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Interaction of Age and APOE Genotype on Cerebral Blood Flow at Rest

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Abstract

We investigated the impact of APOE genotype on cerebral blood flow (CBF) in older and younger adults. Forty cognitively normal older adults (16 $\epsilon 4$ carriers, 24 non- $\epsilon 4$ carriers) and 30 younger adults (15 $\epsilon 4$ carriers, 15 non- $\epsilon 4$ carriers) completed a resting-state whole-brain pulsed arterial spin labeling magnetic resonance scan. Main effects of aging were demonstrated wherein older adults had decreased gray matter CBF corrected for partial volume effects compared to younger adults in widespread brain regions. Main effects of APOE genotype were also observed wherein $\epsilon 4$ carriers displayed greater CBF in the left lingual gyrus and precuneus than non-carriers. An interaction between age and APOE genotype in the left anterior cingulate cortex (ACC) was characterized by reduced CBF in older $\epsilon 4$ carriers and increased CBF in young $\epsilon 4$ carriers. Increased CBF in the left ACC resulting from the interaction of age group and APOE genotype was positively correlated with executive functioning in young $\epsilon 4$ adults ($r = 0.61$, $p = 0.04$). Results demonstrate APOE genotype differentially impacts cerebrovascular function across the lifespan and may modify the relationship between CBF and cognition. Findings may partially support suggestions that the gene exerts antagonistic pleiotropic effects.

Keywords

Aging; antagonistic pleiotropy; apolipoprotein E; cerebral blood flow; cerebrovascular function

Introduction

Certain genes are thought to exert different survival effects throughout the lifespan, and this evolutionary biological concept is termed antagonistic pleiotropy [1]. The apolipoprotein (APOE) $\epsilon 4$ allele on chromosome 19 has been proposed as one such gene given its observed beneficial effects on cognitive and brain response in early life and deleterious effects in late life [2]. For instance, compared to non-carriers, younger $\epsilon 4$ carriers show cognitive advantages including higher IQ [3], higher educational achievement [4], and better performance on certain cognitive tasks [5]. Despite these early benefits, the APOE $\epsilon 4$ allele is best known as a susceptibility gene associated with a three to four-fold increased risk of developing Alzheimer's disease (AD) [6] and is second only to advancing age in conferring AD risk. With advancing age, healthy nondemented older adults with the APOE $\epsilon 4$ allele exhibit subtle cognitive impairments, particularly in episodic memory [7–9], as well as increased blood oxygenation level dependent (BOLD) response during memory and language tasks [10–15]. This increased BOLD response is thought to reflect compensatory mechanisms indicating greater cognitive effort required to maintain performance at an equivalent level to healthy control groups [10, 11, 14].

Although it is unclear exactly how the APOE $\epsilon 4$ allele confers risk of AD, the allele is thought to play a role in cerebrovascular integrity given its association with small vessel arteriosclerosis, microinfarcts of the deep nuclei, neuritic plaque density, and amyloid angiopathy in autopsy-confirmed AD patients [16, 17]. Furthermore, recent evidence of decreased BOLD signal in individuals with autosomal dominant familial AD suggests the elevated BOLD signal in older nondemented $\epsilon 4$ carriers may be an artifact of altered vascular reactivity and cerebral blood flow (CBF) related to the APOE $\epsilon 4$ allele. Such alterations in cerebrovascular function could influence the BOLD signal independent of neural activity [18]. Because APOE $\epsilon 4$ may influence cerebrovascular function, and because this allele may exert dynamic effects throughout the lifespan, examining the effects of advancing age and the APOE $\epsilon 4$ allele on CBF may provide important information about distinct underlying neurovascular changes associated with normal aging and neurodegenerative disease risk. CBF specifically refers to the rate of delivery of blood to a capillary bed in the brain and is typically quantified as milliliters of blood/100 grams of tissue/minute [19]. Reductions of CBF during normal aging have been consistently demonstrated using PET and SPECT methods [20–24]. However, the recent development of arterial spin labeling (ASL) MRI techniques provide a non-invasive method to obtain a quantitative measure of CBF by magnetically labeling arterial water and using it as an endogenous tracer, ultimately helping to disentangle neural and vascular changes [19].

Several regions of reduced CBF have been implicated in older adults including the frontal cortex, superior temporal cortex, precuneus, hippocampus, and the parahippocampal gyrus [25–27], with more pronounced reductions in AD in the parietal cortex [28–30], temporal

and occipital cortices [28, 31], precuneus/posterior cingulate [28–30, 32, 33], parahippocampal gyrus and hippocampus [32], left inferior lateral frontal, and the orbitofrontal cortex [29]. Although regional effects of age on CBF appear independent of gray matter atrophy [34], the incorporation of sophisticated methods to correct for partial volume effects has resulted in lower age-related reductions in gray matter CBF (by up to 15%) than previous reports with age-related differences occurring primarily in frontal and parietal regions [35]. These findings suggest that increased atrophy may confound CBF measurement if partial volume effects are not taken into account. Only a few studies have examined CBF in healthy individuals at genetic risk for AD based on possession of the APOE $\epsilon 4$ allele. In nondemented older adults, greater CBF decline was observed in $\epsilon 4$ carriers in the frontal, parietal, and temporal cortices over an eight year interval compared to non-carriers [36]. In a cross-sectional design, reduced CBF was found in nondemented older $\epsilon 4$ carriers relative to non-carriers in the anterior and posterior cingulate cortices and the cerebellum despite preserved gray matter volume [37]. These findings suggest greater CBF reductions in individuals at genetic risk for AD than non-carriers, perhaps reflecting early neurovascular changes associated with AD. Conversely, a study focused on medial temporal lobe (MTL) CBF in middle-aged adults revealed greater resting CBF in individuals at high risk for AD (family history of AD and at least one copy of the APOE $\epsilon 4$ allele) compared to a low risk group (no family history of AD and no APOE $\epsilon 4$ allele) [38]. These latter findings raise the possibility that very early manifestations of AD may be associated with greater baseline blood flow in the MTL in an effort to compensate for functionally impaired regions and that these differences may be observed decades prior to clinical symptoms. Establishing the relationship between CBF and cognition would support this hypothesis.

To our knowledge, only a few studies have examined the relationship between age-related changes in CBF and cognitive performance [39, 40]. A couple of recent studies suggest that the relationship between cognition and CBF may differ across the lifespan (e.g., decreased CBF across grossly-defined regions of interest comprised of lobes and hemispheres was correlated with improved selective attention in young adults and improved tonic alertness in older adults, while increased hippocampal CBF was correlated with improved spatial memory only in older adults [41, 42]). However, a more focused approach that assesses regionally specific differences in CBF and their association to distinct cognitive domains is needed to elucidate the relationship of CBF and cognition in aging and genetic risk of AD.

Taken together, evidence supports reduced CBF in normal aging with a greater reduction in older APOE $\epsilon 4$ carriers. Further, cognitive functions decline with aging and more so in those at genetic risk for AD, though evidence that younger $\epsilon 4$ carriers outperform their non-carrier peers raises concerns regarding the possible interaction of age and the APOE $\epsilon 4$ allele on cognitive and cerebrovascular changes. The current study aimed to clarify the influence of age and APOE genotype on resting CBF as well as explore the relationship between altered CBF patterns and cognitive performance. We hypothesized that 1) older adults would demonstrate lower resting CBF compared to younger adults, 2) the APOE $\epsilon 4$ allele would exert regionally-specific and differential effects on CBF in older and young adults whereby older $\epsilon 4$ carriers would show lower resting CBF (consistent with vascular compromise) and younger $\epsilon 4$ carriers would display increased resting CBF (consistent with early genetic

benefit) in medial anterior and/or posterior regions, and 3) there would be a differential relationship between CBF and cognition across age and genetic risk groups that may inform the role of CBF on cognition.

Materials and Methods

Participants

Forty cognitively normal older adults (OA; 16 $\epsilon 4$ carriers, 24 non- $\epsilon 4$ carriers) and 30 younger adults (YA; 15 $\epsilon 4$ carriers, 15 non- $\epsilon 4$ carriers) completed a resting-state whole-brain pulsed ASL magnetic resonance (MR) scan. Data from 37 of OA participants were previously reported [43]. Younger participants were recruited through San Diego State University and the University of California, San Diego campuses (e.g., classrooms and flyers), and all older adults completed this study as part of their participation in a larger longitudinal study of healthy aging. To ensure similar sample sizes of APOE $\epsilon 3$ and $\epsilon 4$ carriers, we initially conducted a widespread screening procedure to collect demographic and DNA data from young adult participants. Thirty participants considered eligible for the study based on inclusion/exclusion criteria and genotype were followed up and enrolled in the MR scan procedure. All OA participants were considered normal based on extensive medical, neurologic, laboratory, and neuropsychological evaluations. Potential participants were excluded if they had a history of severe head injury, uncontrolled hypertension, the APOE $\epsilon 2$ isoform, or a Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition Axis 1 diagnosis of learning disability, attention deficit disorder, psychiatric disorder, or substance abuse. Older adults with significant cerebrovascular disease, defined by modified Hachinski Ischemia Scale [44] scores greater than 4 were excluded. Of the 40 older adult participants, 18 participants were taking anti-hypertensive medications (11 $\epsilon 3$, 7 $\epsilon 4$), two had history of stroke or TIA (1 $\epsilon 3$, 1 $\epsilon 4$), three had history of atrial fibrillation (3 $\epsilon 3$), five had evidence of cardiovascular disease (4 $\epsilon 3$, 1 $\epsilon 4$), and no participants had diabetes. The $\epsilon 3$ and $\epsilon 4$ carriers also did not differ in 10 year probability of stroke risk on the Framingham Stroke Risk Profile [45] ($p = 0.41$) or on blood pressure ($p = 0.94$). In addition, participants were excluded if they had contraindications to MRI scans such as metal in their body other than dental fillings, or if they were taking prescription psychoactive medications. No participant reported a significant level of depressive symptoms on the Geriatric Depression Scale (i.e., GDS >11) [46].

This research was approved by the Ethics Committee and Institutional Review Board at the University of California at San Diego and VA San Diego Healthcare System. Written informed consent was obtained from all participants according to guidelines established by the Declaration of Helsinki.

Apolipoprotein E genotyping

Genotyping for APOE alleles was performed using PCR Restriction Fragment Length Polymorphism analysis. Genomic DNA was collected from participants using buccal swab and extracted using Qiamp DNA blood mini kit (Qiagen) followed by PCR amplification. The amplification reaction contained 5 μ l genomic DNA, 2.5 units of Taq DNA Polymerase (New England Biolab), 1 $\times$ ThermoPol Reaction Buffer (New England Biolab), 0.3mM

dNTPs, 10% DMSO, and 0.3 μ M of each primer (forward primer: 5'-ACG-CGGGCACGGCTGTCCAAGGA-3'; reverse primer: 5-GCGGGCCCCGGCCTGGTACAC-3'). PCR cycling conditions were as follows: initial denaturation at 94°C for 3 min followed by 30 cycles of 94°C for 30 s and 72°C for 90 s with a final extension at 72°C for 4 min. Amplification was performed on BioRad C1000 Thermocycler. After PCR, the amplicon products were digested with 10 units of restriction enzyme HhaI (New England Biolab) at 37°C for >4 h. The resulting DNA fragments were analyzed by separation on a 12% acrylamide gel. After electrophoresis, the gel was incubated in ethidium bromide and visualized under UV illumination. APOE genotyping was confirmed using Applied Biosystems TaqMan SNP Genotyping Assay (C_3084793_20 and C_-904973_10 corresponding to APOE SNPs rs429358 and rs7412, respectively). The assay was run on a Bio-Rad CFX96 Touch Real Time PCR Detection System, using a cycling program of 98°C for 2 min and 39 cycles of 98°C for 15 s and 62°C for 45 s. Five positive controls for each genotype and one negative control were included in each plate to ensure accurate determination.

Image acquisition

Imaging data were acquired on a GE Signa Excite 3-T whole body system with a body transmit coil and an 8-channel receive-only head coil. A high-resolution T1-weighted 3D FSPGR scan was obtained to provide anatomic reference: 25 cm FOV, 256 $\times$ 192 matrix, TR = 8 ms, TE = 3.1 ms, flip angle = 12°, T1 = 600 ms, bandwidth = 31.25 kHz, 172 1mm sagittal slices. To assess CBF differences across participants, three sequences were acquired to obtain an absolute CBF measurement. Resting brain blood perfusion was measured with pulsed arterial spin labeling (PASL) using a modified flow-sensitive alternating inversion recovery (FAIR) sequence with both presaturation pulses and PICORE QUIPSS 2 post-inversion saturation pulses and a spiral readout with 4 interleaves to reduce signal dropout due to susceptibility effects [47, 48]. Imaging parameters of the ASL scan were: 22 $\times$ 22 cm field of view, a 64 $\times$ 64 matrix, 3.2 ms echo time, 2500 ms repetition time, post-saturation and inversion times of TI1 = 600 ms and TI2=1600 ms, tag thickness 10 cm, tag to proximal slice gap 1 cm, 20 5 mm axial slices, and 40 volumes for 20 tag + control image pairs [49]. A scan with the 90° excitation pulse turned off for the first eight repetitions was acquired to obtain the equilibrium magnetization of cerebrospinal fluid (CSF) (a 36-s scan with TR = 4s, TE = 3.4 ms, NEX = 9). The CSF signal was used to estimate the equilibrium magnetization of blood, which in turn was used to convert the perfusion signal into calibrated CBF units (ml blood/100 ml tissue-min). A 32 s minimum contrast scan was acquired using an eight-shot acquisition with TR = 2000 ms, TE = 11 ms, NEX = 2 to estimate the combined transmit and receive coil inhomogeneities [50]. The two images were averaged to create the minimum contrast image. The ASL image was then divided by the minimum contrast image to remove the effect of coil inhomogeneity during the CBF quantification step [51].

Data processing and analyses

Structural and CBF processing—Image processing was performed with Analysis of Functional NeuroImages (AFNI, <http://afni.nimh.nih.gov/> [52]), FMRIB Software Library (FSL, Oxford, United Kingdom), and locally created MatLab scripts. Each ASL dataset was reconstructed using the SENSE algorithm [53, 54] to reduce sensitivity to the modulations

that occur between shots caused by physiological fluctuations or motion. An automated MatLab script was used to preprocess the ASL data using AFNI and FSL tools. The ASL time series was co-registered to the middle time point to minimize the effects of participant motion. For each subject, a mean ASL image was formed from the average difference of the control and tag images using surround subtraction to create an uncorrected perfusion time series, and slice timing delays were accounted for, making the inversion time (TI2) slice specific [47]. This mean ASL image was then converted to absolute units of CBF (mL/100 g tissue/min) using an estimate of the equilibrium magnetization of CSF as a reference signal [55]. This procedure resulted in a calibrated perfusion value for each voxel. Skull stripping of the high-resolution T1-weighted image was performed using Brain Surface Extractor [56, 57], shown to outperform other methods in older adults [58]. Scans were manually edited to remove residual non-brain material when necessary. Tissue segmentation was performed using FSL's Automated Segmentation Tool (FAST) algorithm to define CSF, gray matter, and white matter regions. The high-resolution T1-weighted image and partial volume segmentations were registered to ASL space, and partial volume segmentations were down-sampled to the resolution of the ASL data.

Corrections for partial volume effects—To correct the CBF measures for partial volume effects and ensure that CBF values were not influenced by known decreased perfusion in white matter or increased volume of CSF [27, 59], we used the method previously reported by Johnson and colleagues [30]. These calculations assume that CSF has zero CBF and that CBF in gray matter is 2.5 times greater than that in white matter. The following formula was used to compute partial volume corrected CBF signal intensities: $CBF_{corr} = CBF_{uncorr} / (GM + 0.4 * WM)$. CBF_{corr} and CBF_{uncorr} are corrected and uncorrected CBF values, respectively. GM and WM are gray matter and white matter partial volume fractions, respectively. Information from the high resolution structural image and the FSL FAST was used to determine the tissue content of each perfusion voxel. Using AFNI, a 4.0 mm full-width, half-maximum Gaussian filter was applied to the CBF_{corr} data. Voxels with negative intensities were replaced with zero [60]. CBF_{corr} data were warped to standard Talairach space [61] and resampled to a $4 \times 4 \times 4$ mm resolution grid. Data were then screened for data quality and outlying values deviating by more than 3 SDs of the mean were eliminated.

Age group by APOE genotype analyses—Quantified CBF corrected for partial volume effects was compared using a 2 group (YA, OA) $\times$ 2 APOE genotype ($\epsilon 3$, $\epsilon 4$) voxel-wise analysis of variance (ANOVA). Type I error was controlled based on Monte Carlo simulation results using AFNI's *AlphaSim* set with an individual voxel alpha of 0.05. Based on an averaged gray matter mask created from all the participants, a minimum cluster volume threshold of 1344 μ l or 21 contiguous voxels was chosen and this threshold/volume combination protected a whole-brain probability of false positives of $p < 0.05$. CBF values were extracted from significant clusters and subjected to planned follow-up two tailed *t*-tests using a significance cutoff of $p < 0.05$. Effect sizes were calculated according to the following equation: $\eta^2 = (t^2 / (t^2 + df))$ where t = *t*-value and df = degrees of freedom.

Associations with cognition—First, independent samples *t*-tests were conducted to compare raw and standardized neuropsychological scores between the young and old groups and between the $\epsilon 4$ and $\epsilon 3$ carriers within each group. Second, blood flow values from significant clusters were extracted for exploratory partial correlations (including age and education as covariates) between CBF and neuropsychological composite scores for each of the four subgroups. A Bonferroni correction was applied for each region to correct for multiple comparisons. For the interaction of age and APOE genotype and the main effect of APOE genotype correlations were considered significant at $p < 0.05$ ($p = 0.05/1$ region), and for the main effect of age correlations were only considered significant at $p < 0.005$ ($p = 0.05/9$ regions). Composite scores were created for two neuropsychological domains hypothesized to be related to gray matter CBF: memory and executive functions. Composite scores were calculated for the entire sample using principal component analysis (PCA) on age-corrected standardized scores from the following six tests with oblique rotation (direct oblimin): California Verbal Learning Test-2 (CVLT-2; List A trials 1–5 T-score, short delay free recall and long delay free recall z-scores; [62]) and Delis-Kaplan Executive Function System (scaled scores from the Trail Making Letter-Number Switching, Verbal Letter Fluency, and Design Fluency Switching subtests; [63]). We chose to include the immediate (learning) and delayed recall measures, rather than the recognition measure, because these have been shown to exhibit associations with the APOE $\epsilon 4$ genotype in prodromal AD [64]. Additionally, we did not expect much variability on the recognition measure in our cognitively normal sample. The Kaiser-Meyer-Olkin measure verified the sampling adequacy for the analysis, KMO = 0.74, and Bartlett's test of sphericity $X^2(15) = 226.92$, $p < 0.001$, and indicated that correlations between items were sufficiently large for PCA. An initial analysis was run to obtain eigenvalues for each component in the data. Two components had eigenvalues over Kaiser's criterion of 1 and in combination these two factors explained 75.9% of the variance. Table 1 shows the factor loading after rotation. The items that cluster on the same components suggest that component 1 represents verbal memory and component 2 represents executive functions. The resulting factor scores from each PCA were saved and submitted to correlation analyses.

Results

The demographic characteristics and neuropsychological performances of the participants are shown in Table 2. Within each age group, the $\epsilon 4$ -carriers did not differ significantly from the non-carriers, with the exception that the young $\epsilon 4$ -carriers performed better on the CVLT-2 (raw scores on List A Trials 1–5, short delay free recall, long delay free recall; [62]). This latter finding is consistent with literature of improved cognitive performance in $\epsilon 4$ carriers compared to non-carriers in childhood and young adulthood [5]. The groups based on age (YA, OA) did not differ in terms of gender distribution ($\chi^2 = 0.01$, $p = 0.94$) or APOE $\epsilon 4$ distribution ($\chi^2 = 0.70$, $p = 0.41$), but the OA group was significantly more educated than the YA group, had reduced gray matter CBF averaged across the whole brain, performed more poorly on tests of memory (CVLT-2 List A Trials 1–5, long delay free recall) and cognitive switching (Delis-Kaplan Executive Function System Trail Making Test Number-Letter Switching and Design Fluency Switching subtests; [63]), but performed better on Verbal Letter Fluency based on raw scores. However, when age-appropriate

normative data were used to calculate standardized scores, the older adults out-performed the younger adults on all measures except for CVLT-2 long delay free recall in which they performed equally well, and all performances were within normal limits.

Main effects and interaction of age and APOE genotype

Whole brain voxel-level gray matter CBF comparisons revealed a main effect of age whereby older adults demonstrated lower CBF in a large cluster encompassing most of the brain. Applying a more conservative statistical thresholding procedure ($p = 0.001$) resulted in the emergence of 9 clusters for the main effect of age. Specifically, older adults demonstrated lower CBF in the following regions: the left and right medial and lateral prefrontal cortex including the medial frontal gyrus, anterior cingulate, superior and inferior frontal gyri, and insula; left and right thalamus, caudate, and left hippocampus; left parahippocampal gyrus and fusiform gyrus; left precuneus and cuneus extending to the middle occipital gyrus; left and right cerebellum; and right lateral temporal lobe including the middle and superior temporal gyri extending to the supramarginal gyrus (Fig. 1).

A main effect of APOE genotype was revealed in the left occipital lobe involving the lingual gyrus, cuneus, and middle occipital gyrus, in which $\epsilon 4$ carriers had elevated CBF compared to $\epsilon 3$ carriers (Fig. 2). Follow-up planned independent samples t -tests revealed that this effect was driven by the contrast between $\epsilon 3$ and $\epsilon 4$ older adults [$t(38) = -3.49, p < 0.01$] as there were no differences between younger $\epsilon 3$ and $\epsilon 4$ adults [$t(28) = -1.97, p = 0.06$].

Notably, an interaction of APOE genotype ($\epsilon 3, \epsilon 4$) and age (YA, OA) on quantified CBF corrected for partial volume effects was found in the left anterior cingulate cortex (ACC) (Table 3 and Fig. 3). Simple main effects analysis showed that CBF was significantly higher in young $\epsilon 4$ carriers compared to young non-carriers ($t(28) = -3.51, p < 0.01$), and that CBF was lower in older $\epsilon 4$ carriers compared to old non-carriers, but this latter difference did not quite reach significance ($t(38) = 1.89, p = 0.07$). Young and older $\epsilon 3$ carriers did not differ in CBF in this region ($t(37) = -0.60, p = 0.55$) but CBF for young $\epsilon 4$ carriers was significantly greater than for older $\epsilon 4$ carriers ($t(29) = 4.84, p = 0.001$).

Relationship between CBF and cognition

After controlling for age and education, increased CBF in the left ACC resulting from the interaction of age group and APOE genotype was positively correlated with executive functioning in young $\epsilon 4$ adults ($r = 0.61, p = 0.04$), but not for older $\epsilon 4$ carriers ($r = 0.15, p = 0.60$), younger $\epsilon 3$ carriers ($r = -0.25, p = 0.40$), or older $\epsilon 3$ carriers ($r = -0.09, p = 0.70$). In contrast, there were no significant correlations between verbal memory performance and left ACC CBF in young $\epsilon 3$ ($r = -0.49; p = 0.09$) or $\epsilon 4$ ($r = 0.35; p = 0.27$) carriers or old $\epsilon 3$ ($r = -0.28, p = 0.22$) or $\epsilon 4$ ($r = 0.15, p = 0.61$) carriers. No correlations between cognitive performance and CBF in regions identified in the main effect of age or APOE group reached statistical significance after controlling for multiple comparisons.

Discussion

Results supported our prediction that older adults would demonstrate lower resting CBF compared to younger adults despite higher educational attainment and age-normed cognitive

performance. Age had the largest impact on CBF changes, whereby older adults had decreased gray matter CBF compared to younger adults in widespread brain regions including the left and right medial and lateral prefrontal cortex, left and right thalamus, caudate, and left hippocampus, left and right precuneus and cuneus, left parahippocampal gyrus and fusiform gyrus, and right lateral temporal lobe. This finding is consistent with prior studies showing a significant decrease in resting CBF in the cortical and subcortical parenchyma with selective reduction in CBF in the temporal and prefrontal cortices as well as in large cerebral arteries in aging [20, 65, 66]. Despite the strong effect of age, APOE $\epsilon 4$ genotype exerted an independent effect on CBF such that $\epsilon 4$ carriers had greater CBF in the left lingual gyrus and cuneus than non-carriers. Follow-up tests revealed that the CBF elevations in these posterior regions were greater in the older $\epsilon 4$ carriers than the younger carriers.

Notably, an interaction between age and APOE genotype in the left ACC was found, characterized by lower CBF in older $\epsilon 4$ carriers and higher CBF in young $\epsilon 4$ carriers. In contrast to the resulting main effect of APOE, this finding appeared to be driven by differences between $\epsilon 4$ carriers and non-carriers in the younger adult group. Specifically, although older and younger non-carriers had similar levels of CBF in this region, young $\epsilon 4$ carriers had significantly elevated CBF compared to young non-carriers, whereas older $\epsilon 4$ carriers had reduced CBF compared to older non-carriers (the latter difference did not quite reach significance). The reduced ACC resting CBF in older $\epsilon 4$ carriers relative to older non-carriers confirms prior brain imaging results [37], and extends findings to the comparison of young $\epsilon 3$ and $\epsilon 4$ carriers. The finding that resting CBF in the ACC differs between young and older adult $\epsilon 4$ carriers, but does not differ between young and older non-carriers suggests that the influence of the APOE $\epsilon 4$ allele on neurovascular function may change with progressing age and results in a striking elevation in young adults.

Few studies have examined CBF in healthy young adults, with the exception of one study that did not find any differences between young $\epsilon 4$ and non- $\epsilon 4$ carriers [67]. Our findings may differ from this previous study due to our inclusion of both young and older adults in the analysis, as well as different analytic methods (e.g., voxel-wise approach). Previous studies that investigated the effect of APOE genotype on BOLD response in fMRI have reported that young $\epsilon 4$ carriers exhibit increased coactivation in MTL regions, medial-prefrontal, and retrosplenial regions at rest and show task-related elevations in the hippocampus [67, 68] and the left precuneus, bilateral mid-frontal gyrus, right inferior frontal/medial frontal gyrus, right inferior occipital gyrus, right mid-temporal gyrus, right precentral gyrus, left lingual gyrus, right parahippocampal gyrus, and right cingulate gyrus [69]. However, prior PET studies demonstrated lower cerebral glucose metabolism in both young and middle-aged $\epsilon 4$ carriers in the posterior cingulate, and parietal, temporal, and lateral prefrontal cortices compared with non-carriers [70–72]. Our results are consistent with previous literature indicating that the APOE genotype influences brain function in young adulthood, and adds evidence that the effect of APOE on CBF varies from increased flow to decreased flow throughout the lifespan.

As an exploratory analysis, we examined the relationship between CBF and cognition as a function of age and APOE group. Correlations performed between CBF and cognitive

performance revealed that CBF in the left ACC was positively associated with executive functioning in young $\epsilon 4$ carriers, supporting our prediction of a differential relationship between CBF and cognition across age and genetic risk groups. The significant positive association between CBF and executive function in the left ACC for young $\epsilon 4$ carriers is consistent with the established role of the anterior cingulate in functions such as error detection, monitoring conflict between competing responses, and initiation of behavior [73, 74]. Our executive function factor was comprised of the Trail Making Letter-Number Switching Test, Verbal Letter Fluency Test, and Design Fluency Switching Test from the D-KEFS, and thus represents a measure of internally guided generative behavior and cognitive switching consistent with the function of the ACC. However, this relationship was only observed in the younger $\epsilon 4$ carriers, suggesting that the decreased ACC CBF in older $\epsilon 4$ carriers may reflect a disruption in the neural substrates supporting cognitive switching and monitoring conflicting responses. In light of the lack of relationship between left ACC CBF and executive functioning in young and older $\epsilon 3$ carriers, elevated CBF in young $\epsilon 4$ carriers may be associated with early neuropsychological benefits in genetically at-risk young adults. However, as CBF is typically tightly coupled with metabolic demands in healthy subjects, it is possible that utilization of greater CBF than is needed for metabolic demand may signify uncoupling of flow from metabolism in young $\epsilon 4$ carriers. This atypical elevated flow, although potentially beneficial to executive functioning, may be detrimental to cerebrovascular health and could possibly predispose $\epsilon 4$ carriers to develop AD neuropathology in later life [37].

Within the context of reductions in resting CBF that accompany normal aging, the APOE $\epsilon 4$ allelic variant appears to amplify this age-related difference, resulting in elevated ACC CBF in young adults but greater CBF reductions in older adults. In a normal physiological state, CBF is typically maintained through autoregulation of the brain at a relatively constant level at approximately 50 ml per 100 g of brain tissue per minute. Uncertainty remains regarding exactly what the elevated and reduced CBF patterns observed in the APOE $\epsilon 4$ carriers reflect in terms of specific brain processes; however, previous reports of older adults with AD or mild cognitive impairment (MCI) suggest that both higher and lower CBF may be associated with neurodegenerative disease processes. For example, older adults with AD and individuals with preclinical AD, such as those with MCI, often demonstrate patterns of hypoperfusion compared to their cognitively normal peers [75]. However, regional patterns of hyperperfusion have also been implicated in MCI and early AD, such as in medial temporal regions, subcortical regions, and in the anterior cingulate [29, 43]. Additionally, in a previous study, our group found reduced CBF in cognitively normal older $\epsilon 4$ carriers, but greater CBF in $\epsilon 4$ carriers with MCI. However, the specific etiology of this observed cerebrovascular alteration in APOE $\epsilon 4$ carriers remains unclear. The anterior cingulate cortex is supplied by the anterior cerebral artery, the anterior pericallosal artery, and the cingulocallosal arteries [28, 76], raising questions of whether the effects of APOE on CBF are due to structural changes in these feeding arteries or reflect changing hemodynamics due to distal cerebrovascular alterations. The inclusion of an angiogram would further clarify whether a structural etiology contributes to the CBF changes resulting from APOE genotype. Alternatively, it is possible that older APOE $\epsilon 4$ carriers have increased amyloid- β (A β) deposition that leads to reduced CBF [77]. Evidence suggests that the APOE $\epsilon 4$ allele

is more strongly associated with increased $A\beta$ burden than with higher levels of tau (a marker of neurofibrillary tangle pathology) in preclinical AD [77], raising the possibility of a link between a breakdown in the blood-brain barrier and alterations in brain perfusion [78] that interferes with $A\beta$ clearance mechanisms [79]. Future work will need to include a CSF assay or cerebral amyloid imaging to delineate the relationship between $A\beta$ and CBF. However, recent fMRI BOLD studies concluded that changes in functional connectivity between genotype groups are directly related to APOE and not its effects on AD pathology [80, 81], suggesting that there may be independent effects of APOE on functional connectivity. Additionally, recent animal studies indicate potential mechanisms associated with APOE $\epsilon 4$ that can be detrimental to cerebrovascular function. For example, a recent study demonstrated that possession of the APOE $\epsilon 4$ allele in mice triggered an inflammatory response that damaged the blood-brain barrier and led to cerebrovascular damage including small vessel damage [82].

Additionally, the differences in resting CBF between younger and older adults and the interaction of age and APOE genotype on CBF may have serious implications for fMRI studies that make comparisons between age groups. For example, reduced CBF in widespread regions in aging may partially account for increased fMRI BOLD activation seen in aging [83–86] since decreases in baseline CBF significantly increase the amplitude of the BOLD response [38, 87]. Because differences in baseline CBF between groups could account for differences in BOLD activation, future fMRI studies would benefit from integrating simultaneous measurements of CBF and BOLD response to cognition as well as a separate hypercapnic challenge (e.g., CO_2 inhalation) to reduce the potential ambiguity of interpreting BOLD signal differences between age groups by disentangling the relative contribution of vascular versus neuronal components to the fMRI signal and how these may change with age [88].

In summary, results demonstrate that APOE genotype differentially impacts cerebrovascular function across the lifespan as well as the relationship between CBF and cognition. Our study has several strengths, including whole brain voxel-wise CBF comparisons in a neuropsychologically healthy and well-characterized sample with associations between CBF and cognitive performance using partial volume corrected quantitative ASL-MR imaging. Additionally, to our knowledge, this is one of the first studies to compare resting CBF in young $\epsilon 3$ and $\epsilon 4$ carriers to an older adult sample. Despite these strengths, there are several remaining limitations of our ASL technique. For instance, because the overall MR signal caused by the delivered arterial blood is only about 1% of the total signal due to the tissue, ASL has reduced sensitivity to weak activation resulting in a low intrinsic perfusion signal-to-noise ratio compared with that of exogenous tracers (e.g., xenon) [19]. The perfusion quantification in ASL also relies on several assumptions (e.g., tagging efficiency, transit delay). Nevertheless, results further establish the role of CBF in AD risk even in young adults and support the use of ASL MRI as a viable, non-invasive, and relatively simple alternative to the more complicated BOLD methods and more invasive FDG-PET techniques for detecting early changes in the central nervous system associated with AD risk. Refinements in ASL MRI methods such as the recent advent of more optimized pseudo-continuous ASL sequences [51] that continue to improve upon signal-to-noise

characteristics will further advance the applicability of this MRI technique to the question of neurovascular changes in those at risk for the development of AD and vascular dementia. Additionally, future studies that investigate the association between CBF, APOE, vascular risk factors, and neuroimaging markers of cerebrovascular disease may provide additional information about the interaction of APOE and neurovascular changes on brain function in older adults at risk for dementia.

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Fig. 1.

The effect of age on resting gray matter cerebral blood flow. Thresholded and clustered results (protecting a whole-brain voxel-wise $p = 0.05$; red: $p = 0.001$, orange: $p = 0.0005$, yellow: $p = 0.0001$) for a two-way ANOVA indicating a main effect of age with corresponding graphical presentation of significant CBF differences. Error bars represent the standard error of the mean. Results are overlaid onto sagittal slices of a high-resolution anatomical image averaged across all participants (L, left; R, right; c, cluster). For main effect of age: C1: YA = 60.8 ± 14.4 , OA = 31.4 ± 8.7 , $F(1,68) = 113.3$, $p < 0.001$; C2: YA = 63.5 ± 15.3 , OA = 32.4 ± 12.2 , $F(1,68) = 90.2$, $p < 0.001$; C3: YA = 81.0 ± 21.8 , OA = 36.7 ± 15.5 , $F(1,68) = 98.4$, $p < 0.001$; C4: YA = 75.7 ± 41.2 , OA = 41.8 ± 30.9 , $F(1,68) = 15.6$, $p < 0.001$; C5: YA = 70.3 ± 19.0 , OA = 35.2 ± 10.5 , $F(1,68) = 97.3$, $p < 0.001$; C6: YA = 75.5 ± 17.6 , OA = 39.5 ± 19.8 , $F(1,68) = 62.4$, $p < 0.001$; C7: YA = 50.5 ± 15.1 , OA = 26.0 ± 12.7 , $F(1,68) = 54.3$, $p < 0.001$; C8: YA = 56.2 ± 19.3 , OA = 30.3 ± 11.9 , $F(1,68) = 47.9$, $p < 0.001$; C9: YA = 75.0 ± 17.2 , OA = 40.7 ± 18.9 , $F(1,68) = 61.1$, $p < 0.001$.

Fig. 2.

The effect of APOE on resting gray matter cerebral blood flow. Thresholded and clustered results (protecting a whole-brain voxel-wise $p = 0.05$; red: $p = 0.05$, orange: $p = 0.025$, yellow: $p = 0.01$) for a two-way ANOVA indicating a main effect of APOE with corresponding graphical presentation of significant CBF differences. Error bars represent the standard error of the mean. Results are overlaid onto a sagittal slice of a high-resolution anatomical image averaged across all participants (L, left). For main effect of APOE: $\epsilon 3 = 55.7 \pm 24.5$, $\epsilon 4 = 90.4 \pm 45.7$, $F(1,68) = 16.6$, $p < 0.001$.

Fig. 3.

The bottom left panel depicts the interaction of age (YA, OA) and APOE genotype ($\epsilon 3$, $\epsilon 4$) on resting gray matter cerebral blood flow. Thresholded and clustered results (protecting a whole-brain voxel-wise $p = 0.05$; red: $p = 0.05$, orange: $p = 0.025$, yellow: $p = 0.01$) for a two-way ANOVA indicating an interaction of age (YA, OA) and APOE genotype ($\epsilon 3$, $\epsilon 4$) with corresponding graphical presentation of significant CBF differences in the top panel. Error bars represent the standard error of the mean. Results are overlaid onto sagittal and axial slices of a high-resolution anatomical image averaged across all participants (L, left; A, anterior). For interaction of age by APOE genotype: YA $\epsilon 3 = 63.1 \pm 19.3$, YA $\epsilon 4 = 95.7 \pm 30.4$, OA $\epsilon 3 = 68.0 \pm 27.5$, OA $\epsilon 4 = 53.8 \pm 14.5$, $F(3,66) = 8.5$, $p < 0.001$. The bottom right panel depicts the relationship between CBF in the ACC and executive function in young APOE $\epsilon 4$ carriers ($r = 0.61$, $p = 0.04$) and young $\epsilon 3$ carriers ($r = -0.25$, $p = 0.40$).

Table 1

Summary of principal component analysis performed on standardized scores to extract neuropsychological composite scores for the entire sample.

Neuropsychological Test	Rotated Factor Loadings	
	Verbal Memory	Executive Functions
CVLT-2 Long delay free recall	0.98	−0.10
CVLT-2 Short delay free recall	0.95	−0.01
CVLT-2 list a trials 1–5	0.88	0.15
D-KEFS trail making letter-number switching	−0.01	0.82
D-KEFS verbal letter fluency	0.02	0.82
D-KEFS design fluency switching	−0.00	0.72
% of variance	50.80	25.12

Factor loadings over 0.40 appear in bold CVLT, California Verbal Learning Test; D-KEFS, Delis-Kaplan Executive Function System.

Table 2

Demographic information and neuropsychological raw and standardized test scores of the young and older adults by APOE genotype.

Variables	Young (N=30)						Old (N = 40)						Young-Old						
	$\epsilon 3$ (n = 15)			$\epsilon 4$ (n = 15)			Young $\epsilon 3$ - $\epsilon 4$			$\epsilon 3$ (n = 24)				$\epsilon 4$ (n = 16)			Old $\epsilon 3$ - $\epsilon 4$		
	Mean	SD		Mean	SD		p-value			Mean	SD			Mean	SD		p-value		
	Mean	SD		Mean	SD		p-value			Mean	SD			Mean	SD		p-value		p-value
Demographics																			
Age (years)	23.3	(3.0)		23.6	(3.1)		0.81			72.4	(6.0)		75.1	(7.9)		0.23		<0.001	
Education (years)	15.0	(0.5)		14.9	(0.3)		0.67			16.1	(2.0)		16.6	(1.6)		0.47		<0.001	
Women/men	8	7		12	3		0.12			16	8		11	5		0.89		0.94	
Whole brain gray matter CBF	66.2	(15.3)		70.3	(9.9)		0.39			53.5	(14.3)		54.0	(21.7)		0.94		<0.001	
Learning and Memory																			
CVLT-2 Trials 1–5 raw	52.5	(9.8)		60.1	(8.0)		0.03			50.7	(10.2)		50.6	(11.3)		0.99		<0.05	
CVLT-2 Trials 1–5 T-score	50.1	(10.9)		57.5	(9.6)		0.06			59.0	(9.9)		59.5	(12.3)		0.91		<0.05	
CVLT-2 SD free recall raw	10.7	(2.7)		12.8	(2.7)		0.04			10.6	(3.2)		10.6	(4.0)		0.99		0.13	
CVLT-2 SD free recall z-score	−0.3	(1.0)		0.4	(1.0)		0.10			0.7	(1.1)		0.6	(1.3)		0.89		<0.05	
CVLT-2 LD free recall raw	11.7	(3.0)		13.7	(2.1)		0.04			10.9	(3.2)		10.8	(3.9)		0.88		<0.05	
CVLT-2 LD free recall z-score	−0.1	(1.2)		0.5	(0.9)		0.10			0.5	(1.0)		0.5	(1.4)		0.98		0.31	
Executive Function																			
D-KEFS TMT switching raw	54.0	(13.8)		56.0	(19.0)		0.75			81.1	(23.1)		80.3	(15.9)		0.91		<0.001	
D-KEFS TMT switching ss	11.7	(1.5)		11.3	(2.0)		0.60			12.7	(1.6)		12.9	(1.8)		0.63		<0.01	
D-KEFS verbal fluency raw	38.2	(9.6)		35.9	(12.5)		0.57			48.8	(13.8)		44.1	(9.6)		0.30		0.001	
D-KEFS verbal fluency ss	10.4	(2.9)		9.7	(3.9)		0.60			13.8	(3.5)		13.1	(2.6)		0.47		<0.001	
D-KEFS design fluency raw	9.4	(2.4)		8.9	(2.4)		0.53			7.0	(2.1)		8.3	(1.9)		0.11		<0.01	
D-KEFS design fluency ss	11.4	(2.3)		10.5	(2.5)		0.28			11.7	(2.3)		13.8	(2.2)		<0.01		0.01	

CVLT, California Verbal Learning Test; SD, short delay; LD, long delay; D-KEFS, Delis-Kaplan Executive Function System; TMT Switching, Trail Making Test number-letter switching condition; Verbal Fluency, Letter fluency condition; Design Fluency, Switching condition; ss, scaled score.

Table 3

Clusters showing significant interactions and main effects for age (young, old) and APOE ($\epsilon 3$, $\epsilon 4$) for resting gray matter CBF resulting from whole brain voxel-wise two-way ANOVA.

Direction of response	Hemisphere	Subregion (Brodmann's area)	Volume (mm ³)	Coordinates of maximum intensity voxel	F for maximum intensity voxel	η^2 (Mean±SEM)
<i>Main effect of age</i>						
YA>OA	L & R	C1. Medial frontal gyrus, anterior cingulate, superior & inferior frontal gyri, insula (10, 32, 47, 13)	69120	2L, 47A, 12S	62.3	0.83±0.003
	L & R	C2. Cuneus, precuneus, middle occipital gyrus (7, 19)	47872	26L, 81P, 28S	56.7	0.82±0.003
	L & R	C3. Thalamus (pulvinar), caudate, hippocampus	33792	18L, 29P, 12S	91.0	0.86±0.004
	R & L	C4. Cerebellum	11584	22R, 73P, 32I	27.8	0.79±0.005
	R	C5. Inferior frontal gyrus, insula (45, 44, 13)	10304	50R, 11A, 0	54.0	0.83±0.01
	L	C6. Fusiform gyrus, parahippocampal gyrus, cerebellum (37)	4096	38L, 41P, 28I	60.6	0.81±0.01
	R	C7. Middle temporal gyrus (21)	2304	62R, 41P, 8I	33.7	0.82±0.01
	R	C8. Superior temporal gyrus, supramarginal gyrus (40, 42, 22)	2304	62R, 45P, 16S	23.4	0.79±0.01
	L	C9. Parahippocampal gyrus, fusiform gyrus (20)	1536	42L, 25P, 20I	25.7	0.79±0.01
<i>Main Effect of APOE</i>						
$\epsilon 4 > \epsilon 3$	L	C1. Left lingual gyrus, cuneus & middle occipital gyrus (18, 19)	3008	10L, 89P, 16S	11.7	0.35±0.02
<i>Interaction of Age × APOE</i>						
	L	C1. Anterior cingulate (32)	1600	14L, 35A, 12S	14.3	0.37±0.03

Clusters shown survived our cluster threshold alpha-protection procedure ($p < 0.05$, volume $> 1344 \text{ mm}^3$; see text for details). L, left; R, right; A, anterior; P, posterior; S, superior; I, inferior; C, cluster