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

Nicolas Cherbuin,[1] Kaarin J. Anstey,[1] Perminder S. Sachdev,[2,3] Jerome J. Maller,[4] Chantal Meslin,[1] Holly A. Mack,[1] Wei Wen,[2,3] and Simon Easteal[5]

[1]Centre for Mental Health Research, Australian National University, Canberra.
[2]School of Psychiatry, University of New South Wales, Sydney, Australia.
[3]Neuropsychiatric Institute, Prince of Wales Hospital, Sydney, Australia.
[4]The Alfred & Monash University, Department of Psychological Medicine, Melbourne, Australia.
[5]John Curtin School of Medical Research, Australian National University, Canberra.

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.

A POLIPOPROTEIN 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,

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Table 1. Descriptive Characteristics of the Studied Sample Compared to the Subsample Selected to Undergo an MRI Scan and the Randomly Selected Population-Based Cohort

Sample	Population-Based Cohort ($N = 2551$)	Selected for MRI ($N = 622$)	Present Study ($N = 331$)
Age, y	62.5 (1.5)	62.6 (1.5)*	62.6 (1.4)
Gender, % male	1313 (51.6)	323 (51.9)	177 (53.5)
English-speaking background, %	2226 (87.4)	550 (88.7)	302 (91.8)[†,‡]
Education, y	13.8 (2.8)	13.9 (2.80)	14.1 (2.6)*
MMSE	29.1 (1.5)	29.2 (1.3)[†]	29.3 (1.2)*
APOE*E4 carriers, %	641 (27.0)	166 (27.9)	89 (26.9)
Self-rated health (SF 12)	48.1 (10.2)	49.0 (9.6)[†]	49.9 (8.7)[†,§]

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 subsamples and excluded main cohort participants.

[§]$p < .05$, [‡]$p < .01$; significant difference between present study and excluded MRI sample participants.

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 H_2O 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

by *Cfo*I 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 SPMT1 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 2. Descriptive Characteristics of the Study Sample by APOE Genotype Group

APOE Sample	*E4− (N = 242)	*E4+ (N = 89)	df	t/χ²	p
Age, y	62.7 (1.4)	62.4 (1.5)	329	1.708	.090
Gender, % male	129 (53.3)	48 (53.9)	1	.010	.919
Education, y	14.0 (2.7)	13.7 (2.7)	329	.979	.328
MMSE	29.3 (1.1)	29.1 (1.3)	329	1.485	.139

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

APOE Sample	*E4− (N = 242)	*E4+ (N = 89)	df	t	p
Left hippocampus, mL	28.68 (4.37)	28.81 (4.13)	329	−.235	.814
Right hippocampus, mL	29.13 (4.45)	29.22 (4.64)	329	−.148	.882
Left amygdala, mL	12.58 (2.64)	12.20 (2.66)	329	1.16	.247
Right amygdala, mL	12.11 (2.58)	12.23 (2.63)	329	−.386	.700
Gray matter, mL	712 (087)	710 (090)	329	.200	.841
White matter, mL	479 (558)	480 (553)	329	−.162	.872
ICV, mL	1513 (164)	1505 (176)	329	.400	.690

Note: APOE = apolipoprotein E; df = degrees of freedom; ICV = intracranial volume.

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 was 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) = −.162 (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, β = .019; right hippocampus, t(1,325) = .326, p = .745, β = .016; left amygdala, t(1,325) = −1.385, p = .167, β = −.068; and right amygdala, t(1,325) = .377, p = .707, β = .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

amygdalar 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), non-demented 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.

Correspondence

Address correspondence to Nicolas Cherbuin, PhD, Centre for Mental Health Research, Building 63, Australian National University, Canberra, ACT 0200, Australia. E-mail: nicolas.cherbuin@anu.edu.au

References

1. Savitz J, Solms M, Ramesar R. Apolipoprotein E variants and cognition in healthy individuals: a critical opinion. *Brain Res Rev.* 2006;51:125–135.
2. Hashimoto M, Yasuda M, Tanimukai S, et al. Apolipoprotein E epsilon 4 and the pattern of regional brain atrophy in Alzheimer's disease. *Neurology.* 2001;57:1461–1466.
3. Bronge L, Fernaeus SE, Blomberg M, et al. White matter lesions in Alzheimer patients are influenced by apolipoprotein E genotype. *Dement Geriatr Cogn Disord.* 1999;10:89–96.
4. Hogh P, Knudsen GM, Kjaer KH, Jorgensen OS, Paulson OB, Waldemar G. Single photon emission computed tomography and apolipoprotein E in Alzheimer's disease: impact of the epsilon 4 allele on regional cerebral blood flow. *J Geriatr Psychiatry Neurol.* 2001;14:42–51.
5. Cherbuin, N., Leach, L.S., Christensen, H., Anstey, K.J. Neuroimaging and APOE genotype: a systematic qualitative review. *Dementia and Geriatric Cognitive Disorders.* 2007;24:348–362.
6. Lemaitre H, Crivello F, Dufouil C, et al. No epsilon(4) gene dose effect on hippocampal atrophy in a large MRI database of healthy elderly subjects. *Neuroimage.* 2005;24:1205–1213.
7. Ohm TG, Muller H, Braak H, Bohl J. Close-meshed prevalence rates of different stages as a tool to uncover the rate of Alzheimer's disease-related neurofibrillary changes. *Neuroscience.* 1995;64:209–217.
8. Ohm TG, Scharnagl H, März W, Bohl J. Apolipoprotein E isoforms and the development of low and high Braak stages of Alzheimer's disease-related lesions. *Acta Neuropathol.* 1999;V98:273–280.
9. Plassman BL, Welsh-Bohmer KA, Bigler ED, et al. Apolipoprotein E epsilon 4 allele and hippocampal volume in twins with normal cognition. *Neurology.* 1997;48:985–989.
10. Tohgi H, Takahashi S, Kato E, et al. Reduced size of right hippocampus in 39- to 80-year-old normal subjects carrying the apolipoprotein E epsilon 4 allele. *Neurosci Lett.* 1997;236:21–24.
11. Enzinger C, Fazekas F, Matthews PM, et al. Risk factors for progression of brain atrophy in aging: six-year follow-up of normal subjects. *Neurology.* 2005;64:1704–1711.
12. Reiman EM, Uecker A, Caselli RJ, et al. Hippocampal volumes in cognitively normal persons at genetic risk for Alzheimer's disease. *Ann Neurol.* 1998;44:288–291.
13. Sachdev S, Wen W, Christensen H, Jorm AF. White matter hyperintensities are related to physical disability and poor motor function. *J Neurol Neurosurg Psychiatry.* 2005;76:362–367.
14. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res.* 1990;31:545–548.
15. Watson C, Jack CR, Cendes F. Volumetric magnetic resonance imaging. Clinical applications and contributions to the understanding of temporal lobe epilepsy. *Arch Neurol.* 1997;9:97–113.
16. Maller JJ, Reglade-Meslin C, Anstey KJ, Sachdev P. Sex and symmetry differences in hippocampal volumetrics: before and beyond the opening of the crus of the fornix. *Hippocampus.* 2006;16:80–90.
17. Good CD, Johnsrude IS, Ashburner J, Henson RN, Friston KJ, Frackowiak RS. A voxel-based morphometric study of ageing in 465 normal adult human brains. *Neuroimage.* 2001;14:21–36.
18. Jorm AF, Mather KA, Butterworth P, Anstey KJ, Christensen H, Easteal S. APOE genotype and cognitive functioning in a large age-stratified population sample. *Neuropsychology.* 2007;21:1–8.
19. Chen X, Wen W, Anstey KJ, Sachdev PS. Effects of cerebrovascular risk factors on gray matter volume in adults aged 60-64 years: a voxel-based morphometric study. *Psychiatry Res.* 2006;147:105–114.
20. Ashburner J, Friston KJ. Voxel-based morphometry—the methods. *Neuroimage.* 2000;11:805–821.
21. Ware J, Jr., Kosinski M, Keller SD. A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. *Med Care.* 1996;34:220–233.