Mapping the Effect of the Apolipoprotein E Genotype on 4-Year Atrophy Rates in an Alzheimer Disease–related Brain Network[1]

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Purpose: To determine the effect of the apolipoprotein E *(APOE)* genotype on atrophy rates of specific brain gray matter regions hypothesized to be key components of cognitive networks disrupted in Alzheimer disease.

Materials and Methods: The Alzheimer's Disease Neuroimaging Initiative (ADNI) was approved by the institutional review boards of all participating sites. All subjects and their legal representatives gave written informed consent prior to data collection. The authors analyzed data from 237 subjects (mean age, 79.9 years; 40% female) with mild cognitive impairment (MCI) in the ADNI database and assessed the effect of the *APOE* ε4 and ε2 alleles on regional brain atrophy rates over a 12–48-month period. Brain regions were selected a priori: 15 experimental and five control regions were included. Regional atrophy rates were derived by using a fully automated algorithm applied to T1-weighted magnetic resonance (MR) imaging data. Analysis consisted of mixed-effects linear regression with repeated measures; results were adjusted for multiple testing with Bonferroni correction.

Results: Thirteen of 15 experimental regions showed a significant effect of ε4 for higher atrophy rates ($P < .001$ for all). Cohen d values ranged from 0.26 to 0.42, with the largest effects seen in the amygdalae and hippocampi. The transverse temporal cortex showed a trend ($P = .02$, but did not survive Bonferroni correction) for a protective effect (Cohen d value = 0.15) of ε2. No control region showed an *APOE* effect.

Conclusion: The *APOE* ε4 allele is associated with accelerated rates of atrophy in 13 distinct brain regions in limbic and neocortical areas. This suggests the possibility of a genotype-specific network of related brain regions that undergo faster atrophy in MCI and potentially contribute to cognitive decline.

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Apolipoprotein E has a well-established function as a protein involved in the transport and normal metabolism of lipids. In addition, it plays a substantial role in several neuron-specific functions, including normal neuronal development, neuron repair in response to inflammation or oxidative damage (1), and breakdown of extracellular amyloid-β peptide in the brain (2). In fact, variant apolipoprotein E isoforms have been implicated in the pathophysiology of several central nervous system disorders, including Alzheimer disease (AD) (3). Investigators have previously demonstrated that the ε4 isoform of apolipoprotein E is associated with several deleterious-cell physiologic characteristics that may mediate its apparent influence on AD pathology, including inhibition of neurite-sprouting processes (4), predisposition of neuronal tissue to neurofibrillary tau tangle deposits (5), and direct neurotoxic effects (6). These findings are consistent with what is known epidemiologically about the apolipoprotein E gene *(APOE)* as a risk factor for late-onset, sporadic AD—namely, of *APOE* alleles (ε2, ε3, and ε4). Presence of ε4 is associated with not only a higher baseline risk for development of AD, but also an earlier age of onset and faster progression in those affected (7) as compared with *APOE* ε3 homozygotes. *APOE* ε2, on the contrary, is thought to play a more neuroprotective role: In comparison with *APOE* ε3 homozygotes, carriers are less likely to develop AD; those carriers who do develop AD are more likely to do so later in life, and carrier brain autopsy specimens show a lower burden of AD-specific cortical pathology (8–10). *APOE* ε3—the most prevalent isoform, with 93% of the U.S. population carrying at least one copy—is considered neutral with respect to AD pathophysiology.

For the current study, we analyzed longitudinal data in persons at risk for progression to clinical AD—subjects who met criteria for mild cognitive impairment (MCI) (11)—to quantify the degree to which *APOE* genotype influences region-specific brain atrophy as measured via a fully automated, rater-free algorithm applied to 1.5-T MR images. Given previous findings in studies focused on more limited and localized regions of interest—such as the hippocampus (12,13)—our hypothesis was that regions comprising an AD-related gray matter network would display differing rates of atrophy according to *APOE* genotype; and, moreover, we hypothesized that this effect would not be seen in control regions typically spared by AD-related pathology. Specifically, we hypothesized that those individuals carrying at least one copy of the ε4 variant of *APOE* would show accelerated rates of atrophy in these regions and that individuals bearing the ε2 variant would show slower atrophy. Thus, our purpose was to determine the effect of the *APOE* genotype on atrophy rates of specific gray matter regions in the brain that are hypothesized to be key components of cognitive networks disrupted in AD.

Advances in Knowledge

- The apolipoprotein E *(APOE)* ε4 allele is associated with accelerated rates of atrophy in a specific gray matter network of vulnerable brain regions among subjects at risk for Alzheimer disease (AD) ($P < .001$ for 13 of 15 regions examined), and this effect is not seen in control regions typically spared in AD.
- The additional rate of atrophy in vulnerable brain regions—regions that typically display marked degeneration in AD—that is directly attributable to *APOE* ε4 is comparable in magnitude to the underlying age-related, baseline rate of atrophy observed for these areas in ε4 noncarriers.

Implication for Patient Care

- Secondary prevention trials in mild cognitive impairment should be stratified by *APOE* ε4 status to determine potential treatment effects on brain regions that are vulnerable to degeneration.

Materials and Methods

Subjects

The data used in this study were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database *(adni.loni.ucla.edu)* (14), a large multicenter natural history trial in which the goal has been to examine the ability of MR imaging, positron emission tomography (PET), biologic markers, and clinical and neuropsychological assessments to be used in combination to measure progression in MCI and AD. See *www.adni-info.org* for up-to-date information. ADNI was approved by the institutional review boards of all participating sites, including the home institution of the current study. All subjects and, if applicable, their legal representatives gave written informed consent prior to the collection of clinical, genetic, and imaging data.

Study subjects whose data would be eligible for analysis included only those subjects who were classified by the ADNI as having a baseline diagnosis of MCI. Subjects were required to have data for all of the following parameters available in the ADNI database: age, race, patient sex, and years of education;

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Abbreviations:
AD = Alzheimer disease
ADNI = Alzheimer's Disease Neuroimaging Initiative
MCI = mild cognitive impairment
MMSE = Mini–Mental State Examination

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Conflicts of interest are listed at the end of this article.

baseline Mini–Mental State Examination (MMSE) score; *APOE* genotyping results; baseline 1.5-T MR imaging data, which were analyzed to derive cortical thickness and subcortical volume data for the ADNI by using FreeSurfer software (version 4.4); and a minimum of two other follow-up time points (6, 12, 18, 24, 36, or 48 months from baseline) with MR imaging data that had been analyzed by using FreeSurfer. All data from MR imaging examinations that were not deemed a "full pass" by ADNI quality-control evaluators were excluded (15).

Of 397 subjects who had MCI and data in the ADNI, 71 were excluded, owing to an inadequate number of time points (fewer than three) with FreeSurfer-analyzed MR imaging data, which met ADNI quality-control standards. Of the remaining 326 subjects, 81 were eliminated owing to failure to pass MR imaging quality control screening for one or more brain regions. Remaining subjects were split into three groups on the basis of their *APOE* genotype: Those who carried at least one ε2 allele and no ε4 alleles were classified as "ε2 carriers;" subjects with two copies of the ε3 allele were classified "ε3/ε3" or "ε3 homozygotes;" and those with at least one ε4 allele and no ε2 alleles were classified as "ε4 carriers." A subset of eight subjects with the *APOE* ε4/ε2 or ε2/ε4 genotype was excluded, owing to the putative opposing effects of the ε4 and ε2 alleles. As of November 30, 2011, 237 subjects from the ADNI-1 study arm met the criteria and were thus included: There were nine ε2 carriers, 102 ε3 homozygotes, and 126 ε4 carriers. See Table 1 for a summary.

The length of follow-up in months (mean ± standard deviation) was *(a)* ε2 carriers, 27.33 ± 12.4 (range, 12–48); *(b)* ε3 homozygotes, 27.40 ± 9.7 (range, 12–48); and *(c)* ε4 carriers, 27.6 ± 11.3 (range, 12–48). See Table 2 for a follow-up summary.

Table 1

Subject Demographics and Baseline MMSE Score

Parameter	*APOE* ε2 Carriers	*APOE* ε3/ε3 Homozygotes	*APOE* ε4 Carriers	*P* Value
No. of subjects	9	102	126	
Mean age at baseline (y)*	82 ± 2.4	81 ± 0.7	79 ± 0.7	.24
Mean years of education*	15.5 ± 1.0	15.7 ± 0.3	15.6 ± 0.3	.94
Baseline MMSE score*	28.0 ± 0.6	27.2 ± 0.2	27.0 ± 0.2	.19
Patient sex				.16
No. of men	3	66	74	
No. of women	6	36	52	
Race				.45
No. of Asian subjects	1	5	3	
No. of black subjects	0	2	4	
No. of white subjects	8	95	119	

Note.—No baseline characteristics or demographics are different according to *APOE* genotype. All tests were used to assess for significance at a level of $\alpha = .05$. *P* values for age, years of education, and MMSE were all determined by using analysis of variance; patient sex and race were determined by using χ^2 analysis or the Fisher exact test, as appropriate.

* Data are means ± standard deviations.

Table 2

Summary of Subject Follow-up by Genotype

Subject Group	24-Month Follow-up	36-Month Follow-up	48-Month Follow-up	Total No. of Subjects
APOE ε2 carriers	6 (67)	4 (44)	1 (11)	9
APOE ε3/ε3 homozygotes	71 (69.6)	42 (41.2)	4 (3.9)	102
APOE ε4 carriers	90 (71.4)	53 (42.1)	14 (11.1)	126

Note.—Data are numbers of subjects. Numbers in parentheses are the percentage of the total number of subjects in that genotypic group with at least that many months of follow-up MR imaging data. There was no difference in mean follow-up between genotypic groupings (*P* = .99, analysis of variance).

Classification, Clinical Diagnosis, and *APOE* Genotyping

For the ADNI, to be classified in the MCI group, a subject needed an MMSE score between 24 and 30 (inclusive), a memory complaint, objective evidence of memory loss as measured by education-adjusted scores of the Wechsler Memory Scale Logical Memory II, a Clinical Dementia Rating score of 0.5, absence of substantial levels of impairment in cognitive domains other than memory, preserved activities of daily living, and absence of dementia. For specific exclusion criteria for the ADNI-1 study arm, please refer to Appendix E1 (online) or the ADNI-1 procedures manual (16).

Genotyping of all subjects for *APOE* allele status was performed by using DNA extracted from peripheral blood cells. The cells were collected in plastic tubes (10 mL) coated with ethylenediaminetetraacetic acid and sent at room temperature via overnight delivery to the University of Pennsylvania AD Biofluid Bank Laboratory. Please see the ADNI-1 procedures manual for more detailed information (16).

The goal of this study was to examine genotype effects on MR imaging changes over time. We did not extract longitudinal cognitive or clinical conversion data, since other published data from this cohort have already documented the effect of *APOE* on cognitive changes and conversion rates (17,18).

MR Imaging Acquisition

For the ADNI, 1.5-T magnetization-prepared rapid gradient-echo MR images were preprocessed by undergoing *(a)* correction for gradient nonlinearity via "GradWarp," *(b)* intensity nonuniformity by using B1 calibration

acquisitions, and *(c)* residual intensity nonuniformity by using "N3." The images also underwent scaling based on acquisitions of a phantom device. All acquisitions underwent rigorous vetting for quality-control purposes and were performed by using a standardized protocol specifically developed for the ADNI, tailored for use with each model of imager used at the different data collection sites (15). More detailed information on the specific MR acquisition protocols for each type of imager used can be found at *http://adni.loni.usc.edu/* (19).

MR Cortical Thickness and Volume Derivations

Cortical thickness derivations and volumetric segmentation were performed with the FreeSurfer image analysis suite. The technical details of these procedures are described in prior publications (20–26). FreeSurfer has been shown previously to possess good test-retest reliability for cortical thickness derivations, as well as subcortical volume determinations (21,26,27). See Appendix E1 (online) for more details.

Study Design and Statistical Analyses

Statistical analyses were performed by using the R Statistical Package (R Foundation, Vienna, Austria; *www.R-project.org*). All comparisons and test statistics were assessed for significance by using $\alpha = .05$, subject to Bonferroni correction for multiple testing.

Subjects were tested for differences in age, years of education, and baseline MMSE according to *APOE* genotype by using one-way analysis of variance. Differences in patient sex and race were assessed by using χ^2 analysis or the Fisher exact test.

For the primary analysis, the authors selected 20 brain regions of interest to be examined. Fifteen regions that have been implicated previously in the pathologic progression of AD were selected, and it was hypothesized they would show an effect of *APOE* on their rate of atrophy ("experimental" regions); and five regions thought to not play a role in AD pathophysiology were selected and were thus not expected to show an effect of *APOE* ("control" regions). The experimental regions examined focused on those demonstrated in prior studies to be involved in early gray matter atrophy in AD, such as the entorhinal cortex, the parahippocampal cortex, and the hippocampi (28–30). Control regions examined included those that have been demonstrated previously to not be involved in AD gray matter loss early in the disease (29,30) and have been treated as control regions in prior studies (31). See Table 3 for a complete listing of regions.

Data analysis consisted of a single-stage mixed-effects linear regression model (model 1.1), incorporating repeated measures to control for within-subject variation over multiple time points—that is, multivariate regression for a cortical thickness or volume involving the following predictors: time point, age at first imaging examination, race, patient sex, years of education, MMSE score, left- or right-sided measurement, *APOE* genotype, and any significant cross-products. The model is fit separately to each region by the method of restricted maximum likelihood by using the "nlme" package (Pinheiro and Bates) in the R statistical analysis platform:

$$\begin{aligned}\log y_i(x,t) = {} & [a_0(x) + a_i(x)] \\ & + a_{g2i}(x) + a_{g4i}(x) + a_{Mi}(x) \\ & + a_{rAi}(x) + a_{rBi}(x) + \gamma_a(x)\,Age_i \\ & + \gamma_e(x)\,Educ_i + \gamma_M(x)\,MMSE_i \\ & + \delta_{aMi}(x)\,Age_i + \delta_{eMi}(x)\,Educ_i \\ & + \delta_{MMi}(x)\,MMSE_i + \delta_{ag2i}(x)\,Age_i \\ & + \delta_{eg2i}(x)\,Educ_i + \delta_{Mg2i}(x)\,MMSE_i \\ & + \delta_{ag4i}(x)\,Age_i + \delta_{eg4i}(x)\,Educ_i \\ & + \delta_{Mg4i}(x)\,MMSE_i + \delta_{aAi}(x)\,Age_i \\ & + \delta_{eAi}(x)\,Educ_i + \delta_{MAi}(x)\,MMSE_i \\ & + \delta_{aBi}(x)\,Age_i + (x)\,\delta_{eBi}Educ_i \\ & + \delta_{MBi}(x)\,MMSE_i + [\beta_0(x) + b_i(x)]t \\ & + \beta_{g2i}(x)\,t + \beta_{g4i}(x)\,t + \beta_{Mi}(x)\,t \\ & + \beta_{Ai}(x)\,t + \beta_{Bi}(x)\,t + \varepsilon_i(x,t).\end{aligned}$$

All variables in this model are defined in Appendix E1 (online); see Appendix E1 for more detailed information.

Results

Results for the analysis of baseline MMSE and demographic characteristics according to *APOE* genotype are summarized in Table 1. There was no difference with *APOE* in MMSE or any other demographic characteristics.

Of the experimental regions, 13 of 15 exhibited a substantial effect of the *APOE* ε4 allele on atrophy rates that survived Bonferroni correction (new $\alpha = .0025$) ($P < .001$ for all). For all of these regions, the effect of ε4 was a marked increase in the rate of atrophy. Only the posterior cingulate cortex ($P = .0146$, did not survive Bonferroni correction) and the transverse temporal cortex ($P = .7638$) did not show an effect of ε4. In brief, of all 15 regions, the annualized rates of change (percentage) observed for subjects homozygous for ε3 ranged from −0.50% in the transverse temporal cortex to −2.76% in the hippocampi. The magnitude of additional annualized percentage atrophy directly attributable to ε4 ranged from 0.72% in the superior parietal cortex (Cohen *d* value = 0.27) to 2.14% in the entorhinal cortex (Cohen *d* value = 0.42). None of the control regions showed an effect of the ε4 allele on atrophy rate.

Table 3 summarizes the modifying effect of ε4 carrier status on atrophy rates for each region. Figure 1 illustrates *APOE* ε4 effect sizes.

There was no effect of *APOE* ε2 that survived correction for multiple testing for any region, including the hippocampus ($P = .38$), amygdala ($P = .18$), entorhinal cortex ($P = .36$), or total cerebral cortical gray matter volume ($P = .89$). The transverse temporal cortex did show a trend for a protective effect of ε2 carrier status on its rate of atrophy ($P = .02$); however, this did not survive Bonferroni correction (corrected $\alpha = .0025$). No other region approached significance ($P > .10$ for all). No control regions showed an effect of ε2.

Figure 2 depicts the annualized percentage change of the representative measure for each region by *APOE* genotype.

Table 3

***APOE* ε4 Effects on Region-specific Atrophy Rates in MCI**

Brain Region Studied	Baseline Rate of Atrophy $\beta_0(x)$	Additional Effect of *APOE* ε4 $\beta_{g4i}(x)$	Cohen *d* Value for *APOE* ε4 Effect	*P* Value
Control regions				
Cerebellar cortex (volume)*	−0.53	−0.33	−0.010	.14
Cerebellar white matter (volume)*	−0.16	0.33	0.063	.34
Pericalcarine cortex*	−0.13	0.15	0.054	.41
Postcentral gyrus*	−0.58	−0.34	−0.103	.13
Precentral gyrus*	−0.93	−0.72	−0.161	.02
Experimental regions				
Amygdala (volume)	−1.51	−2.40†	−0.376†	<.001†
Cerebral cortex (volume)	−1.47	−0.84†	−0.301†	<.001†
Entorhinal cortex	−2.46	−2.14†	−0.420†	<.001†
Fusiform gyrus	−1.83	−1.02†	−0.268†	<.001†
Hippocampus (volume)	−2.76	−1.53†	−0.368†	<.001†
Inferior parietal cortex	−1.26	−1.04†	−0.295†	<.001†
Inferior temporal cortex	−1.95	−1.34†	−0.335†	<.001†
Middle temporal cortex	−1.94	−1.28†	−0.307†	<.001†
Parahippocampal cortex	−2.11	−1.26†	−0.286†	<.001†
Posterior cingulate cortex	−1.36	−0.64	−0.165	.02
Precuneus cortex	−0.78	−0.95†	−0.294†	<.001†
Superior parietal cortex	−0.72	−0.95†	−0.266†	<.001†
Superior temporal cortex	−1.63	−0.97†	−0.283†	<.001†
Temporal pole cortex	−2.00	−1.58†	−0.301†	<.001†
Transverse temporal cortex	−0.50	−0.09	−0.020	.76

Note.—Estimated effects are given in units of percentage increase in log volume per year: A value of 1.00 (approximately) represents a 1% increase in volume per year. Negative values indicate atrophy. The coefficient $\beta_0(x)$ denotes the (baseline) mean rate of atrophy (percentage change in volume or cortical thickness per month) in region *x* for a white woman aged 80 years, with 15.6 years of education, an MMSE score of 27.13, and the ε3/ε3 *APOE* genotype. The coefficient $\beta_{g4i}(x)$ denotes the differential level of atrophy in a subject with the *APOE* ε4 alleles, respectively.

* Control regions not expected to be significantly affected by *APOE*.

† Indicates highly significant values that will survive a Bonferroni correction across regions.

There were no significant interactions between *APOE* and any other variables for any region (see Appendix E1 [online] for more detailed results).

Discussion

Our study of subjects with MCI demonstrated that *APOE* ε4 carriers exhibited markedly greater 1–4-year atrophy rates than ε3/ε3 homozygotes in 13 of 15 AD-related brain regions. The fact that the largest effects were seen in regions previously demonstrated to display the greatest atrophy in AD—entorhinal cortex, amygdala, and hippocampus—is consistent with what is known about this allele as a major risk factor for nonfamilial AD (29,30). Additionally, although *APOE* ε2 did show a trend for a protective effect against atrophy in the transverse temporal cortex, this result does not survive correction for multiple testing. No other region exhibited an effect of ε2 carrier status on atrophy rate, with or without Bonferroni correction. These results may be applied uniformly across the population being studied, given the lack of interaction between *APOE* and any other population characteristic.

Findings of previous studies have suggested that progression of AD can be correlated with specific patterns of atrophy measured at MR imaging in an interrelated brain network of cortical and subcortical limbic areas with memory functions (32–35). Further, this regional atrophy has been shown to map fairly closely to the typically observed brain distribution of neuropathologic tau and β-amyloid deposits seen in AD; and, what is more, the degree of atrophy in these regions correlates with neuropsychological symptom burden in affected patients (36–38). Because of these observed relationships between imaging and both clinical and pathologic disease, MR imaging is already being used in MCI clinical trials and being studied for utility in the clinical setting. For example, hippocampal volumetry measured with MR imaging is already routinely included as an outcome measure of drug effectiveness in clinical trials of potential disease-modifying agents (39). Given that this and other MR imaging–based volumetrics will likely become increasingly used in this capacity, knowledge of gene-specific effects on structural brain changes would enhance the research and eventual clinical value. Thus, these results are meaningful in that they further inform our current understanding of how *APOE*—thus far the most important genetic factor known in nonfamilial AD—independently influences structural brain changes in subjects at risk for progression to AD. Identification and examination of such confounding effectors, which, as the current study demonstrates, can introduce large variance in MR imaging–derived quantitative brain measures, will be crucial in building statistical models that can accurately filter out the background "noise" inherent in attempting to measure such subtle changes in structure.

Among previous studies of *APOE* ε4, while there are dozens of reports of cross-sectional differences in brain volumes and cortical thicknesses measured at MR imaging (40–43), only a handful of investigators have examined ε4 effects on longitudinal atrophy rates in MCI (12,44–47). Our findings confirm and extend the findings of these few prior longitudinal studies on *APOE* ε4: Van de Pol and colleagues examined 2-year MR imaging data in 323 subjects with MCI and showed that ε4 was an independent predictor of increased rates of hippocampal atrophy (44); Morra et al explored hippocampal volumes measured at MR imaging in 245 subjects

Figure 1

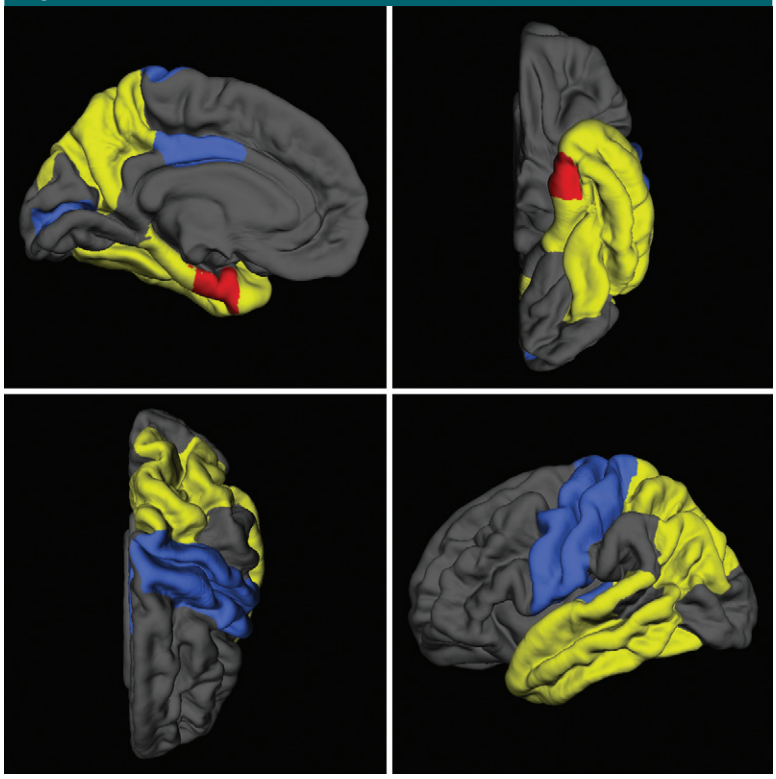

Figure 1: Medial, inferior, superior, and lateral images of a three-dimensional FreeSurfer reference brain model show regions of accelerated atrophy in the presence of *APOE* ε4 in subjects with MCI. Effect size of the *APOE* ε4 acceleration of cortical atrophy is depicted by color: Blue signifies a magnitude of Cohen *d* value of less than 0.25; yellow, 0.25–0.35; and red, more than 0.35. Gray areas were not examined.

with MCI over 1 year and found that ε4 carriers exhibited accelerated left hippocampal atrophy rates compared with ε3/ε3 subjects (45). Spampinato et al studied MR imaging in 95 subjects with MCI over 2 years and found that ε4 was associated with accelerated atrophy of several gray matter regions, including the temporoparietal cortex and bilateral hippocampi (46). Tosun and colleagues found that ε4 was associated with significantly higher rates of cortical thinning in the temporoparietal and entorhinal cortex, precuneus, and temporal pole of subjects with MCI, as measured at serial MR imaging performed over a 2-year period (47). Risacher et al found that ε4+ subjects with MCI showed higher 1-year atrophy rates than noncarriers in the hippocampus and entorhinal cortex (48). Our results are also consistent with prior studies, such as that of Moffat et al (49), in cognitively normal subjects. However, these findings and our results stand in contrast to those of Schuff et al, who found that only ε4+ subjects with AD—not MCI—exhibited accelerated atrophy of the hippocampus over a 12-month period (12). The strengths of our study in comparison to these other studies include our large population of subjects with MCI, who were recruited from more than 50 sites across the country; the heavily vetted MR imaging protocols and precise, rater-free algorithms used to derive volumetrics; the longitudinal nature of the data, with up to 48 months of follow-up; and, finally, the wide net we attempted to cast with regard to brain regions studied.

Compared with *APOE* ε4, even fewer previous studies have been conducted to examine any structural effects of *APOE* ε2 on the brain. We hypothesized a protective effect of ε2 on this brain memory network, given what is known epidemiologically about the allele (50). However, our analyses did not demonstrate any significant morphometric effect. In point of fact—with the exception of one cross-sectional study in which only the entorhinal cortex was examined in adolescents (51) and another longitudinal study of hippocampal atrophy rates in cognitively normal elderly subjects (13)—there have been no studies to our knowledge that have demonstrated a significant protective morphometric effect of *APOE* ε2.

The current study has some limitations. Though the ADNI database allows for examination of large study populations, this particular cohort has previously been shown to have a higher proportion of white subjects and to be more educated than community-based samples (16). Also, the presence of only nine *APOE* ε2 carriers is less than ideal for study when attempting to elucidate an effect of this allele. Hence, our findings with regard to the ε2 allele are insufficient to draw any firm conclusions and should be viewed cautiously. Further, this study was focused solely on genotype effects on atrophy rates measured at MR imaging in subjects with MCI; we did not examine any potential interactions with rate of conversion from MCI to AD or with other biomarkers (eg, cerebrospinal fluid–amyloid or tau) changes, nor did we investigate these effects in cognitively normal subjects or subjects with AD, which limits generalization of our findings to undifferentiated community samples. Despite these limitations, to our knowledge, this is the largest and longest study in which the opposing effects of the *APOE* ε4 and ε2 alleles on the atrophy rates of multiple brain regions were examined in subjects at risk for AD.

Future directions should include reexamination of this issue with even more general, whole-brain techniques, with a study population possessing a larger concentration of the ε2 and ε4 alleles. If an eventual goal of using automated and objective MR imaging–derived

Figure 2

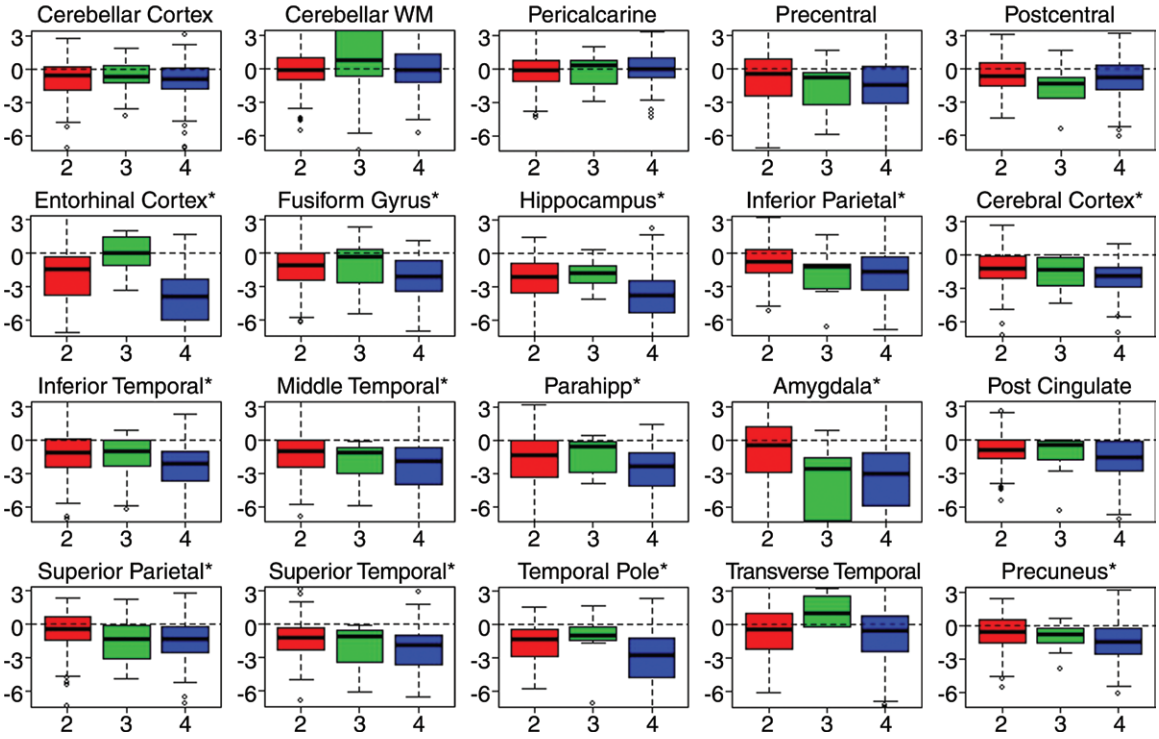

Figure 2: Whisker box plots of regionwise atrophy rates according to *APOE* genotype in subjects with MCI. *Top row:* Control regions. *Bottom three rows:* Experimental regions. For the x-axis, "2" denotes $\varepsilon 2$ carrier, "3" denotes $\varepsilon 3/\varepsilon 3$ homozygote, and "4" denotes $\varepsilon 4$ carrier. The y-axis values represent log volume per month: A value of 1.00 (approximately) represents a 1% increase in volume annually. Negative values indicate atrophy. ∗ = regions that showed significantly higher atrophy rates in $\varepsilon 4$ carriers than in $\varepsilon 3/\varepsilon 3$ homozygotes. *WM* = white matter.

measures of brain atrophy in the clinical setting is to be realized, it will be instructive to catalog as precisely as possible which genetic and lifestyle factors have an influence on the observed variance of such measures. Such knowledge will also be integral to expanding the role of MR imaging measures in clinical trials on the investigation of novel drugs with potential disease-modifying capabilities.

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