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Journal

Journal of Alzheimers Disease, 73(1)

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Publication Date

2020

DOI

10.3233/JAD-190504

Peer reviewed

HHS Public Access

Author manuscript

J Alzheimers Dis. Author manuscript; available in PMC 2020 June 23.

Published in final edited form as:

J Alzheimers Dis. 2020 ; 73(1): 87–101. doi:10.3233/JAD-190504.

Anterior Cingulate Structure and Perfusion is Associated with Cerebrospinal Fluid Tau among Cognitively Normal Older Adult APOEε4 Carriers

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Abstract

Evidence suggests the ε4 allele of the apolipoprotein E gene (APOE) may accelerate an age-related process of cortical thickening and cerebral blood flow (CBF) reduction in the anterior cingulate cortex (ACC). Although the neural basis of this association remains unclear, evidence suggests it might reflect early neurodegenerative processes. However, to date, associations between cerebrospinal fluid (CSF) biomarkers of neurodegeneration, such as CSF tau, and APOE-related alterations in ACC cortical thickness (CTH) and CBF have yet to be explored. The current study explored the interaction of CSF tau and APOE genotype (ε4+, ε4−) on FreeSurfer-derived CTH and arterial spin labeling MRI-measured resting CBF in the ACC (caudal ACC [cACC] and rostral ACC [rACC]) among a sample of 45 cognitively normal older adults. Secondary analyses also examined associations between APOE, CTH/CBF, and cognitive performance. In the cACC, higher CSF tau was associated with higher CTH and lower CBF in ε4+, whereas these relationships were not evident in ε4−. In the rACC, higher CSF tau was associated with higher CTH for both ε4+ and ε4−, and with lower CBF only in ε4+. Significant interactions of CSF tau and APOE on CTH/CBF were *not* observed in two posterior reference regions implicated in Alzheimer's disease. Secondary analyses revealed a negative relationship between cACC CTH and executive functioning in ε4+ and a positive relationship in ε4−. Findings suggest the presence of an ε4-related pattern of increased CTH and CBF reduction in the ACC that is associated with biomarkers of neurodegeneration and subtle decrements in cognition.

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Conflict of interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Keywords

aging; Alzheimer's disease; ApoE; cerebral blood flow; cognition; cognitive decline; grey matter; magnetic resonance imaging; tau proteins

INTRODUCTION

Age-related cognitive decline is heterogeneous and may occur with varying accompanying brain pathology. With the progressive accumulation of neuropathology, subtle cognitive changes and early symptoms of dementia may eventually develop [1,2]. Identifying mechanisms of risk and biomarkers of structure and function during the earliest stages may provide a clearer understanding of the transition to mild cognitive impairment (MCI) and dementia, thus enabling early, personalized intervention to prevent damage and neurodegeneration. The most well-known genetic risk factor for age-related cognitive decline is the ε4 allele of the apolipoprotein (APOE) gene. Possession of this allele increases risk for Alzheimer's disease (AD) by 3-8 fold and lowers the age of disease onset in a dose-dependent fashion [3–6]. Older APOE ε4 carriers also show increased vulnerability to other neurologic conditions that affect cognition, such as vascular dementia and dementia with Lewy bodies [7,8], and appear to be at increased risk for cognitive decline even in the absence of disease or disorder [9–11]. The apparent broad sweeping effects of APOE on cognitive functioning may be related to its role in a diverse range of biological processes, including glucose metabolism, mitochondrial function, synaptic function, neurogenesis, tau phosphorylation, neuronal atrophy, neuroinflammation, and amyloid-β (Aβ) metabolism and aggregation [5,12,13]. The reason why APOE ε4, rather than other isoforms, confers increased risk for cognitive decline is still unknown, but it has been proposed that structural differences result in lower lipidation and lipoprotein stability and the production of toxic APOE ε4 fragments, leading to impaired cellular recycling and altered receptor activity [14,15]. Characterization of the early brain changes that accompany the possession of this allele could provide critical insight into the mechanistic link between APOE ε4 and cognitive decline.

APOE and ACC thickness

Against the backdrop of widespread age-related cortical thinning and volume reduction, there is accumulating evidence of increased medial prefrontal cortical thickness in older age, most notably in the anterior cingulate cortex [16–21]. More importantly, this age-related increase in ACC thickness appears to be exaggerated and/or accelerated among cognitively normal older adult carriers of the APOE ε4 allele [22–25]. Although most of this evidence is cross-sectional in nature, it is suggestive of an age-related process of cortical thickening in the ACC and has led to debate within the field regarding whether this could reflect a beneficial or detrimental brain response. For example, some have suggested that cortical thickening could reflect neuroplastic adaptations to increased environmental demands [19], while others have argued that this process could reflect chronic low-grade inflammation [17] or nuclear hypertrophy in response to cellular injury [26]. The few studies that have explored associations between APOE ε4-related increases in ACC thickness and cognition in aging populations have shown negative associations with executive functioning and memory

performance [24,25]. Moreover, there is cross-sectional evidence suggesting that increased thickness of the cerebral cortex could reflect a very early stage of neurodegeneration [27]. This growing body of literature suggests that higher ACC thickness in older adults could reflect a dysfunctional age-related process that is exacerbated by the APOE ε4 isoform. Linking increased ACC thickness among older adult ε4 carriers to known biomarkers of neural damage and degeneration could provide compelling support for this hypothesis and help to elucidate the neural basis of this association.

APOE and CSF tau

Both animal and human investigations suggest that APOE ε4 is associated with greater tau accumulation [28–31] and with a pro-neurodegenerative response to tau in the brain [31]. Tau aggregation in AD involves mechanisms such as cleavage and phosphorylation. Fragments of tau are detectable in cerebrospinal fluid (CSF) that do not include the aggregation-prone microtubule binding region or C-terminal, and are increased in AD, likely as a result of active release associated with neurodegeneration or neuronal damage [32,33]. Together, these data suggest that increased ACC thickness seen on MRI among cognitively normal APOE ε4 carriers may be accompanied by subtle damage to neurons that may contribute to an increase in CSF tau concentration. However, to our knowledge, there are currently no published studies exploring this proposed relationship.

APOE and ACC cerebral blood flow

Cerebral blood flow (CBF), or the rate of delivery of arterial blood to the capillary bed in a volume of tissue, is an indirect measure of neural function [34] that has been implicated in both normal aging and AD [35–37]. Regional measures of CBF demonstrate reliable correlations with cognition across the lifespan [35,38–40] and can distinguish between normal controls and those with AD and predict conversion to MCI and AD, suggesting its usefulness as a biomarker of cognitive decline [37,41]. More importantly, APOE genotype appears to modify age-related changes in CBF in the ACC, with younger ε4 carriers demonstrating higher ACC CBF compared to younger non-carriers, and older ε4 carriers demonstrating lower ACC CBF compared to older non-carriers [42]. Older adult APOE ε4 carriers also show greater longitudinal reductions in CBF than do non-carriers [43]. In fact, theories of early vascular dysregulation in AD posit that regional reductions in cerebral perfusion can ultimately lead to neuronal damage and degeneration [1,44–47].

Taken together, this evidence suggests that characterization of concurrent associations between CSF tau and ACC CTH and CBF, in cognitively normal older adult APOE ε4 carriers could lead to a more complete understanding of how these brain alterations together, might negatively impact cognition. However, to date, interactions among these variables have yet to be explored. To bridge this gap, the current study explored the interaction of CSF tau concentration and APOE genotype (ε4+, ε4−) on FreeSurfer-derived CTH and arterial spin labeling (ASL) MRI-measured resting CBF in the ACC (caudal ACC [cACC], rostral ACC [rACC]) among a sample of 45 cognitively normal older adults. Caudal and rostral aspects of the ACC were examined separately due to evidence that these two sub-territories are distinguishable with regard to cytoarchitecture, function, and connectivity [48]. Building on previous literature, we hypothesized that higher CSF tau would be associated with *higher*

ACC CTH and with lower CBF in ε4 carriers, and this relationship would differ in rostral and caudal ACC. Exploratory analyses also investigated these same relationships in the posterior cingulate cortex (PCC) and precuneus (Pc) as reference regions to determine the specificity of findings. In these regions, thought to demonstrate cortical thinning/volume reduction and metabolic disturbance early in the AD process [49,50], we hypothesized that higher CSF tau would be associated with *lower* CTH and lower CBF in ε4 carriers.

MATERIALS AND METHODS

Participants

See Table 1 for participant demographic and cognitive characteristics. Participants were community-dwelling older adult volunteers enrolled in a longitudinal study of aging at the VA San Diego Healthcare System (VASDHS). A total of 45 cognitively normal participants between the ages of 65 and 83 (mean age = 72, SD = 4.6) with available data were included in the current analyses. Fourteen participants were carriers of the APOE ε4 allele (ε3/ε4 = 13, ε4/ε4 = 1) and 31 were non-carriers (ε3/ε3 = 29, ε2/ε3 = 2). All participants had available CSF tau data and were administered a full neuropsychological battery and an MRI scan at study entry (mean interval between CSF-tau measurement and baseline neuropsychological/MRI testing = 258 days). Of note, CSF-tau levels show high intra-individual stability longitudinally over at least 1–2 years [51,52]. Normal cognitive function was determined based on a comprehensive neuropsychological test battery. Participants were excluded if performance on more than one measure within a cognitive domain was more than one standard deviation below age-appropriate norms, consistent with the empirically-derived criteria for diagnosis of MCI developed by Jak and colleagues [53], or if overall performance on the Dementia Rating Scale (DRS) was more than 1 standard deviation below age-appropriate norms (see Table S1 in the supplement for specific cognitive tests, domains, and normative data). Potential participants were excluded if they had a dementia or MCI diagnosis, a history of severe head injury, uncontrolled hypertension, or a DSM-IV Axis I diagnosis of learning disability, attention deficit disorder, mood disorder, or substance abuse. In addition, participants were excluded if they had contraindications to MRI scanning or lumbar puncture to obtain CSF measures of tau. All participants provided written informed consent prior to enrollment and data were collected in accordance with all ethical standards as stipulated by the UCSD and VASDHS institutional review board-approved procedures.

Cognitive Composites

All participants were administered a full neuropsychological battery. A verbal memory composite score was created using trials 1-5, short delay free-recall, and long delay free-recall raw scores from the California Verbal Learning Test – Second Edition (CVLT-II) [54], and the Logical Memory immediate and delayed recall subtests of the Wechsler Memory Scale-Revised (WMS-R) [55]. An executive functioning composite score was created using color word inhibition and color word inhibition switching subtests from the Delis-Kaplan Executive Function System (DKEFS) [56], digit span backward from the Wechsler Adult Intelligence Scale-Revised (WAIS-R) [57], and Trail Making Test Part B [58]. These tests were selected based on results from a principal component analysis previously reported by our group on a similar sample of older adults [39]. Verbal memory and executive functioning

composite scores were derived by averaging the *z*-scores for each of the tests within each composite for the entire sample. Although the cognitive composite scores themselves did not adjust for age, gender, or education; these variables were statistically adjusted for within the regression models (see statistical analyses).

CSF procedures

CSF was drawn by lumbar puncture as a research procedure in the early morning. Samples were gently mixed, centrifuged at 1500g and the supernatant aliquoted into 0.5 mL fractions into polypropylene cryotubes, then snap frozen and stored at −80 degrees until analyzed. CSF tau and p-tau were measured using the xMAP-Luminex platform with INNOBIA AlzBio3 immunoassay kit-based reagents (Fujirebio-Innogenetics, Ghent, Belgium), as described previously [59,60]. CSF amyloid beta ($A\beta_{42}$) was measured by liquid chromotography/tandem mass spectrometry (http://www.questdiagnostics.com/testcenter/TestDetail.action?ntc=94627). Of note, the current study explored CSF tau, rather than p-tau or $A\beta_{42}$, for several reasons: 1) evidence suggests that CSF tau best reflects intensity of neurodegeneration or neural injury, whereas CSF p-tau and $A\beta_{42}$ are more specific for AD-type pathology accumulation (i.e., neurofibrillary tangles, Aβ plaques) [61]; 2) Measures of tau consistently demonstrate stronger correlations with cognition than do measures of Aβ [62,63]; and 3) Aβ biomarkers tend to be bimodally distributed [64,65] while neurodegenerative biomarkers typically are not.

APOE genotyping

Genotyping was performed by the ADCS Biomarker Core at UCSD using real-time PCR Restriction Fragment Length Polymorphism analysis. Genomic DNA was collected from participants using a buccal swab and extracted using Qiamp DNA blood mini kit (Qiagen) followed by PCR amplification (Wierenga, et al., 2012). Those with at least one ε4 allele (i.e., ε3/ε4, ε4/ε4) were classified as APOE ε4 carriers (ε4+) and those without an ε4 allele (i.e., ε2/ε3, ε3/ε3) were classified as non-carriers (ε4−). Given that the APOE ε2 allele is thought to have protective effects [66], all analyses were run including and excluding the two ε2 carriers in the sample and the pattern of results was similar (see Table S2 in the supplement). Similarly, given evidence of dose-dependent effects of the APOE ε4 allele[4], all analyses were run including and excluding the one homozygous ε4 carrier in the sample and the results were similar (see Table S3 in the supplement). Therefore, results from the entire sample are presented.

MRI acquisition

Imaging data were acquired on a GE Discovery MR750 3T whole body system with a body transmit coil and an 8-channel receive-only head coil at the University of California San Diego Center for Functional MRI. The structural brain sequence consisted of a high-resolution T1-weighted Fast Spoiled Gradient Recall (3D FSPGR) scan: 172 1 mm contiguous sagittal slices, FOV = 25 cm, TR = 8 ms, TE = 3.1 ms, flip angle = 12, T1 = 600 ms, 256 x 192 matrix, Bandwidth = 31.25 kHz, frequency direction = S-I, NEX = 1, scan time = 8 min and 13 sec. Resting CBF was acquired with the Multiphase Pseudocontinuous Arterial Spin Labeling (MPPCASL) sequence, which is optimized for robust CBF quantification [67]: tagging duration = 2000 ms, post-labeling delay = 1600 ms, TR = 4200

ms, TE = 3 ms, reps = 64, FOV = 24 x 24 cm, 64x64 matrix, 3.75 mm x 3.75 mm in-plane resolution, 20 5 mm axial slices with a single shot 2D spiral acquisition, collecting 8 cycles where each cycle consists of 8 images acquired with unique phase offsets, acquisition time = 4:46 minutes. A CSF calibration scan was also obtained using a spiral readout with TR = 4000 ms and TE = 3.4 ms and comprised nine 90○ excitation pulses which were turned off for the first eight repetitions to generate PD-weighted contrast (scan time: 36 sec) to obtain an estimate of the equilibrium magnetization of cerebral spinal fluid, which is used to convert the perfusion signal into calibrated CBF units (mL blood/100g tissue/min). Finally, a minimum contrast image (TE = 11 ms, TR = 2000 ms, 8-shot interleaved spiral acquisition) was acquired to adjust for transmit and receive coil inhomogeneities. Two field map scans were also acquired and used for off-line field map correction to help correct for signal bunching and dropouts in the frontal/medial temporal lobes.

MRI pre-processing

CTH—Cortical thickness and volume analysis were performed using FreeSurfer version 5.3 (http://surfer.nmr.mgh.harvard.edu), with the technical details of these procedures described in prior publications [68–77]. Cortical thickness was extracted from the left and right cACC, rACC, PCC, and Pc. A quality assurance protocol was performed before analysis using the ENIGMA guidelines (http://enigma.usc.edu/protocols/imaging-protocols) and included visual checks of the cortical segmentations and region-by-region removal of values for segmentations found to be incorrect. Histograms of all regions' values for each site were also computed for visual inspection. This did not result in any removal of data. In order to reduce the number of comparisons and increase power, CTH values were averaged across left and right hemispheres of these midline structures.

CBF—ASL image processing was performed with Analysis of Functional NeuroImages (AFNI, afni.nimh.nih.gov)[78], FMRIB Software Library (FSL, Oxford, United Kingdom), and locally created Matlab scripts (MATLAB 2018a, The MathWorks, Inc., Natick, Massachusetts, United States).

Field map correction was applied to the ASL time series prior to co-registration to the middle time point to minimize the effects of participant motion. Coil inhomogeneities were corrected using data from the minimum contrast image and the approach described in [79]. For each participant, a mean ASL difference image was formed from the average difference of the control and tag images and slice timing delays were accounted for in order to make the post-labeling delay time slice specific [80]. In other words, with the slices acquired in an ascending sequential fashion and an inter-slice timing delay of 28 ms, the slice-specific post-labeling delays ranged from 1600 ms for the most inferior slice to 2132 for the most superior slice. The mean ASL image was then converted to absolute units of CBF (mL/100g tissue/min) using an estimate of the equilibrium magnetization of CSF as a reference signal [81]. This procedure resulted in a calibrated perfusion value for each voxel. Additional details on the CBF quantification process, including the equation used, are provided in [67]. Skull stripping of the high-resolution T1-weighted image was performed using AFNI's 3dSkullStrip. Tissue segmentation was performed using FSL's Automated Segmentation Tool (FAST) algorithm (http://fsl.fmrib.ox.ac.uk/fsl/) to define cerebrospinal fluid (CSF),

gray matter (GM) and white matter (WM) regions. The high-resolution T1-weighted image and partial volume segmentations were registered to ASL space using AFNI's 3dAllineate program and the CBF data were resampled to the same resolution as the T1-weighted image using AFNI's 3dresample program. The partial volume estimates were used to perform partial volume correction of the high-resolution CBF data using a linear regression approach with kernel size of 3x3 [82,83] as implemented by the ASL file function in the BASIL toolset of FSL [84]. The choice of the 3x3 kernel size was motivated the findings of [83] indicating that a 3x3 kernel provides higher accuracy as compared to a larger 5x5 kernel. The partial volume corrected gray matter CBF data were then resampled back to their native resolution and registered to FreeSurfer space. CBF values were extracted from anatomically defined FreeSurfer ROIs (i.e., cACC, rACC, PCC, Pc; see below for FreeSurfer methods). Quality assurance of ASL data was performed prior to analysis using outlier detection, an inspection of CBF histograms, and visual checks of the CBF maps, with the removal of values for regions with poor CBF map coverage. This resulted in the removal of 3% of the data (3.2% ε4−, 3.6% ε4+; see Table S4 in the supplement). In order to reduce the number of comparisons and increase power, CBF values were averaged across the left and right hemispheres of these midline structures.

Statistical analyses

t-tests were used to compare groups on age, years of education, brain variables (CTH, CBF), and cognitive variables. A χ2-test was used to compare groups on sex. CTH and CBF were extracted from FreeSurfer-derived regions of interest (i.e., cACC, rACC) and two sets of multiple linear regression models were employed in R: 1) Two-way interaction of tau and APOE ε4 +/− on ACC CTH (cACC CTH, rACC CTH), statistically adjusting for the effects of age, sex, education, estimated 10-year risk for future stroke, as measured by the Framingham Stroke Risk Profile (D'Agostino, Wolf, Belanger, & Kannel, 1994; Wolf, D'Agostino, Belanger, & Kannel, 1991), ACC CBF, and the time interval between CSF biomarker collection and MRI. 2) Two-way interaction of tau and APOE ε4 +/− on ACC CBF (cACC CBF, rACC CBF), statistically adjusting for the effects of age, sex, education, ACC CTH, the time interval between CSF biomarker collection and MRI, and stroke risk. A Bonferroni correction was applied to correct for multiple comparisons, and results were considered significant at $p<0.0125$. Exploratory analyses explored these same relationships in the PCC and Pc, as reference regions, and were considered significant at $p<0.0125$ (Bonferroni corrected). Post hoc analyses also employed a set of linear regression models exploring the two-way interaction of APOE and cACC brain measures (CTH, CBF) on cognitive performance (executive functioning composite, memory composite), statistically adjusting for the effects of age, sex, education, and stroke risk percent. A Bonferroni correction was applied to correct for multiple comparisons, and results were considered significant at $p<0.0125$. CSF biomarker variables were natural log-transformed and all continuous predictor variables were standardized prior to regression analyses. Non-multicollinearity between independent variables was confirmed by application of the multicollinearity index VIF (all VIF<2), linearity was confirmed by residuals versus fits plots, normality was confirmed by Q-Q plots, non-heteroskedasticity was confirmed by scale location plots, and no influential cases/outliers were identified through examinations of residuals versus leverage plots. Of note, the current investigation was interested in exploring

neurodegeneration/neural injury and thus focused primarily on CSF tau. However, due to evidence of the potential effects of CSF $A\beta_{42}$ on brain changes in clinical populations (e.g., AD) we reran all analyses with CSF $A\beta_{42}$ concentration as a covariate. Results were equivalent and $A\beta_{42}$ did not explain significant variance in any of the models tested (all $t < 0.27$; all $p > 0.785$); therefore, results are presented without $A\beta_{42}$.

RESULTS

Group differences in demographic, cognitive, and brain variables

Groups (ε4+, ε4−) did not differ significantly on age, sex, or years of education, nor did they differ significantly in the time interval between CSF biomarker collection and baseline testing (see Table 1). APOE ε4 carriers demonstrated marginally higher cACC CTH (see Table 1); no other differences in brain variables (CTH, CBF, CSF tau, $A\beta_{42}$) were observed by group (see Table 1). With regard to cognition, ε4 carriers demonstrated worse performance on the memory composite than did non-carriers, with significantly worse performance on CVLT trials 1–5 and marginally worse performance on CVLT long delay free recall and WMS Logical Memory Immediate Recall (see Table 1). Groups did not differ in performance on the executive functioning composite nor any of the subtests that were used to create the composite (see Table 1).

Effects of CSF tau and APOE on ACC CTH

In the cACC, there was a significant interactive effect of CSF tau and APOE on cACC CTH (see Table 2), whereby higher CSF tau concentration was associated with higher ACC CTH among ε4 carriers but not non-carriers (see Figure 1). In the rACC, a significant two-way interaction of CSF tau and APOE on rACC CTH was not observed (see Table 2). However, we observed a marginal main effect of CSF tau on rACC CTH that became statistically significant after removing the non-significant interaction term from the model post hoc (see Table 2 and Figure 2).

Effects of CSF tau and APOE on ACC CBF

There was also a significant interactive effect of CSF tau and APOE on cACC CBF (see Table 2), whereby higher CSF tau concentration was associated with lower ACC CBF among ε4 carriers but not non-carriers (see Figure 1). Although not statistically significant, there was also a trend toward the same interactive effect of CSF tau and APOE on CBF in the rACC that was seen in the cACC (see Table 2 and Figure 2).

Exploratory analyses within the PCC and Pc

Significant interactive effects of CSF tau and APOE on PCC and Pc CTH were *not* observed (see Table 3). Although not statistically significant, there were trends toward interactive effects of CSF tau and APOE on CBF in the PCC and the Pc, such that higher CSF tau concentration was associated with lower CBF in the PCC and in the Pc among ε4 carriers but not non-carriers (see Table 3).

Secondary analyses with cognition

An interaction of APOE and cACC CTH on executive function (β= −0.66, t= 2.47, p= 0.0184) was observed, whereby ε4 carriers showed a trend toward a negative association between cACC CTH and performance on the executive functioning composite and non-carriers showed a trend toward a positive association between cACC CTH and performance on the executive functioning composite (see Figure 3). However, this interaction did not survive correction for multiple comparisons. No interaction of APOE and cACC CBF on executive function was observed (p= 0.3550). Interactions of APOE and cACC CTH or CBF on memory function were also not observed (all *ps*> 0.8893). There was a significant interaction of APOE and Pc CTH on executive function (β= 0.78, t= 3.05, p= 0.0043), whereby ε4 carriers showed a positive association between Pc CTH and performance on the executive function composite and non-carriers showed a negative association between Pc CTH and performance on the executive function composite (see Figure 4). Similarly, there was an interaction of APOE and Pc CTH on memory function (β= 0.66, t= 3.12, p= 0.0036), whereby ε4 carriers showed a positive association between Pc CTH and performance on the memory composite and non-carriers showed a negative association between Pc CTH and performance on the memory composite (see Figure 4). Interactions of APOE and Pc CBF on executive function or memory were not observed (all *ps*> 0.3909), nor were interactions of APOE and PCC CTH or CBF on executive functioning or memory (all *ps*> 0.2130).

DISCUSSION

Results showed that independent associations of CSF tau on CTH and CBF in the cACC differed by APOE ε4 status, despite largely equivalent CBF, CTH, and CSF tau across groups. For ε4 carriers, higher CSF tau concentration was associated with higher cACC CTH and lower cACC CBF, whereas these associations were not observed in non-carriers. These findings suggest the presence of an APOE ε4-related pattern of increased CTH and reduced CBF in the ACC that is associated with biomarkers of neurodegeneration. Moreover, the observed positive association between CSF tau and CTH in the rACC for both ε4 carriers *and* non-carriers suggest that increased ACC thickness could reflect a normal age-related process that is accelerated by, rather than specific to, possession of the APOE ε4 allele. The lack of a significant interactive effect of CSF tau and APOE on CTH in the Pc or PCC could suggest that this relationship is largely specific to the ACC, though future research utilizing more widespread ROIs in larger samples is needed to confirm this.

It is important to note that CSF tau levels reflect the global release of tau by the brain into the CSF, rather than being regionally specific. Therefore, current results do *not* support the notion of associations between ACC CTH/CBF and localized tau release. In fact, studies of PET-derived regional tau concentration suggest that the ACC tends to be relatively spared by accumulation of this protein, even in neurodegenerative populations [85], suggesting that increases in CSF tau could reflect distant, rather than local, responses to ACC thickening and/or blood flow reduction. Although speculative, it is possible that vascular dysregulation among ε4 carriers could explain associations between CSF tau and increased ACC CTH among ε4 carriers. While larger sample sizes and longitudinal data are needed to test this hypothesis directly, our results suggest that these three different aspects of brain health (e.g.,

ACC CTH, ACC CBF, CSF tau) are concurrently altered in the context of APOE ε4. The notion of reduced CBF among APOE ε4 carriers having negative downstream effects on tau and underlying structural integrity is largely consistent with the Capillary Dysfunction Hypothesis of Alzheimer's disease, which posits that compensatory reductions in CBF in response to increased heterogeneity of capillary blood flow eventually lead to oxidative stress, activation of inflammatory pathways, and neurodegeneration [46]. Within this theoretical framework, vascular dysregulation among ε4 carriers could accelerate, or even initiate, tau accumulation in the brain. Similarly, vascular dysfunction could promote local neuroinflammation and conceivably lead to an initial increase in CTH, followed by atrophy with continued inflammation and/or damage.

The current findings, particularly with respect to the observed positive relationship between CSF tau and CTH, could also be understood in the context of a compensatory increase in nuclear volume in the ACC in response to vascular aging or neuropathological damage. The later notion is supported by a recent stereological study by Riudavets and colleagues demonstrating that cognitively normal older adults who demonstrate significant AD pathology at autopsy (i.e., neuritic plaques) show nuclear hypertrophy in the ACC and hippocampus when compared to age-matched controls, MCI and AD. The authors suggest that this increase in nuclear volume may reflect reactive and compensatory processes at the cellular level that allow the brain to function normally despite the presence of AD pathology, or that it may represent the earliest stages of a neurodegenerative response. The current findings suggest that this same cellular process, whether compensatory or dysfunctional in nature, may also be present in cognitively normal older adult ε4 carriers, though future histological research is needed to test this hypothesis.

Although the current study focused solely on CSF tau, given evidence that it best reflect neurodegeneration and is less specific to AD pathology than is CSF p-tau and $A\beta_{42}$ [61,86], it may be important to consider the current findings in light of results from a recent study of preclinical AD [27] that examined effects of CSF p-tau and $A\beta_{42}$ on cortical thickness. In comparison to our observation of an association between higher CSF tau and higher cortical thickness, Fortea and colleagues observed associations between CSF $A\beta_{42}$ and p-tau and cortical thickness. More specifically, in regions typically associated with AD (e.g., medial temporal lobe), higher cortical thickness was associated with the combination of CSF $A\beta_{42}$ positivity and CSF p-tau negativity, whereas lower cortical thickness was associated with positivity in both CSF $A\beta_{42}$ and p-tau, leading the authors to suggest that cortical thickening is more closely associated with increases in CSF $A\beta_{42}$ and thinning more closely associated with increases in CSF p-tau [27]. However, it is important to note that this prior study did not examine or adjust for the effects of APOE genotype or CSF tau concentration. Therefore, in light of the current findings, it is plausible that cortical thickening in this prior study could have been associated with CSF tau concentration, particularly among APOE ε4 carriers. It is also possible that the neural basis of cortical thickening differs by clinical populations (e.g., APOE ε4 carriers versus preclinical AD), or by anatomical region (e.g., ACC versus regions typically implicated in AD). Indeed, our post hoc analyses with cognition showed differential effects of APOE and CTH on cognitive performance depending on the brain area explored, with higher CTH in the ACC among ε4 carriers being associated with *worse* cognitive performance, and higher CTH in the Pc being associated

with *better* cognitive performance. Although it is difficult to impute the accountability of a single region with regard to complex neuropsychological changes, these cross-sectional findings could be explained by a dysfunctional APOE ε4-related process that is associated with neural damage and/or degeneration, though longitudinal studies among larger samples and clinical populations (e.g., MCI), and across more widespread brain regions are needed to validate these assumptions. Future studies should also explore the role of additional CSF biomarkers within these models (e.g., p-tau, $A\beta_{42}$, $A\beta_{42}$/p-tau) to better characterize ε4 carriers and the contribution of AD pathological processes.

Strengths and limitations

The strongest limitation of the current study is its small sample size (n=45), which included only 14 APOE ε4 carriers. As such, findings need to be replicated in a larger sample. The current study is also cross-sectional in nature; therefore, we cannot rule out the possibility that relative increases in cortical thickness and blood flow reduction in the ACC could represent long-standing differences between APOE ε4 carriers and non-carriers, rather than longitudinal changes. In this context, the current observation of higher CTH and lower CBF in this brain region could represent aspects of a neural endophenotype that increase susceptibility to neurodegeneration later in life. However, it should be noted that this interpretation is less supported by the literature, as evidence suggests a reverse pattern in younger ε4 carriers (i.e., lower CTH and higher CBF). Evidence also suggests that cross-sectional observations of relatively increased ACC CTH may reflect decelerated cortical thinning over time, rather than absolute thickening [21]. Another possibility that remains is that the observed associations between CSF tau and ACC CTH/CBF among ε4 carriers are only epiphenomenal and that CSF tau increase is due to other pathological changes affecting other areas that are not explored in this study. Of note, secondary analyses revealed groups did not differ in hippocampal volume, suggesting differences in stages of neurodegeneration likely did not contribute to our findings. However, longitudinal investigations should seek to characterize the order in which these variables (i.e., CBF, CTH, tau) might become abnormal across widespread brain regions to establish causality and regional specificity. Moreover, although the current study focused exclusively on morphology and blood flow in cortical regions, exploring the concurrent effects of APOE ε4 on white matter integrity and/or associated markers of white matter damage (e.g., CSF neurofilament light) could lead to better characterization of the detrimental effects of APOE ε4 in the aging brain. Our sample also reported relatively high levels of education, and although this demographic factor was not associated with APOE genotype, its limited range may reduce the generalizability of these findings. Moreover, although groups (ε4+/ ε4−) did not differ significantly in the average time interval between CSF biomarker collection and MRI scanning, decreasing the time interval between biomarker collection and MRI may improve the accuracy of associations among these brain measures. Of note, averaging CBF and CTH values across the left and right hemispheres limited our ability to detect potential literalized effects. Future studies with larger sample sizes should consider exploring left and right hemispheres separately. Lastly, the method of averaging standardized cognitive scores to create a composite score that represents a particular cognitive domain, though fairly standard in the field, may pose some additional limitations. Selection of our individual tests was based on a prior principal component analyses; future research is needed to determine whether

statistical approaches that optimize the contribution or weight of data from individual neuropsychological tests into composite scores (e.g., scaling tests proportionally to factor loadings) may further increase sensitivity. Lastly, in this work we used a linear regression approach with a 3x3 kernel for partial volume correction of the CBF estimates. As noted in Zhao et al. [83], the evaluation of partial volume correction approaches is an evolving area of research and it may be helpful to consider alternate approaches in future studies.

Strengths of the current study include the use of non-invasive ASL MRI to measure partial volume corrected CBF and the use of a high-resolution structural MRI scan to examine CTH. Furthermore, use of FreeSurfer offers advantages over traditional voxel-based morphometry methods, allowing for examination of the components of volume separately (thickness and surface area), as it has been found that these two measures are distinct and do not necessarily track with one another [21,87,88]. Although the current study focused solely on CTH in an attempt to build on accumulating literature and because CTH thickness is thought to contribute more to age-related decline in cortical volume [21], future studies with larger sample sizes should also consider exploring ACC surface area as it relates to aging and APOE. The extraction of CBF from FreeSurfer-derived brain regions also represents a strength of the current study, allowing us to directly investigate CBF (and brain structure) in regions that are defined by each individual's anatomy, rather than atlas-defined regions which are less sensitive to individual anatomical differences because they require that data are first aligned and warped to a generic anatomic template. Lastly, the current study benefitted from the inclusion of a well-controlled and well-characterized sample of cognitively normal older adults, which included the use of several cognitive test performances to characterize cognitive status.

Conclusions

Results suggest the presence of an ε4-related pattern of increased CTH and reduced CBF in the ACC that is associated with biomarkers of neurodegeneration and subtle decrements in cognition. These cross-sectional findings could lend support to the notion of a dysfunctional age-related process of cortical thickening and vascular dysregulation in the ACC that is exacerbated by the APOE ε4 allele, thus, future longitudinal investigations utilizing larger sample sizes and clinical populations should seek to test this hypothesis directly. Future studies are also needed to confirm and further elucidate the neural basis of cortical thickening with age (e.g., inflammation, nuclear hypertrophy). On a broader scale, this convergence of markers of neurodegeneration, vascular dysfunction and morphological alteration in those at increased risk for AD add to accumulating evidence supporting the early contribution of vascular dysregulation in AD pathogenesis [1,44,45,47] and could lead to the identification of vasoprotective treatments with the potential to delay or prevent the onset of age-related cognitive decline and/or AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

This work was supported by VA CSR&D Merit Award [5I01CX000565 C.E.W.], the National Science Foundation Graduate Research Fellowship Program [2015207525 C.C.H.], and the National Institute on Aging of the National Institutes of Health [K23AG049906 Z.Z.Z.]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the VA, National Science Foundation, or National Institutes of Health. We would also like to acknowledge Dr. Robert Rissman, Associate Professor of Neurosciences at the University of California San Diego for his contributions to this project.

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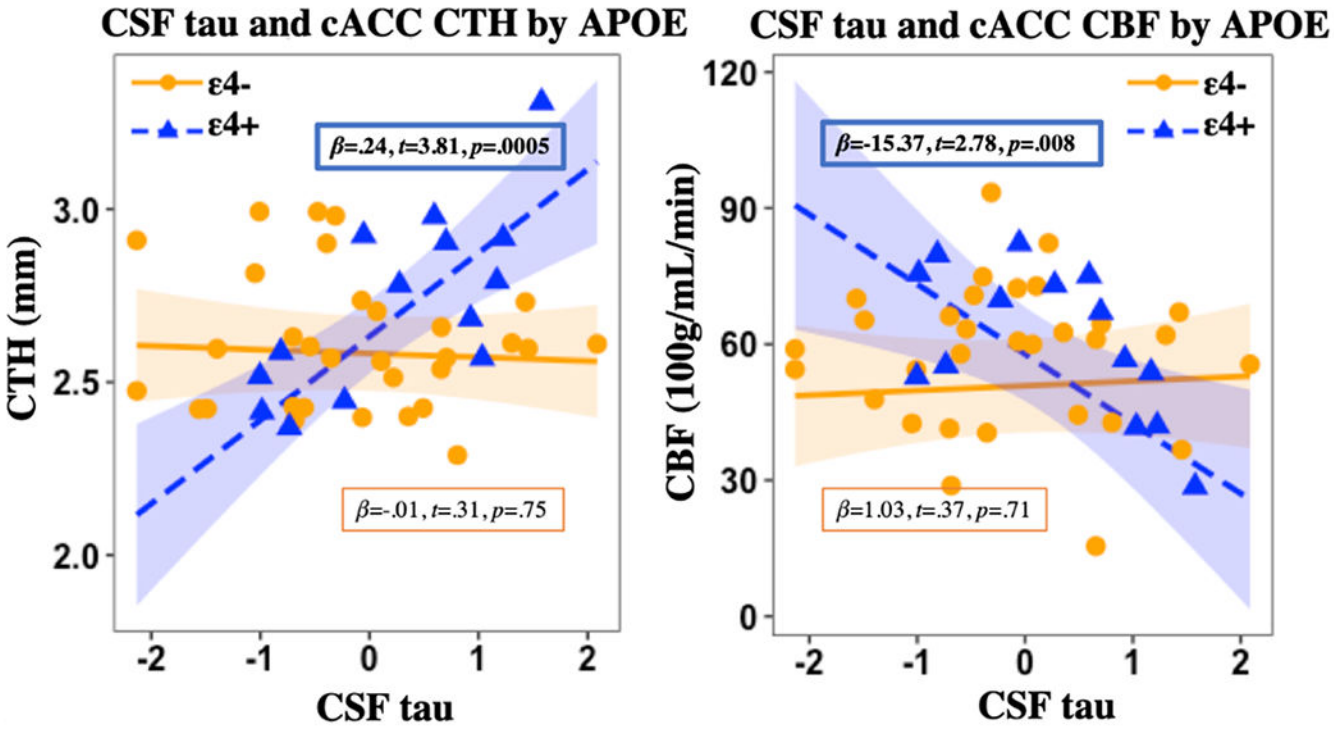

Figure 1. Effect of CSF tau and APOE on cACC CTH and CBF.

Higher CSF tau was associated with higher cACC CTH and with lower cACC CBF among APOE ε4 carriers, with no effect of CSF tau on either brain measure among non-carriers. *Note: CSF tau= cerebrospinal fluid tau; cACC= caudal anterior cingulate cortex; APOE= apolipoprotein E gene; CBF= cerebral blood flow; CTH= cortical thickness; CSF tau values are natural log-transformed and standardized; bolded font denotes simple slope significance at p<0.05*

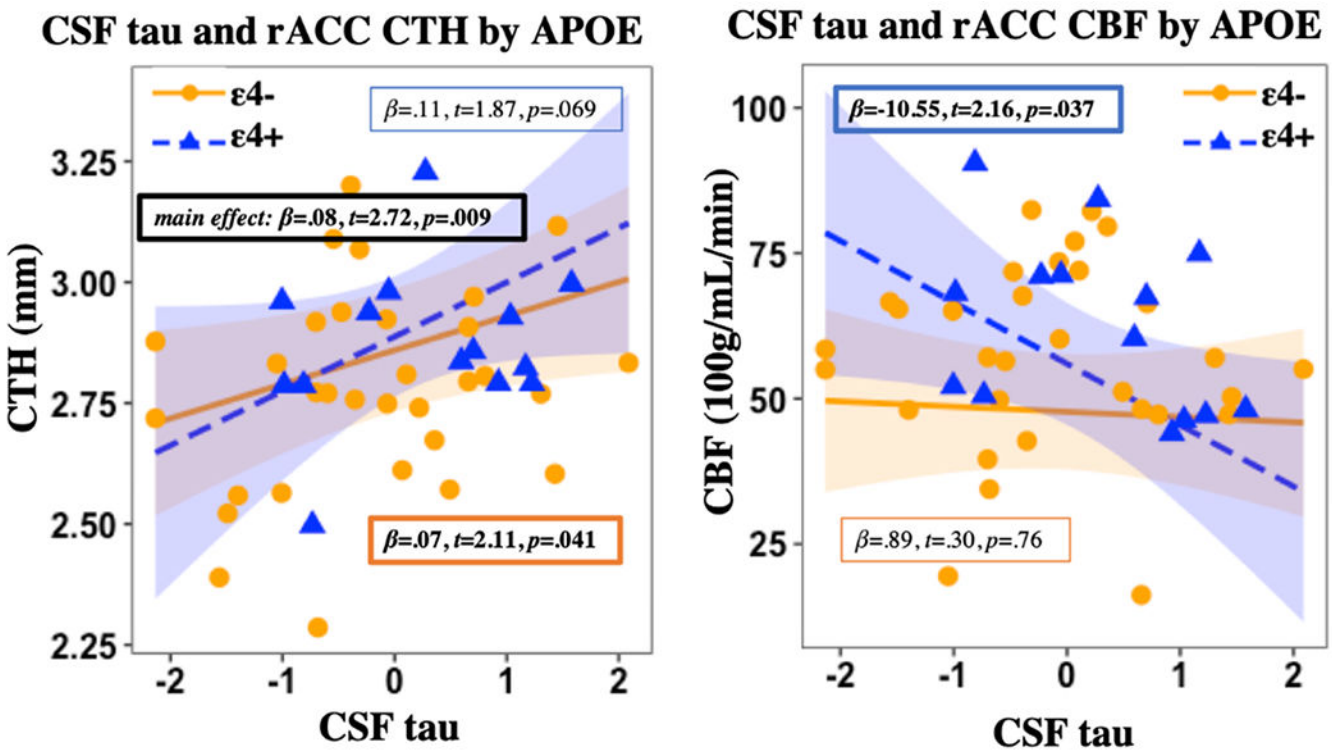

Figure 2. Effect of CSF tau and APOE on rACC CTH and CBF.

Higher CSF tau was associated with higher rACC CTH in both ε4 carriers and non-carriers, and with lower rACC CBF only in non-carriers. *Note: CSF tau= cerebrospinal fluid tau; rACC= rostral anterior cingulate cortex; APOE= apolipoprotein E gene; CBF= cerebral blood flow; CTH= cortical thickness; CSF tau values are natural log-transformed and standardized; bolded font denotes simple slope significance at p<0.05*

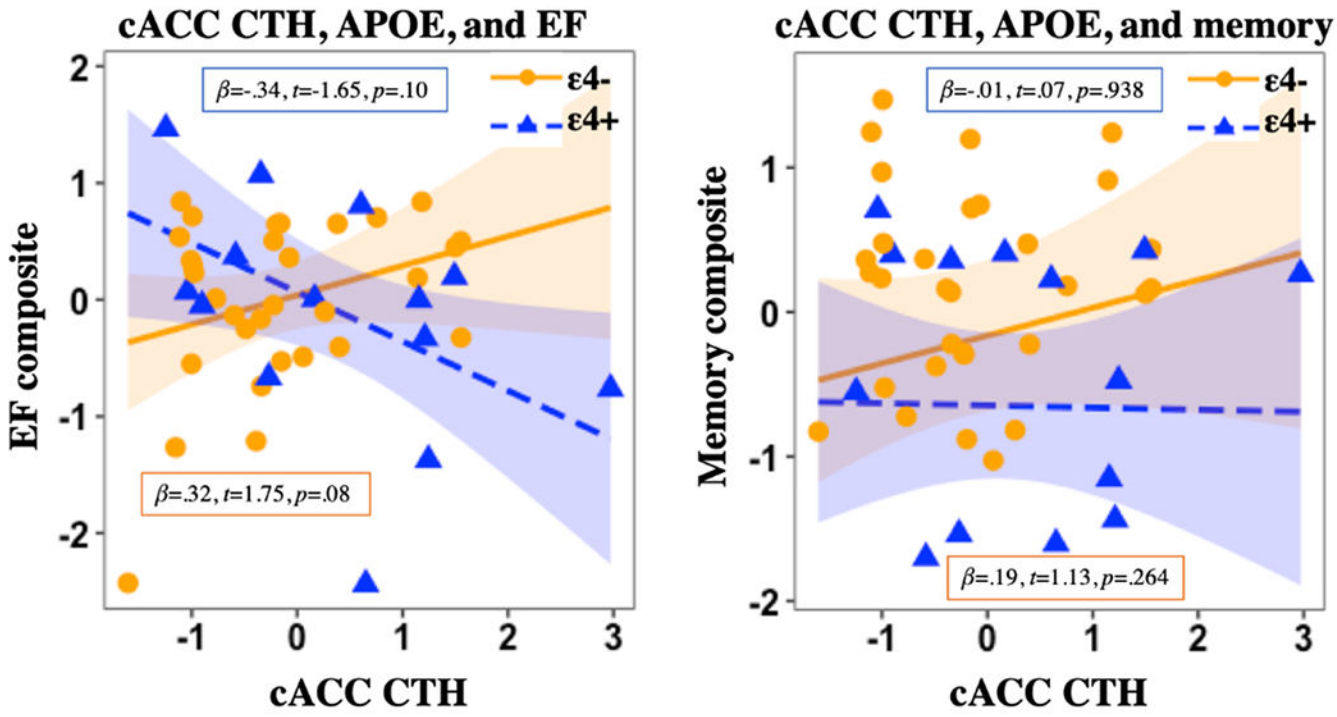

Figure 3. Effect of APOE and cACC CTH on cognition.

The effect of cACC CTH on executive function differs significantly by APOE status, such that the association between cACC CTH and executive function performance is negative among ε4 carriers and positive among non-carriers. There was not a significant interaction of APOE and cACC CTH on memory function. *Note: cACC= caudal anterior cingulate cortex; APOE= apolipoprotein E gene; CBF= cerebral blood flow; CTH= cortical thickness; EF= executive functioning; bolded font denotes simple slope significance at p<0.05*

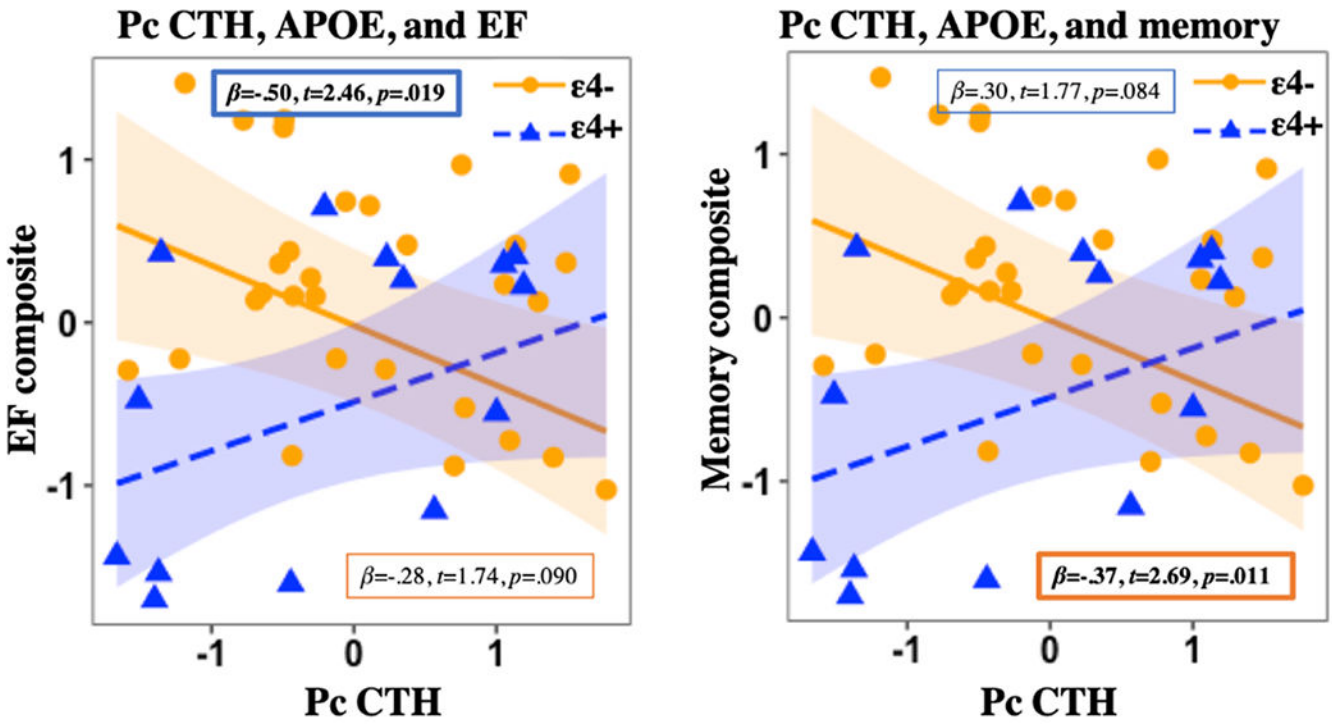

Figure 4. Effect of APOE and Pc CTH on cognition.

The effect of Pc CTH on cognition differs significantly by APOE status, such that the association between Pc CTH and both executive functioning and memory is positive among ε4 carriers and negative among non-carriers. *Note: Pc= precuneus; APOE= apolipoprotein E gene; CBF= cerebral blood flow; CTH= cortical thickness; EF= executive functioning*; *bolded font denotes simple slope significance at p<0.05*

Table 1.

Participant demographic and assessment characteristics.

	ε4− (*n*=31)		ε4+ (*n*=14)				
Variable	**Mean**	**SD**	**Mean**	**SD**	***t* or χ^2**	***df***	***p-value***
Age (years)	72.22	4.35	72.57	5.15	0.23	43	0.816
Sex (male/female)	7/24	--	6/8	--	1.93	1	0.164
Education (years)	16.48	2.45	16.35	2.45	0.16	43	0.871
FSRP Stroke Risk %	8.16	4.24	9.21	7.04	0.62	43	0.536
CSF-B and MRI interval (days)	258.5	374	258.8	167	0.002	43	0.998
Caudal Anterior Cingulate CBF	57.80	16.0	61.0	16.2	0.61	43	0.541
Caudal Anterior Cingulate CTH	2.60	0.19	2.72	0.25	1.78	43	0.081+
Rostral Anterior Cingulate CBF	56.89	16.0	62.58	16.2	1.11	43	0.271
Rostral Anterior Cingulate CTH	2.77	0.19	2.87	0.26	1.30	43	0.145
Posterior Cingulate Cortex CBF	69.96	15.7	71.24	13.3	0.26	43	0.793
Posterior Cingulate Cortex CTH	2.43	0.15	2.45	0.11	0.32	43	0.745
Precuneus CBF	72.43	10.9	69.76	11.6	0.74	42	0.461
Precuneus CTH	2.26	0.19	2.23	0.11	0.78	43	0.439
CSF tau pg/mL (ln)	4.06	0.44	4.29	0.38	1.35	43	0.182
$A\beta_{42}$ pg/mL (ln)	7.81	0.64	7.70	0.45	0.52	41	0.603
DRS Total Score	140.9	2.24	140.7	2.36	0.20	43	0.838
Memory Composite	0.18	0.68	−0.40	0.90	2.41	43	0.020 *
WMS-R LM Immediate Recall	31.25	5.16	27.9	5/95	1.80	43	0.062+
WMS-R LM Delayed Recall	28.25	5.19	25.07	8.02	1.60	43	0.116
CVLT-II List 1–5 total	52.87	8.53	42.7	10.5	3.43	43	0.001 **
CVLT-II SD Free Recall	10.58	2.90	9.93	2.84	0.70	43	0.485
CVLT-II LD Free Recall	11.61	2.68	9.85	2.82	1.00	43	0.051+
Executive Function Composite	0.00	0.68	−0.11	0.90	0.42	43	0.675
DKEFS CW Inhibition	63.16	9.81	65.5	14.0	0.64	42	0.525
DKEFS CW Inhibition Switch	68.6	15.3	67.3	17.8	0.25	42	0.802
WAIS-R Digit Span Backward Span	5.30	1.19	5.31	1.38	0.00	37	0.864
Trail Making Test-B	70.20	19.5	77.28	30.4	0.93	42	0.355

Note: FSRP= Framingham Stroke Risk Profile; CSF-B= Cerebrospinal fluid biomarker; MRI= magnetic resonance imaging; CBF= cerebral blood flow; CTH= cortical thickness; CSF tau= Cerebrospinal fluid tau protein; $A\beta_{42}$= amyloid-β 42 protein; pg= picogram; mL= milliliter; ln= natural log-transformed; DRS= Mattis Dementia Rating Scale; WMS-R= Wechsler Memory Scale-Revised (WMS-R); LM= logical memory; CVLT-II= California Verbal Learning Test – Second Edition; SD= short delay; LD= long delay DKEFS= Delis-Kaplan Executive Function System; CW= color word; WAIS-R= Wechsler Adult Intelligence Scale-Revised; *df= degrees of freedom; +marginal significance at p<0.10;*

* significance at p<0.05;

** significance at p<0.01; all scores represent raw scores unless otherwise stated

Table 2.

Interaction of CSF tau and APOE on ACC CTH and CBF.

DV: cACC CTH (mm^3)	**β**	**s.e.**	**t**	**p-value**
APOE	0.049	0.065	0.756	0.455
CSF tau	−0.011	0.034	0.319	0.752
APOE * CSF tau	0.253	0.071	3.558	0.001*
DV: cACC CBF (100g/mL/min)				
APOE	7.009	5.152	1.360	0.182
CSF tau	1.036	2.773	0.374	0.711
APOE * CSF tau	−16.414	6.113	2.685	0.011*
DV: rACC CTH (mm^3)	**β**	**s.e.**	**t**	**p-value**
APOE	0.028	0.064	0.440	0.663
CSF tau	0.071	0.033	2.116	0.042+
APOE * CSF tau	0.042	0.068	0.620	0.539
APOE	0.038	0.061	0.631	0.532
CSF tau	0.080	0.029	2.722	0.009*
DV: rACC CBF (100g/mL/min)				
APOE	8.264	5.078	1.628	0.112
CSF tau	−0.893	2.916	0.306	0.761
APOE * CSF tau	−9.660	5.354	1.804	0.079+

Note: Only variables of interest are included in table; CSF tau is natural log-transformed and standardized; cACC= caudal anterior cingulate; rACC= rostral anterior cingulate; APOE= apolipoprotein E gene; CTH= cortical thickness; CBF= cerebral blood flow; DV= dependent variable; β= Standardized regression coefficient; se= standard error; t= t-statistic;

+ marginal significance at p<0.10;

* significance at p<0.0125

Table 3.

Interaction of CSF tau and APOE on PCC and Pc CTH and CBF.

DV: PCC CTH (mm^3)	**β**	**s.e.**	**t**	**p-value**
APOE	0.008	0.055	0.156	0.877
CSF tau	−0.021	0.029	0.733	0.468
APOE * CSF tau	0.037	0.060	0.628	0.534
DV: PCC CBF (100g/mL/min)				
APOE	6.150	5.084	1.210	0.234
CSF tau	1.331	2.798	0.476	0.637
APOE * CSF tau	−10.38	5.438	1.909	0.064 +
DV: Pc CTH (mm^3)	**β**	**s.e.**	**t**	**p-value**
APOE	−0.036	0.038	0.961	0.343
CSF tau	−0.009	0.020	0.440	0.663
APOE * CSF tau	0.050	0.042	1.186	0.244
DV: Pc CBF (100g/mL/min)				
APOE	0.768	3.908	0.197	0.845
CSF tau	1.633	2.107	0.775	0.443
APOE * CSF tau	−9.686	4.092	2.367	0.023 +

Note: Only variables of interest are included in table; CSF tau is natural log-transformed and standardized; Pc= Precuneus; APOE= Apolipoprotein E gene; CBF= Cerebral blood flow; CTH= Cortical thickness; DV= Dependent variable; β= Standardized regression coefficient; se= standard error; t= t-statistic;

+ marginal significance at $p<0.10$;

* Significance at $p<0.0125$.