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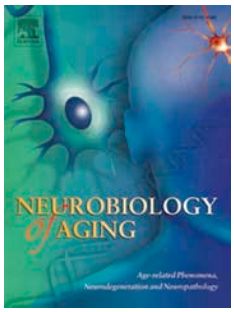

APOE-epsilon4 and aging of medial temporal lobe gray matter in healthy adults over 50

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APOE-epsilon4 and aging of medial temporal lobe gray matter in healthy adults over 50

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Abstract

Atrophy of the hippocampus and surrounding temporal regions occurs in Alzheimer's disease (AD). APOE ε4, 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 ε4 effects in relation to aging, we performed a longitudinal MRI study involving cognitively normal adults (25 APOE ε4 carriers and 31 ε3 homozygotes), initially aged 51-75 years. We used growth curve analyses, which can provide information about APOE ε4-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 ε4 carriers had significantly smaller initial hippocampal volumes than ε3 homozygotes. Rate of hippocampal atrophy was not greater in the APOE ε4 group, even though age-related atrophy was detected in the overall sample. The findings add to the growing evidence that effects of APOE ε4 on hippocampal size begin early in life, underscoring the importance of early interventions to increase reserve.

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

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1. Introduction

After age, the APOE ε4 genetic variant is the major risk factor for late onset Alzheimer's disease (LOAD) (Bertram et al., 2010). Furthermore, APOE ε4 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 ε4 influences risk and age of onset of LOAD are unclear. Reports that APOE ε4 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 ε4 in prolonging clearance of beta amyloid (Aβ) (Castellano et al., 2011) and impairing hippocampal neurogenesis (Li et al., 2009).

In humans, APOE ε4 has been frequently associated with earlier accumulation of Aβ, as assessed by PET imaging of fibrillar amyloid or by cerebrospinal fluid (CSF) assay of Aβ42. The earliest indication of Aβ positivity may begin around age 55 among APOE ε4 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 ε4 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 ε4 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 ε4 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 ε4 on early brain morphology, influences of APOE ε4 on brain function have been seen. As revealed by FDG PET imaging, young adult APOE ε4 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 ε4 carriers, including those who did not show evidence of amyloidosis (Sheline et al., 2010), showed functional connectivity differences. How should we interpret these APOE ε4 differences in brain structure and function? Do these APOE ε4 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 ε4 affects both early brain development and Aβ-mediated risk of AD, we performed a longitudinal MRI study involving subjects of a wide age range. We examined effects of APOE ε4 on hippocampal volume and nearby medial temporal gray matter, using a growth curve approach, which can provide information on ε4-related differences initially and later in life. We expected that APOE ε4 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 ε4 carriers might show progressive differences later in life, which would be consistent with reports of greater atrophy rates over time in older nondemented ε4 carriers (Lu et al., 2011; Risacher et al., 2010) and with the view that greater rates of MRI atrophy occur after Aβ 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 ε4 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 ε4 carriers. Twenty-five participants were APOE ε4 carriers (23 APOE ε3/ε4 heterozygotes; 2 APOE ε4/ε4 homozygotes); the remaining 31 were APOE ε3/ε3 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, Ottowa, 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 x 1.00 mm^2 in plane resolution, acquisition time = 17 min); c) a 3D fast spoiled gradient recall acquisition, TR/TE = 9/2 ms, 15º flip angle, perpendicular to the long axis of the hippocampi (1 x 1 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 ε4 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 ε4 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 ε4, and BDNF *met* status. Age was centered at 61.25 yrs, the median age of this sample at the first scan. APOE ε4 status (ε3/ε3 vs. ε4 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 ε4 carriers and ε3 homozygotes differ in initial volume "I_i". The β_{S1} term is an estimate of how much APOE ε4 carriers and ε3 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 ε4 carrier status. There were no significant differences between APOE ε4 carriers and ε3 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 ε3/ε3 group than the ε4 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 ε3 homozygotes (M = 10.6 ± 2.8) and *APOE* ε4 carriers (M = 9.2 ± 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 ε4 on 'Initial Volume (I_i).' The APOE ε4 group had significantly smaller initial hippocampal volumes than the ε3 homozygotes (β_{I1} = -0.25 cc; p = .042; estimated standardized effect size "d" = -.56). As illustrated in Figure 1, the mean trend line of the APOE ε4 group lies below that of the ε3/ε3 group. There was significant age-related atrophy in the sample as a whole (mean Change over Age η_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 ε4 carriers and ε3 homozygotes (β_{S1} = 0.006 cc; p =.74; d = .09). As can be seen in Figure 1, the mean trend lines for APOE ε4 carriers and ε3 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 non-significant 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 ε4 values (.5, if *APOE* ε4 or -.5 if non ε4). The predicted hippocampal volume at the midpoint age of 61 years is 5.04 cc (η_I). At age 61, APOE ε4 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 η_I = 5.04 cc and β_{I1} = -0.25*(+.5. if *APOE* ε4 or -.5 if non ε4). This APOE ε4-related difference of 0.25 cc in hippocampal volume is roughly equivalent being five

years older (η_S * 5 years, or -0.05 cc/year * 5 years = -0.25 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 ε4 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 (β_{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 ε4 carriers had hippocampal volumes that were 5% smaller than ε3 homozygotes on average, with no evidence of a significantly greater rate of atrophy with increasing age. This pattern of APOE ε4 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 ε4 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 ε4 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 ε4 allele have been subtle in that reductions were reported only for the right hippocampus (Lind et al., 2006; Tohgi et al., 1997), only among ε4 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 non-significant 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 ε4 (and aging) with greater power.

One mechanism of APOE ε4 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 ε4 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 ε4 on hippocampal volume.

Even though APOE ε4 carriers had significantly smaller initial hippocampal volumes than ε3 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 Aβ is crucial for the rate of MRI atrophy to increase. Thus, Aβ 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 Aβ positivity (Aβ+) among APOE ε4 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 Aβ+ is rare among cognitively normal noncarriers younger than 75 (Fleisher et al., 2013). Among cognitively normal APOE ε4 carriers, the prevalence of Aβ+ 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 ε4 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 ε4 carriers in our study cohort, we could estimate that two-thirds of the ε4 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 ε4 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 ε4 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 ε4 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 ε4 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 ε4 homozygotes (Crivello et al., 2010) and three studies did not detect atrophy-rate differences between ε4 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 ε4 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 ε2/ε3 subjects were selected as the non-carrier group (Lu et al., 2011), or the ε4 carrier and noncarrier groups were enriched with APOE ε4/ε4 and APOE ε2/ε3 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 ε4 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 Aβ and directs it to synapses, leading to toxic accumulation of Aβ and synapse shrinkage (Koffie et al., 2012). Blocking oligomeric Aβ-apoE4 interactions has been offered as a therapeutic strategy for preventing this AD-related process (Koffie et al., 2012).

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Table 1
Baseline demographic and health characteristics of the 56 participants, grouped according to APOE status.

Characteristic	*APOE* ε3/ε3 homozygotes *n* = 31	*APOE* ε4 carriers *n* = 25
Age , *M* ± *SD*	62.2 ± 6.3	59.4 ± 5.7
(range in years)	(51 - 75)	(51 - 70)
Rey AVLT delayed recall, *M* ± *SD*	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, *M* ± *SD*	16.9 ± 1.6	17.6 ± 2.0
Women, *n* (%)	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, *n* (%)	18 (58%)	12 (48%)
n (%) 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 2
Growth Curve Analysis of Hippocampal Volume in Relation to APOE, BDNF and Age.[a]

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

[a] The model for the outcome at a given age was: $Y_{it} = I_i + S*(\text{age centered}) + e_{it}$, 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.

[b] The model for initial volume was:
$I_i = \eta_I + \beta_{I1}*(\text{APOE centered}) + \beta_{I2}*(\text{BDNF centered}) + \zeta I_i$
The random effect residual ζI_i is assumed to be normally distributed.

[c] The model for change over age (slope) was:
$S = \eta_S + \beta_{S1}*(\text{APOE centered}) + \beta_{S2}*(\text{BDNF centered})$

APOE ε3/3 and BDNF *val/met* were coded as -.5; APOE ε4 and BDNF *val/val* were coded as +.5

Fig. 1. Observed individual trajectories and model-estimated mean trajectories for *APOE* ε4 carriers and ε3/ε3 homozygotes

<insert Figure 1 here>