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Review

Presence of ApoE ε4 Allele Associated with Thinner Frontal Cortex in Middle Age

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Abstract. The presence of an ApoE ε4 allele (ε4+) increases the risk of developing Alzheimer's disease (AD). Previous studies support an adverse relationship between ε4+ status and brain structure and function in mild cognitive impairment and AD; in contrast, the presence of an ε2 allele may be protective. Whether these findings reflect disease-related effects or pre-existing endophenotypes, however, remains unclear. The present study examined the influence of ApoE allele status on brain structure solely during middle-age in a large, national sample. Participants were 482 men, ages 51–59, from the Vietnam Era Twin Study of Aging (VETSA). T1-weighted images were used in volumetric segmentation and cortical surface reconstruction methods to measure regional volume and thickness. Primary linear mixed effects models predicted structural measures with ApoE status (ε3/3, ε2/3, ε3/4) and control variables for effects of site, non-independence of twin data, age, and average cranial vault or cortical thickness. Relative to the ε3/3 group, the ε3/4 group demonstrated significantly thinner cortex in superior frontal and left rostral and right caudal midfrontal regions; there were no significant effects of ε4 status on any temporal lobe measures.

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The ε2/3 group demonstrated significantly thicker right parahippocampal cortex relative to the ε3/3 group. The ApoE ε4 allele may influence cortical thickness in frontal areas, which are later developing regions thought to be more susceptible to the natural aging process. Previous conflicting findings for mesial temporal regions may be driven by the inclusion of older individuals, who may evidence preclinical manifestations of disease, and by unexamined moderators of ε4-related effects. The presence of the ε2 allele was related to thicker cortex, supporting a protective role. Ongoing follow-up of the VETSA sample may shed light on the potential for age- and disease-related mediation of the influence of ApoE allele status.

Keywords: Magnetic resonance imaging, cerebral cortex, brain, frontal lobe, apolipoproteins E, apolipoprotein E2, apolipoprotein E3, apolipoprotein E4, genetic association studies, aging

INTRODUCTION

The ApoE ε4 allele is studied within imaging genetics as the most common polymorphism associated with late-onset Alzheimer's disease (AD) [1–4]. ApoE is thought to play a role in lipoprotein transport and cell maintenance and repair, including amyloid clearance, and is bound to senile plaques and neurofibrillary tangles [5–7]. Of the three alleles (ε2, ε3, ε4), the ε3/3 pairing is the most common phenotype in the U.S. population (~60%), while the presence of ε2 and ε4 alleles is less frequent [8]. There is an increased prevalence of the ε4 allele in disease populations relative to healthy controls [1, 4, 9–12], and individuals carrying at least one ε4 allele (ε4+) are at an increased risk for developing AD [13–15]. In contrast, the presence of an ε2 allele may impart protection from AD-related neurodegeneration [8, 15–21].

In combination with the risk conferred by ApoE allele status, neuroimaging biomarkers may improve the identification of individuals at risk for AD and the potential for successful intervention in the earliest stages. Studies in AD and mild cognitive impairment (MCI) often demonstrate more significant mesial temporal lobe (MTL) atrophy in ε4+ individuals relative to non-carriers [22–29]. In a positron emission tomography (PET) study using a marker of amyloid and tau proteins (FDDNP), ε4+ MCI demonstrated abnormally high binding in the MTL [30]. Neuropathological studies of ε4 carriers support earlier and greater amyloid deposition in AD, as well as in MCI and in older healthy individuals [20]. Further evidence of an earlier and faster rate of cognitive decline also has been demonstrated in MCI and AD ε4+ individuals [15]. These and other studies support strong disease-related effects within ε4+ MCI and AD individuals.

Studying individuals earlier in life, prior to the development of MCI or AD, is critical to understanding the influence of ApoE allele status. PET studies have shown glucose metabolism reductions in ε4+ late-middle-aged individuals with a positive family history for AD [31, 32] and an accelerated rate of decline in regional cerebral blood flow for ε4 carriers [33]. The affected areas overlap with AD-related regions supporting the potential for a pre-symptomatic endophenotype. Of particular interest, however, a recent PET FDDNP study [30] found higher amyloid and tau binding in frontal areas for ε4+ relative to ε4- healthy individuals, in contrast to an increased temporal lobe binding in the ε4+ MCI group [30]. Structural neuroimaging studies also have been somewhat inconsistent, with reports of smaller MTL structures, including the hippocampus [34–36] and entorhinal cortex [37, 38], in ε4+ carriers, alongside other reports of no significant ε4-related effect in these or other areas [24, 37, 39]. Studies beyond the MTL that have included younger-old and middle-aged individuals are varied, reporting thickening of small cortical areas [40], thinning in medial orbitofrontal areas [24], and lower gray matter density in small anterior frontal and temporal regions [36]. Several reports, however, have suggested that such effects may be driven by older individuals in the samples, rather than reflecting an early ε4-related endophenotype [35, 41].

Fewer studies have examined the potential protective influence of the ε2 allele, particularly in healthy individuals, in part due to the lower prevalence of this allele in the U.S. population. Previous work has provided neuropathological evidence for less cortical amyloid and fewer plaques and neurofibrillary tangles in ε2 carriers (ε2+) [16–18, but see 21]. In addition, ε2 carriers may evidence a reduced rate of cognitive decline [8, 15, 19–21] and fewer are diagnosed with AD [8]. Neuroimaging corroboration for such a protective effect is rarer. A recent study of older individuals reported larger cortical gray matter volume and smaller ventricles in MCI and AD but found no significant effect related to the ε2 allele in healthy older individuals; the sample sizes for ε2 carriers, however, were quite small across all groups studied [42]. A study of adolescents suggested a tendency for thicker mesial temporal and medial orbitofrontal cortex in a larger ε2+ group [38]. An investigation of the ε2 allele in a large community sample may provide complementary insight into the potentially opposing influences of ApoE ε4 and ε2 alleles.

The present study examined the influence of ApoE allele status on brain structure solely during middle age in a national sample from the Vietnam Era Twin Study of Aging (VETSA). This cohort captures individuals in their 6^{th} decade of life likely prior to the onset of AD or other age-related complications [43]. We examined *a priori* AD-related regions of interest (ROIs) as well as regions expected to be influenced by normal aging, which tend to follow an anterior–posterior gradient, exhibiting the greatest rates of decline in frontal areas [44, 45]. Relative to ε3/3 carriers, we expected the ε3/4 group to show the smallest and thinnest MTL areas, most affected in AD, and we also proposed that this group would demonstrate thinner frontal cortex, associated with normal aging. In contrast, the ε2/3 group may evidence larger, thicker MTL areas, supporting a potential protective effect. Continuous surface maps were also generated to explore the extent of effects without the constraints of predefined boundaries.

MATERIALS AND METHODS

Participants

Data were obtained in the first wave of VETSA, a longitudinal study of cognitive and brain aging beginning in midlife [46]. Participants were randomly sampled from over 3,300 Vietnam Era Twin (VET) Registry twin pairs with the constraint that they were in their 50s at the time of recruitment into VETSA. The VET Registry is a nationally distributed sample of male-male twin pairs who served in the U.S. military sometime between 1965 and 1975; descriptions of the composition and method of ascertainment have been reported elsewhere [47]. Importantly, these are Vietnam era, not necessarily Vietnam, veterans; the large majority did not serve in combat. In comparison to census data, VETSA participants are similar in demographic and health characteristics to American men in their age range [48]. Aside from standard exclusion criterion for MRI studies (e.g., metal in the body), there were no additional eligibility requirements for selection into the MRI component.

Participants traveled either to Boston University or the University of California, San Diego (UCSD) for a series of physical, psychosocial, and neurocognitive assessments. Informed consent was obtained from all participants prior to data collection, and the scanning protocol was approved of by the Institutional Review Boards at UCSD, Boston University, and the Massachusetts General Hospital (MGH).

A subset of the 1237 VETSA participants underwent structural MRI, and the present non-twin analyses include data from 482 participants for whom neuroimaging data and APOE genotyping were adequate and available. The dataset included 205 twin pairs (119 monozygotic and 86 dizygotic twin pairs) and 72 unpaired individuals with an average age of 55.7 years (sd = 2.6; range 51–59). Participants in this MRI study were similar to the larger VETSA sample with respect to education (mean=13.8; sd=2.1), ethnicity (85.7% Caucasian), employment (75% employed full-time), and self-reported health status.

ApoE genotype was determined from blood samples using established methods [49, 50]. All genotypes were independently determined twice by laboratory personnel at the VA Puget Sound Healthcare System who were blind to the initial genotype and the identity of the co-twin. Of the 482 participants, 2 (0.4%) possessed a ε2/2 genotype, 67 (13.9%) ε2/3, 18 (3.7%) ε2/4, 288 (59.8%) ε3/3, 94 (19.5%) ε3/4, and 13 (2.7%) ε4/4 (Table 1). These rates are roughly equivalent to those found in the general population [14, 51]. Because the proportion of individuals with ε2/2, ε2/4, and ε4/4 parings were small, these cases were not included in the primary models, however, a secondary overall analyses comparing ε4+ and non-ε4 carriers was completed using all available data.

Participants studied for the primary model were classified as ε2/3, ε3/3, or ε3/4 (see Table 1). These groups did not differ on age (F=1.4, p>.05). General cognitive ability was assessed by the Armed Forces Qualification Test (AFQT), a well-validated test that also was given to VETSA participants in early adulthood [52]. The mean for the entire sample was 63.1 (sd=20.8); this AFQT score is slightly above the mean and would be comparable to an average IQ of approximately 105. The mean across the three primary groups was 63.2 (sd=20.6) and the means did not differ between these groups (Table 1; $F < 1.0$, $p > .05$).

Table 1

Participant characteristics. For all ApoE allele pairings, sample size, mean age in years, and mean Armed Forces Qualifications Test (AFQT) score are provided, along with the standard deviation (sd) and *range*. The primary model considered the first three groups (ε2/3, ε3/3, ε3/4) with sufficient power to examine influence on brain structure

	ε2/3	ε3/3	ε3/4	ε2/2	ε2/4	ε4/4
n	67	288	94	2	18	13
age	56.2 (2.5)	55.7 (2.6)	55.5 (2.8)	55.0 (4.2)	55.1 (2.5)	56.2 (2.6)
	52–59	*51–59*	*51–59*	*52–58*	*52–58*	*51–58*
AFQT (%)	63.2 (22.7)	62.8 (19.8)	64.4 (21.5)	71.5 (27.6)	55.0 (24.4)	70.9 (18.9)
	15–97	*4–95*	*15–98*	*52–91*	*14–94*	*28–89*

MR Image Acquisition

As described previously [53], images were acquired on 1.5 Tesla scanners (255 at UCSD; 227 at MGH). Sagittal T1-weighted MPRAGE sequences were employed with TI=1000ms, TE=3.31ms, TR=2730ms, flip angle=7degrees, slice thickness = 1.33 mm, voxel size 1.3 × 1.0 × 1.3 mm. Raw DICOM MRI scans (two T1 volumes per case) were transferred to MGH for image processing. These raw data were reviewed for quality, registered, and averaged to improve signal-to-noise.

Image processing

As described elsewhere [53], we employed volumetric segmentation [54] and cortical surface reconstruction [55–57] methods based on the publicly available FreeSurfer software package (http://surfer.nmr.mgh.harvard.edu/fswiki; Version 3.0.1b). The 3D whole-brain segmentation procedure [54] uses a probabilistic atlas and applies a Bayesian classification rule to assign a neuroanatomical label to each voxel. The atlas consists of a manually-derived training set created by the Center for Morphometric Analysis (http://www.cma.mgh.harvard.edu/) from 20 unrelated, randomly selected VETSA participants. Use of this study-specific atlas produced more accurate measurements than more commonly used atlases [53]. Estimated total cranial vault (eTIV) volume was calculated to control for differences in head size for volumetric measures. Based on Buckner et al. [58], FreeSurfer provides an eTIV volume derived from the atlas scaling factor on the basis of the transformation of the full brain mask into atlas space. Although this estimate is not a direct volume, this eTIV measure has been shown to correlate well with other cranial vault volumes incorporating T2-weighted information, including manual tracings in controls and individuals with Alzheimer's Disease (r=0.93) [58] and multi-channel tissue segmentations [as in 44] in older controls and individuals with Alzheimer's disease (r=0.87) [59]. The primary volumetric ROI was the hippocampus; exploratory ROIs included amygdala, caudate nucleus, putamen, nucleus accumbens, and thalamus.

The cortical surface was reconstructed to measure thickness at each surface location, or vertex [described in 53, 55, 56]. The explicit reconstruction of the cortical surface requires inhomogeneity corrections, creation of a normalized intensity image, and removal of non-brain. The resulting surface is covered with a polygonal tessellation and smoothed to reduce metric distortions. The gray/white boundary surface is deformed outwards to obtain a representation of the pial surface; the surface model is manually reviewed and edited for technical accuracy in alignment with standard, objective editing rules. Each individual surface is non-rigidly aligned to an atlas in a spherical surface-based coordinate system and divided into distinct ROIs [57], with each vertex assigned a neuroanatomical label [60], to estimate average thickness in each ROI. Primary cortical thickness ROIs included mesial temporal (entorhinal, parahippocampal); lateral temporal (inferior, middle, and superior temporal); and frontal (caudal and rostral middle; superior; inferior; orbitofrontal) cortex (Fig. 1). Exploratory ROIs included superior and inferior parietal, supramarginal, lingual, fusiform, cingulate, and precuneus cortex. Cortical thickness was also estimated over continuous maps on the surface with no predefined regional boundaries as described in Statistical Analysis; smoothing of volumes was done prior to the vertex-wise analyses using a 30 mm FWHM Gaussian kernel.

Statistical analysis

Although the study participants were twins, all analyses in this article are non-twin analyses. Derived ROI values (thickness in mm or volume in mm^3) were submitted to linear mixed effects models with fixed effects of site, ApoE allele status (ε2/3 and ε3/4 were compared to ε3/3), and age. Importantly, site was included

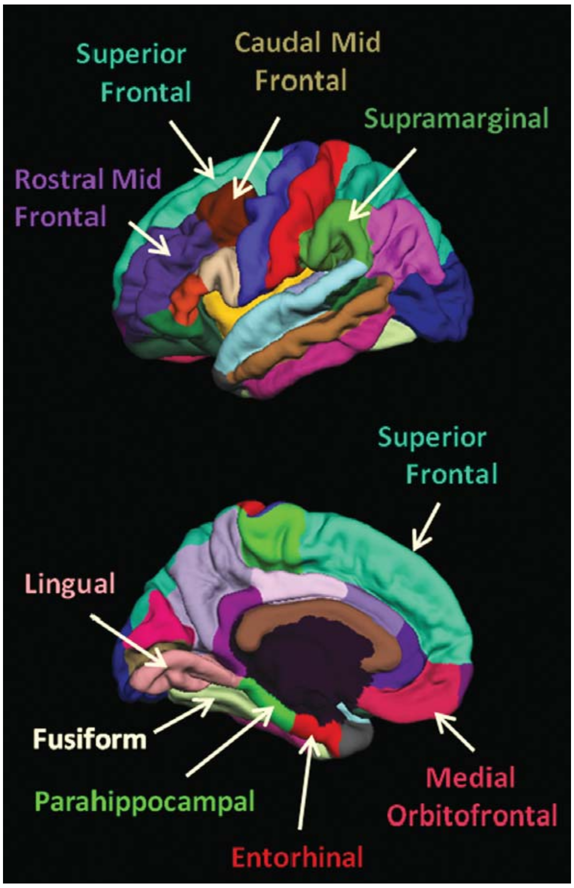

Fig. 1. Cortical region of interest parcellation (30). Primary ROIs include superior frontal (*teal*), rostral (*purple*) and caudal (*brown*) mid frontal, parahippocampal (*green*), entorhinal (*red*), and medial orbitofrontal (*rose*) cortex. Additional exploratory ROIs include fusiform (*yellow*), supramarginal (*olive green*), and lingual (*pink*) gyrus. *Top row:* lateral views; *bottom row:* medial views.

in the model to control for effects related to differences in scanner hardware, known to differentially influence morphometric measures of volume and thickness [e.g., 59, 61–63]. Because twins within pairs are not independent observations, it is necessary to adjust for this non-independence when performing non-twin analyses in a twin sample. Therefore, the "family ID" of each member of a twin pair was entered as a random effect in the model. Doing so adjusts the degrees of freedom and makes it more difficult to attain statistical significance. Finally, to adjust for individual differences in overall head size or thickness of the cortical ribbon, an additional fixed effect was included in each model: eTIV for volumetric measures and average cortical thickness for thickness measures.

Planned comparisons included ROIs implicated in AD: hippocampus, entorhinal cortex, parahippocampal cortex, and lateral temporal gyri; and regions susceptible to the effects of normal aging: superior frontal gyrus, middle frontal gyrus (rostral and caudal), inferior frontal (pars opercularis, pars orbitalis and pars triangularis), and orbitofrontal cortex (medial and lateral). Planned comparisons were limited to these predefined ROIs driven by prior work, and we employed an alpha level of 0.05. Effect sizes were calculated by ROI using Cohen's *d* and were based on estimated marginal means resulting from the full model. In general, a Cohen's *d* of 0.2–0.3 is considered a small, 0.5 a medium, and 0.8 a large effect size.

In a secondary analysis, the same model was modified to utilize the entire cohort of 482 participants to compare ε4+ (n=125) and non-ε4 (n=357) carriers, as has been done in some previous studies. That is, the variables for ApoE allele status were replaced by ApoE ε4 allele status. Given the small sample of homozygous ε4/4 genotype, a dose effect of ε4 (0, 1, or 2 alleles) was not examined due to insufficient power.

To further explore the statistical findings based on our *a priori* ROI analyses, the same model was implemented at each vertex, or point on the cortical surface, resulting in a continuous surface map of cortical thickness without the predefined constraints of ROI boundaries. The resulting map is exploratory in nature and provides guidance for future studies.

RESULTS

There was no significant effect of ApoE allele status on eTIV or average cortical thickness (all $t \leq 1.0$, $p > 0.05$). Relative to the ε3/3 group, the ε3/4 group demonstrated significantly thinner cortex in bilateral superior frontal, left rostral midfrontal, and right caudal midfrontal regions (Table 2; Fig. 2). Although the right rostral midfrontal and left caudal midfrontal ROIs tended to be thinner, these effects were not significant (Table 2). No temporal areas or any other frontal regions were significantly related to ε4 status. Analysis of the entorhinal cortex did not reveal any significant influence of ε4 status, although the variability of thickness in this area was larger than in other ROIs (Table 2). Exploratory cortical analyses suggested thicker fusiform cortex in the ε3/4 group (Table 2, bottom). Primary volumetric analyses did not reveal any significant effect of ε4 status on the hippocampus (Table 2). Exploratory volumetric analyses

Table 2
Effect of ApoE allele status by Regions of Interest. Controlling for other variables in the model, the estimated marginal mean (in mm for cortical thickness and in mm^3 for volumetric ROIs) and standard deviation (sd) are reported by ROI for each primary ApoE allele group. Based on results of the full statistical model, within which the Ɛ3/4 and Ɛ2/3 groups were compared to the Ɛ3/3 group, the resultant *t*-value, level of significance, and the associated effect size (Cohen's *d*) are reported. Negative *t*-values reflect an effect thinner or smaller than the Ɛ3/3 group; positive t-values reflect thicker or larger effects relative to the Ɛ3/3 group

Region of Interest	*Hemi-sphere*	Ɛ3/3 mean (sd)	Ɛ3/4 mean (sd)	*t* value	*d*	Ɛ2/3 mean (sd)	*t* value	*d*
Superior Frontal Gyrus	*right*	2.204 (0.080)	2.179 (0.087)	–2.80**	–0.30	2.202 (0.085)	$<1^{ns}$	–0.02
	left	2.195 (0.084)	2.173 (0.091)	–2.42*	–0.26	2.189 (0.089)	$<1^{ns}$	–0.07
Rostral Mid Frontal Gyrus	*right*	1.819 (0.080)	1.803 (0.090)	$–1.73^{ns}$	–0.19	1.820 (0.088)	$<1^{ns}$	0.01
	left	1.851 (0.075)	1.833 (0.085)	–1.95*	–0.21	1.859 (0.083)	$<1^{ns}$	0.11
Caudal Mid Frontal Gyrus	*right*	2.052 (0.121)	2.018 (0.133)	–2.49*	–0.27	2.023 (0.129)	$–1.80^{ns}$	–0.23
	left	2.042 (0.112)	2.028 (0.126)	$–1.12^{ns}$	–0.12	2.029 (0.122)	$<1^{ns}$	–0.11
Entorhinal Cortex	*right*	2.800 (0.385)	2.883 (0.426)	$+1.89^{ns}$	0.20	2.816 (0.415)	$<1^{ns}$	0.04
	left	2.553 (0.336)	2.574 (0.378)	$<1^{ns}$	–0.06	2.628 (0.367)	$+1.68^{ns}$	0.21
Hippocampal Volume	*right*	4216 (444)	4168 (479)	$<1^{ns}$	–0.11	4289 (466)	$+1.28^{ns}$	0.16
	left	3988 (399)	3954 (430)	$<1^{ns}$	–0.08	4067 (419)	$+1.54^{ns}$	0.19
Parahippocampal Gyrus	*right*	1.901 (0.242)	1.922 (0.266)	$<1^{ns}$	–0.09	1.967 (0.259)	+2.10*	0.27
	left	1.900 (0.260)	1.894 (0.288)	$<1^{ns}$	–0.02	1.916 (0.280)	$<1^{ns}$	0.06
Medial Orbitofrontal	*right*	1.847 (0.159)	1.838 (0.175)	$<1^{ns}$	–0.06	1.851 (0.170)	$<1^{ns}$	0.02
	left	1.849 (0.156)	1.838 (0.175)	$<1^{ns}$	–0.06	1.882 (0.170)	$+1.59^{ns}$	0.20
Fusiform Gyrus	*right*	2.011 (0.101)	2.035 (0.114)	+2.04*	0.22	2.000 (0.110)	$<1^{ns}$	–0.11
	left	1.975 (0.106)	2.000 (0.119)	$+1.88^{ns}$	0.20	1.968 (0.115)	$<1^{ns}$	–0.06
Putamen Volume	*right*	5002 (558)	4846 (582)	–2.60*	–0.27	5010 (567)	$<1^{ns}$	0.01
	left	4942 (582)	4788 (598)	–2.50*	–0.26	4868 (582)	$–1.04^{ns}$	–0.13
Supramarginal Gyrus	*right*	2.085 (0.110)	2.089 (0.124)	$<1^{ns}$	0.04	2.054 (0.120)	–2.08*	–0.26
	left	2.071 (0.099)	2.076 (0.112)	$<1^{ns}$	0.05	2.071 (0.109)	$<1^{ns}$	0.00
Lingual Gyrus	*right*	1.703 (0.093)	1.715 (0.104)	$+1.12^{ns}$	0.12	1.700 (0.101)	$<1^{ns}$	–0.03
	left	1.654 (0.095)	1.655 (0.105)	$<1^{ns}$	0.01	1.630 (0.102)	–1.97*	–0.25

**$p < 0.01$, *$p < 0.05$, ns = $p > 0.05$

suggested a significantly smaller putamen volume in ε4 carriers (Table 2, bottom).

In the secondary ROI analyses utilizing the entire 482 datasets, a comparison of all ε4+ with all non-ε4 carriers provided similar results. Given the significant pattern of effects in the ROI analyses, we reviewed the influence of ε4 allele status on the continuous cortical surface map to explore effects without the predefined constraints of ROI boundaries. Continuous maps of the cortical surface supported a broad distribution of thinner lateral and mesial superior frontal, and thicker fusiform cortex in the ε4+ relative to the non-ε4 group (Fig. 3). In the context of Fig. 1, Fig. 3 shows that some regional effects (e.g., left middle frontal area) fall across the confines of predefined ROIs. The thinnest areas (in orange/yellow) may lie across the intersection of a number of ROIs, to include the more lateral, inferior extent of the caudal midfrontal gyrus, posterior rostral midfrontal gyrus, posterior inferior frontal gyrus, and inferior portions of the pre-central gyrus. With respect to the unexpectedly thicker regions in the ε4+ group suggested by exploratory ROI analyses, posterior regions may be more broadly affected. This map provides guidance for future studies.

Relative to the ε3/3 group, our investigation of the ε2/3 group demonstrated significantly thicker right parahippocampal cortex (Table 2; Fig. 2) and non-significant tendencies towards thicker left entorhinal cortex, left medial orbitofrontal cortex, and hippocampal volumes (Table 2). Exploratory analyses suggested significantly thinner right supramarginal and left lingual gyri (Table 2, bottom).

DISCUSSION

This study of a large, community-dwelling sample provides a comprehensive view of the influence of ApoE allele status on brain structure in men. Few previous studies have captured such a broad description particularly within a solely middle-aged sample. The findings suggest that carriers of the ε4 allele on average have thinner frontal cortices in middle age, without direct evidence of any significant ε4 effect on MTL regions commonly affected in AD. These frontal effects were widespread, although the effect sizes were small, suggesting that studies with smaller sample sizes may not have sufficient power to reliably detect such

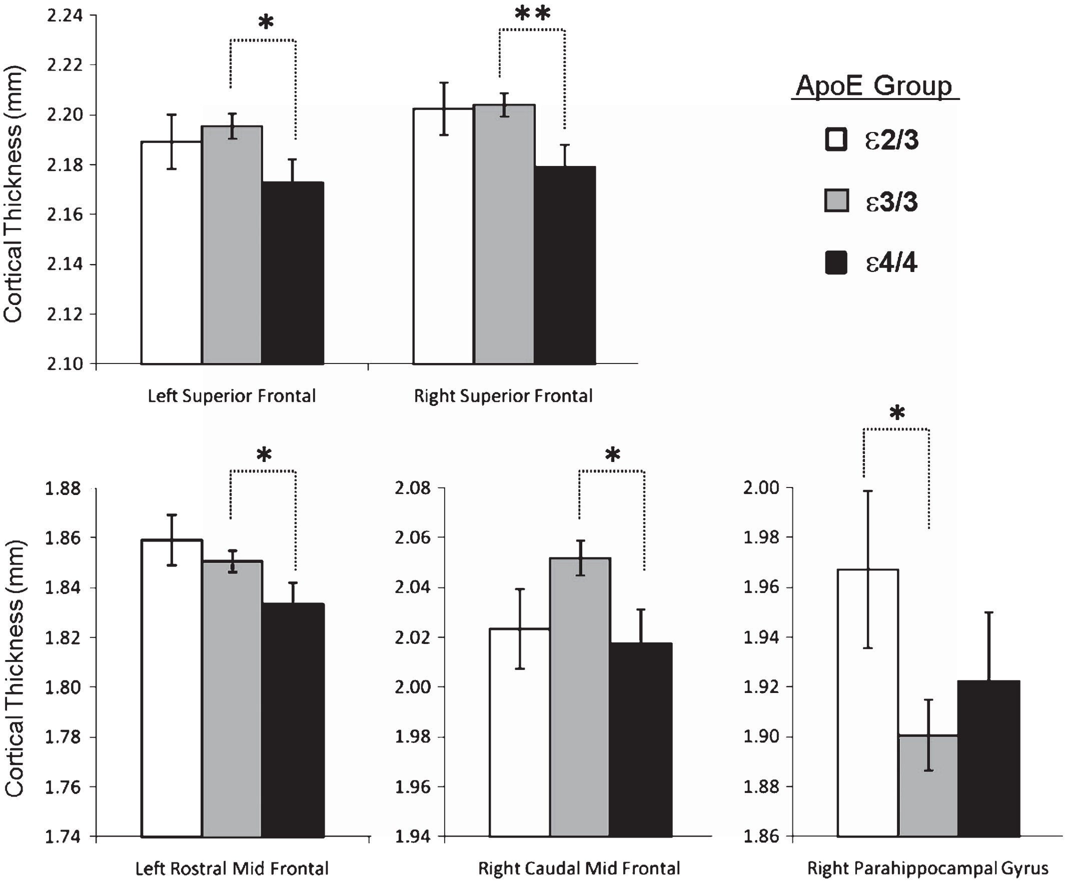

Fig. 2. Cortical thickness in ROIs with significant effects of ApoE allele status. Estimated marginal means (with standard error bars), controlling for all variables in the full model, are shown for cortical thickness (mm) in frontal and temporal ROIs that demonstrated a significant effect of ApoE allele status. Significance levels are reported in Table 2 and denoted in the graph with ** for p<.01 and * for p<.05.

effects. Exploratory analyses also suggested thicker fusiform cortex in ε4+ carriers, in line with a previous study [40]. Potential protective effects of the ε2/3 genotype were supported in part by thicker left parahippocampal cortex and broader MTL and medial orbitofrontal tendencies towards thicker cortex, relative to the ε3/3 group.

Our findings of ε4-related differences in superior and middle frontal cortical thickness are relatively unique and of interest in the context of normal aging. One cross-sectional study including middle-aged and older individuals suggested accelerated age-related thinning in ε4 carriers in the superior medial frontal gyrus; however, the majority of the participants were over the age of 60, limiting the inference of effects in middle age [40]. Within Shaw et al.'s study of children and adolescents [38], there were potential ε4 status effects in frontal regions, with continuous maps showing small areas of thinner orbitofrontal cortex in the ε4+ group. While the present study does not show significant orbitofrontal ROI effects, the continuous surface maps (Fig. 3) further explore patterns without the predefined constraints of ROI boundaries, which may be relatively arbitrary with respect to the underlying cellular, functional, or developmental aspects of the brain. The maps support widespread frontal effects, and the potential influence on frontal cortex development into the adult age range may inform these regional differences.

The ε4-related effects on frontal cortical thickness are bolstered by findings from other modalities and disorders. Amyloid and tau binding PET studies in healthy individuals suggest that binding is higher for ε4+ carriers in frontal areas, as opposed to commonly reported increased temporal lobe binding in ε4+ MCI individuals [30]. In addition, ε4 status may influence dendritic density and complexity in the cortex [64], and may differentially influence cortical patterns of

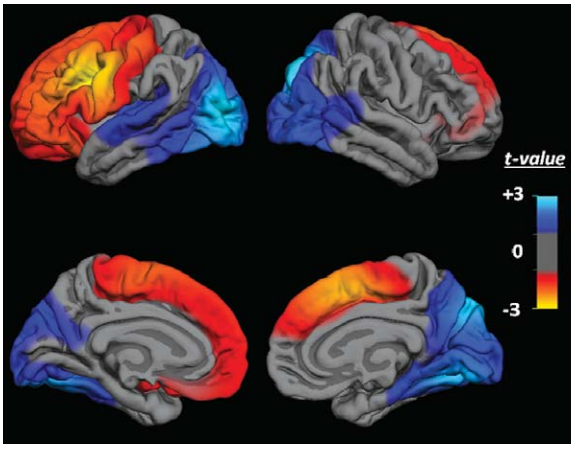

Fig. 3. Continuous surface maps of the estimated ApoE allele status *effect on cortical thickness*. Using the entire available sample (n=482), the *t*-statistic for the effect of carrying the ε4 allele, from the full statistical model, was applied vertex-wise on the pial surface. The color scale denotes effects for the ε4+ relative to the non-ε4 group as follows: thinner cortex in orange/yellow areas (larger negative *t*-values) and thicker cortex in areas with bright blue (cyan) (larger positive *t*-values). Both left (*left column*) and right (*right column*) hemispheres are presented.

thinning based on mediating factors. In a study of AD and frontotemporal dementia, cortical atrophy was greater in both ε4+ subgroups; however, the pattern of thinning in AD represented known neuropathological areas such as the mesial temporal lobe, whereas in frontotemporal dementia, the ε4+ group evidenced greater frontal atrophy [65]. The broad, frontal findings support the relationship between the ε4 allele and increased amyloid deposition in these areas with normal aging, although any progressive nature of such effects must be demonstrated in a longitudinal study, currently underway.

The lack of significant MTL ε4 related effects is not unexpected given conflicting previous reports and may reflect studies including a low proportion of individuals in a preclinical phase of AD and, importantly, other mediating influences on the impact of ε4 status, such as gender and hormones. While substantial support exists for ε4-related MTL effects in MCI and AD, findings in healthy individuals are inconsistent, even in older adults [23, 37, 39, 40, 66]. There is some evidence suggesting the influence of ε4 status on MTL structures in middle age [34] and in children and adolescents [38]; however, other studies including middle-aged individuals have not found the same effects [35, 40] or have found that effects across a broad age range were driven by individuals over 60 or 65 years of age [35]. Some of these older individuals may demonstrate poor cognitive performance relative to their non-ε4 counterparts and some may be in the prodromal stages of AD. Indeed, a recent study of cognition suggests that family history of AD and ε4 status may be additive factors, and that, with the removal of individuals known to convert to AD, only individuals with both a positive family history of AD and ε4+ status demonstrate a more rapid cognitive decline [67]. The present sample represents individuals in their 6th decade of life, when few are likely to be affected by dementia, although we do not have data on family history at this time. In contrast, the unique study of children and adolescents (n=174 non-ε4, n=65 ε4+; 8–21 years) provides support for the thinner left entorhinal cortex for ε4+ individuals [38], although these effects were subtle and the variability in thickness was slightly larger within the ε4+ relative to the non-ε4 group, similar to the present study. These findings together support the hypothesis that additional factors likely mediate the influence of ε4 status on brain structure.

Other studies have demonstrated differences in ε4-related effects by gender and report potential mediating or moderating factors such as hormones. There may be an interaction between gender and ApoE ε4 status [68] such that, in general, females are more influenced by ε4 status than males. In MCI, female ε4+ carriers have a higher risk of developing AD than men of the same genotype [14]. A neuroimaging study reported that female, but not male, ε4+ carriers had significantly smaller hippocampal volumes relative to non-ε4 individuals; the authors suggested the potential for hormonal mediation of the influence of ε4 status [69]. It is possible, then, that in the present male sample, ε4-related MTL effects may be reduced and/or obscured by other factors. In fact, a study of VETSA participants revealed a significant interaction between testosterone and ε4 status indicating that ε4+ men who also had low levels of testosterone have smaller hippocampal volumes [70]. A similar interaction between ε4 status and cortisol levels or patterns also has been observed with respect to cognition in older adults [71].

The present study also included a larger sample, relative to published reports [e.g., 42], that allowed for a characterization of the influence of carrying an ε2 allele in middle-aged individuals. In contrast to ε4 status, the ε2 allele appears to have a subtle impact on thickness in MTL and medial orbitofrontal areas. The significantly thicker right parahippocampal cortex and broader tendencies for thicker cortex in these areas lend support to findings in adolescents [38] and corroborate the protective influence of ε2 demonstrated in neuropathological and cognitive studies [8, 15–21]. Exploratory analyses suggesting thinner right supramarginal and left lingual cortex are intriguing but require further replication.

The unique VETSA cohort provided significant power to examine the influence of ApoE allele status, although the study presents some limitations to generalizability. Because our sample was solely male and largely Caucasian, we cannot be certain of the generalizability of these findings to women or ethnic minorities. Furthermore, although the sample is quite similar in health and demographics to comparably-aged men in the U.S., a minority of them did experience varying amounts of combat exposure 35 years earlier. Thus, concerns might be raised as to the effect of combat exposure or possible posttraumatic stress disorder (PTSD) on the results. As of their mid-40s, 7.7% had a lifetime diagnosis of PTSD, slightly higher than the 5.0% prevalence for men nationally [72]. Importantly, this is unlikely to be create a confound in the present study because previous co-twin control findings indicate that smaller hippocampal volume may be a risk factor for PTSD, rather than a consequence [73]. Another potential limitation of our study is that, with T1-based image processing approaches, it is difficult to distinguish tentorium cerebelli from cortex in some mesial and inferior temporal regions. That is, while we have made every effort to separate cortical gray matter from tentorium, thickness estimates in these regions, such as the entorhinal cortex, may be more variable than in other areas. Such an increase in variability may result in less power to detect significant effects of ApoE allele status on thickness, although we would not expect differential effects across ApoE groups.

CONCLUSION

This study of middle-aged men suggests that the presence of the ApoE ε4 allele may influence cortical thickness in frontal areas, later developing regions thought to be more susceptible to natural aging. In contrast, previous conflicting findings of ε4 effects on MTL regions may be driven by the inclusion of older individuals who may evidence preclinical manifestations of neurodegenerative disease, and by moderators of ε4-realted effects, such as hormone levels. The finding of unexpectedly thicker fusiform cortex in the ε4+ group needs to be explored further and replicated. The examination of the ε2 allele supports a protective role, suggesting tendencies for thicker cortex in some MTL and orbitofrontal areas, although some exploratory areas were thinner. Whether these ε2 and ε4 related findings reflect pre-existing endophenotypes or early neurodegeneration is not clear in these cross-sectional data. Ongoing follow-up studies of the VESTA sample may shed light on the potential for age- and disease-related mediation of the influence of ApoE allele status, as these participants enter the age range within which normal age-related neurodegeneration along with memory decline in ε4+ individuals may accelerate [43, 74].

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