



Published in final edited form as:

*J Alzheimers Dis.* 2011 January 1; 23(3): 433–442. doi:10.3233/JAD-2010-101398.

## Apolipoprotein E Genotype is Associated with Temporal and Hippocampal Atrophy Rates in Healthy Elderly Adults: A Tensor-Based Morphometry Study<sup>1</sup>

Po H. Lu<sup>a,\*</sup>, Paul M. Thompson<sup>a,b</sup>, Alex Leow<sup>a,b,d</sup>, Grace J. Lee<sup>a</sup>, Agatha Lee<sup>a,b</sup>, Igor Yanovsky<sup>c</sup>, Neelroop Parikshak<sup>a,b</sup>, Theresa Khoo<sup>a</sup>, Stephanie Wu<sup>a</sup>, Daniel Geschwind<sup>a,f</sup>, and George Bartzokis<sup>b,d,e</sup>

<sup>a</sup> Department of Neurology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

<sup>b</sup> Laboratory of Neuroimaging, Department of Neurology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

<sup>c</sup> Department of Mathematics, University of California, Los Angeles, CA, USA

<sup>d</sup> Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

<sup>e</sup> Greater Los Angeles VA Healthcare System, West Los Angeles, CA, USA

<sup>f</sup> Neurogenetics Program, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

### Abstract

Apolipoprotein E (ApoE)  $\epsilon 4$  genotype is a strong risk factor for developing Alzheimer's disease (AD). Conversely, the presence of the  $\epsilon 2$  allele has been shown to mitigate cognitive decline. Tensor-based morphometry (TBM), a novel computational approach for visualizing longitudinal progression of brain atrophy, was used to determine whether cognitively intact elderly participants with the  $\epsilon 4$  allele demonstrate greater volume reduction than those with the  $\epsilon 2$  allele. Healthy "younger elderly" volunteers, aged 55–75, were recruited from the community and hospital staff. They were evaluated with a baseline and follow-up MRI scan (mean scan interval = 4.72 years, s.d. = 0.55) and completed ApoE genotyping. Twenty-seven participants were included in the study of which 16 had the  $\epsilon 4$  allele (all heterozygous  $\epsilon 3\epsilon 4$  genotype) and 11 had the  $\epsilon 2\epsilon 3$  genotype. The two groups did not differ significantly on any demographic characteristics and all subjects were cognitively "normal" at both baseline and follow-up time points. TBM was used to create 3D maps of local brain tissue atrophy rates for individual participants; these spatially detailed 3D maps were compared between the two ApoE groups. Regional analyses were performed and the  $\epsilon 4$  group demonstrated significantly greater annual atrophy rates in the temporal lobes ( $p = 0.048$ ) and hippocampus ( $p = 0.016$ ); greater volume loss was observed in the right hippocampus than the left. TBM appears to be useful in tracking longitudinal progression of brain atrophy in cognitively asymptomatic adults. Possession of the  $\epsilon 4$  allele is associated with greater temporal and hippocampal volume reduction well before the onset of cognitive deficits.

<sup>1</sup>This data was presented in part at the 11th annual meeting of the International Conference for Alzheimer's Disease, Chicago, IL.

© 2011 – IOS Press and the authors. All rights reserved

\*Correspondence to: Po H. Lu, Psy.D., Mary S. Easton Center for Alzheimer's Disease Research, 10911 Weyburn Avenue, Suite 200, Los Angeles, CA 90095-7226, USA. Tel.: +1 310 794 3601; Fax: +1 310 794 3148; plu@mednet.ucla.edu.

Authors' disclosures available online (<http://www.jalz.com/disclosures/view.php?id=639>).

## Keywords

Aging; Alzheimer's disease; Apolipoprotein E; asymmetry; healthy elderly; hippocampus; magnetic resonance imaging; tensor-based morphometry; temporal lobe; white matter

---

## INTRODUCTION

Apolipoprotein E (ApoE)  $\epsilon 4$  genotype, coded on chromosome 19, is a strong risk factor for developing Alzheimer's disease (AD), second only to advancing age. The lifetime risk of AD for an individual without the  $\epsilon 4$  allele is approximately 10%, whereas the lifetime risk for an individual carrying at least one  $\epsilon 4$  allele is 30% [1]. Epidemiological studies have demonstrated that ApoE  $\epsilon 4$  genotype shifts the age of onset for AD by more than a decade, thus accounting for the vast majority of observed cases of AD that occur before age 80 [2–5]. Conversely, presence of an ApoE  $\epsilon 2$  allele has been shown to mitigate cognitive decline [6–8] and delay the onset age for dementia [2, 5, 9, 10].

Several magnetic resonance imaging (MRI) studies have attempted to map brain structural changes associated with the ApoE gene in non-demented elderly subjects. These investigations, focused primarily on the hippocampus, have yielded mixed results. Within cross-sectional designs, a handful of studies have reported reduced hippocampal volume in carriers of the  $\epsilon 4$  allele relative to non-carriers [11–13], but many others have failed to detect an ApoE effect on hippocampal size [14–21]. Longitudinal reports were more consistent in finding an association between accelerated hippocampal atrophy and ApoE  $\epsilon 4$  genotype [15–17] although not all investigators have confirmed these findings [18].

Whole brain volume has thus far not been shown to be sensitive to the effects of ApoE in multiple cross-sectional [11, 13, 14] and longitudinal [17] studies, as global brain atrophy likely occurs in later stages of AD. A recent longitudinal report did show greater annual rate of whole brain atrophy for homozygous but not heterozygous  $\epsilon 4$  carriers when compared to non-carriers [22]. The effect of the ApoE gene on white matter integrity has also been investigated. Using diffusion tensor imaging (DTI), lower fractional anisotropy (FA) values were obtained for  $\epsilon 4$  carriers, compared to non-carriers, in the posterior regions of the corpus callosum [23]. More recently, Honea et al. [24] and Nierenberg et al. [25] both reported decreased FA in the left parahippocampal gyrus white matter. We previously demonstrated, using transverse relaxation rate ( $R_2$ ), that ApoE genotype can affect the trajectory of age-related myelin breakdown in healthy, cognitively-intact older adults [26]. Specifically, individuals with the  $\epsilon 4$  allele displayed a steeper slope of age-related decline in  $R_2$  of frontal white matter and genu of the corpus callosum than subjects with the  $\epsilon 2$  genotype.

Tensor-based morphometry (TBM) is a novel computational approach that can compare longitudinally acquired images and visualize the spatial profile of brain atrophy over time, including estimates of tissue volume loss rates at each voxel in the brain (reviewed in [27]). This approach has been successfully used to study neurodegenerative and psychiatric disorders, as well as normal brain development [28–30]. We applied the TBM method to characterize and compare the pattern of brain atrophy in cognitively intact elderly adults who are either  $\epsilon 2$  or  $\epsilon 4$  carriers. We specifically chose to study these two groups because of the contrasting effects of these two genotypes on the development of incipient AD. We hypothesized that  $\epsilon 4$  carriers would demonstrate greater rate of brain atrophy than  $\epsilon 2$  carriers, specifically in the temporal lobes and hippocampus, regions associated with AD pathology.

## MATERIALS AND METHODS

### Participants

Normal adult volunteers between the ages of 55 and 75 were recruited from the community and hospital staff for a study on healthy aging. All subjects received written and oral information about the study and signed written informed consents approved by the local institutional review board prior to study participation. Potential subjects were excluded if they had a history of neurological disorder or a family history of AD or other neurodegenerative disorder, psychiatric illness (including drug or alcohol abuse), or head injury resulting in loss of consciousness for more than 10 minutes. The subjects were physically very healthy and were excluded if they were obese (defined as body mass index [BMI] of  $>30 \text{ kg/m}^2$ ), or if they had a history of diabetes, cardiovascular disease, or difficult to control hypertension. Determination of normal cognition and independent functioning was based on semi-structured clinical interview that included questions directed to subjects regarding performance of basic and instrumental activities of daily living, neurological examination by the study physician (GB), and formal neuropsychological testing. None of the participants met the diagnosis of dementia by Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria [31] or mild cognitive impairment based on Petersen criteria [32]. Specifically, none of the subjects had any subjective cognitive complaints or performed  $\geq 1.5$  s.d. below published normative means [33] on objective memory testing (California Verbal Learning Test; CVLT); all had normal global cognitive ability ( $\geq 27$ ) on the Mini-Mental State Examination [34] and intact activities of daily living.

At baseline and at the 5-year follow-up visit, the subjects underwent MRI scan and a battery of neuropsychological tests; all subjects were genotyped at baseline. Eleven subjects with the ApoE  $\epsilon 2$  allele (all with the  $\epsilon 2\epsilon 3$  genotype) and 16 heterozygous  $\epsilon 4$  carriers (all with  $\epsilon 3\epsilon 4$  genotype) completed the 5-year follow-up visit. The demographic characteristics and cognitive test data are summarized in Table 1, indicating that the two ApoE groups were well-matched on demographic variables and exhibited intact memory functioning across time. There were 15 women and 12 men in the sample, and the racial composition was comprised of 22 (82%) Caucasians, 2 (7%) Asians, 1 (4%) African-American, and 2 (7%) Hispanics.

### MRI acquisition

At baseline, all the subjects were scanned using a single 1.5 Tesla (T) MR instrument with a T1-weighted spoiled gradient recalled (SPGR) 3D MRI sequence. The following scanning parameters were used: repetition time (TR) of 24 ms, echo time (TE) of 7 ms, flip angle of  $35^\circ$ , 25 cm field of view (FOV),  $208 \times 208$  acquisition matrix with slice thickness of 1.5 mm, and acquired resolution of  $1.2 \times 1.2 \times 1.5$  mm. All follow-up scans were conducted on a different 1.5 T MRI scanner using T1-weighted spoiled GRASS (SPGR) sequences. A sagittal plane image acquisition protocol was used with TR = 25 ms; TE = 5 ms; flip angle of  $35^\circ$ ; acquisition matrix =  $256 \times 192 \times 25$  with slice thickness of 1.4 mm; and FOV = 25 cm. The image voxel size was  $0.98 \times 0.98 \times 1.4$  mm.

### Image preprocessing

An automated Brain Surface Algorithm (BSE) from Brainsuite [35] was applied, along with manual editing, to remove skull and other non-brain tissues. We then corrected intensity inhomogeneity caused by nonuniformities in the radio frequency (RF) receiver coils using the N3 bias field algorithm proposed by Sled et al. [36]. To adjust for global differences in brain positioning, orientation, and scaling across individuals, all scans were affinely registered to the stereotactic space defined by the International Consortium for Brain Mapping (ICBM-53) [37] using a 9-parameter (9P) transformation (3 translations, 3

rotations, and 3 scales). All follow-up scans were then aligned to the same subject's baseline scan, and both were aligned to the ICBM space. Globally aligned images were resampled in an isotropic space of 230 voxels along each axis (x-, y-, and z-dimensions) with an interpolated voxel size of 1 mm<sup>3</sup>.

To inspect the quality of the 9-parameter registrations, we used a 3D visualization tool called REGISTER, which automatically overlays the arbitrary slice geometry of each scan pair in ICBM space. All scans were confirmed to have satisfactory alignment without noticeable distortion or mismatch.

### Jacobian maps quantifying structural changes over time

To quantify 3D patterns of volumetric brain atrophy over time for each subject, an individual change map, or Jacobian map, was computed by non-linearly registering the follow-up scan to the baseline scan with mutual information based unbiased registration algorithm [38]. Mutual information is a measure of how much information one random variable has about another and it can be used to align images of different modalities, without requiring knowledge of the relationship (joint intensity distribution) of the two registered images [39, 40]. The unbiased image registration technique computes deformation fields by penalizing statistical bias in the resulting Jacobian maps, thus eliminating skew from the distribution of the corresponding Jacobian determinants, and has been shown to perform favorably in recovering true physiological changes in serial MRI data [41].

For each subject, a local tissue growth/atrophy map was obtained by calculating the local Jacobian determinant (i.e., "expansion factor") of the deformation field, which measures progressive volume contraction (Jacobian < 1) or volume expansion (Jacobian > 1). To adjust for variable time differences between scans, individual Jacobian maps were normalized by dividing each map (percent tissue change) by its corresponding inter-scan interval (in years) to create the annualized Jacobian maps, which represent the average change over 1 year. All results and statistical analyses are based on the annualized Jacobian maps.

### Regions of interest

The regions of interest (ROI), comprised of frontal, temporal, parietal, and occipital lobes, and the hippocampus, were manually hand-traced by a trained anatomist on the ICBM template using the Brainsuite software program [35]. Brain slices depicting the ROIs for the temporal lobe regions and the hippocampus for both hemispheres are shown in Fig. 1.

### Statistical analyses

To illustrate systematic difference in atrophic rates between  $\epsilon 2$  and  $\epsilon 4$  carriers, we constructed voxel-wise statistical maps based on the Student's *t*-statistic. The annualized Jacobian maps of  $\epsilon 2$  and  $\epsilon 4$  groups were compared using permutation-based two sample *t* tests to assess overall significance of group differences inside each ROI, corrected for multiple comparisons [42–44]. In brief, a null distribution for the group differences in atrophic rates (Jacobian values) at each voxel was constructed using 10,000 random permutations of the data. For each test, the subjects' genetic status ( $\epsilon 2$  vs.  $\epsilon 4$  carriers) was randomly permuted and voxel-wise *t*-tests were conducted to identify voxels more significant than  $p = 0.05$ . The volume of voxels inside a mask (i.e., temporal lobes) more significant than  $p = 0.05$  was computed for the real experiment and for the random assignments. A ratio, describing the fraction of the time the *t*-statistic was more extreme in the randomized tests than the original test, was calculated to yield an overall *p*-value for the significance of the map (corrected for multiple comparison by permutation). A numeric summary, the mean atrophy rate for all voxels within each ROI, was computed for each

person, to summarize the annual change within the ROI. This approach has been used extensively in prior work [28, 29].

## RESULTS

Individual Jacobian maps were averaged for the  $\epsilon 2$  and  $\epsilon 4$  groups to demonstrate the average amount of annual brain tissue loss (in blue colors) and ventricular enlargement (in red colors) for each group (Fig. 2). As expected, the maps displaying the pattern of progressive brain changes were not clearly distinguishable between the  $\epsilon 2$  and  $\epsilon 4$  carriers since both groups were comprised of healthy, cognitively intact elderly subjects. However, closer inspection reveals subtle but visible changes showing greater ventricular expansion and temporal lobe atrophy in the  $\epsilon 4$  group relative to the  $\epsilon 2$  carriers.

Mean annualized Jacobian values within each ROI, normalized to indicate annualized rates of atrophy (in percent volume loss per year), were computed for each subject, and group averages for each genotype are graphically depicted in Fig. 3. In general, a consistent trend was observed with the  $\epsilon 4$  group demonstrating greater rates of atrophy across all regions of interest; however, independent *t*-tests of these numerical values did not reveal any statistically significant group differences ( $p > 0.06$ ).

Permutation tests, using suprathreshold percentages, provide a detailed 3D visualization of the local atrophy profile. Significantly greater atrophy rates were observed for  $\epsilon 4$  carriers compared to  $\epsilon 2$  group for temporal lobes ( $p = 0.048$ ) and more specifically, the hippocampus ( $p = 0.016$ ). The *p*-value maps representing the regions of significant genotypic group difference are presented in Fig. 4. Interestingly, when the right and left hippocampus were examined separately, the difference between  $\epsilon 4$  and  $\epsilon 2$  groups was greater in the right hippocampus ( $p = 0.008$ ) than the left hippocampus ( $p = 0.096$ ). This is consistent with our previous findings that permutation testing, using the suprathreshold volume of statistics in a map, may be more powerful for detecting group differences than performing univariate tests on regional averages (numeric summaries) derived from the maps.

## DISCUSSION

Using TBM, the results from the present longitudinal study demonstrate that cognitively intact adults with the ApoE  $\epsilon 4$  allele showed significantly increased rate of temporal lobe and hippocampal atrophy than those with the  $\epsilon 2$  genotype. Furthermore, the rate of volume loss in the right hippocampus was significantly greater than the left hippocampus. While not statistically significant, greater percent volume loss was observed in the frontal, parietal, and occipital lobes of the  $\epsilon 4$  group, indicating that ongoing atrophy is widely distributed but significantly greater in areas that are among the first to be involved with AD pathology.

Longitudinal neuroimaging studies of the effects of ApoE  $\epsilon 4$  genotype on brain structural changes typically combine individuals with the  $\epsilon 3\epsilon 3$  and  $\epsilon 2\epsilon 3$  genotypes into a single “non-carrier” group with inter-scan intervals ranging from 17 months to 3.5 years [15–18]. The present study focused on the comparison between ApoE  $\epsilon 2$  and  $\epsilon 4$  carriers due to the contrasting effects of these two genotypes on the age of onset for AD. The extended time interval of approximately 5 years between MRI examinations also allows for brain structural volume differences to emerge in cognitively intact, high functioning “younger-elderly” individuals. Present findings confirm and extend existing literature reporting accelerated hippocampal atrophy in  $\epsilon 4$  carriers [15, 17] as well as contribute to the mounting evidence of decreased brain atrophy in  $\epsilon 2$  carriers. While these results may be suggestive of a protective role of the  $\epsilon 2$  genotype against the development of AD [2, 5, 9, 10], this cannot be conclusively demonstrated without the inclusion of an  $\epsilon 3\epsilon 3$  group, since it is possible that



individuals with the  $\epsilon 3\epsilon 3$  genotype may show the same attenuated atrophy rate as the  $\epsilon 2$  group or they may atrophy at a rate that is intermediate to that of  $\epsilon 2$  and  $\epsilon 4$  carriers. Future studies that include  $\epsilon 3\epsilon 3$  subjects can better elucidate the effects of each specific genotype on hippocampal volume and allow more definitive conclusions regarding the potential protective effect of the  $\epsilon 2$  allele.

The asymmetric finding of more severe right hippocampal atrophy in association with the  $\epsilon 4$  allele has been repeatedly demonstrated in non-demented subjects [11–13, 45–47] and patients with AD [48–51]. However, some studies have reported the absence of hemispheric differences in hippocampal volume in relation to ApoE genotype [20, 21]. The observation of a “normal” asymmetry favoring the right hippocampus has been reported in several MRI studies, and a recent meta-analysis confirmed that the right hippocampal volume is reliably larger than the left in normal adults [52]. Therefore, the absence or reversal of such a discrepancy was postulated to be a possible indicator of existing or impending pathology [45, 46, 48] although the functional implication for the differential hemispheric vulnerability in the hippocampus remains incompletely understood.

The finding of significantly greater volume loss associated with ApoE  $\epsilon 4$  genotype in this healthy “younger-elderly” sample cannot be easily explained given that neuronal loss is not associated with aging [53, 54]. On the other hand, the process of age-related myelin breakdown and loss has been thoroughly demonstrated [55–61]. When compared with other structural indices of brain health, age-related degenerative changes are most pronounced in late-myelinating regions [62], such as the frontal lobe white matter (FLwm) that contain higher proportions of smaller thinly-myelinated axons [58, 63, 64].

Histopathological studies have demonstrated white matter degeneration in AD, independent of gray matter lesions or vascular disease [65, 66]. Specifically, myelin staining is reduced in the perforant pathway, the main input fibers projecting neocortical information from the entorhinal cortex to the granule cells of the dentate gyrus in the hippocampal formation [66]. Absence of myelin due to poor development can reduce overall hippocampal volume despite normal numbers of neurons [67, 68], and the reverse is also possible as hippocampal volume can increase despite neuronal loss [69]. ApoE is the principal brain cholesterol transporter and is essential to the process of “recycling” cholesterol by helping reclaim it from damaged myelin and supplying it for rapid membrane biogenesis during re-myelination [70, 71]. The number of ApoE molecules necessary for this recycling process is lowest in  $\epsilon 4$  carriers and highest in individuals with the  $\epsilon 2$  allele [72, 73]. Therefore, the mechanism underlying the connection between the ApoE gene and the pathophysiology of AD, namely hippocampal atrophy, may be better understood by examining the role of ApoE in myelin maintenance and repair.

The strengths of the present study include the longitudinal design in which each subject acts as his/her own control and measurement of intra-individual rates of change yields greater sensitivity for detection of subtle brain changes over time. Furthermore, TBM may have an advantage over standard ROI-based, manual tracing methods for detecting subtle or highly localized differences. Several study limitations should also be acknowledged. The absence of a  $\epsilon 3\epsilon 3$  group limits the interpretation on the protective effects of the  $\epsilon 2$  allele. Another important limitation is the small sample size, thus replication with larger sample sizes is warranted; however, the number of participants in the present study is comparable to most longitudinal MRI studies of the ApoE effect on brain changes with sample sizes ranging from 25 to 42 participants [15–18]. The heterozygous makeup of the  $\epsilon 4$  group may limit the generalizability of the findings to homozygous  $\epsilon 4$  carriers, but several cross-sectional studies have demonstrated a dose effect such that homozygous  $\epsilon 4$  groups exert a greater influence on brain changes than heterozygous  $\epsilon 4$  carriers and non-carriers [13, 22]. Another limitation

is the difference in MR instrument for the baseline and follow-up scans due to machine change, but the registration method for fluid alignment in our version of TBM optimizes the mutual information between images, which is a cost function that is deliberately designed to be robust under changes in image contrast. It is also important to emphasize that the study design is balanced such that the scanner change affects all subjects and is not specific to one genetic group. The maps of annual change thus reflect both aging and the scanner effect; this would be a concern for interpreting age-related change but should not be a problem for interpreting modulators or correlates of change including ApoE genotype. In addition, while the relatively strict inclusion criteria (no AD family history, no cardiovascular disease, no obesity) reduce the number of factors that may contribute to the brain changes, it also makes the results less generalizable to the overall US population.

Analysis of serial MRI scans using TBM appears to be sensitive in tracking age-related progression of brain changes and the modifying effects of the ApoE gene on this process. The ApoE  $\epsilon 4$  gene appears to exert its impact via accelerated atrophy that is not reliably detected upon cross-sectional study. Additionally, greater right hippocampal reduction may suggest that ApoE genotypic risk is manifested in a reduction in normal hippocampal asymmetry. Therefore, longitudinal decline in hippocampal volume as well as hippocampal asymmetry may hold more promise as possible biomarkers of an approaching cognitive decline than absolute differences within either left or right hippocampus examined at only one time point.

## Acknowledgments

This work was supported by grant K23-AG028727 from the National Institute of Aging (NIA), a grant from the Alzheimer's Association (NIRG-07-60424), the Alzheimer's Disease Research Center grant P50 AG-16570, and Alzheimer's Disease Research Center of California grant. Further support for this study came from NIH grants (MH51928; MH6357-01A1; MH066029-01A2; AG027342-01A2; U54 RR021813 to GB). Algorithm development for this study was also funded by the NIA, NIBIB, the National Library of Medicine, and the National Center for Research Resources (AG016570, EB01651, LM05639, RR019771 to PT).

## References

1. Seshadri S, Drachman DA, Lippa CF. Apolipoprotein E epsilon4 allele and the lifetime risk of Alzheimer's disease. What physicians know, and what they should know. *Arch Neurol.* 1995; 52:1074–1079. [PubMed: 7487559]
2. Ashford JW. Apoe genotype effects on Alzheimer's disease onset and epidemiology. *J Mol Neurosci.* 2004; 23:157–166. [PubMed: 15181244]
3. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N, van Duijn CM. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. Apoe and Alzheimer Disease Meta Analysis Consortium. *JAMA.* 1997; 278:1349–1356. [PubMed: 9343467]
4. Poirier J, Davignon J, Bouthillier D, Kogan S, Bertrand P, Gauthier S. Apolipoprotein E polymorphism and Alzheimer's disease. *Lancet.* 1993; 342:697–699. [PubMed: 8103819]
5. Raber J, Huang Y, Ashford JW. ApoE genotype accounts for the vast majority of AD risk and AD pathology. *Neurobiol Aging.* 2004; 25:641–650. [PubMed: 15172743]
6. Deary IJ, Whiteman MC, Pattie A, Starr JM, Hayward C, Wright AF, Visscher PM, Tynan MC, Whalley LJ. Apolipoprotein E gene variability and cognitive functions at age 79: a follow-up of the Scottish mental survey of 1932. *Psychol Aging.* 2004; 19:367–371. [PubMed: 15222832]
7. Staehelin HB, Perrig-Chiello P, Mitrache C, Miserez AR, Perrig WJ. Apolipoprotein E genotypes and cognitive functions in healthy elderly persons. *Acta Neurol Scand.* 1999; 100:53–60. [PubMed: 10416512]
8. Wilson RS, Bienias JL, Berry-Kravis E, Evans DA, Bennett DA. The apolipoprotein E epsilon2 allele and decline in episodic memory. *J Neurol Neurosurg Psychiatry.* 2002; 73:672–677. [PubMed: 12438469]

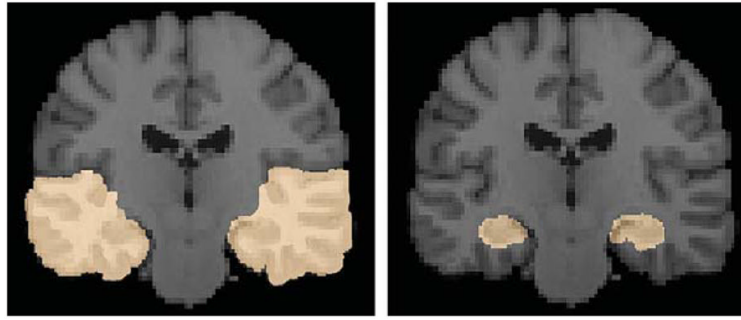
9. Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, Gaskell PC Jr, Rimmler JB, Locke PA, Conneally PM, Schmader KE, Small GW, Roses AD, Haines JL, Pericak-Vance MA. Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nat Genet.* 1994; 7:180–184. [PubMed: 7920638]
10. Khachaturian AS, Corcoran CD, Mayer LS, Zandi PP, Breitner JC. Apolipoprotein E epsilon4 count affects age at onset of Alzheimer disease, but not lifetime susceptibility: The Cache County Study. *Arch Gen Psychiatry.* 2004; 61:518–524. [PubMed: 15123497]
11. den Heijer T, Oudkerk M, Launer LJ, van Duijn CM, Hofman A, Breteler MM. Hippocampal, amygdalar, and global brain atrophy in different apolipoprotein E genotypes. *Neurology.* 2002; 59:746–748. [PubMed: 12221169]
12. Tohgi H, Takahashi S, Kato E, Homma A, Niina R, Sasaki K, Yonezawa H, Sasaki M. Reduced size of right hippocampus in 39- to 80-year-old normal subjects carrying the apolipoprotein E epsilon4 allele. *Neurosci Lett.* 1997; 236:21–24. [PubMed: 9404942]
13. Lemaitre H, Crivello F, Dufouil C, Grassiot B, Tzourio C, Alperovitch A, Mazoyer B. No epsilon4 gene dose effect on hippocampal atrophy in a large MRI database of healthy elderly subjects. *Neuroimage.* 2005; 24:1205–1213. [PubMed: 15670698]
14. Tupler LA, Krishnan KR, Greenberg DL, Marcovina SM, Payne ME, MacFall JR, Charles HC, Doraiswamy PM. Predicting memory decline in normal elderly: genetics, MRI, and cognitive reserve. *Neurobiol Aging.* 2007; 28:1644–1656. [PubMed: 16916565]
15. Cohen RM, Small C, Lalonde F, Friz J, Sunderland T. Effect of apolipoprotein E genotype on hippocampal volume loss in aging healthy women. *Neurology.* 2001; 57:2223–2228. [PubMed: 11756601]
16. Jak AJ, Houston WS, Nagel BJ, Corey-Bloom J, Bondi MW. Differential cross-sectional and longitudinal impact of Apoe genotype on hippocampal volumes in nondemented older adults. *Dement Geriatr Cogn Disord.* 2007; 23:382–389. [PubMed: 17389798]
17. Moffat SD, Szekely CA, Zonderman AB, Kabani NJ, Resnick SM. Longitudinal change in hippocampal volume as a function of apolipoprotein E genotype. *Neurology.* 2000; 55:134–136. [PubMed: 10891924]
18. Du AT, Schuff N, Chao LL, Kornak J, Jagust WJ, Kramer JH, Reed BR, Miller BL, Norman D, Chui HC, Weiner MW. Age effects on atrophy rates of entorhinal cortex and hippocampus. *Neurobiol Aging.* 2006; 27:733–740. [PubMed: 15961190]
19. Schmidt H, Schmidt R, Fazekas F, Semmler J, Kapeller P, Reinhart B, Kostner GM. Apolipoprotein E ε4 allele in the normal elderly: neuropsychologic and brain MRI correlates. *Clin Genet.* 1996; 50:293–299. [PubMed: 9007313]
20. Reiman EM, Uecker A, Caselli RJ, Lewis S, Bandy D, de Leon MJ, De Santi S, Convit A, Osborne D, Weaver A, Thibodeau SN. Hippocampal volumes in cognitively normal persons at genetic risk for Alzheimer's disease. *Ann Neurol.* 1998; 44:288–291. [PubMed: 9708558]
21. Bigler ED, Tate DF, Miller MJ, Rice SA, Hessel CD, Earl HD, Tschanz JT, Plassman B, Welsh-Bohmer KA. Dementia, asymmetry of temporal lobe structures, and apolipoprotein E genotype: relationships to cerebral atrophy and neuropsychological impairment. *J Int Neuropsychol Soc.* 2002; 8:925–933. [PubMed: 12405544]
22. Chen K, Reiman EM, Alexander GE, Caselli RJ, Gerkin R, Bandy D, Domb A, Osborne D, Fox N, Crum WR, Saunders AM, Hardy J. Correlations between apolipoprotein E epsilon4 gene dose and whole brain atrophy rates. *Am J Psychiatry.* 2007; 164:916–921. [PubMed: 17541051]
23. Persson J, Lind J, Larsson A, Ingvar M, Cruts M, van Broeckhoven C, Adolfsson R, Nilsson LG, Nyberg L. Altered brain white matter integrity in healthy carriers of the Apoe epsilon4 allele: a risk for AD? *Neurology.* 2006; 66:1029–1033. [PubMed: 16606914]
24. Honea RA, Vidoni E, Harsha A, Burns JM. Impact of Apoe on the healthy aging brain: a voxel-based MRI and DTI study. *J Alzheimers Dis.* 2009; 18:533–564. [PubMed: 19584451]
25. Nierenberg J, Pomara N, Hoptman MJ, Sidtis JJ, Ardekani BA, Lim KO. Abnormal white matter integrity in healthy apolipoprotein E epsilon4 carriers. *Neuroreport.* 2005; 16:1369–1372. [PubMed: 16056141]



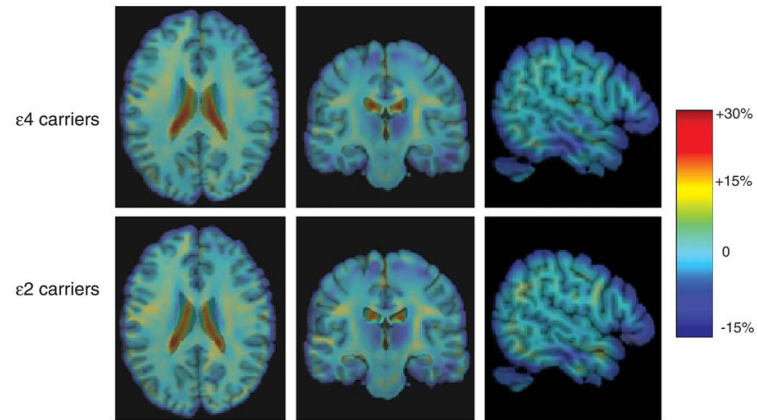
26. Bartzokis G, Lu PH, Geschwind DH, Edwards N, Mintz J, Cummings JL. Apolipoprotein E genotype and age-related myelin breakdown in healthy individuals: implications for cognitive decline and dementia. *Arch Gen Psychiatry*. 2006; 63:63–72. [PubMed: 16389198]
27. Ashburner, J.; Friston, KJ. *Human Brain Function*. Academic Press; 2003. Morphometry.
28. Hua X, Leow AD, Levitt JG, Caplan R, Thompson PM, Toga AW. Detecting brain growth patterns in normal children using tensor-based morphometry. *Hum Brain Mapp*. 2007; 30:209–219. [PubMed: 18064588]
29. Hua X, Leow AD, Lee S, Klunder AD, Toga AW, Lepore N, Chou YY, Brun C, Chiang MC, Barysheva M, Jack CR Jr, Bernstein MA, Britson PJ, Ward CP, Whitwell JL, Borowski B, Fleisher AS, Fox NC, Boyes RG, Barnes J, Harvey D, Kornak J, Schuff N, Boreta L, Alexander GE, Weiner MW, Thompson PM. Alzheimer's Disease Neuroimaging, Initiative. 3D characterization of brain atrophy in Alzheimer's disease and mild cognitive impairment using tensor-based morphometry. *Neuroimage*. 2008; 41:19–34. [PubMed: 18378167]
30. Gogtay N, Lu A, Leow AD, Klunder AD, Lee AD, Chavez A, Greenstein D, Giedd JN, Toga AW, Rapoport JL, Thompson PM. Three-dimensional brain growth abnormalities in childhood-onset schizophrenia visualized by using tensor-based morphometry. *Proc Natl Acad Sci U S A*. 2008; 105:15979–15984. [PubMed: 18852461]
31. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 4. Washington: APA; 1994.
32. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol*. 1999; 56:303–308. [PubMed: 10190820]
33. Delis, DC.; Kramer, JH.; Kaplan, E.; Ober, B. *The California Verbal Learning Test*. The Psychological Corporation; San Antonio: 1987.
34. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state." A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*. 1975; 12:189–198. [PubMed: 1202204]
35. Shattuck DW, Leahy RM. BrainSuite: an automated cortical surface identification tool. *Med Image Anal*. 2002; 6:129–142. [PubMed: 12045000]
36. Sled JG, Zijdenbos AP, Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans Med Imaging*. 1998; 17:87–97. [PubMed: 9617910]
37. Mazziotta J, Toga A, Evans A, Fox P, Lancaster J, Zilles K, Woods R, Paus T, Simpson G, Pike B, Holmes C, Collins L, Thompson P, MacDonald D, Iacoboni M, Schormann T, Amunts K, Palomero-Gallagher N, Geyer S, Parsons L, Narr K, Kabani N, Le Goualher G, Boomsma D, Cannon T, Kawashima R, Mazoyer B. A probabilistic atlas and reference system for the human brain: International Consortium for Brain Mapping (ICBM). *Philos Trans R Soc Lond B Biol Sci*. 2001; 356:1293–1322. [PubMed: 11545704]
38. Leow AD, Yanovsky I, Chiang MC, Lee AD, Klunder AD, Lu A, Becker JT, Davis SW, Toga AW, Thompson PM. Statistical properties of Jacobian maps and the realization of unbiased large-deformation nonlinear image registration. *IEEE Trans Med Imaging*. 2007; 26:822–832. [PubMed: 17679333]
39. D'Agostino E, Maes F, Vandermeulen D, Suetens P. A viscous fluid model for multimodal non-rigid image registration using mutual information. *Med Image Anal*. 2003; 7:565–575. [PubMed: 14561559]
40. Wells WM 3rd, Viola P, Atsumi H, Nakajima S, Kikinis R. Multi-modal volume registration by maximization of mutual information. *Med Image Anal*. 1996; 1:35–51. [PubMed: 9873920]
41. Yanovsky I, Leow AD, Lee S, Osher SJ, Thompson PM. Comparing registration methods for mapping brain change using tensor-based morphometry. *Med Image Anal*. 2007; 13:679–700. [PubMed: 19631572]
42. Bullmore ET, Suckling J, Overmeyer S, Rabe-Hesketh S, Taylor E, Brammer MJ. Global, voxel, and cluster tests, by theory and permutation, for a difference between two groups of structural MR images of the brain. *IEEE Trans Med Imaging*. 1999; 18:32–42. [PubMed: 10193695]
43. Nichols TE, Holmes AP. Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum Brain Mapp*. 2002; 15:1–25. [PubMed: 11747097]

44. Thompson PM, Hayashi KM, de Zubicaray G, Janke AL, Rose SE, Semple J, Herman D, Hong MS, Dittmer SS, Doddrell DM, Toga AW. Dynamics of gray matter loss in Alzheimer's disease. *J Neurosci*. 2003; 23:994–1005. [PubMed: 12574429]
45. Soininen H, Partanen K, Pitkanen A, Hallikainen M, Hanninen T, Helisalmi S, Mannermaa A, Ryyanen M, Koivisto K, Riekkinen P Sr. Decreased hippocampal volume asymmetry on MRIs in nondemented elderly subjects carrying the apolipoprotein E epsilon4 allele. *Neurology*. 1995; 45:391–392. [PubMed: 7854548]
46. Geroldi C, Laakso MP, DeCarli C, Beltramello A, Bianchetti A, Soininen H, Trabucchi M, Frisoni GB. Apolipoprotein E genotype and hippocampal asymmetry in Alzheimer's disease: a volumetric MRI study. *J Neurol Neurosurg Psychiatry*. 2000; 68:93–96. [PubMed: 10601411]
47. Plassman BL, Welsh-Bohmer KA, Bigler ED, Johnson SC, Anderson CV, Helms MJ, Saunders AM, Breitner JC. Apolipoprotein E epsilon4 allele and hippocampal volume in twins with normal cognition. *Neurology*. 1997; 48:985–989. [PubMed: 9109888]
48. Barnes LL, Wilson RS, Bienias JL, Schneider JA, Evans DA, Bennett DA. Sex differences in the clinical manifestations of Alzheimer disease pathology. *Arch Gen Psychiatry*. 2005; 62:685–691. [PubMed: 15939846]
49. Bigler ED, Lowry NJ, Anderson CV, Johnson SC, Terry J, Steed M. Dementia, quantitative neuroimaging, and apolipoprotein E genotype. *Am J Neuroradiol*. 2000; 21:1857–1868. [PubMed: 11110538]
50. Hashimoto M, Yasuda M, Tanimukai S, Matsui M, Hirono N, Kazui H, Mori E. Apolipoprotein E epsilon4 and the pattern of regional brain atrophy in Alzheimer's disease. *Neurology*. 2001; 57:1461–1466. [PubMed: 11673590]
51. Lehtovirta M, Laakso MP, Soininen H, Helisalmi S, Mannermaa A, Helkala EL, Partanen K, Ryyanen M, Vainio P, Hartikainen P, Riekkinen PJ Sr. Volumes of hippocampus, amygdala and frontal lobe in Alzheimer patients with different apolipoprotein E genotypes. *Neuroscience*. 1995; 67:65–72. [PubMed: 7477910]
52. Pedraza O, Bowers D, Gilmore R. Asymmetry of the hippocampus and amygdala in MRI volumetric measurements of normal adults. *J Int Neuropsychol Soc*. 2004; 10:664–678. [PubMed: 15327714]
53. Gomez-Isla T, Hollister R, West H, Mui S, Growdon JH, Petersen RC, Parisi JE, Hyman BT. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzheimer's disease. *Ann Neurol*. 1997; 41:17–24. [PubMed: 9005861]
54. Peters A, Morrison JH, Rosene DL, Hyman BT. Are neurons lost from the primate cerebral cortex during normal aging? *Cereb Cortex*. 1998; 8:295–300. [PubMed: 9651126]
55. Peters A. Structural changes in the normally aging cerebral cortex of primates. *Prog Brain Res*. 2002; 136:455–465. [PubMed: 12143402]
56. Kemper, T. Neuroanatomical and neuropathological changes during aging and dementia. In: Albert, M.; Knoefel, J., editors. *Clinical Neurology of Aging*. 2. Oxford University Press; New York: 1994.
57. Tang Y, Nyengaard JR, Pakkenberg B, Gundersen HJ. Age-induced white matter changes in the human brain: a stereological investigation. *Neurobiol Aging*. 1997; 18:609–615. [PubMed: 9461058]
58. Marner L, Nyengaard JR, Tang Y, Pakkenberg B. Marked loss of myelinated nerve fibers in the human brain with age. *J Comp Neurol*. 2003; 462:144–152. [PubMed: 12794739]
59. Peters A, Rosene DL, Moss MB, Kemper TL, Abraham CR, Tigges J, Albert MS. Neurobiological bases of age-related cognitive decline in the rhesus monkey. *J Neuropathol Exp Neurol*. 1996; 55:861–874. [PubMed: 8759775]
60. Peters A, Sethares C, Killiany RJ. Effects of age on the thickness of myelin sheaths in monkey primary visual cortex. *J Comp Neurol*. 2001; 435:241–248. [PubMed: 11391644]
61. Bartzokis G, Sultzer D, Lu PH, Nuechterlein KH, Mintz J, Cummings J. Heterogeneous age-related breakdown of white matter structural integrity: implications for cortical “disconnection” in aging and Alzheimer's disease. *Neurobiol Aging*. 2004; 25:843–851. [PubMed: 15212838]
62. Kochunov P, Thompson PM, Lancaster JL, Bartzokis G, Smith S, Coyle T, Royall DR, Laird A, Fox PT. Relationship between white matter fractional anisotropy and other indices of cerebral

- health in normal aging: tract-based spatial statistics study of aging. *Neuroimage*. 2007; 35:478–487. [PubMed: 17292629]
63. Bartzokis G. Age-related myelin breakdown: a developmental model of cognitive decline and Alzheimer's disease. *Neurobiol Aging*. 2004; 25:5–18. [PubMed: 14675724]
64. Salat DH, Tuch DS, Greve DN, van der Kouwe AJ, Hevelone ND, Zaleta AK, Rosen BR, Fischl B, Corkin S, Rosas HD, Dale AM. Age-related alterations in white matter microstructure measured by diffusion tensor imaging. *Neurobiol Aging*. 2005; 26:1215–1227. [PubMed: 15917106]
65. Brun A, Englund E. A white matter disorder in dementia of the Alzheimer type: a pathoanatomical study. *Ann Neurol*. 1986; 19:253–262. [PubMed: 3963770]
66. Hyman BT, van Hoesen GW, Kromer LJ, Damasio AR. Perforant pathway changes and the memory impairment of Alzheimer's disease. *Ann Neurol*. 1986; 20:472–481. [PubMed: 3789663]
67. Beck KD, Powell-Braxton L, Widmer HR, Valverde J, Hefti F. *Igf1* gene disruption results in reduced brain size, CNS hypomyelination, and loss of hippocampal granule and striatal parvalbumin-containing neurons. *Neuron*. 1995; 14:717–730. [PubMed: 7718235]
68. Abraham J, Vincze A, Jewgenow I, Veszpremi B, Kravjak A, Gomori E, Seress L. Myelination in the human hippocampal formation from midgestation to adulthood. *Int J Dev Neurosci*. 2010; 28:401–410. [PubMed: 20417266]
69. Cotel MC, Bayer TA, Wirths O. Age-dependent loss of dentate gyrus granule cells in APP/PS1KI mice. *Brain Res*. 2008; 1222:207–213. [PubMed: 18585693]
70. Boyles JK, Zoellner CD, Anderson LJ, Kosik LM, Pitas RE, Weisgraber KH, Hui DY, Mahley RW, Gebicke-Haerter PJ, Ignatius MJ. A role for apolipoprotein E, apolipoprotein A-I, and low density lipoprotein receptors in cholesterol transport during regeneration and remyelination of the rat sciatic nerve. *J Clin Invest*. 1989; 83:1015–1031. [PubMed: 2493483]
71. Goodrum JF. Cholesterol from degenerating nerve myelin becomes associated with lipoproteins containing apolipoprotein E. *J Neurochem*. 56:2082–2086. [PubMed: 2027015]
72. Poirier J. Apolipoprotein E, cholesterol transport and synthesis in sporadic Alzheimer's disease. *Neurobiol Aging*. 2005; 26:355–361. [PubMed: 15639314]
73. Riddell DR, Zhou H, Atchison K, Warwick HK, Atkinson PJ, Jefferson J, Xu L, Aschmies S, Kirksey Y, Hu Y, Wagner E, Parratt A, Xu J, Li Z, Zaleska MM, Jacobsen JS, Pangalos MN, Reinhart PH. Impact of apolipoprotein E (ApoE) polymorphism on brain ApoE levels. *J Neurosci*. 2008; 28:11445–11453. [PubMed: 18987181]

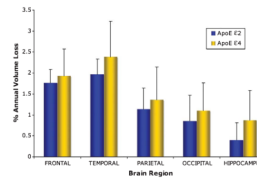


**Fig. 1.** Regions of interest of the temporal lobe regions (left panel) and the hippocampus (right panel) of both hemispheres are shown. The ROIs were manually delineated on the ICBM template by a trained anatomist using the Brainsuite software program [32].

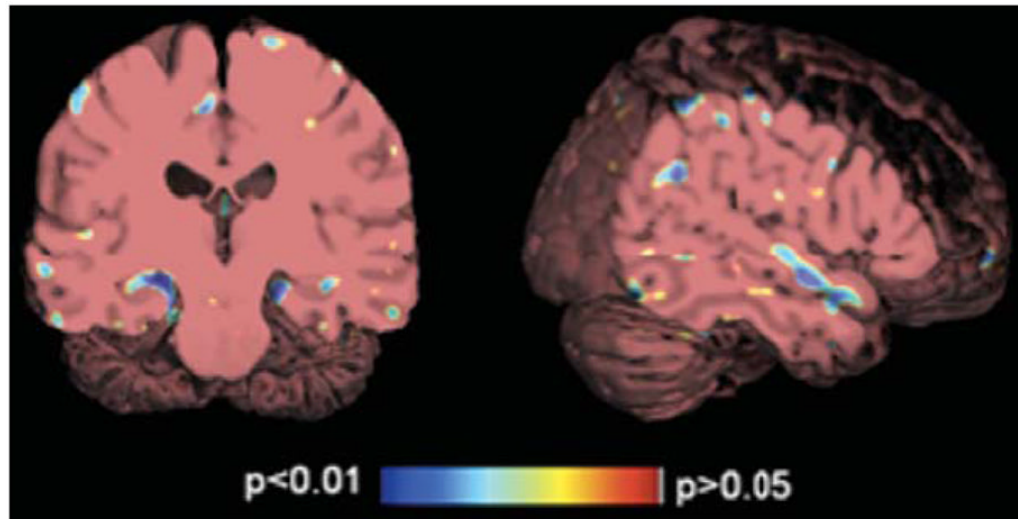


**Fig. 2.** Jacobian maps showing the mean annual rate of atrophy of brain tissue (in blue color) and ventricular enlargement (in red color) for the  $\epsilon 4$  carriers (top row) and the  $\epsilon 2$  carriers (bottom row). These tissue changes are shown as percentages, relative to the baseline scan, and are computed within each individual before averaging across subjects in the group.





**Fig. 3.** Bar plots showing percent volume loss per year in the brain regions of interest for ApoE  $\epsilon 2$  and  $\epsilon 4$  groups. The group differences were not statistically significant for any region ( $p > 0.06$ ).



**Fig. 4.** Significance maps showing that the  $\epsilon 4$  carriers demonstrate significantly greater annual atrophy rate (as indicated in blue) in the hippocampus (left panel) and the superior temporal gyrus (right panel) than the  $\epsilon 2$  group.

**Table 1**

Demographic and cognitive variables by ApoE group

Demographic variables	ApoE 2/3 ( <i>n</i> = 11)	ApoE 3/4 ( <i>n</i> = 16)	<i>t</i> or $\chi^2$	<i>p</i>
	Mean (s.d.)	Mean (s.d.)		
Age, y	67.0 (5.2)	65.0 (4.5)	1.06	0.301
Education, y	14.2 (1.9)	15.6 (2.4)	-1.65	0.112
Gender (m/f)	5m/6f	7m/9f	0.008	0.930
% white	64%	94%	3.92	0.048
Time between scans, y	4.8 (0.5)	4.8 (0.5)	0.30	0.770
Cognitive variables	Mean $\pm$ s.d. (range)	Mean $\pm$ s.d. (range)	<i>t</i>	<i>p</i>
MMSE	28.2 $\pm$ 1.1 (27–30)	28.7 $\pm$ 0.9 (27–30)	-0.92	0.376
CVLT-long delay-baseline				
Raw scores	11.5 $\pm$ 1.5 (9–14)	11.6 $\pm$ 2.0 (8–15)	0.05	0.960
<i>z</i> -scores	0.36 $\pm$ 0.50 (0.0 to +1.0)	0.31 $\pm$ 0.79 (-1.0 to +1.0)	0.19	0.852
CVLT-long delay-follow-up				
Raw scores	11.3 $\pm$ 3.1 (7–16)	11.2 $\pm$ 1.9 (9–15)	-0.19	0.854
<i>z</i> -scores	0.60 $\pm$ 1.07 (-1.0 to +2.0)	0.56 $\pm$ 0.81 (-1.0 to +2.0)	0.10	0.920