



Longitudinal changes in medial temporal cortical thickness in normal subjects with the APOE-4 polymorphism

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ABSTRACT

People with the apolipoprotein-Eε4 (APOE-4) genetic risk for Alzheimer's disease show morphologic differences in medial temporal lobe regions when compared to non-carriers of the allele. Using a high-resolution MRI and cortical unfolding approach, our aim was to determine the rate of cortical thinning among medial temporal lobe subregions over the course of 2 years. We hypothesized that APOE-4 genetic risk would contribute to longitudinal cortical thickness change in the subiculum and entorhinal cortex, regions preferentially susceptible to Alzheimer's disease related pathology. Thirty-two cognitively intact subjects, mean age 61 years, 16 APOE-4 carriers, 16 non-carriers, underwent baseline and follow-up MRI scans. Over this relatively brief interval, we found significantly greater cortical thinning in the subiculum and entorhinal cortex of APOE-4 carriers when compared to non-carriers of the allele. Average cortical thinning across all medial temporal lobe subregions combined was also significantly greater for APOE-4 carriers. This finding is consistent with the hypothesis that carrying the APOE-4 allele renders subjects at a higher risk for developing Alzheimer's disease.

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Introduction

Alzheimer's disease (AD) has multiple and interacting etiologic factors. The ε4 allele of the apolipoprotein E gene (APOE-4) on chromosome 19 is the only well-established genetic risk factor for sporadic AD (Brouwers et al., 2008). Carrying the APOE-4 allele is associated with higher risk for developing AD and earlier age of onset (Corder et al., 1993). However, the APOE-4 allele does not cause the disease, and the relatively high prevalence in the general population suggests antagonistically pleiotropic effects, which can be found in many genetic variants (Wright et al., 2003).

Neuroimaging studies have revealed structural and functional brain differences among cognitively healthy APOE-4 carriers when compared to non-carriers. Using functional magnetic resonance imaging (fMRI), Bookheimer et al. (2000) found increased magnitude and extent of brain activation during tasks requiring memory in healthy APOE-4 carriers compared to non-carriers. Other fMRI data

support these findings (Bondi et al., 2005). Positron emission tomography (PET) reveals reduced glucose metabolism associated with APOE-4 genetic risk in healthy people (Reiman et al., 2005). Cognitively normal APOE-4 carriers also show increased uptake of the amyloid binding ligand PIB (Reiman et al., 2009) and the amyloid and tau binding ligand FDDNP (Small et al., 2009) when compared to subjects not carrying the risk allele.

Advances in spatial resolution and image analysis techniques allow examining regional brain characteristics that might be associated with AD risk in the normal population. This work may contribute to identifying people at greater risk for future cognitive decline. It may also enhance understanding of the relationship between AD risk factors, regional brain structure, and cognitive performance. Hippocampal atrophy is known to be associated with memory impairment and AD (de Leon et al., 1996; Rusinek et al., 2003), but it remains controversial whether hippocampal atrophy is present in healthy people due to APOE-4 genetic risk (Cherbuin et al., 2008; Jack et al., 1998; Mueller et al., 2008). Recent data have shown that when compared to volumetry, medial temporal lobe (MTL) cortical thickness measures may provide better representation of associations between APOE genetic variants and MTL structure changes, given the

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laminar organization of the cerebral cortex, and the expected subtlety of changes within healthy subjects (Burggren et al., 2008). Cortical thickness measurements have been used to assess structural cortical changes with reasonable accuracy and reliability across many diseases, including memory disorders (Bakkour et al., 2009; Thompson et al., 2003). While several MRI studies have documented reduced volume (Apostolova et al., 2006; Devanand et al., 2007) and cortical thinning (Morra et al., 2009; Thompson et al., 2007) in mild cognitive impairment (MCI) and AD subjects, there has been very little work done examining longitudinal MRI changes in healthy volunteers with a genetic risk for AD.

One approach developed in our laboratory examines subregions of the MTL system using high-resolution MRI combined with cortical unfolding. First applied to the visual cortex (Engel et al., 1997), we adapted this procedure to the MTL (Ekstrom et al., 2009a; Zeineh et al., 2000, 2001). This approach uses high-resolution MRI images and increases the visibility of the convoluted MTL cortex while allowing us to measure cortical thickness directly and separately across subregions of the MTL. Using this approach, Burggren et al. (2008) identified reduced cortical thickness in the subiculum (SUB) and entorhinal cortex (ERC) among healthy elderly APOE-4 carriers.

Because APOE proteins are differentially involved in neuronal repair and plasticity processes (Teter et al., 2002), we predict greater decline in neural integrity during the aging process in APOE-4 carriers. In the present study, we used the cortical unfolding approach to identify cortical thinning in a 2-year follow-up among cognitively intact elderly subjects with and without the APOE-4 allele. We hypothesized that subjects with the APOE-4 genetic risk would show increased cortical thinning over time in SUB and ERC, regions susceptible to AD pathology.

Materials and methods

Subjects

Thirty-two subjects (16 APOE-4 carriers, 16 non-carriers) participated in this study, which was performed at the Semel Institute for Neuroscience and Human Behavior and the Ahmanson-Lovelace Brain Mapping Center, University of California, Los Angeles. All participants gave written informed consent in accordance with the UCLA Human Subjects Protection Committee procedures. Participants were selected from a pool of 130 control, MCI and AD subjects recruited through advertisements that underwent baseline and follow-up MRI scans and clinical/neuropsychological examinations from 2004 to 2010. From this sample we excluded 48 subjects showing MCI or dementia at either baseline or follow-up, according to NINCDS-ADRDA criteria (McKhann et al., 1984), scoring <27 points on the Mini Mental State Exam (Folstein et al., 1975), and >0 points on the Clinical Dementia

Rating Scale (Hughes et al., 1982). Subjects underwent diagnostic evaluation including physical and neurological examination, medical history assessment, laboratory screening tests that ruled out medical illnesses possibly affecting cognition, APOE genotyping and neuropsychological testing. General exclusion criteria were a history of psychiatric or neurological disorder, alcohol or substance abuse, head trauma, epilepsy or major systemic disease affecting brain function, as well as arterial hypertension and cardiovascular disease. Three of the excluded subjects meeting criteria for dementia showed signs of extensive white matter disease. Three subjects were excluded because they did not participate in APOE genotyping. Thirty-nine subjects were excluded because they did not have complete MRI data at both timepoints (either did not complete the scan or scans were artifactual). Eight subjects' data were lost due to computer hardware failure in 2005.

Both neuropsychological examination and MRI scans were acquired twice for each subject, with 25.5 ± 8.9 months between scans. A neuroradiologist reviewed the MRI scans to rule out medical conditions that would lead to exclusion of subjects. Demographic parameters and neuropsychological test results are given in Table 1. Among the 16 subjects not carrying the APOE-4 allele were 15 subjects with the APOE-3/3 genotype and one subject with the APOE-2/3 genotype. Among the 16 APOE-4 allele carriers were 15 subjects with the APOE-3/4 genotype and one homozygous APOE-4 subject. 12 non-APOE-4 carriers and 10 APOE-4 subjects had a first-degree relative diagnosed with AD.

Neuroimaging

MRI scanning was performed on a Siemens Allegra 3T head-only MRI scanner. We obtained sagittal T1 weighted magnetization prepared rapid acquisition gradient-echo (MPRAGE) volumetric scans [TR 2300 ms, TE 2.93 ms, slice thickness 1 mm, 160 slices, in-plane voxel size 1.3×1.3 mm, FOV 256 mm] for volumetric measurements and high-resolution oblique coronal T2 weighted fast spin echo (FSE) sequences for structural segmentation and unfolding procedures [TR 5200 ms, TE 105 ms, slice thickness 3 mm, spacing 0 mm, 19 slices, in-plane voxel size 0.39×0.39 mm, FOV 200 mm]. The first scans of three subjects in each group were obtained with different FSE parameters [TR 4000 ms, TE 100 ms, slice thickness 3 mm, spacing 1 mm, 19 slices, in-plane voxel size 0.43×0.43 mm, FOV 200 mm]. The new parameters improved the spatial resolution both within and through plane and were adopted for all subsequent scans; the corresponding thickness values did not significantly differ in mean and distribution from those obtained with the former parameter set. Excluding these subjects did not change our results.

Cortical unfolding (Fig. 1) is aimed at enhancing the visibility of the convoluted MTL cortex by flattening of the entire MTL gray matter

Table 1
Demographic and clinical characteristics.

Characteristic (mean $\pm$ SD)	Non-APOE-4, baseline	Non-APOE-4, follow-up	APOE-4, baseline	APOE-4, follow-up
N	16		16	
Age (years)	60.1 $\pm$ 7.1		61.7 $\pm$ 11.5	
Female sex (no.)	12		10	
Education (years)	16.6 $\pm$ 2.1		17.2 $\pm$ 2.9	
Left-handedness (no.)	2		1	
First-degree family history of AD (no.)	12		10	
Time between scans (months)	25.3 $\pm$ 9.3		25.6 $\pm$ 8.8	
MMSE (score range 0–30)	29.3 $\pm$ 0.9	29.2 $\pm$ 1.3	29.1 $\pm$ 0.8	29.0 $\pm$ 0.8
Delay Total LM (0–50)	28.4 $\pm$ 6.9	28.6 $\pm$ 6.5	27.4 $\pm$ 4.4	30.1 $\pm$ 9.4
CLTR (0–144)	68.4 $\pm$ 39.6	66.4 $\pm$ 32.6	65.9 $\pm$ 32.3	63.6 $\pm$ 37.5
Delay ROF (0–36)	14.6 $\pm$ 5.3	15.5 $\pm$ 5.5	15.0 $\pm$ 5.2	16.1 $\pm$ 5.1
Total VP (0–32)	22.1 $\pm$ 6.6	25.1 $\pm$ 6.6	23.3 $\pm$ 7.7	24.0 $\pm$ 7.0

MMSE = Mini Mental State Examination (Folstein et al., 1975); Delay Total LM = Wechsler memory scale, logical memory delayed recall portion (Wechsler, 1997); CLTR = Buschke-Fuld selective reminding test, consistent long-term retrieval section (Buschke and Fuld, 1974); Delay ROF = Rey-Osterrieth Complex Figure, delayed recall (Lezak, 1976); Total VP = Wechsler memory scale, verbal paired associations II (Wechsler, 1997).

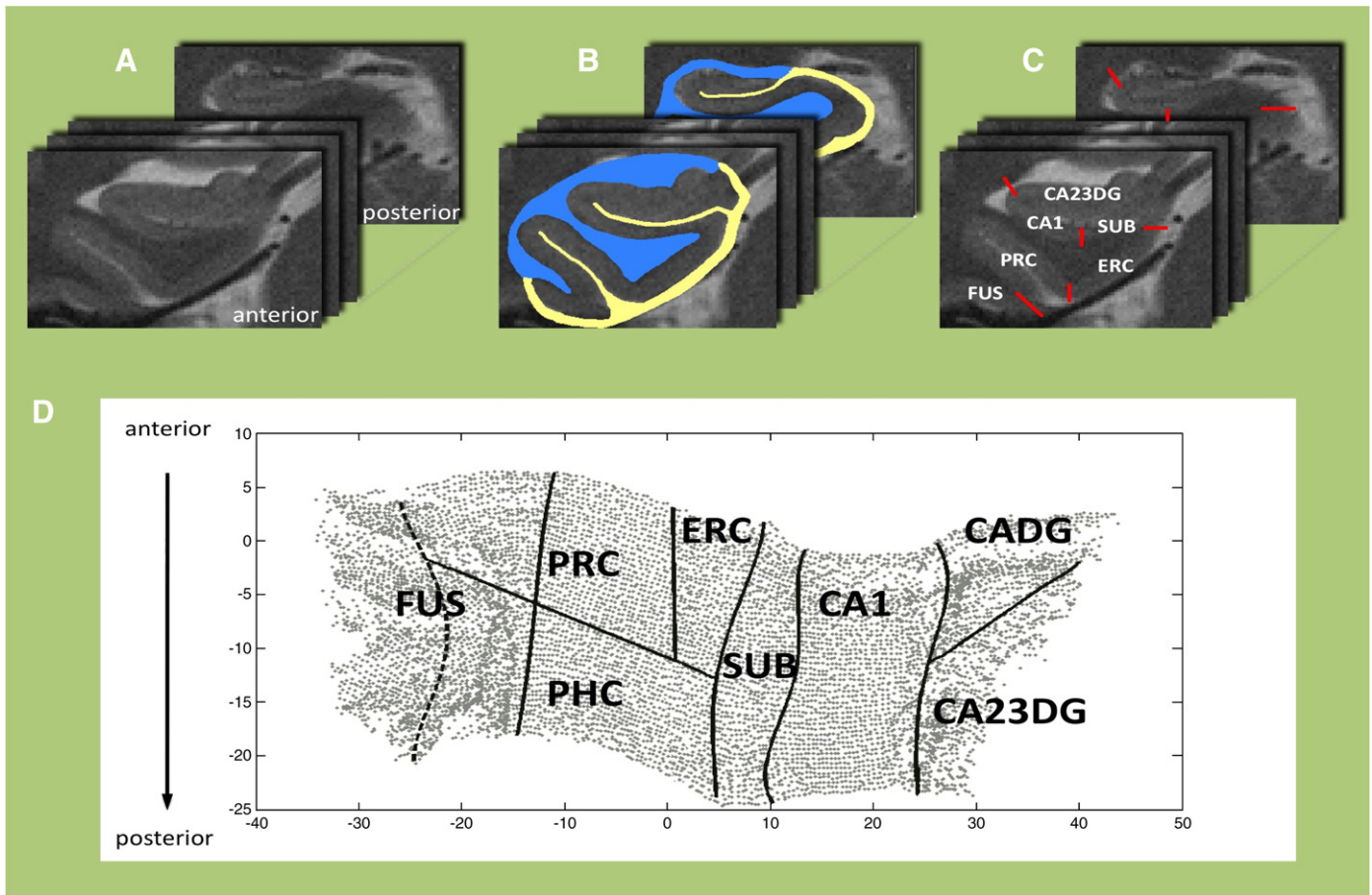


Fig. 1. Cortical unfolding method. Using high-resolution magnetic resonance images (A), white matter (B, blue) and cerebrospinal fluid (B, yellow) are manually segmented. The resulting gray matter strip is then computationally unfolded and flattened. Boundaries (C) between subregions are delineated on the original images and then projected onto the flat map (D, right side shown). CADG = anterior CA fields and dentate gyrus, CA23DG = CA fields 2, 3 and dentate gyrus, CA1 = CA field 1, SUB = subiculum, ERC = entorhinal cortex, PRC = perirhinal cortex, PHC = parahippocampal cortex, FUS = fusiform cortex.

volume to 2D-space (Zeineh et al., 2000, 2001). Gray matter is first specified by manually defining white matter and cerebrospinal fluid (CSF) on an oblique coronal T2 FSE structural MRI sequence with high in-plane resolution. High in-plane resolution is critical for the accuracy of the manual cortical segmentation. Greater slice thickness allowed the achievement of 0.39×0.39 mm in-plane resolution within a reasonable acquisition time and excellent tissue contrast. However, boundary changes between slices will be affected by the larger through-plane resolution. This effect is minimized by acquiring images perpendicular to the long axis of the hippocampal system, as the anatomy is most variable in cross sections and least variable along the long axis. After the segmentation step, the original images are interpolated by a factor of 7, yielding a final voxel size of $0.39 \times 0.39 \times 0.43$ mm. Then, up to 18 connected layers of gray matter are grown out using a region-expansion algorithm to cover all pixels defined as gray matter, resulting in a gray matter strip containing cornu ammonis (CA) fields 1, 2, and 3, the dentate gyrus (DG), subiculum (SUB), entorhinal cortex (ERC), perirhinal cortex (PRC), parahippocampal cortex (PHC), and the fusiform gyrus (FUS). Due to limits in resolution CA fields 2, 3, and DG are treated as a single entity (CA23DG). The unfolding procedure, an iterative algorithm based on multidimensional scaling, was performed with modified mrUnfold software (<http://airto.hosted.ats.ucla.edu/Hippocampus/>; Engel et al., 1997). Boundaries between subregions were delineated on the original in-plane MRI images, based on histological and MRI atlases (Amaral and Insausti, 1990; Duvernoy, 1998; Mai, 1997) and then projected mathematically to their flat map space coordinates. Cortical thickness was calculated in 3D-space in the following regions:

CA23DG, CA1, SUB, ERC, PRC, PHC, and FUS. We also calculated overall MTL cortical thickness ("Global") by averaging thickness across subregions. For each gray matter voxel the distance to the closest non-gray matter voxel was computed. In 2D-space, for each voxel, the maximum distance value of the corresponding 3D voxels across all layers was taken and multiplied by two. Mean thickness in each subregion was calculated by averaging thickness of all 2D voxels within each region of interest.

The same person performed the manual procedures for baseline and follow-up MRI scans and was unaware of the participants' APOE genotype and all other demographic and clinical information. All 64 MRI scans were processed in random order. Processing of each scan pair was therefore independent. Manual image segmentations were performed in native space in line with previous studies using the cortical unfolding technique (Burggren et al., 2008; Ekstrom et al., 2009a; Suthana et al., 2009). Registration to an anatomical template could have introduced another confounder possibly influencing the detection of subtle group differences in cortical morphology. Inter-rater and test-retest reliability analyses have been reported previously for the manual procedures involved (Burggren et al., 2008; Ekstrom et al., 2009b). To assess reliability in this sample given a single rater, we additionally performed the procedure twice in three subjects on two separate scans acquired 3 weeks apart. Test-retest results showed average measurement differences ranging from 0.1% to 1.2% across subregions, suggesting that the method is reliable, with sufficiently small variability to enable detection of longitudinal changes. The procedure's ability to provide topographically correct flat maps has been demonstrated in several ways, including accurate

reconstruction of the hippocampus from a flat map (Ekstrom et al., 2009a,b; Zeineh et al., 2000, 2001).

Comparison of brain volumes between subjects and time points were performed using SIENA image analysis (Smith et al., 2002) within the Analysis Group at the Oxford Centre for Functional MRI of the Brain (FMRIB) software library (FSL) to obtain volumetric data from T1 weighted MPAGE MRI scans. For longitudinal analysis two brain images were aligned to each other and resampled into the space halfway between the two. After tissue-type segmentation to determine brain/non-brain edge points, perpendicular edge displacement between timepoints was estimated at these edge points. Mean edge displacement was converted into an estimate of percentage brain volume change between timepoints (Smith et al., 2002; <http://www.fmrib.ox.ac.uk/analysis/research/siena/>). MTL volumes were obtained by averaging the volume of each unfolded region's voxels in 3D-space (Burggren et al., 2008). We measured brain volumes from the T1 scans rather than using high in-plane resolution T2 data, since these images are easily affected by subtle differences in slice orientation and field of view. Both brain volume-corrected and raw cortical thickness data have been analyzed. Since no statistically significant difference in any of the analyses occurred due to volume correction we always refer to raw data.

Statistical analysis

We estimated mixed general linear models for baseline cortical thickness and percent of change in cortical thickness over time. We used subregions as a within-group factor, and conducted post hoc univariate tests only after significance was established by the multivariate F tests, to determine the subregions that contributed to the significant findings. Using the interval between scans as a covariate did not change the results. Additional ANOVAs have been performed for MTL and brain volume comparisons as well as analyses of neuropsychological data. Statistical analyses used a significance level of $p < 0.05$ (two-tailed).

Results

There were no significant differences in the neuropsychological test scores between groups at baseline. As shown in Table 1, 2 years later no performance changes could be detected. APOE-4 carriers and non-carriers were equivalent in cortical thickness at baseline, which allowed us to specifically examine the effects of genotype on longitudinal change. We did not find an association between cortical thickness change and cognitive change.

Within-group cortical thickness results: subjects not carrying the APOE-4 allele did not show cortical thinning over time within group overall ($F(1,30) = 0.9$, $p = 0.3$) or within subregions (all p -values > 0.05). In contrast, APOE-4 subjects showed a significant longitudinal thickness decrease overall ($F(1,30) = 6.0$, $p = 0.02$) due to significant simple effects in CA1 ($p = 0.02$), SUB ($p < 0.001$), ERC ($p = 0.002$), PRC ($p = 0.002$), PHC ($p < 0.001$), FUS ($p < 0.001$). When the entire medial temporal region was considered as a whole there was also a significant decrease in cortical thickness among APOE-4 subjects (Global thickness; $p < 0.001$).

Between-group cortical thickness results: In the longitudinal analysis, APOE-4 carriers showed a significantly greater decrease in cortical thickness over time than did non-carriers ($F(1,30) = 6.9$, $p = 0.01$) in SUB (7% greater decrease in APOE-4 carriers, $p = 0.02$) and ERC (4%, $p = 0.03$), Fig. 2. Global thickness also decreased significantly more in APOE-4 carriers (4%, $p = 0.01$).

Because of the small sample size we additionally performed non-parametric statistics (Wilcoxon Mann–Whitney tests for between-group analyses; signed rank tests for within-group analyses). Using non-parametric statistics did not change the pattern of significant results between or within groups.

Volumetric analysis: In addition to calculating regional cortical thickness, we also performed a more traditional volumetric analysis on the MTL gray matter as well as the whole brain volume (gray and white matter). APOE-4 carriers showed 2.8% MTL volume loss and 0.2% whole brain volume loss over the 2-year interval, whereas non-

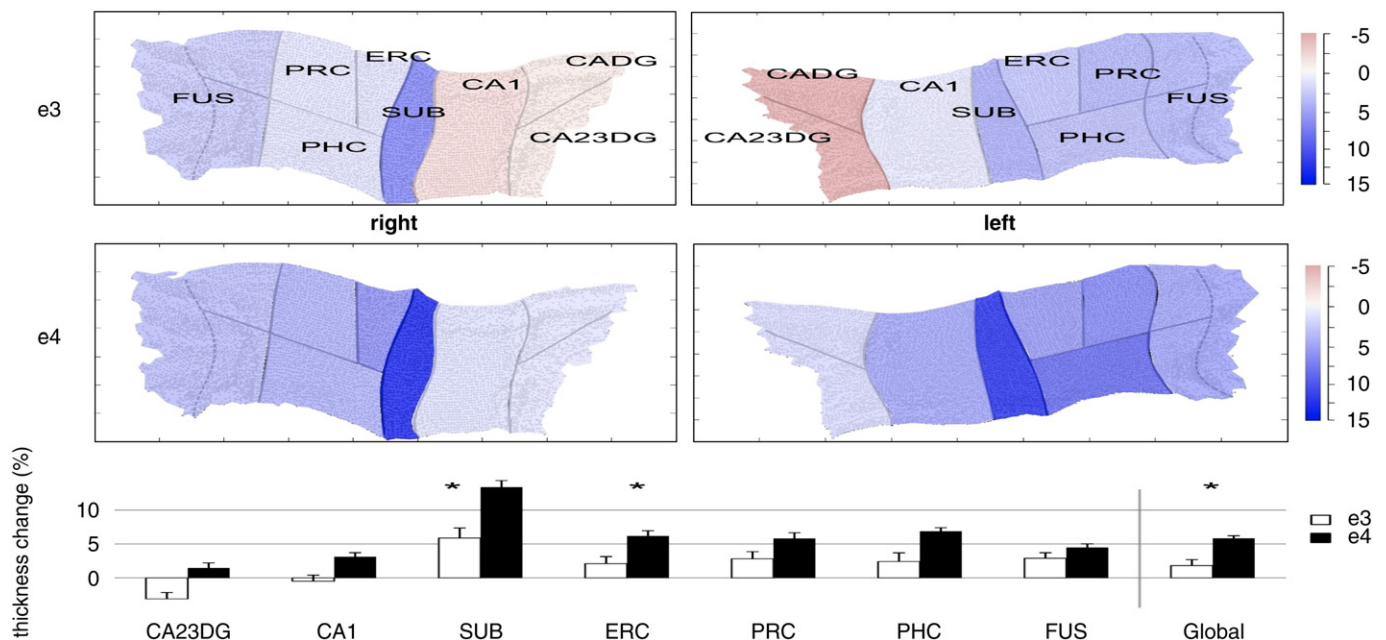


Fig. 2. Cortical thickness change over time (%) across subregions. The color-coded flat maps visualize cortical thickness change over the 2-year follow-up period in APOE-4 carriers and non-carriers for both hemispheres separately. The graph below provides average (across both hemispheres) cortical thickness change for all subregions over time (mean $\pm$ SE). Significant between-group differences are indicated (*, $p < 0.05$, two-tailed). APOE-4 carriers compared to non-carriers showed significant greater cortical thinning in SUB, ERC, and Global. CADG = anterior CA fields and dentate gyrus, CA23DG = CA fields 2, 3 and dentate gyrus, CA1 = CA field 1, SUB = subiculum, ERC = entorhinal cortex, PRC = perirhinal cortex, PHC = parahippocampal cortex, FUS = fusiform cortex, Global = average thickness across all subregions, e3 = non-APOE-4 carriers, e4 = APOE-4 subjects.

carriers showed 1.1%/0.1% volume loss, respectively. However, there was no statistically significant volume difference between groups at baseline (MTL: $p = 0.9$, whole brain $p = 0.3$) or for percent of volume loss over time (MTL: $p = 0.7$, whole brain $p = 0.9$).

Discussion

Our data indicate that among cognitively healthy APOE-4 carriers the rate of cortical thinning over time is significantly greater in SUB and ERC when compared to non-carriers of the APOE-4 allele. Global cortical thinning (average across subregions) was also significantly greater among APOE-4 carriers. Within group, non-APOE-4 carriers did not show cortical thinning over time whereas APOE-4 carriers showed decrease in cortical thickness in all subregions except CA23DG.

The APOE-4 allele is recognized as the most important genetic risk factor for sporadic AD known today (Brouwers et al., 2008). Using different neuroimaging techniques several studies have revealed structural and functional brain characteristics among cognitively healthy APOE-4 allele carriers that may be associated with AD risk (e.g., Bookheimer et al., 2000; Burggren et al., 2008; Reiman et al., 2005). However, APOE-4 associated characteristics are already present in children and young adults (Reiman et al., 2004; Shaw et al., 2007). For example, Shaw et al. (2007) found a thinner ERC in children carrying the APOE-4 allele. This could represent a genetically determined neuroanatomic property, effectively a static risk factor, such that less thinning may be required to develop clinical symptoms once a neurodegenerative process starts later in life (Shaw et al., 2007). It is unlikely that in this case the thinner ERC itself reflects pathology. Cortical thinning in children and adolescents is associated with physiological cortical maturation (Sowell et al., 2001). The thinner ERC may represent a more mature cortex, which could be beneficial with respect to cognitive performance. There is evidence for early developmental benefits associated with the APOE-4 genotype, resulting in greater verbal fluency abilities (Alexander et al., 2007), or a more economic use of learning-related neural resources (Mondadori et al., 2007). The relatively high prevalence of the APOE-4 allele in the general population is also in line with the allele's possible antagonistically pleiotropic effects (Wright et al., 2003). However, Sowell et al. (2001) demonstrated that in children frontal lobe gray matter thinning/maturation was more predictive for cognitive performance than cortical structure changes within the MTL.

Few studies have demonstrated longitudinal changes in brain structure and function in cognitively intact APOE-4 carriers. It has been shown that there is greater decline in brain glucose metabolism when comparing middle-aged healthy APOE-4 carriers and non-carriers in a 2-year follow-up (Small et al., 2000). Chen et al. (2007) reported greater whole brain atrophy in 47- to 68-year-old cognitively normal homozygous but not heterozygous APOE-4 subjects compared to subjects not carrying the APOE-4 allele within a 2-year period, suggesting that APOE-4 gene dose contributes to longitudinal morphological changes. The authors used two different image analysis methods (brain boundary shift integration/iterative principal component analysis; Chen et al., 2007) and reported annual atrophy rates of 0.08%/0.43% for non-APOE-4 carriers, 0.18%/0.58% for heterozygous APOE-4 carriers, and 0.37%/0.76% for homozygous APOE-4 carriers. Cohen et al. (2001) found significantly greater percent of change in hippocampal volume due to APOE-4 genetic risk (annual rate: 2.3% for APOE-4 carriers, and 0.77% for non-carriers). Our volumetric data show a similar magnitude of volume loss over time, although we did not find significant volumetric effects. However, studies examining the association between APOE genotype and hippocampal volume have shown mixed results (Cherbuin et al., 2008; Jack et al., 1998). Burggren et al. (2008) demonstrated that measuring cortical thickness might better reflect morphological changes of the layered cortical architecture than hippocampal

volume. They showed that cortical thickness but not volume was reduced in SUB and ERC among cognitively healthy APOE-4 participants when compared to non-APOE-4 carriers. The present study similarly indicates greater sensitivity of subregional thickness measures compared to overall volume in longitudinal changes in an at-risk population. Our data suggest a dynamic process of thickness decline in elderly subjects. Aging is associated with decreased synaptic plasticity and reduced hippocampal neurogenesis (Cameron and McKay, 1999). APOE proteins are involved in neuronal plasticity and repair processes, possibly through isoform-specific functions in cholesterol and phospholipid metabolism. The presence of the APOE-3 allele stimulates neuronal growth in cell cultures, whereas the APOE-4 allele is associated with decreased or inhibited neuronal sprouting (Teter et al., 2002). It is therefore likely that age-related decline in MTL thickness would be more pronounced in APOE-4 carriers due to lower neuronal repair efficiency. This would suggest vulnerability for AD but does not necessarily reflect AD pathology. Alternatively, greater cortical thickness decline in ERC and SUB of older APOE-4 carriers may be due to cellular processes related to AD pathology. ERC is the first region showing AD-related neurofibrillary tangle deposition, perhaps decades before the onset of clinical symptoms (Ohm et al., 1995), that later spreads to SUB and CA fields (Braak and Braak, 1991).

Although between-group patterns in baseline cortical thickness differ from our previous study (Burggren et al., 2008), we suggest that our recruitment strategy, which was to select subjects that were cognitively healthy at two time points, could contribute to this finding. Subjects that were cognitively healthy at baseline but later developed MCI were excluded from the analysis as we wanted to focus on subjects in the "normal" range of cognition. The rigorous selection of longitudinally stable healthy subjects may have biased our sample towards finding study participants that were equivalent in cortical thickness metrics at baseline; a more random selection of subjects might have shown greater differences in hippocampal structure at baseline. Additionally, more non-APOE-4 carriers and fewer APOE-4 subjects in this study compared to our previous study (Burggren et al., 2008) had a family history of AD; there was no overlap in the subject population between these studies. Family history risk of AD may reflect yet unknown genetic and non-genetic risks for AD. We recently demonstrated that family history of AD is associated with reduced cortical thickness of medial temporal lobe subregions independent of APOE genotype (Donix et al., 2010). Our data suggest that if we had assessed subjects at a slightly later date we would have found baseline cortical thickness differences. It is unclear, however, whether this would have affected the annual rate of longitudinal cortical thinning. The possible relationship between baseline thickness and rate of longitudinal change should be investigated in future studies.

This study has several limitations: Our subject sample size is relatively small, limiting our ability to draw broader conclusions across a range of subject characteristics including age of assessment and family history, to name a few. AD risk factors possibly contributing to having a family history for AD, such as cerebral vascular risk and shared environmental factors could differ between subjects. It is also not possible to determine whether these patterns would be representative for the general population, as our APOE-4 subjects may represent a uniquely healthy risk group and our APOE-3 subjects have a relatively high rate of family history risk. Future examination of cognitively impaired people differing in APOE genotype status using the same image analysis technique could help determine whether the effects seen in this study are driven by sub-clinical disease. The causes of cortical thinning cannot be revealed using MRI alone. Further longitudinal investigations should examine whether specific patterns of cortical thickness change are associated with the subsequent decline into AD and which anatomical features might be related to pathological versus normal aging.

In summary, we found that subjects carrying the APOE-4 allele when compared to non-carriers show greater decline in cortical thickness in SUB and ERC over a 2-year interval. Our results contribute

to the hypothesis that the APOE-4 allele determines a neuroanatomic endophenotype that renders individuals at a higher risk for developing AD due to its different cortical architecture. These structural characteristics could be more dynamic as we age, when APOE-4 related impairment in synaptogenesis and neuronal repair is likely to have greater impact. Other variables, such as a family history of AD could be additionally modulating factors that may contribute to reach a critical threshold in neuronal integrity. Whether this is a necessary condition for the development of AD or may itself represent a first stage of a neurodegenerative disorder remains unknown.

Disclosure statement

Dr. Small reports having served as a consultant and/or having received lecture fees from Abbott, Brainstorming Co., Dakim, Eisai, Forest, Myriad Genetics, Novartis, Ortho-McNeil, Pfizer, Radica, Siemens, and Medivation. Dr. Small also reports having received stock options from Dakim. Dr. Ercoli reports having received lecture fees from the Alzheimer's Association Speakers Bureau and Keiro Senior Health Services. Other investigators have no financial interests.

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References

- Alexander, D.M., Williams, L.M., Gatt, J.M., Dobson-Stone, C., Kuan, S.A., Todd, E.G., Schofield, P.R., Cooper, N.J., Gordon, E., 2007. The contribution of apolipoprotein E alleles on cognitive performance and dynamic neural activity over six decades. *Biol. Psychol.* 75, 229–238.
- Amaral, D.G., Insausti, R., 1990. Hippocampal formation. In: Praxinos, G. (Ed.), *The human nervous system*. Academic Press, San Diego, pp. 711–755.
- Apostolova, L.G., Dinov, I.D., Dutton, R.A., Hayashi, K.M., Toga, A.W., Cummings, J.L., Thompson, P.M., 2006. 3D comparison of hippocampal atrophy in amnesic mild cognitive impairment and Alzheimer's disease. *Brain* 129, 2867–2873.
- Bakkour, A., Morris, J.C., Dickerson, B.C., 2009. The cortical signature of prodromal AD. Regional thinning predicts mild AD dementia. *Neurology* 72 (12), 1048–1055.
- Bondi, M.W., Houston, W.S., Eyler, L.T., Brown, G.G., 2005. fMRI evidence of compensatory mechanisms in older adults at genetic risk for Alzheimer disease. *Neurology* 64, 501–508.
- Bookheimer, S.Y., Strojwas, M.H., Cohen, M.S., Saunders, A.M., Pericak-Vance, M.A., Mazziotta, J.C., Small, G.W., 2000. Patterns of brain activation in people at risk for Alzheimer's disease. *N Engl J. Med.* 343, 450–456.
- Braak, H., Braak, E., 1991. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol. (Berl)* 82, 239–259.
- Brouwers, N., Sleegers, K., Van Broeckhoven, C., 2008. Molecular genetics of Alzheimer's disease: an update. *Ann. Med.* 40, 562–583.
- Burggren, A.C., Zeineh, M.M., Ekstrom, A.D., Braskie, M.N., Thompson, P.M., Small, G.W., Bookheimer, S.Y., 2008. Reduced cortical thickness in hippocampal subregions among cognitively normal apolipoprotein E4 carriers. *Neuroimage* 41, 1177–1183.
- Buschke, H., Fuld, P.A., 1974. Evaluating storage, retention, and retrieval in disordered memory and learning. *Neurology* 24, 1019–1025.
- Cameron, H.A., McKay, R.D., 1999. Restoring production of hippocampal neurons in old age. *Nat. Neurosci.* 2, 894–897.
- Chen, K., Reiman, E.M., Alexander, G.E., Caselli, R.J., Gerkin, R., Bandy, D., Domb, A., Osborne, D., Fox, N., Crum, W.R., Saunders, A.M., Hardy, J., 2007. Correlations between apolipoprotein E epsilon4 gene dose and whole brain atrophy rates. *Am. J. Psychiatry* 164, 916–921.
- Cherbuin, N., Anstey, K.J., Sachdev, P.S., Maller, J.J., Meslin, C., Mack, H.A., Wen, W., Eastel, S., 2008. Total and regional gray matter volume is not related to APOE*E4 status in a community sample of middle-aged individuals. *J. Gerontol. A Biol. Sci. Med. Sci.* 63, 501–504.
- Cohen, R.M., Small, C., Lalonde, F., Friz, J., Sunderland, T., 2001. Effect of apolipoprotein E genotype on hippocampal volume loss in aging healthy women. *Neurology* 57, 2223–2228.
- Corder, E.H., Saunders, A.M., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C., Small, G.W., Roses, A.D., Haines, J.L., Pericak-Vance, M.A., 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 261, 921–923.
- de Leon, M.J., Convit, A., George, A.E., Golomb, J., de Santi, S., Tarshish, C., Rusinek, H., Bobinski, M., Ince, C., Miller, D., Wisniewski, H., 1996. In vivo structural studies of the hippocampus in normal aging and in incipient Alzheimer's disease. *Ann. NY Acad. Sci.* 777, 1–13.
- Devanand, D.P., Pradhaban, G., Liu, X., Khandji, A., De Santi, S., Segal, S., Rusinek, H., Pelton, G.H., Honig, L.S., Mayeux, R., Stern, Y., Tabert, M.H., de Leon, M.J., 2007. Hippocampal and entorhinal atrophy in mild cognitive impairment: prediction of Alzheimer disease. *Neurology* 68, 828–836.
- Donix, M., Burggren, A., Suthana, N., Siddarth, P., Ekstrom, A., Krupa, A., Jones, M., Martin-Harris, L., Ercoli, L., Miller, K., Small, G., Bookheimer, S., 2010. Family history of Alzheimer's disease and hippocampal structure in healthy people. *Am. J. Psychiatry* accepted manuscript. doi:10.1176/appi.ajp.2010.0911575.
- Duvernoy, H.M., 1998. *The human hippocampus: functional anatomy, vascularization, and serial sections with MRI*. Springer, Berlin.
- Ekstrom, A.D., Bazih, A.J., Suthana, N.A., Al-Hakim, R., Ogura, K., Zeineh, M.M., Burggren, A.C., Bookheimer, S.Y., 2009a. Advances in high-resolution imaging and computational unfolding of the human hippocampus. *Neuroimage* 47, 42–49.
- Ekstrom, A.D., Suthana, N.A., Millett, D., Fried, I., Bookheimer, S.Y., 2009b. Correlation between BOLD fMRI and theta-band local field potentials in the human hippocampal area. *J. Neurophysiol.* 101, 2668–2678.
- Engel, S.A., Glover, G.H., Wandell, B.A., 1997. Retinotopic organization in human visual cortex and the spatial precision of functional MRI. *Cereb. Cortex* 7, 181–192.
- Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. Mini-Mental-State: a practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* 12, 189–198.
- Hughes, C.P., Berg, L., Danziger, W.L., Coben, L.A., Martin, R.L., 1982. A new clinical scale for the staging of dementia. *Br. J. Psychiatry* 140, 566–572.
- Jack Jr., C.R., Petersen, R.C., Xu, Y.C., O'Brien, P.C., Waring, S.C., Tangalos, E.G., Smith, G.E., Ivnik, R.J., Thibodeau, S.N., Kokmen, E., 1998. Hippocampal atrophy and apolipoprotein E genotype are independently associated with Alzheimer's disease. *Ann. Neurol.* 43, 303–310.
- Lezak, M.D., 1976. *Rey-Osterrieth complex figure test*. Neuropsychological assessment. Oxford University Press, New York.
- Mai, J.K., 1997. *Atlas of the human brain*. Academic Press, San Diego.
- McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., Stadlan, E.M., 1984. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 34, 939–944.
- Mondadori, C.R., de Quervain, D.J., Buchmann, A., Mustovic, H., Wollmer, M.A., Schmidt, C.F., Boesiger, P., Hock, C., Nitsch, R.M., Papassotiropoulos, A., Henke, K., 2007. Better memory and neural efficiency in young apolipoprotein E epsilon4 carriers. *Cereb. Cortex* 17, 1934–1947.
- Morra, J.H., Tu, Z., Apostolova, L.G., Green, A.E., Avedissian, C., Madsen, S.K., Parikshak, N., Toga, A.W., Jack Jr., C.R., Schuff, N., Weiner, M.W., Thompson, P.M., 2009. Automated mapping of hippocampal atrophy in 1-year repeat MRI data from 490 subjects with Alzheimer's disease, mild cognitive impairment, and elderly controls. *Neuroimage* 45, S3–S15.
- Mueller, S.G., Schuff, N., Raptentsetsang, S., Elman, J., Weiner, M.W., 2008. Selective effect of Apo e4 on CA3 and dentate in normal aging and Alzheimer's disease using high resolution MRI at 4 T. *Neuroimage* 42, 42–48.
- Ohm, T.G., Muller, H., Braak, H., Bohl, J., 1995. Close-meshed prevalence rates of different stages as a tool to uncover the rate of Alzheimer's disease-related neurofibrillary changes. *Neuroscience* 64, 209–217.
- Reiman, E.M., Chen, K., Alexander, G.E., Caselli, R.J., Bandy, D., Osborne, D., Saunders, A.M., Hardy, J., 2004. Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia. *Proc. Natl. Acad. Sci. U. S. A.* 101, 284–289.
- Reiman, E.M., Chen, K., Alexander, G.E., Caselli, R.J., Bandy, D., Osborne, D., Saunders, A.M., Hardy, J., 2005. Correlations between apolipoprotein E epsilon4 gene dose and brain-imaging measurements of regional hypometabolism. *Proc. Natl. Acad. Sci. U. S. A.* 102, 8299–8302.
- Reiman, E.M., Chen, K., Liu, X., Bandy, D., Yu, M., Lee, W., Ayutyanont, N., Keppler, J., Reeder, S.A., Langbaum, J.B., Alexander, G.E., Klunk, W.E., Mathis, C.A., Price, J.C., Aizenstein, H.J., DeKosky, S.T., Caselli, R.J., 2009. Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A.* 106, 6820–6825.
- Rusinek, H., De Santi, S., Frid, D., Tsui, W.H., Tarshish, C.Y., Convit, A., de Leon, M.J., 2003. Regional brain atrophy rate predicts future cognitive decline: 6-year longitudinal MR imaging study of normal aging. *Radiology* 229, 691–696.
- Shaw, P., Lerch, J.P., Pruessner, J.C., Taylor, K.N., Rose, A.B., Greenstein, D., Clasen, L., Evans, A., Rapoport, J.L., Giedd, J.N., 2007. Cortical morphology in children and adolescents with different apolipoprotein E gene polymorphisms: an observational study. *Lancet Neurol.* 6, 494–500.
- Small, G.W., Ercoli, L.M., Silverman, D.H., Huang, S.C., Komo, S., Bookheimer, S.Y., Lavretsky, H., Miller, K., Siddarth, P., Rasgon, N.L., Mazziotta, J.C., Saxena, S., Wu, H.M., Mega, M.S., Cummings, J.L., Saunders, A.M., Pericak-Vance, M.A., Roses, A.D., Barrio, J.R., Phelps, M.E., 2000. Cerebral metabolic and cognitive decline in persons at genetic risk for Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A.* 97, 6037–6042.
- Small, G.W., Siddarth, P., Burggren, A.C., Kepe, V., Ercoli, L.M., Miller, K.J., Lavretsky, H., Thompson, P.M., Cole, G.M., Huang, S.C., Phelps, M.E., Bookheimer, S.Y., Barrio, J.R., 2009. Influence of cognitive status, age, and APOE-4 genetic risk on brain FDDNP

- positron-emission tomography imaging in persons without dementia. *Arch. Gen. Psychiatry* 66, 81–87.
- Smith, S.M., Zhang, Y., Jenkinson, M., Chen, J., Matthews, P.M., Federico, A., De Stefano, N., 2002. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. *Neuroimage* 17, 479–489.
- Sowell, E.R., Delis, D., Stiles, J., Jernigan, T.L., 2001. Improved memory functioning and frontal lobe maturation between childhood and adolescence: a structural MRI study. *J. Int. Neuropsychol. Soc.* 7, 312–322.
- Suthana, N.A., Krupa, A., Donix, M., Burggren, A., Ekstrom, A.D., Jones, M., Ercoli, L.M., Miller, K.J., Siddarth, P., Small, G.W., Bookheimer, S.Y., 2009. Reduced hippocampal CA2, CA3, and dentate gyrus activity in asymptomatic people at genetic risk for Alzheimer's disease. *NeuroImage* Electronic publication ahead of print. doi:10.1016/j.neuroimage.2009.12.014.
- Teter, B., Xu, P.T., Gilbert, J.R., Roses, A.D., Galasko, D., Cole, G.M., 2002. Defective neuronal sprouting by human apolipoprotein E4 is a gain-of-negative function. *J. Neurosci. Res.* 68, 331–336.
- Thompson, P.M., Hayashi, K.M., de Zubicaray, G., Janke, A.L., Rose, S.E., Semple, J., Herman, D., Hong, M.S., Dittmer, S.S., Doddrell, D.M., Toga, A.W., 2003. Dynamics of gray matter loss in Alzheimer's disease. *J. Neurosci.* 23, 994–1005.
- Thompson, P.M., Hayashi, K.M., Dutton, R.A., Chiang, M.C., Leow, A.D., Sowell, E.R., De Zubicaray, G., Becker, J.T., Lopez, O.L., Aizenstein, H.J., Toga, A.W., 2007. Tracking Alzheimer's disease. *Ann. NY Acad. Sci.* 1097, 183–214.
- Wechsler, D., 1997. Wechsler Memory Scale, 3. ed. The Psychological Corporation, San Antonio.
- Wright, R.O., Hu, H., Silverman, E.K., Tsaih, S.W., Schwartz, J., Bellinger, D., Palazuelos, E., Weiss, S.T., Hernandez-Avila, M., 2003. Apolipoprotein E genotype predicts 24-month bayley scales infant development score. *Pediatr. Res.* 54, 819–825.
- Zeineh, M.M., Engel, S.A., Bookheimer, S.Y., 2000. Application of cortical unfolding techniques to functional MRI of the human hippocampal region. *Neuroimage* 11, 668–683.
- Zeineh, M.M., Engel, S.A., Thompson, P.M., Bookheimer, S.Y., 2001. Unfolding the human hippocampus with high resolution structural and functional MRI. *Anat. Rec.* 265, 111–120.