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

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Abstract

Functional magnetic resonance imaging (fMRI) of older adults at risk for Alzheimer's disease (AD) by virtue of their cognitive (i.e., mild cognitive impairment [MCI]) and/or genetic (i.e., apolipoprotein E [APOE] $\epsilon 4$ allele) status demonstrate divergent brain response patterns during memory encoding across studies. Using arterial spin labeling MRI, we examined the influence of AD risk on resting cerebral blood flow (CBF) as well as the CBF and blood oxygenation level dependent (BOLD) signal response to memory encoding in the medial temporal lobes (MTL) in 45 older adults (29 cognitively normal [14 APOE $\epsilon 4$ carriers and 15 noncarriers]; 16 MCI [8 APOE $\epsilon 4$ carriers, 8 noncarriers]). Risk groups were comparable in terms of mean age, years of education, gender distribution, and vascular risk burden. Individuals at genetic risk for AD by virtue of the APOE $\epsilon 4$ allele demonstrated increased MTL resting state CBF relative to $\epsilon 4$ noncarriers, whereas individuals characterized as MCI showed decreased MTL resting state CBF relative to their cognitively normal peers. For percent change CBF, there was a trend toward a cognitive status by genotype interaction. In the cognitively normal group, there was no difference in percent change CBF based on APOE genotype. In contrast, in the MCI group, APOE $\epsilon 4$ carriers demonstrated significantly greater percent change in CBF relative to $\epsilon 4$ noncarriers. No group differences were found for BOLD response. Findings suggest that abnormal resting state CBF and CBF response to memory encoding may be early indicators of brain dysfunction in individuals at risk for developing AD.

Keywords

Apolipoprotein E; cerebral blood flow; functional; hippocampus; magnetic resonance imaging; memory; mild cognitive impairment

INTRODUCTION

Neuropathological studies of cognitively normal older adults suggest that the Alzheimer's disease (AD)-related pathophysiological process begins during a presymptomatic stage of the disease [1, 2]. Identifying sensitive markers that can detect early alterations in brain structure or function that occur prior to irreversible neuropathological damage and while individuals are still functioning independently in their daily lives is essential for early detection of disease and, ultimately, early intervention with disease-modifying therapy to slow or prevent decline and thereby preserve quality of life [3, 4]. Both the apolipoprotein E (APOE) $\epsilon 4$ allele and mild cognitive impairment (MCI) are related to increased risk of developing AD and have been associated with structural and functional brain changes in nondemented older adults. Recent evidence suggests that functional brain changes may precede structural changes indicating that functional magnetic resonance imaging (fMRI), in particular, has great potential as a non-invasive technique for detecting early brain changes *in vivo* [5].

Findings from fMRI studies comparing brain response during memory encoding among individuals with MCI relative to cognitively normal older adults have been variable [6] and include findings of both increases in activation [7, 8] and decreases in activation [9–11]. It has been proposed that variability in observed activation patterns may be related to severity of cognitive deficits across MCI patients (e.g., 'early' versus 'late' MCI) [6]. Twamley et al. [12] reviewed studies of preclinical AD and proposed a nonlinear trajectory of episodic memory decline in which there is a long period of lowered but stable memory capacity in individuals with preclinical AD [13]—perhaps due to neural compensatory mechanisms—that is followed by a relatively precipitous decline in the period immediately preceding the development of overt dementia [14, 15]. Smith and colleagues [16] directly tested whether a plateau versus linear model of decline better fit the longitudinal course of decline in episodic memory in nearly 200 persons followed from normal or MCI status to clinically probable AD. Modeling these data demonstrated that the plateau model did in fact better fit the data for episodic memory but not for other cognitive domains. Dickerson and colleagues, with functional MRI data, have also suggested that brain response during memory tasks follow a pattern during the course of MCI in which there is an early phase involving greater activation in individuals who are very mildly impaired, potentially reflecting an inefficient compensatory response, and a later phase involving decreased activation as medial temporal lobe (MTL) atrophy and memory difficulties progress and compensatory mechanisms are fully exhausted [6, 17, 18].

In terms of genetic risk, fMRI studies examining brain activation during memory encoding by APOE genotype have often demonstrated patterns of greater activation in APOE $\epsilon 4$ carriers relative to noncarriers [19–21]. Such findings have 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. However, other recent work demonstrates increased blood oxygenation level dependent (BOLD) response in APOE $\epsilon 4$ carriers but decreased BOLD response in presymptomatic carriers of familial AD mutations during a novelty encoding task, raising the possibility that the increased response associated with the APOE $\epsilon 4$ allele may not be related to cognitive compensation or reserve but rather to an unidentified effect of the $\epsilon 4$ allele on cerebral vascular reactivity [22].

In addition to alterations in brain response during memory encoding, studies have demonstrated abnormalities in resting cerebral blood flow (CBF) in adults at cognitive and genetic risk. However, results have been mixed and have included both increases and decreases in CBF across different studies but also within the same participants across

different brain regions. Among individuals with MCI, relative to their cognitively normal peers, reduced CBF has been reported in regions including the anterior cingulate, posterior cingulate, medial temporal lobe, cuneus, precuneus, and parietal association areas [23–28], whereas increased CBF has been reported in the hippocampus, amygdala, and basal ganglia [24]. Based on their findings of increases and decreases in CBF across different regions within the same participants, Dai and colleagues [24] argued that the transition from normal aging to dementia involves dynamic pathophysiologic changes.

Similarly, findings from studies examining differences in resting CBF based on APOE genotype have been mixed. Positron emission tomography (PET) studies have demonstrated reduced metabolism in regions affected in AD (e.g., parietal regions) in cognitively normal older adult carriers of the APOE $\epsilon 4$ allele relative to noncarriers [29]. In contrast, Fleisher and colleagues [30] reported elevated resting state perfusion in the MTL in older individuals at risk for AD, as defined by both the presence of a positive family history of AD and at least one APOE $\epsilon 4$ allele, suggesting that preclinical AD may involve increases in resting CBF in an effort to compensate for altered metabolism. Further, in a longitudinal PET study of nondemented older adults, APOE $\epsilon 4$ carriers showed greater regional CBF at baseline but greater decline over a follow-up period of approximately eight years relative to $\epsilon 4$ noncarriers in frontal, parietal, and temporal regions [31].

fMRI is a promising approach with many strengths for studying individuals at risk for AD. Most fMRI studies examining brain response to cognitive activation are based on the BOLD signal, an indirect measure of neural activity that reflects changes in deoxyhemoglobin content, which depends on a complex function of CBF, cerebral blood volume, and cerebral metabolic rate of oxygen (CMRO₂) [32]. The BOLD signal can be affected by cerebrovascular changes associated with aging or disease including changes in ultrastructure of cerebral vessels due to atherosclerosis, decreased resting CBF, altered vascular reactivity, and reduced resting CMRO₂ [33]. Given these issues, it may be difficult to determine whether differences observed in the BOLD signal in aging or disease reflect differences in neural activity or other cerebrovascular factors that influence the BOLD signal. Arterial spin labeling (ASL) MRI offers a number of advantages beyond that provided by BOLD fMRI, including: 1) ASL measures a well defined physiological quantity (CBF typically in units of milliliters of blood per 100 grams of tissue per minute); 2) ASL processing approaches involve a differencing technique that results in reduced sensitivity to low-frequency drifts and make it useful in experiments with long stimulus durations [34]; 3) ASL can be used with imaging techniques that reduce susceptibility-related signal losses (e.g., spin-echo readouts); and 4) the ASL perfusion signal is well localized to the capillary bed 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 [35, 36]. Therefore, the use of ASL MRI, which provides both baseline and functional changes in CBF, may provide a richer context for interpretation of the BOLD signal change [36] and better elucidate the complex relationship between neural and vascular mechanisms in both healthy aging and disease.

Thus, the aim of this study was to examine CBF and BOLD response in nondemented older adults using ASL fMRI in order to characterize the influence of cognitive (i.e., MCI) and genetic risk (i.e., APOE genotype) for AD on MTL CBF during rest as well as MTL response during picture encoding. We predicted that individuals with MCI would demonstrate hypoperfusion in the MTL during rest when compared to their cognitively normal counterparts. In contrast, we hypothesized that APOE $\epsilon 4$ carriers would show increased resting CBF relative to $\epsilon 4$ noncarriers. Based on previous findings suggesting the potential establishment of compensatory mechanisms prior to the development of AD, we predicted that individuals at risk would exhibit greater MTL response during memory

encoding compared to their counterparts without these risk factors. To our knowledge, this is the first study to date to use ASL to examine memory encoding in older adults at risk for AD by virtue of these risk factors.

MATERIALS AND METHODS

Participants

Forty-five community-dwelling adults aged 60 or older were recruited from ongoing research studies. All participants underwent neurological, medical, laboratory, and neuropsychological examinations. MCI diagnoses were made based on the consensus of two neuropsychologists using the empirically-validated criteria proposed by Jak and colleagues [37]. Based on these criteria, 29 individuals were classified as cognitively normal (14 ϵ 4 carriers and 15 noncarriers) and 16 met criteria for MCI (8 ϵ 4 carriers and 8 noncarriers). In the MCI group, individuals met criteria for the following subtypes: five single domain amnesic MCI; two single domain non-amnesic MCI; four multiple domain amnesic MCI; and five multiple domain non-amnesic MCI. Vascular risk was assessed using the Framingham Stroke Risk Profile [38] to estimate the 10-year probability for risk of stroke using gender-corrected scores based on the following risk factors: age, systolic blood pressure, diabetes mellitus, cigarette smoking, history of cardiovascular disease, atrial fibrillation, left ventricular hypertrophy, and use of antihypertensive medications. Individuals with a history of dementia, significant cerebrovascular disease (e.g., stroke), other neurological disorders, major psychiatric disorders, or contraindications to MRI (e.g., pacemaker) were excluded. All data were obtained in accordance with UCSD institutional review board-approved procedures and the guidelines of the Helsinki Declaration. Written informed consent was obtained from all participants.

Neuropsychological assessment

Neuropsychological measures were divided into five cognitive domains: 1) memory, 2) attention, 3) language, 4) visuospatial functioning, and 5) executive functioning (Table 2a and 2b). In addition, the Independent Living Scales [39], a performance-based measure designed to assess an individual's ability to independently complete complex activities of daily living, was administered in order to verify the absence of functional impairment. In an attempt to minimize the effect of demographic characteristics (e.g., age, education, gender) on neuropsychological test performance, raw scores from these measures were converted to demographically-corrected standard scores using the best available normative data. Of note, some participants did not complete the entire neuropsychological battery. Approximately 10% of the neuropsychological data were missing. However, analyses to determine whether there were differences between the risk groups in terms of degree of missing data revealed that there were no significant differences between the MCI and cognitively normal groups ($\chi^2 = 3.10$, $df = 1$, $p = 0.08$, $\phi = 0.26$) or between the APOE ϵ 4 carriers and non-carriers ($\chi^2 = 0.04$, $df = 1$, $p = 0.83$, $\phi = 0.03$).

Picture encoding scanner task

During scanning, participants completed a memory-encoding task consisting of the presentation of novel and familiar landscape images (adapted from Stern and colleagues [40]; Fig. 1). Prior to 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. The scanner task involved a blocked design in order to maximize detection power. Each block consisted of either ten familiar (i.e., repeated) or ten novel images. Each image was displayed for two seconds with a half second interval between images. Five blocks of novel and five blocks of familiar scenes were presented per run with three runs per subject. To ensure that participants maintained attention, they were

instructed to determine whether each image had a horizontal or vertical aspect ratio and make an appropriate response using a response box. Although accuracy was not recorded for later statistical analysis, visual monitoring of responses by co-authors during scanning revealed that all participants performed this task with a high degree of accuracy. In addition, a subset of participants ($n = 21$ including 16 cognitive normal, 5 MCI, 7 APOE $\epsilon 4$ noncarriers, 14 APOE $\epsilon 4$ carriers) completed a two-alternative forced choice recognition task immediately after scanning. The mean accuracy level on the post-scanning recognition task was 88.37% (range = 82 to 92%).

Image acquisition

Participants were scanned in a 3.0 Tesla General Electric EXCITE whole body imager with an 8-channel receive-only head coil. A high-resolution T1-weighted Fast Spoiled Gradient Recall scan was acquired: 172 1-mm thick contiguous sagittal slices, FOV = 25 cm, TR = 8 ms, TE = 3.1 ms, flip angle = 12° , T1 = 450, 256×192 matrix, Bandwidth = 31.25 kHz. Functional BOLD and CBF data were simultaneously acquired using a pulsed ASL sequence (QUantitative Imaging of Perfusion with a Single Subtraction, version II; PICORE QUIPSS II) [41] with a dual-echo single-shot spiral acquisition. Imaging parameters of the ASL scan were as follows: 5 6-mm thick contiguous oblique slices acquired at the level of the hippocampus, FOV = 24 cm, TR = 3000 ms, TE1 = 2.4, TE2 = 24 ms, flip angle = 90° , TI1 = 700 ms, TI2 = 1400 ms, 64×64 matrix, tag thickness 20 cm, tag to proximal slice gap 1 cm. One resting-state and three functional scans during which participants completed the picture encoding task were obtained. In addition, a cerebrospinal fluid (CSF) reference scan was acquired for use in CBF quantification. During the ASL scans, cardiac oximetry and respiratory effort signals were recorded using a pulse oximeter and a respiratory effort transducer, respectively, in order to reduce physiological noise in the ASL data [42].

Data processing and analyses

Structural imaging processing—Structural and functional imaging data were processed using Analysis of Functional NeuroImages (AFNI) [43], FMRIB Software Library (FSL) [44], and in-house MATLAB scripts. Anatomical scans were skull stripped using Brain Surface Extractor [45] and then manually edited. The structural image was then segmented into gray, white, and CSF compartments using FSL's FAST program [46]. For use in the fMRI analyses, an automated subcortical segmentation program (Freesurfer ASEG) [47] was applied to the anatomical image to derive a region of interest (ROI) containing the hippocampus. Then a region-growing algorithm was used to delineate an ROI containing the parahippocampal gyrus. Both ROIs were visually inspected and manually edited in to increase precision. The Freesurfer-derived MTL ROI consisting of bilateral hippocampal and parahippocampal structures was then down-sampled to the resolution of the functional images. However, to further improve precision for volumetric analyses, hippocampal and parahippocampal ROIs (Fig. 2) were also manually outlined in the coronal plane using methods previously published [48]. High levels of inter-rater reliability for the manual procedure were established (intraclass correlation coefficients >0.90). The tissue compartments and ROIs were normalized by dividing each value by total brain volume.

Functional image processing—Each participant's functional datasets were motion corrected and the anatomical volume was aligned with the functional volume. Functional CBF images were computed from the running subtraction of the control and tag images [49]. Functional BOLD images were created from the running average of the second echo [49]. For each participant, a mean ASL image was formed from the average difference of the control and tag images from the resting scan. This mean ASL image was converted to absolute units of CBF (mL/100 g tissue/minute) using the CSF signal to estimate the equilibrium magnetization of blood [50]. For the functional scans, CBF 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. Pre-whitening was performed to account for temporal autocorrelations [51, 52]. An independent set of physiological noise and low frequency nuisance term regressors was used for each functional run within the concatenated GLM [53]. The down-sampled Freesurfer-derived MTL ROI consisting of bilateral hippocampal and parahippocampal structures was applied to the functional data to extract CBF and BOLD signal data. Contrasts between novel and repeated landscape images were calculated. Voxels were considered activated using overall threshold of $p < 0.05$ (with correction for multiple comparisons). To be included in further analyses, it was required that at least one voxel in each activated CBF cluster be contiguous with one voxel in an activated BOLD cluster.

To correct the CBF measures for partial volume effects, we used previously published methods [26] that assumed that CSF has zero CBF and that CBF in gray matter is 2.5 times greater than that in white matter. Partial volume corrected CBF signal intensities were calculated with the following formula: $CBF_{corr} = CBF_{uncorr} / (GM + 0.4 * WM)$. CBF_{corr} and CBF_{uncorr} are corrected and uncorrected CBF values. GM and WM represent gray matter and white matter partial volume fractions, respectively.

Statistical analyses

Independent samples t -tests for continuous variables and χ^2 tests for categorical variables were performed to compare risk groups in terms of demographic, vascular risk, and cognitive variables; linear regression analyses were conducted to examine the relationship between neuroimaging variables and risk; bivariate correlational analyses were conducted to examine the associations among resting CBF, MTL CBF, and BOLD response during picture encoding, and performance on standardized memory measures administered outside of the scanner (i.e., DRS Memory subscale, WMS-R Logical Memory Delayed Recall, WMS-R Visual Reproduction Delayed Recall, and CVLT Trials 1–5 Total Learning); and finally, supplemental analyses examined cognitive by genetic status interaction effects on resting CBF and brain response during memory encoding.

Each set of analyses comparing the risk groups on the same type of dependent variable (e.g., neuropsychological tests) was treated as an omnibus test with Bonferroni corrections for multiple comparisons separately applied for each set of analyses. The resulting adjusted alpha values controlling for multiple comparisons ranged from 0.002 (i.e., 0.05/23, for the t -tests comparing risk groups on neuropsychological test performance) to 0.05 (e.g., for the regression analyses comparing risk groups on resting CBF) and are included in the description of results for each group of tests.

Given the small number of participants in some cells, the statistical analyses did not compare four participant groups (i.e., MCI/non- $\epsilon 4$, MCI/ $\epsilon 4$, cognitively normal/non- $\epsilon 4$, and cognitively normal/ $\epsilon 4$). Instead, all analyses were run twice: once comparing MCI to cognitively normal participants and again comparing APOE $\epsilon 4$ carriers to noncarriers. Data from one MCI APOE $\epsilon 4$ carrier was excluded from group analyses examining percent change CBF and BOLD during picture encoding due to technical difficulties during scan acquisition. All statistical analyses were performed in SPSS (version 18.0).

RESULTS

Demographic and neuropsychological variables: Cognitively normal versus MCI

The cognitively normal and MCI participants did not differ significantly in terms of demographic variables (Table 1a). As expected, using the Bonferroni adjusted alpha level of 0.002 (i.e., 0.05/23) for 23 neuropsychological variables, MCI participants (collapsed across

genotype) performed significantly more poorly relative to their cognitively normal counterparts on various neuropsychological measures including those assessing global cognitive functioning, memory, language, and visuospatial functioning (p -values <0.002 ; Table 2a).

Given that there was a trend toward a significant difference between the MCI and cognitively normal groups in terms of gender distribution, we performed analyses to determine whether men and women differed on the primary variables of interest: resting state CBF, percent change CBF, and percent change BOLD. There were no significant differences between men and women across these variables (all p -values >0.05).

Demographic and neuropsychological variables: APOE $\epsilon 4$ carriers versus noncarriers

The APOE genotype groups did not differ significantly in terms of demographic variables (Table 1b). Using the Bonferroni adjusted alpha level of 0.002 (i.e., $0.05/23$) for 23 neuropsychological variables, the APOE genotype groups did not differ in terms of cognitive abilities (all p -values >0.04 ; Table 2b).

Structural MRI indices: Cognitively normal versus MCI

Using the Bonferroni adjusted alpha level of 0.01 (i.e., $0.05/5$) for five structural MRI comparisons, the MCI and cognitively normal groups did not significantly differ in terms of normalized volumes of the whole brain gray matter ($t = 0.93$, $df = 43$, $p = 0.36$, Cohen's $d = 0.30$) or whole brain white matter ($t = 0.39$, $df = 43$, $p = 0.70$, Cohen's $d = 0.12$). There was a trend toward reduced MTL volumes among the MCI individuals relative to their cognitively normal counterparts ($t = 2.34$, $df = 43$, $p = 0.02$, Cohen's $d = 0.74$). When the two regions constituting the MTL ROI were examined individually, there were trends toward reduced volumes of both the hippocampus ($t = 2.04$, $df = 43$, $p = 0.05$, Cohen's $d = 0.64$) and parahippocampus ($t = 2.18$, $df = 43$, $p = 0.04$, Cohen's $d = 0.71$; Table 3a) among the MCI group.

Structural MRI indices: APOE $\epsilon 4$ carriers versus noncarriers

Using the Bonferroni adjusted alpha level of 0.01 (i.e., $0.05/5$) for five structural MRI comparisons, the APOE genotype groups did not significantly differ in terms of any of the structural MRI measures (all p -values >0.01 ; Table 3b).

Resting CBF: Cognitively normal versus MCI

Individuals with MCI demonstrated significantly reduced bilateral MTL resting state CBF relative to their cognitively normal counterparts ($t = 2.23$, $df = 43$, $p = 0.03$, Cohen's $d = 0.72$; Table 4a; Fig. 3). When left and right hemispheres were examined separately, individuals with MCI demonstrated significantly reduced right MTL resting state CBF compared to their cognitively normal peers ($t = 2.49$, $df = 43$, $p = 0.02$, Cohen's $d = 0.78$). There was a trend toward the MCI group demonstrating reduced left MTL resting state CBF ($t = 1.88$, $df = 43$, $p = 0.07$, Cohen's $d = 0.61$).

Resting CBF: APOE $\epsilon 4$ carriers versus noncarriers

APOE $\epsilon 4$ carriers demonstrated significantly increased bilateral MTL resting state CBF compared to noncarriers ($t = 2.25$, $df = 43$, $p = 0.03$, Cohen's $d = 0.67$; Fig. 3). When left and right hemispheres were examined separately, the APOE $\epsilon 4$ carriers demonstrated increased resting CBF in the left ($t = 2.18$, $df = 43$, $p = 0.04$, Cohen's $d = 0.65$) and right MTL compared to noncarriers ($t = 2.56$, $df = 43$, $p = 0.02$, Cohen's $d = 0.76$; Table 4b).

Functional CBF and BOLD response during picture encoding: Cognitively normal versus MCI

Cognitive status did not predict bilateral, left, or right MTL percent change CBF or BOLD during picture encoding (all p -values >0.05 ; Table 4a).

Functional CBF and BOLD response during picture encoding: APOE $\epsilon 4$ carriers versus noncarriers

Genotype did not account for a significant amount of overall variance in bilateral, left, or right MTL percent change CBF or BOLD during picture encoding (all p -values >0.05 ; Table 4b).

Supplemental analyses: Interaction between cognitive status and APOE genotype

The interaction between cognitive status and APOE genotype was explored using hierarchical regression. When entered on step 1, cognitive status or APOE genotype did not individually account for a significant amount of overall variance in right MTL percent change CBF ($F = 0.66$, $p = 0.52$, $R^2 = 0.03$). Neither cognitive status nor genotype was significantly associated with right MTL percent change CBF (cognitive status: $\beta = -0.01$, $p = 0.93$; genotype: $\beta = 0.18$, $p = 0.26$). When the interaction term was entered on step 2, the model was nearly significant ($F = 2.63$, $p = 0.07$, $R^2 = 0.14$). Further, the interaction term accounted for a significant increase in explained variance ($\Delta F = 6.37$, $p = 0.02$, $\Delta R^2 = 0.14$, $\beta = 0.57$, $p = 0.02$). Among the cognitively normal adults, there was not a significant difference in CBF response based on APOE genotype ($t = 0.42$, $p = 0.68$; means = 95.13 for $\epsilon 4$ carriers and 104.47 for noncarriers). However, in the MCI group, $\epsilon 4$ carriers demonstrated significantly greater CBF response relative to noncarriers ($t = 3.00$, $p = 0.02$; means = 165.00 for $\epsilon 4$ group and 55.22 for non- $\epsilon 4$ group; Fig. 4; Table 5). When the same regression analyses were conducted with bilateral and left percent change CBF as well as resting CBF and bilateral, left, and right percent change BOLD serving as dependent variables, there were no significant interactions (all p 's > 0.05).

Associations between cognition and CBF and BOLD

Using the Bonferroni adjusted alpha level of 0.013 (i.e., $0.05/4$) for analyses correlating four memory variables with resting CBF, across all participants, there were trends toward increased resting CBF and better performance on memory measures administered outside of the scanner (DRS Memory subscale T score: $r = 0.36$, $p = 0.02$; WMS-R Logical Memory delayed recall MOANS scaled score: $r = 0.28$, $p = 0.07$). When these analyses were performed separately for the different risk groups, among MCI participants, increased resting CBF was associated with better performance on the word list-learning task (CVLT List A Trials 1–5 Total T-score: $r = 0.53$, $p = 0.04$). For cognitively normal adults, there were no significant correlations among resting CBF and memory performance (all p -values >0.05). Among the APOE $\epsilon 4$ carriers, greater resting CBF was associated with better non-verbal memory abilities (WMS-R Visual Reproduction immediate recall MOANS scaled score: $r = 0.61$, $p = 0.02$). Among noncarriers, increased resting CBF was significantly correlated with better story memory performance (WMS-R Logical Memory delayed recall MOANS scaled score: $r = 0.47$, $p = 0.03$).

Analyses correlating memory abilities with MTL response during picture encoding revealed that, across all participants, there were no associations among CBF or BOLD response during picture encoding and memory abilities (all p -values for percent change CBF >0.16 ; all p -values for percent change BOLD >0.40). When these analyses were performed separately for the different risk groups, among MCI participants, greater percent change CBF during picture encoding was associated with poorer performance on memory measures

completed outside of the scanner (DRS Memory T-score: $r = -0.77$, $p = 0.002$). There were no other significant associations among memory abilities and MTL response to picture encoding (all p -values >0.013).

DISCUSSION

To summarize our findings, older adults at increased risk for AD showed alterations in resting CBF in the medial temporal lobes. The nature of these changes differed depending on the type of risk factor. Specifically, individuals at genetic risk for AD by virtue of the APOE $\epsilon 4$ allele demonstrated increased resting CBF in the MTL relative to their non- $\epsilon 4$ counterparts. In contrast, individuals characterized as MCI showed decreased MTL resting state CBF compared to their cognitively normal peers. Across all participants, there were positive associations between performance on verbal and nonverbal memory measures completed outside of the scanner and resting CBF. Further, although there were no significant main effects based on AD risk in terms of CBF or BOLD signal response to memory encoding, there was 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 $\epsilon 4$ carriers demonstrated greater percent change CBF relative to noncarriers.

Our finding of reduced resting state CBF in individuals with MCI is consistent with an accumulating body of evidence from ASL MRI studies [24, 26, 28]. Similar to these prior studies, we found MCI participants to have reduced perfusion in the MTL, which is consistent with the notion that MCI individuals show decreases in CBF in the regions first affected in AD and before the clinical onset of dementia. A recent longitudinal study involving ASL MRI demonstrated that resting hypoperfusion of the right inferior parietal cortex and right middle frontal cortex at baseline predicted progression from MCI to dementia at three-year follow-up [54]. One limitation of Chao et al.'s [54] study was that the scanning acquisition did not examine more inferior regions including the MTL. Nevertheless, their finding is consistent with other prior FDG-PET and SPECT studies reporting reduced metabolism in right temporoparietal cortex [55], bilateral temporoparietal regions and precuneus [25], and right inferior parietal cortex [56] at follow-up periods ranging from 18 months to three years. In the present study, among the MCI participants, decreased resting CBF was associated with poorer verbal learning suggesting that reduced perfusion is associated with decreased cognitive functioning in this group.

The finding of increased resting state perfusion in APOE $\epsilon 4$ carriers relative to noncarriers corroborates previous work using ASL MRI to examine MTL resting perfusion in individuals at increased risk for AD by virtue of the presence of the APOE $\epsilon 4$ allele [30]. Preclinical AD may involve increases in resting CBF in an effort to compensate for altered metabolism [30] and/or increased demand for oxygen and glucose due to compensatory changes in neuronal activity.

In the present study, two risk factors for the development of AD resulted in opposite patterns of resting CBF alterations. In general, increases in CBF have been interpreted as reflecting cellular and/or vascular compensatory mechanisms whereas decreases in CBF have typically been interpreted as indicating reduced brain functioning. As previously noted, functional MRI studies of MCI have suggested that brain response during memory processing follows a pattern during the course of MCI in which there is an early phase involving greater activation, potentially reflecting a compensatory response, and a later phase involving decreased activation as MTL neuropathology and memory difficulties progress and MTL regions are no longer able to activate [18]. It is possible that alterations in resting CBF are also associated with severity of MTL compromise and that cognitively intact APOE $\epsilon 4$

carriers with no significant MTL atrophy may demonstrate more effective compensatory mechanisms relative to individuals with MCI who show MTL compromise as evidenced by memory difficulties and atrophy. Alternatively, recent evidence suggests that the frequently observed pattern of increases in BOLD fMRI signal among APOE $\epsilon 4$ carriers may not be related to cognitive compensation but rather to an unidentified effect of the APOE $\epsilon 4$ allele on cerebral vascular reactivity [22]. Given that in this recent study APOE $\epsilon 4$ carriers showed increased BOLD response during memory encoding but presymptomatic carriers of familial AD mutations showed decreased BOLD response, it appears that alterations in fMRI signal among APOE $\epsilon 4$ carriers may be related to changes in cerebrovascular functioning that may be partly independent of AD pathology. Therefore, risk factors for the development of AD may relate to various pathological processes some of which are partly independent of AD pathology potentially leading to divergent patterns of CBF alterations depending on the particular risk factors examined.

The presence of resting CBF differences between groups may also have important implications for the interpretation of fMRI activation results. For instance, based on 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 [30] argued that, when assessed as changes from baseline values, percent change CBF and BOLD signal may be influenced by differences in resting state. As Fleisher and colleagues [30] argue, BOLD activation should be interpreted as reflecting a complex relationship among vascular reactivity, CBF, oxygen metabolism, and the baseline state rather than as a direct measure of neuronal activity.

In the present study, there were no significant main effects for CBF or BOLD response during memory encoding. However, the 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 [57, 58]. Such 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 [57].

Individuals with MCI who are $\epsilon 4$ carriers convert to dementia more quickly than noncarriers [59, 60]. 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 might be suggestive of “excitotoxicity and impending neuronal failure” [61].

Given that those MCI with poorer memory abilities showed greater task-related CBF response during picture encoding, it is possible that the MCI $\epsilon 4$ carriers may have recruited additional resources in order to complete the memory tasks. It would be expected that the right MTL would demonstrate a greater response relative to the left MTL during encoding of visual/nonverbal information [62], such as the landscape images used as stimuli during the present study. In addition, consistent with past studies showing that, regardless of whether stimuli were verbal or pictorial, APOE $\epsilon 4$ carriers have been shown to activate right hemisphere structures, including the hippocampus, to a greater degree as part of a potential compensatory mechanism [19, 21], there was a nearly significant interaction indicating that those at highest risk demonstrated increased activation in the right MTL.

There are limitations that need to be considered when interpreting the present findings and that should be addressed in future studies. First, as is often 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 $\epsilon 4$ allele and cognitive status except in a supplemental or exploratory manner. In addition, despite heterogeneity in terms of cognitive characterization, our sample was generally relatively well-educated and medically healthy. The lack of variability may have attenuated our ability to detect group differences and may also limit the generalizability of our results. Based on visual monitoring of scanner task responses by the co-authors as well as the low difficulty level of the task, it is likely that all participants performed the task with a similar high degree of accuracy. However, given that accuracy data were not recorded for later statistical analysis, it is possible that there were undetected differences in scanner task performance between risk groups. Nonetheless, because the task involved an implicit encoding task with a simple visual discrimination task to ensure adequate attention, errors in task performance (if they occurred) likely do not reflect problems with picture encoding. Further, there are 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. Regarding the latter, we do not know how non-MTL regions (e.g., frontal regions) may be activating or deactivating in response to the picture encoding task. Further, given the cross-sectional design of the present study, we do not yet know which individuals will eventually progress to dementia. The clinical significance of the present study will be elucidated by longitudinal studies monitoring individuals at risk for developing AD over time. Finally, future studies using calibrated fMRI, an approach measuring percent change CBF and BOLD responses to a functional task as well as to a separate hypercapnic challenge (e.g., 5% CO₂ inhalation) will allow for the estimation of functional CMRO₂ changes [63]. This technique will be useful in further elucidating the underpinnings of the BOLD and CBF response to memory encoding. Future studies designed to examine mechanisms of risk-related differences should integrate multiple imaging methods including calibrated fMRI and involve a larger sample size, thereby allowing for separation into MCI subtypes as well as examination of additional risk factors.

In conclusion, our findings provide support for the notion that individuals at risk for AD demonstrate changes in brain function occurring in the preclinical or early periods prior to the onset of dementia. Our findings suggest that distinct alterations in resting CBF and CBF response patterns in the MTL are related to different risk factors for developing AD and may be early indicators of brain dysfunction. Therefore, ASL MRI measures may have potential to serve as sensitive biomarkers 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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Fig. 1.
Picture encoding task.

Fig. 2.
Coronal sections displaying the manually-outlined hippocampal and parahippocampal regions of interest.

Fig. 3. Means and standard errors of medial temporal lobe resting state cerebral blood flow by risk group.

Fig. 4. Cognitive status by genotype interaction for left and right medial temporal lobe percent change cerebral blood flow. Bars represent standard error.

Demographic characteristics for individuals with mild cognitive impairment and cognitively normal participants

Table 1a

	CN, <i>n</i> = 29 mean (sd)	MCI, <i>n</i> = 16 mean (sd)	df	<i>t</i> or χ^2	<i>p</i>	Cohen's <i>d</i> /phi*
Age	74.79 (7.98)	76.88 (7.31)	43	0.86	0.39	0.27
Education	15.86 (2.33)	15.56 (2.53)	43	0.40	0.69	0.12
Gender (male/female)	10/19	10/6	1	3.28	0.07	0.27
APOE genotype (e4+/e4-)	14/15	8/8	1	0.01	0.91	0.02
Vascular risk (10-year probability of stroke)	9.59 (5.06)	12.75 (6.68)	43	1.79	0.08	0.53

CN = cognitively normal; MCI = mild cognitive impairment; df = degrees of freedom; APOE = apolipoprotein E.

* Cohen's *d* values are reported for *t*-tests and phi coefficients are reported for χ^2 tests.

Table 1b

Demographic characteristics for APOE ε4 carriers and noncarriers

	ε4 noncarriers, <i>n</i> = 23 mean (sd)	ε4 carriers, <i>n</i> = 22 mean (sd)	df	<i>t</i> or χ^2	<i>p</i>	Cohen's <i>d</i>/phi
Age	76.35 (7.71)	74.68 (7.71)	43	0.72	0.86	0.22
Education	15.96 (2.50)	15.55 (2.28)	43	0.58	0.57	0.17
Gender (male/female)	11/12	9/13	1	0.22	0.64	0.07
Cognitive status (CN/MCI)	15/8	14/8	1	0.01	0.91	0.02
Vascular risk (10-year probability of stroke)	10.13 (4.96)	11.32 (6.66)	43	0.68	0.50	0.20

See Table 1a for list of abbreviations.

* Cohen's *d* values are reported for *t*-tests and phi coefficients are reported for χ^2 tests.

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

	CN, <i>n</i> = 29 mean (sd)	MCI, <i>n</i> = 16 mean (sd)	df	<i>t</i>	<i>p</i>	Cohen's <i>d</i>
DRS Total T-score	55.10 (5.06)	47.87 (6.58)	42	4.05	<0.001	1.23
ILS Managing Money T-score	56.58 (5.31)	52.00 (6.36)	23	1.76	0.09	0.78
ILS Health and Safety T-score	56.16 (5.98)	53.17 (4.36)	23	1.13	0.27	0.57
Geriatric Depression Scale raw score	4.63 (4.46)	4.73 (2.72)	33	0.07	0.95	0.03
MEMORY						
DRS Memory T-score	53.89 (7.11)	39.93 (16.00)	41	3.21	0.005	1.13
WMS-R Logical Memory I MOANS SS	12.93 (3.16)	8.87 (3.24)	41	3.98	<0.001	1.27
WMS-R Logical Memory II MOANS SS	12.82 (3.03)	8.67 (3.70)	41	3.97	<0.001	1.23
WMS-R Visual Reproduction I MOANS SS	12.55 (3.29)	10.50 (4.38)	28	1.38	0.18	0.53
WMS-R Visual Reproduction II MOANS SS	12.55 (2.70)	7.63 (5.04)	28	3.47	0.002	1.22
CVLT 1-5 total T-score	58.46 (10.69)	44.56 (7.75)	42	4.56	<0.001	1.49
ATTENTION						
DRS Attention T-score	55.64 (4.11)	55.00 (4.90)	41	0.46	0.65	0.14
WAIS-R Digit span MOANS SS	12.21 (2.90)	9.77 (2.09)	40	2.06	0.05	0.97
Trails A MOANS SS	12.50 (3.17)	10.29 (2.79)	40	1.76	0.09	0.74
LANGUAGE						
BNT MOANS SS	14.54 (2.43)	11.31 (3.38)	39	3.49	0.001	1.10
D-KEFS Letter Fluency SS	13.14 (3.05)	11.07 (3.95)	41	1.92	0.06	0.59
D-KEFS Category Fluency SS	13.29 (2.85)	11.00 (2.32)	40	2.60	0.01	0.88
VISUALSPATIAL						
WISC-R Block Design T-score	55.78 (8.05)	43.00 (14.34)	39	3.67	0.001	1.10
D-KEFS Visual Scanning SS	11.22 (2.10)	11.33 (1.84)	40	0.17	0.87	0.06
DRS Construction T-score	52.43 (3.02)	44.27 (13.72)	41	2.28	0.04	0.82
Clock Drawing (Command) raw score	2.96 (0.19)	2.47 (0.74)	41	2.55	0.02	0.91
EXECUTIVE FUNCTIONS						
WCST categories T-score	54.70 (4.87)	48.29 (11.06)	39	2.07	0.06	0.75
WCST perseverative errors T-score	49.04 (3.42)	46.64 (6.86)	39	1.23	0.24	0.44
Trails B MOANS SS	12.32 (2.13)	10.36 (2.82)	40	2.53	0.02	0.78

	CN, <i>n</i> = 29 mean (sd)	MCI, <i>n</i> = 16 mean (sd)	df	<i>t</i>	<i>p</i>	Cohen's <i>d</i>
D-KEFS Color Word Interference Inhibition SS	12.17 (2.21)	10.80 (2.49)	31	1.58	0.12	0.58

CN = cognitively normal; MCI = mild cognitive impairment; df = degrees of freedom; APOE = apolipoprotein E; DRS = Mattis Dementia Rating Scale [64]; ILIS = Independent Living Scales [39]; WMS-R = Wechsler Memory Scale-Revised [65]; normative data drawn from Mayo's Older Americans Normative Studies [MOANS] [66]; CVLT = California Verbal Learning Test [67]; WAIS-R = Wechsler Adult Intelligence Scale-Revised [68]; normative data from MOANS [66]; Normative data for Trail Making Test [69] from MOANS [66]; BNT = Boston Naming Test [70], normative data from MOANS [66]; D-KEFS = Delis-Kaplan Executive Function System [71]; WISC-R=Wechsler Intelligence Scale for Children – Revised [72], age and education adjusted norms drawn from unpublished data derived from the UCSD Alzheimer Disease Research Center; WCST = Wisconsin Card Sorting Test 48-card version [73].

Table 2b

Cognitive performance of APOE ε4 carriers and noncarriers

	ε4 noncarriers, n = 23 mean (sd)		ε4 carriers, n = 22 mean (sd)	df	t	p	Cohen's d
DRS total T-score	54.21 (4.16)		50.90 (8.18)	42	1.66	0.11	0.51
ILS Managing Money T-score	56.33 (4.68)		54.69 (6.75)	23	0.70	0.49	0.28
ILS Health and Safety T-score	55.50 (6.80)		55.38 (4.75)	23	0.05	0.96	0.02
Geriatric Depression Scale raw score	4.65 (3.95)		4.67 (4.07)	33	0.01	0.99	0.005
MEMORY							
DRS memory T-score	50.36 (9.31)		47.62 (15.73)	41	0.69	0.49	0.21
WMS-R Logical Memory I MOANS SS	11.41 (3.97)		11.62 (3.51)	41	0.18	0.86	0.06
WMS-R Logical Memory II MOANS SS	10.95 (3.93)		11.81 (3.71)	41	0.73	0.47	0.23
WMS-R Visual Reproduction I MOANS SS	12.33 (3.87)		11.67 (3.52)	28	0.49	0.63	0.18
WMS-R Visual Reproduction II MOANS SS	11.13 (4.09)		11.33 (4.13)	28	0.13	0.90	0.05
CVLT 1-5 total T-score	53.73 (10.92)		53.09 (12.81)	42	0.18	0.86	0.05
ATTENTION							
DRS Attention T-score	56.55 (3.02)		54.24 (5.23)	41	1.78	0.08	0.54
WAIS-R Digit span MOANS SS	11.68 (2.66)		11.45 (3.35)	40	0.25	0.80	0.08
Trails A MOANS SS	11.86 (3.20)		11.95 (3.25)	40	0.09	0.93	0.03
LANGUAGE							
BNT MOANS SS	13.91 (3.04)		13.05 (3.22)	39	0.88	0.39	0.27
D-KEFS Letter Fluency SS	12.00 (3.39)		12.86 (3.62)	41	0.80	0.43	0.25
D-KEFS Category Fluency SS	12.24 (2.81)		12.81 (2.98)	40	0.64	0.53	0.20
VISUALSPATIAL							
WISC-R Block Design T-score	52.91 (10.53)		49.68 (13.80)	39	0.85	0.40	0.26
D-KEFS Visual Scanning SS	11.86 (1.73)		10.60 (2.09)	40	2.15	0.04	0.66
DRS Construction T-score	51.55 (4.71)		47.52 (12.03)	41	1.43	0.16	0.44
Clock Drawing (Command) raw score	2.77 (0.62)		2.81 (0.40)	41	0.23	0.82	0.08
EXECUTIVE FUNCTIONS							
WCST categories T-score	52.95 (7.44)		52.00 (8.87)	39	0.38	0.71	0.12
WCST perseverative errors T-score	48.14 (4.99)		48.32 (4.98)	39	0.12	0.91	0.04
Trails B MOANS SS	11.95 (2.36)		11.35 (2.72)	40	0.77	0.45	0.24

	e4 noncarriers, <i>n</i> = 23 mean (sd)	e4 carriers, <i>n</i> = 22 mean (sd)	df	<i>t</i>	<i>p</i>	Cohen's <i>d</i>
D-KEFS Color Word Interference Inhibition SS	11.38 (2.19)	12.11 (2.50)	31	0.91	0.37	0.31

See Table 2a for list of abbreviations.

Table 3a

Group comparisons of brain volume indices by cognitive status

	CN, <i>n</i> = 29 mean (sd)	MCI, <i>n</i> = 16 mean (sd)	<i>t</i>	<i>p</i>	Cohen's <i>d</i>
Normalized whole brain gray matter	0.434 (0.028)	0.426 (0.026)	0.93	0.36	0.30
Normalized whole brain white matter	0.342 (0.026)	0.345 (0.025)	0.39	0.70	0.12
Normalized MTL volume *	0.580 (0.085)	0.521 (0.075)	2.34	0.02	0.74
Normalized hippocampal volume *	0.348 (0.051)	0.316 (0.049)	2.04	0.05	0.64
Normalized parahippocampal volume *	0.233 (0.043)	0.205 (0.035)	2.18	0.04	0.71

CN = cognitively normal; MCI = mild cognitive impairment; MTL = medial temporal lobe. Degrees of freedom for all *t*-tests = 43.

* Values multiplied by 100.

Table 3b

Group comparisons of brain volume indices by APOE genotype

	e4 noncarriers, n = 23 mean (sd)	e4 carriers, n = 22 mean (sd)	t	p	Cohen's d
Normalized whole brain gray matter	0.431 (0.020)	0.432 (0.034)	0.15	0.88	0.04
Normalized whole brain white matter	0.339 (0.021)	0.347 (0.029)	1.11	0.27	0.32
Normalized MTL volume *	0.563 (0.093)	0.556 (0.079)	0.27	0.79	0.08
Normalized hippocampal volume *	0.338 (0.055)	0.335 (0.050)	0.13	0.89	0.06
Normalized parahippocampal volume *	0.225 (0.046)	0.220 (0.039)	0.38	0.70	0.12

APOE = apolipoprotein E. See Table 3a for list of other abbreviations. Degrees of freedom for all t-tests = 43.

* Values multiplied by 100.

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

	CN, <i>n</i> = 29 mean (sd)	MCI, <i>n</i> = 16 mean (sd)	<i>t</i>	<i>p</i>	Cohen's <i>d</i>
Bilateral MTL Resting State CBF	65.98 (24.80)	49.69 (19.87)	2.23	0.03	0.72
Bilateral MTL Percent Change CBF	100.58 (62.60)	91.67 (61.92)	0.44	0.67	0.14
Bilateral MTL Percent Change BOLD	0.56 (0.24)	0.63 (0.27)	0.92	0.36	0.27

CBF = cerebral blood flow; BOLD = blood oxygenation level dependent; CN = cognitively normal; MCI = mild cognitive impairment; MTL = medial temporal lobe. Degrees of freedom for all *t*-tests = 43.

Group comparisons of resting state CBF and CBF and BOLD response to picture encoding by APOE genotype

Table 4b

	e4 noncarriers, n = 23	e4 carriers, n = 22	t	p	Cohen's d
Bilateral MTL Resting State CBF	52.57 (18.21)	68.14 (27.48)	2.25	0.03	0.67
Bilateral MTL Percent Change CBF	84.71 (49.98)	111.79 (71.18)	1.44	0.16	0.44
Bilateral MTL Percent Change BOLD	0.57 (0.24)	0.60 (0.27)	0.34	0.74	0.12

APOE = apolipoprotein E. See Table 4a for list of other abbreviations. Degrees of freedom for all t-tests = 43.

Table 5

Results from hierarchical regression analyses of cognitive status, APOE genotype, and their interaction predicting right MTL percent change CBF during picture encoding

Variable	Model 1					Model 2				
	B	SE B	β	t	p	B	SE B	β	t	p
Intercept	89.42	15.34		5.83	<0.001	104.47	15.55		6.72	<0.001
Cognitive status	-1.95	21.59	-0.01	-0.09	0.92	-49.25	27.56	-0.36	-1.79	0.08
APOE genotype	23.08	20.15	0.18	1.15	0.26	-9.34	22.82	-0.07	-0.41	0.69
Cognitive status $\times$ APOE genotype						102.29	40.53	0.57	2.52	0.02
R ²			0.034					0.175		
F for change in R ²			0.66					6.37		
Cohen's f ²									0.17	

APOE = apolipoprotein E; MTL = medial temporal lobe; CBF = cerebral blood flow; B = unstandardized coefficients; SE = standard error; β = standardized coefficients.