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Differentiating between healthy control participants and those with mild cognitive impairment using volumetric MRI data

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DIFFERENTIATING BETWEEN HEALTHY CONTROL PARTICIPANTS AND THOSE WITH MILD COGNITIVE IMPAIRMENT USING VOLUMETRIC MRI DATA

by

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ABSTRACT

Objective: To determine whether volumetric measures of the hippocampus or entorhinal cortex in combination with other cortical measures can differentiate between cognitively normal individuals and participants with amnestic mild cognitive impairment (MCI).

Methods: T1-weighted magnetic resonance imaging (MRI) data acquired from 46 cognitively normal participants and 50 participants with amnestic MCI as part of the Boston University Alzheimer's Disease Center research registry and the Alzheimer's Disease Neuroimaging Initiative were used in this cross-sectional study. Cortical and subcortical volumes, including hippocampal subfield volumes, were automatically generated from each participant's structural MRI data using FreeSurfer v6.0. Nominal logistic regression models containing these variables were used to evaluate their ability to identify participants with MCI.

Results: A model containing 11 regions of interest (insula, superior parietal cortex, rostral middle frontal cortex, middle temporal cortex, pars opercularis, paracentral lobule, whole hippocampus, subiculum, superior temporal cortex, precentral cortex and caudal anterior cingulate cortex) fit the data best ($R^2 = 0.7710$, whole model test chi square = 102.4794, $p < 0.0001$).

Conclusions: Volumetric measures acquired from MRI were able to correctly identify most healthy control subjects and those with amnestic MCI using measures of selected medial temporal lobe structures in combination with those from other cortical areas yielding an overall classification of 95.83% for this dataset. These findings support the notion that while clinical features of amnestic MCI may reflect medial temporal atrophy, differences that can be used to distinguish between these two populations are present elsewhere in the brain. This finding further affirming that atrophy can be identified before clinical features are expressed. Additional studies are needed to assess how well other imaging modalities, such as resting state functional connectivity, diffusion imaging, and amyloid and tau position emission tomography (PET), perform in classifying participants who are cognitively normal versus those who are amnestic MCI.

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LIST OF ABBREVIATIONS

Aβ .....Amyloid-Beta

AD..... Alzheimer's Disease

ADC .....Alzheimer's Disease Center

ADNI.....Alzheimer's Disease Neuroimaging Initiative

ANCOVA ..... Analysis of Covariate

APOE .....Apolipoprotein E

BU ..... Boston University

CA .....Cornu Ammonis

CDR ..... Clinical Dementia Score

CSF .....Cerebrospinal Fluid

DICOM .....Digital Imaging and Communications in Medicine

eTIV .....Estimated Total Intracranial Volume

FDR……..False Discovery Rate

FOV..... Field of View

GDS.....Geriatric Depression Scale

HOPE ..... Health Outreach Program for the Elderly

IRB ..... Institutional Review Board

MCI……Mild Cognitive Impairment

MM .....Millimeters

MMSE..... Mini Mental Status Examination

MPRAGE..... Magnetization-Prepared Rapid Acquisition of Gradient Echo

MRI.....Magnetic Resonance Imaging

MS.....Milliseconds

NACC ..... National Alzheimer's Coordinating Center

NIA .....National Institute on Aging

PET .....Position Emission Tomography

ROI.....Region of Interest

T .....Tesla

TBI ..... Traumatic Brain Injury

TE .....Echo Time

TR ..... Repetition Time

WMS-R.....Wechsler D. Wechsler Memory Scale-Revised

BACKGROUND

In 2017, approximately 5.5 million people in the United States were living with a diagnosis of Alzheimer's disease (AD). This means that roughly 1 in 10 persons above 65 years of age had been diagnosed with AD while perhaps an equal number are now playing the role as primary caregiver for a spouse with AD. The risk of AD increases with age as currently 3% of people between ages 65-74 have been diagnosed with AD, 17% of people between ages 75-84 have been diagnosed with AD, and 32% of people over 85 have been diagnosed with AD (Alzheimer's Association, 2017). Given that the first members of the baby boomer generation turned 70 in 2016, the number of adults 65 and older in America is expected to nearly double by the year 2020 and thus, the incidence of AD and other forms of dementia will likely grow as well. The Alzheimer's Association (2017) estimates by the year 2025, roughly 7.1 million Americans 65 and older will have some form of dementia.

In addition to the growing proportion of the population diagnosed with dementia, another 15-20% of people age 65 and older have been diagnosed with mild cognitive impairment (MCI) (Alzheimer's Association, 2017). People who have MCI are thought to suffer mild, yet measurable changes in their cognitive function that are noticeable to the person affected and often to close friends and family, but not to an extent that fully disrupts daily life. However, people with MCI are more likely to develop AD dementia, and it is estimated that in any given year 15% of all individuals with MCI will progress to AD (Davatzikos et al., 2012). As a result, much research in recent years has focused upon this population in hopes to better identify biomarkers and ultimately treatments for people

suffering from the earliest effects of AD. Such information is critical for accuracy of diagnosis and family/caregiving planning, as well as for future medical intervention and treatment.

Epidemiology

In recent years, researchers have put forth a great deal of effort in identifying risk factors for AD with mixed results. At this time, the largest risk factors are thought to be age, family history, and the presence of apoliprotein E (APOE) ε4 (Alzheimer's Association, 2017). As described in the previous section, the incidence of AD is known to increase with age making older adults more suspectible. In regards to the other two factors, neither is required to develop AD, but having either a family history of AD or a copy of the APOE ε4 gene is known to increase the lifetime risk for developing AD. In terms of family history, people with a sibling or parent with AD (i.e. first degree relative) are more likely to develop the disease, and people with one or more first-degree relatives with AD are at an even higher risk (Alzheimer's Association, 2017). The APOE gene has received much attention in recent years as scientists speculate whether certain combinations of the gene can actually increase or decrease one's risk of AD. Everyone inherits a copy of the gene, in forms ε2, ε3, or ε4, from each parent. At this time, researchers believe the ε4 copy increases one's lifetime risk of developing AD and those with two copies of ε4 are at an even higher risk (Reitz & Mayeux, 2014). Additionally, it is thought that people who have ε4 copies may progress from MCI to AD in a shorter time frame and show signs of cognitive decline faster than those who do not (Aisen et al.,

2017). Conversely, people with copies of the ε2 gene seem to have a lowered lifetime risk though this finding continues to be explored (Alzheimer's Association, 2017).

Other modifiable risk factors for AD are lifestyle factors. It is thought that maintaining a healthy body weight, good cerebrovascular health, a healthy diet, and engaging in both physical and intellectual activity throughout one's lifetime are helpful measures that may reduce one's risk of developing AD (Aisen et al., 2017). Other factors like smoking, having above average blood pressure between the ages of 40-60, developing type 2 diabetes or other metabolic syndromes, or suffering a traumatic brain injury (TBI) may increase the likelihood of AD in one's lifetime (Reitz & Mayeux, 2014).

At this time, most suggested treatment methods for AD fall into two categories: pharmacologic therapies and non-pharmacologic therapies. Unfortunately, the pharmacologic treatments available at this time are symptom modifying, but unable to stop or slow the neuronal damage that occurs in AD. The six drugs currently marketed for AD serve to increase the lifespan of the neurotransmitter, acetylcholine, in the brain by reducing the action of the compound (acetylcholinesterase) designed to stop the neurotransmitter's action. Sadly, the effectiveness of such medications has been variable per individual and is limited in the amount of time that it remains effective (Alzheimer's Association, 2017). Similar to its' pharmacologic alternatives, no direct links have been found between non-pharmacologic therapies and decreasing the risk or severity of AD, but many research studies have found activities such as exercise and cognitive stimulation prove beneficial for those exhibiting symptoms of AD.

Neuropathology

Interestingly, some of the first neuropathological changes in the brain are thought to occur roughly 15 years before the overt onset of cognitive impairment (Alzheimer's Association, 2017; see review Aisen et al., 2017). The two most prominent features that characterize AD dementia are amyloid-beta (Aβ) plaques and neurofibrillary tangles. Generally, it is believed that changes in Aβ deposition occur first and are followed by the build-up of tau pathology resulting in subsequent neurodegeneration over time. Neurodegeneration and atrophy of the affected regions is thought to occur due to the presence of both Aβ plaques and neurofibrillary tangles that disrupt synaptic structures and cause neurons to die (Aisen et al., 2017; Spires-Jones & Hyman, 2014). It should be noted that the presence of Aβ plaques and neurofibrillary tangles alone is not proof of AD as many cognitively normal adults also have the formation of Aβ plaques in their brain as they age despite the absence of dementia (Aisen et al., 2017; Gomez-Isla et al., 1996).

Studies conducted in the past 20-30 years have attempted to further investigate the distribution pattern of how Aβ plaques and neurofibrillary tangles spread throughout the brain (Braak & Braak, 1991). Amyloid deposits are thought to first appear in basal portions of the cortex, before spreading into various cortical association areas with limited involvement of the hippocampus, then lastly into the motor and sensory cortices (Braak & Braak, 1991). As these Aβ deposits accumulate, they form aggregates termed "plaque-like" structures. The neurofibrillary changes that follow Aβ plaque formation are thought to start in the locus coeruleus and then spread to the transentorhinal region, referred to as stages I-II, before spreading further to the limbic regions (stages III-IV) and

lastly into cortical regions (stages V-VI) (Braak & Braak, 1991). The hippocampus and entorhinal cortex are primarily effected in stages III-IV, but initial changes may begin to occur as early as stages I-II. Specifically, the CA1 subfield of the hippocampus is thought to show the earliest changes. Motor regions of the brain remain spared until relatively late in the disease (Pini et al., 2016). Given that Aβ deposits are believed to occur in the earliest phases of the continuum, many researchers believe it is the presence of neurofibrillary tangles that are more directly correlated to symptoms of cognitive decline (Aisen et al., 2017). Specifically, tau deposits in the entorhinal cortex often involve layer II and cause a disruption between this region of the neocortex and the hippocampus. This disconnection hinders the transfer of information from the cortex to the hippocampus and is thought to clinically manifest as episodic memory deficits (Pini et al., 2016). In years to come, it can be expected that biomarkers, such as Aβ plaques and neurofibrillary tangles, will continue to be highly studied with measures such as magnetic resonance imaging (MRI), cerebrospinal fluid (CSF), and amyloid beta and tau position emission tomography (PET) as all forms allow good visibility of brain structures and tissues without being overly invasive. At the same time, it is expected scientists will continue to use these forms of imaging in hopes of detecting other telling biomarkers that have not yet have been discovered (Alzheimer's Association, 2017).

Symptomology

Reasons the treatment and prevention of AD remains so elusive to researchers are because (1) there is no single diagnostic test for AD, (2) the way it presents in individuals differs widely and with a variable time frame and (3) irreversible damage may be present

in the brain by the time the clinical symptoms of AD manifest. The process of diagnosing AD is a lengthy one as it involves a thorough medical and family history, neuropsychological assessment of all cognitive domains, blood tests and imaging, as well as help from a close family member or friend who is able to provide insight about the individual's daily life and behaviors. This extensive information is needed because the clinical diagnosis of AD is based more on exclusionary criteria than inclusionary ones. Thus, there needs to be an impairment of memory as well as an impairment in another cognitive domain (i.e. executive function, attention, language or visuospatial abilities) with no other medical reason for these impairments. Once someone is diagnosed with AD, the time frame in which symptoms escalate can greatly vary depending on the severity of the disease, confounding health factors, age, education, and overall intelligence as some individuals are able to mask symptoms for longer periods of time and maintain seemingly normal daily functioning (Alzheimer's Association, 2017; see review Aisen et al., 2017).

As mentioned, the initial symptoms of AD often vary per individual, but are usually characterized by problems with episodic memory and a rapid rate of forgetting (Alzheimer's Association, 2017; National Institute on Aging, 2017). While some age-related changes in memory are to be expected and even considered a "normal" part of the aging process, changes in memory become a problem when they begin to effect daily life and it is these types of changes that are associated with AD. Such changes include forgetting recently learned information, having to ask for the same information repeatedly, relying on others for memory, or having to write down excessive notes in

order to remember something. Cognitive changes can also be reflected in language, speaking, writing, or completing tasks as people have difficulty finding the "right" word, or struggle to explain the sequence of instructions necessary to complete a task.

Other non-memory impairments can be seen in visuospatial orientation or impaired reasoning and judgement (Alzheimer's Association, 2017). People in early stages of AD often have trouble orienting themselves to less familiar places, confusion regarding time or place, or difficulty gauging distances and other visual problems. Additional trouble with problem solving, impaired reasoning, and judgement can be reflected in poor concentration, tasks taking longer to perform, difficulty handling money, or poor decision making (Alzheimer's Association, 2017).

As the disease progresses, these symptoms are magnified and accompanied by more outward social and personality changes (National Institute on Aging, 2017). People suffering moderate AD often become more withdrawn in social situations, have increased anxiety and aggression, and are more prone to irritability and depression. New compulsive or repetitive behaviors may be noticed as well. Memory loss at this time also worsens, and begins to include more autobiographical facts about one's own life instead of forgetting more trivial information recently learned. The last and final symptoms to present are the most devastating as many are not compatible with life. In the very late stages of AD, people often require around-the-clock care as they can no longer take care of themselves. Often times, people in the most severe stages of AD eventually lose the ability to communicate with others and their bodies lose the ability to fight viruses,

perform physical movement, and even swallow -- all of which can ultimately lead to death (National Institute on Aging, 2017).

INTRODUCTION

The overall prevalence of AD continues to rise as the average human life span grows and thus the global population is aging. In recent years, researchers have come to view AD as a continuum, rather than a sequence of distinct phases of cognitive and neuropathological changes (Aisen et al., 2017). The earliest parts of the continuum are referred to as "preclinical" and individuals are characterized as cognitively asymptomatic regardless of having AD pathology. However, it is recognized many "preclinical" individuals may progress to a symptomatic presentation, and when symptoms, such as episodic memory loss and other cognitive dysfunction, become apparent, this phase is referred to as MCI. In recent years, MCI has been clinically characterized by criteria such as: self- or informant-reported cognitive complaints, objective cognitive impairment, preserved independence in functional abilities, and the absence of dementia (Petersen et al., 2014). As the disease continues to progress, cognitive impairment worsens and functional impairment becomes increasingly apparent in everyday life, and at this point, a person is considered to have AD dementia (Aisen et al., 2017). Thus, with this growing understanding of AD as a continuum, the need to identify biomarkers indicative of the pathophysiological changes that occur prior to cognitive and functional impairment is crucial to develop better diagnostic and treatment techniques.

Morphometric MRI studies have established that the areas of the brain often first damaged in MCI and AD dementia are the hippocampus and the entorhinal cortex (Du et

al., 2001; Killiany et al., 2000; see reviews Pini et al., 2016; Zhou et al., 2016). Aside from their roles in memory, these regions are thought to be some of the first areas to show the impact of aging (Thaker et al., 2017). Amyloid beta (Aβ) accumulates and forms plaques outside of nerve cells, while tau proteins aggregate within neurons and form neurofibrillary tangles that are thought to cause neuronal death and reduced volume in affected regions (Gomez-Isla et al., 1996; see review Spires-Jones & Hyman, 2014). In an effort to obtain more sensitive and specific measures of medial temporal lobe structures, researchers who use structural MRI are encouraging the segmentation of the hippocampus into subfields (De Flores, LaJoie, & Chetelat, 2015, Pini et al., 2016).

To date, numerous studies have found reductions in the volumes of the whole hippocampus, hippocampal subfields, and entorhinal cortex in the brains of MCI and AD patients when compared to control subjects (Mueller et al., 2010; Pennanen et al., 2004; see review De Flores et al., 2015). Such studies often utilize a cross-sectional approach in which they identify previously diagnosed subjects as controls, MCI, or AD based solely upon the characteristics of various regions of interest (ROIs) (Colliot et al., 2008; Du et al., 2001; Hanseeuw et al., 2011; Khan et al., 2015; Mueller et al., 2010, Xu et al., 2000). However, it should be noted that many of these studies largely focus on subjects who already have MCI in order to best predict who with MCI will convert to AD, (Khan et al., 2015; Killiany et al., 2000; Plant et al., 2010; Westman et al., 2011) with less emphasis on creating fit models that can accurately discriminate between control subjects and MCI. Those that do examine these two populations often use the characteristics of only one ROI as a predictor variable, such as the hippocampus, entorhinal cortex, or a specified

hippocampal subfield, and have classification rates that rarely exceed 80% (Colliot et al., 2008; Du et al., 2001; Hanseeuw et al., 2011; Mueller et al., 2010; Pennanen et al., 2004; Westman et al., 2011, see review Weiner et al., 2015). Thus, there remains significant room for models that can predict classification of subjects in earlier stages of the AD continuum with greater accuracy, and for further exploration of regions implicated outside of the hippocampus and entorhinal cortex.

The goal of the present study was to utilize volumetric MRI measures to identify a broader set of variables that would better distinguish controls and amnestic MCI participants using logistic regression. The first step was to systematically determine the utility of using the whole hippocampus, hippocampal subfields, and entorhinal cortex as predictors. Next, less-studied cortical regions outside the medial temporal lobe were added to the model to determine whether any of these regions could improve the model fit and classification accuracy. While these regions are not as commonly used in classification models, researchers have been finding more consistent patterns of atrophy in MCI and AD subjects in surrounding regions of the medial temporal lobe including the frontal, parietal, and temporal lobes (Hangii et al., 2011). Knowledge regarding the discriminatory value of these regions in cognitively normal subjects and those with MCI could serve as valuable information to aid the diagnostic process of MCI and AD dementia at an earlier time point as well as focus treatment therapies.

METHODS

Participants

This study utilized the MRI scans of 96 subjects selected from two sources. Data from 42 subjects were obtained from the Boston University Alzheimer's Disease Center (BU ADC) Clinical Core Registry. The BU ADC is one of 30 centers funded by the National Institute on Aging (NIA) that contributes data to the National Alzheimer's Coordinating Center (NACC). The BU ADC registry, including participant recruitment and inclusion/exclusion criteria, has been described elsewhere (e.g., Ashendorf et al. 2017; Galetta et al., 2017). Subjects' diagnoses were made at multidisciplinary consensus conferences, following presentation and discussion of all history and evaluation results. Subjects were determined to have normal cognition (n=19) if their objective neuropsychological test scores were within the normal range, they had a Clinical Dementia Rating (CDR; Morris, 1993) Global Score of 0.0, and were determined by the consensus panel to be cognitively normal. MCI diagnoses (n=23) followed criteria outlined by Petersen and colleagues (2014). All 23 MCI subjects included in this study were amnestic MCI. Of the 23 MCI subjects, 15 had decreased abilities in one cognitive domain (memory) and 8 were affected in one or more cognitive domains.

Data from the remaining 54 subjects were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (for more information, refer to adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principle Investigator, Michael W. Weiner MD. The primary goal of the ADNI has been to elucidate clinical, genetic, imaging, and biochemical biomarkers of AD and to

better understand the progression from normal cognition to MCI to AD (Weiner et al., 2015). Twenty-seven of these subjects were cognitively normal controls and the other 27 subjects were individuals with amnestic MCI in a single domain. ADNI participants were selected based on utility of a Philips 3T Scanner (to ensure comparable imaging parameters used to collect data from HOPE participants) and criteria that would properly balance the demographic data of the HOPE participants. Procedures conducted in both HOPE and ADNI were approved by local IRBs and participants gave informed consent at the time of their enrollment in both studies.

The study was comprised of 42 males and 54 females. Both HOPE and ADNI collect demographic data including age, education and APOE ε4 status as well as neuropsychological test scores from the Mini-Mental State Exam (MMSE), Geriatric Depression Scale (GDS), Logical Memory recall (modified from the Wechsler D. Wechsler Memory Scale-Revised (WMS-R) San Antonio, Texas: Psychological Corporation; 1987), and Part B of the Trailmaking Test. This data was collected from all participants (Tables 1 and 2).

Table 1. Demographic Data of Control and MCI Groups
p value < 0.01, MCI = Mild Cognitive Impairment, APOE = Apolipoprotein E

(n = 96)	Means for Control Group ± Standard Deviation	Means for MCI Group ± Standard Deviation	*p* value *(* denotes significance)*
Age (in years)	75.24 (8.50)	74.48(6.76)	0.63
Education (in years)	16.02 (2.44)	16.56 (2.57)	0.3
APOE ε4 Status (n=94)	37.78% of Controls have at least 1 ε4 allele	32.65% of MCI have at least 1 ε4 allele	0.87

Table 2. Neuropsychological Test Performance of Control and MCI Groups
p value < 0.01, MCI = Mild Cognitive Impairment, MMSE = Mini Mental State Examination, GDS = Geriatric Depression Scale

	Mean Control Raw Score ± Standard Deviation	Mean MCI Raw Score ± Standard Deviation	*p* value *(* denotes significance)*
Logical Memory Immediate (n = 87)	15.79 (2.97)	10.82 (4.71)	3.99 x E-08*
Logical Memory Delayed (n = 87)	14.86 (3.41)	9.25 (5.04)	1.70 x E-08*
MMSE (n = 96)	29.27 (0.96)	28.00 (1.99)	9.76 x E-05*
GDS (n = 95)	0.78 (1.06)	1.56 (2.45)	0.025
Part B of Trailmaking Test (n = 96, Time to completion in seconds)	70.48 (21.05)	130.24 (82.88)	3.64 x E-06*

Imaging Assessments

In this study, we used the 3D magnetization-prepared rapid acquisition of gradient echo (MPRAGE) sequence scans from all subjects. These scans were acquired on 3T Philips scanners. For the BU ADC scans, a 32-channel headcoil and sense factor of 2 was used with the following imaging parameters: TR = 6.7 ms, TE = 3.1 ms, flip angle = 9°, reconstructed and acquisition voxel size = 0.98 x 0.98 x 1.2 mm, FOV = 250 mm x 250 mm x 180 mm, 150 sagittal slices. Full details of the MRI acquisition parameters used in ADNI have been discussed elsewhere (Jack et al., 2008). All ADNI scans utilized for this study were acquired with an 8-channel headcoil and a sense factor of 1.8 with the following imaging parameters: TR = 6.8 ms, TE= 3.1 ms, flip angle = 9°, reconstructed voxel size = 1.05 mm x 1.05 mm x 1.20 mm, acquisition voxel size = 1.11mm x 1.11mm x 1.20mm, FOV = 270mm x 252 mm x 240 mm, 170 sagittal slices. DICOM scans were downloaded from the ADNI database.

The MRI data from both databases were automatically segmented with Freesurfer v6.0 (http://surfer.nmr.mgh.harvard.edu; for additional details see Desikan et al., 2006; Iglesias et al., 2015). Freesurfer v6.0 utilizes an improved atlas that can automatically segment hippocampal regions into a greater number of subfields than previous versions have allowed (Iglesias et al., 2015).

Statistical Analysis

Independent samples t tests were used to assess whether significant differences existed between the control and MCI groups in terms of demographic factors, neuropsychological outcome measures, and MRI outcome measures. A chi square test

was performed to determine whether there were any significant differences in APOE ε4 status between the groups. To control for the number of comparisons, a Bonferroni corrective value of $p = 0.01$ was used. Volumes generated from Freesurfer v6.0 included estimated total intracranial volume (eTIV), third and fourth ventricle volume, 68 cortical volumes (34 from each hemisphere), 12 subcortical volumes (6 from each hemisphere), the right and left hippocampal volumes, 24 hippocampal subfields (12 in each hemisphere; for additional segmentation details see Iglesias et al., 2015). For a complete list of the variables collected from FreeSurfer v6.0, see Appendix. A subset of the data was visually inspected for errors and upon finding no significant errors, cortical surfaces were not edited. An ANCOVA was performed on the data to determine whether factors such as eTIV, age, gender, education, APOE ε4 status, or study (i.e. HOPE or ADNI) had a significant effect on any volumetric MRI variables. Age and eTIV had a significant effect on majority of the ROI volumes, while gender, education, APOE ε4, and study had no significant impact. In order to correct for age and eTIV, residuals were computed based on the control population data for each of the ROI volumes.

Using these residuals, separate nominal logistic regression models were created to determine how well the volumes of individual regions such as the entorhinal cortex, whole hippocampus, and hippocampal subfields could identify group membership. Subsequently, stepwise variable logistical models (mixed, probability to enter $p < 0.25$) were run using subgroups of ROIs (subcortical, cortical, and hippocampal subfields) to see which ROIs classified participants best. Whole hippocampal formation was included with the subfields and the entorhinal cortex was included with the cortical regions. A

final nominal logistic regression model using a compilation of the significant volumetric MRI variables from all three stepwise analyses was created in order to create an optimal classification model of group membership. Following this analysis, a leave-one-out prediction of the one-out validation technique was conducted to verify the model was transferrable to another data set (Fan et al., 2008; Misra et al., 2009).

RESULTS

Demographic Data

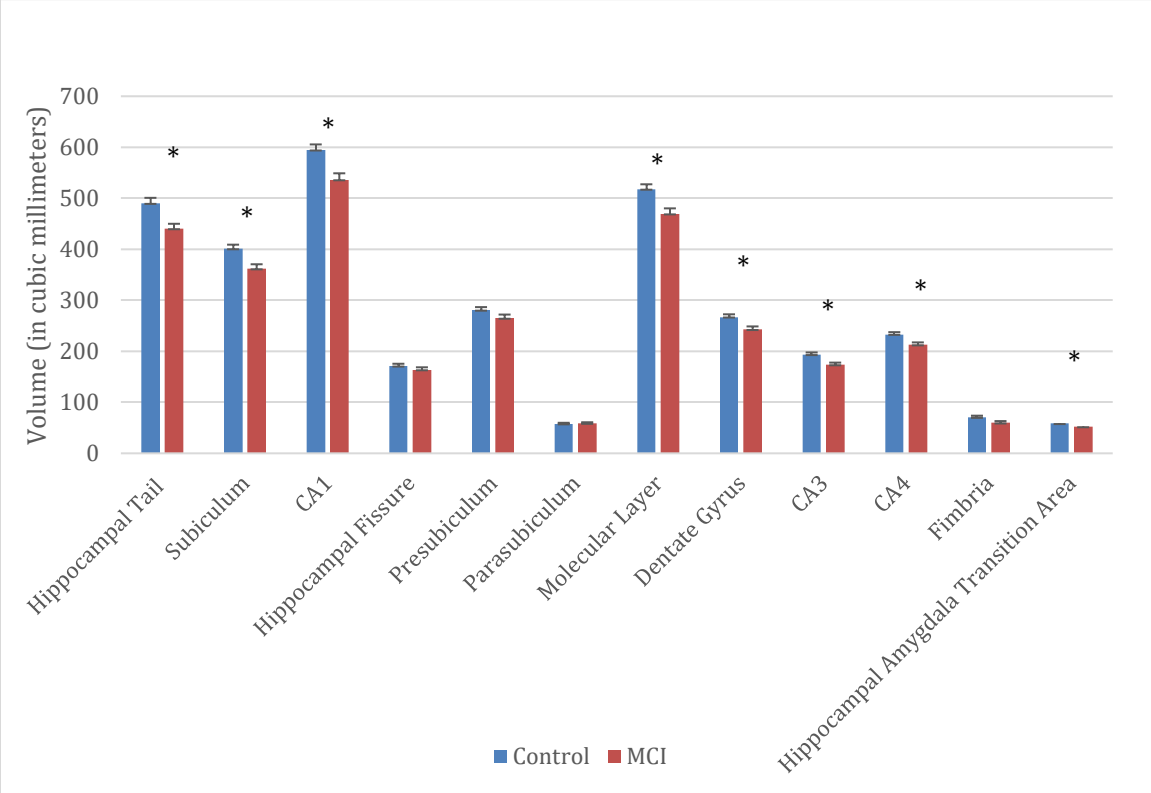
Tables 1 and 2 show the demographic and cognitive data from the control and MCI groups. There were no significant differences between the groups in terms of age, years of education, or APOE ε4 status (*p's* > 0.01*)*. As expected, significant differences were found between the control and MCI groups for MMSE score, Logical Memory Immediate Raw Score, Logical Memory Delayed Raw Score, and Part B of the Trailmaking Test (*p's* < 0.01). There was a trend for the MCI participants to have higher GDS scores ($p = 0.025$) though neither group expressed clinically relevant scores on the GDS.

MRI Data

Significant differences (*p's* < 0.01) observed in uncorrected ROI volumes are illustrated in Figures 1-4. When comparing uncorrected volumes, the MCI group had smaller volumes in 15 of the 24 hippocampal subfields, the right and left whole hippocampal formations, and the right entorhinal cortex, when compared to the control group. In comparing the residual data between the control and MCI groups, the same 15/24 hippocampal subfields, both hippocampal formations, and the right entorhinal

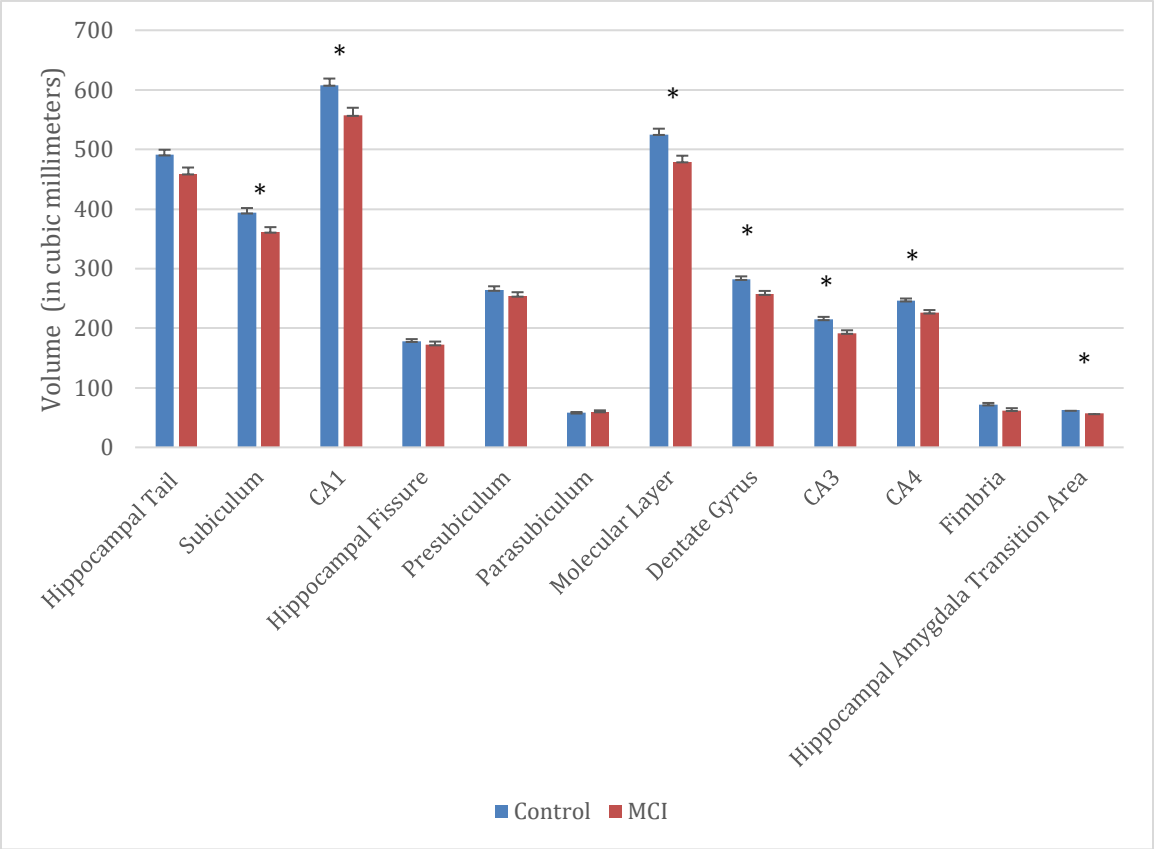
cortex remained significantly smaller with the addition of three additional hippocampal subfields (left fimbria, right hippocampal tail, and right fimbria) and the left entorhinal cortex.

Figure 1. Mean Volume of Left Uncorrected Hippocampal Subfields



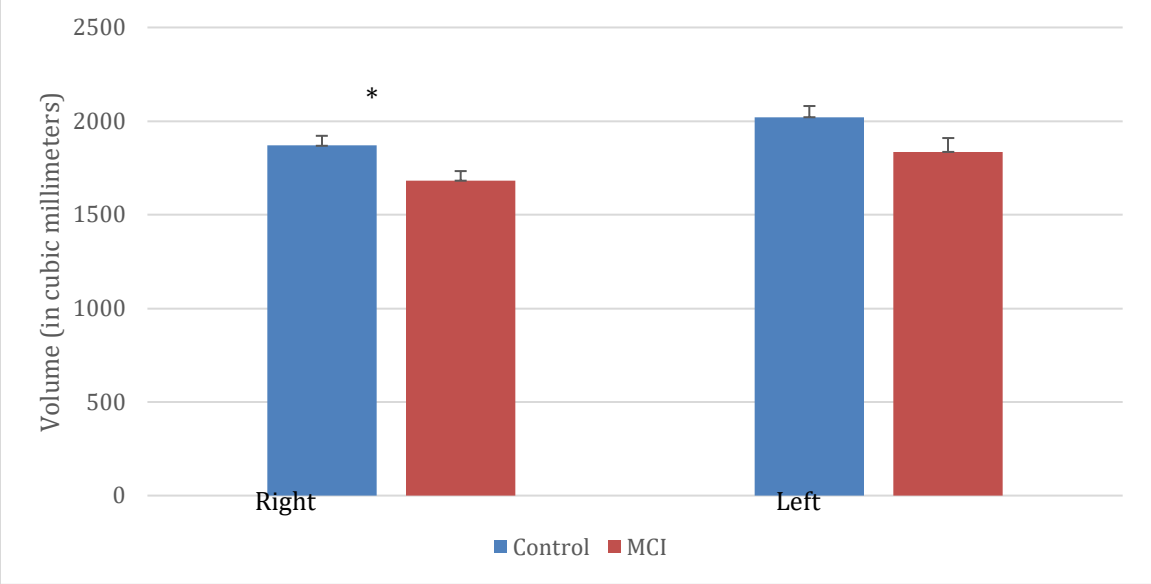
The mean volume of 12 identified hippocampal subfields was measured in control and MCI subjects. Regions showing significant differences ($p < 0.01$) are denoted with a (*) and can be seen in 8 of the 12 subfields of the left hippocampus where the MCI subjects had reduced volume in comparison to the control subjects. Error bars represent standard error of the mean. MCI = Mild Cognitive Impairment, CA = Cornu Ammonis

Figure 2. Mean Volume of Right Uncorrected Hippocampal Subfields



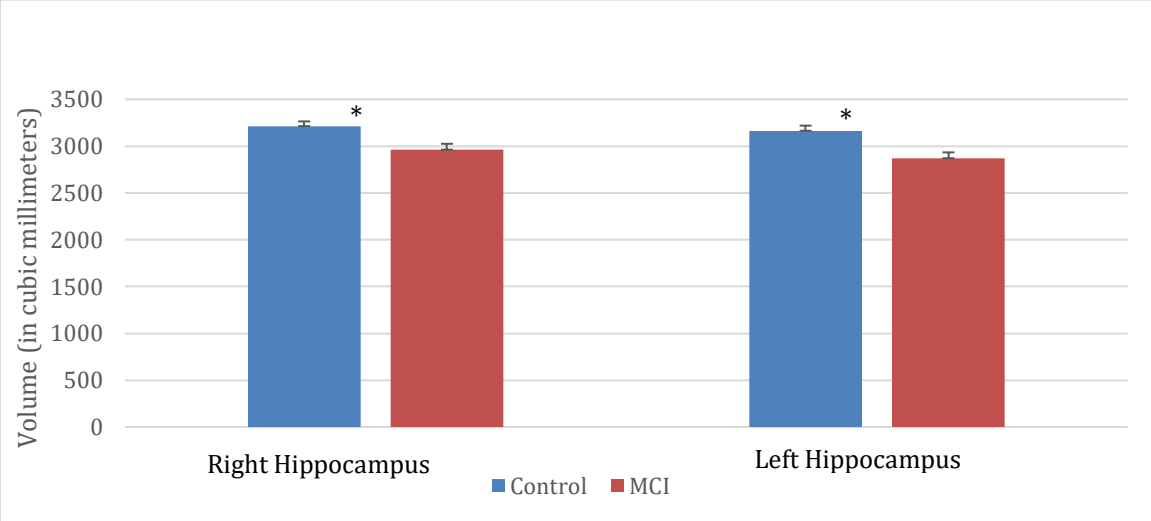
The mean volume of 12 identified hippocampal subfields was measured in control and MCI subjects. Regions showing significant differences ($p < 0.01$) are denoted with a (*) and can be seen in 7 of the 12 subfields of the right hippocampus where MCI subjects had reduced volume in comparison to the control subjects. Error bars represent standard error of the mean. MCI = Mild Cognitive Impairment, CA = Cornu Ammonis

Figure 3. Mean Volume of Uncorrected Entorhinal Cortices



The mean volume of the right and left entorhinal cortices were measured in control and MCI subjects. The right entorhinal cortex in MCI subjects showed a significant reduction in volume compared to control subjects and this loss is indicated by a (*) ($p < 0.01$). Error bars represent standard error of the mean. MCI = Mild Cognitive Impairment

Figure 4. Mean Volume of Uncorrected Whole Hippocampal Formations



The mean volume of the right and left hippocampal formations were measured in control and MCI subjects. Both right and left hippocampal formations in MCI subjects showed a significant reduction in volume compared to control subjects and this loss is indicated by a (*) ($p < 0.01$). Error bars represent standard error of the mean. MCI = Mild Cognitive Impairment

To determine how well the volumes of the entorhinal cortex, hippocampus, and hippocampal subfield identified group membership, these variables were entered into three separate nominal logistic regression models consisting of both the right and left volumes of each region. Though some were significant, none of these models provided a good model fit according to the R^2 values obtained: hippocampal subfields (R^2 = 0.3629, whole model test chi square = 48.2361, p = 0.0024), whole hippocampus (R^2 = 0.1817, whole model test chi square = 24.1557, p < 0.0001), and entorhinal cortex (R^2 = 0.0688, whole model test chi square = 9.1421, p = 0.0103). Thus, subgroup ROI stepwise variable models were conducted. ROIs that were significant in these stepwise variable models (p's <0.01 FDR corrected) were entered into a final nominal logistic regression model which showed that 11 variables were significant (R^2 = 0.7710, whole model test chi square = 102.4794, p < 0.0001) (Table 3). This model had an overall classification rate of 0.9583 (misclassification rate = 0.0417) as 44 out of 46 control participants and 48 out of 50 MCI participants were classified correctly. Surprisingly, entorhinal volume was not one of the factors selected into the final nominal logistic model and when forced into the final model, it did not have a significant effect and was ultimately excluded. Additionally, a leave-one-out prediction of the one-out validation technique was conducted and reached an average classification rate of 0.7742 and drew upon the same top five predictor variables as the original model (Table 3).

Table 3. ROI Predictors of Group Membership
Significant differences ($p < 0.01$) are denoted with a (*). CA = Cornu Ammonis.

Region	*FDR P Value Effect Likelihood Ratio Test (* denotes significance)*
Left Insula	0.0003*
Left Superior Parietal Cortex	0.0003*
Left Rostral Middle Frontal Cortex	0.00039*
Right Middle Temporal Cortex	0.00059*
Right Pars Opercularis	0.00059*
Right Paracentral Lobule	0.00086*
Left Whole Hippocampus	0.00281*
Right Subiculum	0.00281*
Left Superior Temporal Cortex	0.00281*
Right Precentral Cortex	0.00281*
Right Caudal Anterior Cingulate Cortex	0.00281*
Left Putamen	0.01446
Right Pericalcarine Cortex	0.02955
Left Fusiform Cortex	0.04350
Right Parasubiculum	0.10144
Left Hippocampal Amygdala Transition Area	0.14714
Right Thalamus Proper	0.15060
Right CA3	0.18569

DISCUSSION

Demographics

In this study, the groups were well matched in terms of their demographics (i.e. age, education, APOE ε4 status, and gender) (Table 1). Neuropsychological measures revealed expected differences between the control and MCI groups. For example, the control group performed better than the MCI group on four of the five neuropsychological tasks assessed (Logical Memory Immediate Recall, Logical Memory

Delayed Recall, MMSE, and Part B of the Trailmaking Test) (Table 2). A difference in GDS score approached significance ($p = 0.025$) with the MCI group endorsing, on average, 0.75 points higher on this scale. It is feasible this difference is a result of the GDS specifically asking about a decrease in memory, rather than a reflection of true depression symptoms.

MRI

In this study, we found smaller volumes in almost all of the residual ROI volumes examined in the MCI group compared to the control group. Specifically, smaller residual volumes were found in the MCI group in 18 of the 24 hippocampal subfields, bilateral hippocampal formations, and the bilateral entorhinal cortices. These findings are consistent with other reports in the literature *(*Hanseeuw et al., 2011; La Joie et al., 2013; Du et al., 2001; Killiany et al., 2000; Pennanen et al., 2004; see review Zhou et al., 2016).

Hippocampal Subfields

In recent years, attention has shifted from examining whole hippocampal volume to examining the volume of specific hippocampal subfields. Proponents of this shift have argued that since hippocampal subfields are smaller, functionally distinct, and differ in neuroplasticity, they may better differentiate normal aging and the presence of age-related disease (La Joie et al., 2013; Mueller et al., 2010; see review Pini et al., 2016). In terms of the progression of Alzheimer's disease, neurofibrillary tangles in the medial temporal region are initially found in the CA1 region and later are found in the subiculum, CA2, CA3, then the CA4 and dentate gyrus *(*De Flores et al., 2015; Pini et al., 2016*).*

Numerous studies have confirmed that MCI subjects have smaller volumes in the CA1 when compared to controls (La Joie et al., 2013; Mueller et al., 2010; see review De Flores et al., 2015). Further evidence of the CA1 being closely related to memory dysfunction was noted in the case of R.B, a famous neurological patient who suffered from several episodes of ischemia that led to pronounced memory deficits as a result of lesions primarily in the CA1 region of the hippocampus (Zola-Morgan, Squire, & Amaral, 1986). Notably, the present study also found significantly smaller residual volume in the bilateral CA1 subfields in individuals with MCI when compared to control subjects. Likewise, a study done by Khan and colleagues (2015) confirmed smaller volume in the bilateral CA1 subfields as well as the bilateral subiculum and presubiculum in stable MCI subjects when compared to controls. Volume reduction in the subiculum and presubiculum is becoming more widely recognized in MCI while volume loss in the CA2, CA3, and the dentate gyrus is not as consistently observed in MCI (Khan et al., 2015; Li et al., 2016). In the present study, significantly smaller residual volume was found bilaterally in the subiculum, CA3, and dentate gyrus along with other less-studied subfields of the hippocampus (Figures 1 and 2). More longitudinal research is needed on subfields in order to determine the timing of neuropsychological and neuropathological changes in relation to disease progression (De Flores et al., 2015; Pini et al., 2016).

Entorhinal Cortex

Histological studies in the 1990's established that neurofibrillary tangles likely first appear in the entorhinal cortex before progressing to other areas of the medial temporal lobe (Gomez-Isla et al., 1996). These studies suggest the entorhinal cortex is

one of the first regions to experience pathological changes in the progression of cognitive impairment in AD (Pini et al., 2016; Zhou et al., 2016). Several studies have since found smaller entorhinal cortex volumes exist in AD subjects compared to controls, and more recent studies are confirming smaller entorhinal volume in MCI subjects compared to controls (Pennanen et al., 2004). Less work has been done analyzing whether the left or right entorhinal cortex is more suspectible to atrophy, but in studies analyzing both healthy young and elderly adults, the right entorhinal cortex has been found to be larger than the left in both populations (De Toledo-Morrell et al., 2000; Insauti et al., 1998). The study conducted by De Toledo-Morrell and colleagues (2000) also found solely the right entorhinal cortex was significantly reduced in volume in elderly subjects when compared to younger adults. Both these observations have led some researchers to believe the right entorhinal cortex may be more vulnerable to aging and atrophy than the left (Zhou et al., 2016). The present study also found significant differences in the residual data of both the left and right entorhinal cortices of the control group in comparison to the MCI group.

Whole Hippocampus

Researchers heavily study the hippocampus due to its known role in episodic memory, decreases in which are a hallmark sign of cognitive impairment (Mueller et al., 2010; Slavin et al., 2007). It has been established that even in older healthy adults, hippocampal volume decreases with age, making it a region that is susceptible to atrophy (Driscoll et al., 2009; Raz et al., 2004). As with the entorhinal cortex, some debate remains as to whether one hemisphere is more vulnerable than the other, or if both are

equally vulnerable. A study by Slavin and colleagues (2007) found significant decreases in only the left hippocampal formation of amnestic MCI participants in comparison to controls. Other studies, including this one, have found no significant differences between the volumes of the right and left hippocampi (Elshafey et al., 2014), but that the residual volumes of both regions are significantly smaller in MCI subjects when compared to healthy controls (Du et al., 2001; Pluta et al., 2012; Shi et al., 2009).

Nominal Logistic Model of Group Membership

The present study sought to expand current knowledge regarding what regions of the brain are most influential in classifying those who are cognitively normal versus those with amnestic MCI. Some studies have reported the entorhinal cortex to be most effective at discriminating between controls and subjects with cognitive impairment (Killiany et al., 2001; Pennanen et al., 2004). Yet, others have found the entire hippocampal formation or various hippocampal subfield volumes to perform best (Du et al., 2001; Hanseeuw et al., 2011; Mueller et al., 2010; Xu et al., 2000). Regardless, a majority of cross-sectional studies attempting to build similar classification models utilize only one region to classify individuals as controls or MCI and as a result, these studies create models that identify subjects correctly 60-81% of the time, and solely examine the discriminatory value of one region of the brain (Hanseeuw et al., 2011; Mueller et al., 2010; Pennanen et al., 2004). Therefore, some studies are attempting to include multiple ROI volumes in their models to achieve higher classification rates (Colliot et al., 1997; Colliot et al., 2000; Hangii et al., 2011; Killiany et al., 2001).

Initially, the present study utilized hippocampal volume, hippocampal subfield volumes, and entorhinal cortex volume to build three separate classification models and individually, these ROI subgroups performed poorly at classification. However, when other cortical ROIs were added to the classification model, we were able to correctly identify 44 out of 46 control participants and 48 out of 50 MCI participants for an overall classification accuracy of 95.83% with 96% sensitivity and 95.65% specificity. Table 3 shows the 11 volumes that contributed most to this model. Notably, additional cortical regions beyond the canonical medial temporal regions were added to our model in order to see which other structures contribute to identification outside those most commonly studied.

Interestingly, the present study found both whole left hippocampal formation and right subiculum volume to be significant predictors (*p's* = 0.00281*)* in our model. However, similar cross-sectional studies comparing the discriminatory value of hippocampal subfields versus whole hippocampal volumes have found single subfields often better differentiate between control and MCI groups (Hanseeuw et al., 2011; LaJoie et al., 2013, Pluta et al., 2012). Hanseeuw and colleagues (2011) found the subiculum to be a more effective predictor than the whole hippocampus and both La Joie et al. and Pluta et al. found the CA1 to be a stronger predictor than whole hippocampal volume. Regardless, the present study found the left whole hippocampal volume to be selected into the final stepwise variable logistic model while neither the right nor the left CA1 were selected.

Thus far, less research has been done analyzing the influence of cortical structures in classification models for control and MCI populations. However, a three-year study by Killiany and colleagues (2001) found the baseline volume of the caudal portion of the anterior cingulate to be one of the top three predictors for discriminating control subjects, subjects who maintained stable mild cognitive impairment during the duration of the study, and subjects that converted to AD from MCI by the end of the three-year period. In the present study, we also found the caudal portion of the right anterior cingulate (p = 0.00281) to be one of the 11 selected predictors for group membership. Similarly, a study conducted by Hängii and colleagues (2011) found the left superior parietal gyrus to achieve high diagnostic accuracy in identifying controls versus MCI subjects. The present study also found the left superior parietal cortex to be a significant predictor in the classification model (p = 0.00003).

A cross-sectional study conducted by Convit and colleagues (1997) initially built a model that attempted to classify controls and MCI subjects solely based on hippocampal volume and obtained a classification accuracy of 73.4% (Convit et al., 1997). The addition of temporal lobe regions did not improve classification between these two groups, however, future studies conducted by Convit and colleagues continued to include less-studied regions of the temporal lobe into their models. Three years later, Convit and colleagues (2000) found that adding the fusiform gyrus and combined middle and inferior temporal gyrus improved classification rates in identifying who with MCI would further decline to AD (Convit et al., 2000). Notably, the present study also found the right middle temporal gyrus (p = 0.00059) to be a significant predictor in group

membership and the left fusiform gyrus approached significance (p = 0.0435). While it should be noted that these studies investigated group membership of differing cognitive capacities, the overlapping ROIs of significance in both studies may suggest some implications of the middle temporal gyri and the fusiform gyri in the progression of cognitive impairment.

Aside from creating classification models, other studies focus on identifying regions that show the greatest amount of volume loss in MCI subjects compared to controls. In many of these studies, researchers point to regions such as the parietal and lateral temporal lobes as among the first to be implicated in the early stages of AD along with the medial temporal lobe (Desikan et al., 2009; Fan et al., 2008). Interestingly, these studies discuss volume reductions in many of the regions that were chosen to be significant predictors in our classification model. Such include the left insula (p = 0.0003*)*, left superior parietal cortex (p = 0.0003), right middle temporal cortex (p = 0.00059), and right paracentral lobule (p = 0.00086). For example, studies by both Fan and colleagues (2008) as well as Karas and colleagues (2004) reported the insula to be one of the most affected structures in MCI subjects compared to controls. Likewise, the middle temporal gyrus was also reported to have greater atrophy in MCI subjects compared to controls in the study conducted by Fan and colleagues (2008). Therefore, while these studies do not attempt to make classification models like the present study, the reoccurring significance of regions such as the insula and middle temporal cortex may suggest such regions have some role in the progression of cognitive impairment and disease. Further research creating classification models of control and MCI subjects

utilizing both medial temporal regions as well as less-studied cortical regions will likely help clarify which regions in the present study model are truly predictors of cognitive impairment versus characteristics of this particular sample. Additionally, it should be noted that of the 50 amnestic MCI subjects used in this study, 42 were amnestic in a single domain while 8 were amnestic in multiple domains such as memory and language, executive function, visuospatial functioning or a combination of these skills. It is possible the utilization of these eight participants could have influenced the findings of this study. Therefore, future studies creating similar classification models should aim to use solely single domain amnestic subjects to help solidify whether the 11 significant regions reported in this study are true predictors or if these findings reflect a characteristic of this sample.

Limitations

While the best fit model created in this study is promising, there are limitations that must be considered. This study utilized sufficient data to meet the intended goals, but the sample size is nonetheless modest. This was driven by a desire to make optimal use of MCI and control participants from our local ADC population as it more closely resembles a clinical population. We supplemented the subject number using participants from the ADNI study, though we did not want to overwhelm the study with the "clinical trials population" found in ADNI (Petersen et al., 2013). Furthermore, when working with classification models such as nominal logistic regression, the ultimate goal is to build a model using one dataset and then apply it to a parallel dataset. Since we aimed to

investigate regions that are less-studied in MCI, we used all our available subjects to build the model. As such, we realize the potential to be able to refine our findings in a future study as more local participants become available.

Conclusion

The results of the present study confirm many previous findings regarding reduced volume in the hippocampus, entorhinal cortex, and hippocampal subfields in MCI subjects when compared to controls. Additionally, our findings provide further evidence of the value of less-studied regions outside the medial temporal lobe and their ability to aid discrimination models of those who exhibit normal aging and those who do not. We anticipate that future work will continue analyzing which cortical measures in combination with whole hippocampal, hippocampal subfields, and entorhinal cortex volumes contribute most to classification models of group membership. This can provide a basis for assessing disease progression and efficacy of potential therapeutic interventions. Additionally, future studies will likely need to include PET and CSF measures of amyloid and tau along with MRI measures in their statistical models in order to continue improving such models.

APPENDIX

Cortical Volumes (68 total, 34 per hemisphere):

Right Banks of the Superior Temporal Sulcus
Right Caudal Anterior Cingulate
Right Caudal Middle Frontal
Right Cuneus
Right Entorhinal
Right Fusiform
Right Inferior Parietal
Right Inferior Temporal
Right Isthmus Cingulate
Right Lateral Occipital
Right Lateral Orbitofrontal
Right Lingual
Right Medial Orbitofrontal
Right Middle Temporal
Right Parahippocampal
Right Paracentral
Right Pars Opercularis
Right Pars Orbitalis
Right Pars Triangularis
Right Pericalcarine
Right Postcentral
Right Posterior Cingulate
Right Precentral
Right Precuneus
Right Rostral Anterior Cingulate
Right Rostral Middle Frontal
Right Superior Frontal
Right Superior Parietal
Right Superior Temporal
Right Supramarginal
Right Frontal Pole
Right Temporal Pole
Right Transverse Temporal
Right Insula
Left Banks of the Superior Temporal Sulcus
Left Caudal Anterior Cingulate
Left Caudal Middle Frontal
Left Cuneus
Left Entorhinal
Left Fusiform

Left Inferior Parietal
Left Inferior Temporal
Left Isthmus Cingulate
Left Lateral Occipital
Left Lateral Orbitofrontal
Left Lingual
Left Medial Orbitofrontal
Left Middle Temporal
Left Parahippocampal
Left Paracentral
Left Pars Opercularis
Left Pars Orbitalis
Left Pars Triangularis
Left Pericalcarine
Left Postcentral
Left Posterior Cingulate
Left Precentral
Left Precuneus
Left Rostral Anterior Cingulate
Left Rostral Middle Frontal
Left Superior Frontal
Left Superior Parietal
Left Superior Temporal
Left Supramarginal
Left Frontal Pole
Left Temporal Pole
Left Transverse Temporal
Left Insula

Subcortical Volumes (12 total, 6 per hemisphere):

Total Left Lateral Ventricle
Left Thalamus
Left Caudate
Left Putamen
Left Pallidum
Left Amygdala
Total Right Lateral Ventricle
Right Thalamus
Right Caudate
Right Putamen
Right Pallidum
Right Amygdala

Hippocampal Formation (2 total, 1 per hemisphere) and Hippocampal Subfields (24 total, 12 per hemisphere):

Right Hippocampus
Left Hippocampus
Left Hippocampal Tail
Left Subiculum
Left CA1
Left Hippocampal Fissure
Left Presubiculum
Left Parasubiculum
Left Molecular Layer
Left Dentate Gyrus
Left CA3
Left CA4
Left Fimbria
Left Hippocampal Amygdala Transition Area
Right Hippocampal Tail
Right Subiculum
Right CA1
Right Hippocampal Fissure
Right Presubiculum
Right Parasubiculum
Right Molecular Layer
Right Dentate Gyrus
Right CA3
Right CA4
Right Fimbria
Right Hippocampal Amygdala Transition Area

Other (3):

Estimated Intracranial Volume (eTIV)
3rd Ventricle
4th Ventricle

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