

POTENTIAL BIOMARKERS FOR ALZHEIMER'S DISEASE AND MILD COGNITIVE
IMPAIRMENT

by

MEGHAN B. MITCHELL

(Under the Direction of L. Stephen Miller)

ABSTRACT

Previous work from our group has demonstrated a relationship between dementia severity and blood plasma levels of the immunoglobulins for A β and RAGE in a mixed neurological sample of older adults. In this study, we compared non-diabetic older adults with normal cognition, mild cognitive impairment (MCI), and Alzheimer's disease (AD). We found significant group differences between control and AD groups as well as between MCI and AD groups, but no group differences were found between the control and MCI groups. A trend towards significance was found when comparing lower IgG values in nonamnestic versus amnestic MCI groups. In addition, we found both IgGs to be significantly related to global performance on cognitive measures, as well as domain-specific performance on an immediate memory measure. However, IgG levels were not related to language or delayed memory measures. These findings are discussed in terms of their implications regarding these IgGs as potential biomarkers that could be used for early detection of AD, as well as a tool to monitor cognitive decline in AD.

INDEX WORDS: Alzheimer's disease, biomarkers, aging, immunoglobulins, RAGE, A β , mild cognitive impairment, MCI

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B.A., Emory University, 2002

M.S., University of Georgia, 2006

A Dissertation Submitted to the Graduate Faculty of the University of Georgia in Partial
Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2009

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DEDICATION

This dissertation is dedicated to my grandparents, Natalie Reid Walsh, William F. Walsh, Nell Benston Mitchell, and William M. Mitchell.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. L. Stephen Miller, for his mentoring and support while completing this project, and throughout my graduate career. I would also like to thank Rose Schade, Scott Webster, and Dr. Jerry Buccafusco at the Medical College of Georgia for their assistance and guidance. This collaborative project would not have been possible without their help. I would like to thank my dissertation committee, Dr. Steven Beach, Dr. Jerry Buccafusco, and Dr. Joan Jackson for their invaluable insight. Finally, this project was supported by an Achievement Rewards for College Scientists (ARCS) scholarship, a University of Georgia Dissertation Completion Award, and a University of Georgia Graduate School Dean's Award in Social Sciences.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Overview and Aims

Recent Alzheimer's disease (AD) research indicates that blood plasma levels of immunoglobulins (IgGs) may be a marker of amyloid plaques that characterize the progression of AD (Mruthinti et al, 2004). Amyloid peptides, in particular beta amyloid (A β), accumulate in brain tissue at receptor sites called receptors for the advanced glycation end products (RAGE; Emanuele et al., 2005). The amyloid plaques formed in this process are associated with Alzheimer's disease and cognitive impairment (Berg et al., 1998).

The immune system develops IgGs in response to the binding of A β to RAGE in the brain, and these IgGs have been found to be elevated peripherally in the bloodstream (Nath et al., 2003). By obtaining a blood sample, levels of IgGs could potentially be used to detect the presence of AD brain pathology. Measuring IgGs in the blood has the benefit over current methods of probable AD diagnosis (e.g., neuropsychological testing, CSF testing, MRI) because it is a relatively painless, short, and inexpensive procedure. In addition, this method of detecting AD has the added advantage of potentially detecting AD in its pre-clinical form of Mild Cognitive Impairment (MCI).

The purpose of this study is to determine the ability of blood serum levels of the immunoglobulins for A β and RAGE to predict dementia severity in older adults across the spectrum of cognitively unimpaired older adults, older adults with MCI, and older adults with AD. There are three main hypotheses of this study: 1) When divided into groups (i.e., control

group, MCI, AD), the AD group will have significantly higher levels of anti-A β and anti-RAGE IgGs than the MCI group, which will have significantly higher levels of anti-A β and anti-RAGE IgGs than the control group. 2) When all index scores from the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) are entered into two separate multiple regression analyses with anti-A β and anti-RAGE IgGs as the dependent variables, the best predictors of the two IgGs will be Language and Delayed Memory ($p < .05$). 3) When using the conventional categorization of a CDR score of 0.5 as the criteria for MCI, the majority of the participants categorized in that group will meet the commonly-used neuropsychological criteria of having an RBANS Delayed Memory score that is equal to or more than 1.5 standard deviations below the mean.

This dissertation is structured in the manuscript chapter style, in which the first chapter is a combined introduction and literature review, the second chapter is a general description of the method, chapters three and four are manuscripts to be submitted, and chapter five is a general discussion.

Prevalence of AD

Alzheimer's disease (AD) is the most common form of dementia, with a national estimate of 4.5 million cases in 2000 (Herbert, Scherr, Bienias, Bennett, & Evans, 2003). While estimates vary, probable AD represents approximately 60 percent (Skoog & Blennow, 2001) to 80 percent (Mesulam, 2000) of all dementia diagnoses in the United States. It is estimated that the cost to U.S. society for the treatment and management of AD is approximately \$100 billion annually (DeKosky & Orgogozo, 2001). In addition to these figures, the demographic trends in the U.S. indicate that we are undergoing a population shift with an increasingly large population of older adults. It is projected by the U.S. Census Bureau (2005) that by the year 2050, there will be

approximately 78.9 million adults ages 65 and older, many of whom will require medical treatment and long-term care for AD.

Definition of AD

Alzheimer's disease (AD) is characterized by a primary impairment in memory that has an insidious onset, followed by progressive worsening of memory and one or more other domains of cognition, most commonly, language, attention, and/or executive functioning. The two most commonly used diagnostic criteria for AD are the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR; American Psychiatric Association, 2000) and the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann et al., 1984). The DSM-IV-TR criteria for AD require that the patient has impairment in two domains of cognition, one of which must be memory. According to the DSM-IV-TR, these cognitive deficits must be marked by a history of gradual decline from previous functioning that causes impairment in daily functioning, and exclusionary criteria include any other neurological, psychiatric, or other medical condition that could better account for cognitive deficits (e.g., Parkinson's disease, chronic substance abuse).

The NINCDS-ADRDA criteria (McKhann et al., 1984) for AD include criteria for possible, probable, and definite AD. Possible AD according to these criteria is defined as dementia with an atypical onset, presentation, or disease progression that is not known to be caused by another neurologic, psychiatric or medical condition. Typically a diagnosis of possible AD is only given when all other forms of dementia are ruled out, but the full criteria for probable AD are not met. Probable AD is defined as deficits in two or more areas of cognition, one of which must be memory, which are documented by neuropsychological testing and are observed

to progressively worsen over time. NINCDS-ADRDA criteria also require the dementia onset to occur between the ages of 40 to 90 and exclusionary criteria are that the patient has not had alterations in consciousness, and there are no other neurological diseases that could better account for dementia.

In addition to these criteria, NINCDS-ADRDA has also established a list of other criteria that support a diagnosis of probable AD, which include family history of dementia, impairments in daily functioning, or brain imaging results that suggest cerebral atrophy. Finally, the NINCDS-ADRDA criteria for definite AD require that the patient met criteria for probable AD and biopsy or autopsy results also show evidence of the disease by the presence of amyloid plaques, neurofibrillary tangles, and gross cortical atrophy with pronounced cortical atrophy of the hippocampus.

Definition and Prevalence of Mild Cognitive Impairment

Mild Cognitive Impairment (MCI) is a term that is used to describe clinical populations whose cognitive abilities fall between demented and normally-functioning older adults (Chertkow, Verret, & Bergman, 2001). Perhaps because of its relatively recent emergence as a topic of research and clinical diagnosis, there are divergent views on its definition. Knopman, Boeve, and Petersen (2003) argue that people with MCI exhibit cognitive impairment on neuropsychological testing, but do not exhibit functional impairment in their day-to-day lives. In contrast to this definition, Tabert et al. (2002) argue that people with MCI do, in fact, show impairment in daily functioning.

One possibility for why there is controversy surrounding the definition of MCI is that researchers are attempting to characterize a heterogeneous group of people. Petersen et al. (2001a) propose in their review of the literature on MCI that there are three subtypes of MCI that

have distinct characteristics. The first type of MCI proposed in their work is called amnesic Mild Cognitive Impairment. This type of MCI is characterized by preserved general cognitive functioning, but marked memory impairment, along with memory complaints. Additionally, Petersen et al. (2001a) describe amnesic MCI patients as having unimpaired daily functioning. They propose that amnesic MCI is the most common form of MCI, and patients with this type of MCI are the most likely to progress to Alzheimer's disease.

Petersen et al. (2001a) describe two other types of MCI, which are not as common. MCI with multiple domains slightly impaired is described as possibly leading to Alzheimer's Disease, Vascular Dementia, or it is found in normal aging. MCI with a single non-memory domain impaired is described as possibly leading to frontotemporal dementia, Lewy Body Dementia, Vascular Dementia, Primary Progressive Aphasia, or Parkinson's Disease. Clearly, this three-type model is lacking in clarity, as one of its subtypes is proposed to be a precursor to five distinct types of cognitive decline. This lack of clarity again reflects the state of confusion in the fields of psychology and neurology regarding the definition of MCI, as well as the heterogeneity of case presentations in preclinical AD.

In 2001, the American Academy of Neurology published a practice parameter for the early detection of dementia (Petersen et al., 2001b), in which they recognize Petersen and colleagues' (1999) criteria for MCI as the commonly-accepted criteria for MCI, which are as follows: 1) memory complaint, preferably corroborated by an informant; 2) objective memory impairment; 3) normal general cognitive function; 4) intact activities of daily living; 5) not demented. While Petersen and colleagues' (1999) criteria for MCI are indeed the most commonly recognized criteria for MCI, there are a number of issues with the operationalization of the criteria. For example, criterion 2, "objective memory impairment," is not explicitly defined.

While most researchers operationalize "objective impairment" in memory as any score that is 1.5 standard deviations below the mean of the population (Hänninen, Hallikainen, Tuomainen, Vanhanen, & Soininen, 2002), Petersen's group themselves do not use an objective cutoff score of -1.5 standard deviations. Instead, Petersen's group recommends consensus among expert clinicians with regard to what qualifies as "objective memory impairment" stating that "clinical judgment is required" (Petersen et al., 2001a, p. 1988) due to the influence that age, education, race, and other medical factors can have on standardized test scores. Thus, "objective memory impairment" by Petersen's use of the phrase is actually somewhat subjective.

Regardless of the ambiguity of the term MCI, it is an important condition to examine, as, in many cases, it leads to Alzheimer's disease. For example, one study found that every year, 10 to 15 percent of people with MCI convert to Alzheimer's disease (Petersen et al., 1999). Another study found that over a three year period, approximately 44 percent of individuals who met criteria for MCI converted to AD (Grundman, Petersen, & Morris, 1996). However, another study found that even after a ten year follow-up, roughly 25 percent of individuals meeting criteria for MCI still did not meet criteria for AD (Chertkow, Verret, & Bergman, 2001). Again, these divergent findings suggest that there are multiple operational definitions for MCI, and also that MCI patients are a heterogeneous group.

A topic of great interest in MCI research is to determine what areas of cognition are earliest to become impaired. Determining particular areas of cognition that show impairment early on in patients who later develop Alzheimer's disease would allow preclinical AD patients to begin pharmacological intervention early on in the course of their dementia.

Acetylcholinesterase inhibitors have been shown to be modestly effective in slowing down the dementing process in patients with preclinical Alzheimer's disease (Thal, 2003). Given the

clinical implications, it is important to detect early and subtle changes in cognition in preclinical AD patients.

Disease Process of AD

The fundamental cognitive characteristic of AD that is captured by both sets of diagnostic criteria for AD described above is that the disease involves a primary gradual decline in memory and one or more other domains of cognition. These cognitive features of AD occur secondary to three core characteristics of the AD brain: 1) aggregated extracellular plaques, primarily consisting of beta amyloid (A β); 2) cytoskeletal neurofibrillary tangles (NFTs) and neuropil threads (NTs), primarily consisting of tau proteins; and 3) neuronal death in the hippocampus, frontal cortex, and some subcortical areas. A definitive AD diagnosis can thus only be made postmortem (Small et al., 1997). Prior to summarizing the neuropathology of AD, a brief summary of the neuroanatomy and neurotransmitter systems involved in memory is warranted.

Neuroanatomy and Neurotransmitter Systems in Memory

The two general regions of the brain that play a central role in the processing of information in order to form memories, consolidate information, and retrieve information from memory are (1) the regions in and around the medial temporal lobe and (2) the medial diencephalic memory areas (Blumenfeld, 2002). The medial temporal lobe is a general region of the brain that includes the hippocampal formation and the parahippocampal gyrus (Martin, 2003). The medial diencephalic memory areas include several different areas located in the thalamus and hypothalamus (Martin, 2003).

To broadly summarize the manner in which memory is represented structurally and functionally in the brain, memory function involves reciprocal communication between the regions in and around the medial temporal lobe, the medial diencephalic memory areas, and the

association cortex (i.e., disparate neural networks in the cortex that connect to the memory centers of the brain). For memory to function properly, all parts of the two general memory areas of the brain must be neuroanatomically intact and functioning properly, the interconnections between each must be intact and functioning properly (i.e., white matter tracts and other neurons connecting these structures to one another must be functioning), and the connections between each structure and the rest of the brain must be intact and functioning properly (Blumenfeld, 2002). A summary of the structure and function of each of the aforementioned memory-related structures of the brain follows.

The hippocampal formation consists of three neuroanatomical structures: the dentate gyrus, the hippocampus, and the subiculum (Woolsey, Hanaway, & Gado, 2003). These three structures are layered on top of one another and form a tight roll of neurons with the dentate gyrus layer as the innermost portion of the cylindrical formation and hippocampus and subiculum creating the surrounding layers of the hippocampal formation (Blumenfeld, 2002). The hippocampal formation is involved in the processing of information from short-term memory and consolidating that information into long-term explicit memory (Martin, 2003). Thus, individuals with damage to the hippocampal formation, as was the case with the famous patient H.M., are not able to form new explicit memories (Milner, 1965).

The parahippocampal gyrus, which is ventral to the hippocampus, contains several neuroanatomical structures, the most notable of which is the entorhinal cortex (Woolsey, Hanaway, & Gado, 2003). The entorhinal cortex consists of six cellular layers (annotated as layers I-VI) and contains eight different cytoarchitectonic subfields (Insausti, Tunon, Sobreviela, Insausti, & Gonzalo, 1995). The six layers of the entorhinal cortex are also found in the entorhinal cortex of monkeys, making monkey models ideal for the study of human memory

function (Amaral, Insausti, & Cowan, 1987). The entorhinal cortex is important for memory function in general because it is the area of the brain that acts as the major communication center between the hippocampus and other cortical areas, both sending information to the hippocampus from multiple cortical areas, and receiving information from the cortex and sending it to the hippocampus (Blumenfeld, 2002). In the AD brain, one of the earliest pathological changes (to be discussed further in the section on neuropathology below) is found in layers II and V of the entorhinal cortex and the transentorhinal cortex (i.e., the transition area between the entorhinal cortex and adjacent parahippocampal areas; Braak & Braak, 1991).

The medial diencephalic memory areas are connected to the hippocampal formation by axons that form bilateral bundles of white matter called the fornix. The fornix forms bilateral arcs that reach rostrally from the hippocampal formation, run under the curve of the corpus callosum, and connect caudally to areas in the thalamus and hypothalamus. The bilateral fornices are interconnected by white matter called the hippocampal commissure, which allows for communication between the right and left hippocampi (Martin, 2003). The fornix connects to the mammillary bodies of the hypothalamus, which in turn connect to the anterior nuclei of the thalamus. This provides an anatomical loop, as the anterior nuclei have projections to the cingulate gyrus, which connects back to the entorhinal cortex and the hippocampus (Blumenfeld, 2002).

Another important area of the brain for learning, memory, and attention to function properly is the basal forebrain, which is located subcortically (Martin, 2003). Within the basal forebrain is the nucleus basalis of Meynert, a group of cells responsible for the production of the neurotransmitter acetylcholine (ACh; Martin, 2003). ACh has been shown to be deficient in the AD brain (Cummings, 2003). ACh levels are decreased in the AD brain due to the degeneration

of ACh-producing neurons in the nucleus basalis of Meynert. The basal forebrain has diffuse projections to the cortex and hippocampus, thus providing cholinergic projections that are essential for memory functioning (Martin, 2003). Multiple studies have demonstrated an association between lowered Ach transmission and deficits in learning, memory, and attention (Fracis, Palmer, Snape, & Wilcock, 1999). Both on biopsy and autopsy, AD patients evidence neurodegeneration in the basal forebrain and consequential decreases in levels of Ach (Bowen, Benton, Spillane, Smith, & Allen, 1982; Rossor, Garret, Johnson, Mountjoy, Roth, et al., 1982).

Acetylcholinesterase inhibitors, or drugs that increase synaptic levels of ACh by inhibiting the neurotransmitter's breakdown, have been shown to slow down the cognitive decline associated with AD (Benzi & Moretti, 1998). Importantly, current acetylcholinesterase inhibitor therapy for AD, while helpful in slowing down cognitive decline, does not change the end result of the disease (Cummings, 2004), indicating that the mechanism of disease process is only indirectly affected by cholinesterase inhibitor therapy.

Neuropathology of AD

Neuropathological research indicates that while there are individual differences in the precise distribution of A β plaques, neurofibrillary tangles, and neuronal degeneration, there is a general pattern across individuals both in distribution and progression of these neuropathological changes (Braak et al., 1999). The pathology of neurofibrillary tangles (NFTs) and neuropil threads (NTs) has been extensively studied by Braak and Braak (1991), who have proposed disease staging of AD based on the progression of cytoskeletal alterations involving NFTs and NTs.

In a review article summarizing their stages, Braak et al. (1999) indicate that the general progression from the earliest NFT and NT changes in the AD brain to the end stage of AD

involves six stages, identified as stages I-VI. In stage I, NFT and NT lesions are confined to only a few neuronal cells in the transentorhinal region, while in stage II, these lesions have spread to more cells within the transentorhinal region and beginning signs of lesions appear in the hippocampus. In general, stages I and II are considered preclinical in that individuals in these stages do not exhibit overt signs of memory impairment or personality change. Stage III is characterized by severe lesions in the transentorhinal region and entorhinal cortex, and a moderate level of lesions in the hippocampus, temporal, and insular cortices. In addition, in stage III, lesions begin to appear in a few subcortical areas. Stage IV is characterized by a spread of lesions from the medial temporal lobe areas to the adjoining areas of the cortex (i.e., frontal lobes). Individuals in stages III and IV typically evidence overt signs of AD brain pathology (e.g., impaired memory on testing, personality changes). Finally, in stage V, lesions are found in all areas of the cortex with the exception of the relatively preserved primary motor and sensory cortices, while lesions are found in all areas of the cortex in stage VI of AD.

Tau protein deposits have been found as early as 30 years prior to emergence of amyloid plaques in AD brains (Delatour, Blanchard, Pradier, & Duyckaerts, 2002). In addition, while tau protein abnormalities are first found in memory-related brain areas, amyloid plaques tend to be found first in a diffuse form in the neocortex, and tend to transition from the diffuse form to a neuritic form (i.e., the plaques thicken and some form a dense core with extending neurites; Price & Morris, 1999). As AD progresses, amyloid plaques spread from the neocortex to memory related areas, including the hippocampus, but plaque distribution is typically denser in diffuse cortical areas than in the hippocampus and entorhinal cortices (Price & Morris, 1999). Thus, it appears that the time course and location of amyloid plaques and neurofibrillary tangles are distinct. While the two disease process appear to occur in parallel at least initially, in later stages

of AD, it appears that increases in amyloid plaque deposition accelerate the formation of neurofibrillary tangles (Price & Morris, 1999), thus suggesting a relationship between the two aspects of AD brain pathology.

Amyloid cascade hypothesis of AD

The most widely supported theory of the disease process of AD, termed the “amyloid cascade hypothesis” by Hardy and Higgins (1992) theorizes that the core mechanism initiating AD brain pathology is aggregation of A β plaques. To understand how A β plaques can occur in a diseased brain, one must first understand how A β proteins are formed. The precursor to A β is amyloid precursor protein (APP), a large transmembrane protein that can be cleaved by three different types of enzymes, referred to as α , β , and γ -secretases (Selkoe & Schenk, 2003). In the normally functioning brain, most APP proteins are cleaved by α -secretase, forming APP_s- α , which is thought to have important roles in regulating neuronal survival, synaptic plasticity, the growth of neurites, and cell adhesion (Mattson, 2004). It has been argued that it is not only A β plaque formation that is detrimental to the AD brain, but also the decreased production of APP_s- α , as neuronal activity and the activation of muscarinic Ach receptors have a demonstrated association with increased levels of APP_s- α production (Mattson, 1997).

In the AD brain, however, an abnormal amount of APP is sequentially cleaved first by an enzyme located on the cellular membrane called β -secretase (Mattson, 2004). β -secretase cleaves amyloid β protein precursor into one protein fragment that is secreted from the cell membrane, and a smaller protein fragment that remains bound to the cell membrane. The membrane-bound protein fragment, termed an APP carboxy-terminal fragment, or CTF β , is then cleaved by a second enzyme called γ -secretase, resulting in the production of amyloid β proteins (Selkoe &

Schenk, 2003). There are thought to be two main γ -secretases that are essential for this final enzymatic step that produces $A\beta$, and they are called presenilins 1 and 2 or PS1 and PS2.

In a review of current treatment and pathology literature that uses the amyloid cascade hypothesis as a theoretical framework, Golde (2003) summarizes the current understanding of the altered processing of APP that results in $A\beta$ plaque formation. Golde (2003) explains that roughly 80 percent of amyloid β proteins produced by a normal cell are chains of 40 peptides, and are thus termed $A\beta_{40}$. The remaining 20 percent of amyloid β proteins are chains consisting of 42 peptides and are thus termed $A\beta_{42}$ (Golde, 2003). While both $A\beta_{40}$ and $A\beta_{42}$ have been shown to be prone to aggregation in the extracellular space surrounding brain cells, $A\beta_{42}$ has been shown to be particularly prone to aggregation (Jarrett, Berger, & Lansbury, 1993) because they are more fibrillogenic (i.e., more fibrous and insoluble; Selkoe, 1996). It is thus proposed in the amyloid cascade hypothesis that a complex interaction of genetic and environmental factors make the AD brain susceptible to excessive amounts of $A\beta_{42}$ protein production, which initiates the aggregation of plaques, which then lead to a cascade of abnormal biological changes, including oxidative stress (Varadarajan, Yatin, Aksenova, & Butterfield, 2000), changes in cellular homeostasis of calcium (Arispe, Rojas, & Pollard, 1993), and changes in the organization of the neuronal cytoskeleton and development of tauopathy (Götz, Chen, van Dorpe, & Nitsch, 2001).

As outlined in Golde's (2003) above-mentioned review paper, there are multiple lines of evidence to support the amyloid cascade hypothesis of AD pathology. There are several areas of research on genetic contributions to AD pathology that support the amyloid cascade hypothesis. Price and Sisodia's (1998) review of work with transgenic mice shows that in mice with genetic mutations that cause overexpression of mutated forms of APP, their brains show AD-like

pathology in the form of excessive A β aggregation. Similarly, in another line of transgenic mice with mutations causing overexpression of mutated APP along with mutations in presenilin 1 and 2 (PS1 and PS2), their brains showed similar A β aggregation and AD-like pathology (Price & Sisodia, 1998).

Individuals with the apolipoprotein E (ApoE) e4 allele, one of the three variants of the ApoE allele on chromosome 19, are at an increased risk of developing late-onset AD. The ApoE allele is a protein that is important in regulating the metabolism of cholesterol and phospholipids (Mahley, 1988). Compared to individuals with no e4 allele, the risk of developing AD is roughly ten times greater in person with two e4 alleles and three to four times greater in a person with one e4 allele (Corder et al., 1993).

Holtzman et al. (2000) demonstrated that there is a strong association between ApoE genotype and amount of A β plaque accumulation in the brain parenchyma and vasculature. In their study, Holtzman and colleagues used a previously developed transgenic mouse model for AD (APP^{sw}; Hsiao, et al., 1996) with a genetic mutation in the amyloid β precursor protein (APP) that leads to overproduction of A β . In order to examine the relationship between ApoE genotype and level of A β plaques in the brain, Holtzman and colleagues generated three groups of APP^{sw} transgenic mice, one group with two ApoE alleles (ApoE +/+), one group with one ApoE allele (ApoE +/-), and one group of ApoE genetic knockout mice (ApoE -/-). Findings revealed that after the mice had aged (12 months), the ApoE +/+ mice had significantly more A β protein deposits in hippocampus, parietal association cortex, and piriform cortex, when compared to both ApoE +/- and ApoE -/- mice. ApoE +/+ and ApoE +/- mice also developed fibrillar A β in the brain, which was not found in the ApoE -/- group. These findings suggest that ApoE regulates not only the levels of A β in the brain, but also has a regulatory role in the

development of fibrillar A β , which is more neurotoxic than non-fibrillar A β (Holtzman et al., 2000).

Another line of genetics research that supports the amyloid cascade hypothesis is a group of studies that have found that genetic mutations in the gene that codes for the production of APP, located on chromosome 21 (Andreasson, 2001), are linked to early-onset familial forms of AD. Nilsberth and colleagues (2001) found a genetic mutation in the A β protein domain of the APP gene, termed the “Arctic mutation,” in a northern Swedish family that leads to excessive production of A β 42. Findings revealed that those family members who were carriers of the “Arctic mutation” develop early-onset progressive dementia consistent with the clinical features of Alzheimer’s disease. There have been multiple studies finding similar forms of early-onset familial AD in individuals with genetic mutations in the APP gene (e.g., Goate et al., 1991; Mullan et al., 1992), all of which are associated with large accumulations of A β plaques in the brain.

Mutations in two other genes, presenilins 1 and 2 (PS1 and PS2), are additionally associated with early-onset familial AD (Price & Sisodia, 1998). The presenilin-1 (PS1) gene, located on chromosome 14, and the presenilin-2 (PS2) gene, located on chromosome 1 (Andreasson, 2001), are the genetic code for presenilin proteins, which are transmembrane proteins found peripherally and in the central nervous system on neuronal and glial cells (Lee et al., 1996). Approximately 30 mutations in the PS1 gene and two mutations in the PS2 gene have been identified to cause early-onset familial AD (Price & Sisodia, 1998). In all cases of familial AD associated with PS1 and PS2 mutations, large deposits of A β 42 have been found in the brain parenchyma, which are thought to be caused by PS1 and PS2’s influence on APP production (Price & Sisodia, 1998).

Finally, in in-vitro experiments in which A β is used, it has been shown to self-aggregate, supporting the idea that it is highly prone to form plaques (Yankner, 1996). Once A β aggregates are formed in vitro, they are neurotoxic (Yankner, 1996).

In contrast to the abundance of literature supporting the amyloid cascade hypothesis, there is less understanding about the mechanisms through which A β causes the other pathological features of AD (i.e., neurofibrillary tangles and neuronal death). Golde (2003) and others postulate that there are likely multiple mechanisms through which A β aggregations lead to the development of neurofibrillary tangles and neuronal death. As discussed above, there is evidence to suggest that A β plaques are directly neurotoxic (Yankner, 1996) and thus may be responsible for immediate cell death of surrounding neurons.

A β plaques are also associated with longer-term induction of oxidative stress, leading to neuronal cell death (Varadarajan, Yatin, Aksenova, & Butterfield, 2000). Related to the premise of this proposal, it has also been postulated that the presence of A β induces an inflammatory response in the brain, and the immune system, once activated, actively attacks the A β plaques and surrounding neurons, thus exacerbating the disease process (Akiyama et al., 2000).

Although the formation of neurofibrillary tangles is not well characterized in AD, it is clear from autopsy examinations of patients with probable AD that neurofibrillary tangles are a core pathological feature of Alzheimer's disease. Neurofibrillary tangles are composed of tau proteins, which bind to the microtubules inside neurons in a normally functioning brain (Lee & Trojanowski, 1992). In the brains of individuals with AD and other forms of dementia with tau protein tangles, it is thought that a dysfunction in the tau RNA protein transcription process leads to an abundance of tau proteins that are unable to bind to microtubules (Alonso, Zaidi, Novak, Grundke-Iqbal, & Iqbal, 2001). Left as free-floating proteins within the cell, these tau proteins

have then been shown to undergo a process of hyperphosphorylation (i.e., the addition of excessive phosphates to the protein) which in turn leads to the development of various formations of protein filaments which are collectively termed “tauopathy” (Spillantini et al., 1998). Tauopathies interfere with neuronal functioning and ultimately lead to cell death. Tauopathies are not pathologically unique to Alzheimer’s disease. They are one of the core pathological features of Frontotemporal Dementia, Parkinson’s Disease, Dementia with Lewy Bodies, and Cortico-Basal Ganglionic Degeneration (Spillantini et al., 1998). Importantly, tau mutations do not affect the production of A β in Frontotemporal Dementia, thus suggesting that tau pathology, while likely linked to A β pathology in AD, is also a distinct disease process leading to cognitive impairment in other forms of dementia (Golde, 2003).

It is currently postulated by some that neurofibrillary tangles are formed secondary to A β plaque formation in Alzheimer’s disease, but the mechanism through which this occurs is yet to be discovered (Golde, 2003). Others argue that A β plaque formation is a downstream effect of abnormalities in tau, citing studies that demonstrate that tauopathy is found in AD brains prior to A β plaque formation (e.g., Delatour, Blanchard, Pradier, & Duyckaerts, 2002). While there is evidence that tauopathy predates A β plaque formation in the AD brain, there is also evidence that introduction of A β 42 into the brain can accelerate tauopathy (Götz, Chen, van Dorpe, & Nitsch, 2001).

From the above review of the literature on the disease process of AD, there is clear evidence that A β plaque formation is an essential component to the disease. However, there are many obstacles to detecting when these plaques have formed, and whether or not their formation is significant enough to cause cognitive or functional deficits. The following section is a

summary of neuropsychological, neuroimaging, and biological areas of research on potential early markers for MCI and AD.

Detection of AD and MCI

Neuropsychological Measures

As discussed in the above section on the definition of MCI, there is some debate about whether or not it is advisable to use cutoff scores (e.g., -1.5 SD) to operationalize impairment on neuropsychological tests for the classification of MCI. Similarly, there is no consensus on a clear cutoff to distinguish MCI from AD using neuropsychological testing alone (although a general rule of thumb of -2.0 SD is commonly used). There is a large body of literature to support neuropsychological tests as useful in detecting MCI and predicting conversion to AD. For example, Arnáiz and colleagues (2004) examined the predictive ability of the number of cognitive domains below a -1.5 SD cutoff to predict time to AD diagnosis in two separate samples of MCI patients. Findings were that the number of cognitive domains below -1.5 SD was a significant predictor of time to conversion to AD, beyond the variance accounted for by age, education, and APOE e4 genotype. Logistic regression analysis revealed that the only neuropsychological tests that were significantly predictive of conversion to AD were measures of learning and memory when compared to measures of verbal comprehension, attention, and perceptual organization (Arnáiz et al., 2004).

Cerhan and colleagues (2007) compared cognitive profiles of 80 older adults who were longitudinally followed beginning when they were all classified as control participants without cognitive impairments. Twenty of these participants were later diagnosed with possible or probable AD. When time one test profiles were compared, the only significant differences between patients who remained cognitively normal at follow-up and those who converted to AD

were in the domains of learning and memory. There were no group differences in test performance in the domains of verbal comprehension, perceptual organization, or attention (Cerhan, et al., 2007).

In a recent review of studies of preclinical AD, Twamley, Legendre Ropacki, and Bondi (2006) found that cognitive profiles of preclinical AD are not always focally amnesic. In fact, their review of 46 longitudinal studies and 26 cross-sectional studies suggested that older adults with preclinical AD are more likely to have mild decrements in multiple neuropsychological domains, including attention, learning and memory, executive functioning, processing speed, and language. The study authors found that attention was the most frequently impaired cognitive ability, whereas learning and memory was the second most frequent cognitive impairment in preclinical AD. Of note, this review excluded studies whose samples were obtained at memory assessment clinics (i.e., excluding most MCI studies and studies by the Petersen group at Mayo Clinic), because their objective was to examine studies that did not have a sampling bias of individuals who were treatment-seeking for memory concern (Twamley, Legendre Ropacki, & Bondi, 2006).

Sampling bias will undoubtedly skew the results of cognitive testing in a clinical sample of older adults presenting with memory concerns. However, the early neuroanatomical changes found in the temporal lobe of MCI patients (Twamley, Legendre Ropacki, & Bondi, 2006) does, in fact, support the Mayo Clinic view that memory functioning is the most sensitive predictor of AD in an MCI population.

Imaging Techniques

There are a number of existing and developing imaging technologies that have been shown to be useful in detecting early changes in brain structure and function in MCI and AD

patients. The most commonly used technologies are structural and functional magnetic resonance imaging (MRI and fMRI, respectively) and positron emission tomography (PET). The above mentioned review by Twamley, Legendre Ropacki, & Bondi (2006) additionally reviewed imaging changes in preclinical AD. It should again be noted that this review excluded studies using memory clinic samples. Of the 18 imaging studies reviewed, eleven were structural MRI studies and seven were functional imaging studies (four PET studies and three fMRI studies). Across all imaging studies, the most common abnormalities in the preclinical AD brain were medial temporal lobe atrophy (structural imaging) and hypoperfusion in temporoparietal areas (functional imaging; Twamley, Legendre Ropacki, & Bondi, 2006).

Biomarkers

In 1998, the Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging published a consensus statement entitled "Molecular and Biochemical Markers of AD" in which they outline criteria for an ideal biomarker for AD and review the research on potential biomarkers for AD. In their consensus statement, the group proposed the following criteria for the ideal biomarker: 1) able to detect a fundamental feature of Alzheimer's neuropathology; 2) validated in neuropathologically confirmed AD cases; 3) precise (able to detect AD early in its course and distinguish it from other dementias); 4) reliable; 5) non-invasive; 6) simple to perform; and 7) inexpensive (The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group, 1998, p. 110). The work group additionally proposed the following recommended steps in the process of establishing a biomarker: 1) there should be at least two independent studies that specify the biomarker's sensitivity, specificity, and positive and negative predictive values; 2) sensitivity and specificity should be no less than 80%; positive predictive value should approach

90%; 3) the studies should be well powered, conducted by investigators with expertise to conduct such studies, and the results published in peer-reviewed journals; 4) the studies should specify type of control subjects, including normal subjects and those with a dementing illness but not AD; and 5) once a marker is accepted, follow-up data should be collected and disseminated to monitor its accuracy and diagnostic value (The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group, 1998, p. 111). The work group reported that at the time of publication, no proposed biomarkers for AD had met their criteria, and to date there are no identified biomarkers for AD that meet these criteria.

CSF biomarkers.

While none of the identified potential biomarkers for AD meet the above described criteria, there are a number of potential biomarkers for AD that can be used in addition to other clinical information (e.g., neuropsychological testing, MRI, patient, and collateral report) to assist in diagnosis. The most commonly accepted of these biomarkers is cerebrospinal fluid (CSF) testing for levels of tau protein and A β 42 (Growdon, 1999). Several studies have found that A β 42 levels in the CSF of AD patients is significantly lower when compared to levels in normal controls (e.g., Andreasen et al, 1999; Clark et al., 2003; Galasko et al., 1998). The proposed mechanism of lowered A β 42 levels in the CSF of AD patients is that in the central nervous system of an AD individual, the A β 42 that is freely circulating in the CSF becomes increasingly insoluble as the disease progresses. According to this proposed mechanism, as the A β 42 becomes insoluble, it forms aggregated plaques in the brain parenchyma and levels of A β 42 in the CSF are thus decreased (Kuo et al., 1990).

Tau protein levels in the CSF of AD patients have been demonstrated to be elevated when compared to normal controls (Trojanowski, Clark, Arai, & Lee, 1996). Clark and colleagues (2003) used both tau and A β CSF levels to predict autopsy-confirmed AD diagnosis and found that tau levels were a better predictor of AD than A β levels. However, Andreasen and colleagues (2001) found that when using CSF levels of both tau and A β 42, the combined use of both biomarkers was a strong predictor of AD, with 94% sensitivity for probable AD, 88% for possible AD, and 75% for MCI. Their findings suggested questionable specificity of the CSF markers, as patient groups with other dementing illnesses (e.g., Lewy Body Dementia, Vascular Dementia) had specificities ranging from 29-88% (Andreasen et al., 2001). While there is conflicting evidence as to which CSF biomarker (i.e., tau or A β 42) is "better" at predicting AD, both low A β and high tau levels in the CSF are consistently found in AD patients when compared to normal controls. CSF markers, while a relatively direct measure of central nervous system neurobiology, has a distinct disadvantage as a potential biomarker for AD because the procedure for obtaining CSF is painful and distressing.

Blood plasma biomarkers.

Identifying a biomarker for AD in the blood would be ideal, as the procedure is short, simple, familiar to patients, and low in cost. There has been inconsistent evidence that plasma A β levels are a useful biomarker for AD. One study measured plasma levels of A β 40 and A β 42 in 78 AD patients and 61 controls, and found A β 40 to be significantly higher in AD patients, but there were no group differences in A β 42 plasma levels (Mehta et al., 2000). Mehta and colleagues (2000) also noted that although plasma A β 40 was significantly higher in their AD group, there was considerable group overlap in individual participants' plasma A β 40 levels. In another study by the same research group, plasma levels of A β 40 and A β 42 were measured in 50

AD patients to determine if there was a relationship between the plasma biomarkers and CSF levels of A β 40, A β 42, age, sex, and Mini-Mental State Examination (MMSE) score (Mehta, Pirttila, Patrick, Barshatzky, & Mehta, 2001). Findings revealed no significant relationship between either plasma or CSF levels of A β 40 or A β 42 and age, sex, and MMSE score. Further, Mehta and colleagues did not find a relationship between plasma and CSF levels of A β 40 or 42 in their sample of AD patients, thus suggesting that plasma A β 40 and A β 42 levels are not representative of CSF levels. In contrast, Kuo and colleagues (1999) found that there were high levels of plasma A β 42 in AD patients compared to controls, but that the majority of the A β 42 was bound to plasma proteins, and would thus be undetected in previous studies that did not use a purification process to separate the A β 42 from the plasma proteins.

Nonetheless, the majority of cross-sectional studies comparing AD and control groups' plasma levels of A β have not found significant group differences (e.g., Kosaka et al, 1997; Scheuner et al., 1996; Tamaoka et al., 1996). With regard to The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Work Group guidelines for an AD biomarker, plasma A β levels clearly fall short of an ideal biomarker. In fact, one study concluded that age, as opposed to AD diagnosis, is the main predictor of plasma A β levels in their comparison of 371 AD patients, 146 MCI patients, 37 Parkinson's disease patients, and 92 normal controls (Fukumoto, et al., 2003).

While plasma A β levels may not be useful as a diagnostic tool for AD, at least two longitudinal studies have found high plasma A β 42 levels to be a risk factor for future conversion to AD. In one study, 169 non-demented older adults were followed longitudinally, and baseline levels of plasma A β 42 were found to be higher in those individuals who were diagnosed with AD at an average of 3.6 years follow-up (Mayeux, et al., 1999). In a second study by the same

research group, 79 AD patients and 451 cognitively normal older adults were followed longitudinally and plasma A β 40 and A β 42 was measured at baseline and at 3 years follow-up. Findings revealed that baseline plasma levels of A β 42, but not A β 40 were significantly higher in the older adults with AD at baseline and in those older adults who were diagnosed with AD at follow-up when compared to older adults who remained cognitively unimpaired throughout the study (Mayeux, et al., 2003). Overall, results of studies examining the ability of plasma A β levels to differentiate older adults with normal cognitive functioning, those with MCI, and those with AD, suggest a high level of A β 42 is a good predictor of future conversion to AD, but it does not consistently differentiate between AD and non-demented individuals in cross-sectional designs.

More recently, a large group of researchers in California developed a technique to quantify the amount of 120 different plasma signaling proteins in order to determine which proteins had statistically significant levels in an AD group compared to a control group (Ray et al, 2007). Findings revealed that there were 18 plasma signaling proteins that were able to correctly classify blinded AD samples (n=42) with 90% accuracy and control samples (n=39) with 88% accuracy. Further, in a cohort of individuals diagnosed with MCI who later converted to AD (n=22), the proteins identified 91% as AD, whereas in samples of participants with MCI who later converted to non-AD dementia (n=8), all eight samples were correctly classified as non-AD. As this investigation was exploratory in nature, the combined 18 proteins, while statistically able to differentiate groups, did not necessarily have a shared mechanism by which they were biologically related to AD. Thus, the team employed several strategies of investigation to determine potential mechanisms through which the proteins were both related to one another and related to the disease process of AD, using both computational models for relationships

between genes and traditional literature review. Findings revealed that some of the 18 signaling proteins may be related to one another and also possibly to the disease process of AD via a number of cellular pathways, including systematic dysfunction in the production of blood cells, immune system dysfunction, cell death, and neuronal support. These data warrant further investigation with larger numbers of participants, as well as further investigation into the potential cellular mechanisms by which these biomarkers are related to the disease process of AD.

Immunoglobulin biomarkers.

The immune system response to A β aggregation in the AD brain is another promising biological marker for AD. Given the central role of A β aggregation in the disease process of AD, coupled with evidence that the immune system inflammatory response is a key process in age-related chronic diseases (Licastro et al, 2005), the immune response to A β is a promising target for disease detection and possibly disease prevention. Further, there is a preponderance of evidence suggesting that chronic inflammation caused by a microglial response to A β in the AD brain is an important aspect of the disease process that ultimately leads to neurotoxicity (El Khoury et al., 1996; McGeer & McGeer, 1998; Pasinetti, 1996; Yan et al., 1997). An advantage of measuring immune responses to AD is that immune system-produced immunoglobulins (IgGs) have been demonstrated to pass through the blood-brain barrier from brain to blood (Bouras, Riederer, Kovari, Hof, & Giannakopoulos, 2005; Zhang & Pardridge, 2001), thus circumventing the methodological issue of detecting brain pathology in the blood.

One aspect of the immune response to AD that has been identified involves a neuronal membrane receptor for advanced glycation end products (RAGE). This receptor binds advanced glycation end products (AGEs) in the normal aging process, but has been demonstrated to bind to

A β in the AD brain at a high affinity (Lue et al., 2001). The gene for RAGE, located on chromosome 6 (Sugaya et al., 1994), was found in one study to be over-expressed in neurons and microglia located in the hippocampus, entorhinal cortex, and superior frontal gyrus of AD brain tissue, all of which are brain areas strongly associated with AD brain pathology (Lue et al., 2001). RAGE has also been demonstrated to be present in neurons from the CA3 and CA4 regions of hippocampi of AD brains in close proximity to A β (Sasaki, et al., 2001). The RAGE-A β binding process was characterized by Yan and colleagues (1997), and found to induce macrophage-colony stimulating factor, which then triggers a number of cellular responses, all of which lead to a generalized inflammatory response. Mruthinti, Capito, Sood, & Buccafusco (2007) demonstrated that A β and RAGE peptides bind to one another and form a complex that is more cytotoxic to neuronal cells than either individual protein.

Given the key role that the binding of A β to RAGE has in the inflammatory response associated with AD, Mruthinti, Hill, Swamy-Mruthinti, and Buccafusco (2003) investigated the RAGE-A β interaction in a mouse model of AD in which the mice were immunized with a human neurofilament-derived AGE. They unexpectedly found that the mice developed immunoglobulins (IgGs) to both RAGE and A β , suggesting that the RAGE-A β interaction not only results in the previously characterized generalized inflammatory response of the immune system (Yan et al., 1997), but also results in IgGs specific to the disease process that could potentially be used as biomarkers for AD (Mruthinti, Hill, Swamy-Mruthinti, and Buccafusco, 2003).

While antibodies to A β have been previously demonstrated to be elevated in some AD patient samples (e.g., Lopez, Rabin, Huff, Rezek, & Reinmuth, 1992; Nath et al., 2003),

differences between A β antibody levels in AD and healthy older adults have not always been demonstrated (Hyman et al., 2001), possibly due to differences in methods of detecting A β IgGs.

Using a purification process to separate A β from plasma proteins, Mruthinti and colleagues demonstrated that plasma A β and RAGE IgG levels were significantly higher ($p < 0.0001$) in a sample of 33 AD patients when compared to a group of 42 control patients (Mruthinti et al., 2004). Further analyses revealed that the levels of both A β and RAGE IgGs were negatively correlated with gross cognitive status, as measured by the Mini Mental State Examination (MMSE), indicating that across both AD and control groups, those individuals with higher levels of A β and RAGE IgGs tended to exhibit more cognitive impairment. The A β IgG and RAGE IgG correlation coefficients with MMSE scores were -0.71 and -0.60, respectively, indicating a strong association between IgG levels of the biomarkers and cognitive status (Mruthinti et al., 2004). In a more recent study by the same group, Wilson and colleagues (2009) demonstrated a relationship between dementia severity and A β and RAGE IgG levels in a mixed neurological sample.

These findings, while compelling on their own, warrant further investigation into the potential for A β and RAGE IgGs to differentiate between cognitively normal patients, patients with MCI, and patients with AD. This work is ongoing at the Medical College of Georgia (MCG) as part of the Neurological Disorders Database Repository (NDDR), and this dissertation is in collaboration with the NDDR research group. Below is a summary of preliminary findings from the ongoing NDDR project.

Review of Preliminary Findings

Preliminary analyses of the current research participants in the NDDR project indicate that when dividing the sample into three groups based on their Clinical Dementia Rating

(CDR; Hughes, Berg, Danziger, Coben, & Martin, 1982; see Method section for an explanation of exclusionary criteria and all measures used in this study), there was a total sample of 73 with 31 classified as normal controls (CDR = 0), 8 classified as MCI (CDR = 0.5), and 34 classified as AD (CDR \geq 1). Two separate ANCOVA analyses were conducted with group status as the independent variable and anti-RAGE and anti-A β as the respective dependent variables for each analysis. Age and total IgG were used as covariates in both the anti-RAGE and anti-A β models. In the ANCOVA analysis with anti-A β as the dependent variable, group status was a significant predictor of anti-A β levels ($F(2,68) = 11.74, p < .001, \text{partial } \eta^2 = .257$). Age was not a significant covariate ($p > .05$), but total IgG did show significant group differences ($F(1,68) = 15.01, p < .001, \text{partial } \eta^2 = .181$). Post-hoc pairwise comparisons using Bonferroni's adjustment for multiple comparisons revealed that there was a significantly higher mean anti-A β level in the AD versus control group ($p < .001$) and in the AD versus MCI group ($p < .05$), but there was not a significant difference in mean anti-A β levels in the control versus MCI group ($p > .05$). Similarly, the ANCOVA analysis with anti-RAGE as the dependent variable indicated that group status was a significant predictor of anti-RAGE levels ($F(2,68) = 11.98, p < .001, \text{partial } \eta^2 = .261$). Age was not a significant covariate ($p > .05$), but again, total IgG was ($F(1,68) = 16.09, p < .001, \text{partial } \eta^2 = .192$). Post-hoc pairwise comparisons using Bonferroni's adjustment for multiple comparisons revealed that there was a significantly higher mean anti-RAGE level in the AD versus control group ($p < .001$), but not in the AD versus MCI group ($p = .06$), or the control versus MCI group ($p > .05$).

Clearly, these ANCOVA results were influenced by the highly unequal group sizes in the preliminary data set, which had relatively large and equal samples of normal controls and AD participants, but a small sample of MCI participants.

Preliminary analysis of the relationship between cognitive functioning and the two biomarkers was performed via multiple regression analyses (Wilson et al., 2007). When simultaneously entering all cognitive domain scores (i.e., Attention, Language, Immediate Memory, Delayed Memory, Visuospatial/Construction) derived from the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS; Randolph, 1998) to predict anti-RAGE IgG levels, the only significant predictors were language ($t=-3.80$, $p<0.001$) and delayed memory ($t=-2.48$, $p<0.02$). Language was the only significant cognitive predictor of anti-A β IgG levels ($t=-3.96$, $p<0.001$), while delayed memory approached significance ($p=0.16$).

Study Aims and Hypotheses

The purpose of this study was to focus on the critical MCI patient group to elucidate the current preliminary findings. This was done by obtaining a larger sample size of MCI patients in order to more accurately compare equal groups of patients classified into three groups: normal controls, MCI, and AD. While the above summarized preliminary findings provide evidence that there is clearly a relationship between anti-A β and anti-RAGE IgGs and dementia severity, this omnibus effect does not indicate whether or not anti-A β and anti-RAGE IgGs are able to differentiate diagnostic groups with a good degree of sensitivity. Similarly, the statistical effect in the multiple regression analyses reviewed above relating IgG levels to language and delayed memory could largely be driven by the large sample of cognitively impaired AD patients, whereas the smaller sample of MCI patients may not be contributing largely to the relationship between IgG levels and cognitive functioning. Finally, given the large amount of controversy surrounding the diagnosis of MCI, this study examined the relationship between two methods of diagnostic classification of MCI in order to compare an interview technique for dementia

classification, the Clinical Dementia Rating (CDR), and a psychometric approach using the RBANS.

There were three main hypotheses in this dissertation. First, it was hypothesized that when the dependent variables of anti-A β and anti-RAGE IgGs were entered into two ANCOVA analyses with age and total IgG as covariates, there would be statistically significant differences in IgG levels of anti-A β and anti-RAGE between all three groups, with controls having the lowest levels of the two IgGs, MCI patients having higher levels of the IgGs compared to controls, and AD patients having the highest levels of the IgGs. Second, it was hypothesized that when all RBANS index scores were entered into two separate multiple regression analyses with anti-A β and anti-RAGE IgGs as the dependent variables, the best predictors of the two IgGs would be Language and Delayed Memory ($p < .05$). Finally, it was predicted that when using the conventional categorization of a CDR score of 0.5 as the criteria for MCI, the majority of the patients categorized in that group of patients would meet the commonly-used neuropsychological criteria of having a RBANS Delayed Memory score that was less than 1.5 standard deviations below the mean.

CHAPTER 2

METHOD

NDDR Database

The Neurological Disorders Database Repository (NDDR) at the Medical College of Georgia (MCG) is an ongoing research collaboration between MCG, the University of Georgia, and community physicians. The purpose of the NDDR is to collect data from older adults with AD, MCI, other neurological disorders, and normal cognitive functioning. Participants enrolled in the NDDR are tested at 6 months to 1 year increments. Local physicians in the Augusta area are the primary referral source of NDDR participants with neurological conditions. Healthy control participants are primarily recruited through caregivers of neurological patients, as well as local advertisements and talks in retirement centers and hospitals.

Power Analysis

A total of 78 participants, with 26 participants in each group, was estimated to be necessary to find statistically meaningful group differences. The number of participants in this study was arrived at by using G*Power 3 (Faul, Erdfelder, Lang, & Buchner, 2007) to compute the number of participants needed. The effect sizes found in the preliminary ANCOVA analyses with anti-A β and anti-RAGE IgG as the dependent variables described earlier (partial $\eta^2 = .257$ and $.261$, respectively) were used to compute the amount of participants required to find a statistically significant difference between 3 groups (normal controls, MCI, AD) using one dependent variable (anti-A β and anti-RAGE IgG for each respective ANCOVA), and age and total IgG as covariates. The parameters used for this analysis were a statistically significant

cutoff of $p=0.01$ and a power of 0.95. Because the sample size of the AD ($n=34$) and control group ($n=31$) in the preliminary dataset already exceeded the necessary sample size for each group, the aim of this project was to achieve approximately equal sample sizes in each group by increasing the number of participants in the MCI group (initial n was 8).

Participants

Participants were drawn from the existing NDDR database and additional participants were recruited based on the following inclusion/exclusion criteria. Participants were adults age 60 and older whose primary language was English. Participants with neurologic diagnoses other than Alzheimer's disease (e.g., Parkinson's disease, stroke, Dementia with Lewy Bodies) were excluded. Participants with psychiatric diagnoses (e.g., Schizophrenia, Bipolar Disorder) were excluded. In addition, because diabetes has been demonstrated to increase levels of both IgGs under investigation (Mruthinti et al., 2006), individuals with diabetes were excluded. Medical and psychiatric diagnoses were assessed for inclusion/exclusion criteria by reviewing the Initial Encounter Form (see Appendix) completed by the examiner with assistance from the participant or caregivers (see Procedure section below for a description of the Initial Encounter Form). Participants were assigned to the following three groups according to their Clinical Dementia Rating score (CDR, see description in Clinical Dementia Rating section below): Normal Control Group ($CDR=0$), MCI Group ($CDR=0.5$), AD Group ($CDR\geq 1$).

Recruitment

Participants were recruited through physician referrals, the use of fliers, and recruitment talks at local retirement centers. Enrollment was initiated by participants upon receiving information about the study. Potential participants contacted the study coordinator via telephone,

and a two-hour appointment time was scheduled with each interested participant at the Medical College of Georgia.

Procedure

Test sessions were approximately two hours and began with obtaining informed consent, followed by the completion of an Initial Encounter Form which included demographic information, medical history, current medical diagnoses, self-reported cognitive status, family history, and current medications. This form was completed by the participant with assistance from caregivers and was verified for completeness by the study coordinator. Participants also completed a release to verify their medications and medical diagnoses with their physician. Next, each participant and caregiver participated in a semi-structured interview from which the participant's Clinical Dementia Rating (CDR) was derived. Participants were then administered a cognitive test battery by a trained test administrator. Finally, each participant provided a blood sample which was drawn by a trained phlebotomist.

Materials

Clinical Dementia Rating

The Clinical Dementia Rating (CDR, Morris, 1993) is an algorithm-derived scale for rating the severity of dementia that uses a semi-structured interview of the patient and caregiver to assess six domains of the patient's cognitive and functional abilities. The CDR provides an overall summary score on a 5-point scale of either "normal" (CDR=0), "questionable/very mild dementia" (CDR=0.5), "mild dementia" (CDR=1), "moderate dementia" (CDR=2), or "severe dementia" (CDR=3). The overall CDR score is derived from ratings given on each of six domains assessed in a semi-structured interview, including memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care. Each of the six

domains assessed by the CDR is rated on a 5-point scale as follows: 0=no impairment, 0.5=questionable or very mild impairment, 1=mild impairment, 2=moderate impairment, and 3=severe impairment (note: the personal care score is limited to a score of 0, 1, 2, or 3). Each domain score is entered into the CDR scoring algorithm, which weights the memory score as the primary contributor to the overall CDR score (Morris, 1993). Clinicians are trained in the CDR interview and scoring techniques by the CDR author at CDR training workshops or online (<http://alzheimer.wustl.edu/cdr/default.htm>). The CDR raters in this study completed online training and were certified in administering the CDR.

The CDR is widely used in Alzheimer's disease research to classify patient populations into categories based on dementia severity (Morris, 1993). Within the trained CDR raters at Washington University, where the measure was developed, inter-rater reliability has been demonstrated to be 80% or greater for physicians (Burke et al., 1988) and master's level clinicians (McCulla et al., 1989). In a multi-center study comparing the CDR scores of 82 CDR-trained investigators, the CDR was demonstrated to have an overall rating agreement of 83% between trained clinicians and the "gold standard" CDR scores (i.e., CDR scores rated by the CDR author, John Morris; Morris et al., 1997).

While a classification of CDR=0.5 has been noted by Petersen (2000) to *not* be synonymous with a diagnosis of mild cognitive impairment, there are several advantages to using the CDR for the purposes of group classification in this study. First, because the formal criteria for MCI published by Petersen et al. (2001a) are ambiguous in their definition of "objective memory impairment," it is difficult to operationalize these criteria for MCI. Second, the CDR has an established reliability of 80% or higher while the diagnostic criteria for MCI do not have a clearly defined method for diagnosis, nor is there published reliability data on the diagnostic

criteria. Finally, it was important that group classification for this study be made independent of performance on cognitive testing, as the cognitive measures used in this study were collected as outcome measures in order to examine the relationship between IgG levels and cognitive functioning. The CDR is designed to rate individuals without considering cognitive test performance, and is thus an ideal tool for classification. Individuals will be classified into three groups as follows: normal controls = CDR of 0, MCI = CDR of 0.5, AD = CDR of 1, 2, or 3.

Neuropsychological Test Battery

As this dissertation was part of a larger research project, there were measures administered to participants that were not analyzed in this dissertation. The measures of interest for this dissertation are described below.

Each study participant in the NDDR study was administered a neuropsychological test battery including the Geriatric Depression Scale (GDS; Yesavage et al., 1983) and the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS; Randolph, 1998).

The Geriatric Depression Scale-Short Form (GDS; Sheikh & Yesavage, 1986) is a 15-item inventory of depression consisting of yes/no questions that is commonly used in geriatric care to assess for symptoms of depression common in older adults. Because depression has been shown to attenuate cognitive performance in geriatric populations (Elderkin-Thompson, Mintz, Haroon, Lavretsky, & Kumar, 2007), GDS score was initially entered in all regression models to control for the relationship between depression and cognition. However, GDS score was not a significant predictor in all regression models, and was thus dropped from the models.

The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS; Randolph, 1998) is a 30-minute neuropsychological test that was designed to assess cognitive decline in older adults and serve as a screening tool for cognitive functioning in younger adults

(Randolph, Tierney, Mohr, & Chase, 1998). The RBANS consists of 12 subtests that generate 5 Index Scores: Visuospatial/Constructional, Attention, Language, Immediate Memory, and Delayed Memory. The RBANS additionally generates a global score (the Total Scale score), which is derived from the raw scores on all 12 subtests. The 12 subtests of the RBANS are as follows: Figure Copy, Line Orientation, Digit Span, Coding, Picture Naming, Semantic Fluency, List Learning, Story Memory, List Recall, List Recognition, Story Recall, and Figure Recall.

The 12 RBANS subtests include two tests that are the basis for the Visuospatial/Constructional Index Score: the Figure Copy and the Line Orientation subtests. The Figure Copy subtest is a measure of visual construction on which the participant is given four minutes to copy a complex line drawing. The Line Orientation subtest is a measure of visuospatial ability on which the participant is asked to match the spatial position of two lines on the bottom of a page to the corresponding lines in the identical spatial orientations in an array of lines at the top of a page.

The two Attention subtests of the RBANS are the Digit Span subtest and the Coding subtest. The Digit Span subtest is a measure of verbal working memory on which the participant is asked to repeat verbally presented number sequences of increasing difficulty. The Coding subtest of the RBANS is a measure of visual attention and processing speed on which the participant is asked to transpose numbers according to a number-symbol code.

The two Language subtests on the RBANS are the Picture Naming subtest and the Semantic Fluency subtest. The Picture Naming subtest is a 10-item measure of confrontational naming on which the participant is shown pictures of objects (e.g., a chair, a pencil) and asked to name them. The Semantic Fluency subtest is a measure of verbal fluency on which the participant is given instructions to name as many fruits and vegetables as possible in one minute.

The Immediate Memory subtests on the RBANS are the List Learning subtest and the Story Memory subtest. The List Learning subtest is a measure of verbal learning on which the participant is given four trials to learn a ten-item word list. The Story Memory subtest is a measure of verbal learning on which the participant is read a short story and then immediately asked to repeat as many details of the story as possible.

The Delayed Memory score of the RBANS is derived from four delayed recall subtests: List Recall, List Recognition, Story Recall, and Figure Recall, all of which are 20-minute delayed recall tests of previously learned information from the Immediate Memory and Figure Copy subtests.

The RBANS author and colleagues (Randolph et al., 1998) first demonstrated clinical validity of the RBANS in a clinical sample of older adults consisting of a group of patients with probable Alzheimer's disease (AD, n=20), a group with Huntington's disease (HD, n=20), and a group of normal controls (n=40). Randolph and colleagues demonstrated that the RBANS is good at differentiating between individuals with distinct forms of dementia, as the average cognitive profile across all five Index Scores of the RBANS was distinct in each of the three groups studied. Findings revealed that the AD group obtained the lowest scores on Language and Delayed Memory Index Scores compared to the other two groups, whereas the HD group obtained the lowest Index Scores on Attention and Visuospatial/Constructional.

Randolph and colleagues (1998) used the Index Score discrepancies between the AD group and the HD group to create a summary score that differentiates "cortical" dementia (i.e., AD in this study) from "subcortical dementia" (i.e., HD in this study). The authors created this "cortical/subcortical" discrepancy score by subtracting the mean of the scaled scores of the Delayed Memory and Language indices from the mean of the Attention and

Visuospatial/Constructional indices, yielding a summary score with a cutoff point of above zero for a “cortical” score and below zero for a “subcortical” score. Findings revealed that this single cortical/subcortical discrepancy score correctly classified 95% of the AD group as “cortical” and 90% of the HD group as “subcortical” (Randolph et al., 1998).

A later study by Beatty and colleagues (2003) demonstrated clinical validity of the RBANS to differentiate AD from Parkinson’s disease (PD). Using the same cortical/subcortical discrepancy score proposed by Randolph and colleagues (1998), Beatty’s group demonstrated 87% of AD (n = 23) patients correctly classified as “cortical” and 78% of demented PD patients (n=27) as “subcortical.” Findings revealed problems with using the cortical/subcortical discrepancy score to correctly classify non-demented PD patients, as the score only correctly classified 39% of non-demented PD patients (n=23).

The RBANS has been demonstrated to have good reliability, with split-half reliability coefficients in the .80s for the Index Scores and the average reliability for the Total Scale score ranging from .86-.94 across age groups.

Blood Plasma Measures

RAGE and A β IgG purification from plasma.

Blood plasma analysis was performed by trained technicians in psychopharmacology at the Medical College of Georgia. Plasma (0.2 ml) IgG was purified via a purification process involving initial ammonium sulfate precipitation, followed by dialyzation overnight against 1x PBS at 5°C. Next, IgGs were purified via protein A/G isolation and dialyzed overnight against 1xPBS at 5°C. Non-specific total IgG was quantified after protein A/G isolation using bicinchoninic acid (BCA) assay for protein quantification.

Quantifying specific IgGs.

RAGE peptide or A β 1-42 (1 μ g/ 100 μ l/well) were coated to MaxiSorp™ ELISA plates and incubated overnight at 5°C. Antigen was discarded and wells were washed once with 150 μ l of 2% milk in PBS, and then wells were blocked with 150 μ l of 2% milk in PBS for 2 hours. Wells were washed three times with 150 μ l of 2% milk in PBS, and purified anti-A β 1-42 or anti-RAGE IgG (10 μ g) derived from participants were added to each well and incubated overnight at 5°C. After washing four times with 2% milk in Tris buffered saline + Tween 20 (TBST), 200 μ l of donkey anti-human IgG(F(ab')₂)-HRP conjugated secondary antibody (1:1000) were added and incubated for 3 hours at 37°C. Plates were washed three times with 2% milk in TBST followed by adding 100 μ L of ready-made Tetramethylbenzidine (TMB) substrate (Sigma-Aldrich) for blue color development, which was stopped after 15 minutes by adding 100 μ L 1M HCL. Absorbance was read at 450nm providing the titer values used in this study. Each sample was run in triplicate.

In order to control for nonspecific binding, three wells containing the antigen and secondary antibody were run on each ELISA plate, the average value of which was subtracted from all titer values. In addition to patient samples, a control sample of plasma was purified in each purification and ELISA assay and used to create standardized values in order to compare values from different assays. These standardized values were used in all statistical analyses.

Peptides.

RAGE peptide was synthesized according to the following sequence (Neeper et al., 1992): “DQNITARIGKPLVLNCKGAPKKPPQLEWKLN” representing the nucleotide and amino acid sequence of human RAGE. The peptide was synthesized by the Molecular Biology Central Core facility at MCG. A β 1-42 was purchased from Sigma-Aldrich.

Statistical Analysis

To test the first hypothesis that there was a statistically significant difference in IgG levels of anti-A β and anti-RAGE between all three groups, with controls having the lowest levels of the two IgGs, MCI patients having higher levels of the IgGs compared to controls, and AD patients having the highest levels of the IgGs, two separate ANCOVA analyses were performed with the grouping variable defined as group status (i.e., normal control, MCI, AD), the respective dependent variables defined as anti-A β and anti-RAGE blood serum levels, and age and total IgG entered as covariates. To compare dependent variables between each group, post-hoc analyses of between-group differences on each dependent variable were performed.

To test the second hypothesis that the RBANS Language and Delayed Memory scores were the RBANS Index Scores that are most strongly related to anti-A β and anti-RAGE IgG levels, two separate multiple regression analyses with anti-A β and anti-RAGE IgGs as the dependent variables were performed. In each regression analysis, the dependent variable was either anti-A β or anti-RAGE IgG level, and the predictor variables were age, total IgG and the five RBANS Index Scores: Visuospatial/Constructional, Attention, Language, Immediate Memory, and Delayed Memory. The predictor variables for each regression model were entered in a three-step hierarchical regression model, with age entered in the first step, total IgG entered in the second step, and RBANS Index Scores in the third step.

Finally, it was predicted that when using the categorization of a CDR score of 0.5 as the criteria for MCI, the majority of the participants categorized in that group would meet the commonly-used neuropsychological criteria for MCI of having a RBANS Delayed Memory score that was more than 1.5 standard deviations below the mean (i.e., a Delayed Memory Standard Score of 77 or lower). The accuracy of the CDR 0.5 to categorize individuals as having

a RBANS Delayed Memory score of 77 or lower was represented in findings simply as a percentage of participants correctly classified.

CHAPTER 3

RAGE AND A β IMMUNOGLOBULINS: POTENTIAL BIOMARKERS FOR ALZHEIMER'S DISEASE^{1,2}

¹ Mitchell, M.B., Buccafusco, J., Schade, R., Webster, S., Mruthinti, S., & Miller, L.S. To be submitted to *Journal of the American Geriatrics Society*.

² A portion of the data in this study was previously published in a study using a mixed neurological sample (Wilson et al., 2009). This investigation uniquely improves upon this previous work because it has a substantially increased sample of individuals with mild cognitive impairment, has a clearly-defined group of individuals with probable Alzheimer's disease, and excluded individuals with other forms of dementia, psychiatric conditions, and diabetes.

Abstract

Previous work from our laboratory has demonstrated a relationship between dementia severity and blood plasma levels of the immunoglobulins for A β and RAGE in a mixed neurological sample of older adults. In this study, we compared non-diabetic older adults with normal cognition, mild cognitive impairment (MCI), and Alzheimer's disease (AD). We found significant group differences between control and AD groups as well as between MCI and AD groups, but no group differences were found between the control and MCI groups. A trend towards significance was found when comparing lower IgG values in nonamnestic versus amnestic MCI groups. Results provide further evidence that A β and RAGE IgG levels are elevated in AD. A longitudinal study examining changes over time in non-demented individuals who convert to AD is necessary to determine the utility of these IgGs as a marker for early signs of AD.

Introduction

Alzheimer's disease (AD) is the most common form of dementia, with a national estimate of 4.5 million cases in 2000 (Herbert, Scherr, Bienias, Bennett, & Evans, 2003). While estimates vary, probable AD represents approximately 60 percent (Skoog & Blennow, 2001) to 80 percent (Mesulam, 2000) of all dementia diagnoses in the United States. It is estimated that the cost to U.S. society for the treatment and management of AD is approximately \$100 billion annually (DeKosky & Orgogozo, 2001). In addition to these figures, the demographic trends in the U.S. indicate that we are undergoing a population shift with an increasingly large population of older adults. It is projected by the U.S. Census Bureau (2005) that by the year 2050, there will be approximately 78.9 million adults ages 65 and older, many of whom will require medical treatment and long-term care for AD.

Recent Alzheimer's disease (AD) research indicates that blood plasma levels of immunoglobulins (IgGs) may be a marker of amyloid plaques that characterize the progression of AD (Mruthinti et al, 2004). Amyloid peptides, in particular beta amyloid ($A\beta$), accumulate in brain tissue at receptor sites called receptors for the advanced glycation end products (RAGE; Emanuele et al., 2005). The amyloid plaques formed in this process are associated with Alzheimer's disease and cognitive impairment (Berg et al., 1998). The immune system develops IgGs in response to the binding of $A\beta$ to RAGE in the brain, and these IgGs have been found to be elevated peripherally in the bloodstream (Nath et al., 2003). By obtaining a blood sample, levels of IgGs could potentially be used to detect the presence of AD brain pathology. Measuring IgGs in the blood has the benefit over current methods of probable AD diagnosis (e.g., neuropsychological testing, CSF testing, MRI) because it is a relatively painless, short, and inexpensive procedure. In addition, this method of detecting AD has the added advantage of

potentially detecting AD in its pre-clinical form of Mild Cognitive Impairment (MCI). Given the central role of A β aggregation in the disease process of AD (Golde, 2003), coupled with evidence that the immune system inflammatory response is a key process in age-related chronic diseases (Licastro et al, 2005), the immune response to A β is a promising target for disease detection and possibly disease prevention. Further, there is a preponderance of evidence suggesting that chronic inflammation caused by a microglial response to A β in the AD brain is an important aspect of the disease process that ultimately leads to neurotoxicity (El Khoury et al., 1996; McGeer & McGeer, 1998; Pasinetti, 1996; Yan et al., 1997). An advantage of measuring immune responses to AD is that immune system-produced immunoglobulins (IgGs) have been demonstrated to pass through the blood-brain barrier from brain to blood (Bouras, Riederer, Kovari, Hof, & Giannakopoulos, 2005; Zhang & Pardridge, 2001), thus circumventing the methodological issue of detecting brain pathology in the blood.

One aspect of the immune response to AD that has been identified involves the neuronal membrane receptor for advanced glycation end products (RAGE). This receptor binds advanced glycation end products (AGEs) in the normal aging process, but has been demonstrated to bind to A β in the AD brain at a high affinity (Lue et al., 2001). The gene for RAGE, located on chromosome 6 (Sugaya et al., 1994), was found in one study to be over-expressed in neurons and microglia located in the hippocampus, entorhinal cortex, and superior frontal gyrus of AD brain tissue, all of which are areas strongly associated with AD brain pathology (Lue et al., 2001). RAGE has also been demonstrated to be present in neurons from the CA3 and CA4 regions of hippocampi of AD brains in close proximity to A β (Sasaki, et al., 2001). The RAGE-A β binding process was characterized by Yan and colleagues (1997), and found to induce macrophage-colony stimulating factor, which then triggers a number of cellular responses, all of which lead to

a generalized inflammatory response. Mruthinti, Capito, Sood, & Buccafusco (2007) demonstrated that A β and RAGE peptides bind to one another and form a complex that is more cytotoxic to neuronal cells than either individual protein.

Given the key role that the binding of A β to RAGE has in the inflammatory response associated with AD, Mruthinti, Hill, Swamy-Mruthinti, and Buccafusco (2003) investigated the RAGE-A β interaction in a mouse model of AD in which the mice were immunized with a human neurofilament-derived AGE. They unexpectedly found that the mice developed immunoglobulins (IgGs) to both RAGE and A β , suggesting that the RAGE-A β interaction not only results in the previously characterized generalized inflammatory response of the immune system (Yan et al., 1997), but also results in IgGs specific to the disease process that could potentially be used as biomarkers for AD (Mruthinti, Hill, Swamy-Mruthinti, and Buccafusco, 2003).

While antibodies to A β have been previously demonstrated to be elevated in some AD patient samples (e.g., Lopez, Rabin, Huff, Rezek, & Reinmuth, 1992; Nath et al., 2003), differences between A β antibody levels in AD and healthy older adults have not always been demonstrated (Hyman et al., 2001), possibly due to differences in methods of detecting A β IgGs.

Using a purification process to separate A β from plasma proteins, Mruthinti and colleagues demonstrated that plasma A β and RAGE IgG levels were significantly higher ($p < 0.0001$) in a sample of 33 AD patients when compared to a group of 42 control patients (Mruthinti et al., 2004). In a more recent study by the same group, Wilson and colleagues (2009) demonstrated a relationship between dementia severity and A β and RAGE IgG levels in a mixed neurological sample.

These findings, while compelling on their own, warrant further investigation into the potential for A β and RAGE IgGs to differentiate between cognitively normal patients, patients with MCI, and patients with probable AD. It is important to determine not only if there is an omnibus relationship between dementia severity and A β and RAGE IgGs, but also if there are significant differences between diagnostic categories. It is particularly important to determine if A β and RAGE IgGs can distinguish between older adults with normal cognition and those with MCI, as this would be a potentially useful diagnostic tool to guide early intervention to decelerate the progression of cognitive decline. In this study, we sought to improve upon our previous work by comparing IgG levels for A β and RAGE in an elderly control group, an MCI group, and an AD group, excluding individuals with other neurological conditions and those with diabetes, as these conditions also have autoimmune components to their disease processes (Mruthinti et al., 2006).

Method

Participants

Participants in this study were drawn from the Neurological Disorders Database Repository (NDDR) at the Medical College of Georgia (MCG), an ongoing research collaboration between MCG, the University of Georgia, and community physicians. The purpose of the NDDR is to collect data from older adults with AD, MCI, other neurological disorders, and normal cognitive functioning. Participants enrolled in the NDDR are tested at 6 months to 1 year increments. This investigation examined cross sectional data, representing the first study visit for all control and AD participants. In order to achieve approximately equal numbers in each diagnostic group, data from a later time point at which the participant converted from control to MCI was used for five participants in the MCI category in this investigation. These

five participants were not additionally included in the control group. Local physicians in the Augusta area were the primary referral source of NDDR participants with neurological conditions. Healthy control participants and those with MCI were primarily recruited through caregivers of neurological patients, as well as local advertisements in retirement centers and hospitals.

Participants were adults age 60 and older whose primary language was English. Participants with neurologic diagnoses other than Alzheimer's disease (e.g., Parkinson's disease, stroke, Dementia with Lewy Bodies) or psychiatric diagnoses (e.g., Schizophrenia, Bipolar Disorder) were excluded. Due to the demonstrated relationship between anti-A β and anti-RAGE IgGs and diabetes (Mruthinti et al. 2006), participants with diabetes were excluded. Participants were assigned to the following three groups according to their Clinical Dementia Rating score (CDR; Morris, 1993): Normal Control Group (CDR=0), MCI Group (CDR=0.5), AD Group (CDR \geq 1). All participants in the AD group additionally had physician's diagnoses of probable AD.

Procedure

Test sessions were approximately two hours and began with obtaining informed consent, followed by the completion of an Initial Encounter Form which included demographic information, medical history, current medical diagnoses, self-reported cognitive status, family history, and current medications. This form was completed by the participant with assistance from caregivers and was verified for completeness by the study coordinator. Participants also completed a release to verify their medications and medical diagnoses with their physician. Next, each participant and caregiver participated in a semi-structured interview from which the participant's Clinical Dementia Rating (CDR) was derived by a CDR-certified rater. Participants

were then administered a cognitive test battery by a trained test administrator. Finally, each participant provided a blood sample which was drawn by a trained phlebotomist.

Materials

Repeatable Battery for the Assessment of Neuropsychological Status.

Each study participant was administered a neuropsychological test battery that included the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS; Randolph, 1998), a 30-minute neuropsychological test battery that consists of 12 subtests which generate 5 index scores: Visuospatial/Constructional, Attention, Language, Immediate Memory, and Delayed Memory (Randolph, Tierney, Mohr, & Chase, 1998). The RBANS additionally generates a global score (the Total Scale score). Reliability of the RBANS has been established, with split-half reliability coefficients in the .80s for the Index Scores and the average reliability for the Total Scale score ranging from .86-.94 across age groups (Randolph, Tierney, Mohr, & Chase, 1998).

Clinical Dementia Rating.

The Clinical Dementia Rating (CDR, Morris, 1993) is an algorithm-derived scale for rating the severity of dementia that uses a semi-structured interview of the patient and caregiver to assess six domains of the patient's cognitive and functional abilities. The CDR provides an overall summary score on a 5-point scale of either "normal" (CDR=0), "questionable/very mild dementia" (CDR=0.5), "mild dementia" (CDR=1), "moderate dementia" (CDR=2), or "severe dementia" (CDR=3). The overall CDR score is derived from ratings given on each of six domains assessed in a semi-structured interview, including memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care. Each of the six domains assessed by the CDR is rated on a 5-point scale as follows: 0=no impairment,

0.5=questionable or very mild impairment, 1=mild impairment, 2=moderate impairment, and 3=severe impairment (note: the personal care score is limited to a score of 0, 1, 2, or 3). Each domain score is entered into the CDR scoring algorithm, which weights the memory score as the primary contributor to the overall CDR score (Morris, 1993). Administrators were trained in the CDR interview and scoring techniques by the CDR author at the CDR training course online (<http://alzheimer.wustl.edu/cdr/default.htm>).

The CDR is widely used in Alzheimer's disease research to classify patient populations into categories based on dementia severity (Morris, 1993). Inter-rater reliability has been demonstrated to be 80% or greater for physicians (Burke et al., 1988) and master's level clinicians (McCulla et al., 1989). In a multi-center study comparing the CDR scores of 82 CDR-trained investigators, the CDR was demonstrated to have an overall rating agreement of 83% between trained clinicians and the "gold standard" CDR scores (i.e., CDR scores rated by the CDR author, John Morris; Morris et al., 1997). In our study, we established inter-rater reliability by independently scoring a subset of the CDR interviews used in this paper (n=12) and established consistent inter-rater reliability across domains (Cronbach's α range = .83-1.0). Overall rating agreement for the global CDR score in this subset was 100%.

Group classification

The primary method of group classification was CDR staging. Individuals were classified into three groups as follows: normal controls = CDR of 0, MCI = CDR of 0.5, AD = CDR of 1, 2, or 3. While a classification of CDR=0.5 has been noted by Petersen (2000) to *not* be synonymous with a diagnosis of mild cognitive impairment, there are several advantages to using the CDR for the purposes of group classification in this study. First, because the formal criteria for MCI published by Petersen et al. (2001) are ambiguous in their definition of "objective

memory impairment,” it is difficult to operationalize these criteria for MCI. Second, the CDR has an established reliability of 80% or higher while the diagnostic criteria for MCI do not have a clearly defined method for diagnosis, nor is there published reliability data on the diagnostic criteria.

For exploratory analyses, we further classified individuals into groups based on their CDR scores, as specified above, in addition to their performance on the RBANS. To be classified as control, individuals had to have a CDR of 0, in addition to having all RBANS index scores greater than -1.5 standard deviations below the mean. The MCI group was further divided into amnesic and non-amnesic MCI based on cognitive test scores. We classified individuals as having amnesic MCI when their CDR score was 0.5, and Immediate and/or Delayed Memory scores on the RBANS were between 1.5 and 2 standard deviations below the mean, and all other cognitive domain scores were no lower than 2 standard deviations below the mean. We classified individuals as having non-amnesic MCI when their CDR score was 0.5, and one or more of their non-learning and memory scores were between 1.5 and 2 standard deviations below the mean, and all other cognitive domain scores were no lower than 2 standard deviations below the mean. To be classified as having AD, individuals had to have a global CDR of 1 or greater, and two or more indices on the RBANS had to be greater than 2 standard deviations below the mean. Due to incomplete RBANS data on some participants, the n’s in each group were reduced in the control and AD groups using this classification system.

RAGE and A β IgG purification from plasma

Blood plasma analysis was performed by trained technicians in psychopharmacology at the Medical College of Georgia. Plasma (0.2 ml) IgG was purified via a purification process involving initial ammonium sulfate precipitation, followed by dialyzation overnight against 1x

PBS at 4°C. Next, IgGs were purified via protein A/G isolation and dialyzed overnight against 1xPBS at 4°C. Non-specific total IgG was quantified after protein A/G isolation using bicinchoninic acid (BCA) assay for protein quantification.

Quantifying specific IgGs

RAGE peptide or A β 1-42 (1 μ g/ 100 μ l/well) were coated to MaxiSorp™ ELISA plates and incubated overnight at 4 °C. Antigen was discarded and wells were washed once with 150 μ l of 2% milk in PBS, and then wells were blocked with 150 μ l of 2% milk in PBS for 2 hours. Wells were washed three times with 150 μ l of 2% milk in PBS, and affinity purified anti-A β 1-42 or anti-RAGE IgG (100 μ l) derived from participants were added to each well and incubated overnight at 5°C. After washing four times with 2% milk in Tris buffered saline + Tween 20 (TBST), 200 μ l of donkey anti-human IgG(F(ab')₂)-HRP conjugated secondary antibody (1:1000) was added and incubated for 3 hours at 37°C. Plates were washed three times with 2% milk in TBST followed by adding 100 μ L of ready-made Tetramethylbenzidine (TMB) substrate (Sigma-Aldrich) for blue color development, which were stopped after 15 minutes by adding 100 μ L 1M HCL. Absorbance was read at 450nm providing the titer values used in this study. Each sample was run in triplicate.

In order to control for nonspecific binding, three wells containing the antigen and secondary antibody were included on each ELISA plate, the average value of which was subtracted from all titer values. In addition to patient samples, a control sample of plasma was purified in each purification and ELISA assay and used to create standardized values in order to compare values from different assays. These standardized values were used in all statistical analyses.

Peptides

RAGE peptide was synthesized according to the following sequence (Neeper et al., 1992): “DQNITARIGKPLVLNCKGAPKKPPQQLLEWKLN” representing the nucleotide and amino acid sequence of human RAGE. The peptide was synthesized by the Molecular Biology Central Core facility at MCG. A β 1-42 was purchased from Sigma-Aldrich.

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS 16.0 for Windows, SPSS, Chicago, IL). Analyses of covariance (ANCOVA) were used to test group differences in anti- A β and RAGE IgG levels with age and total IgG entered as covariates in each model.

Results

Demographic information and descriptive statistics for diagnostic groups are summarized in Table 3.1. To test our hypothesis that there would be group differences in anti-A β and RAGE IgGs, we conducted two separate ANCOVA analyses, each entering age and total IgG as covariates. As depicted in Table 3.2, for the anti-A β model, both the covariates of age ($F[1, 107] = 4.61, p < .05$) and total IgG ($F[1, 107] = 5.96, p < .05$) accounted for a significant portion of the variance in anti-A β . However, group status additionally accounted for a significant portion of the variance ($F[2, 107] = 10.91, p < .001$) beyond that of age and total IgG level. Pairwise comparisons using Bonferroni’s adjustment for multiple comparisons revealed that there was a significant difference in anti-A β levels between the AD group and the control group ($p < 0.001$). There was also a significant difference between the MCI and the AD group in anti-A β ($p = 0.001$). There was *not* a significant difference between the control group and the MCI group in anti-A β ($p > .05$). Similarly, as depicted in Table 3.3, in the anti-RAGE model, both the

covariates of age ($F[1, 107] = 4.03, p < .05$) and total IgG ($F[1, 107] = 4.72, p < .05$) accounted for a significant portion of the variance in anti-RAGE. However, group status additionally accounted for a significant portion of the variance ($F[2, 107] = 11.94, p < .001$) beyond that of age and total IgG level. Pairwise comparisons using Bonferroni's adjustment for multiple comparisons revealed that there was a significant difference in anti-RAGE levels between the AD group and the control group ($p < 0.001$). There was also a significant difference between the MCI and the AD group in anti-RAGE ($p = 0.001$). There was *not* a significant difference between the control group and the MCI group in anti-RAGE ($p > .05$).

Next, we conducted exploratory analyses to determine if control-MCI group differences in anti-A β and anti-RAGE could be better captured using a different operational definition of MCI (Tables 3.4 & 3.5). When further categorizing the MCI patients into amnesic and nonamnesic MCI (see group classification description above), we again did not find significant group differences between control and amnesic or non-amnesic MCI for both anti-A β and anti-RAGE (all pairwise comparison p 's $> .05$). There were significant group differences in anti-A β and anti-RAGE levels between the control and AD group ($p < .001$) the non-amnesic MCI group and the AD group ($p < .001$). Figure 1 illustrates levels of anti-RAGE and anti-A β IgGs across groups using both classification systems.

Discussion

Our findings support previous findings that both anti-A β and anti-RAGE IgGs can be used to distinguish between older adult controls and those with AD. The main aim of this study, however, was to determine the ability of these potential biomarkers to distinguish between normal and MCI groups, thus indicating a potential tool for early disease detection and prevention. Unfortunately, our investigation did not find a significant difference between control

and MCI groups. As figure 3.1 illustrates, when further dividing MCI patients into amnestic and non-amnestic subtypes, we did find trends in the predicted direction, with controls have the lowest mean values for both IgGs, the non-amnestic MCI group having slightly higher values, the amnestic MCI group having still higher mean values, and the AD group having the highest mean values.

A second issue that figure 3.1 illustrates is the limitation with this and other previously identified biomarkers, which is that there is a considerable amount of variability in IgG levels within diagnostic groups. Further refinement of the protein purification process used in our methodology may reduce this variability, but it may be the case that the level of variability found in our investigation reflects true variability in these potential biomarkers, thus limiting their ability to be used as diagnostic tools. Another likely possibility is that our MCI groups, regardless of classification systems, are heterogeneous groups, and some individuals in these groups will not progress to AD. Thus, it is possible that the reason for our nonsignificant group differences between control and MCI groups are attributable to the inherent heterogeneity in this population.

However, as figure 3.1 illustrates, there were considerably higher mean values for both IgGs in the amnestic MCI group when compared to the control group. It is quite possible that future studies using the psychometrically-driven approach to categorizing amnestic MCI as we described in our method section may find that with a larger n in this sub-group of individuals, there are statistically significantly group differences relative to controls. It is additionally important to note that we used a community-based recruitment method for our MCI participants. As has been demonstrated in the literature, many studies studying MCI recruit their samples from memory assessment clinics, and thus have an inherent sampling bias in their MCI cohorts

which may misrepresent the true range of heterogeneity in MCI (Twamley, Legendre, Ropacki, & Bondi, 2006). Thus, a strength of this study is that we do not have this issue of sampling bias towards a more focally amnesic cognitive profile in our MCI cohort, but this again may partially explain our lack of group differences between the control and MCI group.

Future investigations are necessary to determine the potential use of anti-A β and anti-RAGE IgGs as early markers which could potentially detect AD in its preclinical stage. However, we do show strong support for the biomarkers as capable of distinguishing between control and AD groups. A future investigation using a longitudinal design to characterize IgG levels over time in a cohort that progresses from normal cognition, to amnesic MCI, and finally to probable AD is necessary to better evaluate the clinical utility of anti-A β and anti-RAGE IgGs as potential biomarkers. Given our trends indicating that both IgGs do show increased mean values at increasing levels of impairment in our cross-sectional investigation of these IgGs, they remain promising as potential biomarkers for early detection of AD. We recommend that future investigations examine changes in individuals over time using latent growth modeling in order to determine if rate of change in IgGs is the best marker for future conversion to AD. It is possible that the most sensitive tools for detection of changes in AD brain pathology are not based on analysis of group-level data, but instead based on models capable of individual-level prediction of future conversion to AD.

Finally, studies investigating the sensitivity and specificity of these IgGs are necessary in order to establish them as biomarkers for AD, following the criteria described by The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group (1998).

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Table 3.1 Descriptive statistics.

3.1a. Descriptive statistics for groups categorized by Clinical Dementia Rating.

	Controls (n=41)	MCI (n=34)	AD (n=43)
	Mean (SD)	Mean (SD)	Mean (SD)
Age	71(7.9)	76(8.4)	79(6.9)
Years education	14.9(2.83)	13.8(3.61)	11.6(3.97)
Total IgG standardized value	.49(.341)	.62(.465)	.62(.316)
A β IgG standardized value	1.32(.767)	1.81(1.618)	3.71(2.788)
RAGE IgG standardized value	1.44(.901)	2.25(2.053)	4.59(3.433)
MMSE	29(1.2)	27(2.3)	13(7.9)

Table 3.1b. Descriptive statistics for groups categorized by RBANS performance.

	Controls (n=36)	Non-aMCI (n=18)	aMCI(n=15)	AD (n=29)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Age	71(7.9)	76(8.0)	75(9.3)	80(6.4)
Years education	15(3.0)	13(3.7)	14(2.9)	11(4.4)
Total IgG standardized value	.51(.360)	.50(.373)	.73(.518)	.65(.346)
A β IgG standardized value	1.38(.723)	1.38(1.258)	2.10(1.958)	3.49(2.210)
RAGE IgG standardized value	1.55(.930)	1.47(1.142)	2.67(2.742)	4.56(3.378)
MMSE	29(1.2)	28(1.9)	27(2.3)	16(6.8)

Table 3.2. Analysis of Covariance comparing group levels of anti-A β , entering age and total IgG as covariates.

Source	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>p</i>	Partial η^2
Corrected Model	158.63	4	39.66	10.85	.000	.29
Intercept	1.00	1	1.00	.28	.601	.00
Total IgG (covariate)	21.78	1	21.78	5.96	.016	.05
Age (covariate)	16.85	1	16.85	4.61	.034	.04
Group	79.77	2	39.89	10.91	.000	.17
error	391.24	107	3.66			
Total	1174.13	112				
Corrected Total	549.87	111				

Group	Adjusted Mean	<i>SE</i>	95% C.I. for Mean	
			Lower Bound	Upper Bound
Control	1.49	.34	.82	2.16
MCI	1.83	.33	1.17	2.50
AD	3.54	.31	2.93	4.16

Mean comparisons	Difference	<i>p</i> ^a
Control v MCI	-.35	1.000
Control v AD	-2.06	<.001
MCI v AD	-1.71	.001

a. Adjustment for multiple comparisons: Bonferroni.

Table 3.3. Analysis of Covariance comparing group levels of anti-RAGE, entering age and total IgG as covariates.

Source	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>p</i>	Partial η^2
Corrected Model	253.459	4	63.365	11.194	.000	.295
Intercept	1.204	1	1.204	.213	.646	.002
Total IgG (covariate)	26.733	1	26.733	4.723	.032	.042
Age (covariate)	22.835	1	22.835	4.034	.047	.036
Group	135.173	2	67.587	11.940	.000	.182
error	605.660	107	5.660			
Total	1775.223	112				
Corrected Total	859.119	111				

Group	Adjusted Mean	<i>SE</i>	95% C.I. for Mean	
			Lower Bound	Upper Bound
Control	1.644	.422	.808	2.481
MCI	2.272	.415	1.449	3.095
AD	4.393	.387	3.625	5.161

Mean comparisons	Difference	<i>p</i> ^a
Control v MCI	-.628	.888
Control v AD	-2.749	<.001
MCI v AD	-2.121	.001

a. Adjustment for multiple comparisons: Bonferroni.

Table 3.4. Analysis of Covariance comparing group levels of anti-A β further dividing MCI into amnesic and non-amnesic subtypes.

Source	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>p</i>	Partial η^2
Corrected Model	107.633	5	21.527	9.332	.000	.337
Intercept	.400	1	.400	.174	.678	.002
Total IgG (covariate)	17.387	1	17.387	7.538	.007	.076
Age (covariate)	11.124	1	11.124	4.822	.031	.050
Group	59.287	3	19.762	8.567	.000	.218
error	212.221	92	2.307			
Total	758.019	98				
Corrected Total	319.854	97				

Group	Adjusted Mean	<i>SE</i>	95% C.I. for Mean	
			Lower Bound	Upper Bound
Control	1.509	.270	.973	2.045
Non-amnesic MCI	1.252	.360	.537	1.968
Amnesic MCI	2.238	.397	1.451	3.026
AD	3.338	.300	2.741	3.934

Mean comparisons	Difference	<i>p</i> ^a
Control v Non-amnesic MCI	.257	1.000
Control v Amnesic MCI	-.729	.807
Control v AD	-1.829	<.001
Non-amnesic MCI v Amnesic MCI	-.986	.426
Non-amnesic MCI v AD	-2.085	<.001
Amnesic MCI v AD	-1.099	.172

a. Adjustment for multiple comparisons: Bonferroni.

Table 3.5. Analysis of Covariance comparing group levels of anti-RAGE further dividing MCI into amnestic and non-amnestic subtypes.

Source	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>p</i>	Partial η^2
Corrected Model	201.866	5	40.373	8.260	.000	.310
Intercept	.397	1	.397	.081	.776	.001
Total IgG (covariate)	18.878	1	18.878	3.862	.052	.040
Age (covariate)	14.656	1	14.656	2.999	.087	.032
Group	126.435	3	42.145	8.622	.000	.219
error	449.689	92	4.888			
Total	1313.828	98				
Corrected Total	651.556	97				

Group	Adjusted Mean	<i>SE</i>	95% C.I. for Mean	
			Lower Bound	Upper Bound
Control	1.709	.393	.928	2.489
Non-amnestic MCI	1.336	.524	.294	2.377
Amnestic MCI	2.815	.577	1.668	3.961
AD	4.378	.437	3.510	5.247

Mean comparisons	Difference	<i>p</i> ^a
Control v Non-amnestic MCI	.373	1.000
Control v Amnestic MCI	-1.106	.715
Control v AD	-2.670	<.001
Non-amnestic MCI v Amnestic MCI	-1.479	.378
Non-amnestic MCI v AD	-3.043	<.001
Amnestic MCI v AD	-1.564	.194

a. Adjustment for multiple comparisons: Bonferroni.

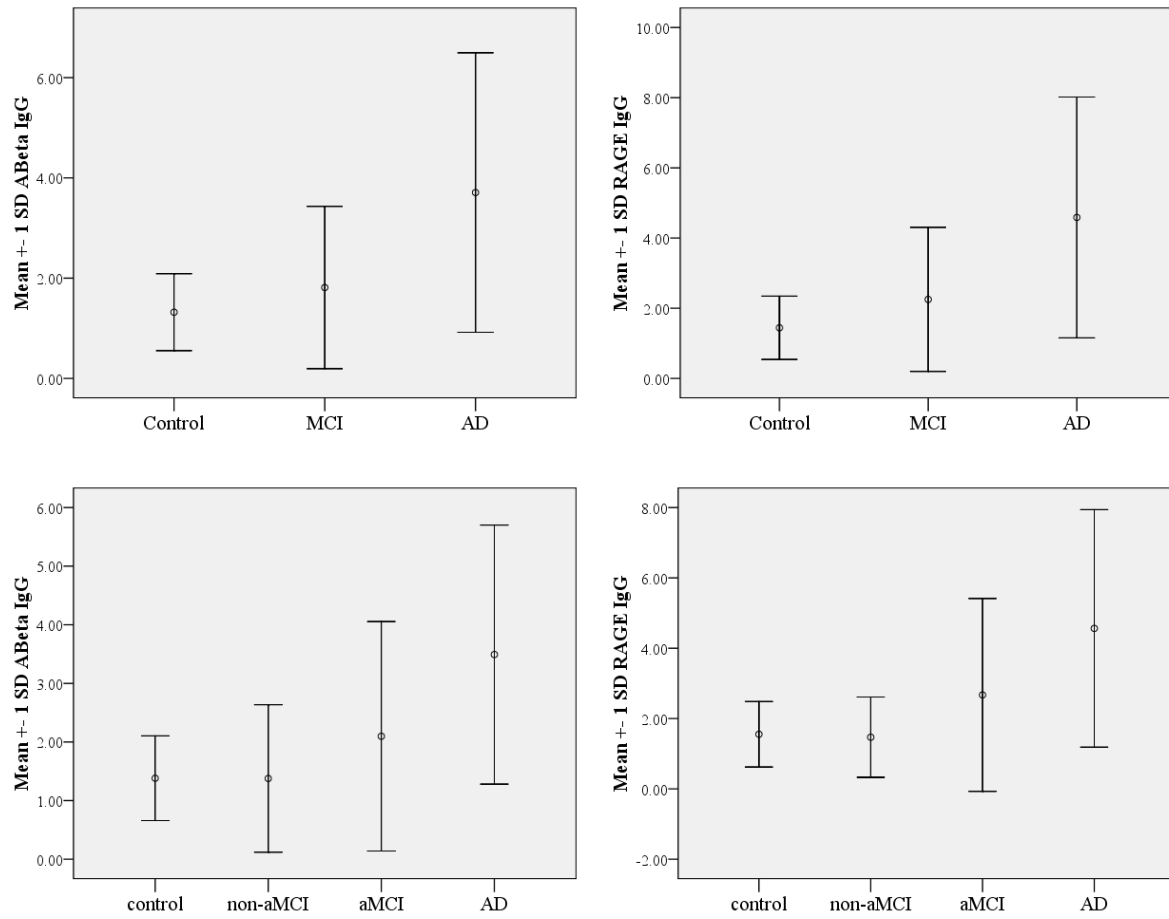


Figure 3.1. Group comparisons of A β and RAGE IgGs.

CHAPTER 4

RAGE AND A β IMMUNOGLOBULINS: RELATION TO ALZHEIMER'S DISEASE-RELATED COGNITIVE FUNCTION^{1,2}

¹ Mitchell, M.B., Buccafusco, J., Schade, R., Webster, S., Mruthinti, S., & Miller, L.S. To be submitted to *Journal of the International Neuropsychological Society*.

² A portion of the data in this study was previously published in a study using a mixed neurological sample (Wilson et al., 2009). This investigation uniquely improves upon this previous work because it has a substantially increased sample of individuals with mild cognitive impairment, has a clearly-defined group of individuals with probable Alzheimer's disease, and excluded individuals with other forms of dementia, psychiatric conditions, and diabetes. This investigation additionally includes some data included in another manuscript (Mitchell, Buccafusco, Schade, Webster, Mruthinti, & Miller, manuscript to be submitted to *Journal of the American Geriatrics Society*). The other manuscript focused on comparison of IgG levels between diagnostic groups. This paper provides a unique contribution to the literature, because it investigates the relationship between IgG levels and cognitive functioning, both using global measures of cognition, and domain-specific measures.

Abstract

The immunoglobulins (IgGs) for A β and RAGE have previously been shown to be related to memory and language measures in a mixed neurological sample of older adults. In this study, we examined the relationship of A β and RAGE to cognitive performance in non-diabetic older adults with normal cognition, mild cognitive impairment (MCI), and Alzheimer's disease (AD). We found both IgGs to be significantly related to global performance on cognitive measures, as well as domain-specific performance on immediate memory measures. However, IgG levels were not specifically related to language or delayed memory measures. Findings support the use of these IgGs as potential biomarkers of cognitive decline in AD.

Introduction

Alzheimer's disease (AD) is the sixth leading cause of death in the United States, and healthcare costs are estimated to be \$148 billion annually in the United States (Alzheimer's Association, 2008). As medical technologies increase the average human lifespan, it is additionally estimated that the amount of older adults with AD will nearly double from an estimate of approximately 377,000 cases in the year 2000, to approximately 959,000 in the year 2050 (Herbert, Beckett, Scherr, & Evans, 2001). Early detection of AD is crucial, as it decreases the financial and emotional burden of the disease on society and the individual (Petersen Stevens, Ganguli, Tangalos, Cummings, & DeKosky, 2001).

One method of early detection under investigation is to detect AD-related immune responses peripherally via blood testing. Recent AD research indicates that immunoglobulins (IgGs) for beta amyloid (A β) and receptors for the advanced glycation end products (RAGE) are produced at increasing levels when comparing older adults with normal cognition to those with probable AD (Mruthinti et al, 2004). Protein plaques containing A β have been previously demonstrated to accumulate in brain tissue at RAGE receptor sites (Emanuele et al., 2005). Formation of these A β plaques is associated with Alzheimer's disease severity, including level of cognitive impairment (Berg et al., 1998). Presumably in response to this plaque buildup in the brain, IgGs for A β and RAGE have been found to be elevated peripherally in the bloodstream (Nath et al., 2003). The implication of this finding is that AD could potentially be detected and its progression monitored through simple blood testing rather than the use of more invasive (e.g., CSF testing), time-consuming (e.g., neuropsychological testing), or expensive (e.g., brain imaging) techniques currently used to diagnose probable AD.

One important aspect of establishing A β and RAGE IgGs as biomarkers for AD is to determine their relation to AD-related cognitive impairment. Mruthinti and colleagues (2004) demonstrated that plasma A β and RAGE IgG levels were negatively correlated with gross cognitive status, as measured by the Mini Mental State Examination (MMSE) in a sample of normal controls and AD participants, indicating that across both AD and control groups, those individuals with higher levels of A β and RAGE IgGs tended to exhibit more cognitive impairment. The A β IgG and RAGE IgG correlation coefficients with MMSE scores were -0.60 and -0.71, respectively, indicating a strong association between IgG levels of the biomarkers and cognitive status (Mruthinti et al., 2004). In a more recent study by the same group, Wilson and colleagues (2009) demonstrated a relationship between global cognition and A β and RAGE IgG levels in a mixed neurological sample. Results further indicated that measures of language and delayed memory were uniquely related to A β and RAGE IgG levels, beyond variance accounted for by age, total IgG level, and the cognitive domains of immediate memory, visuospatial and construction ability, and attention.

These findings indicate that both IgGs are related to global changes in cognition, in addition to specific changes in memory and language functioning. This evidence demonstrates the important link between A β and RAGE IgG levels and performance on language and memory measures, as these are the domains of cognition that are most closely associated with the integrity of temporal lobe structure and function, which has been well established to be the earliest and most profoundly affected brain region in AD (Baron et al., 2001). Further investigation with more refined diagnostic groups is necessary in order to determine the relationship between cognition and A β and RAGE IgGs. In this investigation, we improved upon previous studies by examining the relationship between cognitive test performance and A β and

RAGE IgGs in an elderly control group, an MCI group, and an AD group. Unlike previous investigations, we excluded individuals with other neurological conditions and those with diabetes, as these conditions also have autoimmune components to their disease processes (Mruthinti et al., 2006) which may influence the relationships between cognitive variables and IgG levels.

We hypothesized that consistent with previous findings using mixed neurological samples and samples including diabetics, that in this study, we would similarly find that a global measure of cognition would be significantly related to A β and RAGE IgGs. In addition, we hypothesized that measures of memory and language would be the specific domains of cognition that would be uniquely related to A β and RAGE IgGs, after accounting for shared variance with age, total IgG, visuospatial and construction ability, and attention measures.

Method

Participants

Participants were from the Neurological Disorders Database Repository (NDDR) at the Medical College of Georgia (MCG), the aim of which is to collect data from older adults with AD, MCI, other neurological disorders, and normal cognitive functioning. In this investigation, we used cross sectional data, representing the first study visit for all control and AD participants. However, to approximate equal numbers in each diagnostic group, we used data from a later time point at which the participant converted from control to MCI for some participants. This was used for five participants in the MCI category, and these participants were not included in the control group. Participants with AD and other neurological conditions were primarily recruited via physician referrals from the Medical College of Georgia. Healthy control participants and those with MCI were recruited primarily through caregivers of participants with AD, as well as

local advertisements in retirement centers and hospitals. Participants were adults age 60 and older whose primary language was English. Participants with neurologic diagnoses other than Alzheimer's disease (e.g., Parkinson's disease, stroke, Dementia with Lewy Bodies) or psychiatric diagnoses (e.g., Schizophrenia, Bipolar Disorder) were excluded. Because anti-A β and anti-RAGE IgGs are known to be elevated in diabetes (Mruthinti et al. 2006), we excluded participants with diabetes. After meeting inclusion criteria, participants were assigned to the following three groups according to their Clinical Dementia Rating score (CDR; Morris, 1993): Normal Control Group (CDR=0), MCI Group (CDR=0.5), AD Group (CDR \geq 1). All participants in the AD group additionally had physician's diagnoses of probable AD.

Procedure

Test sessions, lasting approximately two hours, began with obtaining informed consent, and included obtaining demographic information, medical history, current medical diagnoses, self-reported cognitive status, family history, and current medications. This information was obtained by the participant with assistance from caregivers and was verified for completeness by the study coordinator. Participants also completed a release to verify their medications and medical diagnoses with their physician. Each participant and caregiver next participated in a semi-structured interview from which the participant's Clinical Dementia Rating (CDR) was derived by a CDR-certified administrator. Participants were then administered a cognitive test battery by a trained test administrator. Finally, each participant provided a blood sample which was drawn by a trained phlebotomist.

Materials

Repeatable Battery for the Assessment of Neuropsychological Status.

The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS; Randolph, 1998) is a 30-minute neuropsychological test designed to assess cognitive decline in older adults and serve as a screening tool for cognitive functioning in younger adults (Randolph, Tierney, Mohr, & Chase, 1998). Consisting of 12 subtests, the RBANS generates 5 Index Scores: Visuospatial/Constructional, Attention, Language, Immediate Memory, and Delayed Memory. The RBANS additionally generates a global score (the Total Scale score), derived from raw scores on all 12 subtests. The 12 subtests of the RBANS are as follows: Figure Copy, Line Orientation, Digit Span, Coding, Picture Naming, Semantic Fluency, List Learning, Story Memory, List Recall, List Recognition, Story Recall, and Figure Recall. The RBANS has been demonstrated to have good reliability, with split-half reliability coefficients in the .80s for the Index Scores and the average reliability for the Total Scale score ranging from .86-.94 across age groups.

Clinical Dementia Rating.

The Clinical Dementia Rating (CDR, Morris, 1993) is a semi-structured interview designed to rate severity of dementia through the assessment six domains of the participant's cognitive and functional abilities. Domains assessed on the CDR include memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care. Each domain is rated and entered into an algorithm to generate an overall summary score on a 5-point scale of either "normal" (CDR=0), "questionable/very mild dementia" (CDR=0.5), "mild dementia" (CDR=1), "moderate dementia" (CDR=2), or "severe dementia" (CDR=3).

Administrators were trained in the CDR interview and scoring techniques by the CDR author at CDR training course online (<http://alzheimer.wustl.edu/cdr/default.htm>).

The CDR has been well-validated as a classification tool for dementia severity (Morris, 1993), with demonstrated inter-rater reliability of 80% (Burke et al., 1988; McCulla et al., 1989). We established inter-rater reliability by scoring a subset of the CDR interviews used in this paper (n=12) independently and obtained consistent inter-rater reliability across domains (Cronbach's α range = .83-1.0). Overall rating agreement for the global CDR score in this subset was 100%.

RAGE and A β IgG purification from plasma

Blood plasma analysis was conducted at the Medical College of Georgia by trained technicians in psychopharmacology. IgG purification steps followed our procedures described elsewhere (Mitchell, Buccafusco, Schade, Webster, Mruthinti, & Miller, manuscript to be submitted). Briefly, plasma was purified via a purification process involving initial ammonium sulfate precipitation, dialyzation overnight against 1x PBS at 4°C, protein A/G isolation, and a second dialyzation overnight against 1xPBS at 4°C. Total IgG was quantified using a bicinchoninic acid (BCA) assay for protein quantification.

Quantifying specific IgGs

RAGE peptide or A β 1-42 (1 μ g/ 100 μ l/well) were coated to MaxiSorp™ ELISA plates and incubated overnight at 4 °C. Antigen was discarded and wells were washed once with 150 μ l of 2% milk in PBS, and then wells were blocked with 150 μ l of 2% milk in PBS for 2 hours. Wells were washed three times with 150 μ l of 2% milk in PBS, and anti-A β 1-42 or anti-RAGE IgG (100 μ l) derived from participants were added to each well and incubated overnight at 5°C. Wells were washed four times with 2% milk in Tris buffered saline + Tween 20 (TBST), and 200 μ l of donkey anti-human IgG(F(ab')₂)-HRP conjugated secondary antibody (1:1000) was

added and incubated for 3 hours at 37°C. Plates were washed three times with 2% milk in TBST followed by adding 100µL of ready-made Tetramethylbenzidine (TMB) substrate (Sigma-Aldrich) for blue color development, which were stopped after 15 minutes by adding 100µL 1M HCL. Absorbance was read at 450nm providing the titer values used in this study. Each sample was run in triplicate.

To account for nonspecific binding, three wells containing the antigen and secondary antibody were included on each ELISA plate, the average value of which was subtracted from all titer values. A control sample of plasma was purified in each purification and ELISA assay and used to create standardized values in order to compare values from different assays. These standardized values were used in all statistical analyses.

Peptides

RAGE peptide was synthesized according to the following sequence (Neeper et al., 1992): “DQNITARIGKPLVLNCKGAPKKPPQQLEWKLN” representing the nucleotide and amino acid sequence of human RAGE. The peptide was synthesized by the Molecular Biology Central Core facility at MCG. Aβ1-42 was purchased from Sigma-Aldrich.

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS 16.0 for Windows, SPSS, Chicago, IL). Pearson’s correlations were first used to examine the bivariate relationships between each IgG and cognitive variables. To determine the overall relationship between each IgG and cognition, multiple regression analyses evaluated the variance accounted for by global RBANS performance for each IgG. Finally, multiple regression was used to examine the different domains of cognition measured by the RBANS. For each IgG, RBANS index scores—Immediate Memory, Visiospatial/Constructional skills, Language,

Attention, and Delayed Memory—were entered simultaneously as possible predictors of IgG levels. Throughout regression analyses, age and total IgG level were entered into the equations first.

Results

Demographic information and descriptive statistics for diagnostic groups are summarized in Table 4.1. We first conducted correlation analysis to determine the bivariate relationships between age, IgGs, cognitive domain scores, and global cognition (Table 4.2). Bivariate analysis revealed significant correlations between A β and RAGE IgG levels and all cognitive domain scores. We next conducted multiple regression analyses to determine the proportion of unique variance in A β and RAGE IgG levels accounted for global cognition, beyond the variance accounted for by age and total IgG levels (Tables 4.3 and 4.4). In the A β IgG model examining the IgG's unique relationship with global cognition (Table 4.3), we entered age in the first step, which accounted for approximately 10 percent of the variance in A β IgG levels (Adjusted $R^2 = .10$, $F(1, 96) = 11.94$, $p < .001$). Total IgG level accounted for an additional 4 percent of the variance in A β IgG levels in the second step of the model (R^2 change = .04, $F(1, 95) = 4.54$, $p < .05$). Finally, global cognition, as represented by RBANS total score, accounted for an additional 11 percent of the variance in A β IgG levels in the third step of the model (R^2 change = .11, $F(1, 94) = 13.52$, $p = .001$). In the RAGE IgG model examining the IgG's unique relationship with global cognition (Table 4.4), age accounted for 9 percent of the variance in RAGE IgG levels in step one of the model (Adjusted $R^2 = .09$, $F(1, 96) = 10.35$, $p = .002$). Total IgG level accounted for an additional 2 percent of the variance in RAGE IgG in the second step of the model (R^2 change = .02, $F(1, 95) = 1.98$, $p > .05$). Finally, global cognition accounted for an additional 11

percent of the variance in RAGE IgG in the third step of the model (R^2 change = .11, $F(1, 94) = 12.98$, $p = .001$).

To determine which aspects of cognition were uniquely related to A β and RAGE IgG levels, we conducted regression analyses repeating the first two steps of our previous models, but entering the five cognitive domain scores that factor into the global score on the RBANS: Immediate Memory, Visuospatial/Construction, Language, Attention, and Delayed Memory. In the A β IgG model (Table 4.5), in the third step of the model in which RBANS cognitive domain scores were entered, the only index score that was significantly related to A β IgG levels, beyond the variance accounted for by age, total IgG, and the other index scores was the RBANS Immediate Memory score ($t(90) = -2.03$, $\beta = -.49$, $p < .05$). Similarly, in the RAGE IgG model (Table 4.6), Immediate Memory was the only RBANS index score to account for unique variance in RAGE IgG levels, beyond variance accounted for by age, total IgG, and other RBANS indices ($t(90) = -2.23$, $\beta = -.56$, $p < .05$).

We conducted an exploratory analysis to determine the relationship between each IgG and the domains of cognition that we hypothesized to be most related to the IgGs—Language and Delayed Memory. We conducted these regressions due to the large amount of shared variance between immediate and delayed memory measures on the RBANS. These exploratory analyses revealed that in these reduced models, after controlling for age and total IgG, the Delayed Memory index was significantly related to both IgGs, while Language was not (Tables 4.7 & 4.8).

Discussion

Our findings support previous findings that both anti-A β and anti-RAGE IgGs are related to global cognitive measures. A strength of this study was its use of clearly defined diagnostic

groups. While a previous study has examined the relationship of anti-A β and anti-RAGE IgGs to cognition in a mixed neurological sample (Wilson et al., 2009), we have done so using a larger sample of participants with MCI, and we have excluded individuals with non-AD forms of dementia, psychiatric conditions, and diabetes. This method of group definition has allowed us to better characterize the relationship between these two IgGs and AD-specific changes in cognition.

Our hypothesis that delayed memory and language measures would be the only aspects of cognition to be uniquely related to IgG levels was not supported. Nonetheless, our finding that measures of learning and immediate memory were uniquely related to anti-A β and anti-RAGE IgG levels does provide evidence that these IgGs are related to cognitive aspects of the disease process of AD, as immediate memory, in addition to delayed memory, typically declines in MCI and AD (Cerhan et al., 2007). As is demonstrated in Table 4.1, our AD group demonstrated performance on immediate memory measures (AD $m = 50$, $s.d. = 11.5$) that was nearly as low as their performance on delayed memory measures (AD $m = 48$, $s.d. = 11.1$), and as Table 4.2 demonstrates, the correlation between immediate and delayed memory measures was strong ($r(86) = .89$, $p < .001$). Thus, the large amount of shared variance between immediate and delayed memory measures was likely a contributing factor in our finding that our delayed memory measure did not account for unique variance in either anti-A β or anti-RAGE IgG levels. Our exploratory regression analysis suggested this, as we found that when only entering Language and Delayed Memory indices into the model, Delayed Memory was significantly related to both IgGs (Tables 4.7 & 4.8).

Future investigations examining longitudinal changes in cognition in relation to anti-A β and anti-RAGE IgG levels over time will better demonstrate the unique relationships between

different aspects of cognition and these two potential biomarkers for AD. It will be important to determine if elevations in anti-A β and anti-RAGE IgG levels predate changes in cognition in order to establish the clinical utility of these potential biomarkers, beyond being correlates of already evident cognitive impairments. In addition, it will be important to determine if these biomarkers are modulated in conjunction with cognitive changes over time in response to AD drug therapies, again to determine if they serve as a reliable monitor of disease progression.

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Table 4.1 Descriptive statistics for diagnostic groups.

	Controls (n=41)	MCI (n=34)	AD (n=43)
	Mean (SD)	Mean (SD)	Mean (SD)
Age	71(7.9)	76(8.4)	79(6.9)
Years education	14.9(2.83)	13.8(3.61)	11.6(3.97)
Total IgG standardized value	.49(.341)	.62(.465)	.62(.316)
A β IgG standardized value	1.32(.767)	1.81(1.618)	3.71(2.788)
RAGE IgG standardized value	1.44(.901)	2.25(2.053)	4.59(3.433)
MMSE	29(1.2)	27(2.3)	13(7.9)
RBANS Immediate Memory	107(11.2)	89(14.5)	50(11.5)
RBANS Visuospatial/Construction	97(18.5)	77(13.7)	59(10.7)
RBANS Language	105(9.8)	93(12.2)	64(19.4)
RBANS Attention	110(14.6)	96(15.9)	68(14.7)
RBANS Delayed Memory	105(9.7)	78(19.5)	48(11.1)
RBANS Total Scale	106(12.5)	82(11.2)	52(8.6)

Table 4.2 Pearson correlations between age, IgGs, and cognition.

		RBANS Index Scores								
		Total IgG	A β IgG	RAGE IgG	Immediate Memory	Visuospatial/ Construction	Language	Attention	Delayed Memory	Total Scale
Age		.426**	.257*	.228*	-.481**	-.333**	-.393**	-.344**	-.415**	-.445**
	df	95	95	95	87	86	87	86	86	86
Total IgG			-.072	-.138	-.268*	-.264*	-.267*	-.323**	-.265*	-.314**
	df		95	95	82	81	82	81	81	81
A β IgG				.850**	-.470**	-.286**	-.287**	-.200	-.437**	-.395**
	df			95	82	81	82	81	81	81
RAGE IgG					-.429**	-.256*	-.282**	-.260*	-.429**	-.382**
	df				82	81	82	81	81	81
Immediate Memory						.679**	.790**	.725**	.893**	.925**
	df					86	87	86	86	86
Visuospatial/ Construction							.618**	.652**	.620**	.820**
	df						86	86	86	86
Language								.693**	.720**	.834**
	df							86	86	86
Attention									.686**	.839**
	df								86	86
Delayed Memory										.911**
	df									86

Table 4.3 Regression analysis^a examining the relationship between global cognition and A β IgG.

Step 1: $R^2 = .11$ Adj $R^2 = .10$ S.E.E. = .72 $F(1,96) = 11.94$, $p = 0.001$

Step 2: $R^2 = .15$ Adj $R^2 = .13$ S.E.E. = 1.69 R^2 change = .04, F change(1, 95) = 4.54, $p = .036$

Step 3: $R^2 = .26$ Adj $R^2 = .23$ S.E.E. = 1.59 R^2 change = .11, F change(1, 94) = 13.52, $p < .001$

Step	Predictor	Beta	t	Significance of t	Correlations		
					Zero-order	Partial	Part
1	Age	.333	3.455	.001	.333	.333	.333
2	Age	.387	3.954	<.001	.333	.376	.374
	Total IgG Standardized	-.209	-2.131	.036	-.107	-.214	-.201
3	Age	.221	2.151	.034	.333	.217	.191
	Total IgG Standardized	-.253	-2.723	.008	-.107	-.270	-.242
	RBANS Total	-.375	-3.677	<.001	-.420	-.355	-.327

a. Dependent variable: A β IgG Standardized

Table 4.4 Regression analysis^a examining the relationship between global cognition and RAGE IgG.

Step 1: $R^2 = .10$ Adj $R^2 = .09$ S.E.E. = 2.48 $F(1,96) = 10.35, p = 0.002$

Step 2: $R^2 = .12$ Adj $R^2 = .10$ S.E.E. = 2.46 R^2 change = .02, F change(1, 95) = 1.98, $p = .163$

Step 3: $R^2 = .22$ Adj $R^2 = .20$ S.E.E. = 2.32 R^2 change = .11, F change(1, 94) = 12.98, $p = .001$

Step	Predictor	Beta	<i>t</i>	Significance of <i>t</i>	Correlations		
					Zero-order	Partial	Part
1	Age	.312	3.218	.002	.312	.312	.312
2	Age	.349	3.490	.001	.312	.337	.337
	Total IgG Standardized	-.141	-1.407	.163	-.049	-.143	-.136
3	Age	.182	1.733	.086	.312	.176	.158
	Total IgG Standardized	-.185	-1.945	.055	-.049	-.197	-.177
	RBANS Total	-.375	-3.602	.001	-.419	-.348	-.327

a. Dependent Variable: RAGE IgG Standardized

Table 4.5 Regression analysis^a examining the relationship between cognitive domains and A β IgG.

Step 1: $R^2 = .11$ Adj $R^2 = .10$ S.E.E.= 1.72 $F(1,96) = 11.94, p = 0.001$

Step 2: $R^2 = .15$ Adj $R^2 = .13$ S.E.E.= 1.69 R^2 change = .04, F change(1, 95) = 4.54, $p = .036$

Step 3: $R^2 = .31$ Adj $R^2 = .26$ S.E.E.= 1.57 R^2 change = .16, F change(5, 90) = 4.19, $p = .002$

Step	Predictor	Beta	<i>t</i>	Significance of <i>t</i>	Correlations		
					Zero-order	Partial	Part
1	Age	.333	3.455	.001	.333	.333	.333
2	Age	.387	3.954	.000	.333	.376	.374
	Total IgG Standardized	-.209	-2.131	.036	-.107	-.214	-.201
3	Age	.182	1.785	.078	.333	.185	.156
	Total IgG Standardized	-.210	-2.238	.028	-.107	-.230	-.196
	RBANS Imm Mem	-.494	-2.025	.046	-.467	-.209	-.177
	RBANS Vis/Const.	-.104	-.781	.437	-.333	-.082	-.068
	RBANS Language	.097	.624	.534	-.329	.066	.055
	RBANS Attention	.216	1.532	.129	-.215	.159	.134
	RBANS Delayed Memory	-.097	-.502	.617	-.422	-.053	-.044

a. Dependent Variable: A β IgG Standardized

Table 4.6 Regression analysis^a examining the relationship between cognitive domains and RAGE IgG.

Step 1: $R^2 = .10$ Adj $R^2 = .09$ S.E.E.= 2.48 $F(1,96) = 10.35, p = 0.002$

Step 2: $R^2 = .12$ Adj $R^2 = .10$ S.E.E.= 2.46 R^2 change = .02, F change(1, 95) = 1.98, $p = .163$

Step 3: $R^2 = .27$ Adj $R^2 = .21$ S.E.E.= 2.30 R^2 change = .16, F change(5, 90) = 3.83, $p = .003$

Step	Predictor	Beta	<i>t</i>	Significance of <i>t</i>	Correlations		
					Zero-order	Partial	Part
1	Age	.312	3.218	.002	.312	.312	.312
2	Age	.349	3.490	.001	.312	.337	.337
	Total IgG Standardized	-.141	-1.407	.163	-.049	-.143	-.136
3	Age	.146	1.389	.168	.312	.145	.125
	Total IgG Standardized	-.152	-1.568	.120	-.049	-.163	-.141
	RBANS Imm Mem	-.560	-2.230	.028	-.474	-.229	-.201
	RBANS Vis/Const.	.057	.417	.678	-.283	.044	.038
	RBANS Language	.065	.407	.685	-.345	.043	.037
	RBANS Attention	.086	.597	.552	-.266	.063	.054
	RBANS Delayed Memory	-.033	-.165	.870	-.419	-.017	-.015

a. Dependent Variable: RAGE IgG Standardized

Table 4.7 Regression analysis^a examining the relationship between language and delayed memory and Aβ IgG.

Step 1: $R^2 = .11$ Adj $R^2 = .10$ S.E.E.= 1.72 $F(1,96) = 11.94, p = 0.001$

Step 2: $R^2 = .15$ Adj $R^2 = .13$ S.E.E.= 1.69 R^2 change = .04, F change(1, 95) = 4.54, $p = .036$

Step 3: $R^2 = .27$ Adj $R^2 = .23$ S.E.E.= 2.59 R^2 change = .11, F change(2, 93) = 7.19, $p = .001$

Step	Predictor	Beta	t	Sig.	Correlations		
					Zero-order	Partial	Part
1	Age	.333	3.455	.001	.333	.333	.333
2	Age	.387	3.954	.000	.333	.376	.374
	Total IgG Standardized	-.209	-2.131	.036	-.107	-.214	-.201
3	Age	.237	2.347	.021	.333	.236	.209
	Total IgG Standardized	-.251	-2.703	.008	-.107	-.270	-.240
	Language	-.023	-.178	.859	-.329	-.018	-.016
	Delayed Memory	-.359	-2.718	.008	-.422	-.271	-.242

a. Dependent Variable: Aβ IgG Standardized

Table 4.8 Regression analysis^a examining the relationship between language and delayed memory and RAGE IgG.

Step 1: $R^2 = .10$ Adj $R^2 = .09$ S.E.E.= 2.48 $F(1,96) = 10.35, p = 0.002$

Step 2: $R^2 = .12$ Adj $R^2 = .10$ S.E.E.= 2.46 R^2 change = .02, F change(1, 95) = 1.98, $p = .163$

Step 3: $R^2 = .23$ Adj $R^2 = .20$ S.E.E.= 2.32 R^2 change = .11, F change(2, 93) = 6.91, $p = .002$

Step	Predictor	Beta	t	Sig.	Correlations		
					Zero-order	Partial	Part
1	Age	.312	3.218	.002	.312	.312	.312
2	Age	.349	3.490	.001	.312	.337	.337
	Total IgG Standardized	-.141	-1.407	.163	-.049	-.143	-.136
3	Age	.195	1.891	.062	.312	.192	.172
	Total IgG Standardized	-.182	-1.921	.058	-.049	-.195	-.175
	Language	-.064	-.487	.627	-.345	-.050	-.044
	Delayed Memory	-.330	-2.435	.017	-.419	-.245	-.222

a. Dependent Variable: RAGE IgG Standardized

CHAPTER 5

DISCUSSION

As discussed in chapter three of this dissertation, findings revealed control-AD group differences in A β and RAGE IgGs, but there were not control-MCI group differences, regardless of classification method for defining MCI. Nonetheless, trends in the predicted direction were found, with the MCI group having mean levels of both IgGs that were intermediate to control and AD group means. Chapter four summarizes the findings that global cognition and immediate memory measures were related to both IgGs, but language and delayed memory were not significantly related to IgG levels in multiple regression analysis.

One hypothesis not directly addressed in the preceding manuscript chapters was the prediction that a CDR classification of 0.5 would adequately capture the majority of participants with an RBANS profile consistent with amnesic MCI. In order to classify groups by RBANS profile, as discussed in Chapter 3, the CDR-classified MCI group was further divided into amnesic and non-amnesic MCI based on cognitive test scores. We classified individuals as having amnesic MCI when their CDR score was 0.5, and Immediate and/or Delayed Memory scores on the RBANS were between 1.5 and 2 standard deviations below the mean, and all other cognitive domain scores were no lower than 2 standard deviations below the mean. We classified individuals as having non-amnesic MCI when their CDR score was 0.5, and one or more of their non-learning and memory scores were between 1.5 and 2 standard deviations below the mean, and all other cognitive domain scores were no lower than 2 standard deviations below the mean. To be classified as having AD, individuals had to have a global CDR of 1 or greater, and two or

more indices on the RBANS had to be greater than 2 standard deviations below the mean. Due to incomplete RBANS data on some participants, the n's in each group were reduced in the control and AD groups using this classification system.

A total of 15 participants were classified as amnesic MCI using the above-described criteria for RBANS classification. As is depicted in table 5.1, 12 of these individuals were classified as MCI using the CDR = 0.5 criteria, 1 was classified as a control using the CDR = 0 criteria, and 2 were classified as AD using the CDR \geq 1.0 criteria. While the majority of individuals with RBANS profiles consistent with amnesic MCI were correctly categorized in the CDR = 0.5 category (n = 12, 80% of RBANS classified amnesic MCI cases), there were also individuals classified as CDR = 0.5 who had cognitive profiles consistent with non-amnesic MCI (n = 13, 38.2% of CDR classified MCI cases), normal cognition (n = 6, 17.6% of CDR classified MCI cases), and AD (n = 2, 5.9% of CDR classified MCI cases). These results reflect the difficulties in categorizing MCI and the lack of consensus between categorization methods, as have been extensively discussed in the literature on MCI (e.g., Petersen, 2000).

These findings of inconsistency between methods of classifying MCI represent the central finding of this project, which is that individuals with early signs of cognitive decline are a largely heterogeneous group that are difficult to characterize using cognitive measures (e.g., RBANS), interviewing techniques (e.g., CDR staging), and biological markers (e.g., A β and RAGE IgGs). Nonetheless, these individuals are a crucial population to study, as detecting AD in its preclinical stages is crucial to optimize response to therapeutic intervention.

There are several future directions for research that this project suggests. In order for A β and RAGE IgGs to meet the criteria for biomarkers described by The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working

Group (1998), future studies examining the sensitivity and specificity of these IgGs are necessary. Such studies would need to demonstrate that both IgGs are uniquely elevated in AD-type dementia, and not in other forms of dementia (e.g., Parkinson's disease). In addition, to be used as a clinical tool, cutoff values would need to be established to determine the level of both IgGs that was predictive of AD.

Another line of research that would support A β and RAGE IgGs as good biomarkers for AD would be to investigate both IgG levels over time in a cohort of individuals with AD undergoing current psychopharmacological treatments for AD. This line of research would help to determine if A β and RAGE IgG levels decrease in absolute level, or decelerate in rate of increase over time in this cohort, in order to establish the utility of these potential biomarkers as markers of response to intervention.

Future studies can also build on the findings of this study by investigating longitudinal changes in both A β and RAGE IgGs using a cohort of individuals who at initial testing present as normal controls, later convert to amnesic MCI, and finally convert to probable AD. Examining changes over time in both IgGs in this cohort would determine if changes in disease status are related to changes in levels of both IgGs over time.

Table 5.1 Comparison of group classification systems.

RBANS Classified Group	Control		CDR Classified Group MCI		AD	
	Frequency	Percent	Frequency	Percent	Frequency	Percent
Control	34	82.9	6	17.6	0	0
Non-amnestic MCI	6	14.6	13	38.2	0	0
Amnestic MCI	1	2.4	12	35.3	2	4.7
AD	0	0	2	5.9	27	62.8
Missing	0	0	1	2.9	14	32.6
Total	41	100.0	34	100.0	43	100.0

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APPENDIX

INITIAL ENCOUNTER FORM

Questions	Responses
1. What is your current age? DOB?	
2. How many years of education do you have?	
3. Have you had any of the following stressful changes in your family life in the last year?	
a. Death in the family?	
b. Divorce?	
c. Marriage?	
d. Moving?	
e. Accident?	
f. Hospitalizations?	
4. Do you have a family history of AD?	
5. Have you been diagnosed with....	
a. Diabetes? If so when?	
b. Alzheimer's Disease? If so when?	
c. Parkinson's Disease? If so when?	
6. Have you had any illnesses or infections in the last year?	
7. Have you been hospitalized in the last year?	
8. Have you had any surgeries/ Procedures performed in the last year?	
9. Have you had any changes in medication in the last year? If so what are they?	
10. Have there been any changes in your daily activities in the last year?	
11. Do you need more assistance in the home than you did before?	

Medical Information Summary

Vital Signs and Labs for Subject # _____ at _____ month visit.

Current Diagnoses	Current Prescriptions
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Clinical Lab Data for Subject

Hemoglobin

Hematocrit

Glucose

Cholesterol

Triglyceride

LDL

VLDL

HDL

B-12

Folate
HGB A1C

TSH

Free T4

Blood Pressure
