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Reduced cerebrovascular reactivity in young adults carrying the *APOE* ε4 allele

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Abstract

Background: Functional magnetic resonance imaging (MRI) studies have shown that *APOE* ε2- and ε4-carriers have similar patterns of blood-oxygenation-level-dependent (BOLD) activation suggesting that we need to look beyond the BOLD signal to link *APOE*'s effect on the brain to Alzheimer's disease (AD)-risk.

Methods: We evaluated *APOE*-related differences in BOLD activation in response to a memory task, cerebrovascular reactivity using a CO_2-inhalation challenge (CO_2-CVR), and the potential contribution of CO_2-CVR to the BOLD signal.

Results: *APOE* ε4-carriers had the highest task-related hippocampal BOLD signal relative to noncarriers. The largest differences in CO_2-CVR were between ε2- and ε4-carriers, with the latter having the lowest values. Genotype differences in CO_2-CVR accounted for ∼70% of hippocampal BOLD differences between groups.

Conclusion: Because CO_2-CVR gauges vascular health, the differential effect of *APOE* in young adults may reflect a vascular contribution to the vulnerability of ε4-carriers to late-life pathology. Studies confirming our findings are warranted.

Keywords: *APOE* gene; Alzheimer's disease; BOLD; Cerebrovascular reactivity; fMRI

1. Introduction

The human apolipoprotein E gene (*APOE*) has three major alleles (ε2, ε3, and ε4), which differentially influence cognitive health [1]. The ε4 allele is the best-established genetic risk factor for sporadic late-onset Alzheimer's disease (AD) [2], and it has been shown to increase the prevalence and lower the age of onset of AD in a gene-dose dependent way [3]. It has also been associated with less effective responses to AD therapies [4] and faster age-related cognitive decline [5], relative to the ε2 and ε3 alleles. Conversely, the ε2 allele has been associated with lower risk of AD-related pathology, and therefore is believed to confer protection [6]. Because of its association with high risk for AD, most of the *APOE*-research has focused on ε4, with the protective ε2 allele receiving little attention [7].

Neuroimaging studies have reported reduced hippocampal volumes [8,9] and glucose metabolism [10] in both AD patients and healthy ε4-carriers relative to noncarriers. Functional MRI (fMRI) studies based on the blood-oxygenation-level-dependent (BOLD) contrast have shown that the ε4 allele modulates brain function [11,12]. We have previously reported that these effects are already evident in young adults, with *APOE*

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ε4-carriers showing increased resting and task-based hippocampal BOLD activity relative to ε3-homozygotes, suggesting that *APOE* differentially affects brain function decades before any possible cognitive decline [13]. Based on these findings, if the level of BOLD activity were related to AD-risk, one would predict that ε2- (low-risk) and ε4- (high-risk) carriers should show opposite patterns of activation. However, the very few studies that have investigated the effects of *APOE* ε2 on brain function report similar patterns of BOLD activity in both ε2- and ε4-carriers relative to ε3-homozygotes [14,15]. It is therefore evident that interpretations of *APOE*-related effects on the brain are incomplete without including the ε2 allele and that we may need to look beyond the BOLD signal to obtain greater insight into the relationship between *APOE*, brain function, and AD-risk.

In addition to impairments in BOLD activity [16], patients with AD, even at its prodromal stage, also show abnormal changes in neurovascular measures subserving neuronal activity, i.e. brain perfusion [17,18] and cerebrovascular reactivity (CVR) [19]. Interestingly, recent studies have reported that CVR plays a key role in influencing the BOLD signal [20,21]. CVR gauges the cerebral vasodilatory capacity and is a marker of cerebrovascular health [22]. Because the *APOE* alleles have been shown to differentially influence the risk of cerebrovascular disease and play a deleterious role on vascular function [23], we specifically investigated whether *APOE* influences CVR in young healthy individuals.

We set up an MRI protocol to investigate early effects of the three *APOE* alleles on: (a) task-related BOLD signal, using a memory encoding paradigm, (b) small-vessel cerebrovascular reactivity (CO_2-CVR), using a CO_2-inhalation challenge (physiological MRI) [24], and (c) the potential contribution of CO_2-CVR to the BOLD response. Our primary targets were the medial temporal lobes and the hippocampi, because these are the earliest brain regions to show pathologic signs of AD and are modulated by *APOE* [25].

2. Materials and methods

2.1. Participants

A total of 191 right-handed subjects aged 20 to 40 years were recruited in Oxfordshire, United Kingdom. Exclusion criteria were history of neurologic or psychiatric disorders, head injury, substance or alcohol abuse, smoking, corticosteroid, or diabetic treatment. DNA was extracted from buccal swab samples and genotyped for *APOE* using standard methods. Genotyping was successful for 184 of 191 subjects. The *APOE* distribution reflected that expected in a healthy Caucasian population ($\chi^2 = 2.226$, df = 5, $P = 1$) [26] and allele frequencies of the component single nucleotide polymorphisms were in Hardy-Weinberg equilibrium (rs7412:$\chi^2 = 1.88$, df = 1, $P > .1$; rs429358:$\chi^2 = 0.05$, df = 1, $P > .1$). Eighteen ε2-carriers (ε2ε2/ε2ε3), 18 ε3-homozygotes (ε3ε3) and 18 ε4-carriers (ε3ε4/ε4ε4) were matched for age, gender, education level, and family history of dementia, and underwent our neuroimaging protocol. Of the 18 ε3-homozygotes scanned, one was excluded from the analysis because of an incidental finding and was subsequently referred to a neurologist. This was a single-blind study approved by the local Ethics Committee (10/H606/34), and informed consent was obtained from all subjects.

2.2. Neuroimaging protocol

Scanning was carried out at the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB) using a 3T Siemens Verio scanner with a 32-channel head coil. The neuroimaging protocol included the following:

2.2.1. Structural MRI

High-resolution 3D T1-weighted images were acquired using a multiecho magnetization-prepared rapid acquisition with gradient echo (ME-MPRAGE) sequence [27]. Repetition time (TR) = 2,530 ms, echo time (TE) = 1.79/3.65/5.51/7.37 ms, voxel dimension = 1 mm^3.

2.2.2. Functional MRI (task), resting perfusion MRI and physiological MRI

Whole-brain simultaneous perfusion and BOLD data were acquired using a dual gradient echo, pseudocontinuous arterial spin labeling (pCASL) echo planar imaging sequence. The dual-echo provides two images per repetition (TE_1 = 13 ms/TE_2 = 30 ms). The short echo time provides perfusion-weighted images, while the longer provides BOLD-weighted images. A multi-TI MRI sequence was used for the resting scan to allow absolute quantitative measurement of resting cerebral blood flow (CBF_0) and arterial arrival time (AAT). A labeling duration of 1400 ms, and five postlabeling delay (PLD) times (250/500/750/1000/1250 ms) were employed. A single-TI sequence (labeling duration = 1200 ms, PLD = 600 ms) was used for both the task-based and the physiological MRI (pMRI) sequences. TR = 4.370 ms for resting acquisition, TR = 4.170 ms for task and pMRI acquisitions, voxel dimension = 3.3 × 3.3 × 4mm^3

2.3. Image analysis

Data analysis was carried out using FSL tools (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) [28].

2.3.1. Structural MRI

Brain tissues were segmented using FMRIB's Automated Segmentation Tool to extract measures of total brain volume, grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF). An automated model-based segmentation/registration tool (FIRST) was used to extract measures of hippocampal volumes [29]. A whole-brain investigation of *APOE*-related changes in GM volume was carried out using a voxel-based-morphometry-style analysis (FSL-VBM) [30]. Brain extraction and tissue-type segmentation was