found between

ApoE-e4 allele status and cerebral infarcts was from a sample of older persons and not within an AD population (Schneider et al., 2005). Thus, ApoE-e4 allele status and cerebral infarcts have not been consistently correlated with each other and do not meet conditions (a) and (b) of the mediating hypothesis. According to condition (c) of the mediating hypothesis, the relationship between ApoE-e4 allele status and AD should no longer be significant if the mediating relationship (Paths *a* and *b*) is controlled. However, ApoE-e4 allele status will likely always be associated with an increased risk of developing AD, regardless of the status of cerebral infarcts. Therefore, cerebral infarcts are unlikely to be a mediating variable between ApoE-e4 allele status and AD because the conditions of the mediating hypothesis cannot be met.

Figure 3 is a modified version of the moderator hypothesis by Barron and Kenny (1986). "In general terms, a moderator is a . . . variable that affects the direction and/or strength of the relation between an independent or predictor variable and a dependent or criterion variable" (Baron & Kenny, 1986, p. 1174). The moderator hypothesis can also be referred to as effect modification and in statistical terms is represented as an interaction (Baron & Kenny, 1986). It is plausible that the presences of an ApoE-e4 allele may modify the impact that cerebral infarcts have on the likelihood of developing AD. As depicted in Figure 3, there are three causal pathways. ApoE-e4 allele status (Path *a*) represents the moderator (effect modifier) and has the ability to influence the outcome. Cerebral infarcts (Path *b*) represent the predictor variable, which also has the ability to influence the outcome, AD. Path *c* represents the interaction between ApoE-e4 allele status and cerebral infarcts, and, if significant, the moderator hypothesis is supported. Baron and Kenny (1986) suggest that ". . . it is desirable that the moderator variable be uncorrelated with both the predictor and the criterion (the dependent variable) to provide a clearly interpretable interaction term" (p. 1174). However, they also suggest that if the moderator

and predictor are correlated or main effects for the moderator (Path *a*) are significant, these relationships ". . . are not directly relevant conceptually to testing the moderator hypothesis" (Baron & Kenny, 1986, p. 1174). Therefore, it is plausible that ApoE-e4 allele status and cerebral infarcts could be working together through the moderator hypothesis (effect modification) to increase the likelihood of developing AD.

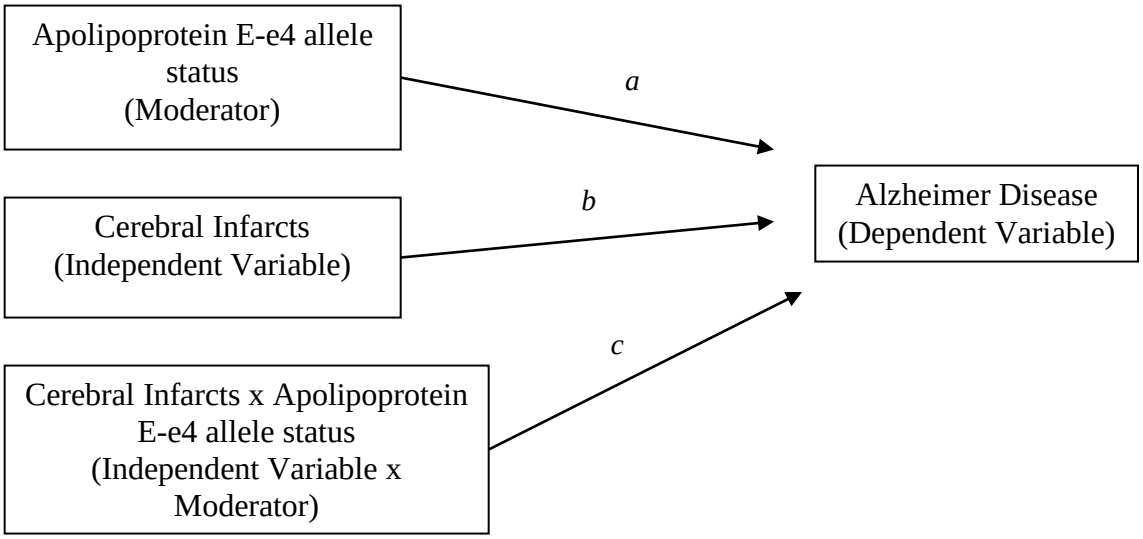

Figure 3. The Modified Moderator Hypothesis

ApoE allele status has been found to modify the relationship between history of stroke and AD; ApoE-e4 carriers with a history of stroke were more likely to have AD than ApoE-e4 carriers without a history of stroke (Johnston et al., 2000). However, this study accounted for history of clinical stroke, instead of both clinical and silent stroke. Silent strokes may be highly

influential in the development of AD because their prevalence increases with age and they have been thought to greatly contribute to cognitive decline. Currently there is no study that examines the moderator hypothesis between ApoE allele status and cerebral infarcts (both silent and clinical stroke) and the likelihood of developing AD.

2.4 Summary

Given the aging population, AD has become an increasing public health concern. The association between cerebral infarcts and AD has been established; however, the exact mechanisms by which cerebral infarcts influence the likelihood of developing AD are unknown. In addition, various studies have examined specific characteristics of cerebral infarcts (e.g., size, location, and type) and their effect on the likelihood of developing AD, but results have varied. The ApoE-e4 allele is an established risk factor for AD; however, the exact mechanisms by which the ApoE-e4 allele influences the likelihood of developing AD are also unknown.

ApoE-e4 allele carriers with a history of stroke are more likely than ApoE-e4 carriers without a history of stroke to develop AD, suggesting that cerebral infarcts and ApoE-e4 allele status are two risk factors that could potentially work together through effect modification to increase the likelihood of developing AD. However, no study has examined the relationship between cerebral infarcts (representing both clinical and silent strokes) and AD in the context of the ApoE-e4 allele.

3.0 Study Rationale and Research Questions

3.1 Rationale

The purpose of this study was to examine if ApoE-e4 allele status influences the strength of the relationship between cerebral infarcts and AD using data from the Nun Study, a longitudinal clinico-pathological study on aging.

Because all neuropathological information concerning cerebral infarcts was collected at post-mortem analysis, this study had information on both clinical and silent strokes. Silent strokes have been shown to be more prevalent in the general and older adult population than clinical strokes. Studies based solely on clinical strokes may underestimate the relationship between all pathologically present infarcts and AD. Thus, this investigation was able to capture the effect of all strokes on the likelihood of developing AD.

This study also had access to specific characteristics about cerebral infarcts (i.e., location and size) that may provide insight into how the infarcts are contributing to AD. Previous studies have reported trends and significant findings for infarct location and size in AD populations. However, none of these studies have examined characteristics of infarcts within the context of ApoE-e4 allele status as an effect modifier.

This investigation will expand our understanding of how cerebral infarcts are associated with AD, especially within the context of ApoE-e4 allele status. If significant associations are found, individuals who have risk factors for cerebral infarcts could be considered at high risk for the development of AD. Given the fact that vascular risk factors for stroke are modifiable through therapy, targeting these therapies towards high-risk individuals may delay the development or progression of AD.

3.2 Research Questions

The outcome of interest is AD, which was defined as meeting both clinical and pathological diagnostic criteria for AD (see section 4.4.2). The exposure of interest varies by research question.

Question 1A: Does the presence of cerebral infarcts increase the risk of developing AD?

Question 1B: Do specific characteristics of the infarcts increase the likelihood of AD?

i) Does the *location* of the cerebral infarct increase the risk of developing AD?

ii) Does the *size* of the cerebral infarct (i.e., large or lacunar) increase the risk of developing AD?

Question 2: Does the presence of an ApoE-e4 allele increase the risk of developing AD?

Question 3: Does the presence of an ApoE-e4 allele increase the risk of developing cerebral infarcts?

Question 4A: Does the presence of an ApoE-e4 allele act as an effect modifier in the relationship between the presence of cerebral infarcts and the risk of developing AD?

Question 4B: Does ApoE-e4 allele status modify the relationship between specific characteristics of cerebral infarcts and the risk of developing AD?

i) Does ApoE-e4 allele status modify the relationship between the *location* of the cerebral infarct and the risk of developing AD?

ii) Does ApoE-e4 allele status modify the relationship between the *size* of the cerebral infarct and the risk of developing AD?

4.0 Methods

4.1 Literature Search

The literature used for the review of research on AD was a collection of seminal articles mixed with current reviews. A search was conducted in Medline (1950-present) using the MeSH term Alzheimer disease. Current reviews were extracted for information and were also used to identify seminal articles. The extraction of articles was not intended to be systematic, but the chosen articles reflect established and current knowledge on AD.

For the literature review of research on cerebral infarcts, four searches were completed using the Medline database (1950-present) in October 2010. Limits applied to every search included human subjects, English language only, and no commentaries or editorials. Inclusion criteria consisted of appropriate exposure (cerebral infarcts or lacunes) and outcome variables (older persons, dementia, AD). The first search was performed using the following MeSH terms: Alzheimer disease AND cerebral infarcts AND causality. This search yielded 115 peer-reviewed results. Of these 115 articles, 66 were excluded based on unrelated title and abstract and 29 were excluded based on irrelevant outcome and exposure variables. Ultimately, 20 articles were selected for review. The second search was performed using the following MeSH terms: Alzheimer disease AND cerebral infarcts AND (senile plaques or neurofibrillary tangles). This search yielded 33 peer-reviewed results. Of these 33 articles, 15 were duplicates from the first search and 14 articles were excluded based on unrelated title or abstract. Ultimately, 4 articles were selected for review. The third search was performed using the following MeSH terms: Alzheimer disease AND cerebral infarcts AND (cognition OR neuropsychological tests). This search yielded 207 peer-reviewed results. Of these 207 articles, 140 were duplicates from the two previous searches, 37 were excluded based on unrelated title or abstract and 20 were excluded

based on irrelevant exposure or outcome variables. Ultimately, 10 articles were selected for review. The fourth search was a comprehensive search of all articles concerning cerebral infarcts, but excluded duplicates from previous searches. The fourth search was performed using the following terms: Alzheimer disease [MeSH] AND cerebral infarcts [MeSH] NOT (#1 OR #2 OR #3). Searches #1, #2, and #3 were described previously. Search #4 yielded 407 peer-reviewed results. Of these 407 articles, 268 were excluded based on unrelated title or abstract and 136 were excluded based on irrelevant exposure or outcome. Ultimately, 3 articles were selected for review.

The literature used for the review of research on ApoE and AD was a collection of seminal articles mixed with current reviews. A search was conducted in Medline (1950-present) in October 2010 using the MeSH term Apolipoprotein E. Current reviews were extracted for information and were also used to identify seminal articles. The extraction of articles was not intended to be systematic, but the chosen articles reflect established and current knowledge on ApoE. For literature regarding ApoE and cerebral infarcts, the Medline database (1950-present) was searched in October 2010 using the following MeSH terms: Apolipoprotein E AND cerebral infarcts. This search yielded 14 peer-reviewed results. After examination of the titles and abstracts, 5 articles were selected for review.

4.2 Data Source: The Nun Study

4.2.1 Study Population

The Nun Study is a longitudinal clinico-pathologic study on aging based in the United States. All participants were female and were members of a religious congregation, the School Sisters of Notre Dame. To be eligible, participants had to be 75 years or older at baseline (1991) and agree to participate in all aspects of the study. Of the 1031 eligible participants, 678 (66%)

agreed to comprehensive annual cognitive and physical assessments, review of medical records and access to convent archives, and brain donation after death. Participants did not differ significantly from non-participants in age, mortality rate, race, or country of birth (Tyas, Snowdon et al., 2007). Since 1991, 606 participants (90%) have died, reflecting the large number of participants who have been followed to completion. Of the 606 participants who have died, 547 eligible brains have complete neuropathologic evaluations.

Few studies in the world have a larger series of comprehensive clinical data collected before death and autopsied brains after death. In addition, the Nun Study is a unique sample because all participants have similar lifestyles, and as a result, the number of confounding factors present is minimized: participants were similar with respect to gender, access to medical care, access to diet, income, marital status, reproductive histories, social support, and alcohol and tobacco consumption.

4.2.2 Data Collection

Cognitive and physical assessments were performed annually. This study had access to twelve waves of these data. Global cognitive functioning was assessed by the MMSE. Cognitive function was evaluated using a battery of seven tests compiled by CERAD, which assessed memory, concentration, language, visuospatial ability, and orientation to time and place (Morris et al., 1989). All cognitive assessments were administered by trained field gerontologists. Physical function was assessed using the ADLs scale, which examines social and daily functioning. The results of the MMSE, CERAD, and ADL assessments were used for the diagnosis of dementia. ApoE genotyping was performed by standard laboratory methods, using buccal cells from living participants or frozen brain samples from deceased participants (Saunders et al., 1996).

Gross and microscopic evaluations were performed by one board-certified neuropathologist. This neuropathologist was blinded to clinical diagnosis, cognitive test scores, and functional assessments. As part of the gross evaluation, each brain was measured, described, and photographed. In addition, coronal sections (1.5 cm thick) of the cerebral hemispheres, brainstem, and cerebellum were cut, measured, described, and photographed. Sections were then taken from the frontal and temporal poles, middle frontal gyrus, superior and middle temporal gyri, inferior parietal lobule, occipital lobe, anterior and posterior cingulate gyri, and hippocampus (at the level of the lateral geniculate nucleus, entorhinal cortex, amygdala, and the basal ganglia). All microscopic specimens were processed in paraffin blocks and stained with hematoxylin and eosin, the modified Bielschowsky stain, and Gallyas stain. For additional information on the microscopic evaluation, see Snowdon et al. (1997). The neuropathologist determined the preliminary diagnosis for each participant based on their gross and microscopic evaluation. The final AD diagnoses were derived in a consensus conference and based on a complete review of the pathologic findings, clinical and functional data collected during the annual assessments, and a review of medical records.

Cerebral infarcts visible to the naked eye were identified, measured, and described during the gross examination of the intact brain and during examination of the coronal sections (1.5 centimeters thick). They were categorized as large (>1.5 centimeters) or lacunar (<= 1.5 centimeters). Cerebral infarct data were abstracted from a narrative of the gross assessment. Data abstraction forms were created in Access software to capture detailed characteristics of the cerebral infarcts. All infarct data abstracted from the gross examination reports were reviewed by at least two coders, one of whom was a board-certified neuropathologist.

4.3 Thesis Project

4.3.1 The Analytic Sample

The analytic sample was comprised of 199 subjects who had complete information on the exposure, outcome, and covariates of interest (see Figure 4). Of the 678 participants who agreed to enroll in the Nun Study, 606 participants have died and 547 brains have had complete neuropathologic evaluations. The remaining 59 brains without neuropathological evaluations are either awaiting assessment or are not eligible for assessment due to issues such as withdrawal of the participant from the study or problems with specimen transportation. From those 547 brains, participants were included if they had been evaluated for AD diagnosis. The last assessment before death and a modified version of the NIA-RI criterion was used to identify AD cases and controls (see Section 4.4.2). Participants were excluded if they did not have information on one or more variables of interest. The remaining participants represent the analytic sample for this research project.

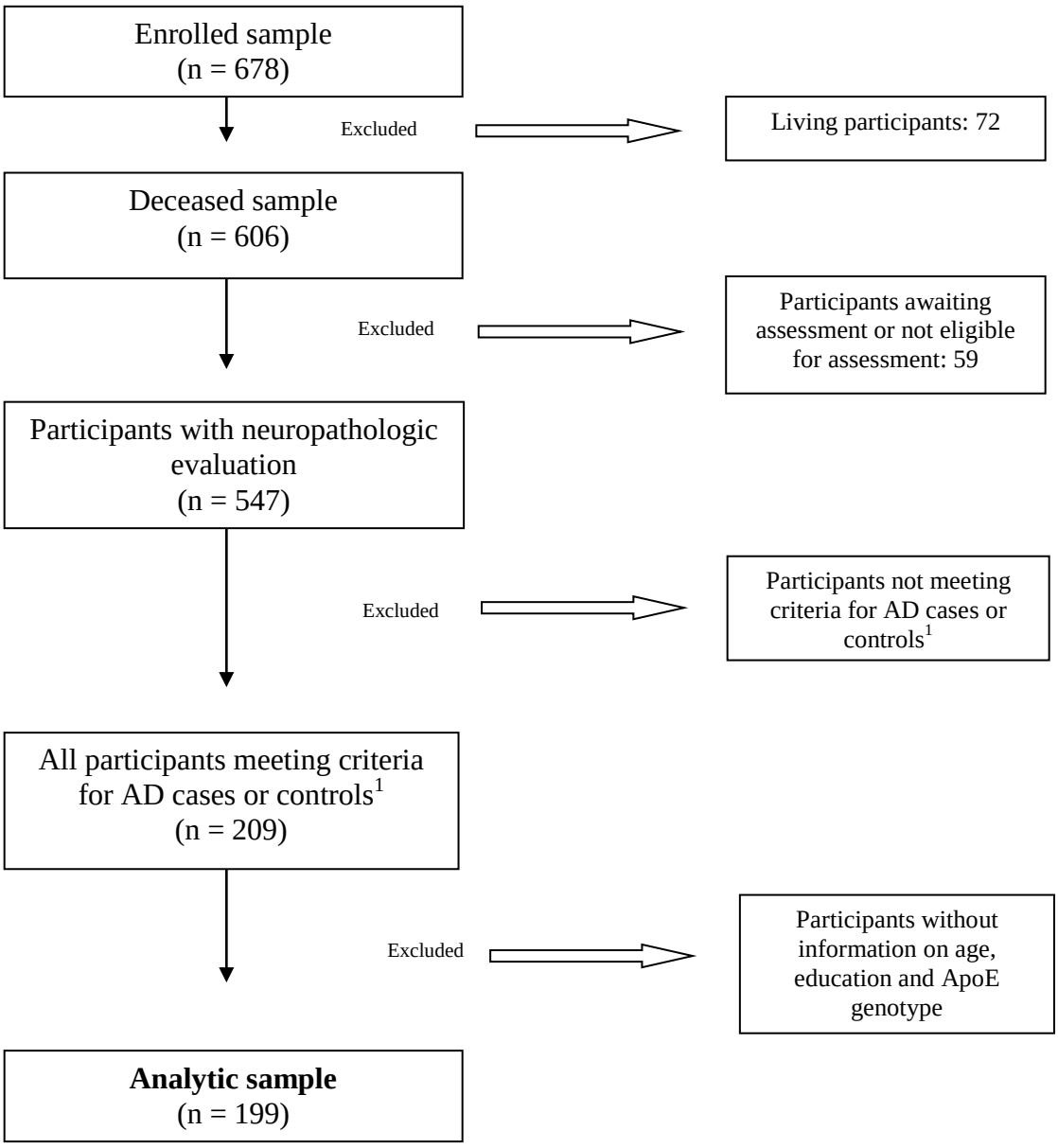

Figure 4. Description of Analytic Sample
[1] See section 4.4.2 for the definition of AD cases and controls

4.3.2 Assessing Non-Response Bias

In order to determine if the analytic sample differed significantly from those participants who were excluded during sample derivation, non-response bias analyses were conducted. Samples were assessed for differences based on presence of infarcts, age at death, educational level, or presence of an ApoE-e4 allele. Table 1 outlines the comparisons that were made.

Table 1. Sample comparisons while assessing non-response bias

	Deceased sample	Analytic sample (n=199)
Enrolled sample[†] (n=678)	✓	✓
Deceased sample (n=606)		✓

[†]Enrolled sample represents both living and deceased participants

The enrolled sample was statistically similar to the deceased and analytic samples with respect to educational level but had a significantly lower presence of the ApoE-e4 allele (see Appendix B, Tables 1 and 2). Presence of infarcts was not significantly different between the enrolled and analytic samples. However, presence of infarcts could not be tested between the enrolled and deceased samples because infarcts were only detected after death in this study.

Comparing the deceased sample with the analytic sample revealed that participants in the analytic sample were significantly younger at death and more likely to have an ApoE-e4 allele, but did not differ based on the presence of infarcts or on educational level (see Appendix, Table 4).

4.4 Measures

This study utilized variables collected from the annual cognitive and physical assessments, convent archives, and neuropathologic assessments. The selection of covariates was guided by

findings from the literature review. Appendix A presents a full list of variables involved in this investigation.

4.4.1 Exposure

The main exposure of interest was cerebral infarcts, which were assessed during the gross neuropathological assessment. The narrative of the gross neuropathologic assessment was transcribed and relevant data were abstracted into a database. The presence of infarcts was determined by a neuropathologist who described any infarcts in the brain visible to the naked eye. These infarct data were then categorized according to specific characteristics: type, size, and location. The type of infarct was classified as hemorrhagic or ischemic. The size of infarct was classified as large (> 1.5 cm) or lacunar (<= 1.5 cm). Finally, the location of the infarct was categorized into cortex (frontal, occipital, parietal, temporal, calcarine, cingulate, and insular cortexes), white matter (internal capsule, external capsule, centrum semiovale, and white matter: area not specified), basal ganglia (caudate nucleus, globus pallidus, putamen, and basal ganglia: area not specified), limbic system (amygdala, anterior commissure, hippocampus, hypothalamus, internal capsule, mammillary bodies, and thalamus), or vascular system (internal carotid artery, middle cerebral artery, and posterior cerebral artery). For the purpose of this proposed project, the main locations (i.e., cortex, white matter, basal ganglia, and limbic system) were analyzed.

4.4.2 Outcome

The outcome of interest for this research project was AD, which was assessed clinically as dementia using the last cognitive assessment before death, and pathologically, through a modified version of the NIA-RI diagnostic criterion. Thus for this study, any reference to the outcome of AD should be interpreted as including both clinical and pathological data. Any reference to the outcome of dementia should be interpreted as a clinical diagnosis without

pathological confirmation. Finally, any reference to the outcome of pathological AD should be interpreted as a pathology-based diagnosis without clinical confirmation.

The last cognitive assessment before death was used to determine a clinical diagnosis of dementia based on the following definition: (1) impairment in memory (defined as a score <4 on the Delayed Word Recall Test); (2) impairment in at least one other area of cognition (defined as scores of <11 on Verbal Fluency, <13 on Boston Naming, or <8 on Constructional Praxis); (3) impairment in social or daily functioning (defined as the inability to use a telephone, handle money, or dress oneself); and (4) decline in function from a previous level attributed to cognitive impairment. Details on the clinical diagnosis of dementia can be found in previous Nun Study publications (e.g. Snowdon et al., 1997; Tyas et al., 2007). Because the focus of this project concerns cerebral infarcts present at death, analyses focused on results from the last cognitive assessment before death. Participants were diagnosed as cognitively intact if they scored within 1.5 standard deviations of the age-appropriate mean for tests in the CERAD neuropsychological battery, scored intact according to the MMSE, and were independent for ADLs (Tyas, Snowdon et al., 2007).

A modified version of the neuropathologic NIA-RI criterion was used to categorize participants based on their AD pathology levels. The NIA-RI criterion categorizes participants into low, intermediate, or high likelihood that dementia during life was due to AD, based on a correlation between Braak staging and CERAD levels (Table 1). Overall, the NIA-RI criterion was selected over other available diagnostic data (e.g., CERAD criterion) because it quantifies both senile plaques (neuritic type) and neurofibrillary tangles. However, one major limitation to the NIA-RI criterion is that it assumes perfect correlation between plaques and tangles and results in many uncategorized participants with unique distributions and densities. As depicted

in Table 2, numerous participants will be left uncategorized if they do not fall within the assumed correlated shaded areas, and, ultimately, will be excluded from diagnosis.

Table 2. Composition of likelihood categories for the original NIA-RI diagnostic criterion

	Braak Staging[2]						
CERAD[1]	0	I	II	III	IV	V	VI
0	No AD Pathology						
A		Low Likelihood					
B				Intermediate Likelihood			
C						High Likelihood	

[1] measures neuritic plaque density (all participants 75+ years): 0 = none; A = sparse; B = moderate; C = frequent

[2] measures neurofibrillary tangle distribution: all six stages represent an increasing degree of severity within specific areas of the brain

Note: Shaded areas represent NIA-RI classification categories and assume correlation between the two criteria; individuals falling outside the shaded areas are uncategorized and thus excluded from diagnosis

Thus, a modified version of the criterion was developed to allow for a larger proportion of participants to be diagnosed and included into the likelihood categories (Table 3). The modified version allows for more participants to fall specifically within the low and high likelihood categories. The intermediate likelihood category was not an overall concern for modification, as participants with Braak stage III or IV and a CERAD level B may represent a transition between silent and clinical periods of the disease and thus reflect a highly variable category. The low likelihood category was expanded to include all participants that had Braak

stages 0, I, or II and CERAD level 0 or A as these categories represent clinically silent periods of the disease and/or provide uncertain evidence of AD (Braak & Braak, 1991). The high likelihood category was expanded to include participants with a CERAD level B. Research has suggested that neuritic plaques and neurofibrillary tangles have a weak association in older populations (90+ years) and that greater densities are not always required for a diagnosis (Haroutunian et al., 2008). In addition, the CERAD neuropathologic criteria have been criticized as being too restrictive, with level B often enough to account for cognitive impairment (Haroutunian et al., 1999). Thus, the high likelihood category was relaxed based on neuritic plaque density to account for potential neuropathologic variability in this older population.

Table 3. Composition of the likelihood categories for the modified NIA-RI diagnostic criterion

	Braak Staging[2]						
CERAD[1]	0	I	II	III	IV	V	VI
0	Low Likelihood						
A							
B				Intermediate Likelihood		High Likelihood	
C							

[1] measures neuritic plaque density (all participants 75+ years): 0 = none; A = sparse; B = moderate; C = frequent

[2] measures neurofibrillary tangle distribution: all six stages represent an increasing degree of severity within specific areas of the brain

Note: Shaded areas represent the modified NIA-RI classification categories and assume correlation between the two criteria; individuals falling outside the shaded areas are uncategorized and thus excluded from diagnosis

Participants that fell into the category of ‘high likelihood’ for the modified NIA-RI criterion were considered pathologically confirmed cases of AD. Participants that fell in the intermediate category were not considered cases or controls since this category could potentially represent a transition period. Participants that fell into the ‘low likelihood’ category were considered pathologically confirmed non-AD controls.

In summation, AD cases were defined as participants who were diagnosed as clinically demented at the last cognitive assessment before death and were diagnosed based on the neuropathologic assessment as having a ‘high likelihood’ of AD according to the modified NIA-RI criterion. Controls were considered participants that were not demented at the last cognitive assessment before death and were diagnosed as ‘low likelihood’ of AD according to the modified NIA-RI criterion.

4.4.2.1 Sensitivity Analyses

The outcome of interest for this study was clinically and pathologically defined AD. To ensure findings were not related to how AD pathology was defined within the AD diagnosis (using the modified NIA-RI neuropathologic diagnostic criteria), two alternative definitions for AD pathology using NIA-RI were also tested. The first alternative of the NIA-RI criterion is outlined in Table 4. The low likelihood and intermediate likelihood categories are the same as the modified definition; however, the high likelihood category represents the original NIA-RI criterion definition. This definition of AD pathology was then combined with dementia status for an AD diagnosis. The second alternative of the NIA-RI criterion is outlined in Table 5. Again, the low likelihood and intermediate likelihood categories are the same as the modified definition; however, the high likelihood category includes Braak stage IV. This definition of AD pathology was then combined with dementia status for an AD diagnosis.

Other studies may define AD based solely on AD pathology or solely on clinical dementia. Another sensitivity analysis was conducted using samples that separated these two criteria out from the original AD definition. To test AD pathology as an outcome, AD cases were defined based on the modified NIA-RI neuopathologic diagnostic criteria. To test dementia as an outcome, AD cases were defined based on dementia status, assessed by the last clinical assessment before death.

Table 4. Composition of likelihood categories for the first alternative version of the NIA-RI diagnostic criterion

	Braak Staging[2]						
CERAD[1]	0	I	II	III	IV	V	VI
0	Low Likelihood						
A							
B				Intermediate Likelihood			
C						High Likelihood	

[1] measures neuritic plaque density (all participants 75+ years): 0 = none; A = sparse; B = moderate; C = frequent
[2] measures neurofibrillary tangle distribution: all six stages represent an increasing degree of severity within specific areas of the brain
Note: Shaded areas represent alternative NIA-RI classification categories and assume correlation between the two criteria; individuals falling outside the shaded areas are uncategorized and thus excluded from diagnosis

Table 5. Composition of likelihood categories for the second alternative version of the NIA-RI diagnostic criterion

	Braak Staging[2]						
CERAD[1]	0	I	II	III	IV	V	VI
0	Low Likelihood						
A							
B				Intermediate Likelihood			
C					High Likelihood		

[1] measures neuritic plaque density (all participants 75+ years): 0 = none; A = sparse; B = moderate; C = frequent
[2] measures neurofibrillary tangle distribution: all six stages represent an increasing degree of severity within specific areas of the brain
Note: Shaded areas represent alternative NIA-RI classification categories and assume correlation between the two criteria; individuals falling outside the shaded areas are uncategorized and thus excluded from diagnosis

4.4.3 Covariates

Covariates are variables that have the ability to modify or confound the association between exposure and outcome. Age is a strong risk factor for both AD and stroke; thus, age at death was included as a covariate. Age was analyzed as a continuous variable. Educational level was also included as a covariate since low educational level is an established risk factor for developing AD. In addition, low educational level may also be related to environmental risk factors that increase the risk of stroke. Educational data were collected from archival records and have been categorized into the following three levels: high school or less, Bachelor's degree, and Masters degree or higher. Finally, the presence of an ApoE-e4 allele, a genetic susceptibility factor for AD, was included as a covariate if not already acting as an exposure variable or effect modifier. ApoE-e4 status was categorized as presence (yes or no), but for descriptive purposes was also categorized as zero, one, or two e4 alleles.

4.5 Ethics

The Nun Study was initiated at the University of Kentucky, where the study obtained original ethics clearance for access to convent archives, annual cognitive and physical assessments during life and for brain donation after death. The Nun Study has since moved to the University of Minnesota. Ethics clearance and confidentiality of the data are maintained at all times. At the University of Waterloo, all participants are coded by study ID number only and there are no personal identifiers (i.e., names). Any paper copies of data are stored in locked filing cabinets and only select staff have access. All electronic data are stored on password-protected computers in locked areas. The Office of Research Ethics at the University of Waterloo has provided ethics clearance for this project (ORE # 16551).

4.6 Data Analysis

All analyses were performed using SAS version 9.2 (SAS Institute Inc, Cary, North Carolina).

4.6.1 Descriptive Analyses

Exploratory analyses were conducted using univariate and bivariate procedures. Univariate analyses were performed on all measures for the enrolled sample (n=678) and the deceased sample (n=606). In the bivariate analyses, Pearson chi-square tests with Yates continuity correction as appropriate were used to measure associations between categorical variables. When more than 25% of the cells had expected values less than 5, the significance level was obtained from the Fisher's exact test. Independent sample t-tests were used to assess the association between continuous and dichotomous variables. To obtain the significance level for the t-tests, the pooled variance method was utilized if variances of a given variable were found to be equal or the Satterthwaite method was utilized if variances of a given variable were found to be unequal. Characteristics of the enrolled, deceased, and analytic samples were all compared to assess non-response bias and are presented in Appendix B. Odds ratios (OR) were calculated with 95% confidence intervals (CI) to test the strength of association between variables when appropriate.

4.6.2 Multivariate Analyses

The plan of multivariate analyses for each research question is outlined in Appendix C. The influence of exposure variables, covariates, and effect modifiers on the outcome of interest was assessed using logistic regression. Hierarchical backward elimination was used as the preferred method of variable selection for the logistic regression models (Tyas, Koval, & Pederson, 2000). This method is preferred over other standard selection methods as it has been

shown to yield a lesser mean squared error, regardless of significance level used, when compared to forward selection (Kennedy & Bancroft, 1971). The significance levels for variable selection in backward elimination regression models were 0.15 for main effects and 0.05 for first-order interaction terms. If the exposure variable of interest or any of the *a priori* covariates were eliminated from the model, these variables were forced into the final regression model to provide estimates of associations. Crude models were calculated first, followed by a model adjusted for the covariates age at death and educational level (shown as adjusted-i in result tables), and then a model adjusted for age at death, educational level, and ApoE-e4 allele status (shown as adjusted-ii in result tables). The two adjusted models were calculated to observe how the association between the exposure and outcome varied with and without the ApoE-e4 allele as a covariate. OR were calculated using the PROC LOGISTIC procedure in SAS with the profile likelihood-based estimate option for 95% confidence intervals (CI).

In order to judge how well the data fit the logistic regression models, lack of fit analyses and regression diagnostics (collinearity and residual tests) were performed. The Hosmer-Lemeshow goodness of fit (H-L GOF) test was performed on each model using the LACKFIT option in the PROC LOGISTIC procedure. Models were rejected when $p<0.05$ for the H-L GOF test. Multicollinearity tests were conducted on each final model using the PROC REG procedure with the COLLIN option. All final models were also analyzed for residual diagnostics using the INFLUENCE option under the PROC LOGISTIC procedure. To determine which observations were influential on the fit of a model, a critical value of ±1.96 was used when examining calculated values of DFBETA, C and CBAR. When an influential observation was found, it was deleted, and the model reran. This method was employed until influential observations were no longer present or until an OR was unable to be computed due to an inadequate sample size.

5.0 Results

5.1 Descriptive Statistics

Of the 199 participants, 114 (55%) were diagnosed with AD, while the remaining 85 (45%) were identified as controls (Table 6). Overall, the presence of cerebral infarcts was 37.9%; there was no significant difference in the presence of cerebral infarcts by AD status. Location of infarcts did not significantly differ between participants in the AD or control groups; however, infarcts were most prevalent in the cortex, white matter, and basal ganglia for all participants. Relatively few AD participants had an infarct in the vascular area (0.9%) and there were no infarcts present in the vascular area for controls. Size of infarcts did not differ based on outcome status, but lacunar infarcts were the most prevalent compared to large or both large and lacunar infarcts. Almost all of the participants in this sample had an ischemic infarct (99.5%). Participants who developed AD were significantly older at death (91.2 years versus 87.6 years; $p<0.0001$), had a lower educational level (24.7% versus 7.1% high school or less; $p<0.001$), and had a higher prevalence of an ApoE-e4 allele (49.2% versus 9.4%; $p<0.001$) than participants without AD.

Table 6. Descriptive characteristics of the sample by Alzheimer's disease status (n=199)

Variable		Total (n=199)	Controls (n=85)	AD (n=114)
Cerebral infarcts (%)	Presence	37.9	35.3	38.6
Location:	Cortex	14.6	11.8	16.7
	White matter area	15.1	14.1	15.8
	Basal ganglia	18.4	22.4	15.8
	Limbic system	7.5	7.1	7.9
	Vascular	0.5	0	0.9
Size:	Large[2]	9.42	9.8	9.1
	Lacunar[3]	25.2	23.6	26.3
	Both large & lacunar[4]	12.6	11.3	13.6
Type:	Ischemic	99.5	100	99.1
	Hemorrhagic	0.5	0	0.9
Age at death ** (mean years [SD])		89.7 (5.33)	87.6 (5.43)	91.2 (4.71)
Educational level[1]*(%)	High school or less	17.1	7.1	24.7
	Bachelors degree	39.2	36.5	41.2
	Masters degree +	43.7	56.5	34.2
ApoE-e4 allele (%)				
Presence**	(1+)	32.2	9.4	49.2
Number of alleles**	Zero	67.8	90.6	50.9
	One	29.2	8.2	44.7
	Two	3.0	1.9	4.4

Abbreviations: ApoE-e4 = Apolipoprotein E e4; AD = Alzheimer's disease

*p<0.001;**p<0.0001

[1]Statistical significance reflects the differences between the sample with AD (cases) and the sample without AD (controls)

[2]n=138

[3]n=167

[4]n=143

5.2 Multivariate Logistic Regression Models

Please see Section 4.6.2 for the model selection techniques that were employed for each research question. An example of the analytic plan for each research question can be found in Appendix C. Logistic regression models, crude and adjusted, were developed for each research question. If backwards elimination did not allow any main effects to stay in the model, they were forced into the model due to *a priori* hypotheses. Unless otherwise stated, all first-order interactions between covariates and the presence of cerebral infarcts were non-significant. Several outliers were present in each model. Please see section 4.6.2 for methods regarding significant outliers. After several rounds of exclusion of individual observations, models did not improve and the number of observations became too small to compute an OR estimate for ApoE-e4 (see Appendix E for example). Since there was no evidence that the data were incorrectly transcribed or translated and the outliers did not have a significant impact on the original interpretation of these models, the influential observations were retained in the final models.

5.2.1 Research Question 1A

Logistic regression models were developed to examine if the presence of cerebral infarcts was associated with an increased risk of developing AD (Table 7). The presence of infarcts was not significantly associated with AD in either the crude (OR = 1.15; 95% CI = 0.65, 2.01) or the final adjusted model (OR = 1.04; 95% CI = 0.51, 2.10) (see Table 8). Age at death (OR = 1.14; 95% CI = 1.06, 1.23) and the presence of an ApoE-e4 allele (OR = 10.6; 95% CI = 4.69, 26.93) were both significantly associated with an increased risk of AD. An educational level of high school or less was associated with a significantly higher risk of AD than a graduate level university education (OR = 5.30; 95% CI = 1.83, 17.22).

Table 7. The association between presence of cerebral infarcts and Alzheimer's disease (n=199)

Model	**Exposure** OR (95% CI)	**Covariates** OR (95% CI)			
	Presence of Infarcts	Age at death	Educational level[1]		ApoE-e4[2]
			High school or less	Bachelors degree	
Crude	1.15 (0.65, 2.01)	-	-	-	-
Adjusted - i	0.99 (0.52, 1.87)	**1.14 (1.07, 1.21)**	**4.12 (1.55, 12.42)**	1.42 (0.73, 2.75)	-
Adjusted - ii	1.04 (0.51, 2.10)	**1.14 (1.06, 1.23)**	**5.30 (1.83, 17.22)**	1.68 (0.80, 3.58)	**10.60 (4.69, 26.93)**

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+
[2] Reference category is the presence of zero ApoE-e4 alleles
Bolded values indicate significance

5.2.2 Research Question 1B – Location

Logistic regression models were developed to examine if location of cerebral infarcts was associated with an increased risk of developing AD. The four locations analyzed were cortex, white matter, basal ganglia, and limbic system.

In the crude and adjusted models, infarcts located in the cortex, white matter, basal ganglia, or limbic system were not associated with an increased risk of AD (Table 8). In all final adjusted models, age at death, educational level of high school or less, and the presence of an ApoE-e4 allele were significantly associated with an increased risk of AD (Table 8). In all final models, the H-L GOF test values were all significant, suggesting the data did not adequately fit the models. Thus, results presented in Table 8 should be interpreted with caution.

Table 8. The association between Alzheimer's disease and the location of at least one cerebral infarct (n=199)

Model	**Exposure**	**Covariates**			
	Infarcts	Age at death	Education[1]		ApoE-e4[2]
			High school or less	Bachelors degree	
Cortex[3]					
Crude	1.41 (0.62, 3.42)	-	-	-	-
Adjusted - i	1.43 (0.59, 3.63)	**1.13 (1.05, 1.22)**	**3.54 (1.20, 12.12)**	1.64 (0.78, 3.48)	-
Adjusted - ii	1.39 (0.52, 3.88)	**1.12 (1.04, 1.22)**	**5.01 (1.54, 18.79)**	1.99 (0.85,4.74)	**11.47 (4.53, 33.91)**
White Matter[3]					
Crude	1.18 (0.53, 2.71)	-	-	-	-
Adjusted - i	1.14 (0.48, 2.77)	**1.14 (1.06, 1.24)**	**4.06 (1.26, 15.80)**	1.69 (0.81, 3.54)	-
Adjusted - ii	1.02 (0.38, 2.77)	**1.14 (1.05, 1.25)**	**5.42 (1.52, 23.06)**	2.06 (0.88, 4.94)	**12.73 (5.01, 37.91)**
Basal Ganglia[3]					
Crude	0.74 (0.35, 1.56)	-	-	-	-
Adjusted - i	0.61 (0.27, 1.37)	**1.14 (1.07, 1.23)**	**4.63 (1.46, 17.91)**	1.16 (0.56, 2.39)	-
Adjusted - ii	0.47 (0.18, 1.19)	**1.14 (1.05, 1.24)**	**6.07 (1.70, 26.41)**	1.73 (0.81, 3.71)	**13.70 (5.54, 38.91)**
Limbic System[3]					
Crude	1.18 (0.40, 3.70)	-	-	-	-
Adjusted - i	0.94 (0.28, 3.21)	**1.12 (1.04, 1.21)**	**5.17 (1.46, 24.51)**	1.58 (0.73,3.42)	-
Adjusted - ii	1.32 (0.37, 4.91)	**1.12 (1.03, 1.22)**	**5.64 (1.44, 28.75)**	1.75 (0.73, 4.26)	**12.00 (4.45, 39.11)**

Note: All final adjusted models had significant Hosmer-Lemeshow goodness of fit test values

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval

[1] Reference category is Masters degree+

[2] Reference category is the presence of zero ApoE-e4 alleles

[3]Comparison group was participants without infarcts

5.2.3 Research Question 1B – Size

Logistic regression models were developed to examine if size of cerebral infarcts was associated with an increased risk of AD. The size of cerebral infarcts were analyzed based on if participants had either large, lacunar, or both large and lacunar infarcts.

In the crude and adjusted models, large, lacunar, or both large and lacunar infarcts were not significantly associated with AD compared to individuals without an infarct (Table 9). In all final adjusted models, age at death, an educational level of high school or less, and the presence of an ApoE-e4 allele were all associated with an increased risk of developing AD (Table 9).

Table 9. The association between size of cerebral infarct and Alzheimer's disease (n=199)

Model	**Exposure**	**Covariates**			
	Infarcts	Age at death	Education[1]		ApoE-e4[2]
			High school or less	Bachelors degree	
Large[3]					
Crude	0.92 (0.29, 3.00)	-	-	-	-
Adjusted - i	1.07 (0.31, 3.79)	**1.13 (1.05, 1.23)**	**4.42 (1.34, 17.76)**	1.73 (0.79, 3.79)	-
Adjusted - ii	1.63 (0.43, 6.24)	**1.12 (1.04, 1.22)**	**4.73 (1.33, 20.08)**	2.01 (0.85, 4.85)	**8.88 (3.41, 26.78)**
Lacunar[3]					
Crude	1.16 (0.57, 2.38)	-	-	-	-
Adjusted - i	0.88 (0.40, 1.92)	**1.14 (1.06, 1.23)**	**3.38 (1.15, 11.44)**	1.34 (0.66, 2.74)	-
Adjusted - ii	0.86 (0.37, 2.03)	**1.15 (1.06, 1.25)**	**3.79 (1.18, 13.86)**	1.48 (0.66, 3.33)	**10.72 (4.44, 29.08)**
Both Large and Lacunar[3]					
Crude	1.24 (0.46, 3.55)	-	-	-	-
Adjusted - i	1.22 (0.41, 3.80)	**1.13 (1.05, 1.23)**	**5.18 (1.47, 24.56)**	1.53 (0.71, 3.33)	-
Adjusted - ii	1.05 (0.31, 3.75)	**1.13 (1.04, 1.24)**	**7.11 (1.80, 36.65)**	1.86 (0.76, 4.69)	**14.76 (5.44, 48.59)**

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval

[1] Reference category is Masters degree+

[2] Reference category is the presence of zero ApoE-e4 alleles

[3]Comparison group was participants without infarcts

5.2.4 Research Question 2

Logistic regression models were developed to examine if the presence of one or more ApoE-e4 alleles was associated with an increased risk of developing AD. Backwards elimination forced all main effects in the model.

The presence of an ApoE-e4 allele was significantly associated with AD in the crude model (OR = 9.30; 95% CI = 4.32, 22.47). When age at death and educational level were added to the model, the presence of an ApoE-e4 allele remained significantly associated with AD (OR = 10.61; 95% CI = 4.68, 26.87). Age at death (OR = 1.14; 95% CI = 1.06, 1.22) and an educational level of high school or less (OR = 5.32; 95% CI = 1.84, 17.24) were both significantly associated with an increased risk of AD (Table 10).

Since the interaction term between presence of an ApoE-e4 allele and educational level was significant, the model was stratified by educational level. There was a significant association between presence of an ApoE-e4 allele and AD when compared to participants without an ApoE-e4 allele, but only in participants with a Bachelors or Masters degree+ level of education (Table 11).

Table 10. The association between presence of an ApoE-e4 allele and Alzheimer's disease (n=199)

Model	**Exposure** OR (95% CI)	**Covariates** OR (95% CI)		
	ApoE-e4[2]	Age at death	Educational level[1]	
			High school or less	Bachelors degree
Crude	**9.30 (4.32, 22.47)**	-	-	-
Adjusted	**10.61 (4.68, 26.87)**	**1.14 (1.06, 1.22)**	**5.32 (1.84, 17.24)**	1.68 (0.80, 3.57)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+
[2] Reference category is the presence of zero ApoE-e4 alleles
Bolded values indicate significance

Table 11. The association between presence of an ApoE-e4 allele and AD, stratified by educational level (n=199)

Model	**Exposure**	**Covariates**
	Presence of an ApoE-e4 allele	Age at death
High school or less (n=34)		
Crude	3.24 (0.44. 66.41)	-
Adjusted	3.30 (0.41, 71.72)	1.16 (0.98, 1.41)
Bachelors degree (n=78)		
Crude	**5.00 (1.63, 18.92)**	-
Adjusted	**5.32 (1.70, 20.62)**	1.09 (0.99, 1.21)
Masters degree+ (n=87)		
Crude	**26.81 (7.96, 125.01)**	-
Adjusted	**24.20 (6.89, 117.15)**	**1.18 (1.04, 1.37)**

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval

5.2.5 Research Question 3

Logistic regression models were developed to examine if the presence of an ApoE-e4 allele was associated with the presence of cerebral infarcts. The presence of an ApoE-e4 allele was not significantly associated with the presence of cerebral infarcts (Table 12, crude OR = 0.92; 95% CI = 0.50, 1.71), and remained non-significant after adjustment for covariates (adjusted OR = 0.88; 95% CI = 0.47, 1.64). Age at death and educational level were also not significantly associated with the presence of infarcts in either crude or adjusted models.

Table 12. The association between presence of an ApoE-e4 allele and cerebral infarcts (n=199)

Model	**Exposure** OR (95% CI)	**Covariates** OR (95% CI)		
	ApoE-e4[2]	Age at death	Educational level[1]	
			High school or less	Bachelors degree
Crude	0.92 (0.50, 1.71)	-	-	-
Adjusted	0.88 (0.47, 1.64)	1.03 (0.97, 1.09)	1.39 (0.60, 3.20)	0.80 (0.41, 1.55)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+
[2] Reference category is the presence of zero ApoE-e4 alleles
Bolded values indicate significance

5.2.6 Research Question 4A

To assess if the ApoE-e4 allele acted as an effect modifier in the association between cerebral infarcts and AD, models were stratified by ApoE-e4 status. The presence of infarcts was not significantly associated with AD among those with or without an ApoE-e4 allele (Table 13).

Table 13. The association between presence of infarcts and Alzheimer's disease, stratified by ApoE-e4 allele status (n=199)

Model	**Exposure**	**Covariates**		
	Presence of Cerebral Infarcts	Age at death	Education[1]	
			High school or less	Bachelors degree
ApoE-e4 + (n = 135)				
Crude	0.93 (0.21, 4.90)	-	-	-
Adjusted	0.86 (0.19, 4.60)	1.01 (0.86, 1.20)	1.31 (0.14, 28.51)	0.59 (0.10, 3.00)
ApoE-e4 – (n = 64)				
Crude	1.31 (0.65, 2.64)	-	-	-
Adjusted	1.05 (0.46, 2.35)	**1.16 (1.07, 1.26)**	**7.38 (2.24, 27.78)**	2.19 (0.92, 5.27)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+

5.2.7 Research Question 4B – Location

When location of infarcts was stratified by presence of an ApoE-e4 allele in the AD sample, all locations were non-significant compared to participants without any infarcts (see Appendix G for results). An OR was unable to be computed for infarcts located in the limbic system due to insufficient sample size. All location models had significant H-L GOF test values, which indicate that the model does not adequately fit the data. The OR and 95% CI for all locations regarding the outcome AD should be interpreted with caution.

5.2.8 Research Question 4B – Size

When stratified by ApoE-e4 allele status, large, lacunar or both large and lacunar infarcts were not significantly associated with AD (see Appendix G for results). These results should be interpreted with caution as the H-L GOF test values were significant, indicating the model had a lack of fit. In addition, an OR was unable to be calculated for the presence of both large and lacunar infarcts when stratified by the presence of an ApoE-e4 allele, likely due to a sample size issue.

5.3 Results from Sensitivity Analyses

The first research question was analyzed using AD cases and controls as defined by the first NIA-RI neuropathologic alternative definition presented in section 4.4.2.1; results were similar to the results obtained using the primary AD definition above (see Appendix D, Table 1). Similarly, the first research question was analyzed using AD cases and controls as defined by the second NIA-RI neuropathologic alternative definition presented in section 4.4.2.1; results were also similar to the results obtained above (see Appendix D, Table 2).

When the outcome represented solely AD pathology status, the presence of infarcts was not significantly associated with AD pathology in the crude or adjusted models (see Appendix D, Table 3). Thus, the results were similar to results presented above. When the outcome represented solely dementia status, the presence of infarcts did significantly increase the risk of dementia in both crude and adjusted models (see section 5.3.2.1). Thus, the research questions were repeated using clinical dementia status as the outcome. Please see section 4.4.2 for the definition of clinical dementia.

5.3.1 Descriptive Statistics

After the exclusion of participants without information on the outcome, exposure, or covariates, 462 participants were included in the dementia sample. Of those 462 participants, 231 (50%) were clinically diagnosed with dementia at their last cognitive assessment, while the remaining 231 (50%) were diagnosed as non-demented (Table 14). Overall, the presence of cerebral infarcts was 34.6%; participants with dementia were significantly more likely to have cerebral infarcts present compared to participants without dementia ($p<0.001$). Infarcts located in the white matter area, basal ganglia, and limbic system did not significantly differ between participants with dementia and participants without dementia. However, participants with

dementia had a significantly higher prevalence of infarcts located in the cortex compared to controls. Cerebral infarcts in the vascular area were relatively rare. Lacunar infarcts were more common than large infarcts. The prevalence of large or lacunar infarcts did not differ significantly by dementia status; however, participants with dementia had a significantly higher prevalence of both large and lacunar infarcts compared to non-demented participants. Almost all of the participants in this sample had an ischemic infarct (96.9%). Participants with dementia were significantly older at death (91.6 years versus 88.9 years; $p<0.0001$), had a lower educational level (25.1% versus 10.0% high school or less; $p<0.01$), and had a higher presence of an ApoE-e4 allele (31.6% versus 18.2%; $p<0.01$).

Table 14. Descriptive characteristics of the participants in the dementia sample (n=462)

Variable		**Total** (n=462)	**No Dementia** (n=231)	**Dementia** (n=231)
Cerebral infarcts[1] (%)	Presence**	34.6	27.3	42.0
Location:	Cortex*	18.2	12.6	23.8
	White matter area	15.8	12.6	19.1
	Basal ganglia	17.8	13.4	22.5
	Limbic system	6.7	5.6	7.7
	Vascular	0.9	0.4	1.30
Size:	Large[2]	8.8	5.6	12.4
	Lacunar[3]	21.4	12.7	25.6
	Both large & lacunar[4]*	13.5	8.7	18.8
Type:	Ischemic	96.9	98.3	95.6
	Hemorrhagic	3.1	1.7	4.4
Age at death*** (mean years [SD])		90.2 (5.29)	88.9 (5.10)	91.6 (5.15)
Educational level[1]*** (%)	High school or less	17.5	10.0	25.1
	Bachelors degree	42.4	42.4	42.4
	Masters degree +	40.0	47.6	32.5
ApoE-e4 allele (%)				
Presence*	(1+)	24.9	18.2	31.6
Number of alleles*	Zero	75.1	81.8	68.4
	One	21.9	15.6	28.1
	Two	3.0	2.6	3.5

Abbreviations: ApoE-e4 = Apolipoprotein E e4
*p<0.01; **p<0.001;***p<0.0001
[1]Statistical significance reflects the differences between the sample with dementia (cases) and the sample without dementia (controls)
[2]n=331
[3]n=384
[4]n=349

5.3.2 Multivariate Logistic Regression Models

Please see section 4.6.2 for the model selection techniques that were employed for each research question. Logistic regression models, crude and adjusted, were developed for each research question. If backwards elimination did not allow any main effects to stay in the model, they were forced into the model due to *a priori* hypotheses. Unless otherwise stated, all first-order interactions between covariates and the presence of cerebral infarcts were non-significant. Several outliers were present in each model. Please see section 4.6.2 for methods regarding significant outliers. After several rounds of exclusion of individual observations, models did not improve and the number of observations became too small to compute an OR estimate for ApoE-e4 (see Appendix E for example). Since there was no evidence that the data were incorrectly transcribed or translated and the outliers did not have a significant impact on the original interpretation of these models, the influential observations were retained in the final models.

5.3.2.1 Research Question 1A

The presence of infarcts was significantly associated with an increased risk of dementia in the crude (OR = 1.93; 95% CI = 1.31, 2.86) and final adjusted model (OR = 1.92; 95% CI = 1.27, 2.93) (see Table 15). Age at death (OR = 1.10; 95% CI = 1.06, 1.15) and the presence of an ApoE-e4 allele (OR = 2.57; 95% CI = 1.62, 4.10) were both significantly associated with an increased risk of dementia. An educational level of high school or less was significantly associated with a significantly higher risk of dementia than a graduate university education (OR = 3.19; 95% CI = 1.77, 5.91).

Table 15. The association between presence of cerebral infarcts and dementia within the dementia sample (n=462)

Model	**Exposure** OR (95% CI)	**Covariates** OR (95% CI)			
	Presence of Infarcts	Age at death	Educational level[1]		ApoE-e4[2]
			High school or less	Bachelors degree	
Crude	**1.93 (1.31, 2.86)**	-	-	-	-
Adjusted - i	**1.85 (1.23, 2.80)**	**1.09 (1.05, 1.14)**	**2.88 (1.61, 5.26)**	1.20 (0.78, 1.85)	-
Adjusted - ii	**1.92 (1.27, 2.93)**	**1.10 (1.06, 1.15)**	**3.19 (1.77, 5.91)**	1.27 (0.82, 1.98)	**2.57 (1.62, 4.10)**

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+
[2] Reference category is the presence of zero ApoE-e4 alleles
Bolded values indicate significance

5.3.2.2 Research Question 1B - Location

In the crude and adjusted models, infarcts located in the cortex (adjusted OR = 2.54; 95% CI = 1.48, 4.43), white matter (adjusted OR = 2.12; 95% CI = 1.27, 3.93), basal ganglia (adjusted OR = 1.96; 95% CI = 1.15, 3.37), or limbic system (adjusted OR = 1.82; 95% CI = 0.82, 4.13) were significantly associated with an increased risk of dementia compared to no infarcts in any region (Table 16). However, the final adjusted model for the basal ganglia area produced a significant H-L GOF test value, indicating that the model did not adequately fit the data. The OR and 95% CI for the basal ganglia area should thus be interpreted with caution. In all final adjusted models, age at death, educational level of high school or less, and the presence of one or more ApoE-e4 alleles were all significantly associated with an increased risk of dementia (Table 16).

Table 16. The association between dementia and the location of at least one cerebral infarct compared to no infarcts (n=462)

Model	**Exposure**	**Covariates**			
	Infarcts	Age at death	Education[1]		ApoE-e4[2]
			High school or less	Bachelors degree	
Cortex[3]					
Crude	**2.41 (1.45, 4.10)**	-	-	-	-
Adjusted - i	**2.41 (1.42, 4.19)**	**1.08 (1.04, 1.13)**	**2.67 (1.42, 5.15)**	1.17 (0.73, 1.88)	-
Adjusted - ii	**2.54 (1.48, 4.43)**	**1.09 (1.04, 1.14)**	**2.97 (1.55, 5.80)**	1.23 (0.76, 2.00)	**2.45 (1.49, 4.09)**
White Matter[3]					
Crude	**1.97 (1.17, 3.36)**	-	-	-	-
Adjusted - i	**2.12 (1.23, 3.72)**	**1.10 (1.05, 1.15)**	**2.94 (1.53, 5.79)**	1.37 (0.85, 2.20)	-
Adjusted - ii	**2.12 (1.27, 3.93)**	**1.10 (1.06, 1.16)**	**3.20 (1.65, 6.39)**	1.42 (0.87, 2.31)	**2.52 (1.53, 4.23)**
Basal Ganglia[3]					
Crude	**1.98 (1.20, 3.31)**	-	-	-	-
Adjusted - i	**1.92 (1.14, 3.29)**	**1.10 (1.05, 1.15)**	**2.74 (1.45, 5.30)**	1.19 (0.74, 1.91)	-
Adjusted - ii	**1.96 (1.15, 3.37)**	**1.11 (1.06, 1.16)**	**3.04 (1.59, 5.98)**	1.27 (0.78, 2.06)	**2.63 (1.60, 4.38)**
Limbic System[3]					
Crude	**1.64 (0.77, 3.56)**	-	-	-	-
Adjusted - i	**1.80 (0.81, 4.08)**	**1.09 (1.04, 1.14)**	**3.01 (1.51, 6.17)**	1.27 (0.77, 2.10)	-
Adjusted - ii	**1.82 (0.82, 4.13)**	**1.10 (1.05, 1.15)**	**3.19 (1.59, 6.60)**	1.29 (0.77, 2.15)	**2.21 (1.32, 3.77)**

Note: The final adjusted model for basal ganglia area had a significant Hosmer-Lemeshow goodness of fit test value and thus should be interpreted with caution.

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval

[1] Reference category is Masters degree+

[2] Reference category is the presence of zero ApoE-e4 alleles

[3]Comparison group was participants without infarcts

Bolded values indicate significance

5.3.2.3 Research Question 1B - Size

In both crude and adjusted models, the presence of at least one large infarct was significantly associated with an increased risk of dementia compared to no infarcts (Table 17, adjusted OR = 3.15; 95% CI = 1.35, 7.74). Lacunar infarcts were not associated with dementia in both crude and adjusted models (adjusted OR = 1.39; 95% CI = 0.82, 2.36). Participants with both large and lacunar infarcts had a significantly higher risk of dementia than participants without any infarcts (adjusted OR = 2.53; 95% CI = 1.30, 5.09). In all final adjusted models, age at death, educational level of high school or less, and the presence an ApoE-e4 allele were all associated with an increased risk of dementia (Table 17).

Table 17. The association between size of cerebral infarcts and dementia (n=462)

Model	**Exposure**	**Covariates**			
	Infarcts	Age at death	Education[1]		ApoE-e4[2]
			High school or less	Bachelors degree	
Large[3]					
Crude	**2.38 (1.09, 5.50)**	-	-	-	-
Adjusted - i	**2.85 (1.24, 6.93)**	**1.10 (1.05, 1.15)**	**3.27 (1.64, 6.72)**	1.44 (0.86, 2.39)	-
Adjusted - ii	**3.15 (1.35, 7.74)**	**1.10 (1.05, 1.15)**	**3.45 (1.71, 7.15)**	1.46 (0.88, 2.46)	**2.09 (1.23, 3.59)**
Lacunar[3]					
Crude	1.60 (0.98, 2.63)	-	-	-	-
Adjusted - i	1.38 (0.82, 2.33)	**1.11 (1.06, 1.16)**	**2.87 (1.51, 5.56)**	1.22 (0.76, 1.95)	-
Adjusted - ii	1.39 (0.82, 2.36)	**1.12 (1.07, 1.17)**	**3.08 (1.60, 6.06)**	1.26 (0.78, 2.05)	**2.48 (1.52, 4.11)**
Both Large and Lacunar[3]					
Crude	**2.43 (1.29, 4.73)**	-	-	-	-
Adjusted - i	**2.46 (1.27, 4.92)**	**1.09 (1.05, 1.15)**	**2.69 (1.38, 5.39)**	1.10 (0.66, 1.80)	-
Adjusted - ii	**2.53 (1.30, 5.09)**	**1.10 (1.05, 1.15)**	**2.99 (1.51, 6.07)**	1.41 (0.69, 1.90)	**2.57 (1.54, 4.38)**

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval

[1] Reference category is Masters degree+

[2] Reference category is the presence of zero ApoE-e4 alleles

[3]Comparison group was participants without infarcts

5.3.2.4 Research Question 2

In both crude and adjusted models, the presence of an ApoE-e4 allele significantly increased the risk of dementia (adjusted OR = 2.49; 95% CI = 1.58, 3.96). Age at death (OR = 1.10; 95% CI = 1.06, 1.15) and an educational level of high school or less (OR = 3.18; 95% CI = 1.77, 5.86) were significantly associated with an increased risk of dementia (Table 18).

Table 18. The association between presence of an ApoE-e4 allele and dementia (n=462)

Model	**Exposure** OR (95% CI)		**Covariates** OR (95% CI)	
	ApoE-e4[2]	Age at death	Educational level[1]	
			High school or less	Bachelors degree
Crude	**2.01 (1.35, 3.23)**	-	-	-
Adjusted	**2.49 (1.58, 3.96)**	**1.10 (1.06, 1.15)**	**3.18 (1.77, 5.86)**	1.27 (0.79, 1.90)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+
[2] Reference category is the presence of zero ApoE-e4 alleles
Bolded values indicate significance

5.3.2.5 Research Question 3

In both crude and adjusted models, the presence of an ApoE-e4 allele was not associated with the presence of cerebral infarcts (Table 19). In addition, age at death and educational level were not associated with cerebral infarcts. Since the backwards procedure retained an interaction term between the presence of an ApoE-e4 allele and educational level, the model was stratified by educational level (Table 20). The relationship between the presence of an ApoE-e4 allele and cerebral infarcts remained non-significant in all three educational strata. Age at death was also not associated with presence of infarcts in the stratified models.

Table 19. The association between presence of an ApoE-e4 allele and presence of cerebral infarcts in the dementia sample (n=462)

Model	**Exposure** OR (95% CI)		**Covariates** OR (95% CI)	
	ApoE-e4[2]	Age at death	Educational level[1]	
			High school or less	Bachelors degree
Crude	0.91 (0.58, 1.42)	-	-	-
Adjusted	0.92 (0.59, 1.44)	1.03 (0.99, 1.07)	1.18 (0.68, 2.05)	0.83 (0.53, 1.29)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+
[2] Reference category is the presence of zero ApoE-e4 alleles
Bolded values indicate significance

Table 20. The association between presence of an ApoE-e4 allele and the presence of cerebral infarcts in the dementia sample (n=274), stratified by educational level

Model	**Exposure**	**Covariates**
	Presence of an ApoE-e4 allele[1]	Age at death
High school or less (n=51)		
Crude	0.27 (0.04. 1.22)	-
Adjusted	0.26 (0.04, 1.19)	1.09 (0.97, 1.23)
Bachelors degree (n=30)		
Crude	3.33 (0.54, 27.78)	-
Adjusted	3.23 (0.51, 27.21)	0.99 (0.86, 1.12)
Masters degree+ (n=193)		
Crude	0.62 (0.28, 1.28)	-
Adjusted	0.67 (0.30, 1.41)	1.05 (0.99, 1.12)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Comparison group is those with an ApoE-e4 allele

5.3.2.6 Research Question 4A

To assess if the ApoE-e4 allele acted as an effect modifier in the association between infarcts and dementia, models were stratified by ApoE-e4 allele status. The presence of infarcts was significantly associated with an increased risk of dementia in both ApoE-e4+ (Table 21, adjusted OR = 2.55; 95% CI = 1.05, 6.68) and ApoE-e4- (adjusted OR = 1.74; 95% CI = 1.08, 2.80) strata indicating a slightly stronger association between infarcts and dementia among those with an ApoE-e4 allele. Age at death was also significantly associated with an increased risk of dementia in both stratified groups but educational level was only significant in participants without an ApoE-e4 allele (Table 21).

Table 21. The association between presence of infarcts and dementia, stratified by ApoE-e4 allele status (n=462)

Model	**Exposure**	**Covariates**		
	Presence of Cerebral Infarcts	Age at death	Education[1]	
			High school or less	Bachelors degree
ApoE-e4 + (n = 115)				
Crude	**2.42 (1.04, 6.04)**	-	-	-
Adjusted	**2.55 (1.05, 6.68)**	**1.10 (1.01, 1.21)**	2.45 (0.64, 12.2)	0.74 (0.31, 1.74)
ApoE-e4 – (n = 347)				
Crude	**1.89 (1.21, 2.96)**	-	-	-
Adjusted	**1.74 (1.08, 2.80)**	**1.10 (1.05, 1.15)**	**3.65 (1.90, 7.24)**	1.58 (0.94, 2.66)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+

5.3.2.7 Research Question 4B - Location

The comparison group for these models was participants without any infarcts present. When stratified by the presence of an ApoE-e4 allele, participants with at least one infarct located in the basal ganglia area were significantly more likely to have dementia (Table 22; OR = 3.37; 95% CI=1.06, 13.26). Infarcts in the basal ganglia were not significantly associated with dementia among participants without an ApoE-e4 allele. Thus, the ApoE-e4 allele modified the relationship between infarcts located in the basal ganglia and dementia. Infarcts located in the cortex were significantly associated with an increased risk of dementia in both ApoE-e4+ (Table 23, OR = 3.68; 95% CI = 1.04, 17.49) and ApoE-e4- strata (OR = 2.21; 95% CI = 1.21, 4.12), indicating that the presence of an ApoE-e4 allele slightly modified the relationship between infarcts located in the cortex and dementia. Infarcts located in the limbic system were not significantly associated with an increased risk of dementia in either ApoE-e4 allele strata (Table 24). Infarcts in the white matter were significantly associated with an increased risk of dementia in both the ApoE-e4+ (Table 25, OR = 3.76; 95% CI = 1.08, 17.70) and ApoE-e4- strata (OR = 1.92; 95% CI = 1.02, 3.66), indicating the presence of an ApoE-e4 allele slightly modified the relationship between infarcts located in the white matter and dementia.

Table 22. The association between infarcts located in the basal ganglia and dementia, stratified by ApoE-e4 allele status (n=382)

Model	**Exposure**	**Covariates**		
	Basal ganglia infarcts	Age at death	Education[1]	
			High school or less	Bachelors degree
ApoE-e4 + (n = 98)				
Crude	**3.19 (1.06, 11.87)**	-	-	-
Adjusted	**3.37 (1.06, 13.26)**	1.10 (0.99, 1.21)	2.07 (0.51, 10.69)	0.70 (0.27, 1.78)
ApoE-e4 – (n = 284)				
Crude	1.79 (0.99, 3.18)	-	-	-
Adjusted	1.59 (0.86, 2.96)	**1.11 (1.05, 1.17)**	**3.67 (1.76, 7.88)**	1.65 (0.93, 2.95)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+

Table 23. The association between infarcts located in the cortex and dementia, stratified by ApoE-e4 allele status (n=381)

Model	**Exposure**	**Covariates**		
	Cortical infarcts	Age at death	Education[1]	
			High school or less	Bachelors degree
ApoE-e4 + (n = 94)				
Crude	**3.50 (1.04, 16.07)**	-	-	-
Adjusted	**3.68 (1.04, 17.49)**	1.05 (0.96, 1.16)	1.86 (0.45, 9.63)	0.67 (0.26, 1.69)
ApoE-e4 – (n = 287)				
Crude	**2.38 (1.34, 4.27)**	-	-	-
Adjusted	**2.21 (1.21, 4.12)**	**1.09 (1.04, 1.15)**	**3.56 (1.72, 7.56)**	1.56 (0.88, 2.79)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+

Table 24. The association between infarcts located in the limbic system and dementia, stratified by ApoE-e4 allele status (n=332)

Model	**Exposure**	**Covariates**		
	Limbic system infarcts	Age at death	Education[1]	
			High school or less	Bachelors degree
ApoE-e4 + (n = 85)				
Crude	1.25 (0.29, 6.44)	-	-	-
Adjusted	1.51 (0.31, 8.62)	1.08 (0.98, 1.20)	1.94 (0.47, 10.15)	0.69 (0.27, 1.78)
ApoE-e4 – (n = 247)				
Crude	1.80 (0.75, 4.43)	-	-	-
Adjusted	1.90 (0.75, 4.97)	**1.09 (1.04, 1.16)**	**3.86 (1.73, 8.88)**	1.66 (0.90, 3.09)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+

Table 25. The association between infarcts located in the white matter and dementia, stratified by ApoE-e4 allele status (n=374)

Model	**Exposure**	**Covariates**		
	White matter infarcts	Age at death	Education[1]	
			High school or less	Bachelors degree
ApoE-e4 + (n = 94)				
Crude	**3.50 (1.04, 16.07)**	-	-	-
Adjusted	**3.76 (1.08, 17.70)**	1.08 (0.98, 1.21)	2.02 (0.49, 10.48)	0.73 (0.29, 1.84)
ApoE-e4 – (n=280)				
Crude	1.80 (0.99, 3.28)	-	-	-
Adjusted	**1.92 (1.02, 3.66)**	**1.10 (1.05, 1.16)**	**3.84 (1.81, 8.40)**	1.84 (1.04, 3.30)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+

5.3.2.8 Research Question 4B - Size

Among participants without an ApoE-e4 allele, large infarcts were significantly associated with an increased risk of dementia compared to no infarcts (Table 26; OR = 4.06, 95% CI = 1.58, 11.24). Among participants with an ApoE-e4 allele, large infarcts were not significantly associated with dementia. Lacunar infarcts were not significantly associated with an increased risk of dementia in either ApoE-e4+ or ApoE-e4- groups (Table 27). Among those with an ApoE-e4 allele present, participants with both large and lacunar infarcts had a significantly higher risk of dementia (Table 28, OR = 8.37; 95% CI = 1.41, 161.27). Among participants without an ApoE-e4 allele, both large and lacunar infarcts had an increased risk for dementia in the crude model; however, the significant association was lost once the model was adjusted for the covariates age at death and educational level.

Table 26. The association between large infarcts and dementia, stratified by ApoE-e4 allele status (n=331)

Model	**Exposure**	**Covariates**		
	Large infarcts	Age at death	Education[1]	
			High school or less	Bachelors degree
ApoE-e4 + (n = 82)				
Crude	1.13 (0.18, 8.91)	-	-	-
Adjusted	1.07 (0.16, 8.83)	1.06 (0.96, 1.17)	2.00 (0.48, 9.87)	0.68 (0.26, 1.76)
ApoE-e4 – (n = 249)				
Crude	**3.00 (1.26, 7.67)**	-	-	-
Adjusted	**4.06 (1.58, 11.24)**	**1.11 (1.05, 1.17)**	**4.25 (1.89, 9.88)**	**1.97 (1.06, 3.71)**

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+

Table 27. The association between lacunar infarcts and dementia, stratified by ApoE-e4 allele status (n=384)

Model	**Exposure**	**Covariates**		
	Lacunar infarcts	Age at death	Education[1]	
			High school or less	Bachelors degree
ApoE-e4 + (n = 98)				
Crude	1.88 (0.68, 5.73)	-	-	-
Adjusted	1.95 (0.67, 6.30)	**1.13 (1.03, 1.27)**	2.49 (0.62, 12.87)	0.80 (0.32, 1.98)
ApoE-e4 – (n = 286)				
Crude	1.55 (0.88, 2.75)	-	-	-
Adjusted	1.24 (0.67, 2.28)	**1.11 (1.06, 1.17)**	**3.47 (1.65, 7.47)**	1.55 (0.87, 2.76)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+

Table 28. The association between both large and lacunar infarcts and dementia, stratified by ApoE-e4 allele status (n=349)

Model	**Exposure**	**Covariates**		
	Both large & lacunar infarcts	Age at death	Education[1]	
			High school or less	Bachelors degree
ApoE-e4 + (n= 88)				
Crude	**7.50 (1.34, 141.08)**	-	-	-
Adjusted	**8.37 (1.41, 161.27)**	1.07 (0.97, 1.19)	1.86 (0.44, 9.86)	0.72 (0.27, 1.90)
ApoE-e4 – (n= 261)				
Crude	**2.10 (1.03, 4.36)**	-	-	-
Adjusted	1.90 (0.89, 4.13)	**1.10 (1.05, 1.17)**	**3.64 (1.69, 8.11)**	1.40 (0.77, 2.58)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+

6.0 Discussion

Few studies have examined the relationship between AD (clinically and pathologically defined) and cerebral infarcts. Instead, the majority of research regarding cerebral infarcts has focused solely on either clinical or pathological definitions of AD. In addition, few studies have examined ApoE-e4 as an effect modifier in the relationship between stroke and AD; these studies were only able to do so in the context of clinical stroke rather than investigating cerebral infarcts, which include both clinical and silent strokes (Johnston et al., 2000; Zhu et al., 2000). Although not clinically overt, silent strokes are hypothesized to have significant impacts on cognitive impairments, especially within older populations where their prevalence is expected to be the highest (Song et al., 2007). This study examined the relationship between cerebral infarcts and AD in the context of the ApoE-e4 allele.

6.1 Study Findings

In this study, the overall presence of cerebral infarcts was not significantly associated with an increased risk of AD. This is one of few studies to report on the relationship between cerebral infarcts and AD that has been clinically and pathologically defined. In contrast, the presence of cerebral infarcts has been demonstrated to be significantly associated with an increased risk of developing dementia (Schneider et al., 2004; Schneider et al., 2005; Snowdon et al., 1997), consistent with the results of this study.

To ensure the lack of association between cerebral infarcts and AD was not due to the modified NIA-RI diagnostic criterion applied when classifying AD cases and controls, a sensitivity analysis was conducted based on two alternative outcomes. These alternative outcomes represented different ways of defining AD pathology based on the NIA-RI diagnostic criterion. The sensitivity analyses indicated that results did not vary based on alternative AD

pathology definitions within the overall AD diagnosis. When a sensitivity analysis was conducted using solely AD pathology as an outcome, based on the modified version of the NIA-RI neuropathologic criteria, results did not vary. However, a sensitivity analysis assessing clinical dementia as an outcome without AD pathology data did produce significantly different results.

The prevalence of cerebral infarcts present in the AD and dementia samples was relatively comparable (37.9% and 34.6%, respectively). These percentages are also consistent with what other studies have reported. However, participants with dementia were significantly more likely to have cerebral infarcts compared to non-demented participants whereas, in the AD sample, there was no difference in the presence of cerebral infarcts between the AD case and control participants. The main difference between the definitions of AD and dementia was the inclusion or exclusion of AD pathology in the diagnosis. Sensitivity analyses showed that cerebral infarcts were not associated with AD pathology, as defined by a modified version of the NIA-RI neuropathologic diagnostic criteria. The lack of association between AD pathology and cerebral infarcts has also been demonstrated consistently across other studies using various neuropathologic criteria (Honig et al., 2003; Schneider et al., 2004; Schneider et al., 2005; Snowdon et al., 1997; Troncoso et al., 2008). Thus, the lack of an association noted in this thesis for the outcome AD in contrast to the presence of an association for the outcome dementia was likely due to the respective inclusion or exclusion of AD pathology.

Cerebral infarcts have been suggested to have a large impact in individuals with dementia who have moderate to low levels of AD pathology (Del Ser, Hachinski, Merskey, & Munoz, 2005; Song et al., 2007). Since neurofibrillary tangles are highly correlated with the cognitive declines apparent during clinical dementia (Riley, Snowdon, & Markesbery, 2002; Wilcock &

Esiri, 1982), individuals who reach advanced Braak stages (i.e., stages V-VI) are unlikely to be further affected by vascular lesions, such as cerebral infarcts (Del Ser et al., 2005). Thus, the lack of a statistically significant association between the presence of cerebral infarcts and AD could be due to the high levels of AD pathology required to be considered an AD case in this study. AD cases were required to have a high Braak stage (V-VI) and a high CERAD level (B or C); therefore, only severe cases of AD pathology were represented in AD cases, potentially creating a ceiling effect and masking any impact cerebral infarcts may have had on those participants.

On the other hand, a measure of AD pathology was not a part of the dementia diagnosis, potentially allowing a spectrum of severities in AD pathology to be represented and the impact of cerebral infarcts to be observed. Inconsistencies between clinical dementia status and severity of AD pathology do occur. Previous research on cerebral infarcts has suggested that they are often associated with lowering the clinical threshold for dementia, especially when low levels of AD pathology are present (Esiri, Nagy, Smith, Barnetson, & Smith, 1999; Geppert, Wroblewska, & Przedpelska-Ober, 2007; Nagy et al., 1997; Snowdon et al., 1997). For example, in the early stages of AD, when clinical symptoms are silent and AD pathology levels are low, cerebral infarcts are able to have significant impacts on cognition; however, they do not appear to equally affect all cognitive abilities (Schneider et al., 2003). Cerebral infarcts have been reported to be strongly associated with declines in perceptual speed or association rather than declines in episodic memory, which is the hallmark clinical symptom of AD (Price et al., 1997; Schneider et al., 2003; Schneider et al., 2007). Even though cerebral infarcts and AD pathology were not associated, the damage caused from vascular pathologies that leads to poor cerebral blood flow could result in accelerated downstream progression of AD pathology, as suggested by the vascular hypothesis (Roher et al., 2011). In the later stages of AD when AD pathology is severe,

large disturbances in memory are prominent (Fleischman et al., 2005). Recognizing the impact AD pathology has towards declines in memory is important; however, identifying upstream factors that potentially initiate cognitive impairment in domains other than memory is just as significant for delaying clinical AD progression.

In addition to their presence, the location and size of cerebral infarcts may influence the risk of AD or dementia (Jellinger & Attems, 2003). Previous research on the Nun Study has reported on the presence of cerebral infarcts (Mortimer et al., 2009; Snowdon et al., 1997), but did not report on specific locations of the infarcts. In addition, various studies have reported size of infarcts based on macroscopic findings, combining large and lacunar infarcts together. However, this study was able to examine the impacts of large and lacunar infarcts independently. Participants who had both large and lacunar infarcts present were also examined independently.

There were no significant differences in location of infarcts between the AD cases and controls. Participants with infarcts located in the cortex, white matter, basal ganglia, or limbic system were not at higher risk of AD when compared to participants without any infarcts present. All logistic models examining the locations had goodness of fit issues; thus, results should be interpreted with caution. Sample sizes within each exposure group were a problem. As well, many participants had infarcts located in more than one area; it was thus not feasible to distinguish the independent effects of infarcts in each location.

Participants with dementia were significantly more likely to have infarcts located in the cortex compared to non-demented participants; however, the remaining three locations did not significantly vary by dementia status. Infarcts located in the cortex, white matter, basal ganglia, or limbic system were significantly associated with an increased risk of dementia compared to no infarcts. Not surprisingly, the odds of dementia was highest (2.5 times) in participants with

infarcts located in the cortex, the area of the brain that is responsible for processing information such as sensory, memory, emotion, and decision-making. Infarcts located in the cortex include those found in any of the following areas: frontal, occipital, parietal, temporal, calcarine, cingulate, and insular cortices.

Size of infarcts (large, lacunar, or both large and lacunar) was not significantly associated with the risk of AD when compared to having no infarcts. However, participants in the dementia sample showed a different pattern of association. The odds of dementia in participants with large infarcts were three times higher than in participants without any infarcts present. Participants with lacunar infarcts showed an increased risk of dementia, but this association was not statistically significant. The odds of dementia in participants with both large and lacunar infarcts present were two times higher than in participants without any infarcts. Since lacunar infarcts were not significant independently, large infarcts likely account for the majority of the risk associated with dementia in participants with both large and lacunar infarcts.

Clinical and silent strokes are claimed to have similar risk factors and overall pathogenesis (Leary & Saver, 2003). However, research examining lacunar infarcts has suggested the risk factors and pathogenesis associated with clinical lacunar stroke and clinically silent lacunes may be different (Adachi, Kobayashi, Yamaguchi, & Okada, 2000; Benisty et al., 2009). Misclassification of lacunar infarcts has been suggested as a problem when examining their association with cognitive outcomes (Potter et al., 2010). Lacunar infarcts are said to become lacunes once healed and are distinguished by fluid filled cavities rather than a distinct lesion (Potter et al., 2010; Wardlaw, 2008). In some studies, lacunes are often counted as old lacunar infarcts, which are considered clinically silent (Potter et al., 2010). The association between lacunar infarcts and lacunes remains controversial because the rate of progression from

a lacunar infarct to a lacune is unknown (Potter et al., 2010). Considering clinical lacunar infarcts and silent lacunes as equal could alter the relationship when examining cognitive outcomes; thus, lacunar infarcts and lacunes should be separated until there is a better understanding of their pathogenesis (Wardlaw, 2008). In this study, lacunar infarcts were not significantly associated with AD or dementia. The lack of association could be because both clinical lacunar stroke and silent lacunes were both included as a measure of lacunar infarcts, potentially weakening the association.

The association between presence of an ApoE-e4 allele and AD was tested to ensure the ApoE-e4 allele was a risk factor in this population. The odds of AD in participants with at least one ApoE-e4 allele were 10 times higher than participants without an ApoE-e4 allele, when adjusted for age at death and educational level. The presence of an ApoE-e4 allele was also significantly associated with an increased risk of dementia. Studies have suggested that the risk related to the ApoE-e4 allele decreases with increasing age and that other risk factors associated with old age may become more important in determining risk (Skoog et al., 1998). The findings from this study show that even in a very old population (mean age 89.7 years for the AD sample and 90.2 years for the dementia sample), the possession of an ApoE-e4 allele may increase the risk of developing AD and dementia. A more plausible hypothesis would be that not only does the ApoE-e4 allele remain a significant risk factor for AD and dementia into old age, but that it may have the ability to interact with other age-related risk factors.

Given evidence of an interaction term between the presence of an ApoE-e4 allele and educational level, the relationship between the ApoE-e4 allele and AD was tested to see if it would vary by educational level. The presence of an ApoE-e4 allele was not significantly associated with AD when participants had an educational level of high school or less. This is

rather surprising because low educational attainment and the presence of an ApoE-e4 allele are both risk factors for developing AD; thus, an interaction between these two risk factors would have been expected to show the highest risk. In contrast, presence of an ApoE-e4 allele was significantly associated with an increased risk of AD in participants with a Bachelors level education and this risk was even greater with a Master level or higher. One possible explanation for these results is that for participants with an educational level of high school or less, the strong risk of this low education (and associated factors, such as occupation) outweighed the usual impact of ApoE-e4 allele status on AD. There are few studies that have examined the interaction between ApoE and educational level on the risk of developing AD and their results have varied (Sando et al., 2008; Seeman et al., 2005; Shadlen et al., 2005).

Since the ApoE gene has been suggested to be related to stroke (Honig et al., 2003; Kalmijn, Feskens, Launer, & Kromhout, 1996), the ApoE-e4 allele was tested to see if it was associated with cerebral infarcts in this sample. In both the AD and dementia samples, the presence of an ApoE-e4 allele was not significantly associated with cerebral infarcts. The covariates present in both models, age at death and educational level, were also not significantly associated with cerebral infarcts. Similar findings have been reported with respect to ApoE-e4 alleles and the risk of clinical stroke (Zhu et al., 2000). Since there is no apparent association between ApoE and cerebral infarcts, this finding confirms that cerebral infarcts are highly unlikely to act as a mediator between ApoE and AD or dementia.

ApoE was tested as an effect modifier in the relationship between presence of cerebral infarcts and the outcomes AD and dementia. Since the ApoE-e4 allele has been shown to be associated with an increased risk of AD and dementia, but not associated with cerebral infarcts, the effect modification hypothesis is plausible. The ApoE-e4 allele did not modify the effect

between cerebral infarcts and AD. However, the ApoE-e4 allele slightly modified the effect between cerebral infarcts and dementia. In participants with an ApoE-e4 allele present, the odds of dementia were 2.5 times higher if cerebral infarcts were present compared to having no cerebral infarcts. In participants without any ApoE-e4 alleles, the odds of dementia were 1.7 times higher if cerebral infarcts were present compared to having no cerebral infarcts.

The results from this study on ApoE as an effect modifier, however, are not consistent with what others have reported. Few studies have examined the ApoE-e4 allele as an effect modifier in the relationship between stroke and AD or dementia; however, no study has examined the effects of both clinical and silent stroke on this relationship. Kalmijn et al. (1996) were one of the first investigators to suggest and prove that ApoE-e4 carriers who suffered a stroke had an increased risk of cognitive decline, as measured by the MMSE. Johnston et al. (2000) further showed that ApoE-e4 carriers with a clinical history of stroke were five times more likely to develop clinical AD. More recently there has been a three-way interaction reported: progression of AD was influenced by ApoE-e4 carrier status and varied through time for individuals with a history of stroke (Mielke et al., 2011). Zhu et al. (2000) did not find a significant interaction between the ApoE-e4 allele and dementia; however, this finding was likely due to their short mean follow-up period of three years.

ApoE-e4 was also examined as an effect modifier of the association between dementia and specific characteristics of the infarcts. When infarcts located in the basal ganglia were stratified by the presence of an ApoE-e4 allele, participants with infarcts had a significantly higher odds of dementia compared to participants without any infarcts. When infarcts were located in either the cortical or white matter areas, the risk of dementia varied slightly by ApoE-

e4 allele status. The ApoE-e4 allele did not significantly modify the relationship between infarcts located in the limbic system and dementia compared to participants without any infarcts.

The presence of an ApoE-e4 allele did not significantly interact with large or lacunar infarcts to increase the odds of dementia. However, the presence of an ApoE-e4 allele did have a significant interaction with infarcts to increase the odds of dementia when participants had both large and lacunar infarcts.

This study combined both clinical and silent stroke into one summary measure for cerebral infarcts. The small effect of ApoE-e4 allele as an effect modifier between cerebral infarcts and dementia may be explained by the use of this summary measure. Studies have shown that people who live to an old age, without any evidence of clinical stroke, have a high prevalence of silent stroke (Song et al., 2007; Vermeer, Longstreth, & Koudstaal, 2007). Due to the summary measure used for infarcts in this study, the independent impacts of clinical and silent stroke were not able to be separated. Despite current theories on the cognitive impact of silent strokes, studies have suggested that clinically evident strokes are more related to dementia (Cho et al., 2011; Schneider et al., 2003). However, inconsistent results across studies could be a result of sample characteristics, sample size, number of participants with infarcts, and the exclusion of lacunar infarcts (Cho et al., 2011).

Clinically evident strokes are more likely to represent large infarcts rather than lacunar infarcts (Schneider et al., 2003). Although large infarcts were significantly associated with dementia and this relationship did vary by ApoE-e4 allele status, there were proportionally more lacunar infarcts present in this sample than large infarcts. Thus, the overall association between infarcts and dementia may have been weakened by the proportion of lacunar infarcts in this study. In addition, given the unique characteristics of the Nun Study participants, this sample

was likely at a low risk for developing stroke compared to the general population (e.g., no tobacco use or heavy alcohol consumption).

Although an association between infarcts and AD (clinically and pathologically defined) was not significant in this study, this relationship needs to be reassessed within the context of independent impacts from both clinical and silent stroke. Individuals with cerebral infarcts represent a heterogeneous group with respect to infarct location and size; thus a larger sample size is needed to accurately study the impacts of infarcts on cognition, dementia, and AD (Cho et al., 2011).

6.2 Study Limitations

When interpreting the results of this study, there are certain limitations that should be considered. The following sections highlight limitations concerning the available data and methodologies employed.

6.2.1 Ascertainment of Cerebral Infarcts

In this study, cerebral infarcts were examined during the gross neuropathologic assessment and macroscopically though 1.5cm thick coronal sections. This type of assessment for cerebral infarcts represents an approximation rather than an exact value. Given issues of feasibility, examining sections of the brain rather than the entire brain is standard. It may be argued that there was an underrepresentation of lacunar infarcts, since they are classified as <=1.5centimeters; however, in our sample lacunar infarcts were more common than large infarcts but were not significantly associated with AD or dementia.

The neuropathologic assessment was able to quantify both clinical and silent strokes creating a total measure for cerebral infarcts, representing a major advantage for this study. Previous studies have only been able to examine ApoE-e4 allele as an effect modifier in the

relationship between clinical stroke and dementia or cognitive decline (Johnston et al., 2000; Kalmijn et al., 1996; Zhu et al., 2000). The risk of developing silent strokes increases with age (Schneider et al., 2003; Vermeer et al., 2003) and, if present, silent strokes have been hypothesized to have an impact on cognitive function (Schmidt et al., 2004; Song et al., 2007; Vermeer et al., 2003). However, including silent strokes in the total measure for cerebral infarcts may have weakened the associations in this study as the association between AD and cerebral infarcts was not statistically significant. The results for dementia were somewhat consistent with other studies: ApoE-e4 carriers with cerebral infarcts had slightly higher risk for dementia compared to non-ApoE-e4 carriers; although, in this study both strata were statistically significant. In addition, the strength of the association (OR = 2.55) was generally smaller compared to what has been previously reported. Johnston et al. (2000) described that ApoE-e4 carriers with a history of stroke were five times more likely to have a history of clinical AD than non-ApoE-e4 carriers. Kalmijn et al. (1996) reported that carriers of the ApoE-e4 allele with cerebrovascular disease were 17 times more at risk of cognitive decline compared to non-ApoE-e4 carriers. Silent and clinical strokes are assumed to have the same risk factors (Leary & Saver, 2003); however, their pathogenesis in relation to cognitive decline may be different depending on their clinical significance (Adachi et al., 2000). If the data on cerebral infarcts could have been separated to examine the independent effects of clinical and silent strokes, it could have been determined if silent strokes were the cause of the non-significant findings for the outcome of AD or the reduced OR for outcome of dementia in this study. Perhaps in a sample with a relatively low prevalence of stroke risk factors, such as smoking and low educational level, silent strokes may not have as large an impact as what would be expected in the general population. In addition, the number of silent strokes versus clinically present strokes was unknown. Potentially

the AD sample could have had a higher prevalence of silent strokes given their advanced age, resulting in non-significant findings, whereas the dementia sample could have had a higher prevalence of clinical stroke, resulting in significant findings. Despite the lack of flexibility within the cerebral infarct measure, this is the first study to include both clinical and silent strokes when examining ApoE-e4 allele status as an effect modifier in the relationship between infarcts and AD or dementia.

6.2.2 Generalizability

The participants in this study were unique as they were relatively free of several common confounding variables associated with both stroke and AD. However, external validity of the results may be affected by the lack of such characteristics that are usually present in the general population. For example, all participants in this study were women, potentially limiting the external validity of study results to men. Although women appear to have a higher risk for AD and dementia compared to men, this association may be distorted by longer life expectancy and disease duration in women. Despite the unknown risks with gender, there is no reason to believe that the pathogenesis for AD or stroke is different in men and women. In addition, all participants in this study had similar lifestyles with respect to nutrition, tobacco use, alcohol consumption, and socio-economic status (SES), all of which may cause variation in the risk of either stroke or AD. However, the results in this study may offer a glimpse of how the prevalence of AD and dementia may decrease if such risk factors as cigarette smoking and alcohol consumption were not present in the general population. For example, in the dementia sample, 50% of participants were diagnosed as non-demented and that group had a mean age of 88.9 years. Living into advanced age without evidence of cognitive decline is remarkable given the exponential increase in risk for dementia after the age of 85 (von Strauss et al., 1999).

Although a lack of common risk factors in the Nun Study population may affect the external validity, the results of this study most likely represent an underestimation of the risks associated with AD and dementia. This unique sample, with few confounding variables, allows for the unique opportunity to examine relationships that population-based studies would not have the ability to test.

6.2.3 Temporal Sequence

The analyses for this project relied on the use of secondary data, which was not originally intended to address the objective of this study. The Nun Study was designed to follow participants longitudinally, representing a major methodological strength; however, the analyses conducted in this study are more representative of prevalent cases in a cross-sectional snap shot. Since cerebral infarcts were measured at autopsy, a clear temporal sequence between clinical symptoms and pathological findings cannot be certain. Despite the inability to determine a true causal relationship, this study was still able to utilize the cross-sectional analyses to indicate possible associations among variables of interest.

6.2.4 Covariates not assessed

Although results in this study were adjusted for age at death, educational level, and ApoE-e4 allele status, additional covariates not available could have added to the interpretation of our results.

Mid-life hypertension is the most common vascular risk factor in the general population and in older adults. Mid-life hypertension has also been found to be associated with late-life cognitive decline (see review by Staessen et al., 2007). The relationship between hypertension and late-life cognitive decline is often misunderstood, as blood pressure tends to decrease in very old adults not allowing associations to become evident (see review by Staessen et al., 2007). The

relationship between hypertension and cerebral infarcts needs to be more clearly understood through additional research (Vermeer et al., 2007). It would have been interesting to see how or if the association between cerebral infarcts and AD may have changed when hypertension was accounted for. Hypertension would be an important confounding variable to consider for future studies examining cerebrovascular disease and AD.

Diabetes is also highly prevalent in adults 65 years or older and is a risk factor for both stroke and dementia (Arvanitakis et al., 2006). Individuals with diabetes have a high risk for stroke and mortality due to the pathophysiological changes in the cerebrovascular vessels (Karsito & Soeatmadji, 2008). More specifically, diabetes has been shown to significantly increase the odds of cerebral infarcts (Arvanitakis et al., 2006). Diabetes increases the risk of clinical dementia, but has been found to not be associated with AD pathology (Arvanitakis et al., 2006). The mechanisms by which diabetes is related to dementia are not clear, but are likely related to the vascular damage caused by diabetes (Arvanitakis et al., 2006). Assessment of diabetes status may have added insight into the relationship between ApoE-e4 allele status, cerebral infarcts, and dementia.

6.2.5 Data Analyses

In relatively few instances, ORs in adjusted models were unable to be calculated due to relatively small sample sizes. In some models the number of individuals with infarcts of a certain size or within a specific area was not large enough to elucidate the association of infarcts with AD. Participants commonly had infarcts of both sizes (large and lacunar) and had infarcts located in more than one area. Although adjusted models could not be calculated, these findings clearly indicate the complexity and heterogeneous nature of cerebral infarcts within AD.

Several models regarding infarct locations within these analyses had significant H-L GOF test values, indicating the data had a lack of fit with these models. The H-L GOF test is a global measure and is unable to specify which predictors or observations are not precisely fitting the model (Kuss, 2002). However, residual deviances were also examined as they represent a more specific measure of how the data fit the model (Kuss, 2002). Several influential outliers were present; however, these outliers were permitted to stay in the model since their deletion did not affect the interpretation of the OR. The sample used in this study represented a homogenous population where all participants are similar with respect to key variables. Thus, any deviation away from the homogenous distribution of the data would be recognized as an influential outlier, even though in the general population similar deviances might fall within a normal distribution. In addition to a possible homogenous population, small sample sizes likely a contribution towards significant H-L GOF test values.

6.3 Study Strengths

6.3.1 Outcome Measure

The Nun Study was able to collect a vast array of robust clinical and pathological information that was used in creating the outcome variables in this study. AD was able to be clinically diagnosed and pathologically confirmed.

Commonly dementia is detected using a global measure of cognitive decline, such as the MMSE or CDR. The Nun Study was able to utilize the MMSE, the CERAD battery of neuropsychological tests, and the ADLs scale to assess dementia status over multiple years of assessments. The robustness of multiple measures over many years ensures that cognitive decline in this sample was due to dementia instead of common age-related changes.

There are many different neuropathologic criteria for assessing AD pathology. One neuropathologic diagnostic criterion is not recommended over another and each has its strengths and limitations. At autopsy, the Nun Study was able to capture and document the burden from amyloid plaques, neurofibrillary tangles, and other lesions or abnormalities present in the brains of participants.

The NIA-RI neuropathologic diagnostic criterion is currently valued as one of the most accurate diagnostic criteria for AD pathology as it incorporates both amyloid plaques and neurofibrillary tangles. However, the NIA-RI criterion has been criticized as being too restrictive because it assumes plaque and tangle burden are ideally correlated with each other. The result of the correlation assumption leaves numerous participants uncategorized because they have a mismatch correlation between plaques and tangles (Geddes et al., 1997). An improvement of the NIA-RI criterion had been suggested by reassessing the correlations required to fit within a diagnostic category (Geddes et al., 1997).

The major correlation limitation of the NIA-RI criterion was recognized in this study and thus, the criterion was modified to allow for additional combinations between plaques and tangles to be made for inclusion into the diagnostic categories. Since the Nun Study collected information on plaque density though the CERAD neuropathologic assessment and collected information on tangle distribution through the Braak staging technique, this study was able to modify the likelihood categories in the NIA-RI criterion to allow for a more inclusive diagnosis of AD pathology. Altering the correlations necessary for each diagnostic category is likely the future for the NIA-RI diagnostic criterion. Due to the vast array of pathological information available, this study had the major strength of modifying the NIA-RI diagnostic criterion.

Another strength related to the pathological outcomes investigated in the Nun Study was that all autopsies were performed by one board-certified neuropathologist. Since examinations of AD pathology are often a semi-quantitative assessment, inter-rater agreement among more than one neuropathologist can become a source of error for many studies. Fortunately, inter-rater agreement was not an issue in the Nun Study. In addition, the neuropathologist was blinded to any clinical information; thus, his AD pathology diagnoses were unbiased with respect to dementia status.

6.3.2 The Nun Study Sample

Although the external validity of study results may have been affected, overall the lack of confounding variables present in the Nun Study sample is a major methodological strength. Typically in studies examining the general population, associations are often misunderstood or misleading due to the presence of confounding variables, such as cigarette smoking and alcohol consumption. However, in this study, such variables were not present and thus not a concern. In addition, participants involved in the Nun Study were similar with respect to other variables such as diet, educational level, reproductive history, access to medical care and occupational level. Due to these similarities, a richer understanding of the etiologic pathway involved in AD can be studied as influences from these variables are likely uniform. Even though the Nun Study characteristics are not typical of the general population, there is no reason to believe that the pathogenesis of AD or dementia would be any different. In addition, the Nun Study sample, which is comprised entirely of females, provides a unique opportunity to examine potential gender specific characteristics of stroke, the major morbidity that affects women over the age of 65.

6.3.3 Study Design

The Nun Study is longitudinal and prospective in design, which offers numerous methodological strengths. Participants were able to be followed through 12 waves of annual cognitive and physical assessments. This prospective design allows for incident cases of dementia to be identified through time. Although this study analyzed Nun Study data in a cross-sectional manner, misclassification of dementia status is highly unlikely due to the rigorous prospective assessments employed. In addition, dementia status was able to be pathologically confirmed as AD at autopsy, allowing for the outcome variable of clinically defined and pathologically confirmed cases of AD.

All participants were examined annually despite their cognitive status, essentially eliminating the chance for information bias. Information about dementia-free participants was valued equally as important as information about participants with dementia. In addition, the prospective design allowed for the assessment of non-response bias between participants included and excluded in the study. Participants in the analytic sample did significantly differ from excluded participants based on ApoE-e4 allele status. Participants in the analytic sample were more likely to have an ApoE-e4 allele; however, this was expected since ApoE-e4 is associated with an increased risk of AD. The ability to test and identify any differences related to non-respondents allows for a more in-depth interpretation of study results.

Cross-sectional studies examining older populations often have the potential limitation of survival bias; older participants who survived may be systematically different compared to participants who have died. The prospective nature of the Nun Study allows for examination of survival bias. Of those enrolled in the Nun Study, presently 90% of participants have been followed to completion. In this study, the deceased sample was significantly different with

respect to ApoE-e4 allele status as they were more likely to have an e4 allele compared to living participants. This result is expected since ApoE-e4 allele status is associated with an increased risk of mortality (Jacobsen et al., 2010) and overall results obtained in this study are unlikely to be affect by survival bias.

6.4 Implications and Future Research Directions

Given the number of Canadians entering into the 65+ cohort and taking into consideration the risks associated with old age, research on stroke and AD is being recognized as an important area for investigation. There is a continuously growing body of evidence that strives to understand the relationship between cerebrovascular disease and AD. This complex relationship is unlikely to be understood within one epidemiologic study. However, this study can confirm existing theories or highlight important issues in the field when assessing the relationship between stroke and AD.

This study found that cerebral infarcts were not significantly associated with AD, suggesting that individuals with severe AD pathology are unlikely to be affected by cerebral infarcts. Although severe AD pathology has consistently been shown to be associated with clinical dementia symptoms, it's unclear how useful this information would be in a general population. AD represents a heterogeneous disease and prevalence of severe AD pathology in the general population versus intermediate or low levels is unknown. Previous studies have also shown that in participants not meeting the neuropathologic criteria for AD, cerebral infarcts increased the risk of dementia. Again, the prevalence of low AD pathology in the general population, especially older adults, is unknown. Future studies should focus on improving imaging techniques for the identification of AD pathology as its use as a diagnostic measure for individuals experience mild cognitive decline may present efficient intervention opportunities. In

addition, there is a need to explore the different impacts cerebral infarcts have within a broader spectrum of AD pathology rather than merely the extremes. This study was able to modify the NIA-RI neuropathologic diagnostic criterion to improve the number of included participants in the diagnosis; however, the AD cases in this study represented a high level of AD pathology. Future research should focus on modifying the levels of AD pathology in the NIA-RI diagnostic criterion required to meet an AD diagnosis, as well as examining different stages of AD in relation to cerebral infarcts.

The presence of cerebral infarcts was significantly associated with dementia in this study. The dementia outcome likely included a more heterogeneous sample with respect to AD pathology and potentially could be more representative of the general population. In addition, this finding confirms the theory suggesting that cerebral infarcts lower the threshold for clinical symptoms of AD (Honig et al., 2003). Cerebral infarcts, regardless of their clinical significance, may be an indicator of early AD (Vermeer et al., 2007). Although infarcts do not always noticeably impact memory they have been found to be associated with more subtle neurological impairments (Vermeer et al., 2007). Individuals diagnosed with mild cognitive impairment, which is classified as the border zone between normal cognition and dementia, have intermediate levels of cerebral infarcts (Bennett, Schneider, Bienias, Evans, & Wilson, 2005). These findings highlight the importance of several cognitive domains other than memory affected during the progression of dementia. Perhaps screening and early detection can identify high-risk individuals based on subtle cognitive impairments other than memory and their burden of cerebral infarcts.

This study confirmed that the ApoE-e4 allele remains a significant risk factor for AD and dementia in older populations; however, this relationship may vary depending on educational level. The mechanism by which the ApoE-e4 allele is related to AD remains

unknown, but several hypotheses have been suggested. This study hypothesized that in older populations, the ApoE-e4 allele may interact with other risk factors to increase the risk of AD. In our sample, results showed that the ApoE-e4 allele was a plausible effect modifier. When tested, the ApoE-e4 allele did not modify the effect of cerebral infarcts on AD and slightly modified the effect of cerebral infarcts on dementia. The lack of a strong association may have been due to the summary measure of cerebral infarcts employed in this study. Given the population is aging and the prevalence of silent stroke increases with age (Leary & Saver, 2003; Song et al., 2007), future studies should aim to identify the impacts of clinical and silent stroke independently. This would allow for the clinical significance of silent strokes with respect to dementia to be highlighted as well as provide a more comprehensive understanding about the possible role of the ApoE-e4 allele as an effect modifier.

Since vascular factors are modifiable with various types of therapies, they may be an influential target when aiming to reduce the incidence and severity of AD. Many studies have examined the influence of vascular medication use and the risk of developing dementia or decreasing its severity. Results have varied based on the age of the sample being studied and are likely due to inconsistencies in measurement of medication use and the duration of follow-up (Guo et al., 2001; Haag, Hofman, Koudstaal, Breteler, & Stricker, 2009). Additionally, these studies were large longitudinal studies that were not originally designed to study the impact that vascular medications may have on dementia. To identify if vascular medications can be used as a primary or secondary prevention tool against dementia, future studies should identify high-risk individuals based on the presence of subtle cognitive impairments typical of cerebral infarcts, apply imaging techniques to identify the burden of cerebral infarcts, and then prescribe medications accordingly. As previously mentioned, AD is a heterogeneous disease, likely

resulting from many possible combinations of risk factors interacting to progress the disease mechanism. Understanding that there is not one risk factor sufficient to cause dementia or AD emphasizes the fact that exploring combinations of risk factors is imperative for the future of prevention.

References

Adachi, T., Kobayashi, S., Yamaguchi, S., & Okada, K. (2000). MRI findings of small subcortical "lacunar-like" infarction resulting from large vessel disease. *Journal of Neurology, 247*(4), 280-285.

Altman, R., & Rutledge, J. C. (2010). The vascular contribution to Alzheimer's disease. *Clinical Science (London, England : 1979), 119*(10), 407-421. doi:10.1042/CS20100094

Alzheimer's Association. (2010). 2010 Alzheimer's disease facts and figures. *Alzheimer's & Dementia : The Journal of the Alzheimer's Association, 6*(2), 158-194. doi:10.1016/j.jalz.2010.01.009

Alzheimer's Society of Canada. (2010). *Rising tide: Impact of dementia in Canada.* Retrieved April 1, 2010, from http://www.Alzheimer.ca/english/rising_tide/rising_tide_summary.htm

American Psychiatric Association. (1994). *Diagnostic and statistical manual of mental disorders.* (4th ed.). Washington, DC: American Psychiatric Association.

Arvanitakis, Z., Schneider, J. A., Wilson, R. S., Li, Y., Arnold, S. E., Wang, Z., & Bennett, D. A. (2006). Diabetes is related to cerebral infarction but not to AD pathology in older persons. *Neurology, 67*(11), 1960-1965. doi:10.1212/01.wnl.0000247053.45483.4e

Ashford, J. W. (2004). APOE genotype effects on Alzheimer's disease onset and epidemiology. *Journal of Molecular Neuroscience : MN, 23*(3), 157-165. doi:10.1385/JMN:23:3:157

Bancher, C., Paulus, W., Paukner, K., & Jellinger, K. (1997). Neuropathologic diagnosis of Alzheimer disease: Consensus between practicing neuropathologists? *Alzheimer Disease and Associated Disorders, 11*(4), 207-219.

Baron, R. M., & Kenny, D. A. (1986). The moderator-mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. *Journal of Personality and Social Psychology, 51*(6), 1173-1182.

Benisty, S., Gouw, A. A., Porcher, R., Madureira, S., Hernandez, K., Poggesi, A., . . . LADIS Study group. (2009). Location of lacunar infarcts correlates with cognition in a sample of non-disabled subjects with age-related white-matter changes: The LADIS study. *Journal of Neurology, Neurosurgery, and Psychiatry, 80*(5), 478-483. doi:10.1136/jnnp.2008.160440

Bennett, D. A., Schneider, J. A., Bienias, J. L., Evans, D. A., & Wilson, R. S. (2005). Mild cognitive impairment is related to Alzheimer disease pathology and cerebral infarctions. *Neurology, 64*(5), 834-841. doi:10.1212/01.WNL.0000152982.47274.9E

Bookheimer, S., & Burggren, A. (2009). APOE-4 genotype and neurophysiological vulnerability to Alzheimer's and cognitive aging. *Annual Review of Clinical Psychology, 5*, 343-362. doi:10.1146/annurev.clinpsy.032408.153625

Braak, H., & Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathologica, 82*(4), 239-259.

Bu, G. (2009). Apolipoprotein E and its receptors in Alzheimer's disease: Pathways, pathogenesis and therapy. *Nature Reviews.Neuroscience, 10*(5), 333-344. doi:10.1038/nrn2620

Castellani, R. J., Zhu, X., Lee, H. G., Moreira, P. I., Perry, G., & Smith, M. A. (2007). Neuropathology and treatment of Alzheimer disease: Did we lose the forest for the trees? *Expert Review of Neurotherapeutics, 7*(5), 473-485. doi:10.1586/14737175.7.5.473

Cho, S., Scarmeas, N., Jang, T., Marder, K., Tang, M., & Honig, L. S. (2011). Importance of symptomatic cerebral infarcts on cognitive performance in patients with Alzheimer's disease. *Journal of Korean Medical Science, 26*(3), 412-416.

Chui, H. (2005). Neuropathology lessons in vascular dementia. *Alzheimer Disease and Associated Disorders, 19*(1), 45-52.

Citron, M. (2010). Alzheimer's disease: Strategies for disease modification. *Nature Reviews.Drug Discovery, 9*(), 387-398. doi:10.1038/nrd2896

Craig, L. A., Hong, N. S., & McDonald, R. J. (2011). Revisiting the cholinergic hypothesis in the development of Alzheimer's disease. *Neuroscience & Biobehavioral Reviews, 35*(6), 1397-1409. doi:DOI: 10.1016/j.neubiorev.2011.03.001

Dai, S., Bancej, C., Bienek, A., Walsh, P., Stewart, P., & Wielgosz, A. (2009). *Tracking heart disease and stroke in Canada.* Ottawa, Canada: Public Health Agency of Canada.

Dal Forno, G., Carson, K. A., Brookmeyer, R., Troncoso, J., Kawas, C. H., & Brandt, J. (2002). APOE genotype and survival in men and women with Alzheimer's disease. *Neurology, 58*(7), 1045-1050.

de la Torre, J. C. (2006). How do heart disease and stroke become risk factors for Alzheimer's disease? *Neurological Research, 28*(6), 637-644. doi:10.1179/016164106X130362

de la Torre, J. C. (2011). Three postulates to help identify the cause of Alzheimer's disease. *Journal of Alzheimer's Disease : JAD, 24*(4), 657-668. doi:10.3233/JAD-2011-101884

Del Ser, T., Hachinski, V., Merskey, H., & Munoz, D. G. (2005). Alzheimer's disease with and without cerebral infarcts. *Journal of the Neurological Sciences, 231*(1-2), 3-11. doi:10.1016/j.jns.2004.08.016

Dickson, D. W. (1998). Aging in the central nervous system. In W. R. Markesbery (Ed.), *Neuropathology of dementing disorders* (pp. 56). New York, NY: Oxford University Press Inc.

Duron, E., Rigaud, A. S., Dubail, D., Mehrabian, S., Latour, F., Seux, M. L., & Hanon, O. (2009). Effects of antihypertensive therapy on cognitive decline in Alzheimer's disease. *American Journal of Hypertension, 22*(9), 1020-1024. doi:10.1038/ajh.2009.119

Esiri, M. M., Nagy, Z., Smith, M. Z., Barnetson, L., & Smith, A. D. (1999). Cerebrovascular disease and threshold for dementia in the early stages of Alzheimer's disease. *Lancet, 354*(9182), 919-920. doi:10.1016/S0140-6736(99)02355-7

Fleischman, D. A., Wilson, R. S., Gabrieli, J. D., Schneider, J. A., Bienias, J. L., & Bennett, D. A. (2005). Implicit memory and Alzheimer's disease neuropathology. *Brain, 128*(Pt 9), 2006-2015. doi:10.1093/brain/awh559

Francis, P. T., Palmer, A. M., Snape, M., & Wilcock, G. K. (1999). The cholinergic hypothesis of Alzheimer's disease: A review of progress. *Journal of Neurology, Neurosurgery, and Psychiatry, 66*(2), 137-147.

Fries, J. F. (2005). The compression of morbidity. 1983. *The Milbank Quarterly, 83*(4), 801-823. doi:10.1111/j.1468-0009.2005.00401.x

Frikke-Schmidt, R., Nordestgaard, B. G., Thudium, D., Moes Gronholdt, M. L., & Tybjaerg-Hansen, A. (2001). APOE genotype predicts AD and other dementia but not ischemic cerebrovascular disease. *Neurology, 56*(2), 194-200.

Geddes, J. W., Tekirian, T. L., Soultanian, N. S., Ashford, J. W., Davis, D. G., & Markesbery, W. R. (1997). Comparison of neuropathologic criteria for the diagnosis of Alzheimer's disease. *Neurobiology of Aging, 18*(4 Suppl), S99-105.

Geppert, A. M., Wroblewska, K. A., & Przedpelska-Ober, E. M. (2007). Greater frequency of subcortical lesions in severely demented patients with early onset Alzheimer's disease. *Alzheimer's & Dementia, 3*(1), 54-57. doi:10.1016/j.jalz.2006.10.007

Good, P. F., Werner, P., Hsu, A., Olanow, C. W., & Perl, D. P. (1996). Evidence of neuronal oxidative damage in Alzheimer's disease. *The American Journal of Pathology, 149*(1), 21-28.

Graves, A. B. (2004). Alzheimer's disease and vascular dementia. In L. Nelson, C. Tanner, S. Van Den Eeden & V. McGuire (Eds.), *Neuroepidemiology* (pp. 102). New York, NY: Oxford University Press, Inc.

Guo, Z., Fratiglioni, L., Viitanen, M., Lannfelt, L., Basun, H., Fastbom, J., & Winblad, B. (2001). Apolipoprotein E genotypes and the incidence of Alzheimer's disease among persons aged 75 years and older: Variation by use of antihypertensive medication? *American Journal of Epidemiology, 153*(3), 225-231.

Haag, M. D., Hofman, A., Koudstaal, P. J., Breteler, M. M., & Stricker, B. H. (2009). Duration of antihypertensive drug use and risk of dementia: A prospective cohort study. *Neurology, 72*(20), 1727-1734. doi:10.1212/01.wnl.0000345062.86148.3f

Halliday, G., Ng, T., Rodriguez, M., Harding, A., Blumbergs, P., Evans, W., . . . Kril, J. (2002). Consensus neuropathological diagnosis of common dementia syndromes: Testing and standardising the use of multiple diagnostic criteria. *Acta Neuropathologica, 104*(1), 72-78. doi:10.1007/s00401-002-0529-5

Hanon, O., Pequignot, R., Seux, M. L., Lenoir, H., Bune, A., Rigaud, A. S., . . . Girerd, X. (2006). Relationship between antihypertensive drug therapy and cognitive function in elderly hypertensive patients with memory complaints. *Journal of Hypertension, 24*(10), 2101-2107. doi:10.1097/01.hjh.0000244961.69985.05

Haroutunian, V., Purohit, D. P., Perl, D. P., Marin, D., Khan, K., Lantz, M., . . . Mohs, R. C. (1999). Neurofibrillary tangles in nondemented elderly subjects and mild Alzheimer disease. *Archives of Neurology, 56*(6), 713-718.

Haroutunian, V., Schnaider-Beeri, M., Schmeidler, J., Wysocki, M., Purohit, D. P., Perl, D. P., . . . Grossman, H. T. (2008). Role of the neuropathology of Alzheimer disease in dementia in the oldest-old. *Archives of Neurology, 65*(9), 1211-1217. doi:10.1001/archneur.65.9.1211

Heyman, A., Fillenbaum, G. G., Welsh-Bohmer, K. A., Gearing, M., Mirra, S. S., Mohs, R. C., . . . Pieper, C. F. (1998). Cerebral infarcts in patients with autopsy-proven Alzheimer's disease: CERAD, part XVIII. Consortium to Establish a Registry for Alzheimer's Disease. *Neurology, 51*(1), 159-162.

Honig, L. S., Kukull, W., & Mayeux, R. (2005). Atherosclerosis and AD: Analysis of data from the US National Alzheimer's Coordinating Center. *Neurology, 64*(3), 494-500. doi:10.1212/01.WNL.0000150886.50187.30

Honig, L. S., Tang, M. X., Albert, S., Costa, R., Luchsinger, J., Manly, J., . . . Mayeux, R. (2003). Stroke and the risk of Alzheimer disease. *Archives of Neurology, 60*(12), 1707-1712. doi:10.1001/archneur.60.12.1707

Huang, Y. (2006). Apolipoprotein E and Alzheimer disease. *Neurology, 66*(2 Suppl 1), S79-85. doi:10.1212/01.wnl.0000192102.41141.9e

Hyman, B. T. (1998). New neuropathological criteria for Alzheimer disease. *Archives of Neurology, 55*(9), 1174-1176.

Hynd, M. R., Scott, H. L., & Dodd, P. R. (2004). Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. *Neurochemistry International, 45*(5), 583-595. doi:10.1016/j.neuint.2004.03.007

Jacobsen, R., Martinussen, T., Christiansen, L., Jeune, B., Andersen-Ranberg, K., Vaupel, J. W., & Christensen, K. (2010). Increased effect of the ApoE gene on survival at advanced age in healthy and long-lived Danes: Two nationwide cohort studies. *Aging Cell, 9*(6), 1004-1009. doi:10.1111/j.1474-9726.2010.00626.x; 10.1111/j.1474-9726.2010.00626.x

Janicki, S. C., & Schupf, N. (2010). Hormonal influences on cognition and risk for Alzheimer's disease. *Current Neurology and Neuroscience Reports, 10*(5), 359-366. doi:10.1007/s11910-010-0122-6

Jellinger, K. A. (2009). Criteria for the neuropathological diagnosis of dementing disorders: Routes out of the swamp? *Acta Neuropathologica, 117*(2), 101-110. doi:10.1007/s00401-008-0466-z

Jellinger, K. A., & Attems, J. (2003). Incidence of cerebrovascular lesions in Alzheimer's disease: A postmortem study. *Acta Neuropathologica, 105*(1), 14-17. doi:10.1007/s00401-002-0634-5

Johnston, J. M., Nazar-Stewart, V., Kelsey, S. F., Kamboh, M. I., & Ganguli, M. (2000). Relationships between cerebrovascular events, APOE polymorphism and Alzheimer's disease in a community sample. *Neuroepidemiology, 19*(6), 320-326.

Jorm, A. F., Korten, A. E., & Henderson, A. S. (1987). The prevalence of dementia: A quantitative integration of the literature. *Acta Psychiatrica Scandinavica, 76*(5), 465-479.

Kalmijn, S., Feskens, E. J., Launer, L. J., & Kromhout, D. (1996). Cerebrovascular disease, the apolipoprotein e4 allele, and cognitive decline in a community-based study of elderly men. *Stroke, 27*(12), 2230-2235.

Karsito, & Soeatmadji, D. W. (2008). Diabetes and stroke. *Acta Medica Indonesiana, 40*(3), 151-158.

Katzman, R. (1993). Education and the prevalence of dementia and Alzheimer's disease. *Neurology, 43*(1), 13-20.

Kennedy, W. J., & Bancroft, T. A. (1971). Model building for prediction in regression based upon repeated significance tests. *The Annals of Mathematical Statistics,* 1273-84. Retrieved from http://www.jstor.org/stable/2240028

Khachaturian, Z. S. (1985). Diagnosis of Alzheimer's disease. *Archives of Neurology, 42*(11), 1097-1105.

Kim, J., Basak, J. M., & Holtzman, D. M. (2009). The role of apolipoprotein E in Alzheimer's disease. *Neuron, 63*(3), 287-303. doi:10.1016/j.neuron.2009.06.026

Kovari, E., Gold, G., Herrmann, F. R., Canuto, A., Hof, P. R., Bouras, C., & Giannakopoulos, P. (2007). Cortical microinfarcts and demyelination affect cognition in cases at high risk for dementia. *Neurology, 68*(12), 927-931. doi:10.1212/01.wnl.0000257094.10655.9a

Kuss, O. (2002). Global goodness-of-fit tests in logistic regression with sparse data. *Statistics in Medicine, 21*(24), 3789-3801. doi:10.1002/sim.1421

Leary, M. C., & Saver, J. L. (2003). Annual incidence of first silent stroke in the United States: A preliminary estimate. *Cerebrovascular Diseases, 16*(3), 280-285. doi:10.1159/000071128

Lee, J. H., Olichney, J. M., Hansen, L. A., Hofstetter, C. R., & Thal, L. J. (2000). Small concomitant vascular lesions do not influence rates of cognitive decline in patients with Alzheimer disease. *Archives of Neurology, 57*(10), 1474-1479.

Li, N. C., Lee, A., Whitmer, R. A., Kivipelto, M., Lawler, E., Kazis, L. E., & Wolozin, B. (2010). Use of angiotensin receptor blockers and risk of dementia in a predominantly male population: Prospective cohort analysis. *British Medical Journal, 340*, b5465.

Lim, A., Tsuang, D., Kukull, W., Nochlin, D., Leverenz, J., McCormick, W., . . . Larson, E. B. (1999). Clinico-neuropathological correlation of Alzheimer's disease in a community-based case series. *Journal of the American Geriatrics Society, 47*(5), 564-569.

Lindsay, J., & Anderson, L. (2003). *Dementia and Alzheimer's disease: Women's health surveillance report.* Ottawa, Ontario, Canada: Public Health Agency of Canada.

Lindsay, J., Laurin, D., Verreault, R., Hebert, R., Helliwell, B., Hill, G. B., & McDowell, I. (2002). Risk factors for Alzheimer's disease: A prospective analysis from the Canadian Study of Health and Aging. *American Journal of Epidemiology, 156*(5), 445-453.

Lindsay, J., Sykes, E., McDowell, I., Verreault, R., & Laurin, D. (2004). More than the epidemiology of Alzheimer's disease: Contributions of the Canadian Study of Health and Aging. *Canadian Journal of Psychiatry, 49*(2), 83-91.

Maccioni, R. B., Farías, G., Morales, I., & Navarrete, L. (2010). The revitalized tau hypothesis on Alzheimer's disease. *Archives of Medical Research, 41*(3), 226-231. doi:DOI: 10.1016/j.arcmed.2010.03.007

Markesbery, W. R. (1998a). Vascular dementia. In W. M. Markesbery (Ed.), *Neuropathology of Dementing Disorders* (pp. 293). New York, NY: Oxford University Press Inc.

Markesbery, W. R. (1998b). Overview of dementing disorders. In W. R. Markesbery (Ed.), *Neuropathology of Dementing Disorders* (pp. 1). New York: Oxford University Press.

McDonald, R. J., Craig, L. A., & Hong, N. S. (2010). The etiology of age-related dementia is more complicated than we think. *Behavioural Brain Research, 214*(1), 3-11. doi:10.1016/j.bbr.2010.05.005

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., & Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA work group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology, 34*(7), 939-944.

McPherson, B. D. (Ed.). (2004). *Aging as a social process: Canadian perspectives* (4th ed.). Toronto: Oxford University Press.

Meyer, M. R., Tschanz, J. T., Norton, M. C., Welsh-Bohmer, K. A., Steffens, D. C., Wyse, B. W., & Breitner, J. C. (1998). APOE genotype predicts when--not whether--one is predisposed to develop Alzheimer disease. *Nature Genetics, 19*(4), 321-322. doi:10.1038/1206

Mielke, M. M., Leoutsakos, J. M., Tschanz, J. T., Green, R. C., Tripodis, Y., Corcoran, C. D., . . . Lyketsos, C. G. (2011). Interaction between vascular factors and the APOE E4 allele in predicting rate of progression in Alzheimer's disease. *Journal of Alzheimer's Disease,* doi:10.3233/JAD-2011-110086

Miklossy, J. (2003). Cerebral hypoperfusion induces cortical watershed microinfarcts which may further aggravate cognitive decline in Alzheimer's disease. *Neurological Research, 25*(6), 605-610.

Mirra, S. S., Heyman, A., McKeel, D., Sumi, S. M., Crain, B. J., Brownlee, L. M., . . . Berg, L. (1991). The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology, 41*(4), 479-486.

Morris, J. C., Heyman, A., Mohs, R. C., Hughes, J. P., van Belle, G., Fillenbaum, G., . . . Clark, C. (1989). The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assessment of Alzheimer's disease. *Neurology, 39*(9), 1159-1165.

Mortimer, J. A., French, L. R., Hutton, J. T., & Schuman, L. M. (1985). Head injury as a risk factor for Alzheimer's disease. *Neurology, 35*(2), 264-267.

Mortimer, J. A., Snowdon, D. A., & Markesbery, W. R. (2009). The effect of APOE-epsilon4 on dementia is mediated by Alzheimer neuropathology. *Alzheimer Disease and Associated Disorders, 23*(2), 152-157.

Mott, R. T., & Hulette, C. M. (2005). Neuropathology of Alzheimer's disease. *Neuroimaging Clinics of North America, 15*(4), 755-65, ix. doi:10.1016/j.nic.2005.09.003

Nagy, Z., Esiri, M. M., Jobst, K. A., Morris, J. H., King, E. M., McDonald, B., . . . Smith, A. D. (1997). The effects of additional pathology on the cognitive deficit in Alzheimer disease. *Journal of Neuropathology and Experimental Neurology, 56*(2), 165-170.

Norrving, B. (2008). Leucoaraiosis and silent subcortical infarcts. *Revue Neurologique, 164*(10), 801-804. doi:10.1016/j.neurol.2008.07.009

Omran, A. R. (1971). The epidemiologic transition. A theory of the epidemiology of population change. *The Milbank Memorial Fund Quarterly, 49*(4), 509-538.

Patterson, C., Feightner, J., Garcia, A., & MacKnight, C. (2007). General risk factors for dementia: A systematic evidence review. *Alzheimer's & Dementia, 3*(4), 341-347. doi:10.1016/j.jalz.2007.07.001

Peila, R., Rodriguez, B. L., Launer, L. J., & Honolulu-Asia Aging Study. (2002). Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: The Honolulu-Asia Aging Study. *Diabetes, 51*(4), 1256-1262.

Pimplikar, S. W. (2009). Reassessing the amyloid cascade hypothesis of Alzheimer's disease. *The International Journal of Biochemistry & Cell Biology, 41*(6), 1261-1268. doi:10.1016/j.biocel.2008.12.015

Pinkston, J. B., Alekseeva, N., & Gonzalez Toledo, E. (2009). Stroke and dementia. *Neurological Research, 31*(8), 824-831. doi:10.1179/016164109X12445505689643

Potter, G. M., Doubal, F. N., Jackson, C. A., Chappell, F. M., Sudlow, C. L., Dennis, M. S., & Wardlaw, J. M. (2010). Counting cavitating lacunes underestimates the burden of lacunar infarction. *Stroke, 41*(2), 267-272. doi:10.1161/STROKEAHA.109.566307

Powers, J. M. (1997). Diagnostic criteria for the neuropathologic assessment of Alzheimer's disease. *Neurobiology of Aging, 18*(4 Suppl), S53-4.

Price, L. D., Thinakaran, G., Borchelt, D. R., Martin, L. J., Crain, B. J., Sisodia, S. S., & Troncoso, J. C. (1998). Neuropathology of Alzheimer's disease and animal models. In W. R. Markesbery (Ed.), *Neuropathology of Dementing Disorders* (pp. 121). New York, NY: Oxford University Press Inc.

Price, T. R., Manolio, T. A., Kronmal, R. A., Kittner, S. J., Yue, N. C., Robbins, J., . . . O'Leary, D. H. (1997). Silent brain infarction on magnetic resonance imaging and neurological abnormalities in community-dwelling older adults. The Cardiovascular Health Study. CHS collaborative research group. *Stroke, 28*(6), 1158-1164.

Regan, C., Katona, C., Walker, Z., Hooper, J., Donovan, J., & Livingston, G. (2006). Relationship of vascular risk to the progression of Alzheimer disease. *Neurology, 67*(8), 1357-1362. doi:10.1212/01.wnl.0000240129.46080.53

Riley, K. P., Snowdon, D. A., & Markesbery, W. R. (2002). Alzheimer's neurofibrillary pathology and the spectrum of cognitive function: Findings from The Nun Study. *Annals of Neurology, 51*(5), 567-577. doi:10.1002/ana.10161

Roher, A. E., Garami, A., Tyas, S. L., Maarouf, C. L., Kokjohn, T. A., Belohlavek, M., . . . Emmerling, M. R. (2011). Transcranial doppler ultrasound blood flow velocity and pulsatility index as systemic indicators for Alzheimer's disease. *Alzheimer's and Dementia*, in press.

Roher, A. E., Tyas, S. L., Maarouf, C. L., Daugs, I. D., Kokjohn, T. A., Emmerling, M. R., . . . Beach, T. G. (2011). Intracranial atherosclerosis as a contributing factor to Alzheimer's disease dementia. *Alzheimer's and Dementia,* in press.

Sabbagh, M. N. (2009). Drug development for Alzheimer's disease: Where are we now and where are we headed? *The American Journal of Geriatric Pharmacotherapy, 7*(3), 167-185. doi:10.1016/j.amjopharm.2009.06.003

Sando, S. B., Melquist, S., Cannon, A., Hutton, M., Sletvold, O., Saltvedt, I., . . . Aasly, J. (2008). Risk-reducing effect of education in Alzheimer's disease. *International Journal of Geriatric Psychiatry, 23*(11), 1156-1162. doi:10.1002/gps.2043

Saunders, A. M., Hulette, O., Welsh-Bohmer, K. A., Schmechel, D. E., Crain, B., Burke, J. R., . . . Rosenberg, C. (1996). Specificity, sensitivity, and predictive value of apolipoprotein-E genotyping for sporadic Alzheimer's disease. *Lancet, 348*(9020), 90-93.

Schmidt, R., Schmidt, H., & Fazekas, F. (2000). Vascular risk factors in dementia. *Journal of Neurology, 247*(2), 81-87.

Schmidt, W. P., Roesler, A., Kretzschmar, K., Ladwig, K. H., Junker, R., & Berger, K. (2004). Functional and cognitive consequences of silent stroke discovered using brain magnetic resonance imaging in an elderly population. *Journal of the American Geriatrics Society, 52*(7), 1045-1050. doi:10.1111/j.1532-5415.2004.52300.x

Schmitt, F. A., Davis, D. G., Wekstein, D. R., Smith, C. D., Ashford, J. W., & Markesbery, W. R. (2000). "Preclinical" AD revisited: Neuropathology of cognitively normal older adults. *Neurology, 55*(3), 370-376.

Schneider, J. A., Bienias, J. L., Wilson, R. S., Berry-Kravis, E., Evans, D. A., & Bennett, D. A. (2005). The apolipoprotein E epsilon4 allele increases the odds of chronic cerebral infarction detected at autopsy in older persons. *Stroke, 36*(5), 954-959. doi:10.1161/01.STR.0000160747.27470.2a

Schneider, J. A., Boyle, P. A., Arvanitakis, Z., Bienias, J. L., & Bennett, D. A. (2007). Subcortical infarcts, Alzheimer's disease pathology, and memory function in older persons. *Annals of Neurology, 62*(1), 59-66. doi:10.1002/ana.21142

Schneider, J. A., Wilson, R. S., Bienias, J. L., Evans, D. A., & Bennett, D. A. (2004). Cerebral infarctions and the likelihood of dementia from Alzheimer disease pathology. *Neurology, 62*(7), 1148-1155.

Schneider, J. A., Wilson, R. S., Cochran, E. J., Bienias, J. L., Arnold, S. E., Evans, D. A., & Bennett, D. A. (2003). Relation of cerebral infarctions to dementia and cognitive function in older persons. *Neurology, 60*(7), 1082-1088.

Seeman, T. E., Huang, M. H., Bretsky, P., Crimmins, E., Launer, L., & Guralnik, J. M. (2005). Education and APOE-e4 in longitudinal cognitive decline: MacArthur studies of successful aging. *The Journals of Gerontology, 60*(2), P74-83.

Seripa, D., Panza, F., Franceschi, M., D'Onofrio, G., Solfrizzi, V., Dallapiccola, B., & Pilotto, A. (2009). Non-apolipoprotein E and apolipoprotein E genetics of sporadic Alzheimer's disease. *Ageing Research Reviews, 8*(3), 214-236.

Shadlen, M. F., Larson, E. B., Wang, L., Phelan, E. A., McCormick, W. C., Jolley, L., . . . van Belle, G. (2005). Education modifies the effect of apolipoprotein epsilon 4 on cognitive decline. *Neurobiology of Aging, 26*(1), 17-24. doi:10.1016/j.neurobiolaging.2004.03.005

Silver, M. H., Newell, K., Brady, C., Hedley-White, E. T., & Perls, T. T. (2002). Distinguishing between neurodegenerative disease and disease-free aging: Correlating neuropsychological evaluations and neuropathological studies in centenarians. *Psychosomatic Medicine, 64*(3), 493-501.

Skoog, I., Hesse, C., Aevarsson, O., Landahl, S., Wahlstrom, J., Fredman, P., & Blennow, K. (1998). A population study of apoE genotype at the age of 85: Relation to dementia, cerebrovascular disease, and mortality. *Journal of Neurology, Neurosurgery, and Psychiatry, 64*(1), 37-43.

Snowdon, D. A., Greiner, L. H., Mortimer, J. A., Riley, K. P., Greiner, P. A., & Markesbery, W. R. (1997). Brain infarction and the clinical expression of Alzheimer disease. The Nun Study. *The Journal of the American Medical Association, 277*(10), 813-817.

Song, I. U., Kim, J. S., Kim, Y. I., Eah, K. Y., & Lee, K. S. (2007). Clinical significance of silent cerebral infarctions in patients with Alzheimer disease. *Cognitive and Behavioral Neurology, 20*(2), 93-98. doi:10.1097/WNN.0b013e31805d859e

Staessen, J. A., Richart, T., & Birkenhager, W. H. (2007). Less atherosclerosis and lower blood pressure for a meaningful life perspective with more brain. *Hypertension, 49*(3), 389-400. doi:10.1161/01.HYP.0000258151.00728.d8

Statistics Canada. (2007). *Portrait of the Canadian population in 2006, by age and sex, 2006 census.* No. 97-551-XIE). Ottawa, Ontario, Canada: Statistics Canada.

Stern, Y., Gurland, B., Tatemichi, T. K., Tang, M. X., Wilder, D., & Mayeux, R. (1994). Influence of education and occupation on the incidence of Alzheimer's disease. *The Journal of the American Medical Association, 271*(13), 1004-1010.

The National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease [NIA-RI working group]. (1997). Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. *Neurobiology of Aging, 18*(4 Suppl), S1-2.

Troncoso, J. C., Zonderman, A. B., Resnick, S. M., Crain, B., Pletnikova, O., & O'Brien, R. J. (2008). Effect of infarcts on dementia in the Baltimore Longitudinal Study of Aging. *Annals of Neurology, 64*(2), 168-176. doi:10.1002/ana.21413

Tyas, S. L., & Gutmanis, I. (2008). Alzheimer's disease. In S. L. Tyas (Ed.), *Managerial Epidemiology: Concepts and Cases* (pp. 441). Chicago, IL: Health Administration Press.

Tyas, S. L., Koval, J. J., & Pederson, L. L. (2000). Does an interaction between smoking and drinking influence the risk of Alzheimer's disease? results from three Canadian data sets. *Statistics in Medicine, 19*(11-12), 1685-1696.

Tyas, S. L., Salazar, J. C., Snowdon, D. A., Desrosiers, M. F., Riley, K. P., Mendiondo, M. S., & Kryscio, R. J. (2007). Transitions to mild cognitive impairments, dementia, and death: Findings from The Nun Study. *American Journal of Epidemiology, 165*(11), 1231-1238. doi:10.1093/aje/kwm085

Tyas, S. L., Snowdon, D. A., Desrosiers, M. F., Riley, K. P., & Markesbery, W. R. (2007). Healthy ageing in The Nun Study: Definition and neuropathologic correlates. *Age and Ageing, 36*(6), 650-655. doi:10.1093/ageing/afm120

Tyas, S. L., Snowdon, D. A., Desrosiers, M. F., Riley, K. P., & Markesbery, W. R. (2009). Early-life linguistic ability, late-life pathology and asymptomatic Alzheimer's disease: Findings from The Nun Study [Abstract]. *Alzheimer's & Dementia, 5*(4) P103-P104.

Tyas, S. L., White, L. R., Petrovitch, H., Webster Ross, G., Foley, D. J., Heimovitz, H. K., & Launer, L. J. (2003). Mid-life smoking and late-life dementia: The Honolulu-Asia Aging Study. *Neurobiology of Aging, 24*(4), 589-596. doi:DOI: 10.1016/S0197-4580(02)00156-2

United Nations Economic and Social Council. (2001). *Demographic dynamics and sustainability: Report of the secretary-general.* No. E/CN.17/2001/PC/2). New York, NY: United Nations.

Vermeer, S. E., Longstreth, W. T.,Jr, & Koudstaal, P. J. (2007). Silent brain infarcts: A systematic review. *Lancet Neurology, 6*(7), 611-619. doi:10.1016/S1474-4422(07)70170-9

Vermeer, S. E., Prins, N. D., den Heijer, T., Hofman, A., Koudstaal, P. J., & Breteler, M. M. (2003). Silent brain infarcts and the risk of dementia and cognitive decline. *The New England Journal of Medicine, 348*(13), 1215-1222. doi:10.1056/NEJMoa022066

von Strauss, E., Viitanen, M., De Ronchi, D., Winblad, B., & Fratiglioni, L. (1999). Aging and the occurrence of dementia: Findings from a population-based cohort with a large sample of nonagenarians. *Archives of Neurology, 56*(5), 587-592.

Wang, S., Oelze, B., & Schumacher, A. (2008). Age-specific epigenetic drift in late-onset Alzheimer's disease. *PloS One, 3*(7), e2698.

Wardlaw, J. M. (2008). What is a lacune? *Stroke, 39*(11), 2921-2922. doi:10.1161/STROKEAHA.108.523795

White, L., Petrovitch, H., Hardman, J., Nelson, J., Davis, D. G., Ross, G. W., . . . Markesbery, W. R. (2002). Cerebrovascular pathology and dementia in autopsied Honolulu-Asia Aging Study participants. *Annals of the New York Academy of Sciences, 977,* 9-23.

Wilcock, G. K., & Esiri, M. M. (1982). Plaques, tangles and dementia. A quantitative study. *Journal of the Neurological Sciences, 56*(2-3), 343-356.

Wimo, A., Winblad, B., & Jonsson, L. (2010). The worldwide societal costs of dementia: Estimates for 2009. *Alzheimer's & Dementia, 6*(2), 98-103. doi:10.1016/j.jalz.2010.01.010

Yach, D., Hawkes, C., Gould, C. L., & Hofman, K. J. (2004). The global burden of chronic diseases: Overcoming impediments to prevention and control. *The Journal of the American Medical Association, 291*(21), 2616-2622. doi:10.1001/jama.291.21.2616

Yip, A. G., McKee, A. C., Green, R. C., Wells, J., Young, H., Cupples, L. A., & Farrer, L. A. (2005). APOE, vascular pathology, and the AD brain. *Neurology, 65*(2), 259-265. doi:10.1212/01.wnl.0000168863.49053.4d

Zekry, D., Duyckaerts, C., Belmin, J., Geoffre, C., Herrmann, F., Moulias, R., & Hauw, J. J. (2003). The vascular lesions in vascular and mixed dementia: The weight of functional neuroanatomy. *Neurobiology of Aging, 24*(2), 213-219.

Zekry, D., Duyckaerts, C., Moulias, R., Belmin, J., Geoffre, C., Herrmann, F., & Hauw, J. J. (2002). Degenerative and vascular lesions of the brain have synergistic effects in dementia of the elderly. *Acta Neuropathologica, 103*(5), 481-487. doi:10.1007/s00401-001-0493-5

Zhu, L., Fratiglioni, L., Guo, Z., Basun, H., Corder, E. H., Winblad, B., & Viitanen, M. (2000). Incidence of dementia in relation to stroke and the apolipoprotein E epsilon4 allele in the very old. Findings from a population-based longitudinal study. *Stroke, 31*(1), 53-60.

Appendices

Appendix A: List of Variables

Variable	Type	Role
Alzheimer's disease (*dementia at last assessment and the modified NIA-RI diagnosis*)	Categorical	Outcome
Dementia (*at last assessment*)	Categorical	Outcome
Alzheimer's disease pathology (*defined by the modified NIA-RI diagnostic criteria*)	Categorical	Outcome
Presence of infarct	Categorical 0=No; 1=Yes	Exposure
Presence of both large and lacunar infarct (*Participants in the sub-analytic sample that have both large or lacunar infarcts*)	Categorical 0=No; 1=Yes	Exposure
Presence of lacunar infarct (*Presence of one or more lacunar infarcts; infarcts anywhere in the brain that are visible to naked eye at gross autopsy and <= 1.5 cm*)	Categorical 0=No; 1=Yes	Exposure
Presence of large infarct (*Presence of one or more large infarcts; infarcts anywhere in the brain, i.e., visible to naked eye at gross autopsy, and > 1.5 cm.*)	Categorical 0=No; 1=Yes	Exposure
Location of infarct(s) (*Cortical, white matter region, basal ganglia, or limbic system*)	Categorical	Exposure
ApolipoproteinE-e4 (*the number of ApoE-e4 alleles*)	Categorical Zero = 0; one = 1; or two = 2 e4 alleles	Exposure/Effect Modifier
Age	Continuous	Covariate
Educational level	Categorical High school or less=1; Bachelors degree=2; or Masters degree+=3	Covariate

Appendix B: Assessing Non-Response Bias

Table 1. Participant characteristics: The deceased sample compared to the living sample[1]

Variable		Deceased Sample (n=606)	Living Sample (n=72)
Cerebral infarcts (%)	Presence	35.1	N/A
Educational level (%)	Grade school	10.4	6.5
	High school	5.9	1.4
	Bachelors degree	40.3	36.1
	Masters degree +	43.4	55.6
ApoE-e4 allele (%)			
Presence*	(1+)	24.2	10.8
Number of alleles*	Zero	75.8	89.2
	One	21.5	9.2
	Two	2.7	1.5

Note: Age at death and presence of cerebral infarcts cannot be compared since not all participants are deceased.
Abbreviations: ApoE-e4 = Apolipoprotein E e4; N/A = information not applicable
*$p<0.05$
[1]Refer to Figure 1 in section 4.3.1 for sample breakdown

Table 2. Participant characteristics: The analytic sample compared to the excluded sample[1]

Variable		Analytic Sample (n=199)	Excluded Sample (n=479)
Cerebral infarcts (%)	Presence	37.2	33.9
Educational level (%)	Grade school	11.1	9.6
	High school	6.0	5.2
	Bachelors degree	39.2	40.1
	Masters degree +	43.7	45.1
ApoE-e4 allele (%)			
Presence*	(1+)	32.2	18.3
Number of alleles*	Zero	67.8	81.7
	One	29.2	16.0
	Two	3.0	2.4

Abbreviations: ApoE-e4 = Apolipoprotein E e4
*$p<0.001$
[1]Refer to Figure 1 in section 4.3.1 for sample breakdown

Table 3. Participant characteristics: The analytic sample compared to the deceased sample[1]

Variable		Analytic Sample (n=199)	Deceased Sample (n=407)
Cerebral infarcts (%)	Presence	37.2	33.9
Age at death**	(mean years [SD])	89.7 (5.33)	90.8 (5.37)
Educational level (%)	Grade school	11.1	10.1
	High school	6.0	5.9
	Bachelors degree	39.2	40.8
	Masters degree +	43.7	43.2
ApoE-e4 allele (%)			
Presence*	(1+)	32.2	19.7
Number of alleles*	Zero	67.8	80.3
	One	29.2	17.2
	Two	3.0	2.5

Abbreviations: ApoE-e4 = Apolipoprotein E e4

*p<0.01; **p<0.05

[1]Refer to Figure 1 in section 4.3.1 for sample breakdown

Appendix C: Logistic Regression Models

Table 1. Analytic Plan for Research Question 1A

Model 1A	Statistical method:	Logistic Regression
	Outcome:	Alzheimer's Disease
	Exposure:	Presence of Cerebral Infarcts
	Covariates:	None
Model 1A-i	Statistical method:	Logistic Regression
	Outcome:	Alzheimer's Disease
	Exposure:	Presence of Cerebral Infarcts
	Covariates:	Age and Education
Model 1A-ii	Statistical method:	Logistic Regression
	Outcome:	Alzheimer's Disease
	Exposure:	Presence of Cerebral Infarcts
	Covariates:	Age, Education, and ApoE-e4 Status

Table 2. Analytic Plan for Research Question 1B

Model 1B - Location[1]	Statistical method:	Logistic Regression
	Outcome:	Alzheimer's Disease
	Exposure:	*Location* of Cerebral Infarcts
	Covariates:	None
Model 1B-i	Statistical method:	Logistic Regression
	Outcome:	Alzheimer's Disease
	Exposure:	*Location* of Cerebral Infarcts
	Covariates:	Age and Education
Model 1B-ii	Statistical method:	Logistic Regression
	Outcome:	Alzheimer's Disease
	Exposure:	*Location* of Cerebral Infarcts
	Covariates:	Age, Education, and ApoE-e4 Status
Model 1B - Size[2]	Statistical method:	Logistic Regression
	Outcome:	Alzheimer's Disease
	Exposure:	*Size* of Cerebral Infarcts
	Covariates:	None
Model 1B-i	Statistical method:	Logistic Regression
	Outcome:	Alzheimer's Disease
	Exposure:	*Size* of Cerebral Infarcts
	Covariates:	Age and Education
Model 1B-ii	Statistical method:	Logistic Regression
	Outcome:	Alzheimer's Disease
	Exposure:	*Size* of Cerebral Infarcts
	Covariates:	Age, Education, and ApoE-e4 Status

[1] Location refers to cortex, white matter, basal ganglia, or limbic system
[2] Size refers to large, lacunar, or both large and lacunar

Table 3. Analytic Plan for Research Question 2

Model 2	Statistical method:	Logistic Regression
	Outcome:	Alzheimer's Disease
	Exposure:	ApoE-e4 Status
	Covariates:	None
Model 2-i	Statistical method:	Logistic Regression
	Outcome:	Alzheimer's Disease
	Exposure:	ApoE-e4 Status
	Covariates:	Age and Education

Table 4. Analytic Plan for Research Question 3

Model 3	Statistical method:	Logistic Regression
	Outcome:	Presence of Cerebral Infarcts
	Exposure:	ApoE-e4 Status
	Covariates:	None
Model 3 - i	Statistical method:	Logistic Regression
	Outcome:	Presence of Cerebral Infarcts
	Exposure:	ApoE-e4 Status
	Covariates:	Age and Education

Table 5. Analytic Plan for Research Question 4A

Model 4a-i	Statistical method:	Logistic Regression
	Outcome:	Alzheimer's Disease
	Exposure:	Presence of Cerebral Infarcts
	Covariates:	None
	Effect Modifier:	ApoE-e4 Status
	Stratified by:	ApoE-e4- & ApoE-e4+
Model 4a-ii	Statistical method:	Logistic Regression
	Outcome:	Alzheimer's Disease
	Exposure:	Presence of Cerebral Infarcts
	Covariates:	Age and Education
	Effect Modifier:	ApoE-e4 Status
	Stratified by:	ApoE-e4- & ApoE-e4+

Abbreviations: ApoE-e4- = zero e4 alleles; ApoE-e4+ = presence of one or more e4 allele

Table 6: Analytic Plan for Research Question 4B[1]

Model 4B- Location[1]	Statistical method:	Logistic Regression
	Outcome:	Alzheimer's Disease
	Exposure:	*Location* of Cerebral Infarcts
	Covariates:	None
	Effect Modifier:	ApoE-e4 Status
	Stratified by:	ApoE-e4-, ApoE-e4+, ApoE-e4++
Model 4B-ii	Statistical method:	Logistic Regression
	Outcome:	Alzheimer's Disease
	Exposure:	*Location* of Cerebral Infarcts
	Covariates:	Age and Education
	Effect Modifier:	ApoE-e4 Status
	Stratified by:	ApoE-e4-, ApoE-e4+, ApoE-e4++
Model 4B-Size[2]	Statistical method:	Logistic Regression
	Outcome:	Alzheimer's Disease
	Exposure:	*Size* of Cerebral Infarcts
	Covariates:	None
	Effect Modifier:	ApoE-e4 Status
	Stratified by:	ApoE-e4-, ApoE-e4+, ApoE-e4++
Model 4B-ii	Statistical method:	Logistic Regression
	Outcome:	Alzheimer's Disease
	Exposure:	*Size* of Cerebral Infarcts
	Covariates:	Age and Education
	Effect Modifier:	ApoE-e4 Status
	Stratified by:	ApoE-e4-, ApoE-e4+, ApoE-e4++

[1] Location refers to cortex, white matter, basal ganglia, or limbic system

[2] Size refers to large, lacunar, or both large and lacunar

Abbreviations: ApoE-e4- = zero e4 alleles; ApoE-e4+ = presence of one or more e4 allele

Appendix D: Results of Sensitivity Analyses

Table 1. The association between presence of cerebral infarcts and Alzheimer's disease among a subset of Nun Study participants defined by the first alternative[3] for NIA-RI neuropathologic AD diagnosis (n=170)

Model	**Exposure** OR (95% CI)	**Covariates** OR (95% CI)			
	Presence of Infarcts	Age at death	Educational level[1]		ApoE-e4[2]
			High school or less	Bachelors degree	
Crude	1.05 (0.56, 1.97)	-	-	-	-
Adjusted - i	0.95 (0.48 ,1.87)	**1.13 (1.06, 1.21)**	**6.57 (1.69, 43.6)**	1.62 (0.39, 7.39)	-
Adjusted - ii	0.89 (0.41, 1.89)	**1.15 (1.07, 1.24)**	**7.55 (1.73, 53.4)**	1.25 (0.26, 6.47)	**10.31 (4.37, 26.93)**

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+
[2] Reference category is the presence of zero ApoE-e4 alleles
[3]See section 4.4.2.1 for first alternative definition
Bolded values indicate significance

Table 2. The association between presence of cerebral infarcts and Alzheimer's disease among a subset of Nun Study participants defined by the second alternative[3] for NIA-RI neuropathologic AD diagnosis (n=179)

Model	**Exposure** OR (95% CI)	**Covariates** OR (95% CI)			
	Presence of Infarcts	Age at death	Educational level[1]		ApoE-e4[2]
			High school or less	Bachelors degree	
Crude	1.14 (0.62, 2.10)	-	-	-	-
Adjusted - i	1.02 (0.53 ,1.98)	**1.14 (1.07,1.22)**	**5.82 (1.50, 38.5)**	1.66 (0.42, 7.32)	-
Adjusted - ii	1.01 (0.49, 2.06)	**1.15 (1.07, 1.23)**	**6.28 (1.47, 43.9)**	1.43 (0.33, 6.88)	**8.76 (3.80, 22.52)**

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+
[2] Reference category is the presence of zero ApoE-e4 alleles
[3]See section 4.4.2.1 for first alternative definition
Bolded values indicate significance

Table 3. The association between presence of cerebral infarcts and Alzheimer's disease pathology among a subset of Nun Study participants, defined by the modified version of the neuropathologic NIA-RI diagnostic criteria[3] (n=255)

Model	**Exposure** OR (95% CI)	**Covariates** OR (95% CI)			
	Presence of Infarcts	Age at death	Educational level[1]		ApoE-e4[2]
			High school or less	Bachelors degree	
Crude	0.95 (0.57, 1.58)	-	-	-	-
Adjusted - i	0.91 (0.54 ,1.53)	**1.07 (1.01, 1.12)**	1.38 (0.67, 2.88)	1.23 (0.70, 2.19)	-
Adjusted - ii	0.90 (0.50, 1.61)	**1.07 (1.01, 1.13)**	1.81 (0.80, 4.13)	1.45 (0.75, 2.79)	**13.82 (6.49, 33.31)**

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval

[1] Reference category is Masters degree+

[2] Reference category is the presence of zero ApoE-e4 alleles

[3]See section 4.4.2 for definition

Bolded values indicate significance

Appendix E: Example of results obtained by deletion of influential outliers

Table 1. Results of model from research question 1A

Effect	OR	95% Confidence Interval
Presence of cerebral infarcts	1.04	0.51, 2.10
Age at death	1.14	1.06, 1.22
Educational level		
High school or less	5.30	1.83, 17.22
Bachelors degree	1.68	0.80, 3.51
ApoE-e4 allele	10.60	4.63, 26.83

Abbreviations: OR = Odds ratio; ApoE-e4 = Apolipoprotein E e4 allele

Table 2. Results of model from research question 1A after deletion of one influential outlier (observation 142)

Effect	OR	95% Confidence Interval
Presence of cerebral infarcts	0.97	0.47, 1.99
Age at death	1.14	1.06, 1.23
Education:		
High school or less	7.09	2.32, 25.28
Bachelors degree	1.70	0.79, 3.68
ApoE-e4 allele	12.76	5.42, 34.28

Abbreviations: OR = Odds ratio; ApoE-e4 = Apolipoprotein E e4 allele

Table 3. Results of model from research question 1A after deletion of two influential outliers (observation 130)

Effect	OR	95% Confidence Interval
Presence of cerebral infarcts	0.89	0.43, 1.86
Age at death	1.16	1.08, 1.25
Education:		
High school or less	9.48	2.88, 38.70
Bachelors degree	1.65	0.77, 3.57
ApoE-e4 allele	12.80	5.40, 34.62

Abbreviations: OR = Odds ratio; ApoE-e4 = Apolipoprotein E e4 allele

Table 4. Results of model from research question 1A after deletion of three influential outliers (observation 51)

Effect	OR	95% Confidence Interval
Presence of cerebral infarcts	0.84	0.39, 1.77
Age at death	1.17	1.09, 1.28
Education:		
High school or less	9.98	2.90, 41.82
Bachelors degree	1.76	0.80, 3.89
ApoE-e4 allele	15.89	6.39, 46.38

Abbreviations: OR = Odds ratio; ApoE-e4 = Apolipoprotein E e4 allele

Table 5. Results of model from research question 1A after deletion of four influential outliers (observation 91)

Effect	OR	95% Confidence Interval
Presence of cerebral infarcts	0.84	0.39, 1.78
Age at death	1.17	1.09, 1.28
Education:		
High school or less	10.00	2.97, 41.86
Bachelors degree	1.77	0.81, 3.91
ApoE-e4 allele	15.60	6.26, 45.59

Abbreviations: OR = Odds ratio; ApoE-e4 = Apolipoprotein E e4 allele

Table 6. Results of model from research question 1A after deletion of five influential outliers (observation 92)

Effect	OR	95% Confidence Interval
Presence of cerebral infarcts	0.79	0.36, 1.69
Age at death	1.19	1.10, 1.30
Education:		
High school or less	9.79	2.85, 41.85
Bachelors degree	1.64	0.74, 3.67
ApoE-e4 allele	19.35	7.36, 62.07

Abbreviations: OR = Odds ratio; ApoE-e4 = Apolipoprotein E e4 allele

Table 7. Results of model from research question 1A after deletion of six influential outliers (observation 96)

Effect	OR	95% Confidence Interval
Presence of cerebral infarcts	0.86	0.39, 1.86
Age at death	1.18	1.09, 1.28
Education:		
High school or less	9.96	2.91, 42.23
Bachelors degree	1.82	0.81, 4.16
ApoE-e4 allele	23.99	8.53, 87.43

Abbreviations: OR = Odds ratio; ApoE-e4 = Apolipoprotein E e4 allele

Table 8. Results of model from research question 1A after deletion of seven influential outliers (observation 16)

Effect	OR	95% Confidence Interval
Presence of cerebral infarcts	0.81	0.36, 1.78
Age at death	1.18	1.09, 1.29
Education:		
High school or less	10.62	3.05, 45.87
Bachelors degree	2.03	0.89, 4.73
ApoE-e4 allele	33.77	10.80, 151.52

Abbreviations: OR = Odds ratio; ApoE-e4 = Apolipoprotein E e4 allele

Table 9. Results of model from research question 1A after deletion of eight influential outliers (observation 174)

Effect	OR	95% Confidence Interval
Presence of cerebral infarcts	0.75	0.33, 1.69
Age at death	1.18	1.09, 1.29
Education:		
High school or less	11.48	3.23, 50.49
Bachelors degree	2.30	0.99, 5.48
ApoE-e4 allele	53.95	14.59, 354.84

Abbreviations: OR = Odds ratio; ApoE-e4 = Apolipoprotein E e4 allele

Table 10. Results of model from research question 1A after deletion of nine influential outliers (observation 171)

Effect	OR	95% Confidence Interval
Presence of cerebral infarcts	0.83	0.36, 1.90
Age at death	1.19	1.09, 1.30
Education:		
High school or less	10.81	3.03, 47.58
Bachelors degree	2.19	0.92, 5.27
ApoE-e4 allele	107.05	20.87, >999.99

Abbreviations: OR = Odds ratio; ApoE-e4 = Apolipoprotein E e4 allele

Table 11. Results of model from research question 1A after deletion of ten influential outliers (observation 171)

Effect	OR	95% Confidence Interval
Presence of cerebral infarcts	0.94	0.40, 2.16
Age at death	1.18	1.09, 1.30
Education:		
High school or less	10.13	2.85, 44.06
Bachelors degree	2.11	0.88, 5.12
ApoE-e4 allele	.	>999.99, 48.32

Abbreviations: OR = Odds ratio; ApoE-e4 = Apolipoprotein E e4 allele

Appendix F: Results for Research Question 4B: The Association Between Location and Size of Cerebral Infarcts and Alzheimer's Disease

Table 1. The association between infarcts located in the basal ganglia and AD, stratified by ApoE-e4 allele status (n=199)

Model	**Exposure**	**Covariates**		
	Basal ganglia infarcts	Age at death	Education[1]	
			High school or less	Bachelors degree
ApoE-e4 +				
Adjusted	0.72 (0.13, 5.66)	1.01 (0.85, 1.22)	0.81 (0.07, 18.82)	0.37 (0.05, 2.17)
ApoE-e4 -				
Adjusted	0.32 (0.09 1.04)	**1.16 (1.06, 1.29)**	**12.1 (2.59, 73.63)**	2.37 (0.86, 6.79)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval

[1] Reference category is Masters degree+

Table 2. The association between infarcts located in the white matter area and AD, stratified by ApoE-e4 allele status (n=199)

Model	**Exposure**	**Covariates**		
	White matter infarcts	Age at death	Education[1]	
			High school or less	Bachelors degree
ApoE-e4 +				
Adjusted	1.35 (0.17, 28.3)	0.94 (0.78, 1.15)	0.35 (0.01, 9.70)	0.22 (0.01, 1.64)
ApoE-e4 -				
Adjusted	0.85 (0.25, 2.82)	**1.18 (1.07, 1.32)**	**10.3 (2.32, 58.71)**	3.76 (1.35, 11.1)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+

Table 3. The association between infarcts located in the limbic system and AD, stratified by ApoE-e4 allele status (n=199)

Model	**Exposure**	**Covariates**		
	Limbic system infarcts	Age at death	Education[1]	
			High school or less	Bachelors degree
ApoE-e4 +				
Adjusted	N/A	0.89 (0.72, 1.08)	0.33 (0.10, 9.82)	0.27 (0.01, 2.57)
ApoE-e4 -				
Adjusted	1.10 (0.25, 4.71)	**1.17 (1.06, 1.30)**	**9.30 (1.85, 71.18)**	2.43 (0.88, 6.91)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+

Table 4. The association between infarcts located in the cortex and AD, stratified by ApoE-e4 allele status (n=199)

Model	**Exposure**	**Covariates**		
	Cortical infarcts	Age at death	Education[1]	
			High school or less	Bachelors degree
ApoE-e4 +				
Adjusted	0.77 (0.07, 17.1)	0.90 (0.74, 1.07)	0.53 (0.04, 13.54)	0.53 (0.06, 3.76)
ApoE-e4 -				
Adjusted	1.36 (0.40, 4.59)	**1.17 (1.07, 1.29)**	**7.85 (2.00, 37.17)**	2.70 (0.99, 7.58)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+

Table 5. The association between lacunar infarcts and AD, stratified by ApoE-e4 allele status (n=199)

Model	**Exposure**	**Covariates**		
	Lacunar Infarcts	Age at death	Education[1]	
			High school or less	Bachelors degree
ApoE-e4 +				
Adjusted	**1.34 (0.17, 28.0)**	0.97 (0.81, 1.17)	0.88 (0.07, 20.68)	0.60 (0.07, 4.10)
ApoE-e4 -				
Adjusted	0.83 (0.30, 2.24)	**1.18 (1.07, 1.30)**	**5.32 (1.40, 23.62)**	2.05 (0.80, 5.33)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+

Table 6. The association between both large and lacunar infarcts and AD, stratified by ApoE-e4 allele status (n=199)

Model	**Exposure**	**Covariates**		
	Both large and lacunar	Age at death	Education[1]	
			High school or less	Bachelors degree
ApoE-e4 +				
Adjusted	N/A	0.89 (0.73, 1.08)	0.36 (0.01, 10.71)	0.28 (0.01, 2.68)
ApoE-e4 -				
Adjusted	0.56 (0.11, 2.70)	**1.18 (1.08, 1.33)**	**15.6 (2.95, 129.4)**	2.86 (0.98, 8.77)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+

Table 7. The association between large infarcts and AD, stratified by ApoE-e4 allele status (n=199)

Model	**Exposure**	**Covariates**		
	Large Infarcts	Age at death	Education[1]	
			High school or less	Bachelors degree
ApoE-e4 +				
Adjusted	**0.10 (0.00, 3.19)**	0.92 (0.76, 1.11)	1.38 (0.09, 51.56)	0.47 (0.05, 3.58)
ApoE-e4 -				
Adjusted	2.97 (0.70, 13.5)	**1.19 (1.08, 1.32)**	**7.07 (1.59, 39.79)**	2.81 (1.00, 8.21)

Abbreviations: ApoE-e4 = Apolipoprotein E e4; OR = odds ratio; CI = Confidence interval
[1] Reference category is Masters degree+