Posterior Cingulate Glucose Metabolism, Hippocampal Glucose Metabolism, and Hippocampal Volume in Cognitively Normal, Late-Middle-Aged Persons at 3 Levels of Genetic Risk for Alzheimer Disease

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**Objective:** To characterize and compare measurements of the posterior cingulate glucose metabolism, the hippocampal glucose metabolism, and hippocampal volume so as to distinguish cognitively normal, late-middle-aged persons with 2, 1, or 0 copies of the apolipoprotein E (APOE) ε4 allele, reflecting 3 levels of risk for late-onset Alzheimer disease.

**Design:** Cross-sectional comparison of measurements of cerebral glucose metabolism using 18F-fluorodeoxyglucose positron emission tomography and measurements of brain volume using magnetic resonance imaging in cognitively normal ε4 homozygotes, ε4 heterozygotes, and noncarriers.

**Setting:** Academic medical center.

**Participants:** A total of 31 ε4 homozygotes, 42 ε4 heterozygotes, and 76 noncarriers, 49 to 67 years old, matched for sex, age, and educational level.

**Main Outcome Measures:** The measurements of posterior cingulate and hippocampal glucose metabolism were characterized using automated region-of-interest algorithms and normalized for whole-brain measurements. The hippocampal volume measurements were characterized using a semiautomated algorithm and normalized for total intracranial volume.

**Results:** Although there were no significant differences among the 3 groups of participants in their clinical ratings, neuropsychological test scores, hippocampal volumes (P=.60), or hippocampal glucose metabolism measurements (P=.12), there were significant group differences in their posterior cingulate glucose metabolism measurements (P=.001). The APOE ε4 gene dose was significantly associated with posterior cingulate glucose metabolism (r=0.29, P=.0003), and this association was significantly greater than those with hippocampal volume or hippocampal glucose metabolism (P<.05, determined by use of pairwise Fisher z tests).

**Conclusions:** Although our findings may depend in part on the analysis algorithms used, they suggest that a reduction in posterior cingulate glucose metabolism precedes a reduction in hippocampal volume or metabolism in cognitively normal persons at increased genetic risk for Alzheimer disease.


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We and others have been using positron emission tomography (PET) measurements of the cerebral metabolic rate for glucose (CMRgl) in the posterior cingulate cortex and other brain regions that are known to be preferentially affected by Alzheimer disease (AD), magnetic resonance imaging (MRI) measurements of hippocampal and other brain tissue volumes, and other biomarker measurements to detect and track some of the brain changes that precede the clinical onset of AD. These findings have led our group and others to consider how these biomarkers could be used for people at increased risk for AD in the accelerated evaluation of presymptomatic AD treatments. They have also led researchers to propose models that characterize the trajectory of different biomarker changes associated with the preclinical stages of the disorder. For instance, 18F-fluorodeoxyglucose (FDG)—
PET studies reveal characteristic and progressive CMRgl reductions in the posterior cingulate, precuneus, and parietal, temporal, and prefrontal brain regions beginning years before the clinical onset of AD. In other studies, we have reported evidence of CMRgl reductions in an automatically characterized hippocampal region of interest; these reductions were associated with apparent normal aging and predicted future cognitive decline. Magnetic resonance imaging studies reveal progressively reduced hippocampal volumes, which tend to parallel the earliest memory changes that herald the clinical onset of AD.

The apolipoprotein E (APOE) ε4 allele is the major genetic risk factor for late-onset AD. Each additional ε4 allele in a person's APOE genotype is associated with a greater risk of AD and a younger average age at clinical onset. We and others have found that cognitively normal, late-middle-aged and young adult APOE ε4 carriers exhibit CMRgl reductions in these AD-affected regions and that these reductions in late middle age are correlated with APOE ε4 gene dose (the number of ε4 alleles in a person's APOE genotype, reflecting 3 levels of genetic risk for AD). In a preliminary study of cognitively normal, late-middle-aged ε4 homozygotes and noncarriers, we were able to detect CMRgl reductions in the posterior cingulate cortex and other brain regions preferentially affected by AD, prior to detectable evidence of hippocampal volumes; CMRgl reductions in the location with maximal posterior cingulate CMRgl reductions were still apparent after controlling for hippocampal volume differences, and smaller hippocampal volumes were associated with poorer long-term memory scores. Together, these and other findings led us to propose that posterior cingulate CMRgl reductions were apparent prior to hippocampal atrophy and that hippocampal atrophy may correspond to the earliest memory declines associated with the clinical onset of AD. In the present study, we sought to extend our previous findings to a much larger group of cognitively normal, late-middle-aged persons with 2 copies, 1 copy, and no copies of the APOE ε4 allele and to compare posterior cingulate CMRgl measurements, hippocampal CMRgl measurements, and hippocampal volumes in automatically selected regions of interest.

METHODS

PARTICIPANTS

Cognitively normal volunteers 47 to 68 years of age were recruited using newspaper advertisements and were enrolled into a longitudinal cohort study as previously described. Participants provided informed consent, agreed not to receive any information about their APOE genotype, and were studied under guidelines approved by the human subjects committees at Banner Good Samaritan Medical Center in Phoenix, Arizona, and the Mayo Clinic in Scottsdale, Arizona. Venous blood samples were obtained, and APOE genotypes were characterized by analysis by restriction fragment-length polymorphisms. APOE ε4 heterozygotes and noncarriers were individually matched to each APOE ε4 homozygotes for their sex, age (within 3 years), and educational level (within 2 years). Individuals who were enrolled reported a first-degree family history of probable AD and denied any cognitive symptoms. Additional inclusion criteria for participation consisted of a Folstein Mini-Mental State Examination score of at least 28, a Hamilton Depression Rating Scale score of less than 10, the absence of a current psychiatric disorder based on a structured psychiatric interview, and normal neurological examination results. Participants with a reported history of coronary artery disease, diabetes mellitus, or cerebrovascular accidents were excluded. Participants with clinically significant abnormalities, including but not limited to the presence of lacunar infarcts on their T1-weighted MRI scans, were also excluded. Note that, at the time these MRI scans were acquired, a complete clinical MRI examination, including T2-weighted images, was not performed; hence, the evaluation of more subtle evidence of cerebrovascular disease was not possible. For the present study, cross-sectional MRI and FDG-PET data from 160 participants were available for analysis. Data from 11 participants were excluded owing to technical MRI failures that resulted in the inability to segment the hippocampus. The remaining 149 participants included 31 APOE ε4 homozygotes, 42 ε4 heterozygotes, and 76 noncarriers, all 49 to 67 years of age.

BRAIN IMAGING

18F-fluorodeoxyglucose PET and volumetric T1-weighted MRI were performed as previously described. A preliminary study of cognitively normal, late-middle-aged ε4 homozygotes and noncarriers, we were able to detect CMRgl reductions in the posterior cingulate cortex and other brain regions preferentially affected by AD, prior to detectable evidence of hippocampal volumes; CMRgl reductions in the location with maximal posterior cingulate CMRgl reductions were still apparent after controlling for hippocampal volume differences, and smaller hippocampal volumes were associated with poorer long-term memory scores. Together, these and other findings led us to propose that posterior cingulate CMRgl reductions were apparent prior to hippocampal atrophy and that hippocampal atrophy may correspond to the earliest memory declines associated with the clinical onset of AD. In the present study, we sought to extend our previous findings to a much larger group of cognitively normal, late-middle-aged persons with 2 copies, 1 copy, and no copies of the APOE ε4 allele and to compare posterior cingulate CMRgl measurements, hippocampal CMRgl measurements, and hippocampal volumes in automatically selected regions of interest.

SPM99 (Wellcome Trust Centre for Neuroimaging; http://www.fil.ion.ucl.ac.uk/spm/) was used by investigators at the Banner Alzheimer's Institute in Phoenix, Arizona, to linearly and nonlinearly deform (normalize) each participant's PET image into the coordinates of a standard brain atlas. The Automated Anatomical Labeling toolbox was used to extract the PET data from the bilateral posterior cingulate. An automated algorithm developed at New York University was used by these investigators to characterize bilateral hippocampal regions of interest and extract CMRgl measurements from each person's FDG-PET image. Posterior cingulate and hippocampal CMRgl measurements were normalized for the individual variation in whole-brain measurements using proportionate scaling. A semiautomated algorithm (Surface Navigator Technologies; Medtronic) was used by investigators at the University of California, San Francisco, to characterize bilateral hippocampal volumes (Medtronic Surgical Navigation Technologies) as previously described. Hippcampal volumes were normal-
ized for the individual variation in total intracranial volumes using proportionate scaling.

A 1-way analysis of variance with a linear trend was used to examine the ability of posterior cingulate CMRgl measurements, hippocampal CMRgl measurements, and hippocampal volume measurements to distinguish among the 3 levels of risk for AD. Two-tailed t tests were subsequently performed to characterize and compare between-group measurements in the \( \varepsilon_4 \) homozygotes, \( \varepsilon_4 \) heterozygote, and noncarrier groups. An analysis of covariance with a linear trend was used to examine \( \text{APOE} \varepsilon_4 \) dose effects on posterior cingulate and hippocampal CMRgl measurements, covarying for hippocampal volume. Using the Fisher z test, we directly compared the correlation coefficients relating \( \text{APOE} \varepsilon_4 \) gene dose to posterior cingulate CMRgl, hippocampal CMRgl, and hippocampal volume. Lastly, the area under the curve of the receiver operating characteristic for the posterior cingulate CMRgl, hippocampal CMRgl, and hippocampal volume was computed using \( U \) statistics in order to characterize and compare the ability of the 3 measurements to distinguish among the 3 levels of genetic risk for AD.

**RESULTS**

The \( \text{APOE} \varepsilon_4 \) homozygote, \( \varepsilon_4 \) heterozygote, and noncarrier group characteristics are reported in Table 1. There were no significant differences in age, sex, educational levels, clinical ratings, or neuropsychological test scores.

Brain imaging measurements are reported in Table 2 and in our Figure. The 3 groups of participants differed from each other in posterior cingulate CMRgl measurements (analysis of variance: \( P = .001 \); pairwise comparisons: \( P < .05 \)) but not in bilateral hippocampal CMRgl measurements (analysis of variance: \( P = .12 \)) or left, right, or total hippocampal volume.
bilateral hippocampal volume measurements (analysis of variance: \(P = .23, .89, \) and \(.60\), respectively). The posterior cingulate and hippocampal CMRgl findings remained unchanged after controlling for hippocampal volumes (analysis of covariance: \(P = .002\) and \(.11\), respectively).

Supporting the between-group differences, the APOE \(\varepsilon 4\) gene dose was more closely correlated with posterior cingulate hypometabolism (\(r = 0.29, P = .0003\)) than with hippocampal hypometabolism or hippocampal volumes (\(r = 0.07\) and \(0.013\), respectively, determined using pairwise Fisher \(z\) tests; \(P < .05\)). Indeed, posterior cingulate CMRgl measurements were significantly better than hippocampal CMRgl or hippocampal volume measurements in distinguishing between the \(\varepsilon 4\) homozygote and noncarrier groups (area under the receiver operating characteristic curve: 0.71, 0.52, and 0.54, respectively, using pairwise comparisons: \(P = .04\)), whereas the hippocampal CMRgl and hippocampal volume measurements did not differ significantly in their ability to distinguish between the groups of participants.

A quadratic model was used post hoc to further explore the relationship between APOE \(\varepsilon 4\) gene dose and each of the imaging measurements following the exploratory finding of lower mean hippocampal CMRgl measurements in the APOE \(\varepsilon 4\) heterozygotes than in \(\varepsilon 4\) homozygotes or noncarriers (Table 2). Although the finding of a significant quadratic relationship between APOE \(\varepsilon 4\) gene dose and baseline measurements of hippocampal CMRgl (\(P = .04\)) may or may not be consistent with greater hippocampal activation in functional MRI studies of cognitively normal older adult APOE \(\varepsilon 4\) carriers during learning and memory tasks,\(^{30}\) this observation must be considered exploratory. We failed to detect a significant difference between carriers and noncarriers using either hippocampal CMRgl (\(P = .30\)) or hippocampal volume (\(P = .32\)).

**COMMENT**

Our study directly compared posterior cingulate CMRgl, hippocampal CMRgl, and hippocampal volume measurements using preselected, automatically or semiautomatically generated regions of interest in a large number of well-matched, late-middle-aged, cognitively normal persons at 3 levels of genetic risk for AD. As expected, a higher APOE \(\varepsilon 4\) gene dose was associated with a lower posterior cingulate CMRgl. These findings were apparent in the absence of detectable hippocampal CMRgl or hippocampal volume differences. Together, these findings confirm and extend our observation that posterior cingulate CMRgl reductions can be detected before hippocampal CMRgl or hippocampal volume alterations in the preclinical stages of late-onset AD, and they support similar findings in early-onset AD-causing mutation carriers.\(^{41}\)

As previously noted, posterior cingulate CMRgl reductions could reflect a reduction in the density activity or metabolism of terminal neuronal fields or perisynaptic astroglial cells;\(^{2}\) as previously shown, these reductions are unlikely to reflect the combined effects of brain atrophy and partial-volume averaging.\(^{9,10}\) Posterior cingulate hypometabolism was also found in young adult carriers several decades before possible dementia;\(^{2}\) indeed, young adult carriers who died were found to have reduced cytochrome oxidase activity, even before they showed evidence of soluble or fibrillar amyloid-\(\beta\) pathology.\(^{12}\)

In comparison with our previous report,\(^{22}\) the present study compared FDG and volumetric MRI measurements in a much larger number of research participants and compared measurements in persons at 3 levels of genetic risk for AD. It compared posterior cingulate and hippocampal measurements in automatically or semiautomatically generated regions of interest, free from the inflated type I error associated with multiple regional comparisons in our previous FDG-PET analysis. It included the additional comparison of hippocampal CMRgl measurements, which had been implicated in the early detection and tracking of AD, and found that the posterior cingulate measurements were more sensitive to detecting this preclinical stage of the disorder. We previously demonstrated reduced posterior cingulate CMRgl measurements in cognitively normal young adult APOE \(\varepsilon 4\) heterozygotes, more than 4 decades before their estimated age at clinical onset.\(^{9,9,4}\) Based on other comparisons,\(^{8,9}\) we found that the reduction in the posterior cingulate CMRgl does not progress between young adulthood and late middle age but that it anticipates progressive...
CMRGlu declines, with some of the earliest fibrillar Aβ deposition starting in late-middle-aged ε4 carriers.

Additional analyses will be needed to compare CMRGlu measurements with regional gray matter volume or cortical thickness measurements using other voxel-based or region-of-interest–based methods because the sensitivity to detect a change using any biomarker method may be at least partly related to technical factors, such as the data analysis technique used, and not solely attributable to the underlying biological process. Although we found that the posterior cingulate CMRGlu was more sensitive than the hippocampal CMRGlu or the hippocampal volume in its ability to discriminate among cognitively normal, late-middle-aged persons at 3 levels of genetic risk for AD, the differential sensitivity could be related to actual differences in the underlying processes, the image acquisition, the region of interest, the analysis techniques used, or a combination of these factors. It is possible that future technical developments could further improve the sensitivity of these biomarker measurements for the preclinical detection of AD.

Additional analyses will also be needed to compare CMRGlu measurements with fibrillar amyloid-β measurements using PET; we have acquired these measurements in a smaller number of participants. As we have stated in the past, these and other biomarker measurements are not yet recommended to predict a cognitively normal person’s clinical course or his or her response to suggested but unproven risk-reducing treatments. Additional studies are needed to characterize and compare the trajectory of these and other biomarker changes during the preclinical stages of AD because these biomarker changes continue to set the stage for the accelerated evaluation of presymptomatic AD treatments.13

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REFERENCES