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APOE-epsilon4 and aging of medial temporal lobe gray matter in healthy adults older than 50 years

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Abstract

Atrophy of the hippocampus and surrounding temporal regions occurs in Alzheimer's disease (AD). APOE ϵ 4, the major genetic risk factor for late-onset AD, has been associated with smaller volume in these regions before amyloidosis can be detected by AD biomarkers. To examine APOE ϵ 4 effects in relation to aging, we performed a longitudinal MRI study involving cognitively normal adults (25 APOE ϵ 4 carriers and 31 ϵ 3 homozygotes), initially aged 51–75 years. We used growth curve analyses, which can provide information about APOE ϵ 4-related differences initially and later in life. Hippocampal volume was the primary outcome; nearby medial temporal regions were secondary outcomes. BDNF val66met was a secondary covariate. APOE ϵ 4 carriers had significantly smaller initial hippocampal volumes than ϵ 3 homozygotes. Rate of hippocampal atrophy was not greater in the APOE ϵ 4 group, even though age-related atrophy was detected in the overall sample. The findings add to the growing evidence that effects of APOE ϵ 4 on hippocampal size begin early in life, underscoring the importance of early interventions to increase reserve.

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Keywords

Aging; Apolipoprotein E4; Alzheimer Disease; Brain-Derived Neurotrophic Factor; Hippocampus; Longitudinal Studies; Magnetic Resonance Imaging

1. Introduction

After age, the APOE ϵ 4 genetic variant is the major risk factor for late onset Alzheimer's disease (LOAD) (Bertram et al., 2010). Furthermore, APOE ϵ 4 is associated with an earlier age of onset (Corder et al., 1993; Khachaturian et al., 2004; Kwon et al., 2010); but the mechanisms by which APOE ϵ 4 influences risk and age of onset of LOAD are unclear. Reports that APOE ϵ 4 carriers frequently have greater accumulation of cerebral amyloid and smaller hippocampal volumes are particularly interesting in light of AD mouse models that suggest roles of APOE ϵ 4 in prolonging clearance of beta amyloid ($A\beta$) (Castellano et al., 2011) and impairing hippocampal neurogenesis (Li et al., 2009).

In humans, APOE ϵ 4 has been frequently associated with earlier accumulation of $A\beta$, as assessed by PET imaging of fibrillar amyloid or by cerebrospinal fluid (CSF) assay of $A\beta$ 42. The earliest indication of $A\beta$ positivity may begin around age 55 among APOE ϵ 4 carriers, 20 years earlier than in noncarriers (Fleisher et al., 2013). APOE's influence on brain development is also increasingly recognized, particularly in regions affected early by AD. In an MRI study involving neonates, APOE ϵ 4 carrier status was associated with reduced temporal lobe gray matter (GM), as analyzed by tensor-based morphometry (Knickmeyer et al., 2013). Similarly, a longitudinal MRI study of adolescents reported that APOE ϵ 4 status was associated with thinner entorhinal cortex (ERC) (Shaw et al., 2007). Shaw et al. (2007) also noted that the APOE genotype differences seemed "fixed and non-progressive" from age 11 to 20 years, with no evidence of accelerated cortical loss. These indications of localized gray matter reduction are suggestive of lower tissue reserve in AD-vulnerable brain regions, and may also help explain why APOE ϵ 4 carriers are at increased risk of AD. In later adult life, less age-related atrophy would need to occur before a critical anatomical threshold associated with cognitive impairment is reached.

In addition to influences of APOE ϵ 4 on early brain morphology, influences of APOE ϵ 4 on brain function have been seen. As revealed by FDG PET imaging, young adult APOE ϵ 4 carriers showed signs of cortical hypometabolism that resembles an AD pattern (Reiman et al., 2004). As assessed by resting-state functional MRI, young (Filippini et al., 2009) and older adult APOE ϵ 4 carriers, including those who did not show evidence of amyloidosis (Sheline et al., 2010), showed functional connectivity differences. How should we interpret these APOE ϵ 4 differences in brain structure and function? Do these APOE ϵ 4 effects reflect "preclinical AD" (i.e., the presence of AD pathology in apparently normal individuals) (Sperling et al., 2011) or do they reflect early developmental effects?

Given the considerable evidence suggesting that APOE ϵ 4 affects both early brain development and $A\beta$ -mediated risk of AD, we performed a longitudinal MRI study involving subjects of a wide age range. We examined effects of APOE ϵ 4 on hippocampal volume and nearby medial temporal gray matter, using a growth curve approach, which can

provide information on $\epsilon 4$ -related differences initially and later in life. We expected that APOE $\epsilon 4$ carriers in our sample of 51- to 78-year-olds would have smaller initial volumes than noncarriers, in agreement with results on young subjects (Knickmeyer et al., 2013; Shaw et al., 2007). Second, APOE $\epsilon 4$ carriers might show progressive differences later in life, which would be consistent with reports of greater atrophy rates over time in older nondemented $\epsilon 4$ carriers (Lu et al., 2011; Risacher et al., 2010) and with the view that greater rates of MRI atrophy occur after A β deposition (Jack et al., 2010). The effect of the val66met variation in brain-derived neurotrophic factor (BDNF) was also included in the growth-curve analyses given its relevance to human brain development (Knickmeyer et al., 2013), hippocampal volume (Hajek et al., 2012), and hippocampal aging (von Bohlen und Halbach, 2010). In summary, our overall approach is relevant to assessing brain reserve in adult life and the age when a faster rate of atrophy may typically begin among APOE $\epsilon 4$ carriers.

2. Method

2.1. Participants

Participants were 56 volunteers (45 men and 11 women) who completed 1 to 3 MRI scans. Participants were selectively recruited into the MRI study with the aim of achieving an enriched proportion of APOE $\epsilon 4$ carriers. Twenty-five participants were APOE $\epsilon 4$ carriers (23 APOE $\epsilon 3/\epsilon 4$ heterozygotes; 2 APOE $\epsilon 4/\epsilon 4$ homozygotes); the remaining 31 were APOE $\epsilon 3/\epsilon 3$ homozygotes. These participants had previously been genotyped as part of the ongoing Stanford/VA Aviation Study, a longitudinal study of recreational and commercial pilots. The MRI study recruited Aviation Study participants who were at least 50 years old, were still actively flying (as assessed by FAA currency rules), and had a current medical certificate. Data for eight other MRI study participants were not included in the present analysis because their BDNF genotype was either unknown ($n = 5$) or it was the rare met/met genotype ($n = 3$). All participants agreed to have genotyping results withheld from them. Informed consent, approved by Stanford University and VA Palo Alto Health Care System Institutional Review Boards, was obtained from all participants.

2.2. Genotyping

APOE genotyping was performed on genomic DNA extracted from samples of frozen whole blood, buccal mucosa, or saliva using restriction isotyping (Hixson and Vernier, 1990) as previously described (Murphy et al., 1997). For blood samples, we used the Gentra PureGene kit (Gentra Systems, Minneapolis, MN); for buccal mucosa samples we used the protocol of Richards *et al.* (1993); for saliva, we extracted DNA from epithelial cells using the Oragene kit (DNA Genotek, Ottawa, ON).

2.3. Image acquisition

All MRI data were acquired on a 1.5 Tesla MRI scanner (General Electric Medical Systems, Milwaukee, WI) at the Veterans Affairs Palo Alto Health Care System. Participants were positioned on the scanner bed so that head movements were restricted. The following structural MR sequences were done on all participants using a standard head coil: a) a spin-echo, sagittal localizer 2D sequence of 5 mm thick slices (acquisition time = 1 min 44 s); b)

a proton density and T2-weighted spin-echo MRI, TR/TE1/TE2 = 5000/30/80 ms, 51 oblique axial 3 mm slices covering the entire brain and angulated parallel to the long axis of the hippocampal formation ($1.00 \times 1.00 \text{ mm}^2$ in plane resolution, acquisition time = 17 min); c) a 3D fast spoiled gradient recall acquisition, TR/TE = 9/2 ms, 15° flip angle, perpendicular to the long axis of the hippocampi ($1 \times 1 \text{ mm}^2$ in plane resolution, 1.5 mm coronal slices covering the entire brain, no skip, acquisition time = 7 min 58 s).

2.4. Hippocampal voluming

Hippocampal volumetry was carried out using a commercially available high dimensional brain mapping tool (Medtronic Surgical Navigation Technologies [SNT], Louisville, CO), that has previously been validated and compared to manual tracing of the hippocampus (Hsu et al., 2002). This semi-automated technique involves manual placement of 22 landmarks by a trained technician. Next, fluid image transformation is used to match the individual brains to a template brain (Christensen et al., 1997). The pixels corresponding to the hippocampus are then labeled and counted to obtain volumes. The SNT method has been validated and compared to manual tracing (left: $r = .92$; right: $r = .91$; $n=60$) (Hsu et al., 2002). Intra- and inter-rater reliability coefficients are .90 or better (Hsu et al., 2002; Schuff et al., 2009b). The SNT method has been implemented by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (Schuff et al., 2009b), and SNT has been used in our prior work showing hippocampal volume correlates with memory performance ($r = .47$) (Adamson et al., 2010). SNT total hippocampal volume, which includes hippocampal gray matter only and no nearby regions such as the parahippocampal gyrus (PHG), was chosen as the *a priori* primary ROI.

2.5. Secondary ROIs

Because APOE $\epsilon 4$ has been found to preferentially influence MTL gray matter more negatively than parietal or frontal regions in both healthy young samples (Knickmeyer et al., 2013; Shaw et al., 2007) and AD samples (e.g., Pievani et al., 2009; Wolk et al., 2010), two MTL regions near the hippocampus were examined using FreeSurfer (FS 4.5) image analysis suite: the ERC and the posterior PHG (labeled by FreeSurfer as "EC" and "PHG" respectively). Details about FS 4.5 are described at <http://surfer.nmr.mgh.harvard.edu/fswiki/LongitudinalChangeLog> (Reuter et al., 2012).

2.6. Statistical analyses

To adjust for differences in head size, each ROI was normalized by dividing the subject's ROI volume by that subject's Total Intracranial Volume (TIV). To convey results that are more meaningful than proportions, the ROI/TIV values were multiplied by the median TIV of the sample. For the longitudinal data analysis of each normalized ROI, we used a mixed-effects growth model (Singer and Willett, 2003), which can provide information about a developmental trajectory in terms of the estimated initial starting point (intercept "I_i") and rate of change (slope "S"). We modeled effects of APOE $\epsilon 4$ and BDNF *met* status on initial volume "I_i" and on rate of change over age "S." We assumed a linear age trend; inclusion of an age*age term did not significantly improve the fit of the growth model (results not reported). Thus, there were three independent variables—age (at time of MRI), APOE $\epsilon 4$,

and BDNF *met* status. Age was centered at 61.25 yrs, the median age of this sample at the first scan. APOE $\epsilon 4$ status ($\epsilon 3/\epsilon 3$ vs. $\epsilon 4$ carrier) and BDNF *met* status (*met/val* vs. *val/val*) were centered around zero as $-.5$ and $+.5$ (Kraemer and Blasey, 2004). In the results below, the β_{I1} term is an estimate of how much APOE $\epsilon 4$ carriers and $\epsilon 3$ homozygotes differ in initial volume “ I_1 ”. The β_{S1} term is an estimate of how much APOE $\epsilon 4$ carriers and $\epsilon 3$ homozygotes differ in rate of change over age “ S ” (i.e., the interaction of APOE with age). Analogously, the β_{I2} and β_{S2} terms indicate how much BDNF *met* carriers and noncarriers differ in initial volume and in change over age. To examine sex effects, the model parameters were initially allowed to reflect the influence of sex. There were no significant effects of sex or interactions of sex with age ($F_s < 1$; data not shown), so sex was dropped as a covariate in the final models of hippocampal, ERC and posterior PHG volume. Growth models were fit using the PROC MIXED procedure in SAS software, version 9.3 (SAS Institute, Cary, NC).

3. Results

3.1. Sample Characteristics

Table 1 summarizes baseline characteristics of the participants, separated by APOE $\epsilon 4$ carrier status. There were no significant differences between APOE $\epsilon 4$ carriers and $\epsilon 3$ homozygotes in memory performance ($p = .29$), as measured by the Rey Auditory Verbal Learning Test delayed recall score (Rey, 1958). No participant performed > 1.5 SDs below the mean of normative data (Geffen, 1995; Ivnik et al., 1992) (range of raw scores = 4 to 15 words recalled; percentile range = 14.5 to 99th percentile). A greater proportion of women were represented in the $\epsilon 3/\epsilon 3$ group than the $\epsilon 4$ carrier group, $p = .049$. Otherwise, the groups were comparable in terms of age, family history of dementia, years of education, and health-related variables; 96.8 % of all participants reported their health to be “good” or “excellent.”

Thirty-nine (69.6%) participants had multiple MRIs; the average interval from the first to the last scan was 3.3 ± 1.0 years (total 116 scans). Participants with follow-up memory assessments continued to perform within 1.5 SD of the recall score expected for their age (range of raw scores = 2 to 15; percentile range = 8th to 99th percentile), with $\epsilon 3$ homozygotes ($M = 10.6 \pm 2.8$) and APOE $\epsilon 4$ carriers ($M = 9.2 \pm 3.7$) performing comparably at the last assessment, $p = .20$. APOE groups did not differ in the percentage that had longitudinal data, $p = .13$ nor did they differ in the mean length of follow-up, $p = .18$.

3.2. Hippocampal Volume

Table 2 lists the parameter estimates of the mixed-effects growth model. The parameter β_{I1} is a test of the effect of APOE $\epsilon 4$ on ‘Initial Volume (I_1).’ The APOE $\epsilon 4$ group had significantly smaller initial hippocampal volumes than the $\epsilon 3$ homozygotes ($\beta_{I1} = -0.25$ cc; $p = .042$; estimated standardized effect size “ d ” = $-.56$). As illustrated in Figure 1, the mean trend line of the APOE $\epsilon 4$ group lies below that of the $\epsilon 3/\epsilon 3$ group. There was significant age-related atrophy in the sample as a whole (mean Change over Age $\eta_S = -0.051$ cc per year; $p < 0.0001$, equivalent to 1% volume loss per year). The rate of atrophy did not differ

significantly between $\epsilon 4$ carriers and $\epsilon 3$ homozygotes ($\beta_{S1} = 0.006$ cc; $p = .74$; $d = .09$). As can be seen in Figure 1, the mean trend lines for APOE $\epsilon 4$ carriers and $\epsilon 3$ homozygotes are nearly parallel (n.s. interaction of APOE with age). BDNF *met* status did not predict initial hippocampal volume or rate of atrophy, as indicated by the nonsignificant β_{I2} and β_{S2} terms in Table 2.

The parameter estimates of the model can be used to predict total hippocampal volume for a particular combination of age and APOE $\epsilon 4$ values (.5, if APOE $\epsilon 4$ or $-.5$ if non $\epsilon 4$). The predicted hippocampal volume at the midpoint age of 61 years is 5.04 cc (η_I). At age 61, APOE $\epsilon 4$ carriers have an expected mean volume of 4.92 cc, and noncarriers have an expected volume of 5.17 cc. (Initial Level (I) = $\eta_I + \beta_{I1}$; where $\eta_I = 5.04$ cc and $\beta_{I1} = -.25*(+.5, \text{ if APOE } \epsilon 4 \text{ or } -.5 \text{ if non } \epsilon 4)$). This APOE $\epsilon 4$ -related difference of 0.25 cc in hippocampal volume is roughly equivalent being five years older ($\eta_S * 5 \text{ years}$, or $-.05 \text{ cc/year} * 5 \text{ years} = -.25 \text{ cc}$).

3.3. Secondary ROIs

There were no significant APOE-related decreases in initial volumes of the ERC or the posterior PHG (p 's $> .30$); nor were there APOE $\epsilon 4$ effects on rates of atrophy (p 's $> .30$). The BDNF *val/val* group had larger initial posterior PHG volumes on average than the *val/met* group ($\beta_{I2} = 0.52$ cc; $SE = 0.16$, $p = .0025$) relative to the midpoint of 5.01 cc. *Val/val* status did not slow the rate of age-related atrophy in either ROI, p 's $> .9$, although, in the sample as a whole, significant age-related atrophy was detected (p 's < 0.004). For the ERC, the average yearly loss was 0.028 cc (0.66%); for the posterior PHG, average loss was 0.038 cc/yr (0.75%).

4. Discussion

APOE $\epsilon 4$ carriers had hippocampal volumes that were 5% smaller than $\epsilon 3$ homozygotes on average, with no evidence of a significantly greater rate of atrophy with increasing age. This pattern of APOE $\epsilon 4$ differences in MTL gray matter is analogous to that observed in a longitudinal study of adolescents, in which the ERC was 4% thinner in APOE $\epsilon 4$ carriers, with no evidence of accelerated thinning across ages 11 to 20 years (Shaw et al., 2007). Less MTL gray matter in association with APOE $\epsilon 4$ has also been reported in cross-sectional studies of neonates (Knickmeyer et al., 2013), young adults (O'Dwyer et al., 2012), and healthy middle-aged to older adults (den Heijer et al., 2002; Potkin et al., 2009; Wishart et al., 2006). Often, MTL gray matter differences related to the $\epsilon 4$ allele have been subtle in that reductions were reported only for the right hippocampus (Lind et al., 2006; Tohogi et al., 1997), only among $\epsilon 4$ homozygotes (Lemaitre et al., 2005), or only in some MTL subregions, specifically the dentate gyrus, CA3 (Mueller et al., 2008), ERC and subiculum (Burggren et al., 2008). The subtleness is also reflected by many nonsignificant trends (e.g., Chen et al., 2012; Richter-Schmidinger et al., 2011; Schmidt et al., 1996; Tupler et al., 2007). In the present study, longitudinal MRI data helped model the effects of APOE $\epsilon 4$ (and aging) with greater power.

One mechanism of APOE $\epsilon 4$ that could account for reduced volume at any early age is decreased hippocampal neurogenesis. As demonstrated in a series of experiments examining

GABAergic interneuron function in apoE4 Knock-In adult mice, neurons in the dentate gyrus did not mature normally or completely (Li et al., 2009). The SNT method we used for hippocampal voluming includes the dentate gyrus, CA1-3 and subiculum. The dentate gyrus, CA3, subiculum and ERC regions appear to be differentially affected in APOE ϵ 4 carriers (Burggren et al., 2008; Mueller et al., 2008). In contrast to SNT, FreeSurfer parcellates the entire brain and is less specific to the hippocampal formation; it generates a larger hippocampal volume that includes not only the dentate gyrus, CA1-3 and subiculum, but also additional white and gray matter (fimbria, alveus, infralimbic gyrus, and parts of the amygdala and parahippocampal gyrus) (Schuff et al., 2009a). SNT is well suited to detect subtle effects of APOE ϵ 4 on hippocampal volume.

Even though APOE ϵ 4 carriers had significantly smaller initial hippocampal volumes than ϵ 3 homozygotes, the rates of hippocampal atrophy did not differ significantly in this study. In the current model of AD biomarkers (Jack et al., 2010), initial deposition of A β is crucial for the rate of MRI atrophy to increase. Thus, A β deposition alone defines “Stage 1- Preclinical AD” and abnormal MRI atrophy is “Stage 2 – Preclinical AD” per recent guidelines for research on preclinical AD (Sperling et al., 2011). Based on these guidelines and estimates of the age-specific prevalence of A β positivity (A β +) among APOE ϵ 4 carriers and noncarriers, the majority of our participants were too young to have preclinical AD at the time of their MRIs. Recent amyloid imaging data suggests that A β is rare among cognitively normal noncarriers younger than 75 (Fleisher et al., 2013). Among cognitively normal APOE ϵ 4 carriers, the prevalence of A β may be 10 to 15% among 50–59 year-olds (Fleisher et al., 2013; Morris et al., 2010); rising to around 50% among 60–79 year-old APOE ϵ 4 carriers (Fleisher et al., 2013; Morris et al., 2010; Reiman et al., 2009; Rowe et al., 2010). By applying these prevalence rates to the age distribution of the ϵ 4 carriers in our study cohort, we could estimate that two-thirds of the ϵ 4 carriers would not have had preclinical AD at the time of their MRIs. By this reasoning and the current rubric for biomarkers of preclinical AD, the non-significant effect of APOE ϵ 4 on the rate of MRI atrophy is not surprising. Two important limitations of the present study are the modest sample size, which limits statistical power to detect an APOE ϵ 4 difference in the slope assessing MRI atrophy rate, and the lack of amyloid imaging to assess preclinical AD.

The likelihood of detecting an effect of APOE ϵ 4 on rate of hippocampal atrophy would be expected to be higher in older-age samples, in line with the increasing prevalence of preclinical AD. Indeed, there are several reports on older nondemented subjects in which hippocampal change was greater in APOE ϵ 4 carriers (mean age 76) (Chiang et al., 2011; Jak et al., 2007; Morra et al., 2009; Risacher et al., 2010). The literature with regard to older-age samples is not entirely consistent, as one study detected faster atrophy only in APOE ϵ 4 homozygotes (Crivello et al., 2010) and three studies did not detect atrophy-rate differences between ϵ 4 carriers and noncarriers (Du et al., 2006; Jack et al., 1998; Schuff et al., 2009b). Also, some studies of late middle-aged subjects have reported significantly greater atrophy in APOE ϵ 4 carriers (e.g., Cohen et al., 2001). It is worth noting however, that these studies may have had greater sensitivity to detect differences, in that APOE ϵ 2/ ϵ 3 subjects were selected as the non-carrier group (Lu et al., 2011), or the ϵ 4 carrier and noncarrier groups were enriched with APOE ϵ 4/ ϵ 4 and APOE ϵ 2/ ϵ 3 subjects, respectively (Moffat et al., 2000). Collectively, the MRI literature is reasonably consistent with the

notion that hippocampal atrophy rate is age- and genotype-dependent in tandem with risk for preclinical AD.

Early interventions that increase tissue reserve in regions such as the hippocampus could potentially delay the onset of AD symptoms. Interventions that have potential to increase hippocampal volume include aerobic exercise and cognitive stimulation (Fotuhi et al., 2012). Two controlled trials of exercise interventions have demonstrated increases in hippocampal volume (Erickson et al., 2011; Pajonk et al., 2010). Because the BDNF val66met polymorphism is relevant to tissue reserve, it was included as a covariate in the mixed-effects growth model. No effect of BDNF val66met on hippocampal volume was detected. A recent meta-analysis reported an effect size of 0.41 for the effect of BDNF on hippocampal volume (Hajek et al., 2012), suggesting that the present study was not powered to detect a significant effect of BDNF on hippocampal volume. The BDNF *val/val* group had more MTL volume near the hippocampus, consistent with studies of young individuals (e.g., Knickmeyer et al., 2013). Even though the effect size of BDNF val66met is small (Hajek et al., 2012), it would be worthwhile to include it as a covariate in future studies because BDNF val66met genotype may interact with physical activity level (Kim et al., 2011).

In conclusion, it is remarkable the same regions that are smaller in APOE ϵ 4 carriers early in life are the regions affected in early AD. Thus, it seems that the biological effects of apoE4 are in play throughout life. Indeed, the mechanisms that lead to less gray matter density early in life may overlap with the mechanisms that lead to earlier onset of preclinical AD. One possible lifelong mechanism could be disruption of synaptic input in the presence of apoE4 (Ji et al., 2003; Koffie et al., 2012; Li et al., 2009). It has been suggested that apoE4 impairs synaptic function via its role as a co-factor that stabilizes oligomeric A β and directs it to synapses, leading to toxic accumulation of A β and synapse shrinkage (Koffie et al., 2012). Blocking oligomeric A β -apoE4 interactions has been offered as a therapeutic strategy for preventing this AD-related process (Koffie et al., 2012).

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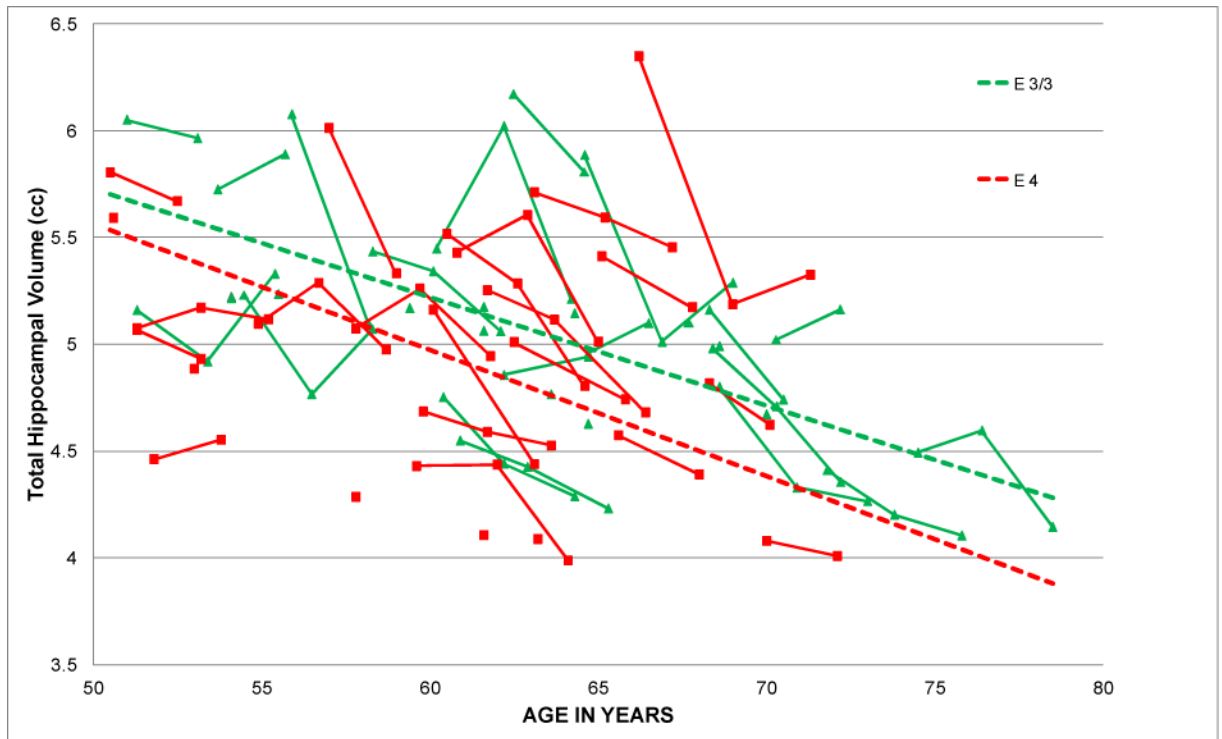


Fig. 1. Observed individual trajectories and model-estimated mean trajectories for *APOE* $\epsilon 4$ carriers and $\epsilon 3/\epsilon 3$ homozygotes

Table 1

Baseline demographic and health characteristics of the 56 participants, grouped according to APOE status.

Characteristic	<i>APOE</i> ε3/ε3 homozygotes <i>n</i> = 31	<i>APOE</i> ε4 carriers <i>n</i> = 25
Age, <i>M</i> ± <i>SD</i>	62.2 ± 6.3	59.4 ± 5.7
(range in years)	(51 – 75)	(51 – 70)
Rey AVLT delayed recall, <i>M</i> ± <i>SD</i>	10.2 ± 2.8	9.4 ± 2.9
% Family history of dementia: “yes/no/not sure”	29/61/10%	32/64/4%
Years of education, <i>M</i> ± <i>SD</i>	16.9 ± 1.6	17.6 ± 2.0
Women, <i>n</i> (%)	9 (29%)	2 (8%)
% Self-rated health “excellent/good/fair”	42/55/3%	44/52/4%
% Cholesterol-lowering medications	23%	32%
% Anti-hypertensive medications	29%	28%
BDNF val/val, <i>n</i> (%)	18 (58%)	12 (48%)
<i>n</i> (%) with multiple scans	19 (61%)	20 (80%)
Duration of follow-up (years) [‡]	3.2 ± 1.0	3.3 ± 1.1

Rey Auditory Verbal Learning Test (Rey, 1958), delayed recall score (max = 15 words).

SD: standard deviation

[‡] Subjects in this category had more than one scan.

Table 2Growth Curve Analysis of Hippocampal Volume in Relation to APOE, BDNF and Age.^a

	Parameter Estimate (SE)	<i>p</i>
Initial Volume ^b (<i>I_i</i>)		
Intercept (mean, η_I)	5.04 (0.06)	<0.0001
<i>APOE</i> (β_{I1})	-0.25 (0.12)	0.042
<i>BDNF</i> (β_{I2})	-0.01 (0.12)	0.93
Change over Age ^c (<i>S</i>)		
Intercept (mean, η_S)	-0.051 (0.01)	<0.0001
<i>APOE</i> (β_{S1})	0.006 (0.02)	0.74
<i>BDNF</i> (β_{S2})	0.002 (0.02)	0.92

^aThe model for the outcome at a given age was: $Y_{it} = I_i + S*(age\ centered) + e_{it}$, where the outcome *Y* for individual *i* at age *t* is a function of random initial level *I_i*. The residual e_{it} is assumed to be normally distributed.

^bThe model for initial volume was:

$$I_i = \eta_I + \beta_{I1} * (APOE\ centered) + \beta_{I2} * (BDNF\ centered) + \zeta I_i$$

The random effect residual ζI_i is assumed to be normally distributed.

^cThe model for change over age (slope) was:

$$S = \eta_S + \beta_{S1} * (APOE\ centered) + \beta_{S2} * (BDNF\ centered)$$

APOE $\epsilon 3/3$ and BDNF *val/met* were coded as -.5; APOE $\epsilon 4$ and BDNF *val/val* were coded as +.5