UNIVERSITY OF CALIFORNIA, SAN DIEGO

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Assessment of Alzheimer's Disease Risk with Structural and Functional Magnetic Resonance Imaging: An Arterial Spin Labeling Study

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy

in

Clinical Psychology

by

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The Dissertation of Katherine J. Bangen is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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ABSTRACT OF THE DISSERTATION

Assessment of Alzheimer's Disease Risk with Structural and Functional Magnetic Resonance Imaging: An Arterial Spin Labeling Study

by

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Doctor of Philosophy in Clinical Psychology

University of California, San Diego, 2010
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BACKGROUND: There are several risk factors for the development of Alzheimer's disease (AD) including the apolipoprotein E (APOE) ε4 allele, an important susceptibility gene for AD, and mild cognitive impairment (MCI). The literature to date generally indicates that nondemented older adults at risk for AD by virtue of their cognitive (i.e., MCI) and/or genetic (i.e., APOE) status demonstrate reduced medial temporal lobe (MTL) volumes and divergent brain response patterns during memory encoding relative to their counterparts not at risk.

METHODS: We used arterial spin labeling (ASL) functional magnetic resonance imaging (FMRI) to examine the influence of AD risk on functional brain responses to memory encoding. Participants were 43 individuals aged 60 or older. Twenty-nine individuals were classified as cognitively normal and 14 met criteria for MCI. Twenty individuals were APOE ε4 carriers whereas 23 were non-ε4 carriers. The risk groups were equivalent in terms of mean age, mean years of education, gender distribution, vascular risk, or medial temporal lobe (MTL) volumes.

RESULTS: Individuals at genetic risk for AD by virtue of the presence of at least one APOE ε4 allele demonstrated increased MTL resting state CBF relative to their non-ε4 counterparts. In contrast, individuals characterized as MCI showed decreased MTL resting state CBF relative to their cognitively normal peers. There was a trend toward a cognitive status by genotype interaction for percent change CBF. In the cognitively normal group there was no difference in percent change CBF based on APOE genotype. In contrast, in the MCI group, APOE ε4 carriers demonstrated significantly greater activation relative to non-ε4 carriers.

CONCLUSIONS: Our findings provide support for the notion that individuals at risk for AD demonstrate changes in brain function in the preclinical period prior to the onset of dementia. Further, our results suggest that abnormal resting state CBF and FMRI response pattern to memory encoding may be early indicators of brain dysfunction in individuals at risk for developing AD and, therefore, ASL MRI may provide a sensitive technique for identifying individuals at risk, monitoring changes in

neural activity due to developing AD neuropathology, and assessing effectiveness of disease-modifying treatments.

Assessment of Alzheimer's Disease Risk with Structural and Functional Magnetic Resonance Imaging: An Arterial Spin Labeling Study

INTRODUCTION

Overview of Alzheimer's disease

Alzheimer's disease (AD) is the most common cause of dementia in adults over the age of 65 in the United States (Evans, 1990) and is a growing public health concern due to the increasing longevity of the U.S. population. It is estimated that the prevalence of AD in the U.S. will increase from its current estimate of 5 million people to approximately 11 – 16 million people by 2050 (Alzheimer's Association, 2008). AD is a debilitating disease and has devastating psychological and financial effects on patients, caregivers, families, and society. It is necessary to reliably identify individuals in the preclinical phase in order to treat them during a period that will have the largest impact in preserving cognitive functioning and quality of life (Solomon & Murphy, 2005).

AD is an age-associated, progressive neurodegenerative disorder characterized by cognitive decline, behavioral disturbances, and the neuropathologic presence of neuritic plaques and neurofibrillary tangles (Katzman, 1986). Early cognitive deficits involve memory (Salmon & Bondi, 1999), an important topic of study due to its necessary presence in AD diagnosis and its significant impact on functioning. Consistent with the early appearance of memory impairment, histological (Braak &

Braak, 1991) and neuroimaging studies (Jack, Petersen, O'Brien, & Tangalos, 1992) indicate that the neuropathology associated with AD begins in the medial temporal lobe (MTL; Braak & Braak, 1991). The neuropathology gradually becomes more widespread, affecting neocortical association areas (Braak, Braak, Bohl, & Bratzke, 1998) and resulting in deficits in multiple cognitive domains. A more thorough understanding of the profile of neuropsychological impairments linked to early AD has lead to earlier and more reliable diagnosis of AD (Salmon et al., 2002) as well as an improvement in the ability to identify individuals in a prodromal phase of the disease (Bondi, Salmon, Galasko, Thomas, & Thal, 1999; Bondi et al., 1995; Jacobson, Delis, Bondi, & Salmon, 2002; Lange et al., 2002; Mickes et al., 2007).

Although AD was first described a century ago, there is still no laboratory test for a definitive biomarker. Rather diagnosis is based on clinical and radiological features and not confirmed until autopsy verifies the presence of AD neuropathology (Kamboh, 2004). There are several non-genetic risk factors for AD including advancing age, female gender, poor education, low lifetime occupational attainment, and cerebrovascular disease. The greatest risk factor is increasing age (Kamboh, 2004) and the prevalence of AD appears to rise in an exponential fashion between the ages of 60 and 90. In fact, Roses (1995) suggests that all individuals would develop AD if they lived to be 140. The causative link between AD and increasing age is unclear. Van Leeuwen and colleagues (2000) proposed that during aging errors in protein synthesis occur and result in the generation and accumulation of abnormal proteins. Taken together, the literature suggests that risk for AD results from interactions

between genetic and environmental factors throughout the lifespan (Borenstein, Copenhaver, & Mortimer, 2006).

Nondemented older adults who subsequently develop AD often demonstrate subtle prodromal memory changes in the years prior to diagnosis (Bondi et al., 1994). Recent evidence indicates that cognitive domains in addition to episodic memory may decline during this prodromal period (see Twamley, Ropacki, & Bondi, 2006, for review). Consistent with the progression of neuropathologic changes in AD, Mickes et al. (2007) have shown that cognitive abilities mediated by the medial and lateral temporal lobes (episodic memory and semantic knowledge, respectively) may be more impaired in prodromal AD than abilities subserved by frontal regions (executive function).

One approach to examining potential cognitive and biomarkers of AD during its prodromal phase involves comparing neuropsychological performance and neuroimaging-based findings between nondemented older adults who have an increased risk for developing AD as a result of their cognitive or genetic status and their counterparts who are not at risk. This approach is predicated on the notion that the group of participants with the risk factor will likely contain more individuals in the prodromal phase of the disease compared to those without the risk factor.

Overview of mild cognitive impairment

Mild cognitive impairment (MCI) was originally conceived to represent a transitional state between normal aging and AD (Petersen et al., 1995). Individuals with MCI perform more poorly on cognitive measures with sensitivity to AD but do

not meet the diagnostic criteria for dementia (Petersen et al., 2001). In fact, individuals with MCI often perform at a level midway between cognitively normal older adults and mild AD patients on measures including the Mini-Mental State Examination (MMSE), Dementia Rating Scale (DRS), and Global Deterioration Scale (GDS; Petersen, 2004). Individuals with MCI and other at-risk groups have generated much research and clinical interest. There is hope that, as new treatments are developed, these individuals can be identified earlier and targeted for treatment in order to prevent or slow progression to AD.

Individuals with MCI convert to AD at a higher rate than cognitively normal adults (Petersen et al., 1999; Tierney et al., 1996). A published study of 220 individuals with a mean age of 79 reported that MCI individuals progressed to dementia at a rate of roughly 12% per year compared to 1-2% per year in normal older adults (Petersen, 2004). However, previously reported rates of progression have varied widely from 1 to 72% (Tuokko & McDowell, 2006) and estimates of instability of the diagnosis (i.e., percentage of individuals who are characterized as MCI but then revert back to normalcy at re-assessment) has ranged from 10-40% (Bickel, Mosch, Seigerschmidt, Siemen, & Forstl, 2006; Petersen, 2004). Variability in conversion rates and reported instability of the diagnosis may be related to differences across studies regarding source of participants (i.e., clinic versus population based), diagnostic criteria employed, strategies implemented (e.g., normal reference standards of optimally healthy older adults, normal young adults, etc.), and length of follow-up. Nevertheless, published reports indicate that one factor that predicts a more rapid

progression from MCI to dementia is the presence of an apolipoprotein E (APOE) ε4 allele (Petersen et al., 1995). Tierney and colleagues (1996) reported that the presence of an APOE ε4 allele was a reliable predictor of conversion from MCI to AD only when combined with performance on memory tests. Similarly, other groups suggest that neuropsychological tests measuring delayed recall and executive functions in particular may be better predictors of conversion to dementia than performance on tests assessing other cognitive abilities (Amieva et al., 2004; Chen et al., 2000; Tabert et al., 2006).

Currently, there is no consensus regarding a single set of diagnostic criteria for MCI. Historically, an MCI patient was one who typically had memory impairment beyond that expected for age but was relatively intact in other cognitive domains and did not have appreciable declines in daily activities. Specific diagnostic criteria included memory complaint corroborated by an informant, objective memory impairment for age, preserved general cognitive abilities, essentially intact functional activities, and no dementia (Petersen, et al., 2001). However, with the explosion of MCI-related research, it became evident that several subtypes may exist and, thus, the construct was expanded to include other types of cognitive impairment in addition to memory (Petersen, 2004). Specifically, Petersen and Morris (2005) proposed four clinical subtypes of MCI: (1) *amnestic MCI-single domain* characterized by isolated impairment in memory (amnesia), (2) *non-amnestic MCI-single domain* involving impairment in a cognitive domain other than memory (e.g., language, executive function, visuospatial skills), (3) *amnestic MCI-multiple domain* involving impairment

in memory and at least one other cognitive domain, and (4) *non-amnestic MCI-multiple domain* characterized by impairment in two or more non-memory cognitive domains.

The distinction between amnestic and non-amnestic MCI subtypes is relevant when considering clinical outcome given recent evidence suggesting that the former group is more likely to progress to AD (Busse, Hensel, Guhne, Angermeyer, & Riedel-Heller, 2006; Petersen et al., 2004) whereas individuals from the latter group are more likely to progress to non-AD types of dementia (e.g., dementia with Lewy bodies; Boeve et al., 2004). However, Storandt, Grant, Miller, and Morris (2006) have shown that memory need not be the first cognitive function affected in MCI patients who progress to AD. Also, another study reported that individuals with amnestic MCI developed both AD and non-AD forms of dementia and many individuals developed AD from non-amnestic MCI (Fischer et al., 2007). Such findings emphasize the heterogeneous nature of MCI.

Despite the proposal of diagnostic schemes for MCI, there are still many challenges related to assessment and diagnosis. This may be due, in part, to the fact that the standards of cognitive changes in normal aging are often unclear. For instance, in normal aging there is commonly some memory decline and, as a result, the delineation between normal aging and early MCI requires clinical judgment (Petersen, 2004). In addition, individuals with MCI frequently show very minor functional impairment and many clinicians have difficulty distinguishing this from such impairment that occurs in normal aging (Petersen, 2004). An important part of the

criteria involves the clinician's judgment as to whether an individual meets criteria for dementia, which is challenging given that there are no established criteria regarding the degree of functional impairment necessary to constitute a dementia diagnosis (Petersen, 2004). Furthermore, a lack of consensus regarding the objective identification of impairment (i.e., types of cognitive tests and cutoff scores that should be used; Luis, Loewenstein, Acevedo, Barker, & Duara, 2003) has likely played a role in the variability across studies regarding conversion rates from MCI to dementia and the instability of the diagnosis. Petersen and colleagues (1999) recommended a cutoff score of 1.5 or greater standard deviations below age and education-adjusted means for defining mild impairment across cognitive domains, however, other groups have utilized cutoffs ranging from 1 standard deviation (Busse et al., 2006) to 1.96 standard deviations (Bickel et al., 2006) below the mean to operationalize or define impairment.

In light of these issues, Jak and colleagues (2009) compared five diagnostic strategies, each of which varied "the cutoff for objective impairment as well as the number of neuropsychological tests considered in the diagnostic process" and quantified the variability in diagnostic outcomes. Ninety nondemented older adults were initially assessed and 72 were seen for follow-up approximately 17 months later. Based on a comprehensive neuropsychological battery and consensus diagnosis, participants were characterized as either neuropsychological normal or MCI. The "historical" criteria operationalized the original criteria proposed by Petersen and colleagues (1999). These criteria required that global cognitive functioning be intact and memory on one measure (Logical Memory) fall 1.5 standard deviations below the

mean. The "typical" criteria were adapted from the scheme proposed by Petersen and Morris (2005). This required one test within a cognitive domain fall 1.5 standard deviations below the mean. The "comprehensive" criteria utilized multiple tests in multiple domains and were similar to the typical criteria approach. However, in an effort to "strike a balance between reliability and sensitivity to detect mild impairment" a lower cutoff score was used to identify impairment (Heaton, Grant, & Matthews, 1991; Heaton, Miller, Taylor, & Grant, 2004). These criteria required two tests in a single domain to be greater than 1 standard deviation below published norms.

Based on the "comprehensive" criteria, four clinical subtypes of MCI were proposed: (1) *single domain amnestic MCI* requires performance on at least two memory measures fall more than 1 standard deviation below age appropriate norms, (2) *single domain non-amnestic MCI* requires performance on at least two measures in one non-memory domain to fall more than 1 standard deviation below age appropriate norms, (3) *multiple domain amnestic MCI* relies on performance on at least two measures in both memory and at least one nonmemory domain to fall more than 1 standard deviation below age appropriate norms or performance on at least one measure each in memory and at least two other non-memory domains to fall more than 1 standard deviation below age appropriate norms, and (4) *multiple domain-amnestic MCI* relies on performance on at least two measures in two non-memory domains be greater than 1 standard deviation below age appropriate norms or performance on one measure within each of three or more non-memory domains to fall at least 1 standard deviation below age appropriate norms. In order to cover the range of possible

diagnostic schemes, a "liberal" criteria and "conservative" criteria were also assessed. The "liberal" criteria rely on one test within any cognitive domain to be at least 1 standard deviation below age appropriate norms and the "conservative" criteria require two tests within a domain to be at least 1.5 standard deviations below normative data.

Notably, Jak and colleagues (2009) reported significant differences in the percentages of individuals classified as MCI versus neuropsychologically normal depending on the diagnostic criteria used. However, providing support for the validity of MCI as a construct, most diagnoses were consistent across diagnostic schemes and remained stable over the follow-up period. Although the historical criteria approach provided the greatest stability over the follow-up period, it likely missed a substantial number of individuals with both amnestic and non-amnestic MCI. Individuals with amnestic MCI may have been missed by requiring only paragraph recall with a 1.5 standard deviations cutoff to identify memory impairment and lack of inspection of other memory tests. Individuals with non-amnestic MCI may have been missed because the criteria did not consider or require detailed assessment of cognitive domains beyond the sole memory test despite evidence that the development of AD need not necessarily begin with memory impairment (Storandt et al., 2006).

Jak and colleagues (2009) noted that "the comprehensive criteria were developed in consideration of the statistical maxim that multiple measures tend to provide a more reliable estimate of a cognitive construct than a single measure (Anastasi & Urbina, 1997)." Moreover, Heaton and colleagues (1991, 2004) reported that on the Halstead-Retain neuropsychological battery the majority of neurologically

normal adults will score within the impaired range on at least one measure. In addition, they found that a cutoff score of 1 SD below the normative mean on a summary score provided the best balance between sensitivity (80%) and specificity (88%). Providing further support for the importance of using a comprehensive battery with multiple measures assessing each domain, Palmer, Boone, Lesser, & Wohl (1998) found that a minority of healthy older adults (approximately 20%) obtained one impaired score in two different cognitive domains but fewer (5% or less) earned two or more impaired scores within the same domain. These findings point to the potential difficulties with interpreting an isolated impaired score. Moreover, a recent neuroimaging study reported brain-based empirical support for the comprehensive criteria in the form of hippocampal volume changes (Jak, Houston, Corey-Bloom, Nagel, & Bondi, 2007).

To summarize, MCI historically represents a transitional state between normal aging and AD. Individuals with MCI perform more poorly on cognitive measures with sensitivity to AD but do not meet the diagnostic criteria for dementia (Petersen et al., 1995) and they convert to AD at a higher rate than cognitively normal older adults (Petersen & Morris, 2003). Research findings have emphasized the heterogeneous nature of MCI and, thus, the construct has been further refined to include four subtypes. Despite advances in MCI-related research, controversy persists regarding diagnostic criteria and the sensitivity of neuropsychological measures and cutoff scores to detect MCI.

Overview of genetically-based risk for Alzheimer's disease

In addition to advancing age and MCI status, the APOE[1] ε4 allele is a risk factor for AD (Corder et al., 1993; Saunders et al., 1993). ApoE is an extracellular cholesterol transport protein abundant in cells in the brain. The human APOE gene is located on chromosome 19 and encodes for the apoE protein. There are three common protein isoforms (apoE2, apoE3, apoE4), which are coded by three alleles (ε2, ε3, and ε4). The three alleles produce six possible APOE genotypes for humans: ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4.

Menzel, Kladetzky, and Assmann (1983) published the largest of the European studies involving 2,000 subjects participating in cardiovascular investigations and reported relative allele frequencies of ε3 = 0.77 > ε4 = 0.15 > ε2 = 0.08. The distribution of APOE allele frequency varies across different ethnic groups and geographic locations (Gerdes, Klausen, Sihm, & Faergeman, 1992; Hallman et al., 1991). These variations in allele frequency raise the question as to whether APOE ε4 plays the same role as a risk factor for AD across different ethnic and geographic groups, although several groups have reported no interaction of ethnicity and APOE genotype (e.g., Blair et al., 2005; Fillenbaum et al., 2001; Jorm et al., 2004).

Although there is variability among findings, it appears that approximately 40% of AD cases are associated with the APOE ε4 allele (Saunders et al., 1993), making it an important susceptibility gene for AD (Katzman, 1994). The presence of an APOE ε4 allele roughly doubles the lifetime risk of developing AD (e.g., Mayeux et al., 1998). A meta-analytic study reported a gene dose effect in which the risk of developing AD is increased three- to fourfold for those who are heterozygous APOE

[1] APOE indicates the apolipoprotein E gene. ApoE indicates the protein.

ε4 carriers and approximately ten- to twelvefold for homozygous APOE ε4 carriers (Farrer et al., 1997). AD cases can be classified into early-onset, which presents before age 60 and involves 1% of cases, or late-onset AD (LOAD), occurring after age 60 (Rocca et al., 1991). The APOE ε4 allele is associated with LOAD (Saunders et al., 1993). With each APOE ε4 allele, risk of developing AD increases and age at disease onset decreases. In contrast, presence of the APOE ε2 allele decreases AD risk and increases age at onset (Corder et al., 1994).

Regarding proposed mechanisms by which apoE may influence neuropathological processes, in the brain apoE plays an important role in lipid transport and in functions related to cell maintenance and repair (Blackman, Worley, & Strittmatter, 2005; Lomnitski, Oron, Sklan, & Michaelson, 1999; Pitas, Boyles, Lee, Hui, D., & Weisgraber, 1987). Evidence from animal models suggests that apoE plays a role in development, remodeling, and regeneration of the CNS (Boyles, Notterpek, & Anderson, 1990) through distributing lipids, repairing injured neurons, maintaining connections between synapses and dendrites, and removing toxins (Mahley, Weisgraber, & Huang, 2006). The various isoforms differ in their abilities to carry out these tasks. Moreover, apoE4 has been linked to various neuropathological processes (Mahley et al., 2006) and several mechanisms of action have been proposed. Two proposed pathways have been called the "Amyloid Hypothesis" and the "Neuronal Maintenance/Repair Hypothesis" (Mahley et al., 2006). In the first pathway, apoE4 is linked to decreased β-amyloid clearance and increased β-amyloid deposition leading to the formation of plaques (Wisniewski, Castano, Golabek, Vogel, T. & Frangione,

1994), increases in β-amyloid production (Ye, et al., 2005), and enhanced β-amyloid-induced lysosomal leakage and apoptosis (Ji et al., 2006). In addition to neurofibrillary tangles, β-amyloid plaques are the hallmark neuropathological feature of AD. The second pathway is independent of β-amyloid and related to inefficient responses to CNS stressors (e.g., oxidative stress, ischemia, inflammation) that can result in neuronal death (Mahley & Huang, 2006; Mahley et al., 2006). With advancing age, neurons require repair to maintain connections between synapses and dendrites. Given its role in lipid transport, apoE plays an important role in these functions and apoeE3 and apoE2 are effective in carrying out such tasks while apoeE4 is less so (Mahley et al., 2006). In both proposed pathways, it is purported that several factors interact to lead to neurodegeneration and cognitive decline.

Regarding apoE's effects on cognition, the literature is somewhat inconsistent regarding the APOE ε4 allele as a risk factor for cognitive decline in normal aging. A recently published study of 6,560 participants stratified across three age groups (20-24, 40-44, and 60-64) found no association between APOE genotype and cognitive functioning (Jorm et al., 2007). In contrast, a meta-analytic study (Small, Rosnick, Fratiglioni, & Backman, 2004) of 38 empirical, cross-sectional studies with over 20,000 cognitively normal older adults reported that APOE ε4 carriers performed significantly more poorly relative to non-ε4 carriers on measures of global cognitive functioning, episodic memory, and executive functioning. In contrast, there were no group differences observed for verbal ability, perceptual speed, primary memory, attention, or visuospatial skills.

Two longitudinal studies that both followed participants for over 25 years corroborated the findings of the meta-analysis of Small and colleagues (2004). A twin study in its 27th year (Haan, Shemanski, Jagust, Manolio, & Kuller, 1999) and a community based study in its 30th year of follow-up (Mortensen & Hogh, 2001) both reported greater cognitive decline in ε4 carriers. The latter study reported that the presence of an APOE ε4 allele was associated with increased risk for cognitive decline in women only. Similarly, a longitudinal study of 5,804 older adults aged 70 to 82 reported that APOE ε4 carriers demonstrated poorer memory performance at baseline as well as greater decline over a mean of 3.2-year follow-up on measures of immediate and delayed memory, and that the decline was larger for ε4 homozygotes relative to heterozygotes (Packard et al., 2007).

Published studies reporting APOE ε4-related decline in cognitive function among cognitively normal older adults have interpreted such findings in divergent ways. For instance, Savitz, Solms, and Rameasar (2006) noted that normal aging and AD are associated with different patterns of neurocognitive changes and, thus, "accelerated aging is unlikely to account for the pattern of deficits observed in nondemented ε4 allele carriers." The authors argued that findings of APOE ε4 allele-related cognitive decline in nondemented older adults are likely due to inclusion of a great number of individuals with incipient AD in the APOE ε4 allele group.

Bondi and colleagues (1999) directly tested this hypothesis by conducting two sets of statistical analyses comparing neuropsychological performance between nondemented older adults with and without an ε4 allele. The first set of analyses

included all participants and the second set excluded individuals who had preclinical dementia. When the participants with preclinical dementia were excluded, there were no longer any differences in cognition between the ε4 and non-ε4 groups. The authors concluded that apparent differences in cognition based on APOE genotype are more likely the result of inclusion of a greater number of individuals with incipient dementia within the APOE ε4 rather than a cognitive phenotype of APOE. These findings were corroborated in a large study of 415 healthy individuals spanning ages 6 to 65 in which there were no APOE ε4 decrements in cognition across many different domains, nor were there any decrements in dynamic neural activity for ε4 subjects relative to ε2 or ε3 groups (Alexander et al., 2007). However, other groups have also attempted to test an APOE phenotype hypothesis and reported results contrasting those of Bondi and colleagues (1999). For instance, Schultz and colleagues (2008) compared APOE ε4 carriers and non-ε4 carriers who were younger (i.e., in their 50s) and evidenced no signs of preclinical dementia. The authors reported that the ε4 allele was associated with poorer verbal but not visuospatial memory performance, greater cognitive asymmetry, or more memory-related complaints when participants were in the mid-50s and before the development of prodromal dementia. In addition, Bretsky, Guralnik, Launer, Albert, and Seeman (2003) demonstrated that APOE ε4 allele is a risk factor for cognitive decline even in older adults in their 70s who were characterized as "high functioning" during a baseline evaluation thereby decreasing the likelihood that the included any individuals with incipient dementia. These "high

functioning" participants exhibited decline in memory, naming, and constructional praxis at a seven-year follow-up evaluation.

Episodic memory decline is one of the most salient markers of preclinical AD, although mild asymmetric cognitive decline may also detect individuals in the preclinical phase. Jacobson and colleagues (2002) reported that the initial presentation of cognitive deficits in AD may have asymmetrical involvement as a common feature (e.g., language impairments greater than visuospatial impairments). The authors derived measures of asymmetric cognitive profiles by using difference scores on tests of verbal and visuospatial skills. Although mean score analyses between the groups revealed similar performance on the individual cognitive tests, the use of difference scores measuring asymmetric cognitive performance revealed consistent evidence of subtle differences in cognition in a subgroup of preclinical AD patients. Specifically, the preclinical AD participants demonstrated significantly greater discrepancies between naming and visuoconstruction skills relative to matched control participants, and a higher frequency of asymmetric cognitive profiles compared to a larger normative group. Further studies have reported such cognitive discrepancies on tests of auditory and spatial attention (Jacobson et al., 2005), verbal and design fluency (Houston et al. 2005), global versus local item processing (Jacobson et al. 2005), response inhibition and cognitive flexibility (Wetter et al. 2005), and heterogeneity in verbal memory (Wetter et al. 2006). In addition, Fine and colleagues (2008) examined 24 normal-functioning older adults, with 16 showing no change in global cognition over a one-year period and 8 showing decline. In the year prior to their decline, this

group of individuals exhibited a greater degree of cognitive discrepancy between higher-level executive functions vs. fundamental skills (i.e., response inhibition/switching vs. basic color naming and word reading) than matched control participants who remained cognitively stable over the same period. Taken together, this line of research suggests that cognitive discrepancy measures not only appear to be a useful method for identifying individuals at risk for cognitive deficits, but they also show promise in predicting those who decline.

In sum, the APOE ε4 allele is a robust risk factor for AD and there is evidence of a gene-dose effect in which, with each APOE ε4 allele, the risk of developing AD increases and age at disease onset decreases. ApoE4 has been linked to various neuropathological processes that may lead to neurodegeneration and cognitive decline (Mahley et al., 2006). In general, published reports suggest that APOE ε4 carriers perform significantly more poorly relative to non-ε4 carriers on measures of cognitive functioning and the most commonly observed impairments are in the domains of episodic memory and executive functioning. Given that poorer performance in APOE ε4 carriers relative to non-ε4 carriers has been reported in middle age and in individuals carefully screened for evidence of cognitive impairment, Schultz and colleagues (2008) suggest that it is unlikely that their findings are simply due to inclusion of ε4 carriers with incipient dementia, although other findings contrast with this notion (Bondi et al., 1999; Alexander et al., 2007). Ghebremedhin, Schultz, Braak, and Braak (1998) report that the APOE ε4 allele promotes the accumulation of neurofibrillary tangles as early as one's 20-40s, suggesting that the neuropathology of

AD may begin decades prior to disease. Variables that have been proposed to influence the association between APOE genotype and cognition include age, APOE ε4 zygosity, APOE ε2 zygosity, (Small et al., 2004) and gender (Mortensen & Hogh, 2001).

Volumetric changes in Alzheimer's disease, mild cognitive impairment, and apolipoprotein E ε4 carriers

Many structural neuroimaging studies of AD patients and individuals at risk for AD have focused on the hippocampus and other related MTL structures as these regions are necessary for the acquisition and retention of new information (Squire, 1992) and are the initial areas affected in AD (Braak & Braak, 1991). Decreased MTL volumes have been consistently reported in individuals with both AD (e.g., Jack et al., 1992) and MCI (e.g., Schott, Kennedy, & Fox, 2006). For instance, Schott and colleagues (2006) reported that participants with MCI demonstrated atrophy of the entorhinal and hippocampo-amygdala regions that was intermediate between normal elderly and AD patients. Moreover, Rusinek and colleagues (2003) reported that MTL atrophy was the best predictor of conversion from MCI to AD and had a specificity of 91% and sensitivity of 85%. Notably, another study demonstrated that the different subtypes of MCI are associated with different types of structural abnormalities. Although both amnestic MCI and multiple domain MCI evidenced MTL and cortical volume loss, individuals with amnestic MCI evidenced greater involvement of the MTL and less involvement of neocortical areas relative to those with multiple domain MCI (Bell-McGinty et al., 2005).

Results from studies examining the association between APOE genotype and MTL volumes have been more equivocal. Several groups have found that cognitively normal older adults with the APOE ε4 allele have reduced hippocampal volume relative to their non-ε4 counterparts (Cohen, Small, Lalonde, Friz, & Sunderland, 2001; den Heijer et al., 2002; Soininen et al., 1995). However, other studies have failed to reveal an effect of APOE genotype on volumetry (e.g., Jernigan, et al., 2001). A recent study involving both cross-sectional and longitudinal components reported that there was no effect of APOE genotype on hippocampal volume when initially examined cross-sectionally. In contrast, APOE ε4 was linked to greater reduction in hippocampal volume over time in the sample of 34 older adults (Jak et al., 2007). The authors concluded that longitudinal decline in hippocampal volume may be a useful biomarker of approaching dementia.

Functional activation patterns in Alzheimer's disease, mild cognitive impairment, and apolipoprotein E ε4 carriers

Recent evidence suggests that functional brain changes may precede structural changes indicating the FMRI has great potential as a non-invasive technique for detecting early and/or subtle functional brain changes in prodromal individuals (see Wierenga & Bondi, 2007, for review). Functional neuroimaging studies have consistently reported hypoperfusion during resting state (Alsop, Detre, & Grossman, 2000; N. Johnson et al., 2005) and decreased activation in the hippocampus and other MTL structures during encoding in AD patients (e.g., Small, Perera, De La Paz, Mayeux, R., & Stern, 1999; Machulda et al., 2003). Sperling and colleagues (2003)

reported decreased hippocampal activation accompanied by greater activation in non-MTL regions including the medial parietal lobe and posterior cingulate in AD patients relative to nondemented elderly participants during an associative encoding task. In contrast, published studies examining individuals at risk for AD by virtue of their characterization as MCI or their APOE genotype have been relatively inconsistent.

FMRI studies of at-risk groups have reported between-group differences in brain activation patterns (usually based on the BOLD response) or metabolic changes during cognitive tasks often in the context of equivalent behavioral performance (e.g., Han et al., 2007). Before reviewing these findings in more detail, the "compensatory hypothesis" will be briefly discussed, because many of these studies have concluded that their findings are consistent with the operation of compensatory brain mechanisms. Individuals in the preclinical stage of AD may experience an initial decline in memory performance due to incipient MTL neuropathology and they may recruit additional brain regions to temporarily prevent or slow memory decline. Such findings have routinely been interpreted as evidence for compensatory neural recruitment in which at-risk individuals employ additional brain regions and/or the same regions as other groups but to a greater degree in order to maintain a certain level of performance. Decreased activation in healthy individuals has been interpreted as efficient processing whereas in at-risk groups with neurodegenerative changes it is often interpreted as cortical compromise or disconnection (Han, Bangen, & Bondi, 2009; Wierenga & Bondi, 2007). Stern (2002) presented a model in which greater response of regions (i.e., quantitative changes) underlying a task in healthy adults

reflects cognitive reserve, and recruitment of additional regions not typically underlying the task (i.e., qualitative changes) represents compensation.

Group differences in brain activation are often interpreted as evidence for compensatory recruitment despite factors that may suggest other alternative explanations, such as dedifferentiation of function related to decreased cortical specialization of cognitive functions (Li & Lindenberger, 1999), altered resting state activity irrespective of the cognitive task performed during scanning (Greicius, Srivastava, Reiss, & Menon, 2004), or greater neural network noise (Beason-Held, Kraut, & Resnick, 2008). Thus, the need is great for standardized criteria for interpreting functional neuroimaging findings as evidence for compensation. We recently proposed the Region-Activation-Performance (RAP) model (Han et al., 2009) which expanded upon earlier schemes (italicized text indicates the modifications to the definition proposed by Dixon and colleagues, 2003) and defined compensation as "a cluster of behaviors and processes that are designed to overcome or mitigate cognitive deficits or declines" (Dixon et al., 2003) *as manifested through either* ***(1) employing additional brain regions,*** *and/or* ***(2) discrepant brain activation patterns****, such that a cognitive ability is either* ***(3) maintained or improved.****"* The RAP model advocates considering issues related to brain regions involved, activation patterns, and behavioral performance before any between-group differences in activation are interpreted as indicative of compensatory neural recruitment. In terms of the brain regions involved, it is reasonable to assume that observed differences in brain activation patterns may be influenced by structural differences between groups and,

therefore, volumetric data and information from segmentation of structural images should be considered. Regarding the consideration of activation patterns, it may be necessary to consider the optimal activation pattern response during a particular task (e.g., activation, deactivation, connectivity patterns) as well as perfusion measurements in order to adjust for resting state metabolic differences. Finally, the RAP model advocates explicit cognitive task control (e.g., involving titration of task difficulty and/or ensuring equivalence of performance between groups) so that group differences in brain activation cannot be attributed to factors including effort and task difficulty.

Resting state

As mentioned above, FMRI studies have often reported divergent brain activation patterns in those at risk for AD. Using ASL techniques, N. Johnson and colleagues (2005) reported that MCI participants demonstrated regional hypoperfusion in the inferior right parietal lobe during a resting state scan relative to healthy older adults, which was similar to the pattern observed in AD patients. Findings from studies examining differences in resting state perfusion based on APOE genotype have been mixed. The initial studies of functional metabolic activity in cognitively normal older adults indicated that, despite equivalent MMSE scores, APOE ε4 allele carriers demonstrated significantly lower parietal lobe metabolism relative to individuals without the ε4 allele (Small et al., 1995). In contrast, Fleisher and colleagues (2009) reported elevated resting state perfusion in the medial temporal lobe in older individuals (aged 50-65) at risk for AD, as defined by both the presence of a positive

family history of AD and at least one APOE ε4 allele, relative to individuals without these risk factors.

Extending these findings to younger age groups, positron emission tomography (PET) studies have demonstrated reduced glucose metabolism in regions affected in AD (e.g., posterior cingulate, parietal, temporal, and prefrontal regions) in cognitively normal middle-aged (Reiman et al., 1996) and young adults (Reiman et al., 2004) with the ε4 allele. Reiman and colleagues have argued that their results provide evidence of alterations in cerebral metabolism occurring years prior to the onset of AD symptomatology and suggest that glucose hypometabolism represents a biomarker for AD. However, since these findings were reported in young adults (mean age = 31 years), it is unclear whether glucose hypometabolism represents a biomarker for AD, or if it is present across the lifespan it may more likely represent a phenotype of APOE independent of underlying neuropathological processes. In contrast, in another study of 18 young adults, Scarmeas and colleagues (2003) reported reduced resting state perfusion in the bilateral inferior temporal gyrus whereas resting CBF was higher in the lest insula, right supramarginal gyrus, and the inferior occipital gyrus in APOE ε4 carriers relative to those without the ε4 allele. The authors concluded that this pattern of hyperperfusion may reflect compensatory mechanisms in a similar manner as those proposed to occur during performance of cognitive tasks (e.g., Bondi et al., 2005; Han et al., 2007). One limitation of the study by Scarmeas and colleagues is related to sample size give that only three of the 18 participants were carriers of the ε4 allele.

Memory encoding

There have been relatively few published reports of functional neuroimaging studies examining memory encoding in subjects at risk for AD based on either their cognitive or genetic status. Several studies have reported divergent brain response patterns in individuals with MCI relative to cognitively normal older adults. However, the findings have been variable (see Sperling, 2007, for review) and range from increases in activation (Dickerson et al., 2004; Hamalainen et al., 2007) to decreases in activation (Johnson et al., 2004; S. Johnson et al., 2005; Machulda et al., 2003). Notably, Machulda and colleagues (2003) reported that differences in activation between MCI and normal elders occurred during a memory encoding task, although the groups demonstrated no activation differences during a sensory task. The authors concluded that because both groups demonstrated the ability to produce a BOLD signal response of equivalent amplitude during the sensory task the reduced activation in the MCI group during memory processing was not due to a global impairment in the BOLD signal response. Notably, the reduced activation was specific to memory, the domain that clinically distinguishes these impaired groups from healthy aging. Furthermore, Machulda and colleagues (2003) concluded that MTL reduced activation "may be a specific marker of medial temporal lobe dysfunction due to a neurodegenerative disease, not a nonspecific marker of old age."

Variability in activation patterns (e.g., increased activation versus decreased activation) during memory encoding in these groups may be related to FMRI task performance as well as severity of cognitive deficits (see Sperling, 2007, for review). Dickerson and colleagues (2005) reported that individuals with very mild MCI showed

increased MTL activation relative to normal older adults despite equivalent recognition memory performance and hippocampal volume. In contrast, AD patients performed more poorly, showed less hippocampal activation, and had smaller MTL volumes relative to both normal older adults and MCI individuals. The location and extent of this increased MTL activation in very mild MCI has been interpreted as compensation. On the other hand, more impaired MCI participants demonstrate significantly reduced activation in the hippocampus during encoding in a pattern similar to mild AD patients (Celone et al., 2006). Based on these findings, a nonlinear trajectory has been proposed in which FMRI responses during memory tasks in MCI and AD follow a pattern in which there is an early phase involving greater activation in individuals who are very mildly impaired and a later phase involving decreased activation as the MTL atrophy and memory difficulties progress (Dickerson et al., 2005; Dickerson & Sperling, 2008; Sperling, 2007).

A recent ASL MRI study comparing 12 amnestic MCI participants and 14 age-matched cognitively normal participants revealed regional cerebral hypoperfusion in right precuneus and cuneus during the control condition (i.e., visual fixation) and extending to the posterior cingulate during picture encoding. Moreover, a percent increase of 22.7% during memory encoding was observed in the control group but no change was observed in the amnestic MCI participants. The authors concluded that MCI participants lacked the ability "to modulate their regional cerebral blood flow (CBF) responses to the challenge of the functional tasks" (Xu et al., 2007).

Similar to studies of MCI participants, published reports of FMRI studies examining brain activation during memory encoding by APOE genotype have often demonstrated patterns of greater activation in APOE ε4 carriers relative to non-ε4 carriers (e.g., Bondi et al., 2005; Han et al., 2007; Bookheimer et al., 2000). For instance, Bookheimer and colleagues (2000) first reported that episodic encoding revealed greater intensity and extent of brain activation in neurologically normal middle aged and older adults with the ε4 allele. In addition, the degree of baseline brain activation correlated with the extent of memory decline at two-year follow-up. The authors proposed that the larger activation in the left hemisphere hippocampus, parietal, and prefrontal regions indicates compensatory mechanisms in which individuals with the ε4 allele recruited additional cognitive resources to bring their memory performance into the normal range. However, the results of this study are clouded because the ε4 carriers performed more poorly than the non-ε4 group on some tests of episodic memory. Thus, it is unclear whether the results are related to APOE genotype, poor general memory performance, or some interaction between these two variables. Additionally, the study did not state whether the results may be due to differential patterns of brain atrophy between the ε4 and non-ε4 groups. Segmentation of whole-brain gray matter, white matter, and CSF compartments and estimation of gray matter MTL volumes would clarify whether the BOLD contrast effect were influenced by differential atrophy across the genotype groups. Nonetheless, these findings are consistent with subsequent studies that report increased brain activation in individuals at risk for developing AD (e.g., Bondi et al., 2005; Han et al., 2007).

In an effort to determine whether increased activation in APOE ε4 carriers is specific to memory processing or generalizable to other cognitive domains, Burggren, Small, Sabb, & Bookheimer (2002) examined the performance of older adults on a digit span task. Regardless of APOE genotype, increasing task difficulty (defined as increasing digit length) was associated with greater magnitude and extent of activation in the prefrontal cortex. The authors concluded that increased brain activation was associated with increased cognitive demand and greater brain activation in ε4 carriers may be specific to memory tasks and not generalizable to other cognitive abilities.

Bondi and colleagues (2005) examined performance and brain activation patterns during a landscape picture encoding task in individuals at genetic risk for AD and those not at risk. APOE ε4 carriers exhibited greater brain activation during novel picture encoding in several regions including bilateral fusiform gyrus, right superior parietal lobule, left middle frontal gyrus, and medial frontal gyrus compared to participants who were homologous for the ε3 allele. The authors concluded that given that both groups had normal learning and memory capabilities; comparable brain volumes; no baseline differences in the control condition; and no broad differences in physiology based on the fact that both groups demonstrated a strong positive bilateral response to the encoding condition in primary visual cortex. Differences appear to be more directly related to APOE genotype.

Another recent study (Han et al., 2007) demonstrated that APOE ε4 carriers evidenced greater activation in several right hemisphere regions compared to their non-ε4 counterparts while viewing previously encoded word pairs. The authors

concluded that additional activation in frontotemporal regions provided evidence for the recruitment of executive functions and semantic memory processes in order to compensate for episodic memory encoding deficits linked to the ε4 allele.

Fleisher and colleagues (2009) examined cognitively normal, middle aged adults using simultaneous BOLD and ASL and found that participants with both a positive family history of AD and at least one copy of the APOE ε4 allele demonstrated greater resting state perfusion and decreased BOLD and perfusion responses to an associative encoding task but no differences in absolute CBF during encoding relative to a "low risk" group with no family history and no ε4 allele. The authors noted that to their knowledge their study is the first to use ASL/BOLD MRI techniques in individuals at risk for AD. Fleisher and colleagues (2009) concluded that at-risk participants have greater MTL CBF during the resting state, which then influences apparent differences in BOLD activations. Thus, findings of BOLD differences between risk groups do not necessarily reflect differences in neuronal activation and should therefore be interpreted with caution. These findings are in contrast to most studies to date, which have reported increased MTL activation to memory encoding in cognitively healthy APOE ε4 carriers.

To summarize, MCI status and the APOE ε4 allele have been associated with structural (e.g., Soininen et al., 1995) and functional changes (e.g., Reiman et al., 1996) as well as with cognitive deficits in nondemented older adults (Bondi et al., 1995, 1999; Petersen et al., 1995). More specifically, a review of the literature reveals that both MCI status and the APOE ε4 allele are linked to decreased MTL volumes

(Cohen, et al., 2001; den Heijer et al., 2002; Jak et al., 2007; Schott et al., 2006; Soininen et al., 1995) and hypoperfusion or reduced metabolism in areas affected by AD (e.g., parietal region) during resting state (e.g., N. Johnson et al., 2005; Small et al., 1995; Reiman et al., 1996). The preponderance of evidence from FMRI studies of memory encoding suggest that individuals with MCI and APOE ε4 carriers demonstrate divergent brain response patterns relative to individuals not at risk. These divergent patterns range from those reporting increases in neural activation (Dickerson et al., 2004; Hamalainen et al., 2007) to those claiming decreases in neural activation (Johnson et al., 2004; Johnson et al., 2005; Machulda et al., 2003). The variability in findings may be related to FMRI memory task performance and degree of cognitive impairment across individuals (Sperling, 2007) as well as differences in the resting state (Fleisher et al., 2009). Furthermore, in young adults, the APOE ε4 allele has been linked to increased accumulation of neurofibrillary tangles in the entorhinal cortex (Ghebremedhin et al., 1998) and functional brain changes (Reiman et al., 2004). Based on histological and neuroimaging studies, it appears that AD-related neuropathology may develop decades prior to the onset of cognitive changes. In addition, the combination of risk factors (e.g., both MCI and APOE ε4 genotype) may confer a greater risk of conversion to AD relative to the risk imparted by either risk factor alone.

Merits and shortcomings of FMRI in at-risk groups

FMRI is a promising approach with many strengths for studying individuals at risk for

AD. For instance, the technique is capable of detecting changes that occur prior to the onset of AD. FMRI can be used to measure brain activation of cognitive processes that are typically difficult to isolate with behavioral assessment, such as encoding and retrieval. Findings suggest that MTL regions are more reliably activated during encoding and frontal areas tend to be activated during retrieval processes (Schacter & Wagner, 1999). Novel picture encoding is a task that reliably activates the hippocampal formation (Stern et al., 1996). Therefore, FMRI activation of at-risk older adults during picture encoding is an appropriate methodology for examining the initial changes in brain function that may occur in preclinical AD.

Despite the strengths of FMRI, studies using this technique to examine at-risk individuals have yielded inconsistent findings. In addition to the potentially confounding variables previously discussed (e.g., FMRI task performance, degree of cognitive impairment), discrepancies across studies may be related to differences across samples regarding the coupling between the hemodynamic response and underlying neurophysiology and neuroanatomy.

Alterations in the cerebrovascular system with aging and disease

Medical illnesses affecting the cerebrovascular system (e.g., hypertension, hyperlipidemia), which are common in older adults, may affect CBF and neurovascular coupling (see D'Esposito, Deouell, & Gazzaley, 2003, for review). The precise mechanisms underlying neurovascular coupling have yet to be elucidated, although there is evidence that such mechanisms may undergo age- or disease-related

alterations. Thus, changes in the BOLD signal may also reflect cerebrovascular alterations due to age, disease, or medication.

Alterations in vascular dynamics that occur in normal aging and disease include ultrastructural changes in the cerebral vasculature, which is often related to atherosclerosis and may result in reduced elasticity and compliance of the affected vessels; reduced resting state CBF; alterations in vascular reactivity that may be secondary to decreased vascular compliance or result from the gliosis that occurs with tissue scarring related to stroke or another injury; and reduced resting cerebral metabolic rate of oxygen consumption ($CMRO_2$). Mechanisms that may affect neurovascular coupling include chronic cerebral ischemia; chronic cerebral vasodilation resulting from carotid stenosis; effects of hypertension, diabetes, or hyperlipidemia on vascular smooth muscle/vasodilation; effects of medications; and abnormal alterations of specific neurotransmitter systems (e.g., acetylcholine). For instance, chronic cerebral ischemia may be accompanied by a compensatory mechanism involving chronic vasodilation distal to occluded vessels (Daut et al., 1990). Although such a situation would allow adequate perfusion, it could reduce or prevent the additional increase in CBF that is "responsible" for the BOLD signal. Despite normal neural activity, any potential difference in CBF and oxygen concentration between resting state and the functional task may be limited and result in misguided conclusions regarding neural activation during the task (D'Esposito et al., 2003). Future research should clarify the neuroanatomical and neurophysiological

contributions to the BOLD signal and implement experimental design and statistical analysis strategies to address these potential mediators of the BOLD signal.

Most FMRI studies, including the vast majority of those reviewed above, are based on the BOLD contrast. BOLD signal represents a complex function of physiological variables that can be affected by vascular and metabolic changes associated with normal and pathological aging (D'Esposito et al., 2003). ASL FMRI allows for simultaneous measurement of BOLD contrast data and CBF. Thus, findings using ASL/BOLD FMRI may better elucidate the complex relationship between neuronal and physiological activity in both healthy aging and disease (e.g., AD).

Arterial spin labeling

ASL is a non-invasive MRI technique in which arterial water is magnetically labeled and used as an endogenous tracer to measure CBF (Detre, Leigh, Williams, & Koretsky, 1992; Williams, Detre, Leigh, & Koretsky, 1992). The magnetization of arterial blood water is altered in a region proximal to the image slice. This is followed by a delay to allow labeled blood to arrive at the capillary bed in the tissue of interest. The labeled blood diffuses into the tissue resulting in the local alteration of the longitudinal magnetization. Difference images are generated by subtracting images acquired with arterial spin labeling from control images that are acquired in the absence of labeling. The resulting MRI difference image is proportional to CBF (Garraux, Hallett, & Talagala, 2005). ASL has been employed to reliably measure resting CBF in AD patients (N. Johnson et al., 2005), nondemented older adults, and young adults (Bangen et al., 2009; Restom, Bangen, Bondi, Perthen, & Liu, 2007).

ASL studies of AD patients demonstrate similar patterns of regional hypoperfusion as those revealed with PET and single photon emission computed tomography (SPECT; Alsop, Detre, & Grossman, 2000; Detre, & Alsop, 1999). ASL has advantages over PET and SPECT including the use of an endogenous tracer (rather than an intravenously administered contrast agent) and relatively brief scan times (typically five to ten minutes). Due to T1 relaxation, the magnetization of the labeled blood water decays within seconds allowing ASL scans to be repeated in short succession (N. Johnson et al., 2005).

ASL can be used as a FMRI technique (e.g., Kim, 1995; Kwong et al., 1992) and is an attractive alternative to BOLD contrast FMRI. The BOLD signal depends on several physiological effects (e.g., CBF, cerebral blood volume (CBV), $CMRO_2$). In contrast, ASL provides a quantitative measure of CBF (typically in physiological units of millimeters of blood per 100 grams of tissue per minute). Several studies suggest that the ASL signal may be more closely related to neuronal activity and may better localize functional activity as it involves the arterial side of the vascular tree whereas BOLD is suggested to mainly involve the venous side (Lee, Duong, Yang, Iadecola, & Kim, 2001). Additionally, ASL processing of CBF data makes use of a differencing technique that results in reduced sensitivity to low-frequency noise and makes it a useful approach in longer experiments (Aguirre, Detre, Zarahn, & Alsop, 2002). ASL allows measurement of resting state, whereas BOLD is sensitive to changes across different conditions rather than baseline neuronal activity. In our preliminary work

(Bangen et al., 2009; Restom et al., 2007) CBF data has provided a valuable complement to the BOLD contrast data.

As mentioned previously, the BOLD signal is an indirect measure of neural activity that depends on several physiological variables (e.g., CBF, CBV, $CMRO_2$) and estimation of these variables can provide for a potentially more meaningful interpretation of the BOLD signal response. A calibrated FMRI approach allows for the estimate of $CMRO_2$ when BOLD and CBF data are collected during both a functional task and a hypercapnic challenge (e.g., inhaling a gas mixture containing 5% CO_2; Davis, Kwong, Weisskoff, & Rosen, 1998). Changes in $CMRO_2$ are closely linked to neural activity changes (Hyder et al., 2001) and, therefore, an estimate of $CMRO_2$ is particularly useful in interpreting brain activation. A recent calibrated FMRI study of young adults performing a picture encoding task found that functional changes in CBF and $CMRO_2$ are more tightly coupled in the MTL relative to sensory regions (Restom, Perthen, & Liu, 2008) and this coupling was relatively insensitive to the precise values of the local BOLD scaling parameter or the BOLD response. The authors concluded that calculating accurate estimates of $CMRO_2$ depends on obtaining robust measurements of CBF during activation whereas conducting a hypercapnic calibration may be less important.

Potential treatments for AD are continuing to emerge. Neuroprotective agents intended to prevent disease progression will be most effective during the incipient phase. Sensitive and reliable indicators of prodromal dementia are necessary to facilitate early detection capabilities. ASL FMRI techniques will be useful paradigms

for assessing the effects of possible treatments including agents designed to enhance cognition (Rombouts, Barkhof, van Meel, & Scheltens, 2002). The ability to detect AD in its earliest phase is an important topic in neuropsychological and neuroimaging research as potential neuroprotective agents continue to be developed (Thal, 1999).

Preliminary studies

In our preliminary work using ASL/BOLD FMRI, we have shown a greater percent CBF increase in the MTL during picture encoding in healthy older adults relative to young adults with lower absolute CBF during resting state and task performance (Bangen et al., 2009; Restom et al., 2007). The older adults also demonstrated a small but nonsignificant increase in BOLD response amplitude. In addition, both cognitive performance and vascular risk were associated with the functional CBF response. Specifically, better memory performance was associated with a larger CBF response during picture encoding across the sample as a whole. In the older adult group, vascular risk was linked to greater CBF and BOLD response amplitude. These findings support the importance of considering cognitive status and vascular risk when interpreting FMRI findings in older adults. Although we did not obtain measures of the CBF and BOLD responses to a hypercapnic challenge, we estimated functional $CMRO_2$ changes based on a mathematical model of the BOLD signal (Davis et al., 1998) and assumptions based on prior studies (Restom et al., 2007). These analyses suggested that, "despite lower baseline metabolic levels, the older subjects are trying to achieve the same overall level of oxygen metabolism as the young subjects during execution of the task." These findings are consistent with a

view in which the significant age-related increase in percent change CBF is accompanied by increases in percent change $CMRO_2$, resulting in a lack of significant change in the BOLD response to memory encoding. These results highlight the difficulties of interpreting the BOLD response in isolation and provide further support for the need to more fully characterize the physiological underpinnings of functional brain response to memory encoding.

Specific aims and hypotheses

The aim of this study was to examine the influence of AD risk on brain activation patterns using simultaneous ASL and BOLD FMRI. The risk factors of interest were cognitive status (i.e., MCI) and the presence of at least one APOE ε4 allele. Changes in the cerebrovascular system due to age or disease can significantly alter the BOLD signal and complicate its interpretation. The simultaneous acquisition of CBF and BOLD data represents a technique to more fully characterize the neurovascular underpinnings of functional brain response to cognition. The study assessed resting state CBF as well as percent change in CBF, and percent change in BOLD response during picture encoding. In addition, the study examined the influence of resting state CBF on both the CBF and BOLD responses during encoding.

Specific Aim 1: CBF and BOLD Response

To measure CBF and BOLD response in nondemented older adults using ASL/BOLD FMRI in order to characterize the influence of cognitive (i.e., MCI) and/or genetic risk for AD (i.e., APOE genotype) on MTL activation during rest as well as during picture encoding.

Hypothesis 1a: Resting state cerebral blood flow

Based on prior evidence from several modalities, we predicted that nondemented adults at risk for AD would demonstrate hypoperfusion in the MTL during rest when compared to older adults not at risk. If demonstrated, reduced resting state CBF may be related to variables including increased atrophy or cerebrovascular alterations.

Hypothesis 1b: Task-related CBF and BOLD response

Based on previous findings from multiple modalities indicating the establishment of compensatory mechanisms prior to the development of AD, we predicted that individuals at risk for developing AD would exhibit greater extent and/or intensity of MTL brain response during memory encoding compared to their counterparts not at risk. As proposed in the RAP Model (Han et al., 2009), issues related to brain regions involved, activation patterns, and behavioral performance would be collectively considered before any between-group differences in brain activation were interpreted as indicative of compensatory neural recruitment.

Specific Aim 2: Brain structural integrity and vascular risk

To better elucidate the relationships among CBF and BOLD response, MTL volumetry, vascular risk, and cognition in individuals at risk for developing AD.

Hypothesis 2a: Medial temporal lobe volume reduction

We predicted that nondemented older adults at risk for developing AD would demonstrate reductions in MTL volume relative to those not at risk. If so, any differences in the CBF and BOLD data could potentially be related to these structural differences. Therefore, a correction for atrophy—if present—should be made when conducting statistical analyses related to Specific Aim 1.

Hypothesis 2b: Vascular risk

We hypothesized that greater vascular risk would be linked to increased CBF and BOLD response during picture encoding. Such a finding may reflect compensatory neural mechanisms in individuals with vascular risk factors or vascular alterations.

METHODS

Participants

Fifty-five individuals aged 60 or older were recruited from the larger cohort of research volunteers of the Alzheimer's Disease Research Center (ADRC) at the University of California, San Diego (UCSD) as well as from a cohort currently serving as control subjects in an NIA-funded longitudinal research project of nondemented older adults (R01 AG12674; PI: Dr. Bondi) and scanned. Twelve individuals were excluded from further analyses as a result of lack of any detectable activation during picture encoding (n = 7), technical difficulties during scanning (i.e., physiological noise data or dual echo data not properly acquired (n = 2), excessive movement (n = 1), poor head placement in coil (n = 1), and lack of neuropsychological or APOE

genotypic data necessary to characterize this participant's cognitive and genotype status (n = 1).

Given small sample sizes in some cells, the participants were not further divided into four groups of older adults stratified by presence or absence of MCI and APOE genotype (presence or absence of at least one ε4 allele): (a) MCI / non-ε4 (n = 8); (b) MCI / ε4 (n = 6); (c) cognitively normal / non-ε4 (n = 15); and (d) cognitively normal / ε4 (n = 14). Using the empirically-validated diagnostic criteria proposed by Jak and colleagues (2009), 29 individuals were classified as cognitively normal and 14 met criteria for MCI. In the MCI group, individuals met criteria for the following subtypes: three *single domain amnestic MCI*; two *single domain non-amnestic MCI*; four *multiple domain amnestic MCI*; and five *multiple domain non-amnestic MCI*. Of the 43 participants, 20 had at least one copy of the APOE ε4 allele whereas 23 did not. In the APOE ε4 group, 16 were heterozygous carriers and four were homozygous carriers.

Measures

Exclusionary criteria included dementia, significant cerebrovascular disease (e.g., cerebrovascular accident) by history or MRI, a history of significant head trauma with residual cognitive deficits, or other neurological or major psychiatric disorders including Schizophrenia, Bipolar Disorder, developmental learning disorder, and alcohol or substance abuse. The Geriatric Depression Scale (Yesavage et al., 1983) was administered to screen for the presence of any significant affective disturbance. Additional exclusionary criteria included MRI contraindications (e.g., pacemaker).

All ADRC participants and individuals in Dr. Bondi's longitudinal study receive annual neurological, medical, and neuropsychological examinations. Participants from both sources receive similar neuropsychological batteries assessing cognitive domains including attention, memory, language, visualspatial skills, executive functioning, and motor skills. The batteries take approximately four hours to administer and are scored by trained psychometrists following test manual procedures. A detailed description of the tests including in the ADRC Core Neuropsychological Battery has been previously published (see Salmon & Butters, 1992). Based on the comprehensive criteria diagnostic scheme for MCI, the neuropsychological tests of interest from the battery administered as part of Dr. Bondi's longitudinal study include measures divided into five cognitive domains with at least three tests from each of these domains: (1) **Memory**: the Logical Memory Subtest of the Wechsler Memory Scale – Revised (WMS-R; Wechsler, 1987; immediate and delayed free recall; normative data drawn from Mayo's Older Americans Normative Studies [MOANS; Ivnik et al., 1992]), the Visual Reproduction subtest of the WMS-R (immediate and delayed free recall), and the California Verbal Learning Test (CVLT-II; Delis, Kramer, Kaplan, & Ober , 1987; Trials 1-5 total recall and long delay free recall; published norms [Delis et al., 1987]); (2) **Attention:** the Attention subscale of the DRS (Mattis, 1988; published norms [Mattis, 1988]), the Digit Span subtest of the Wechsler Adult Intelligence Scale-Revised (WAIS-R; Wechsler, 1981; normative data from the MOANS [Ivnik et al., 1992]), and Trail Making Test Part A (Reitan & Wolfson, 1985; normative data from the MOANS [Ivnik et al., 1992]); (3) **Language:**

the Boston Naming Test (Kaplan, Goodglass, H., & Weintraub, 1983; normative data from the MOANS [Ivnik et al., 1992]), and Letter Fluency and Category Fluency of the Delis-Kaplan Executive Function System (D-KEFS; Delis, Kaplan, & Kramer, 2001; published norms [Delis et al., 2001]); (4) **Visuospatial functioning:** the Block Design subtest of the Wechsler Intelligence Scale for Children-Revised (WISC-R; Wechsler, 1974; age and education adjusted norms drawn from unpublished data derived from the UCSD Alzheimer Disease Research Center), the Visual Scanning condition of the D-KEFS Trail Making Test, D-KEFS Design Fluency Test (empty and filled dot conditions), DRS Construction subscale (Mattis, 1988), and draw-a-clock; (5) **Executive functioning**: the modified Wisconsin Card Sorting Test (WCST-48-card-version; categories achieved and perseverative errors; administration procedure and normative data from Lineweaver, Bondi, Thomas, & Salmon, 1999;), Trail Making Test Part B, D-KEFS Color-Word Interference Test (inhibition and inhibition/switching), and D-KEFS fluency switching conditions (visual and verbal).

Five separate principal components analyses supported the inclusion of the specific tests in each of the five domains (Jak et al., 2009). In addition, the Independent Living Scales (ILS; Loeb, 1996), an ecologically valid measure designed to assess an individual's ability to independently complete complex activities of daily living, was administered in order to verify the presence or absence of functional impairment. In an attempt to minimize the effect of demographic characteristics (e.g., age, education, sex, ethnicity) on neuropsychological test performance, raw scores from these measures were converted to demographically-corrected standard scores

using the best available normative data. The institutional review boards at San Diego State University (SDSU) and UCSD approved the project. Written informed consent was obtained from all participants.

Additional measures of interest

Framingham Stroke Risk Profile

Vascular risk factors are common in older adults in the general population (Gorelick, 2003) and recent studies suggest that they are associated with not only vascular cognitive impairment but also with AD (Korczyn, 2005; Luchsinger & Mayeux, 2004). Historically, there has been much debate surrounding the association between vascular risk and AD, but recent research has increasingly implicated vascular risk factors in the pathogenesis of MCI and AD (see Bondi et al., 2008, for review). Various theories have been proposed to elucidate the complex relationship between vascular disease and AD. For instance, vascular pathology and AD may have an additive effect or a synergistic effect thereby increasing the overall "burden of pathology." Another theory argues that AD itself may be a vascular disorder (de la Torre, 2002).

Vascular risk factors are linked to cognitive decline in normal aging (Elias et al., 2004), increased rate of progression to dementia in nondemented older adults with cognitive impairment (Reitz, Tang, Manly, Mayeux, & Luchsinger, 2007), and greater rate of decline in Alzheimer's disease (AD; Miekle et al., 2007). Some studies report that vascular risk is associated with executive function decline, in particular, while

other studies have found associations with poorer performance in multiple cognitive domains (Elias et al., 2004). As mentioned above, certain vascular risk factors (e.g., hypertension, hyperlipidemia, diabetes) may lead to alterations in neurovascular coupling thereby influencing the BOLD signal during FMRI studies. Therefore, vascular risk factors are important to assess and consider when analyzing and interpreting both neuropsychological and neuroimaging findings in studies of normal aging and disease. In this proposed study, vascular risk was be quantified using the Framingham Stroke Risk Profile (FSRP; D'Agostino, et al., 1994).

The FSRP is a validated scale that was developed to predict a 10-year probability for risk of stroke and is a commonly used clinical estimate of cerebrovascular risk burden. The FSRP provides gender-corrected scores based on the following risk factors which were identified from 36 years of longitudinal study within the Framingham Heart Study: age, systolic blood pressure, diabetes mellitus, cigarette smoking, history of cardiovascular disease, atrial fibrillation, left ventricular hypertrophy as identified by electrocardiogram, and use of antihypertensive medications.

Family history of Alzheimer's disease

Although MCI status and the APOE ε4 allele are robust risk factors for development of AD, first-degree family history of AD (FH) has been rarely studied and is not well understood. Published reports suggest that the FH-related risk of developing AD is additive to that associated with APOE ε4 allele (Cupples et al., 2004). However, there is little known about the mechanisms. As Johnson and

colleagues (2006) noted, it is important to disentangle relative contributions of FH and APOE genotype because these two risk factors commonly co-occur (e.g., in one study 45% of offspring of AD patients also were ε4 carriers; Sager et al., 2005) and it is unclear whether APOE-related findings are confounded by FH. At the time that their study was published, Johnson and colleagues (2006) reported that there were no published reports of FMRI studies examining FH as a risk factor independent of the APOE ε4 allele. The authors reported that during an encoding task in which middle-aged participants were required to discriminate novel from previously learned stimuli FH negative participants demonstrated greater MTL and fusiform activation. In the hippocampus, –FH +ε4 participants demonstrated the greatest response while +FH +ε4 individuals showed the least activation. The authors concluded that FH may play a role in modulating the effect of APOE genotype on the brain. Notably, findings of studies examining the role of FH often involve younger samples (Wierenga & Bondi, 2007). As age is the greatest risk factor for AD, genetic risk factors may interact with age and their deleterious effects on MTL function may accelerate with advancing age. All participants were asked about FH.

Procedures

Functional imaging

Participants were scanned on a 3.0 Tesla General Electric Medical Systems EXCITE whole body imager with an 8-channel receive-only head coil (General Electric Medical Systems, Milwaukee, WI, USA). The scanner is housed at the UCSD Center for Functional Imaging (CFMRI) on the UCSD La Jolla campus. On the day of

scanning, participants underwent careful screening for contraindications for magnetic resonance imaging (e.g., metal in the body, claustrophobia) before entering the magnet room. Individuals requiring vision correction were fitted with plastic framed glasses with interchangeable lenses matching their prescription. After being positioned in the scanner, the participant's head was stabilized to minimize motion. The MRI technologist ensured that the participant could fully view the display screen, and ask the participant to test the response box in the right hand. Task stimuli were presented from a laptop computer through a data projector to a screen in the MRI room positioned near the foot of the scanner bed. The participant viewed stimuli through a mirror mounted on the head coil.

Picture encoding task

The functional scanning involved a memory encoding task consisting of the presentation of novel and familiar landscape images (Stern et al., 1996; see Figure 1). This task was selected because it has been shown to reliably activate MTL regions including the hippocampus and parahippocampal gyrus in cognitively normal adults (e.g., Stern et al., 1996). A task known to activate the MTL in cognitively normal adults will be advantageous in studying the reliability of combined ASL and BOLD in at-risk groups. Additionally, AD has been shown to affect MTL structures early in the course of the disease. Since we hoped to identify differential patterns in brain structure and function in older adults at risk for AD versus those not at risk, we selected a task that is known to activate the MTL and involves a cognitive function (i.e., memory encoding) that is known to decline during the course of AD.

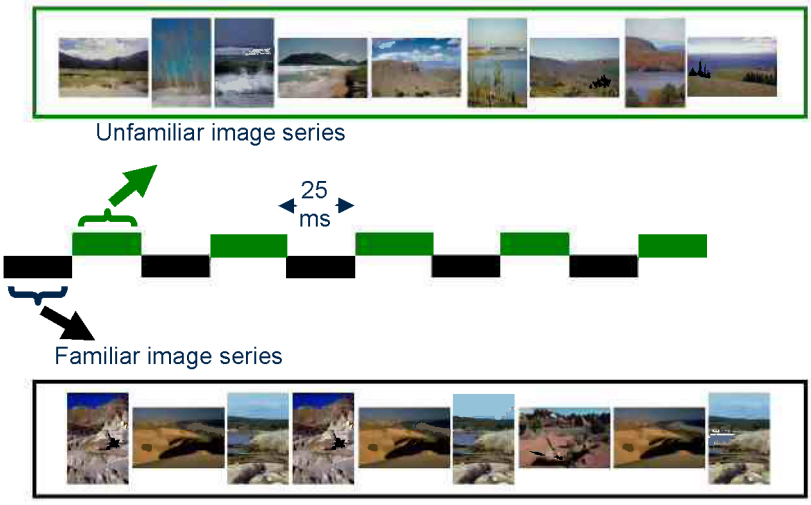

Figure 1. Picture encoding task

Prior to the functional scanning, participants viewed four landscape images (two with horizontal and two with vertical aspect ratio) for approximately ten minutes and these images served as the familiar images. Images were displayed in a blocked design with each block consisting of either ten familiar (i.e., repeated) or ten novel images. Each image was displayed for a duration of two seconds with a half second interval between images. Five blocks of novel and five blocks of familiar scenes were presented per run (250 sec) with three runs per subject. The sequence of familiar images varied between blocks and a single familiar image never occurred twice in a row. To ensure that participants maintained adequate attention, they were instructed to determine whether each image has a horizontal or vertical aspect ratio and make an appropriate response using a two-button response box.

After scanning, subjects completed a two-alternative forced choice recognition task to verify successful encoding of images. Each picture presented during scanning

was paired with a never before seen image. Of the non-target images, one-third were landscape images similar to the targets, one-third were landscape images dissimilar to the targets (e.g., contained buildings or people), and one-third were animals. Participants were instructed to press a button to indicate which picture they viewed during scanning and then asked to make a confidence judgment based on three different ratings (i.e., confident, "so-so," or not confident).

Scanning parameters

A high-resolution structural image was collected: Fast Spoiled Gradient Recall (3D FSPGR), 172 1 mm contiguous sagittal slices, FOV = 25 cm, TR = 8 ms, TE = 3.1 ms, flip angle = 12, T1 = 450, 256 x 192 matrix, Bandwidth = 31.25 kHZ, frequency direction = S-I, NEX =1, scan time = approximately 7 min 30 sec. Functional BOLD and ASL data were simultaneously acquired using a quantitative pulsed ASL sequence (QUantitative Imaging of Perfusion with a Single Subtraction, version II; PICORE QUIPSS II; (Wong, Buxton, & Frank, 1998) with a dual-echo single-shot spiral acquisition (TE1 = 2.4 and TE2 = 24 ms). Five contiguous oblique slices, each 6 mm thick, were acquired at the level of the hippocampus. A tagging slab of 200 mm thickness was placed 10 mm below the most inferior slice. Tag images were interleaved with control images. Other parameters included TI1 = 700 ms, TI2 = 1400 ms, matrix size 64x64, FOV 240 mm, flip angle = 90 degrees, TR = 3000 ms, and 84 repetitions. The total scan time for each ASL run was approximately 4 min 20 sec. One resting-state and three functional scans were acquired. In addition, a cerebral spinal fluid (CSF) reference scan and a minimum contrast scan were acquired for use

in CBF quantification. The CSF scan consisted of a single-echo, single repetition scan acquired at full relaxation and echo time equal to 2.4 ms, while the minimum contrast scan was acquired at TR = 2 s and TE = 11 ms. Both scans used the same in-place parameters as the resting ASL scan, but the number of slices was increased to cover the lateral ventricles. During all four ASL runs, cardiac oximetry and respiratory effort signals were recorded using a pulse oximeter (INVIVO Magnitude 3150M patient monitor, Orlando, FL, USA) and a respiratory effort transducer (TSD201, BioPac Systems, Inc., Goleta, CA, USA) in order to reduce physiological noise and improve the signal-to-noise ratios in our ASL data (Restom, Behzadi, & Liu, 2006).

Data processing and statistical analyses

Structural image processing

Anatomical scans were skull stripped using Brain Surface Extractor (BSE; Version 3.3; Shattuck, Sandor-Leahy, Schaper, Rottenberg, & Leahy, 2001) and manually edited to remove any remaining skull. This approach has been shown to be very effective when working with the images of older adults (Fennema-Notestine et al., 2006). The structural image was then segmented into gray, white, and CSF compartments using FSL's FAST program (FMRIB's Automated Segmentation Tool; Zhang, Brady, & Smith, 2001). Hippocampal, parahippocampal, and entorhinal cortex regions of interest (ROIs; see Figure 2) were manually outlined in the coronal plane for use in the volumetric, resting state, and functional data analysis. Posterior cingulate volumes were also manually traced and used in volumetric analyses, however, given that this structure is only partially covered by the acquired slices during the functional

task and that the FMRI scanning protocol was optimized for the MTL, this structure was not used as an ROI in the functional data analysis. Volumes were delineated using AFNI software and completed by an experienced operator (KJB). High levels of inter-rater reliability for the procedure were established on a separate set of images not among those studied presently (intraclass correlation coefficients [ICC] > .85). The tissue compartments and ROIs were normalized by dividing each value by total brain volume (see Jak et al., 2007).

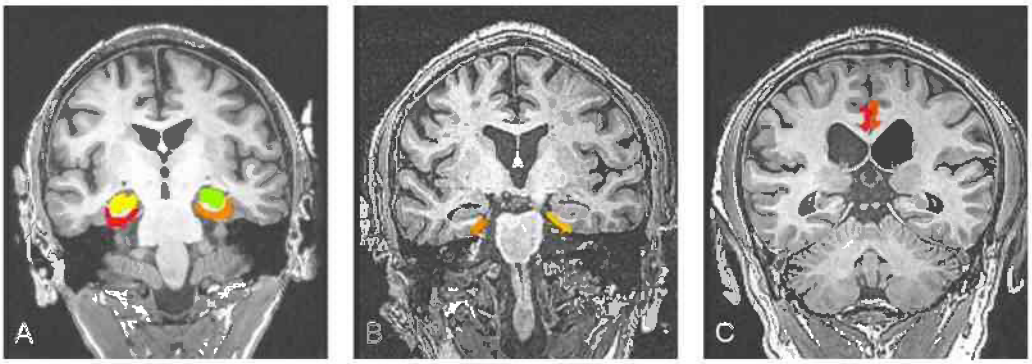

Figure 2. Regions of interest (ROIs). Coronal sections displaying the manually-outlined (A) hippocampal and parahippocampal, (B) entorhinal cortex, and (C) poster cingulate regions of interest ROIs.

Hippocampal volumes were delineated using a stereotactic approach adapted from methods published previously (Bangen et al., 2009; Han et al., 2007; Jak et al., 2007; Nagel et al., 2004). The anterior boundary of the hippocampus was chosen as the coronal slice through the fullest portion of the mammillary bodies. At this level, the posterior boundary was traced on the last coronal slice on which the superior colliculi can be fully visualized. The temporal horn and alveus demarcated the dorsal and lateral boundaries. Ventrally, the hippocampus was bound by the

parahippocampal white matter. Medially, the ambient cistern defined the hippocampal boundary.

Parahippocampal gyrus ROIs were similarly created using a stereotactic approach adapted from methods published previously (Bangen, et al., 2009; Raz et al., 1997). The anterior and posterior bounds of the parahippocampal gyrus were identical to those used to delineate the hippocampus. Dorsally, a horizontal line drawn from the most medial portion of the parahippocampal gyrus white matter to the most lateral point of the collateral sulcus demarcated the parahippocampal gyrus. Ventrally and laterally, the boundary was a line drawn from the most inferior part of the collateral sulcus to the white matter lateral to the collateral sulcus. Medially, the parahippocampal gyrus was bounded by CSF.

Entorhinal cortex ROIs were manually outlined using methods based on previously published methods (Killiany et al., 2002). Specifically, the entorhinal cortex was traced on five consecutive coronal images centered at the level of the mammillary bodies. In cases where more than one image contained the mammillary bodies, then the most anterior image in which the white matter tracts of the fornix were apparent in the mammillary bodies was used as the center image. The outline of the entorhinal cortex began at the inferior boundary, which was defined as the angle formed by the intersection of the rhinal sulcus and the surface of the brain. The outline transected this angle and cut across the gray matter until reaching the white matter, which represented the lateral boundary. The white matter was followed superiorly

until the inferior surface of the hippocampus. The outline then followed the medial surface of the brain until reaching the starting point.

The methods for manually delineating posterior cingulate ROIs were adapted from those published by Jones and colleagues (2006). Posterior cingulate regions located dorsal to the corpus callosum were outlined on coronal slices whereas those located posterior to the corpus callosum were delineated on axial images. The sagittal images also guided the tracing. The anterior boundary of the posterior cingulate was defined as the "pc-line" (i.e., a straight line running dorsally-caudally along the posterior edge of the posterior commissure on the midsagittal image) whereas the posterior boundary was defined by the splenial sulcus as it curves around the most posterior region of the corpus callosum. Dorsally, the structure was bounded by the cingulate sulcus in anterior regions and the splenial sulcus in posterior regions. The ventral boundary of the cingulate gyrus was defined by the callosal sulcus. The most ventral axial slice on which the region was outlined was that on which the "curvature of the splenium of the corpus callosum was visible" as this is the border between the cingulate gyrus and the parahippocampal cortex.

Functional image processing

The procedures for processing of the FMRI data have been previously described (Bangen et al., 2009). Data was processed and analyzed using several different neuroimaging analysis programs. Using Analysis of Functional NeuroImages (AFNI) software, a three-dimensional (3D) brick (or brik) was created from the structural scan slices. A 3D brick of each image file was generated for each TR of the time course

of the PICORE QUIPSS II scanning sequence. Motion in the time series data was corrected by using the AFNI 3D volume registration program to register each acquisition to a selected reference volume with an iterated least squares algorithm to estimate three rotational and three displacement parameters for each participant. The anatomical volume was aligned with the functional volume with a MATLAB program that uses the scanner coordinates of each volume. Functional CBF images were generated from the running subtraction of the control and tag images (Liu & Wong, 2005). Functional BOLD images were computed from the running average (average of each image with the mean of its two nearest neighbors) of the second echo (Liu & Wong, 2005). For each subject, a mean ASL image was formed from the average difference of the control and tag images from the resting state scan data. This mean ASL image was then converted to absolute units of CBF (mL/100 g tissue/minute) with use of the CSF image (Chalela et al., 2000).

For the functional scans, ASL and BOLD runs were concatenated to form one time series per voxel for each type of scan and analyzed with a general linear model (GLM) framework as described in Restom and colleagues (2006). Pre-whitening with the assumption of an autoregressive AR(1) model (Burock & Dale, 2000; Woolrich, Ripley, Brady, & Smith, 2001) was performed to account for temporal autocorrelations. Different physiological noise and low frequency nuisance term regressors were used for each run (Restom et al., 2006). An overall threshold of $p < 0.05$ (with correction for multiple comparisons) was used to define voxels with

significant activation. The AFNI program AlphaSim (Cox, 1996) was used to account for multiple comparisons.

To correct the CBF measures for partial volume effects, we used the method previously reported by N. Johnson and colleagues (2005). These calculations assumed that CSF has zero CBF and that CBF in gray matter is 2.5 times greater than that to white matter. The following formula was used to compute partial volume corrected CBF signal intensities: $CBF_{corr} = CBF_{uncorr}/(GM + 0.4 * WM)$. CBF_{corr} and CBF_{uncorr} are corrected and uncorrected CBF values, respectively. GM and WM are gray matter and white matter partial volume fractions, respectively. Information from the high resolution structural image and the FSL Automated Segmentation Tool (FAST) were used to determine the tissue content of each perfusion voxel (Smith et al., 2004).

Data screening and statistical analyses

Data quality was examined prior to performing statistical analyses. As previously mentioned, participants who had no detectable activation across all CBF and BOLD variables, were excluded from all analyses. However, nine participants had no detectable activation on at least one of the nine FMRI activation variables with valid data on other dependent variables and these individuals were included in the analyses. In addition, what appeared to be unrealistically high percent change CBF values based on previously published studies of ASL MRI response during memory encoding (i.e., values of 267 and higher; Bangen et al., 2009; Fleisher et al., 2009), were deleted and considered missing. There were a total of five participants who had seemingly impossible values across at least one of the 9 dependent variables (i.e.,

bilateral, left, and right resting state CBF, percent change CBF, and percent change BOLD values). A dummy coded variable was created for "missingness" and a one-way ANOVA indicated that there were no differences between those who were missing data versus those who were not in terms of resting state CBF, CBF response during memory encoding, BOLD response during memory encoding, cognitive status, or genotype (all p values > .10). Mean activation values extracted from the ROIs were screened for non-normal distribution and univariate outliers. There were no extremely skewed or kurtotic distributions (i.e., all values between +/-1.5). Next, univariate outliers as defined by 3 standard deviations from mean were examined and there were two additional subjects who had outlier values (i.e., one participant for bilateral percent change BOLD and a second participant for left percent change BOLD).

Given the small number of participants in some cells, the statistical analyses did not compare four participant groups as had originally been proposed (i.e., comparing MCI / non-ε4, MCI / ε4, cognitively normal / non-ε4, and cognitively normal / ε4). Instead, all analyses were run twice: once comparing MCI to NC participants and again comparing APOE ε4 carriers to their non-ε4 counterparts. To test hypothesis 1a we used linear regression. Resting state CBF and participant group served as the dependent and independent variables, respectively. Given that participant groups did not differ on demographic and other variables of interest (e.g., total MTL volume, vascular risk) we did not enter these additional variables into the model. To address hypothesis 1b, we conducted regression analyses similar to those described for hypothesis 1a with two exceptions. First, two separate analyses were conducted: one

with MTL CBF response to picture encoding as the dependent variable and a second with MTL BOLD response as the dependent variable. Second, resting state CBF was added as an independent variable in the model. Furthermore, an interaction between APOE genotype and cognitive status (i.e., MCI versus cognitively normal) was built and tested.

In order to examine hypothesis 2a volume estimates for the manually generated ROIs encompassing the hippocampus, parahippocampus, entorhinal cortex, and posterior cingulate were calculated using AFNI (ROI Average). We expected reduced MTL volumes among MCI patients and perhaps ε4 carriers and performed linear regression to examine the relationship between AD risk and structural volumetry. To test hypothesis 2b two separate regression-based analyses were conducted: one with MTL CBF response to picture encoding as the dependent variable and a second with MTL BOLD response as the dependent variable. In both of these regressions, AD risk served as an independent variable and an interaction was built to determine whether the relationship between AD risk and MTL response varies with depending on vascular risk. Additional bivariate correlational analyses were conducted to further examine the associations among vascular risk, family history of AD, cognitive performance, brain volumes, resting state CBF, and FMRI (CBF and BOLD) response to picture encoding across all participants. All analyses were run in SPSS (version 18.0).

Sample size estimates and statistical power

Preliminary studies (Bondi et al., 2005) reveal several brain sites where the difference in BOLD response to a novel picture encoding task was greater in the ε4 group than in the ε3 group. In all of these regions the effect size for the group difference was larger than .30, which corresponds to a Cohen's *d* of about 1.2 and represents a very large effect. It is assumed that the task used in the present study is as sensitive to group differences as the novel picture encoding task used in the study by Bondi and colleagues. In addition, a second preliminary study using ASL/BOLD FMRI (Restom et al., 2007) identified significant differences between young and older adults in MTL resting state CBF and percent change CBF and BOLD response to picture encoding. The effect sizes for group differences ranged from a Cohen's *d* of .66 to 1.37, which represent medium-to-large to very large effects. Based on these preliminary studies, medium-to-large to very large effect sizes are anticipated for the proposed analyses. Using regression-based models, a total sample size of 80 participants (n = 20 for each group) provides power of approximately .80 to detect medium effect sizes with a 2-tailed alpha of .05 (Cohen, 1988). In order to detect large effect sizes, a total sample size of 36 participants (n = 9 for each group) is needed.

RESULTS

Demographic variables and neuropsychological performance

Cognitively normal versus mild cognitive impairment

The NC and MCI groups did not differ significantly in terms of age ($F_{1,41}$ = .74, p = .39, η_p^2 = .02), education ($F_{1,41}$ = .08, p = .78, η_p^2 = .002), or vascular risk

($F_{1,41} = 3.40$, $p = .07$, $\eta_p^2 = .08$). In addition, the cognitive status groups did not differ in terms of gender (proportion) ($\chi^2 = 3.80$, $p = .05$), APOE genotype distribution ($\chi^2 = .11$, $p = .74$), or family history of AD ($\chi^2 = .05$, $p = .82$). Please see Table 1a for demographic data. As expected, DRS total scores for the MCI group were significantly lower than that for the NC group ($F_{1,41} = 15.03$, $p < .001$, $\eta_p^2 = .27$). Further, the MCI group performed more poorly relative to their cognitively normal counterparts on measures assessing memory, attention, language, visualspatial functioning, and executive functioning. Both the MCI and NC groups performed well on the post-scanning recognition task (i.e., on average better than 85% accuracy) and there were no between group differences in terms of accuracy or confidence ratings (all p values > .05). In addition, across all participants, neither performance accuracy nor confidence rating was significantly associated with MTL CBF (accuracy: r = -.22, p = .40; confidence: r = -.15, p =.57) or BOLD signal response (accuracy: r = -.14, p = .57; confidence: r = .004, p =.98) to picture encoding. See Table 2a for cognitive performance data.

Table 1a. Demographic data for individuals with mild cognitive impairment and cognitively normal participants

	NC $n = 29$	**MCI** $n = 14$	
	Mean (SD)	Mean (SD)	p
Age	74.79 (7.98)	77.00 (7.60)	.39
Education	15.86 (2.33)	15.64 (2.68)	.78
Gender (M/F)	10/19	10/4	.05
APOE Genotype (ε4+/ε4-)	14/15	6/8	.74
Vascular Risk (%)	9.59 (5.06)	12.93 (6.52)	.07
Family History of AD (+/-)	17/11	8/6	.82

Table 2a. Cognitive performance of individuals with mild cognitive impairment and cognitively normal participants

	NC *n* = 29	**MCI** *n* = 14	
	Mean (SD)	Mean (SD)	*p*
DRS Total T-score	55.10 (5.06)	47.93 (6.82)	<.001
ILS Managing Money T-score	56.58 (5.31)	51.20 (6.76)	.07
ILS Health and Safety T-score	56.16 (5.98)	52.20 (4.09)	.18
Geriatric Depression Scale raw score	4.63 (4.46)	4.80 (2.86)	.91
Post-scan Recognition Task Accuracy (%)	87.68 (3.66)	89.24 (2.19)	.43
Post-scan Recognition Task Confidence Rating (correct trials)	1.53 (.29)	1.50 (.33)	.84
MEMORY			
WMS-R Logical Memory I MOANS SS	12.93 (3.16)	8.79 (3.36)	<.001
WMS-R Logical Memory II MOANS SS	12.82 (3.03)	8.71 (3.83)	.001
WMS-R Visual Reproduction I MOANS SS	12.55 (3.29)	10.14 (4.60)	.14
WMS-R Visual Reproduction II MOANS SS	12.55 (2.70)	8.43 (4.86)	.008
CVLT 1-5 total T-score	58.46 (10.69)	45.43 (7.62)	<.001
ATTENTION			
DRS Attention T-score	55.64 (4.11)	54.71 (4.95)	.52
WAIS-R Digit span MOANS SS	12.21 (2.90)	9.77 (2.09)	.01
Trails A MOANS SS	12.50 (3.17)	10.39 (2.81)	.05

Table 2a continued

	NC *n* = 29	**MCI** *n* = 14	
	Mean (SD)	Mean (SD)	*p*
LANGUAGE			
BNT MOANS SS	14.54 (2.43)	11.42 (3.50)	.002
D-KEFS Letter Fluency SS	13.14 (3.05)	10.69 (3.71)	.03
D-KEFS Category Fluency SS	13.29 (2.85)	10.67 (2.31)	.008
VISUALSPATIAL			
WISC-R Block Design T-score	55.78 (8.05)	42.77 (14.90)	.001
D-KEFS Visual Scanning SS	11.22 (2.10)	11.38 (1.94)	.82
DRS Construction T-score	52.43 (3.02)	43.64 (14.01)	.003
Clock Drawing (Command) raw score	2.96 (.19)	2.50 (.76)	.004
EXECUTIVE FUNCTIONS			
WCST categories T-score	54.70 (4.87)	47.38 (10.96)	.005
WCST perseverative errors T-score	49.04 (3.42)	46.15 (6.89)	.08
Trails B MOANS SS	12.32 (2.13)	10.00 (2.58)	.004

Given that there was a trend toward a significant difference between MCI and NC groups in terms of gender distributions, we performed one-way ANOVAs to determine whether men and women differed on our main variables of interest: resting state CBF, percent change CBF, and percent change BOLD for bilateral, left, and right MTL. There were no significant differences between men and women across these variables (all p values > .05).

APOE ε4 carriers versus non-ε4 carriers

The APOE genotype groups did not differ significantly in terms of age ($F_{1,41}$ = .56, p = .46, η_p^2 = .01), education ($F_{1,41}$ = .23, p = .64, η_p^2 = .006), or vascular risk ($F_{1,41}$ = .45, p = .51, η_p^2 = .01). In addition, the APOE ε4 and non-ε4 groups did not differ in terms of gender (proportion) (χ^2 = .03, p = .85), cognitive status (i.e., MCI vs. NC; χ^2 = .11, p = .74), or family history of AD (χ^2 = .004, p = .95). Please see Table 1b for demographic data. The genotype groups did not differ in terms of global cognitive functioning as measured by DRS total score ($F_{1,41}$ = 2.48, p = .12, η_p^2 = .06). APOE ε4 carriers performed significantly more poorly on a measure of visual scanning (D-KEFS Trail Making Test Visual Scanning, $F_{1,38}$ = 4.50, p = .04, η_p^2 = .11), however, their performance was within the average range (i.e., mean score is a scaled score of 10.56) and not indicative of objective impairment. Further, there were no between group differences on any other cognitive measures (all p values > .05). Both genotype groups performed well on the post-scanning recognition task (i.e., on average better than 85% accuracy). There were no between group differences in terms of accuracy of performance (p = .33), although the ε4 carriers were significantly more confident in their responses relative to the non-ε4 carriers (p = .03). See Table 2b for cognitive performance data.

Table 1b. Demographic data for APOE ε4 and non-ε4 groups

	Non-ε4 *n* = 23	**ε4** *n* = 20	
	Mean (SD)	Mean (SD)	*p*
Age	76.35 (7.71)	74.55 (8.07)	.46
Education	15.96 (2.50)	15.60 (2.37)	.64
Gender (M/F)	11/12	9/11	.85
Cognitive Status (NC/MCI)	15/8	14/6	.74
Vascular Risk (%)	10.13 (4.96)	11.31 (6.57)	.51
Family History of AD (+/-)	13/9	12/8	.95

Table 2b. Cognitive performance of APOE ε4 and non-ε4 groups

	Non-ε4 *n* = 23	**ε4** *n* = 20	
	Mean (SD)	Mean (SD)	*p*
DRS Total T-score	54.21 (4.16)	51.10 (8.35)	.12
ILS Managing Money T-score	56.33 (4.68)	54.58 (7.04)	.48
ILS Health and Safety T-score	55.50 (6.80)	55.17 (4.90)	.89
Geriatric Depression Scale raw score	4.65 (3.95)	4.71 (4.19)	.97
Post-scan Recognition Task Accuracy (%)	86.98 (2.81)	88.61 (3.70)	.33
Post-scan Recognition Task Confidence Rating (correct trials)	1.72 (.20)	1.42 (.28)	.03
MEMORY			
WMS-R Logical Memory I MOANS SS	11.41 (3.97)	11.70 (3.59)	.81
WMS-R Logical Memory II MOANS SS	10.95 (3.93)	12.00 (3.70)	.38
WMS-R Visual Reproduction I MOANS SS	12.33 (3.87)	11.57 (3.63)	.59
WMS-R Visual Reproduction II MOANS SS	11.13 (4.09)	12.00 (3.35)	.54
CVLT 1-5 total T-score	53.73 (10.92)	54.55 (12.39)	.82

Table 2b continued

	Non-ε4 *n* = 23	**ε4** *n* = 20	
	Mean (SD)	Mean (SD)	*p*
LANGUAGE			
BNT MOANS SS	13.91 (3.04)	13.22 (3.23)	.49
D-KEFS Letter Fluency SS	12.00 (3.39)	12.79 (3.51)	.47
D-KEFS Category Fluency SS	12.24 (2.81)	12.79 (3.12)	.56
VISUALSPATIAL			
WISC-R Block Design T-score	52.91 (10.53)	49.89 (14.17)	.44
D-KEFS Visual Scanning SS	11.86 (1.73)	10.56 (2.18)	.04
DRS Construction T-score	51.55 (4.71)	47.25 (12.27)	.14
Clock Drawing (Command) raw score	2.77 (.62)	2.85 (.37)	.63
EXECUTIVE FUNCTIONS			
WCST categories T-score	52.95 (7.44)	51.56 (8.91)	.59
WCST perseverative errors T-score	48.14 (4.99)	48.06 (4.99)	.96
Trails B MOANS SS	11.95 (2.36)	11.16 (2.65)	.32
D-KEFS Color Word Interference Inhibition SS	11.38 (2.19)	12.07 (2.31)	.40
LANGUAGE			
BNT MOANS SS	13.91 (3.04)	13.22 (3.23)	.49
D-KEFS Letter Fluency SS	12.00 (3.39)	12.79 (3.51)	.47
D-KEFS Category Fluency SS	12.24 (2.81)	12.79 (3.12)	.56

Structural volumetry

Cognitively normal versus mild cognitive impairment

The MCI and cognitively normal groups did not significantly differ in terms of normalized volumes of the whole brain gray matter ($F_{1,41} = .43$, $p = .52$, $\eta_p^2 = .01$), whole brain white matter ($F_{1,41} = .04$, $p = .85$, $\eta_p^2 = .001$), total medial temporal lobe ($F_{1,41} = 2.96$, $p = .09$, $\eta_p^2 = .07$), hippocampus ($F_{1,41} = 2.08$, $p = .16$, $\eta_p^2 = .05$), parahippocampus ($F_{1,41} = 3.29$, $p = .08$, $\eta_p^2 = .07$), entorhinal cortex ($F_{1,41} = .11$, $p = .74$, $\eta_p^2 = .003$), or posterior cingulate ($F_{1,41} = .63$, $p = .43$, $\eta_p^2 = .02$). When each of

the five ROIs were separated into left and right hemispheres, the MCI group demonstrated significantly reduced right parahippocampal gyrus volumes relative to their cognitively normal counterparts ($F_{1,41} = 4.97$, $p = .03$, $\eta_p^2 = .11$). There were no additional differences between the groups (all p values > .05). Please see Table 3a for structural volumetry descriptive statistics.

Table 3a. Group comparisons of brain volume indices by cognitive status

	NC $n = 29$	**MCI** $n = 14$	p	η_p^2
Normalized whole brain gray matter	.434 (.028)	.428 (.026)	.52	.01
Normalized whole brain white matter	.342 (.026)	.344 (.025)	.85	.001
Normalized hippocampal volume*	.348 (.051)	.325 (.046)	.16	.05
Normalized parahippocampal volume*	.233 (.043)	.208 (.034)	.08	.07
Normalized entorhinal cortex volume*	.021 (.004)	.021 (.005)	.74	.003
Normalized MTL volume*	.601 (.088)	.555 (.074)	.09	.07
Normalized posterior cingulate volume*	.359 (.057)	.374 (.054)	.43	.02

* Values multiplied by 100.

APOE ε4 carriers versus non-ε4 carriers

The APOE genotype groups did not significantly differ in terms of normalized volumes of the whole brain gray matter ($F_{1,41} = .16$, $p = .69$, $\eta_p^2 = .004$), whole brain white matter ($F_{1,41} = .95$, $p = .34$, $\eta_p^2 = .02$), total medial temporal lobe ($F_{1,41} = .05$, $p = .82$, $\eta_p^2 = .001$), hippocampus ($F_{1,41} = .15$, $p = .70$, $\eta_p^2 = .004$), parahippocampus

($F_{1,41} = .005$, $p = .94$, $\eta_p^2 < .001$), entorhinal cortex ($F_{1,41} = .51$, $p = .48$, $\eta_p^2 = .01$), or posterior cingulate ($F_{1,41} = 1.23$, $p = .27$, $\eta_p^2 = .03$). Similarly, when each of the five ROIs was separated into left and right hemispheres, there were no differences between the groups (all p values > .05). Please see Table 3b for structural volumetry descriptive statistics.

Table 3b. Group comparisons of brain volume indices by APOE genotype

	Non-ε4 $n = 23$	**ε4** $n = 20$	p	η_p^2
Normalized whole brain gray matter	.431 (.020)	.434 (.034)	.69	.004
Normalized whole brain white matter	.339 (.021)	.347 (.029)	.34	.02
Normalized hippocampal volume*	.338 (.055)	.344 (.045)	.70	.004
Normalized parahippocampal volume*	.225 (.046)	.224 (.037)	.94	<.001
Normalized entorhinal cortex volume*	.021 (.004)	.022 (.004)	.48	.01
Normalized MTL volume*	.583 (.096)	.589 (.074)	.82	.001
Normalized posterior cingulate volume*	.355 (.060)	.374 (.050)	.27	.03

* Values multiplied by 100.

Resting-state CBF

Cognitively normal versus mild cognitive impairment

A linear regression was conducted with bilateral resting state MTL CBF as the criterion variable and cognitive status (i.e., MCI or NC) as the predictor variable. There were no additional variables entered as predictors given that the two participant

groups did not differ in terms of demographics or other variables of interest (e.g., family history of AD, vascular risk, etc.). Individuals with MCI demonstrated significantly reduced bilateral MTL resting state CBF relative to their cognitively normal counterparts ($F_{1,41} = 5.34$, $p = .03$, $R^2 = .12$, $\beta = -.34$, $p = .03$; See Figure 3). When left and right hemispheres were examined separately, there was a trend toward individuals with MCI demonstrating reduced left MTL resting state CBF ($F_{1,41} = 3.39$, $p = .07$, $R^2 = .08$, $\beta = -.28$, $p = .07$). However, individuals with MCI demonstrated significantly reduced right MTL resting state CBF compared to their cognitively normal peers ($F_{1,41} = 7.09$, $p = .01$, $R^2 = .15$, $\beta = -.38$, $p = .01$). See Table 4a.

APOE ε4 carriers versus non-ε4 carriers

APOE ε4 carriers demonstrated significantly increased bilateral MTL resting state CBF compared to the non-ε4 carriers ($F_{1,41} = 5.35$, $p = .03$, $R^2 = .12$, $\beta = .34$, $p = .03$; See Figure 3). When left and right hemispheres were examined separately, the APOE ε4 carriers demonstrated increased resting state CBF in the left ($F_{1,41} = 5.19$, $p = .03$, $R^2 = .11$, $\beta = .34$, $p = .03$) and right MTL compared to non-ε4 carriers ($F_{1,41} = 6.72$, $p = .01$, $R^2 = .14$, $\beta = .38$, $p = .01$). See Table 4b.

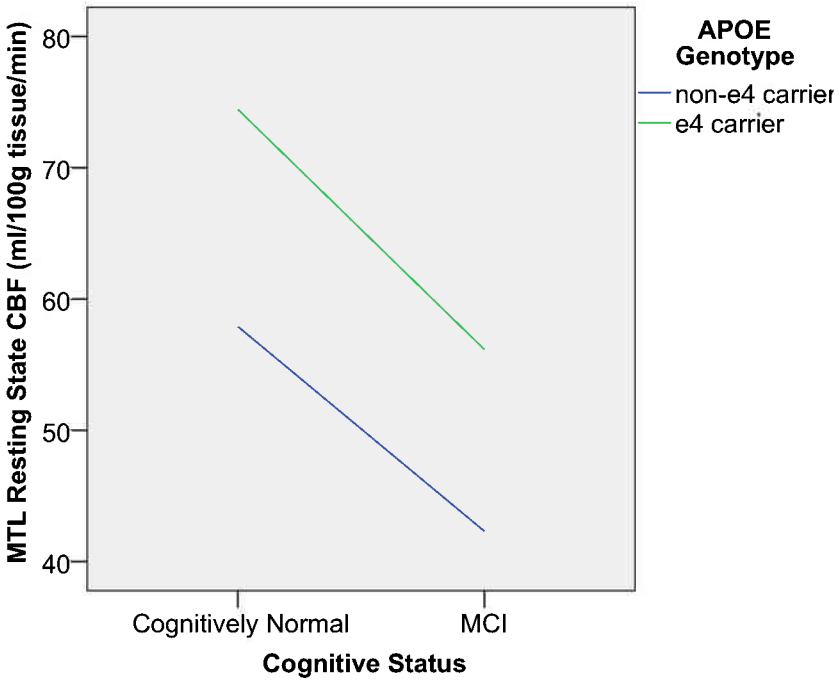

Figure 3. MTL Resting State CBF by participant group

Functional CBF and BOLD response during picture encoding

Cognitively normal versus mild cognitive impairment

Linear regression analyses were conduced with percent change MTL CBF as the criterion and cognitive status (i.e., MCI or NC) as the predictor variable. Cognitive status did not account for a significant amount of overall variance in bilateral MTL percent change CBF during picture encoding ($F_{1,41} = .08$, $p = .78$, $R^2 = .002$, $\beta = -.04$, $p = .78$). When hemispheres were analyzed separately, cognitive status did not predict left MTL percent change CBF ($F_{1,41} = 1.62$, $p = .21$, $R^2 = .04$, $\beta = -.20$, $p = .21$) or right percent change CBF during picture encoding ($F_{1,41} = .007$, $p = .93$, $R^2 < .001$, $\beta = -.01$, $p = .21$).

Similar analyses were conduced with percent change MTL BOLD as the criterion and cognitive status (i.e., MCI or NC) as the predictor variable. Cognitive status did not account for a significant amount of overall variance in bilateral MTL percent change BOLD during picture encoding ($F_{1,41} = .32$, $p = .58$, $R^2 = .008$, $\beta = .09$, $p = .58$). When hemispheres were analyzed separately, cognitive status did not predict left MTL percent change BOLD ($F_{1,41} = .61$, $p = .44$, $R^2 = .02$, $\beta = .12$, $p = .44$) or right percent change BOLD during picture encoding ($F_{1,41} = 1.37$, $p = .25$, $R^2 = .03$, $\beta = .18$, $p = .25$).

Similar analyses were conduced as hierarchical regression analyses with the same percent change MTL CBF and BOLD variables as the criterion variable and resting state CBF and cognitive status (i.e., MCI or NC) entered individually as predictor variables in this order. None of these models were significant and the predictors were not significantly related to the dependent variable (all p values $> .05$), indicating that resting state MTL CBF did not account for a significant amount of overall variance in bilateral, left, or right MTL percent change CBF or BOLD.

Table 4a. Group comparisons of resting state CBF and CBF and BOLD response to picture encoding by cognitive status

	NC *n* = 29	**MCI** *n* = 14
Bilateral MTL Resting State CBF	65.98 (24.80)	48.37 (19.72)
Left MTL Resting State CBF	72.22 (28.47)	56.37 (21.51)
Right MTL Resting State CBF	60.50 (22.77)	41.31 (20.76)
Bilateral MTL Percent Change CBF	100.82 (63.78)	94.83 (63.27)
Left MTL Percent Change CBF	102.88 (73.43)	74.19 (51.81)
Right MTL Percent Change CBF	99.97 (59.01)	98.12 (77.53)
Bilateral MTL Percent Change BOLD	.56 (.24)	.61 (.27)
Left MTL Percent Change BOLD	.52 (.24)	.59 (.24)
Right MTL Percent Change BOLD	.56 (.31)	.70 (.39)

APOE ε4 carriers versus non-ε4 carriers

Regression analyses were conducted with percent change MTL CBF as the criterion variable and genotype as the predictor. Genotype did not account for a significant amount of overall variance in bilateral MTL percent change CBF during picture encoding ($F_{1,41} = 2.56$, $p = .12$, $R^2 = .06$, $\beta = .24$, $p = .12$). When hemispheres were analyzed separately, genotype did not account for a significant amount of overall variance in left ($F_{1,41} = 2.75$, $p = .11$, $R^2 = .06$, $\beta = .25$, $p = .11$) or right MTL percent change CBF ($F_{1,41} = 1.32$, $p = .26$, $R^2 = .03$, $\beta = .18$, $p = .26$).

Regression analyses were then conducted with BOLD percent change CBF during picture encoding as the criterion variable and genotype as the predictor variable. Genotype did not account for a significant amount of overall variance in bilateral MTL percent change BOLD ($F_{1,41} = .002$, $p = .96$, $R^2 < .001$, $\beta = .007$, $p =$

.96). When hemispheres were analyzed separately, genotype did not account for a significant amount of overall variance in left MTL percent change BOLD during picture encoding ($F_{1,41} = 2.57$, $p = .12$, $R^2 = .06$, $\beta = .24$, $p = .12$) or right MTL percent change BOLD during picture encoding ($F_{1,41} = .02$, $p = .90$, $R^2 < .001$, $\beta = -.02$, $p = .90$).

Table 4b. Group comparisons of resting state CBF and CBF and BOLD response to picture encoding by APOE genotype

	Non-ε4 *n* = 23	**ε4** *n* = 20
Bilateral MTL Resting State CBF	52.57 (18.21)	69.07 (28.09)
Left MTL Resting State CBF	58.66 (20.26)	76.73 (31.26)
Right MTL Resting State CBF	46.05 (17.88)	63.68 (26.40)
Bilateral MTL Percent Change CBF	84.71 (49.98)	116.17 (73.51)
Left MTL Percent Change CBF	77.03 (46.58)	113.50 (84.74)
Right MTL Percent Change CBF	88.80 (58.08)	112.30 (71.29)
Bilateral MTL Percent Change BOLD	.57 (.24)	.58 (.27)
Left MTL Percent Change BOLD	.49 (.18)	.61 (.28)
Right MTL Percent Change BOLD	.62 (.31)	.60 (.38)

Supplemental analyses: Interaction between cognitive status and APOE genotype

The interaction between cognitive status and APOE genotype was explored using hierarchical regression. When cognitive status and genotype were entered on step 1, the model was not significant ($F_{2,40} = 1.27$, $p = .29$, $R^2 = .06$) and neither variable was significantly associated with bilateral MTL percent change CBF (cognitive status: $\beta = -.03$, $p = .84$; genotype: $\beta = .24$, $p = .12$). Further, there was no significant interaction between cognitive status and APOE genotype for bilateral MTL

percent change CBF ($F_{3,39} = 1.35$, $p = .27$, $R^2 = .09$) and the interaction term was not significantly associated with bilateral percent change CBF ($\beta = .27$, $p = .23$).

When the hemispheres were analyzed separately, cognitive status and genotype did not account for a significant amount of overall variance in left MTL percent change CBF ($F_{2,40} = 2.13$, $p = .13$, $R^2 = .10$) and neither variable was significantly associated with left MTL percent change CBF (cognitive status: $\beta = -.18$, $p = .23$; genotype: $\beta = .24$, $p = .12$). When the interaction term was entered on step 2, the model was not significant, ($F_{3,39} = 1.49$, $p = .23$, $R^2 = .10$, $\beta = -.12$, $p = .60$). When entered on step 1, cognitive status and genotype did not account for a significant amount of overall variance in right MTL percent change CBF ($F_{2,40} = .64$, $p = .53$, $R^2 = .03$). Neither cognitive status nor genotype was significantly associated with right MTL percent change CBF (cognitive status: $\beta = -.004$, $p = .98$; genotype: $\beta = .18$, $p = .27$). When the interaction term was entered on step 2, the model was nearly significant ($F_{3,39} = 2.57$, $p = .06$, $R^2 = .17$). Further, the interaction term accounted for a significant increase in explained variance ($\Delta F_{1,39} = 6.27$, $p = .02$, $\Delta R^2 = .13$, $\beta = .55$, $p = .02$). In the NC group, there was not a significant difference in CBF response based on APOE genotype ($F_{1,25} = .19$, $p = .67$, $\eta_p^2 = .008$ (Means = 94.35 for ε4 group and 104.47 for non- ε4 group)). However, in the MCI group, ε4 carriers demonstrated significantly greater CBF response relative to non-ε4 carriers ($F_{1,11} = 6.95$, $p = .02$, $\eta_p^2 = .39$ (Means = 148.18 for ε4 group and 55.22 for non-ε4 group)). See Figure 4.

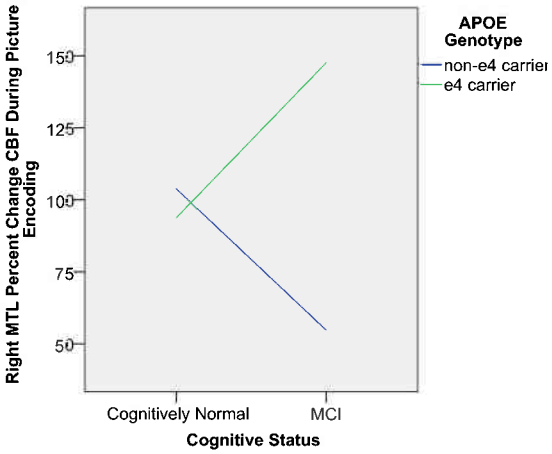

Figure 4. Cognitive status by genotype interaction for right MTL percent change CBF

When the same regression analyses were conducted with percent change BOLD rather than CBF serving as the dependent variable, cognitive status and APOE genotype did not account for a significant amount of overall variance in bilateral percent change BOLD ($F_{2,40}$ = .16, p = .85, R^2 = .008 (cognitive status: β = .09, p = .58; genotype: β = .01, p = .94)). There was no significant interaction between cognitive status and APOE genotype for bilateral MTL percent change BOLD ($F_{3,39}$ = .15, p = .93, R^2 = .01, β = .09, p = .71). When the hemispheres were analyzed separately, cognitive status and genotype did not account for a significant amount of overall variance in left MTL percent change BOLD ($F_{2,40}$ = 1.65, p = .21, R^2 = .09). Neither cognitive status nor genotype was significantly associated with left MTL percent change BOLD (cognitive status: β = .14, p = .41; genotype: β = .27, p = .11). When the interaction term was entered on step 2, the model was not significant ($F_{3,39}$ =

1.26, $p = .30$, $R^2 = .10$, $\beta = .18$, $p = .47$). Cognitive status and genotype did not account for a significant amount of overall variance in right MTL percent change BOLD ($F_{2,40} = .68$, $p = .52$, $R^2 = .04$). Neither cognitive status ($\beta = .19$, $p = .26$) nor genotype ($\beta = -.02$, $p = .89$) was significantly associated with right MTL percent change BOLD. When the interaction term was added on step 2, the model was not significant ($F_{3,39} = .78$, $p = .52$, $R^2 = .06$, $\beta = .25$, $p = .33$).

Regression models examining AD risk by stroke risk interaction

Cognitively normal versus mild cognitive impairment

A hierarchical multiple regression was conducted with bilateral MTL percent change CBF during picture encoding as the dependent variable and cognitive status and stroke risk, and the interaction between the two serving as the predictor variables. When entered on step 1, cognitive status and stroke risk did not account for a significant amount of overall variance in bilateral MTL percent change CBF during picture encoding ($F_{2,40} = 1.17$, $p = .32$, $R^2 = .06$). When the interaction term was entered on step 2, a significant amount of overall variance was not explained ($F_{3,39} = .77$, $p = .52$, $R^2 = .06$). When the same analysis was run with bilateral MTL percent change BOLD during picture encoding served as the dependent variable, cognitive status and stroke risk accounted for a significant amount of overall variance ($F_{2,40} = 3.59$, $p = .04$, $R^2 = .16$). Cognitive status was not related to BOLD ($\beta = -.06$, $p = .73$); however greater stroke risk was associated with increased BOLD response ($\beta = .42$, $p = .01$). When the interaction term was entered on step 2, there was a trend toward a significant amount of overall variance being explained ($F_{3,39} = 2.75$, $p = .06$, $R^2 = .18$)

and the interaction term did not account for a significant increase in explained variance ($\Delta F_{1,39} = 1.04$, $p = .32$, $\Delta R^2 = .02$, $\beta = -.22$, $p = .32$).

APOE ε4 carriers versus non-ε4 carriers

A hierarchical multiple regression was conducted with bilateral MTL percent change CBF during picture encoding as the dependent variable and genotype and stroke risk, and the interaction between the two serving as the predictor variables. When entered on step 1, genotype and stroke risk did not account for a significant amount of overall variance in bilateral MTL percent change CBF during picture encoding ($F_{2,40} = 2.07$, $p = .14$, $R^2 = .10$). When the interaction term was entered on step 2, a significant amount of overall variance was not explained ($F_{3,39} = 1.46$, $p = .24$, $R^2 = .11$). When the same analysis was run with bilateral MTL percent change BOLD during picture encoding served as the dependent variable, genotype and stroke risk accounted for a significant amount of overall variance ($F_{2,40} = 3.53$, $p = .04$, $R^2 = .16$). Genotype was not significantly associated with BOLD ($\beta = -.02$, $p = .89$); however greater stroke risk was associated with increased BOLD response ($\beta = .40$, $p = .01$). When the interaction term was entered on step 2, a significant amount of overall variance was not explained ($F_{3,39} = 2.32$, $p = .09$, $R^2 = .16$, $\beta = .06$, $p = .78$).

Additional bivariate correlations

Additional bivariate correlational analyses were conducted to examine the associations among vascular risk, family history of AD, cognitive performance, brain volumes, resting state CBF, and MTL response to picture encoding. Across all

participants, greater vascular risk was significantly associated with reduced total MTL volumes ($r = -.43$, $p = .004$) and greater MTL activation during picture encoding (bilateral percent change BOLD: $r = .40$, $p = .01$; right percent change CBF: $r = .35$, $p = .03$). However, vascular risk was not significantly related to resting state CBF ($r = -.02$, $p = .92$), global cognitive functioning (DRS total score; $r = -.27$, $p = .08$), or memory performance (CVLT Long Delay Free Recall; $r = -.20$, $p = .21$). Family history was not significantly associated with global cognition or memory performance, MTL volumes, resting state CBF, or MTL activation during picture encoding (all p values $> .05$).

DISCUSSION

To summarize, we found differences between individuals at increased risk for AD and those not at risk in terms of resting state CBF. The nature of these between group differences varied depending on the type of risk factor. Specifically, individuals at genetic risk for AD by virtue of the presence of at least one APOE ε4 allele demonstrated increased MTL resting state CBF relative to their non-ε4 counterparts. In contrast, individuals characterized as MCI showed decreased MTL resting state CBF compared to their cognitively normal peers. Further, although we did not find significant main effects based on AD risk in terms of CBF or BOLD signal response to memory encoding, we did find a trend toward an interaction. In the cognitively normal group there was no difference in percent change CBF based on APOE genotype.

However, in the MCI group, APOE ε4 carriers demonstrated significantly greater percent change CBF relative to non-ε4 carriers.

Our finding of reduced resting state CBF in individuals with MCI compared to their cognitively normal counterparts is consistent with an accumulating body of evidence from ASL MRI studies (Dai et al., 2009; Johnson et al., 2005; Xu et al., 2007). In the present study, the MCI participants demonstrated reduced perfusion in the MTL, which is consistent with the notion that MCI individuals may show regional hypoperfusion in the regions first affected in AD before the clinical onset of dementia. These findings suggest that the measurement of resting state CBF using ASL MRI could assist in the early detection of dementia and monitoring of treatment effectiveness.

In fact, a recent longitudinal study involving ASL MRI demonstrated that resting state hypoperfusion of the right inferior parietal cortex and right middle frontal cortex at baseline predicted conversion from MCI to dementia at three-year follow-up (Chao et al., 2010). One limitation of Chao et al.'s study was that the scanning acquisition did not cover more inferior regions of the brain including the medial temporal lobe. Nevertheless, this finding was consistent with previously reported studies in the FTD-PET and SPECT literature that reported reduced metabolism in areas including right temporoparietal cortex (Chetelat et al., 2003), bilateral temporo-parietal regions and precuneus (Hirao et al., 2005), and right inferior parietal cortex (Mosconi et al., 2004) at follow-up periods ranging from 18 months to three years.

Although the preponderance of evidence to date supports the presence of hypoperfusion in MCI and AD, a recent study utilizing ASL MRI demonstrated hippocampal hyperperfusion in a subset of AD patients (Alsop et al., 2008). Most of the AD patients, who ranged in severity of cognitive impairment, showed temporoparietal hypoperfusion. However, several mild AD patients displayed MTL hyperperfusion, although there were no areas of significant hyperperfusion in this group relative to normal controls. Alsop and colleagues acknowledged that most previous PET and SPECT studies do not report hyperperfusion; however, they noted concerns regarding the methodology and interpretation of findings from many of these studies given that they generally do not correct for MTL atrophy that occurs early in dementia and yet do not show significantly reduced flow in MTL (e.g., Matsuda et al., 2002).

Alsop and colleagues (2008) noted that it is unclear whether the elevated flow they observed in mild AD is coupled to elevated metabolism. This uncertainty results in part from the inconsistencies in reported results across previous FDG PET studies. Specifically, some studies have shown no differences in glucose consumption between those with AD and their cognitively normal counterparts (e.g., Ishii et al., 1998; Jagust et al., 1993) whereas others have demonstrated declines in AD patients (e.g. De Santi et al., 2001). However, significantly reduced medial temporal lobe oxygen metabolism in the absence of significantly decreased CBF has been demonstrated in AD (Ishii et al., 1996) indicating possible decoupling of flow and metabolism. As Aslop and colleagues noted, a possible explanation for decoupling may be release of nitric oxide,

which is a potent vasodilator and can occur during inflammation (Haas et al., 2002). The authors concluded that their findings of hyperperfusion in mild AD may reflect "compensatory or pathological elevation of neural activity, inflammation, or elevated production of vasodilators."

The findings reported by Alsop and colleagues (2008) contrast with not only the majority of existing studies, which describe hypoperfusion in AD, but also with theories of chronic hypoperfusion "as a primary mechanism of AD" (de la Torre, 2002). Nevertheless, there is at least one other study that showed a general pattern of hypoperfusion in MCI and AD compared to cognitively normal adults with the exception of hyperperfusion in frontobasal regions in those with mild AD (Luckhaus et al., 2008). Similar to Alsop and colleagues (2008), Luckhaus et al. noted that one possible interpretation of this finding is that hyperperfusion may reflect functional compensatory mechanisms.

Our finding of increased resting state perfusion in APOE ε4 carriers relative to non-ε4 carriers corroborates the only existing published report using ASL MRI to examine MTL resting state perfusion in individuals at increased risk for AD by virtue of the presence of the APOE ε4 allele (Fleisher et al., 2009). Although somewhat controversial, based on evidence from epidemiological, pharmacotherapetuic, and neuroimaging studies, it has been argued that AD can be conceptualized as a vascular disorder that begins as brain hypoperfusion, which results in a "neuronal energy crisis" and subsequently leads to the cellular and molecular changes observed in AD (see de la Torre, 2009, for review). As Fleisher and colleagues noted, preclinical AD may

involve metabolic changes resulting in increased resting state CBF "in an effort to compensate for functionally impaired metabolic substrate." Given that apoE plays a role in lipid transport and cell maintenance and repair, as well as its link to cardiovascular disease and hypertension (e.g., Niu et al., 2009), it is possible that APOE ε4 carriers, including even those who do not go on to develop dementia, show neurovascular dysfunction.

Further, the presence of resting state CBF differences between groups may have important implications for the interpretation of FMRI activation results. Based on their findings of individuals at "high risk" for AD demonstrating elevated resting CBF and decreased fractional BOLD and CBF responses to encoding relative to those at "low risk" but no between group differences in absolute CBF during the task, Fleisher and colleagues (2009) argued that when assessed as changes from baseline values, percent change CBF may be influenced by differences in resting state. Fractional changes in the BOLD signal may also depend, in part, on baseline CBF. Therefore, BOLD activation "should not be directly interpreted as representing neuronal activity, but reflect a more complex relationship between vascular reactivity, cerebral blood flow, oxygen utilization, and the baseline state."

In the present study, we did not find significant main effects for CBF or BOLD activation during memory encoding. However, our finding of a trend toward a significant interaction may explain, in part, the discrepancies in previously published studies. In addition, this finding corroborates recent FMRI findings demonstrating complex interactions between APOE genotype and other risk factors for AD (e.g.,

family history) on FMRI activation (Johnson et al., 2006; Xu et al., 2009). Taken together, these findings suggest that modeling additional risk factors may help explain inconsistencies among previous studies regarding whether APOE or cognitive status lead to increased or decreased FMRI activation in individuals at risk for AD (Johnson et al., 2006).

Individuals with MCI who are ε4 carriers convert to dementia more quickly than non-ε4 carriers (e.g., Okello et al., 2009; Petersen et al., 1995) and demonstrate accelerated rates of atrophy (e.g., Hamalainen et al., 2008). This increased activation in the individuals who are likely at the greatest risk for developing dementia by virtue of the presence of two separate risk factors may reflect compensatory mechanisms evoked to maintain performance in the context of developing AD pathology or be suggestive of "excitotoxicity and impending neuronal failure" (Sperling et al., 2010).

Given that there were no between group differences in performance on the post-scanning recognition task and all participants performed adequately, it is possible that the MCI ε4 carriers may have recruited additional resources in order to perform the task at a level equivalent to their counterparts who were not at increased risk. It would be expected that the right MTL would demonstrate a greater response relative to the left MTL during encoding of visual/nonverbal information (e.g., Martin, 1999), such as the landscape images used as stimuli during the present study. In addition, consistent with past studies showing that, regardless of whether stimuli was verbal or pictorial, APOE ε4 carriers have been shown to activate right hemisphere structures, including the hippocampus, to a greater degree as part of a compensatory mechanism

(Bondi et al., 2005; Han et al., 2007), and we showed a nearly significant interaction indicating that those at highest risk demonstrated increased activation in the right MTL.

Another possible interpretation of our findings is related to the "default network," a brain network involved in internal modes of cognition (see Buckner, Andrews-Hanna, & Schacter, 2008, for review; Raichle et al., 2001). A growing body of evidence indicates that the default network shows a pattern of "deactivation" characterized by being more active in passive relative to active tasks/conditions. In particular, these regions are thought to normally deactivate during memory encoding or other cognitively demanding tasks relative involving external stimuli. The core regions in this network include the ventral medial prefrontal cortex, posterior cingulate/retrosplenial cortex, inferior parietal lobule, lateral temporal cortex, dorsal medial prefrontal cortex, and hippocampal formation. The increased activation in the APOE ε4 carrier MCI individuals may represent an impaired FMRI deactivation.

Evidence indicating that the default network is disrupted in AD comes from studies demonstrating reduced glucose metabolism (e.g., Benson et al., 1983; Kumar et al., 1991) and brain atrophy (e.g., Braak & Braak, 1991) in this patient group. Further, given that nondemented individuals at risk for AD show reduced glucose metabolism (e.g., Chao et al., 2010) and decreased hippocampal volume (Cohen et al., 2001; den Heijer et al., 2002), it is likely that disruption in the default network occurs during the preclinical period prior to the onset of dementia. Indeed, Persson and colleagues (2008) demonstrated reduced deactivation in nondemented older adult ε4

carriers relative to non-ε4 carriers, indicating that these alterations in deactivation occur in the absence of dementia.

Corroborating the finding reported by Persson et al. (2008), Pihlajamaki and colleagues (2010) recently reported results from a study of 75 older adults including individuals with normal cognition, MCI, and mild AD who underwent a face-name associative memory FMRI task. Even in cognitively normal older adults, ε4 carriers showed decreased deactivation in posteromedial regions, including the precuneus and posterior cingulate, compared to non-ε4 carriers. Posteromedial cortices are core components of the default network, play an important role in memory encoding and retrieval, and have strong functional connections to the MTL (Sperling et al., 2010). Further, "greater failure of posteromedial deactivation was related to worse memory performance across all subjects." It has been postulated that preclinical hippocampal dysfunction may underlie these early changes in deactivation with evidence from studies reporting a relationship between degree of hippocampal activation and deactivation in medial and lateral parietal areas during memory encoding (Celone et al., 2006).

Providing additional evidence for disruption of the default network in older adults at risk for AD, Sperling and colleagues (2009) used in vivo amyloid imaging to demonstrate that nondemented individual with high levels of amyloid deposition also showed reduced deactivation in the default network during memory encoding. This pattern of findings was similar to those found in AD patients and the authors argued that it provides additional support for the notion that amyloid pathology "is related to

disrupted synaptic activity in the networks supporting memory function, even prior to cognitive impairment." Sperling and colleagues reported that, in their sample, amyloid plaques tended to form initially in the posterior cingulate. However, other studies have shown that in early stages of AD amyloid plaque distribution tends to be relatively diffuse and considerably variable across individuals. In contrast, neurofibrillary tangles follow a characteristic distribution pattern initially affecting transentorhinal regions, followed by limbic structures, and, finally, isocortical regions (Braak & Braak, 1991). Nevertheless, taken together, these studies suggest that altered FMRI activity in regions of the default network have potential use as early indicators of AD risk and disease progression.

Given that AD-related neuropathology develops preferentially throughout the default network, it has been proposed that the activity within this system may facilitate the disease process (Buckner et al., 2005). There are several proposed theories attempting to explain why memory structures are susceptible to early neuropathological changes including those based on anatomy (Hyman et al., 1990) and the possibility that these structures are particularly vulnerable to toxicity as a result of their role in plasticity (Mesulam 2000). Buckner et al. suggested a new hypothesis termed the "metabolism hypothesis" that is based on the default network and argues that because this system is continuously active it may facilitate an activity or metabolism-dependent cascade that leads to the development of AD neuropathology. Individuals in the earliest stages of AD show amyloid plaques in a distribution that is very similar to that of the default network. Based on the theory proposed by Buckner

and colleagues, memory systems may be preferentially vulnerable to the AD disease process because these regions are a core component in resting brain activity throughout life.

Finally, in the present study, across all participants, increased vascular risk was associated with decreased MTL volumes and increased MTL response during picture encoding. These findings corroborate previously published reports demonstrating that in nondemented older adults increased vascular risk is associated with atrophy (e.g., Bangen et al., 2009; Knopman et al., 2005). Although the precise mechanisms remain unknown, it has been proposed that vascular risk factors, such as diabetes, have microvascular effects that cause reduced CBF and microinfarction, which then leads to atrophy (Knopman et al., 2005). In our sample we did not find a significant association between vascular risk and resting state CBF. However, increased vascular risk was related to greater FMRI response to picture encoding, which corroborates our previous findings and may reflect upsurges in neural activity related to compensatory mechanisms (Bangen et al., 2009) or vascular or metabolic alterations (D'Esposito, Deouell, & Gazzaley, 2003). Of note, the FSRP assesses a combination of current and cumulative risk factors. Current indices of the cerebrovascular system, such as pulse pressure (systolic blood pressure – diastolic blood pressure), may reflect a better index of current cerebrovascular integrity relative to those factors that emphasize cumulative/lifetime risk (Nation et al., in press). Such indices should be used in future studies examining the contributions of vascular risk factors to age-related atrophy and cognitive decline as well as the pathogenesis of AD. Nevertheless, taken together, the

present findings as well as those from previously published reports indicate that vascular risk factors are important to assess and consider when analyzing and interpreting neuroimaging findings in studies of aging and diseases that may affect cerebrovascular functioning.

There are several limitations that need to be considered when interpreting the present findings and that should be addressed in future studies. First, as is often in the case in neuroimaging studies of this type, our sample size was relatively small. As a result, we did not conduct analyses based on four groups stratified for the presence or absence of the APOE ε4 allele and cognitive status as originally planned. Our sample was heterogeneous in terms of MCI subtypes. Given that amnestic MCI tend to demonstrate demographic, genetic, and MRI findings indicative of AD neuropathology whereas such findings in non-amnestic subtypes are indicative of vascular disease (He et al., 2009), conducting analyses by subtype may have yielded interesting findings. Nevertheless, our ability to detect some group differences with this small sample size suggests that these findings were robust effects. In addition, despite heterogeneity in terms of cognitive characterization, our sample was generally relatively well-educated and medically healthy, which may have attenuated our ability to detect group differences given limited variability and truncated ranges on some variables (e.g., the stroke risk score only ranged from 2% to 22%; performance accuracy on the post-scanning recognition task only ranged from 82% to 92%) and may also limit the generalizability of our findings. Further, the ratio of men to women in our sample is concerning. However, we did run analyses and found no significant

differences in resting state CBF or CBF and BOLD response to picture encoding based on gender.

There are a variety of limitations associated with the ASL MRI technique in general as well as our specific scanning protocol, including low signal-to-noise ratio and an inability to collect whole brain functional data. As a result, we do not know how non-MTL regions may be activating or deactivating in response to our task and we cannot determine whether spared performance resulted from recruitment of frontal regions (e.g., Han et al., 2007). And we cannot rule out the possibility that the participant groups varied in terms of strategy employed or cognitive effort required to complete the task.

In addition, alterations in neurovascular coupling (Girouard & Iadecola, 2006) and brain metabolism (e.g., De Santi et al., 2001) have been demonstrated in AD. Despite equivalent stroke risk profiles and exclusionary criteria including significant cerebrovascular disease, we cannot rule out differential neurovascular coupling across risk groups. In addition, findings from a previous study of normal aging that used the same picture encoding task employed in the present study demonstrated significantly increased percent change CBF in cognitively normal older adults relative to young adults, however, there were no significant group differences in percent change BOLD. This finding was consistent with the possibility of increased $CMRO_2$ response to picture encoding in the older adults, which may reflect increased neural activity as a form of compensation (Restom et al., 2007). Given that we found a trend toward greater percent change CBF in MCI ε4 carriers relative to MCI non-ε4 carriers but no

such trend in BOLD response, it is possible that the risk groups vary in terms of functional $CMRO_2$ changes. However, as noted by Fleisher and colleagues (2009), due in part to a lack of understanding regarding neurovascular responsiveness and metabolism in APOE ε4 carriers and individuals with MCI, we did not mathematically estimate $CMRO_2$ as has been done in normal aging (Restom et al., 2007). Further, given our small sample size in certain cells, another possible explanation for our pattern of findings may be related to a power issue. Future studies with larger sample sizes may detect group differences in the BOLD response to memory encoding based on AD risk.

Studies using calibrated BOLD, an approach measuring percent change CBF and BOLD responses to a functional task and a hypercapnic challenge to allow for the estimation of functional $CMRO_2$ changes, will be useful in further elucidating the underpinnings of the BOLD and CBF response (Davis et al., 1998; Hoge et al., 1999). Future studies to elucidate mechanisms of risk-related differences should integrate multiple imaging methods including amyloid imaging and calibrated BOLD and involve a larger sample size thereby allowing for separation into MCI subtypes as well as examination of additional risk factors. Finally, given the cross-sectional design of the present study, we do not know which individuals will eventually convert to dementia. The clinical significance of the present study will be elucidated by longitudinal studies monitoring individuals at risk for developing AD over time.

In conclusion, despite these limitations, our findings provide support for the notion that individuals at risk for AD demonstrate changes in brain function occurring

in the preclinical period prior to the onset of dementia. Our findings suggest that abnormal resting state CBF and FMRI response pattern in the MTL are related to risk factors for developing AD and may be early indicators of brain dysfunction. Therefore, ASL MRI may provide a sensitive technique for identifying individuals at risk for AD, monitoring changes in neural activity due to developing AD neuropathology, and assessing effectiveness of disease-modifying treatments.

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