Total and Regional Gray Matter Volume Is Not Related to APOE*E4 Status in a Community Sample of Middle-Aged Individuals

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Background. The APOE*E4 allele has been associated with greater gray matter atrophy and with Alzheimer’s disease. The aim of this study was to investigate whether the relationship between cerebral gray matter atrophy and APOE*E4 genotype was also present in a community-dwelling, nondemented 60- to 64-year-old cohort.

Methods. Hippocampal and amygdalar volumes were manually traced and analyzed on 331 cranial T1-weighted magnetic resonance imaging (MRI) scans to detect differences associated with APOE*E4 genotype. Voxel-based morphometric (VBM) analyses were applied to detect regional gray matter volume differences.

Results. No total, hippocampal, or amygdalar gray matter volume difference was detected between APOE*E4 carriers and noncarriers.

Conclusions. In nondemented 60- to 64-year-olds, there was no association between APOE genotype and gray matter volume using both region-of-interest analysis and VBM.

Key Words: APOE—MRI—Cerebral atrophy—Voxel-based morphometry—Hippocampus—Amygdala.

APOIPOPROTEIN E (APOE) is the main known genetic risk factor for late-onset Alzheimer’s disease (AD) and is also a predictor of cognitive deficits in normal aging (for a review, see 1). A growing number of imaging studies has shown associations between APOE genotype and brain structure and function, particularly in relation to increased medial temporal lobe and whole brain atrophy (2), increased white matter (WM) hyperintensities volume (3), and altered cerebral blood flow (4) in AD and mild cognitive impairment (for a review see 5). Similar findings have also been reported in healthy aging populations (6).

Ohm and colleagues (7) have suggested that structural brain changes associated with neurodegenerative processes involved in aging and dementia start in early adulthood and are already detectable in some middle-aged individuals, particularly in the parahippocampal region. It has also been demonstrated, in a postmortem study, that greater levels of neurodegeneration can be detected in APOE*E4 carriers (8).

Some recent studies have shown an association between cerebral morphometry and APOE genotype in healthy young old samples (<65 years) (9,10) supporting the involvement of APOE*E4 allele in early neurodegenerative processes starting in middle age, but others have not [e.g., (11,12)].

The aim of the present study was to investigate a possible effect of APOE genotype on brain structure in a population-based sample of 478 individuals ages 60–64 years and participating in the Personality and Total Health (PATH) study by comparing regions of interest (ROI) (hand-traced hippocampal and amygdalar volumes) and by using voxel-based morphometry (VBM) to assess regional gray matter (GM) differences in APOE*E4 carriers and noncarriers.

METHODS

Participants

The sample was drawn from the PATH Through Life Project designed to study the risk and protective factors for normal aging, dementia, and other neuropsychiatric disorders (13). This PATH Project cohort comprised 2551 individuals 60–64 years old who were residents of the city of Canberra and the adjacent town of Queanbeyan, Australia, and were recruited randomly through the electoral roll. Enrollment to vote is compulsory for Australian citizens. Participants were asked at the initial interview if they would be willing to undergo a magnetic resonance imaging (MRI) brain scan. Those who were unwilling during the initial interview were significantly (p < .05) more likely to be female (53.9%), of non-English-speaking background, were less educated, and had poorer self-rated physical health and lower cognitive test scores. Of those who had indicated willingness for a scan, 622 were approached, and 478 (77%; men = 252) provided written informed consent to undergo an MRI brain scan. Of these,
by Cfo1 restriction digestion of PCR products (14). Genotype scorers were blinded to the identity of the samples.

**MRI Scans**

All participants were imaged with a 1.5 Tesla Philips Gyroscan ACS-NT scanner (Philips Medical Systems, Best, the Netherlands) for T1-weighted three-dimensional structural MRI. The T1-weighted MRI was acquired in coronal orientation using a T1-fast field echo (FFE) sequence with the following parameters: repetition time (TR)/echo time (TE) = 28.05/2.64 ms; flip angle = 30°; matrix size = 256 × 256; field of view (FOV) = 260 × 260 mm; slice thickness = 2.0 mm; and midslice to midslice distance = 1.0 mm, yielding over-contiguous coronal slices.

**Image Analysis**

The volumes of GM, WM, and cerebrospinal fluid (CSF) were calculated after the segmentation of T1-weighted MRI scans using SPM2 (Wellcome Department of Cognitive Neurology, Institute of Neurology, London, U.K.). Intracranial volume (ICV) was measured as the sum of the total GM, WM, and CSF, and total brain volume as the sum of total GM and WM. The volumes of brain anatomical regions were determined by manually outlining the periphery of the ROI on the coronal T1-weighted slices using Analyze 5.0 (Brain Imaging Resource, Mayo Clinic, Rochester, MN). The outlining of the hippocampus and amygdala always proceeded from anterior to posterior, and the amygdala was traced first. Amygdalar tracing began a maximum number of four slices anterior to the slice where the anterior tip of the temporal horn was visible, and was traced according to the protocol outlined by Watson and colleagues (15). In addition, the hippocampal tail was manually traced according to the protocol described in detail in (16). Volume estimations were repeated by two trained researchers on 10 randomly selected scans, and inter-class correlations were 0.997 for the right and 0.995 for the left, indicating high inter-rater reliability.

Optimized VBM analysis (17) was applied using SPM2 (Wellcome Department of Cognitive Neurology, Institute of Neurology, London, U.K.) on Matlab 7.1 (Math Works, Natick, MA). All images were inspected, and 331 images were selected for inclusion in the present study. Reasons for exclusion of images were artefacts and unsatisfactory tissue segmentation due to insufficient gray/white tissue differentiation and/or low signal-to-noise ratio. First, a customized template was created from all included images, thus avoiding any confounding effects from the study population. All structural MR images were spatially normalized by applying a 16-parameter affine transformation to an SPM1 template, then averaged and smoothed with an 8 mm full-width half-maximum (FWHM) smoothing kernel to make a customized T1 template. Second, the structural MR images were spatially normalized with a 16-parameter affine linear transformation into the new template and then segmented into GM, WM, and CSF partitions. GM partitions were smoothed and averaged to create the study-specific GM template. Third, raw structural images were segmented into GM, WM, and CSF partitions. The total GM volumes, WM volumes, CSF volumes, and ICVs were computed for each

<table>
<thead>
<tr>
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<th>Present Study (N = 331)</th>
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<tr>
<td>Age, y</td>
<td>62.5 (1.5)</td>
<td>62.6 (1.5)*</td>
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</tr>
<tr>
<td>Gender, % male</td>
<td>1313 (51.6)</td>
<td>323 (51.9)</td>
<td>177 (53.5)</td>
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<td>English-speaking background, %</td>
<td>2226 (87.4)</td>
<td>550 (88.7)</td>
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<tr>
<td>Education, y</td>
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<td>13.9 (2.80)</td>
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<td>MMSE</td>
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<td>APOE*E4 carriers, %</td>
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<td>Self-rated health (SF 12)</td>
<td>48.1 (10.2)</td>
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Notes: Physical health was measured by the Physical Component Summary of the SF-12. This scale consists of a 12-item subset of the SF-36 (21) with higher scores indicating better health (standardized norm, mean 50, standard deviation 10).

* | p < .05, | p < .01; significant difference between subgroups and excluded main cohort participants.

\[ \text{MRI = magnetic resonance imaging; MMSE = Mini-Mental State Examination.} \]

147 were excluded because of missing data for APOE genotype or poor quality scans, giving a sample of 331 (men = 177; 50.5%). The demographic and health differences between those who refused or were excluded (n = 291) were small (see Table 1) with those included in this study being more likely to be of English-speaking background and with better self-rated health. Approval for the study was obtained from the human research ethics committees of the Australian National University, Canberra, and the University of New South Wales, Sydney, Australia.

**Genetics**

Genomic DNA was extracted from buccal swabs using QIAamp 96 DNA Blood kits (no. 51162; QIAGEN, Hilden, Germany). To determine the APOE genotype composed of the APOE*E2, APOE*E3, and APOE*E4 alleles, two single nucleotide polymorphisms (SNPs; NCBI SNPs rs429358 and rs7412) were genotyped using TaqMan assays (Applied Biosystems, Inc. [ABI], Foster City, CA) in 384-well reaction plates (ABI no. 4309849). Each APOE TaqMan assay contained 1 μL of genomic DNA, 2.0 μL of TaqMan 2 × universal polymerase chain reaction (PCR) master mix (ABI no. 4304437), 0.0625 μL of the appropriate 80 × assay mix containing the SNP-specific primers and probes (TaqMan genotyping assays; ABI) and H2O to a total volume of 5 μL. Plates were sealed with optical adhesive covers (ABI no. 4311971), spun briefly (800 × g), and placed into an ABI 7900HT real-time PCR machine. The cycling program was: 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Allelic discrimination was automated using the manufacturer’s software (SDS v2.2; ABI). Positive controls, consisting of DNA of each of the six possible APOE genotypes (*E2*/E2, *E2*/E3, *E2*/E4, *E3*/E3, *E3*/E4, and *E4*/E4), were included on each genotyping plate. These six controls were genotyped

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participant’s image. As a measure of individual total GM volume corrected for differences in head size, total GM volume ratio (TGVR) was calculated as the ratio of total GM volume to total brain volume. GM partitions were then normalized into the GM template both linearly and non-linearly to estimate optimal normalization parameters, which were reapplied to the original raw images to make whole brain images in stereotactic space. These optimally normalized images were segmented again to make GM partitions, which were modulated by multiplying voxel values with the Jacobian determinants derived from spatial normalization, to obtain the absolute volume of GM (17). Finally, GM images were smoothed with a 12 mm FWHM Gaussian kernel.

**Statistical Analysis**

For GM, WM, ICV, and ROI comparisons, raw volumes were first compared using t tests, and second with multiple regression analyses. Left and right hippocampal and amygdalar volumes were entered separately as dependent variables, and APOE genotype, sex, age, ICV, and education as predictor variables. The value of \( p \) was set at .01.

For VBM, GM volume differences, measured as TGVR, were assessed by applying a general linear model (GLM). TGVR entered the model as a dependent variable, APOE genotype as predictor variable, and sex, age, ICV, and education (measured in years) as covariates. Regional GM volume differences were investigated by applying GLM to form statistical parametric maps, in which statistical significance was tested on a voxel-by-voxel basis. To locate significant regions, the statistical threshold was set at \( p < .05 \) and family-wise-corrected for multiple comparisons.

**RESULTS**

Demographic characteristics of participants in the study are shown in Table 2. Of the 331 participants investigated using VBM, 89 were APOE*E4 carriers and 242 had no *E4 allele. Distribution of the APOE genotype was as follows: *E2*E2, \( n = 3 \); *E2*E3, \( n = 34 \); *E3*E3, \( n = 205 \); *E2*E4, \( n = 9 \); *E3*E4, \( n = 74 \); *E4*E4, \( n = 6 \). Carriers and noncarriers did not differ in their GM volume, \( t(329) = .200 \) (not statistically significant), WM volume, \( t(329) = –1.62 \) (not statistically significant), or ICV, \( t(329) = .400 \) (not statistically significant).

Hippocampal and amygdalar volumes are presented in Table 3. Volumetric measures assessed by multiple regression while controlling for sex, age, ICV, and education, did not differ between carriers and noncarriers for: the left hippocampus, \( t(1,325) = .389, p = .697, \beta = .019 \); right hippocampus, \( t(1,325) = .326, p = .745, \beta = .016 \); left amygdala, \( t(1,325) = –1.385, p = .167, \beta = –.068 \); and right amygdala, \( t(1,325) = .377, p = .707, \beta = .019 \). Voxel-wise statistical analysis controlling for sex, age, ICV, and education did not reveal any APOE genotype effect (carriers vs noncarriers).

**DISCUSSION**

In this study, differences in GM volumes in non-demented, community-based APOE carriers and noncarriers were investigated using both ROI analyses and VBM. Contrary to a recent, large community-based study reporting significant decrease in GM volume in APOE*E4 homozygous persons (6), no differences in GM volumes were found between *E4 carriers and noncarriers in the present study.

The cohort from which this sample was drawn was randomly sampled from the community. These negative results are therefore unlikely to be due to small sample size or sampling bias. However, our cohort differs in a number of ways from that studied by Lemaitre and colleagues (6), which had a higher mean age (69.4 years vs 62.6 years in this cohort), a lower level of education (10.7 years vs 13.9 years), a larger proportion of women (72% vs 53%), and a lower Mini-Mental State Examination score (27.5 vs 29.3). It is consequently possible that the lack of APOE effect is due, at least in part, to the fact that the present sample was younger, with a higher cognitive status, and less affected by processes associated with ageing. Moreover, Lemaitre and colleagues found differences only between homozygous carriers and noncarriers which, in the present study, were too few to be analyzed separately. The present findings are also consistent with the findings of another study based on the larger cohort of 2551 persons from which this sample was drawn. That study found no association between cognitive performance and APOE genotype while controlling for other risk factors such as education, health status, level of activity, and cardiovascular pathologies (18).

In contrast, postmortem (8) and smaller volumetric (9) studies in younger individuals have demonstrated APOE effects. Thus it is possible that small changes might be present in cohorts like ours but only detectable by precise measurement of discrete volumes. Hippocampal and

**Table 2. Descriptive Characteristics of the Study Sample by APOE Genotype Group**

<table>
<thead>
<tr>
<th>APOE Sample</th>
<th>*E4− (N = 242)</th>
<th>*E4+ (N = 89)</th>
<th>df</th>
<th>( \psi^2 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>62.7 (1.4)</td>
<td>62.4 (1.5)</td>
<td>329</td>
<td>1.708</td>
<td>.090</td>
</tr>
<tr>
<td>Gender, % male</td>
<td>129 (53.3)</td>
<td>48 (53.9)</td>
<td>1</td>
<td>.010</td>
<td>.919</td>
</tr>
<tr>
<td>Education, y</td>
<td>14.0 (2.7)</td>
<td>13.7 (2.7)</td>
<td>329</td>
<td>.979</td>
<td>.328</td>
</tr>
<tr>
<td>MMSE</td>
<td>29.3 (1.1)</td>
<td>29.1 (1.3)</td>
<td>329</td>
<td>1.485</td>
<td>.139</td>
</tr>
</tbody>
</table>

*Note: APOE = apolipoprotein E; \( df \) = degrees of freedom; MMSE = Mini-Mental State Examination.*

**Table 3. Hippocampal and Amygdalar Raw Volumes in APOE*E4 Carriers and Noncarriers**

<table>
<thead>
<tr>
<th>APOE Sample</th>
<th>*E4− (N = 242)</th>
<th>*E4+ (N = 89)</th>
<th>df</th>
<th>( t )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left hippocampus, mL</td>
<td>28.68 (4.37)</td>
<td>28.81 (4.13)</td>
<td>329</td>
<td>–2.25</td>
<td>.019</td>
</tr>
<tr>
<td>Right hippocampus, mL</td>
<td>29.13 (4.45)</td>
<td>29.22 (4.64)</td>
<td>329</td>
<td>–1.48</td>
<td>.148</td>
</tr>
<tr>
<td>Left amygdala, mL</td>
<td>12.58 (2.64)</td>
<td>12.20 (2.66)</td>
<td>329</td>
<td>.167</td>
<td>.247</td>
</tr>
<tr>
<td>Right amygdala, mL</td>
<td>12.11 (2.58)</td>
<td>12.23 (2.63)</td>
<td>329</td>
<td>–.386</td>
<td>.700</td>
</tr>
<tr>
<td>Gray matter, mL</td>
<td>712 (87)</td>
<td>710 (90)</td>
<td>329</td>
<td>.200</td>
<td>.841</td>
</tr>
<tr>
<td>White matter, mL</td>
<td>479 (55)</td>
<td>480 (55)</td>
<td>329</td>
<td>–1.62</td>
<td>.100</td>
</tr>
<tr>
<td>ICV, mL</td>
<td>1513 (164)</td>
<td>1505 (176)</td>
<td>329</td>
<td>.400</td>
<td>.690</td>
</tr>
</tbody>
</table>

*Note: APOE = apolipoprotein E; \( df \) = degrees of freedom; ICV = intracranial volume.*
amygldal volumetric analyses suggest that this is not the case in our sample. Hippocampus and amygdala raw volumes did not differ between carriers and noncarriers, nor were significant differences present after controlling for sex, age, intracranial volume, and education. However, the cognitive status of participants in the present study was high and might at least partly explain differences with previous studies. These results should also be assessed in light of the limitation of this study. First, to meet the criteria of image quality for VBM analysis, a number of images were excluded. However, included and excluded persons were similar in load of risk factors (see 19). Therefore, this issue is unlikely to have significantly affected the present findings. Second, the VBM method requires a number of criteria to be met. The images must be of high quality, accurately segmented, and adequately spatially normalized [for a discussion, see (20)]. To meet these criteria we have used a customized template and the optimized method. The use of a customized template and MRI scan processing using the optimized method does not completely exclude the possibility of tissue misclassification but, if it occurred, such an error would be unlikely to systematically affect the results.

Summary

This study investigated the effect of APOE genotype on brain morphometry in a subsample of a randomly sampled cohort of nondemented, community-based individuals. The results suggest that, in middle age (60–64 years), nondemented individuals’ gross cerebral atrophy associated with APOE genotype is not evident, and that the detectable effects of APOE genotype on brain aging may only become apparent in the late 60s in normal adults and with current morphometric measures.

ACKNOWLEDGMENTS

The study was supported by the National Health and Medical Research Council (NHMRC) of Australia Unit Grant No. 973302, Program Grant No. 179805, NHMRC Project Grant No. 157125, and grants from the Australian Rotary Health Research Fund and the Australian Brewers Foundation. Nicolas Cherbuin is funded by Alzheimer’s Australia’s Research and the Centre for Mental Health Research at the Australian National University. Kaarin Anstey is funded by NHMRC Research Fellowship No. 366756.

We thank Anthony Jorm, Helen Christensen, Bryan Rodgers, National Capital Diagnostic Imaging group, Patricia Jacomb, Karen Maxwell, June Cullen, the Neuroimaging Group, Neuropsychiatric Institute, Prince of Wales Hospital, and the PATH interviewers. Susan Tan and Karen Nunweek assisted with the APOE genotyping, which was carried out in the Australian Cancer Research Foundation Biomolecular Resource Facility at the John Curtin School of Medical Research.

CORRESPONDENCE

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Received May 7, 2007
Accepted August 16, 2007
Decision Editor: Luigi Ferrucci, MD, PhD