Apolipoprotein E and Gray Matter Volume Loss in Patients with Mild Cognitive Impairment and Alzheimer Disease[1]

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Purpose: To examine the influence of apoliprotein E ε4 allele (*APOE*4) carrier status on disease progression by evaluating the rate of regional gray matter (GM) volume loss and disease severity in patients with newly diagnosed Alzheimer disease (AD) and stable amnestic mild cognitive impairment (MCI).

Materials and Methods: This study was approved by the institutional review board and was HIPAA compliant. All subjects or their legal representatives gave informed consent for participation. Ninety-five subjects (63 male; average age, 77.1 years; age range, 58–91 years; 51 *APOE4* carriers; 44 noncarriers) with either documented MCI to AD conversion or stable amnestic MCI underwent three yearly magnetic resonance imaging examinations. Voxel-based morphometry for image postprocessing and Clinical Dementia Rating (CDR) scale for cognitive assessment were used.

Results: In *APOE*4 carriers, GM volume loss affected the hippocampi, temporal and parietal lobes, right caudate nucleus, and insulae in patients with MCI to AD conversion and the insular and temporal lobes in patients in whom MCI was stable. In subjects who were not *APOE*4 carriers, there was no significant GM volume change. There were no differences in CDR scores between *APOE*4 carriers and noncarriers.

Conclusion: *APOE*4 carriers with cognitive decline undergo faster GM atrophy than do noncarriers. The involvement of *APOE*4 in the progression of hippocampal atrophy, neocortical atrophy, or both has potential important implications for diagnosis and therapeutic approaches in patients with AD and should be considered in clinical trials. The present results and the results of prior studies indicate that the rate of hippocampal and neocortical atrophy is greater in association with *APOE*4 in nondemented elderly subjects, subjects with MCI, and those with AD.

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Apolipoprotein E (*APOE*) is involved in lipid transfer, cell metabolism, repair of neuronal injury due to oxidative stress, ischemia, inflammation, amyloid-β peptide accumulation, and the aging process (1). *APOE* is synthesized by a gene on chromosome 19 in a locus with three alleles (ε2, ε3, and ε4), and it is expressed in the central nervous system in astrocytes and neurons (2). The *APOE* ε4 allele (*APOE*4) is a genetic risk factor that increases the occurrence and lowers the age of onset of sporadic and some familial forms of Alzheimer disease (AD), while the *APOE* ε2 allele may have a protective effect (3,4). *APOE*4 has been implicated in the pathogenesis of AD through multiple paths, ultimately leading to neurodegeneration in animal models of AD and human subjects with AD who are *APOE*4 carriers (1,5,6).

We used voxel-based morphometry (VBM), a whole-brain neuroimaging technique, and magnetic resonance (MR) imaging (7,8) to detect gray matter (GM) loss, with voxelwise comparison of GM volume in patients with mild cognitive impairment (MCI) and AD. To reduce the confounding effect of different disease stages, we included in this longitudinal study with within-subject comparison *(a)* patients with documented conversion from MCI to AD and *(b)* patients who had received a diagnosis of amnestic MCI but in whom there had been no clinical progression over 3 years. On the basis of prior studies (9,10), we hypothesized that the rate of hippocampal and neocortical volume loss would be greater in *APOE*4 carriers. Our purpose was to examine the influence of *APOE*4 carrier status on disease progression by evaluating the rate of regional GM volume loss and disease severity in patients with newly diagnosed AD and stable amnestic MCI.

Advances in Knowledge

- Not only is apoliprotein E ε4 allele (*APOE*4) carrier status a genetic risk factor for Alzheimer disease (AD), it also appears to be related to accelerated atrophy of the hippocampi and neocortical regions in subjects with amnestic mild cognitive impairment (MCI) and AD.
- In *APOE*4 noncarriers with conversion from MCI to AD, there is dissociation between worsening cognitive status and progression of gray matter atrophy.

Materials and Methods

We used prospectively acquired data obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (*www.loni.ucla.edu/ADNI*), which was launched in 2003 by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, and pharmaceutical companies and nonprofit organizations. The primary goals of the ADNI are to test whether serial imaging, measurement of biomarkers, and clinical and neuropsychologic assessment can be combined to measure the progression of MCI and early AD, develop new treatments for AD, and monitor the effectiveness of these treatments. Details of the ADNI study protocol, including inclusion and exclusion criteria, are publicly available (*http://www.adni-info.org*). Briefly, inclusion criteria require that participants be aged 55–90 years, be in good general health, and have at least 6 years of education. Exclusion criteria are any relevant neurologic condition other than AD and screening MR images that show evidence of infection, infarction, or other focal lesions. After screening, subjects with MCI in the ADNI study undergo clinical and imaging evaluation at baseline and every 6 months thereafter for the first 2 years; thereafter, they undergo annual screening for a total duration of 5 years.

This retrospective study was approved by the institutional review board of the Medical University of South Carolina and was compliant with Health Insurance Portability and Accountability Act regulations. This study was conducted between January 2, 2009, and August 31, 2009. All subjects or their legal representatives gave informed consent for participation. At baseline, the entire ADNI cohort consisted of 818 subjects, 396 of whom had a diagnosis of MCI, 193 of whom had a diagnosis of AD, and 229 of whom were healthy volunteers. The MCI and AD diagnoses were made by a multidisciplinary team that conducted extensive neuropsychologic and neuroimaging assessments. The diagnosis of probable AD was made on the basis of established criteria (11). Clinical criteria for amnestic MCI included *(a)* memory complaint, *(b)* abnormal scores on memory tests, *(c)* normal general mental status, *(d)* normal daily functioning, and *(e)* absence of dementia (12). Subjects with the amnestic form of MCI are considered to be at increased risk of developing dementia (13).

We divided patients with a diagnosis of MCI at baseline into two groups:

Implications for Patient Care

- It is important to understand the role of the apoliprotein E (*APOE*) genotype in the progression of cognitive decline and neurodegeneration to optimize treatment regimens.
- *APOE* genotype testing should be included in clinical trials in which patients with probable AD are targeted.

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Abbreviations:
AD = Alzheimer disease
ADNI = Alzheimer's Disease Neuroimaging Initiative
CDR = Clinical Dementia Rating
GM = gray matter
MCI = mild cognitive impairment
SB = sum of boxes
VBM = voxel-based morphometry

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Guarantors of integrity of entire study, M.V.S., J.E.M.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; manuscript final version approval, all authors; literature research, M.V.S., J.E.M.; clinical studies, J.E.M.; statistical analysis, M.V.S., R.J.H.; and manuscript editing, all authors

Potential conflicts of interest are listed at the end of this article.

See also the editorial by Bizzi in this issue.

Group 1 comprised 125 (31.6%) subjects in whom MCI progressed to AD during participation in the ADNI project. Group 2 comprised 271 (68.4%) subjects with stable MCI. We used the following inclusion criteria for the two groups: In group 1, subjects had *(a)* documented conversion from MCI to probable AD during study participation, *(b)* known *APOE* genotypes, and *(c)* three readily available serial brain MR imaging studies, the first obtained 12 months before the visit in which probable AD was diagnosed; the second, at the time of AD diagnosis; and the third, 12 months after diagnosis of AD. In group 2, subjects had *(a)* clinical diagnosis of amnestic MCI, *(b)* clinical follow-up data obtained at 6-month intervals for 2 years and at 12-month intervals thereafter showing stable cognitive status over the remaining 3 years, *(c)* known *APOE* genotype, and *(d)* three readily available serial brain MR imaging studies, the first obtained at baseline; the second, at 12-month follow-up; and the third, at 24-month follow-up.

We excluded all subjects if all the findings of the required serial clinical and imaging assessments were not available. We excluded subjects in whom at least one of the brain MR data sets was not technically adequate because of macroscopic head motion or technical issues with image postprocessing. We excluded nonwhite subjects.

Subjects with MCI in the ADNI cohort undergo brain MR imaging and a comprehensive clinical evaluation every 6 months for the first 2 years of participation and then every 12 months for the next 3 years. As a result, the date of conversion from MCI to AD is not precisely known, because conversion could have happened during the 6 months between visits. However, we included in group 1 only those patients in whom MCI had converted to AD during the first 2 years of their participation in the ADNI project, when study visits were conducted every 6 months.

Forty-seven subjects with documented conversion from MCI to AD (27 *APOE*4 carriers, 20 noncarriers), representing the totality of the sample in the cohort that met study inclusion and exclusion criteria for MCI to AD conversion at the time of data collection (January and February 2009), were included (Table 1). A total of 78 subjects with conversion from MCI to AD were excluded: Twenty-eight subjects were excluded because of head motion or issues with image postprocessing of at least one MR data set, 47 subjects were excluded because MR imaging or clinical records were incomplete and because conversion from MCI to AD occurred after the first 2 years of study participation, and three subjects were excluded because they were not white. (Details are provided later in this article.)

We included 48 subjects with stable MCI (24 *APOE*4 carriers, 24 noncarriers), representing the totality of the sample in the cohort that met study inclusion and exclusion criteria for stable MCI at the time of data collection (May 2009). Patients with stable MCI whose ADNI baseline visit occurred between September 2005 (when enrollment in the ADNI study began) and May 2006 and who had undergone 3 years of longitudinal clinical follow-up at the time of data collection, revealing stable cognitive impairment, were included. A total of 22 subjects with stable MCI were excluded: Seventeen subjects were excluded because of incomplete MR imaging or clinical data, two were excluded because of head motion or issues with image postprocessing of at least one of the MR data sets, and three were excluded because they were not white.

We included only white patients in the present study because previous studies have reported a weaker association between *APOE*4 status and AD onset in other ethnic groups (14). This resulted in the exclusion of three subjects with conversion from MCI to AD (one Asian subject who was a noncarrier, one African-American subject who was an *APOE*4 carrier, and one African-American subject who was a noncarrier) and three subjects with stable MCI (two Asian subjects who were noncarriers and one African-American subject who was an *APOE*4 carrier).

Three brain MR imaging studies obtained 12 months before conversion from MCI to AD, at the time of AD diagnosis, and 12 months after AD diagnosis were available for each subject in whom MCI converted to AD. Three brain MR imaging studies obtained at baseline and at 12- and 24-month follow-up were available for each subject. Two neuroradiologists (M.V.S., Z.R.; 6 and 10 years, respectively, of experience in neuroradiology) reviewed MR imaging studies; they evaluated head motion and confirmed the absence of other causes of diffuse or focal brain volume loss, including extensive white matter disease or cerebral infarction. Determination of *APOE* genotype of all subjects from peripheral blood DNA was conducted at the ADNI Biomarker Core Laboratory. Cerebrospinal fluid samples were obtained in 52 subjects (11 *APOE*4 carriers and 14 noncarriers with conversion from MCI to AD, 14 *APOE*4 carriers and 13 noncarriers with stable MCI) when they entered the ADNI, as described in the ADNI procedures manual (*http://www.adni-info.org/*). Amyloid-β peptide ($A\beta_1$ to $A\beta_{42}$) levels (measured in picograms per milliliter) were measured in cerebrospinal fluid samples.

Cognitive Testing

Cognitive status was assessed with the Clinical Dementia Rating (CDR) scale during a semistructured interview with the patient and caregiver, in which an index of global functioning is obtained (15). By assigning a severity score for six domains (memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care), a total score known as the CDR sum of boxes (SB) is obtained. The CDR scale has been extensively validated as a clinical tool with which to stage the severity of dementia, and it is considered a reliable predictor of cognitive decline (15–17).

MR Imaging

MR imaging was performed with 1.5-T systems and use of standardized parameters, the details of which are available online (*http://www.loni.ucla.edu/ADNI/Research/Cores*). Volumetric magnetization-prepared rapid gradient echo sequences were used in the analysis. Magnetization-prepared rapid gradient

Table 1

Main Demographic and Clinical Characteristics of *APOE*4 Carriers and Noncarriers Stratified by Diagnostic Group

Characteristic	Stable MCI		Conversion from MCI to AD	
	*APOE*4 Noncarriers ($n = 24$)	*APOE*4 Carriers ($n = 24$)	*APOE*4 Noncarriers ($n = 20$)	*APOE*4 Carriers ($n = 27$)
Age (y)	78.6 ± 7.7	77.7 ± 5.95	76.2 ± 10.5	75.9 ± 5.96
Sex*	6/18	7/17	9/11	10/17
Education (y)	15.9 ± 3.1	15.8 ± 2.4	15.7 ± 3.4	15.4 ± 2.7
CDR SB†				
Baseline	1.0 (0.5–3.0)	1.0 (0.5–2.5)	2.0 (0.5–3.5)	2.0 (0.5–4.0)
12-month follow-up	1.25 (0.5–4.5)	1.0 (0.5–4.0)	3.0 (1.0–8.0)	4.0 (0.5–8.0)
24-month follow-up	1.0 (0–9.0)	2.0 (0–3.5)	4.5 (1.5–9.0)	5.0 (2.5–11.0)
$A\beta_1$ to $A\beta_{42}$ peptide level‡	215.3 ± 55.4	133.3 ± 29.3	160 ± 51.2	127 ± 22.5

Note.—Unless otherwise indicated, data are mean ± standard deviation.

* Data are number of women and men, respectively.

† Data are median, and data in parentheses are the range.

‡ Data were available for 14 *APOE*4 carriers and 13 noncarriers with stable MCI and 11 *APOE*4 carriers and 14 noncarriers with conversion from MCI to AD.

echo images downloaded from the ADNI Web site had been previously submitted for image preprocessing correction to ensure standardized quality across multiinstitutional data.

Statistical Analysis

Differences between *APOE*4 carriers and noncarriers within each group (MCI to AD conversion and stable MCI) were evaluated by using the Pearson χ^2 test for sex and global CDR (baseline and 12- and 24-month follow-up) and one-way analysis of variance for age, education, and CDR SB (baseline and 12- and 24-month follow-up). General linear model repeated-measures analysis was used for analysis of variance of repeated CDR SB measurements (within-subjects factor) over time, stratifying patients on the basis of *APOE* genotype (between-subjects factor).

Analyses were conducted by using statistical software (SPSS 16.0 for Windows; SPSS, Chicago, Ill). A P value of less than .05 indicated a significant difference. None of the MR data sets were excluded because of diffuse or focal brain volume loss or extensive white matter disease. Magnetization-prepared rapid gradient echo images were processed by using VBM with statistical parametric mapping (SPM5; Functional Imaging Laboratory, Wellcome Trust Centre for Neuroimaging, London, England) on Matlab 7.6.0 (Mathworks, Natick, Mass), with the VBM5 toolbox (Christian Gaser, University of Jena, Jena, Germany). Preprocessing included spatial normalization, segmentation, Jacobian modulation, and smoothing with a 12-mm full width at half maximum isotropic Gaussian kernel (final voxel resolution, 1 × 1 × 1 mm). The resulting GM maps were then submitted for statistical analysis with statistical parametric mapping. Comparison between longitudinal data for *APOE*4 carriers and noncarriers acquired at baseline and at 12- and 24-month follow-up within each group was conducted with the paired t test. Familywise error correction for multiple comparisons was applied, and a P value of less than .05 (extent threshold, 10 voxels) indicated a significant difference. MR image postprocessing and statistical analyses were conducted by a neuroradiologist (M.V.S.) with 7 years of experience with brain MR postprocessing with statistical parametric mapping.

Results

Clinical Data

Table 1 summarizes main demographic data and CDR SB of patients stratified by diagnosis and *APOE* status. CDR score was 0.5 for all subjects with stable MCI at baseline, while median CDR score was 0.5 at 12- and 24-month follow-up (range, 0–0.5 for both). CDR score was 0.5 for all subjects with MCI to AD conversion at baseline; median CDR score was 0.5 (range, 0.5–1) when AD conversion was diagnosed and 1 (range, 0.5–2) 12 months after diagnosis in all patients with MCI to AD conversion (both *APOE*4 carriers and noncarriers). There were no significant differences in age, sex distribution, years of education, or disease severity, as measured by using the CDR scale and CDR SB at baseline and 12 and 24 months after diagnosis of AD between *APOE*4 carriers and noncarriers within the MCI and AD groups. There was a trend toward higher CDR SB in *APOE*4 carriers than in noncarriers in the AD group 12 months after diagnosis of AD (P = .065). All *APOE*4 carriers had decreased $A\beta_1$ to $A\beta_{42}$ peptide levels in cerebrospinal fluid on the basis of the 192 pg/mL cutoff proposed by Shaw et al (18), regardless of whether they had stable or worsening cognitive status. Among *APOE*4 noncarriers, 11 (79%) of 14 patients with MCI to AD conversion and four (31%) of 13 patients with stable MCI had decreased $A\beta_1$ to $A\beta_{42}$ peptide levels in cerebrospinal fluid.

The general linear model for repeated measures (tests of within-subjects effects with the Huynh-Feldt epsilon approach) revealed a significant ($P < .001$) progressive cognitive decline in the subgroup of patients in whom MCI progressed

Figure 1

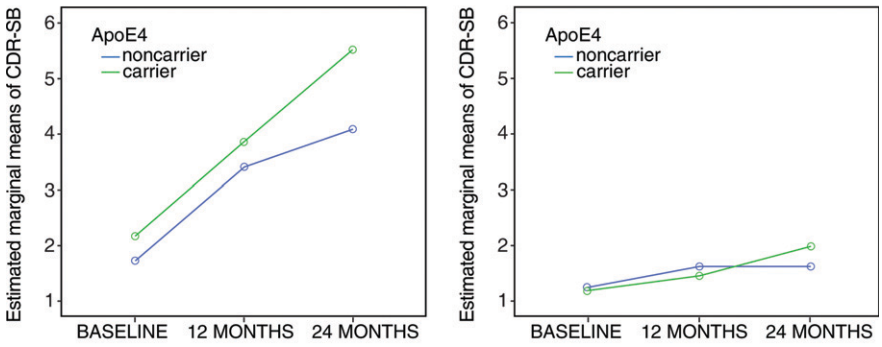

Figure 1: **(a, b)** Graphs show CDR SB of patients stratified by diagnosis and *APOE* status. **(a)** Patients in whom MCI progressed to AD. **(b)** Patients with stable MCI.

to AD, without significant interaction between CDR SB and *APOE*4 status (Fig 1a). In the stable MCI group (Fig 1b), differences in CDR SB over time were significant (P = .049) during the 2-year follow-up period, but there was no significant interaction between CDR SB and *APOE*4.

MR Imaging

Comparison of GM volume in *APOE*4 carriers with that in *APOE*4 noncarriers at baseline did not reveal significant differences between the groups. In *APOE*4 carriers with conversion from MCI to AD, significant GM volume loss was observed during the 12 months before diagnosis of conversion (Table 2, Fig 2a) and during the 12 months after diagnosis of conversion (Table 2, Fig 2b). Significant GM volume loss was seen in the right temporal lobe, right hippocampus, and left insula during the 12 months before diagnosis of conversion. During the 12 months after diagnosis of conversion, GM volume loss was observed in the bilateral hippocampi, bilateral temporal lobes, bilateral parietal lobes, right caudate nuclei, and bilateral insula. Conversely, there were no significant changes in GM volume in noncarriers during the 12 months before and the 12 months after diagnosis of conversion.

In *APOE*4 carriers with stable MCI, areas of GM volume loss were observed in the bilateral insula and temporal lobes during the 1st year of follow-up (Table 3, Fig 2a) but were not observed during the 2nd year of follow-up. In *APOE*4 noncarriers with stable MCI, no significant GM volume loss was observed during the 1st or 2nd year of follow-up.

Discussion

We evaluated the longitudinal effects of the *APOE* genotype on regional GM loss and disease severity in patients with stable amnestic MCI and in those with documented conversion from MCI to probable AD over a 2-year period.

There was significant progression of GM atrophy in the bilateral hippocampi, temporal neocortex, insula, and parietal lobes in *APOE*4 carriers with conversion from MCI to AD. We also found areas of GM volume loss in the bilateral insula and temporal lobes in *APOE*4 carriers with stable MCI. The reason GM volume loss was observed in *APOE*4 carriers with stable MCI during only the 1st year of follow-up remains unclear. This may reflect the nonlinear effect of *APOE*4 on brain volume loss rates in patients with MCI, as previously reported by Jack et al (19).

*APOE*4 noncarriers in each subgroup did not experience any significant GM volume loss during the 2-year follow-up. No significant difference in cognitive status, as measured by using the CDR scale and CDR SB, was observed between *APOE*4 carriers and noncarriers in each group.

*APOE*4 has been linked to the pathogenesis of AD with a "two-switch" mechanism (20). In response to stressors, neurons begin processing intranuclear *APOE* intron 3 into mature *APOE* messenger RNA (4,5). However, *APOE*4, to a greater extent than its isoforms, undergoes proteolytic cleavage (1,5). After proteolysis, carboxyl-terminal-truncated *APOE* fragments have an increased affinity for phospholipids; therefore, they have the potential to destabilize and cross membranes, disrupting the structure and function of neuronal cells and ultimately leading to neuronal death. Preclinical studies suggest that *APOE*4 is responsible for increased accumulation of amyloid-β protein compared with its isoforms; it is less efficient in maintaining cytoskeletal structure and mitochondrial function, it stimulates τ phosphorylation, and it potentiates amyloid-β–induced lysosomal leakage and apoptosis (1,21). The distribution of brain volume loss observed in this study in *APOE*4 carriers is consistent with the results of prior animal and human studies, which have shown an association between *APOE*4 and pathologic changes typical of AD in the limbic system and neocortex (6,22–26). Cross-sectional and longitudinal imaging data revealed a more severe hippocampal, entorhinal, and amygdalar volume loss in nondemented *APOE*4 carriers than in noncarriers (27–29). Moffat et al (29) evaluated longitudinal changes in hippocampal volume in 39 nondemented elderly subjects by comparing two sets of MR images obtained an average of 2.7 years apart and found greater loss of hippocampal volume in *APOE*4 carriers than in noncarriers. Patients with AD who had the E4/E4 genotype had greater volume loss in the amygdala and hippocampus, especially the right hippocampus and amygdala, when compared with those who had other genotypes (9). Mori et al (10) found that the rate of hippocampal atrophy over a 12-month period was significantly more severe in patients with AD who were *APOE*4 carriers than in patients with AD who were *APOE*4 noncarriers.

In prior cross-sectional VBM studies, researchers have investigated the relationship between *APOE*4 status and GM atrophy in patients with AD and

Table 2

Areas of GM Volume Loss during the 12 Months prior to and 12 Months after Clinical Conversion from MCI to AD in *APOE4* Carriers

Location	X Coordinate	Y Coordinate	Z Coordinate	Brodmann Area	T Score*
	GM Volume Loss during the 12 Months prior to Conversion to AD				
Right superior temporal gyrus	41	3	−17	38	9.88
Right middle temporal gyrus	51	5	−36	21	8.57
Left insula	−45	−12	−7	13	7.61
Right hippocampus	28	−15	−12	...	7.32
	GM Volume Loss during the 12 Months after Conversion to AD				
Left superior temporal gyrus and insula	−48	6	−4	22	8.16
Right hippocampus	31	−12	−19	...	7.19
Left supramarginal gyrus	−55	−56	25	40	6.91
Left hippocampus	−33	−12	−20	...	6.71
Right caudate	35	−33	−6	...	6.71
Right insula	43	−1	−4	13	6.57
Right superior temporal gyrus	58	5	−2	22	6.51
Right supramarginal gyrus	40	−57	55	40	6.29
Left middle temporal gyrus	−57	−2	−18	21	6.16

Note.—X, Y, and Z are brain coordinates based on the Montreal Neurologic Institute standard brain template. This format uses three numbers to describe the distance from a point at midline and 4 mm below the anterior commissure. The X, Y, and Z dimensions refer to left-right, posterior-anterior, and inferior-superior, respectively. By convention, the right hemisphere has positive X values; the anterior brain, positive Y values; and the superior brain, positive Z values.

* $P < .05$. Family-wise error correction for multiple comparisons was applied.

Figure 2

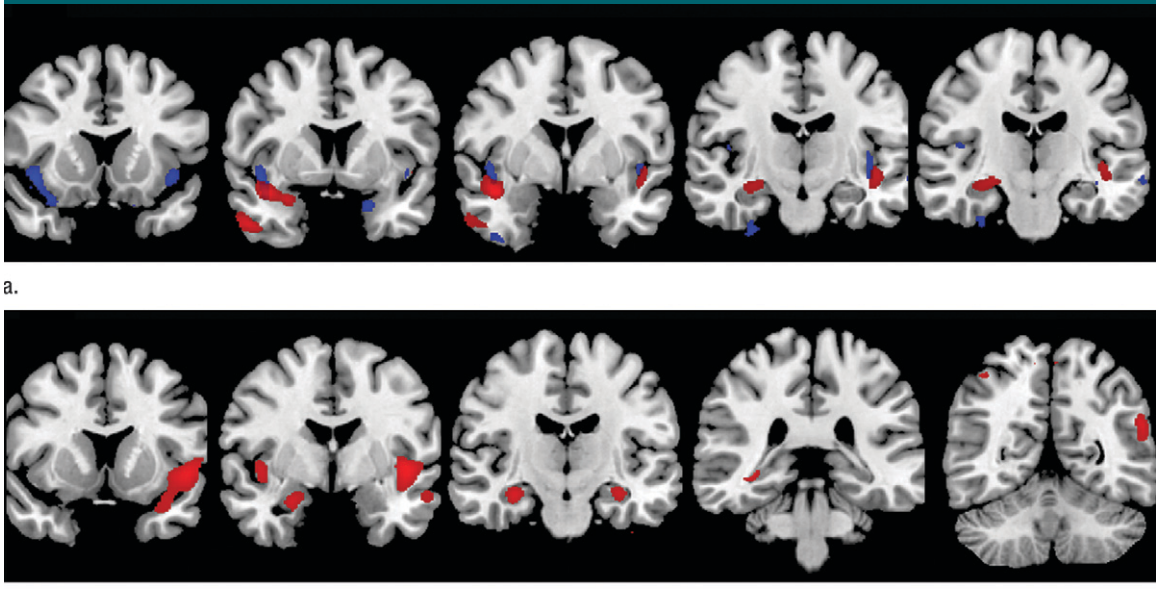

Figure 2: **(a, b)** MR images show GM volume loss in *APOE4* carriers with MCI and those with AD. **(a)** Areas of significant GM volume loss in patients with stable MCI (blue) and those with progression from MCI to AD (red) during 1st year of follow-up. The area of GM volume loss in the region of the right parahippocampal gyrus represents a cluster of cortical loss centered at the level of the uncus (coordinates: 30, −11, −40), which artifactually extends outside the brain. **(b)** Areas of significant GM volume loss in patients in whom MCI progressed to AD during 2nd year of follow-up (red). No areas of significant GM volume loss were observed in patients with stable MCI during the 2nd year of follow-up with the same statistical threshold. Statistical maps are displayed, with a statistical threshold of $P < .05$ (family-wise error-corrected, extent threshold = 10 voxels).

Table 3

Areas of GM Volume Loss during the First 12 Months after Entering the Study in *APOE*4 Carriers with Stable MCI

Location	X Coordinate	Y Coordinate	Z Coordinate	Brodmann Area	T Score*
Left uncus	−21	9	−26	28	8.03
Right insula	42	15	−9	47	8.02
Left insula	−40	17	−8	47	7.03
Right uncus	30	−11	−40	20	6.86
Right middle temporal gyrus	57	−54	−13	37	6.69

Note.—X, Y, and Z are brain coordinates based on the Montreal Neurologic Institute standard brain template. This format uses three numbers to describe the distance from a point at midline and 4 mm below the anterior commissure. The X, Y, and Z dimensions refer to left-right, posterior-anterior, and inferior-superior, respectively. By convention, the right hemisphere has positive X values; the anterior brain, positive Y values; and the superior brain, positive Z values.

* $P < .05$. Family-wise error correction for multiple comparisons was applied.

those with MCI. A cross-sectional VBM study showed that *APOE*4 carriers with stable MCI had greater atrophy in the right hippocampus and amygdala than did noncarriers, while *APOE*4 carriers in whom MCI eventually progressed to dementia at clinical follow-up had atrophy in the inferior frontal gyrus when compared with noncarriers (30). Pievani et al (31) found that *APOE*4 status may have a region-specific effect on brain volume loss, with greater volume loss affecting the temporal lobes and right occipital lobe in *APOE*4 carriers with AD; however, these results were not significant after the researchers applied statistical correction for multiple comparisons. A cross-sectional VBM study revealed significant loss of GM volume in the bilateral parietal cortex, right hippocampus, precuneus, and middle frontal gyrus in *APOE*4 carriers with AD when compared with noncarriers (20). Conversely, another recent cross-sectional VBM study in a group of 32 patients with AD failed to reveal any significant difference in regional brain atrophy between *APOE*4 carriers and noncarriers (32).

Neuropathologic changes typical of AD, particularly neurofibrillary tangles, follow a predictable distribution at different stages of the disease (33). Initially, neurofibrillary tangles occur predominantly in the perirhinal region of the temporal lobe (Braak stages I and II, clinically silent). Later on, they occur in the limbic system (Braak stages III and IV, incipient AD). In advanced stages of the disease, they affect the neocortex (Braak stages V and VI, fully developed AD) (34,35). In agreement with this model of neurodegeneration in AD, we found significant loss of GM volume in the temporal and insular regions in *APOE*4 carriers with stable MCI, while *APOE*4 carriers had significant GM volume loss not only in the temporal lobe and insula but also in the neocortex, specifically, the parietal lobes 1 year after conversion of MCI to AD. In addition, only patients with clinical progression from MCI to AD had significant loss of hippocampi volume during 2-year follow-up.

The association between hippocampal volume loss and conversion to AD is well known and has been recently evaluated with MR morphometric techniques (36,37).

We found significant GM volume loss in the insula in both the stable MCI group and the MCI-to-AD group, with greatest foci of GM loss centered at the level of the anterior insula (right and left anterior and middle short gyrus) in *APOE*4 carriers with stable MCI and at the level of the posterior insula (left inferior periinsular gyrus and right anterior longus insular gyrus) in patients with conversion from MCI to AD (38). The insula has been implicated in a variety of processes, ranging from representation of self-awareness and consciousness to visceral control and sensation, as part of a complex functional network that includes multimodality sensory inputs, subcortical and limbic structures, the anterior and posterior cingulate cortex, and the dorsolateral frontal and parietal cortex (39–41). Previous pathologic analyses and imaging studies have revealed substantial abnormalities in the insular cortex in patients with AD (35,42–44). Although the role of the insular cortex in the pathogenesis of AD is not completely understood, insular disease likely contributes to the corruption of concepts of self and well-being (35).

We also found significant loss of GM volume in the supramarginal gyrus in *APOE*4 carriers during the 12 months after conversion from MCI to AD. The supramarginal gyrus is an area of the posterior cortex that is particularly susceptible to deposition of neurofibrillary tangles and neuronal loss, especially in patients with advanced AD, in whom cognitive deficits in areas other than memory begin to appear (45–47).

Drawbacks of prior VBM studies on the correlation between regional GM volume changes and *APOE*4 status were use of a cross-sectional approach and potential inclusion of patients at different stages of disease. Thus, more rigorous selection criteria and longitudinal observations are needed to reveal differential patterns of GM loss between *APOE*4 carriers and noncarriers.

The main strengths of our study when compared with the existing literature were as follows: We used a whole-brain image analysis technique to detect GM changes, and we were not limited to region-of-interest analysis of selected regions in patients stratified on the basis of *APOE* genotype and disease status. We performed a longitudinal analysis of MR data, which enabled us to perform within-subject comparisons. We selected relatively homogeneous patient groups, with respect to cognitive status and timeline in the natural history of patient condition. We used a stringent statistical

threshold compared with that used in prior VBM cross-sectional studies.

The results of the present study and those of prior studies indicate that the rate of hippocampal and neocortical atrophy is greater in association with *APOE*4 in nondemented elderly subjects, in subjects with MCI, and in those with AD. The greater rate of GM volume loss in *APOE*4 carriers with AD compared with that in noncarriers with AD 1 year before and 1 year after the diagnosis of AD potentially clarifies the known relationship between treatment response and *APOE*4 genotype. *APOE*4 carrier status is thought to be a predictor of poor response to treatment with acetylcholinesterase inhibitors and rosiglitazone in patients with AD (26,48–50). The greater rate of GM volume loss in *APOE*4 carriers suggests that the brains of *APOE*4 carriers may be more vulnerable to endo- and exogenous stressors and more difficult to treat than the brains of noncarriers. Significant GM volume loss was observed only in *APOE*4 carriers, regardless of whether they had stable cognitive impairment or clinically worsening cognitive impairment. On the other hand, there was no significant GM volume loss in *APOE*4 noncarriers with worsening cognitive status. These findings suggest that *APOE*4 is related to progression to AD and GM volume loss; however, they also challenge the postulated relationship between GM atrophy and cognitive decline. Our results in the *APOE*4 noncarriers suggest that accelerated hippocampal and neocortical volume loss cannot entirely account for cognitive deterioration in this clinical setting and that other factors must be investigated to clarify the clinical progression from MCI to AD.

$A\beta_1$ to $A\beta_{42}$ peptide levels measured in the cerebrospinal fluid are promising AD biomarkers. Reduced values of this peptide are found in the cerebrospinal fluid of patients with AD as a result of its accumulation into insoluble plaques in the brain. Shaw et al (18) found that a cutoff value of 192 pg/mL for $A\beta_1$ to $A\beta_{42}$ peptide levels had a sensitivity of 96.4% and a negative predictive value of 95.2% in the identification of subjects with AD versus healthy control subjects. There is also a well-known inverse correlation between levels of $A\beta_1$ to $A\beta_{42}$ peptide and *APOE* genotype with lowest $A\beta_1$ to $A\beta_{42}$ values in patients homozygous for *APOE*4 (51,52). Given the correlation of the *APOE*4 genotype and decreased $A\beta_1$ to $A\beta_{42}$ values with amyloid plaque formation, it would be important to assess the differences in GM volume loss rates in patients with AD who are *APOE*4 carriers and "amyloid positive" (as defined by a cerebrospinal fluid cutoff of 192 pg/mL) and patients who are *APOE*4 carriers and "amyloid negative." We were unable to conduct this subgroup analysis, since all of the *APOE*4 carriers with MCI-to-AD conversion were amyloid positive.

This study had several limitations. An important limitation of the study was the inclusion of only white subjects. Given the small numbers of nonwhite subjects eligible for the study (three patients with conversion to AD, three patients with stable MCI), we decided to focus this investigation on white subjects because a weaker association between *APOE*4 carrier status and onset of AD has been reported in African-American subjects compared with white subjects (14). When more ADNI data on nonwhite subjects becomes available, further analysis of data from nonwhite subjects should be conducted. Sample sizes may have been insufficient to enable us to detect small volume changes in the *APOE*4 noncarrier groups and subtle differences in cognitive performance between groups. These findings were not confirmed with pathologic analysis; nevertheless, a multidisciplinary team evaluated patients, and pathologic findings will eventually become available for some patients.

This study focuses on an important but relatively narrow phase of the natural history of this disease. Future investigations of patterns and rates of brain volume loss during transition from normal aging to MCI are needed. The relationship between *APOE* genotype, brain volume loss, and regional changes in brain metabolism, as measured with positron emission tomography, also requires further investigation. Quantification of longitudinal regional changes in GM volume in *APOE*4 carriers and noncarriers with MCI and comparison of the relative degree of volume loss over time may assist in the identification of morphometric predictors of MCI-to-AD conversion.

In conclusion, we investigated the correlation between *APOE* genotype, disease severity, and patterns of GM volume loss in patients with stable MCI and those with progression from MCI to AD. Not only is the *APOE* genotype linked to the development of AD, it also appears to be specifically related to accelerated atrophy of the hippocampi and neocortical regions in patients with MCI and those with AD. Although the *APOE* genotype does not strongly influence the rate of cognitive decline in patients with AD, the faster rate of hippocampal and cortical atrophy seen in *APOE*4 carriers is likely an important factor in determining and modulating treatment response. It is important to understand the role of the *APOE* genotype in the progression of neurodegeneration to optimize treatment regimens, including therapies that target *APOE* structure and function, and *APOE* genotype testing should be included in clinical trials that target patients with probable AD.

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