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Longitudinal brain imaging in preclinical Alzheimer disease: impact of *APOE* ε4 genotype

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While prior work reliably demonstrates that the *APOE* ε4 allele has deleterious group level effects on Alzheimer disease pathology, the homogeneity of its influence across the lifespan and spatially in the brain remains unknown. Further it is unclear what combinations of factors at an individual level lead to observed group level effects of *APOE* genotype. To evaluate the impact of the *APOE* genotype on disease trajectories, we examined longitudinal MRI and PET imaging in a cohort of 497 cognitively normal middle and older aged participants. A whole-brain regional approach was used to evaluate the spatial effects of genotype on longitudinal change of amyloid-β pathology and cortical atrophy. Carriers of the ε4 allele had increased longitudinal accumulation of amyloid-β pathology diffusely through the cortex, but the emergence of this effect across the lifespan differed greatly by region (e.g. age 49 in precuneus, but 65 in the visual cortex) with the detrimental influence already being evident in some regions in middle age. This increased group level effect on accumulation was due to a greater proportion of ε4 carriers developing amyloid-β pathology, on average doing so at an earlier age, and having faster amyloid-β accumulation even after accounting for baseline amyloid-β levels. *APOE* ε4 carriers displayed faster rates of structural loss in primarily constrained to the medial temporal lobe structures at around 50 years, although this increase was modest and proportional to the elevated disease severity in *APOE* ε4 carriers. This work indicates that influence of the *APOE* gene on pathology can be detected starting in middle age.

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Abbreviations: CDR = Clinical Dementia Rating; PIB = ^{11}C-Pittsburgh compound B; SUVR = standardized uptake value ratio

Introduction

Alzheimer's disease is the most common cause of dementia in individuals above the age of 60. The most significant genetic modifier of late-onset Alzheimer disease is the apolipoprotein E (*APOE*) gene (Corder *et al.*, 1993; Saunders *et al.*, 1993; Strittmatter *et al.*, 1993; Reiman *et al.*, 1996). APOE is a protein that functions as a ligand in receptor-mediated endocytosis of lipoprotein particles in the CNS and the periphery (Holtzman and Herz, 2012). The ε4 allele of *APOE* increases the risk of developing Alzheimer's disease and lowers the age of onset, while the

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ε2 allele is associated with a lower risk of Alzheimer's disease (Corder *et al.*, 1993; Reiman *et al.*, 2009). The association between the presence of an *APOE* ε4 allele and the clinical risk of Alzheimer's disease has been well established in the literature (Farrer *et al.*, 1997). While the exact mechanism of the relationship is still an active area of research (Mahoney-Sanchez *et al.*, 2016), one of the major effects of the APOE protein on Alzheimer's disease risk is that it influences amyloid-β aggregation and clearance (Huynh *et al.*, 2017).

The pathological hallmarks of Alzheimer's disease are neurofibrillary tangles and amyloid-β plaques (Braak and Braak, 1991), and significant work has been done evaluating the effect of *APOE* ε4 allele on these biomarkers. Previous studies of pathology, CSF and PET imaging of the brain consistently find a relationship between *APOE* ε4 genotype and elevated amyloid-β burden (Schmechel *et al.*, 1993; Morris *et al.*, 2010; Rowe *et al.*, 2010; Johnson *et al.*, 2013; Mathis *et al.*, 2013; Murphy *et al.*, 2013; Villemagne and Rowe, 2013; Risacher *et al.*, 2015; Gottesman *et al.*, 2016). Whereas studies overwhelmingly find that the *APOE* ε4 genotype is associated with worse amyloid-β pathology, its effect on markers of neurodegeneration such as CSF tau, fludeoxyglucose PET, and structural MRI are less consistent. Studies have reported both an influence of the *APOE* ε4 allele (Reiman *et al.*, 1996; Galasko *et al.*, 1998; Liu *et al.*, 2010; Jagust *et al.*, 2012; Hostage *et al.*, 2013; Lehmann *et al.*, 2013; Roussotte *et al.*, 2014) and no effect (Soininen *et al.*, 1995; Jack *et al.*, 1998; Reiman *et al.*, 1998; Sunderland *et al.*, 2004; Drzezga *et al.*, 2009; Fan *et al.*, 2010).

Perhaps most importantly, the fundamental interpretation of *APOE* ε4 effects on Alzheimer's disease pathology has focused on general group effects while ignoring parallel ways such effects could arise. For example, it is unclear from the literature whether population level *APOE* ε4 effects on amyloid-β pathology are a result of a higher proportion of *APOE* ε4 carriers developing Alzheimer's disease pathology, ε4 carriers developing Alzheimer's disease pathology at an earlier age, whether ε4 carriers have an increased rate of amyloid-β accrual, or a combination of the three. These questions are critical to understanding the influence of *APOE* on Alzheimer's disease as well as for clinical trials that are using ε4 genotype or PET amyloid-β levels to select participants (Reiman *et al.*, 2011; Sperling *et al.*, 2014). Disentangling these questions may be best addressed through the investigation of *APOE* ε4 effects using longitudinal data in a population that spans both middle and older age.

Reports on the relationship of *APOE* ε4 carrier status on longitudinal accumulation of amyloid-β pathology have been mixed, with studies showing that the *APOE* ε4 genotype increases the rate of change of amyloid-β pathology (Grimmer *et al.*, 2010; Villemagne *et al.*, 2011; Jack *et al.*, 2013*b*), while others find no genotype effect (Vlassenko *et al.*, 2011; Resnick *et al.*, 2015). When examining *APOE* genotype effects across the lifespan, prior works have reported a significant ε4 and age interaction, (Fleisher *et al.*, 2013; Scheinin *et al.*, 2014; Jansen *et al.*, 2015) although this is not always the case (Rodrigue *et al.*, 2012).

The most common neurodegenerative biomarker studies with APOE have been structural MRI. Both cross-sectional (Hashimoto *et al.*, 2001; Liu *et al.*, 2010; Hostage *et al.*, 2013; Manning *et al.*, 2014) and longitudinal (Geroldi *et al.*, 1999; Mori *et al.*, 2002; Van De Pol *et al.*, 2007; Morra *et al.*, 2009; Risacher *et al.*, 2010; Hostage *et al.*, 2014; Manning *et al.*, 2014) studies have reported significant effects of *APOE* genotype on hippocampal volume or cortical thickness in diseased populations, but other studies have shown no effect (Soininen *et al.*, 1995; Jack *et al.*, 1998; Reiman *et al.*, 1998; Cohen *et al.*, 2001; Lemaitre *et al.*, 2005; Drzezga *et al.*, 2009; Schuff *et al.*, 2009; Fan *et al.*, 2010; Leung *et al.*, 2013). A similar pattern is seen within populations of only cognitively normal older adults, with studies finding differences between carriers and non-carriers on rates of change in medial temporal lobe structures (Moffat *et al.*, 2000; Chen *et al.*, 2007; Risacher *et al.*, 2010; Lu *et al.*, 2011; Cohen *et al.*, 2013), while other work does not (Du *et al.*, 2006; Taylor *et al.*, 2014).

This heterogeneity in the literature may be attributed to inconsistencies in the populations studied, insufficient longitudinal follow-up, and relatively modest participant cohorts. The majority of the studies evaluating the effect of *APOE* ε4 on longitudinal changes in amyloid-β have either exclusively looked at impaired (Alzheimer's disease or mild cognitive impairment) patients or combined healthy controls with cognitively impaired individuals (Jack *et al.*, 2009, 2013*b*; Grimmer *et al.*, 2010; Villemagne *et al.*, 2011; Bilgel *et al.*, 2016). Changes in Alzheimer's disease pathology can begin decades before the onset of dementia (Bateman *et al.*, 2012; Jack *et al.*, 2013*a*). The focus of APOE studies on older and already impaired individuals has left the question of how the *APOE* ε4 genotype modulates early changes in Alzheimer's disease pathology largely unanswered.

Pathology is not uniformly distributed, but has a spatial evolution in the brain as the disease progresses (Benzinger *et al.*, 2013; Gordon *et al.*, 2014). The majority of studies in the literature have looked at whole-brain summary measures of amyloid-β (Morris *et al.*, 2010; Rowe *et al.*, 2010; Johnson *et al.*, 2013; Mathis *et al.*, 2013; Risacher *et al.*, 2015; Gottesman *et al.*, 2016). Of those that have used a regional approach, some have not seen regional variability in ε4 effects (Murphy *et al.*, 2013), while others have found effects in primarily frontal cortex regions (Reiman *et al.*, 2009; Scheinin *et al.*, 2014) or more posterior temporal-parietal regions (Fleisher *et al.*, 2013). The *APOE* ε4 relationship on structural MRI has primarily focused on hippocampus or surrounding medial temporal lobe structures, with a minority of studies utilizing a whole-brain voxel-wise or regions of interest approach (Geroldi *et al.*, 1999; Lu *et al.*, 2011; Tosun *et al.*, 2011; Hostage *et al.*, 2014). While the literature demonstrates that the ε4 allele has an overall deleterious effect on amyloid-β pathology

Table 1 Longitudinal cohort demographics table for estimating rates of biomarker change

	PIB	MRI
Participants, *n*	249	497
Age (SD)	64.8 (9.4)	66.8 (10.0)
Gender, *n* male (%)	83 (33.3)	189 (38.0)
Education (SD)[a]	15.9 (2.5)	15.8 (2.5)
MMSE (SD)	29.1 (1.1)	29.1 (1.1)
CDR Sum of Boxes (SD)	0.01 (0.08)	0.02 (0.10)
Scans, *n*, mean (SD)	2.5 (0.6)	3.1 (1.3)
Years of follow-up (SD)	4.9 (2.1)	5.6 (3.1)
n APOE ε4[a] (ε34 or ε44) (%)	74 (29.7)	150 (30.2)
ε22/ε23/ε33/ε24[b]/ε34/ε44, *n* [%]	3/30/135/7/63/11 [1.2/12.0/54.2/2.8/25.3/4.4]	4/59/269/15/127/23 [0.8/11.9/54.1/3.0/25.6/4.6]
n that converted to CDR >0	13 (5.2%)	89 (17.9%)

[a]Education values were not available for 23 individuals.
[b]Individuals with both an ε2 and ε4 allele were excluded from analysis.
MMSE = Mini-Mental State Examination.

and neurodegenerative biomarkers, there are unanswered questions about where in the brain these effects spatially manifest.

The objective of this study was to utilize longitudinal data to characterize the effect of *APOE* ε4 on amyloid-β pathology as measured by PET and neurodegeneration as measured by structural MRI. Here, we attempt to understand the nature of the *APOE* ε4 effects by using a whole-brain regional approach to evaluate the spatial effects of *APOE* ε4 on longitudinal rates of change of pathology. Additionally, we explore whether ε4 carriers develop Alzheimer's disease pathology at a higher proportion, at an earlier time point, or a faster rate than non-carriers. These analyses address shortcomings in the literature to better understand the role that the *APOE* ε4 genotype plays on regional pathology across the lifespan.

Materials and methods

Participants

Participants between the ages of 45 and 90 were included from ongoing studies on ageing and dementia from the Knight Alzheimer Disease Research Center at Washington University. Participants who had one or more ε4 allele were assigned a positive APOE ε4 status, while those with no ε4 allele were assigned a negative APOE ε4 status.

The longitudinal analyses of amyloid-β PET and structural MRI were analysed only in individuals who were cognitively normal at baseline [clinical dementia rating (CDR) of 0 (Morris, 1997)]. In this initial cohort 249 participants were identified that had two or more amyloid-β PET scans and 497 participants had two or more MRI sessions. Demographics for the entire cohort are shown in Table 1. Individuals with both an ε4 and an ε2 allele were excluded, yielding a final sample for statistical analyses of 242 with longitudinal PET and 482 with longitudinal MRI. Demographic comparisons between ε4 carrier and ε4 non-carrier groups are presented in the online Supplementary material. There were no significant demographic differences between carriers and non-carriers.

It was of additional interest to estimate population level frequencies of an abnormal amyloid-β PET scan across middle and older age. To do this we examined the baseline visits from the 242 cognitively normal individuals included in the longitudinal PET data, as well as another 333 (total $n = 575$) individuals with only one PET session. Individuals carrying both an ε4 and an ε2 were excluded from this sample. From this total population 443 were CDR = 0, 70 CDR = 0.5, 40 CDR = 1, 19 CDR = 2, and 3 CDR = 3. Cognitively impaired individuals were included in this one analyses as excluding them would erroneously underestimate the true percentages of the population with abnormal amyloid-β PET scans. Demographics for this cross-sectional cohort are shown in Table 2.

MRI acquisition and processing

Structural magnetization-prepared rapid gradient-echo (MPRAGE) images were acquired on either a 1.5 T ($n = 336$ sessions) or 3 T ($n = 1199$ sessions) Siemens. Scans had a resolution of either 1 × 1 × 1.25 mm or 1 × 1 × 1 mm. Structural scans were processed with FreeSurfer (Fischl, 2012) using the Desikan atlas. For each hemisphere, cortical thickness values were obtained for all FreeSurfer cortical regions of interest, and volumes were obtained for all FreeSurfer subcortical regions of interest. Cortical thickness was calculated as the shortest distance between the cortical grey/white boundary to the grey/CSF boundary (Fischl and Dale, 2000). All subcortical region volumes were adjusted for intracranial volume using a regression approach (Buckner *et al.*, 2004). Left and right values for each region of interest were averaged together.

PIB PET acquisition and processing

Amyloid-β PET imaging was completed using ^{11}C-Pittsburgh compound B (PIB). PET data from the 30–60-min post-injection window were analysed using regions of interests derived from Freesurfer (Fischl, 2012; Su *et al.*, 2013) (PET Unified

Table 2 Cross-sectional demographics table for estimating population rates of amyloid-β positivity

Participants, *n*	575
Age (SD)	67.7 (9.9)
Gender, *n* male (%)	239 (41.6)
CDR	443 CDR 0, 70 CDR 0.5, 40 CDR 1, 19 CDR 2, 3 CDR 3
ε22/ε23/ε33/ε34/ε44, *n* [%]	4/69/297/176/29 [0.7/12.0/51.7/30.6/5.0]
n APOE ε4+ (ε34 or ε44) (%)	205 (35.6)

Pipeline, https://github.com/ysu001/PUP). Regional estimates were transformed into standardized uptake value ratios (SUVRs) with cerebellar cortex as the reference region. Partial volume correction was performed using a regional spread function technique (Rousset *et al.*, 1998; Su *et al.*, 2015). As with the MRI data, regions were averaged across hemispheres before being entered into statistical analyses. For an examination of population level frequencies of abnormal amyloid-β deposition, participants were additionally classified as having abnormal (amyloid-β positive) or normal (amyloid-β negative) levels using a previously established PIB mean cortical SUVR cut-off of 1.42 (Sutphen *et al.*, 2015; Brier *et al.*, 2016; Vlassenko *et al.*, 2016; Mishra *et al.*, 2017), which is the linear projection of a mean cortical binding potential (MCBP) cut-off previously established in this cohort of 0.18 (Mintun *et al.*, 2006; Vlassenko *et al.*, 2011; Gordon *et al.*, 2015).

Genotyping

Genomic DNA was isolated from peripheral blood samples using standard procedures. *APOE* genotyping was performed as previously described (Talbot *et al.*, 1994). The distribution of alleles was in Hardy-Weinberg equilibrium.

Statistical analysis

We used multivariate linear mixed effects models (LME) to evaluate longitudinal changes in amyloid-β PET and structural MRI. LME models provide a statistical approach that is flexible and can accommodate unequal numbers of measurement points or sampling intervals. Considering multiple regions of interest simultaneously in a multivariate approach provides the ability to account for correlations between regional measurements. Within each modality, we modelled the cortical and subcortical regions separately. One model fit the 34 FreeSurfer cortical regions simultaneously and one model fit the seven FreeSurfer subcortical regions simultaneously. For MRI, cortical thickness was used for the cortical regions' model and intracranial volume-corrected volumes were used for the subcortical regions' model. Models were fit on mean-centred and standard deviation (SD) scaled data. Within each model (e.g. cortical PIB) for each individual region of interest, the model included fixed effects of baseline age, time, and *APOE* ε4 status. To allow for non-linear associations with baseline age, we modelled baseline age as a restricted cubic spline with three knots. Restricted cubic spline functions allow for the flexibility to vary non-linearly without forcing the relationship into a particular polynomial fit and have been used previously for modelling imaging biomarkers with respect to age or time in longitudinal analyses (Vemuri *et al.*, 2010; Jack *et al.*, 2015; Jansen *et al.*, 2015). In our final model we included the two terms for baseline age from the spline, *APOE* ε4 status, time, and all two-way and three-way interactions as fixed effects and also subject-varying slope and intercept random effect terms. Both the subject-level intercepts and the subject-level slopes were allowed to correlate across all brain regions included in a model.

To fit each model, we used the software package Stan (mc-stan.org) (Gelman *et al.*, 2015; Carpenter *et al.*, 2017) implemented in R using rstan version 2.14.1, to perform Markov Chain Monte Carlo (MCMC) analyses. A Bayesian approach was selected as the resultant credible intervals provide a richer, more informative estimate than classical confidence intervals obtained from null-hypothesis significance testing approaches. We used a normal distribution (mean = 0, SD = 5) as a prior for the fixed effects beta-estimates and used a normal distribution (mean = 0, SD = 1) as a prior for the random effects gamma-estimates of the scaled data. We used uniform priors for the variances and the lkj correlation density function (mu = 1.0) as a prior for the correlation matrix (Lewandowski *et al.*, 2009). We implemented MCMC with eight chains of 10 000 iterations each (5000 warm-up, thinning 1 in 10 iterations) (Kruschke, 2014; Sorensen and Vasishth, 2016). Credible intervals were defined as the range between the 0.5% and 99.5% estimates to represent 99% credible intervals. To test for convergence, the Gelman-Rubin convergence statistic (Ř), the ratio of between-chain variance to within-chain variance was used (Gelman and Rubin, 1992). A value close to 1 indicates convergence. Ř for each parameter estimate was within 0.01 of 1.0. The code for the models is included in the Supplementary material.

We report the rate of change of PIB SUVR or structural MRI data as a function of baseline age for *APOE* ε4-positive and *APOE* ε4-negative individuals. The ages at which the slopes between groups diverge were determined as the first age at which the 99% credible interval of the distribution of differences between groups did not overlap zero. To improve reliability and avoid spurious points, a time point was only considered significant if the distribution continued to not overlap zero for 2 years. To understand whether ε4 carriers accumulate pathology at a faster rate relative to non-carriers at a similar point in the disease, both the PIB longitudinal rate of change in the precuneus and the volume longitudinal rate of change in the hippocampus were additionally evaluated as a function of baseline disease burden, measured by the mean cortical SUVR (mcSUVR) from the baseline PIB scan. A linear mixed effects model was fit including fixed effects of baseline mcSUVR, time, and *APOE* ε4 status, along with all two-way and three-way interactions and subject-varying slope and intercept random effect terms. To again allow for non-linear associations, mcSUVR was modelled as a restricted cubic spline with three knots. MCMC analyses were completed as previously described to derive PIB slope and volume slope estimates as a function of baseline PIB mcSUVR, with 99% credible intervals. As exploratory analyses, we additionally ran models for precuneus PIB and hippocampal volume stratifying ε4 carriers into homozygotes (ε44) and heterozygotes (ε43 or ε34) and including all appropriate additional terms in the linear mixed effects models. These results were considered exploratory rather than as main analyses due to the small number of homozygotes with PIB ($n = 11$) and MRI ($n = 23$) data.

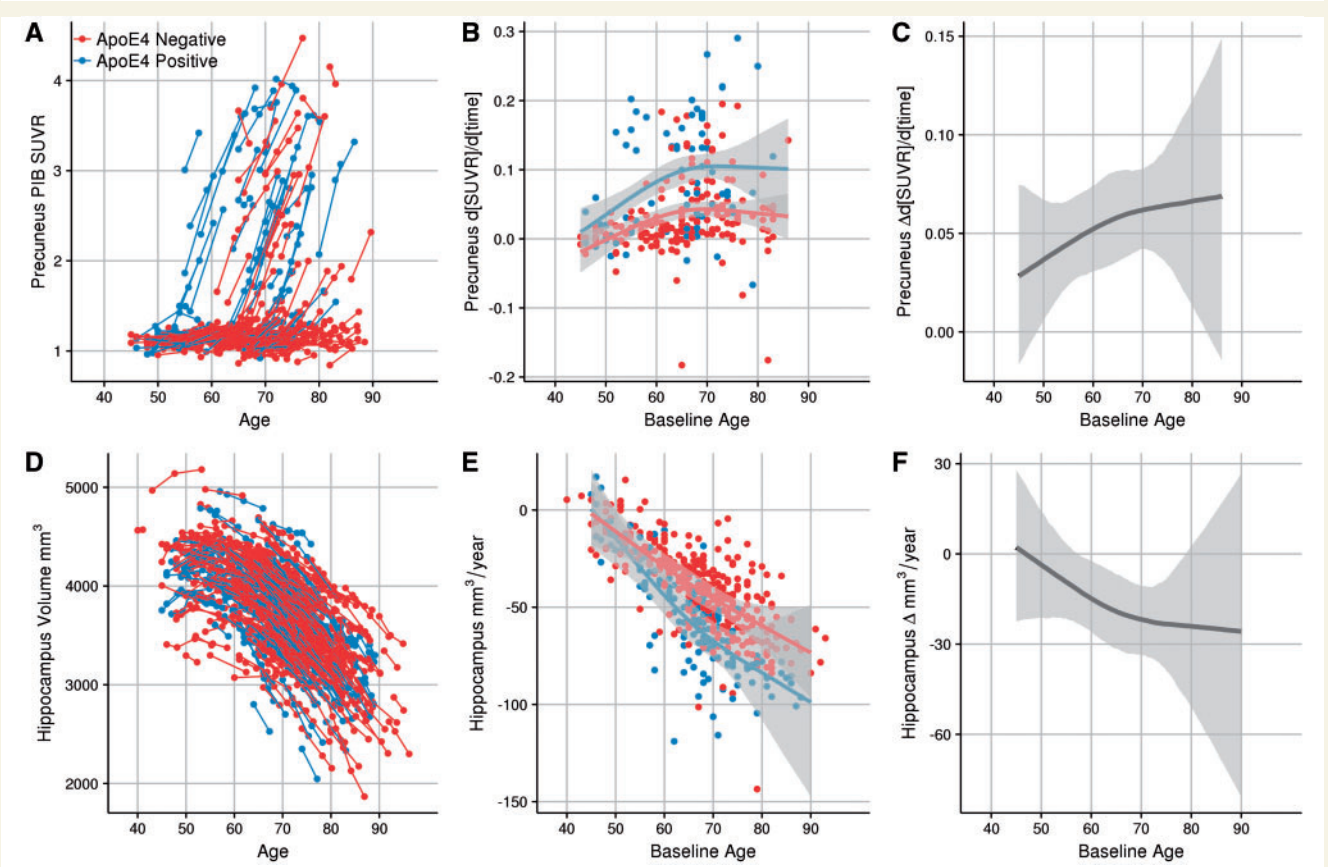

Figure 1 Modelling longitudinal change in precuneus PIB (A–C) and hippocampal volume (D–F). **A** and **D** represent spaghetti plots of the model fits for the individual participant SUVR and volume trajectories, respectively. **B** and **E** show the longitudinal rates of change between the *APOE* ε4 carriers and non-carriers, along with the 99% credible intervals. For reference, individual random effect slope estimates are also plotted. **C** and **F** depict the difference in rate of biomarker change between *APOE* ε4 carriers and non-carriers across the course of the sampled lifespan. Shaded regions represent 99% credible intervals..

Finally, to characterize abnormal levels of amyloid-β PET across the lifespan, the cross-sectional cohort of 575 individuals with at least one PET scan was used. Each participant was characterized as amyloid-β positive or negative based upon previously defined cut-offs. The proportion of all ε4 carriers and all non-carriers who were amyloid-β positive was determined as a function of age across the span of 42 to 89. The resultant curves were smoothed using a local regression method, using the loess method implemented in R, with a span = 0.7.

Results

We found a statistically significant difference in the rate of amyloid-β accumulation in *APOE* ε4 carriers in comparison to non-carriers in 33/34 FreeSurfer cortical regions. Figure 1 shows an example of the results in the precuneus. Figure 1A shows a spaghetti plot of the model fits for the individual participant SUVR trajectories. Figure 1B shows the model fits for the SUVR rate of change as a function of baseline age and *APOE* genotype. Figure 1C shows the difference in the longitudinal rates of change of amyloid-β accumulation between the *APOE* ε4 carriers and non-carriers, along with the 99% credible intervals of this difference. The first point in this difference where credible intervals do not overlap 0 is the first baseline age where rates of accumulation significantly diverge between groups. In our data, *APOE* ε4 carriers demonstrate significantly accelerated accumulation of amyloid-β deposition in the precuneus, in comparison to non-carriers, at a baseline age of 49 years old. The difference in slopes between carriers and non-carriers is significantly greater than zero throughout the majority of the lifespan represented by our cohort. Figure 2 shows the age at which APOE ε4 carriers first have significantly greater longitudinal change in PIB binding than non-carriers for each of the cortical regions. These data are presented numerically in Table 3. In all cortical regions where there was a statistically significant difference between ε4 carriers and non-carriers, the earliest age of divergence in rates of amyloid-β accumulation between carriers and non-carriers varied between 49 and 68 years. Two out of seven of the FreeSurfer subcortical regions showed significant differences between groups in rates of amyloid-β plaque accumulation across the adult

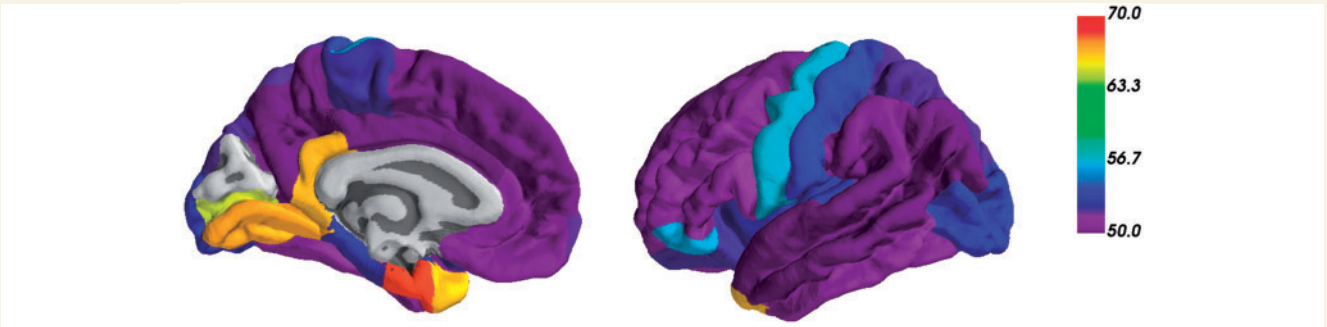

Figure 2 Regional differences in emergence of PIB. The colour scale represents the first age where the rate of amyloid-β accrual in the cortical regions is significantly different between APOE ε4 carriers and non-carriers.

lifespan, the caudate (age 56) and the putamen (age 63). Graphs for all regions are depicted in the Supplementary material.

In contrast to the effects on amyloid-β deposition, only 3/34 cortical regions showed a statistically significant difference in the rate of cortical atrophy, with carriers having faster rates of cortical thinning than non-carriers in the insula (age 47), parahippocampal cortex (age 51), and entorhinal cortex (age 71). All of these effects were very modest and bordered on significance (see regional graphs available in the Supplementary material). For the subcortical analyses 4/7 of the regions showed a significantly greater rate of subcortical atrophy in *APOE* ε4 carriers relative to non-carriers: the hippocampus, amygdala, putamen, and accumbens at ages of 57, 66, 70, and 71, respectively. Plots of the model fits of hippocampal volume are shown in Fig. 1D–F. The non-carriers' hippocampal volumetric rate of change shows a linear acceleration throughout the lifespan, while *APOE* ε4 carriers demonstrate even greater accelerated cortical atrophy in midlife followed by a similar rate of acceleration to that of the non-carriers later in the lifespan (Fig. 1E). The slope difference between carriers and non-carriers significantly differs from zero between ∼55 to 80 years, after which there is not a significant difference in the rates (Fig. 1F). Exploratory analyses examining the effect of ε4 dosage are presented in Supplementary Fig. 1. These results indicate that ε4 homozygotes had earlier and greater rates of amyloid-β accumulation and hippocampal atrophy than *APOE* ε4 heterozygotes and non-carriers.

The temporal evolution of pathology as related to the *APOE* ε4 genotype differs regionally. Figure 3A and B shows the differences in the longitudinal rate of change of amyloid-β deposition and cortical atrophy, respectively, between carriers and non-carriers in two cortical regions prominently studied in Alzheimer's disease. In comparison to the precuneus, which demonstrates early acceleration followed by a plateau in the rate of change of amyloid-β accumulation, the entorhinal cortex shows no difference in amyloid-β accumulation rates between carriers and non-carriers at younger ages but *APOE* ε4 carriers demonstrate increased rates of amyloid-β deposition in the entorhinal cortex at older ages. In contrast, the differences in cortical atrophy between carriers and non-carriers in the entorhinal cortex and precuneus are not significantly different across the lifespan sampled in this population.

Figure 3C and D shows the differences in the longitudinal rate of change of amyloid-β accumulation and structural atrophy, respectively, between carriers and non-carriers in two subcortical regions. The differences in amyloid-β accumulation between carriers and non-carriers in the hippocampus and caudate did not differ significantly across the lifespan. When examining structural MRI, in the hippocampus ε4 carriers have accelerated volumetric loss early in the lifespan, but there is no significant difference in rate of change of volume loss between carriers and non-carriers in the caudate across the sampled lifespan.

The difference in amyloid-β accumulation in the precuneus and atrophy in the hippocampus between *APOE* ε4 carriers and non-carriers was evaluated as a function of disease burden, measured by baseline PIB mean cortical SUVR. Figure 4A shows no difference between hippocampal atrophy as a function of disease burden in ε4 carriers, in comparison to non-carriers. Figure 4B demonstrates greater longitudinal amyloid-β accumulation as a function of disease burden in ε4 carriers, in comparison to non-carriers. These findings show that APOE ε4 carriers experience accelerated amyloid-β accumulation, but not atrophy, relative to non-carriers after matching individuals for baseline levels of pathology.

The population level frequencies of abnormal PIB levels stratified by genotype are shown across the ages of participants in the cross-sectional cohort (Fig. 5). At a population level, *APOE* ε4 carriers begin having elevated amyloid-β pathology at an earlier age, and at higher frequencies (steeper slope) after age 60. The proportion of *APOE* ε4 carriers that eventually develop pathological amyloid-β PET levels approaches 1 by age 90, while the proportion of non-carriers that develop pathological amyloid-β levels plateaus at less than 0.5, or half of the population.

Discussion

The purpose of this study was to better characterize the role of the *APOE* ε4 allele on amyloid-β pathology and

Table 3 First age of accelerated PIB binding in ε4 carriers versus non-carriers

Region of interest	Age of first detectable *APOE* effect
Cortical	
Inferior parietal cortex	49.28
Precuneus cortex	49.32
Middle temporal gyrus	50.21
Superior frontal gyrus	50.29
Superior temporal gyrus	50.6
Supramarginal gyrus	50.69
Caudal anterior cingulate cortex	50.71
Posterior cingulate cortex	50.93
Rostral anterior cingulate cortex	50.94
Banks of the superior temporal sulcus	50.96
Rostral middle frontal cortex	50.99
Medial orbitofrontal cortex	51.1
Pars triangularis	51.29
Caudal middle frontal cortex	51.44
Fusiform gyrus	51.79
Inferior temporal cortex	51.89
Pars opericularis	52.06
Superior parietal cortex	52.32
Frontal pole	52.44
Paracentral cortex	53
Lateral orbitofrontal cortex	53.02
Transverse temporal gyrus	53.51
Parahippocampal cortex	53.67
Insula cortex	54.28
Lateral occipital cortex	54.5
Postcentral gyrus	54.71
Pars orbitalis	56.69
Precentral gyrus	57.19
Pericalcarine cortex	65.28
Isthmus cingulate	66.74
Temporal pole	67.41
Lingual gyrus	67.62
Entorhinal cortex	68.45
Cuneus cortex	-
Subcortical	
Nucleus accumbens	-
Amygdala	-
Caudate	56.76
Hippocampus	-
Pallidum	-
Putamen	64.04
Thalamus	-

neurodegeneration across the lifespan of middle-aged to elderly cognitively normal adults. This study illustrates that the presence of the *APOE* ε4 allele results in increased longitudinal rates of change of amyloid-β plaque accumulation diffusely through the cortex. While *APOE* ε4 carriers show near ubiquitous accelerated amyloid-β accumulation throughout the brain relative to non-carriers, the temporal emergence of this pathological accumulation differs across brain regions (between ages 49 and 70). In addition, the present work is the first to show that *APOE* ε4 carriers have not only increased rates of amyloid-β accumulation earlier but also have faster rates of amyloid-β accumulation in comparison to non-carriers after adjusting for baseline levels of pathology. *APOE* ε4 effects on neurodegeneration were modest, with carriers displaying greater rates of structural loss in medial temporal lobe structures as early as 50 years.

The literature has shown that *APOE* ε4 carriers develop Alzheimer's disease more often and earlier than non-carriers, and the *APOE* ε4 allele has deleterious effects on amyloid-β pathology. The majority of work on amyloid-β pathology has been cross-sectional (for a review see Fouquet *et al.*, 2014), with a limited subset of work examining longitudinal change (Grimmer *et al.*, 2010; Villemagne *et al.*, 2011, 2013; Vlassenko *et al.*, 2011; Jack *et al.*, 2013*b*; Resnick *et al.*, 2015; Bilgel *et al.*, 2016). In general, longitudinal studies have found that *APOE* ε4 status did not significantly affect the rates of amyloid-β accumulation after adjusting for age (Vlassenko *et al.*, 2011; Jack *et al.*, 2013*b*; Villemagne *et al.*, 2013; Resnick *et al.*, 2015; Bilgel *et al.*, 2016). Relatively modest longitudinal follow-ups, and study populations that contain older adults and demented individuals, but do not include middle-aged participants, may drive such null results. While prior work indicates there is an undeniable effect of the ε4 allele on group level measures of amyloid-β pathology, it is unclear how the ε4 allele produces this effect. It has remained to be shown whether these group differences can solely be attributed to a higher proportion of *APOE* ε4 carriers developing the disease, whether they develop it at an earlier age, or whether *APOE* ε4 carriers are developing pathology at a faster rate even relative to individuals with preclinical Alzheimer's disease without the ε4 allele.

The current study found that at the group level ε4 carriers had greater longitudinal accumulation of amyloid-β than non-carriers and that this deposition accelerated with increasing baseline age. When considering population frequencies of PIB positivity (Fig. 5) we found that ε4 carriers had abnormal PIB scans earlier in time and at an elevated population frequency relative to non-carriers. Finally, in addition to looking at slope differences as a function of age, the present study shows that the accumulation of amyloid-β pathology as a function of baseline amyloid-β burden is also higher in *APOE* ε4 carriers than non-carriers (Fig. 4). This study examining cognitively normal middle-aged and older adults provides evidence that *APOE* ε4 positivity not only shifts the onset of amyloid-β deposition earlier, but also accelerates accrual of amyloid-β deposits once this process has begun. In contrast, previous work suggesting that APOE ε4 status primarily affects the age at which people begin to develop amyloid-β pathology, but not the rate of amyloid-β deposition (Jack *et al.*, 2013*b*; Bilgel *et al.*, 2016) has primarily been conducted using older adults and impaired individuals. Our findings suggest that genetic factors in Alzheimer's disease may change the trajectory of disease progression, in contrast to the hypothesis that all individuals at a given disease

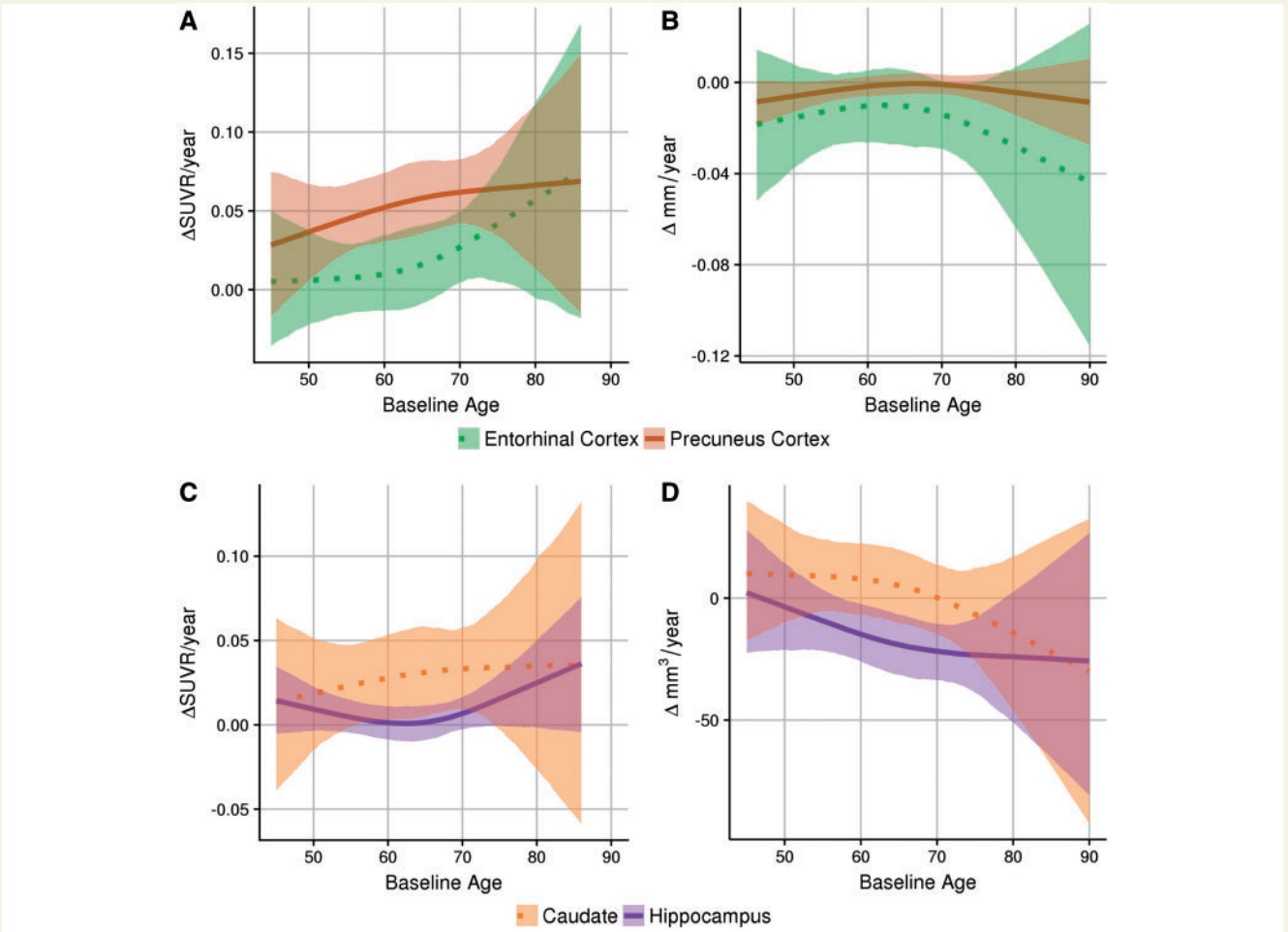

Figure 3 Regional differences in biomarker trajectories. *Top*: The difference in the longitudinal rates of change of PIB measurements (**A**) and cortical thickness (**B**) between *APOE* ε4 carriers and non-carriers in two cortical regions. *Bottom*: The difference in the longitudinal rates of change of PIB (**C**) and volume (**D**) between *APOE* ε4 carriers and non-carriers in two subcortical regions.

burden will progress similarly. This may have important implications for clinical trial selection and therapeutic targets for *APOE* ε4 carriers.

The current study examined regional differences in the temporal evolution of amyloid-β accumulation in *APOE* ε4 carriers versus non-carriers. The vast majority of prior studies have used summary metrics in cross-sectional and longitudinal studies looking at *APOE* ε4 effects. In the present study, we consider the longitudinal rates of change of amyloid-β in all cortical and subcortical FreeSurfer anatomical regions of interest. The present study shows that the age at which *APOE* ε4 carriers display increased rates of accumulation in comparison to non-carriers is as early as 49 years old in regions that are affected early by amyloid-β pathology (i.e. precuneus). The presence of temporal differences in different regions introduces a novel aspect of genetic influences on late-onset Alzheimer's disease.

The relationship between *APOE* ε4 and volumetric changes longitudinally has conflicting evidence in the current literature. Structural changes from neurodegeneration is a late biomarker change in the progression of Alzheimer's disease (Bateman *et al.*, 2012; Benzinger *et al.*, 2013; Jack and Holtzman, 2013; Jack *et al.*, 2013*a*). The present study shows that cognitively normal *APOE* ε4 carriers demonstrate more rapid volume loss in medial temporal lobe structures, in comparison to non-carriers, first detected around 60 years of age. This is consistent with prior work indicating significant effects of *APOE* genotype on structural MRI (Geroldi *et al.*, 1999; Mori *et al.*, 2002; Van De Pol *et al.*, 2007; Morra *et al.*, 2009; Risacher *et al.*, 2010; Hostage *et al.*, 2014; Manning *et al.*, 2014) and in contrast to the studies that have shown no *APOE* ε4 effect on volumetric changes (Jack *et al.*, 1998; Moffat *et al.*, 2000; Du *et al.*, 2006; Fan *et al.*, 2010; Taylor *et al.*, 2014).

Despite the large sample and high number of longitudinal visits the observed *APOE* ε4 effect on neurodegenerative biomarkers was quite subtle and the modest size of this effect may explain the heterogeneity in the field. The ε4 effect on neurodegenerative biomarkers may also be most prominent when including both middle-aged and older adults. Further, when looking at hippocampal atrophy as a function of baseline disease status, there was no difference between *APOE* ε4 carriers than non-carriers. These findings suggest that, in contrast with the *APOE* ε4 effect

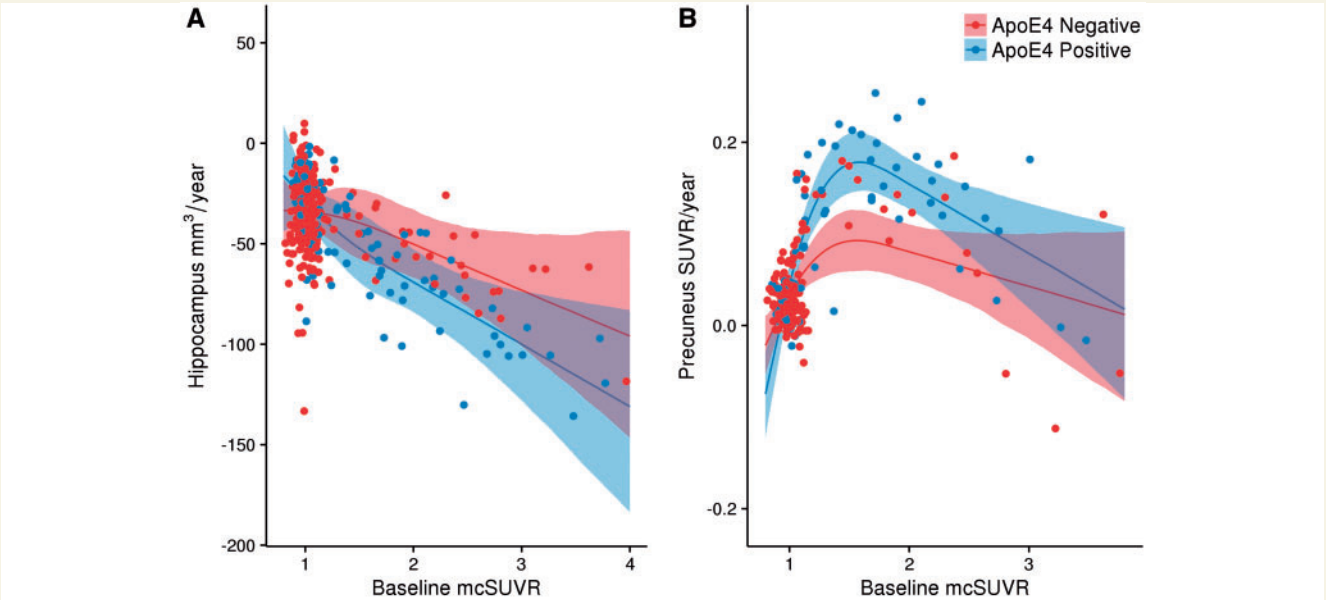

Figure 4 Longitudinal change in PIB as a function of disease status. The longitudinal rates of change of PIB in the hippocampus (**A**) and the precuneus (**B**) for both *APOE* ε4 carriers and non-carriers are shown as a function of mean cortical SUVR from the baseline PIB assessment. Shaded areas represent 99% credible intervals.

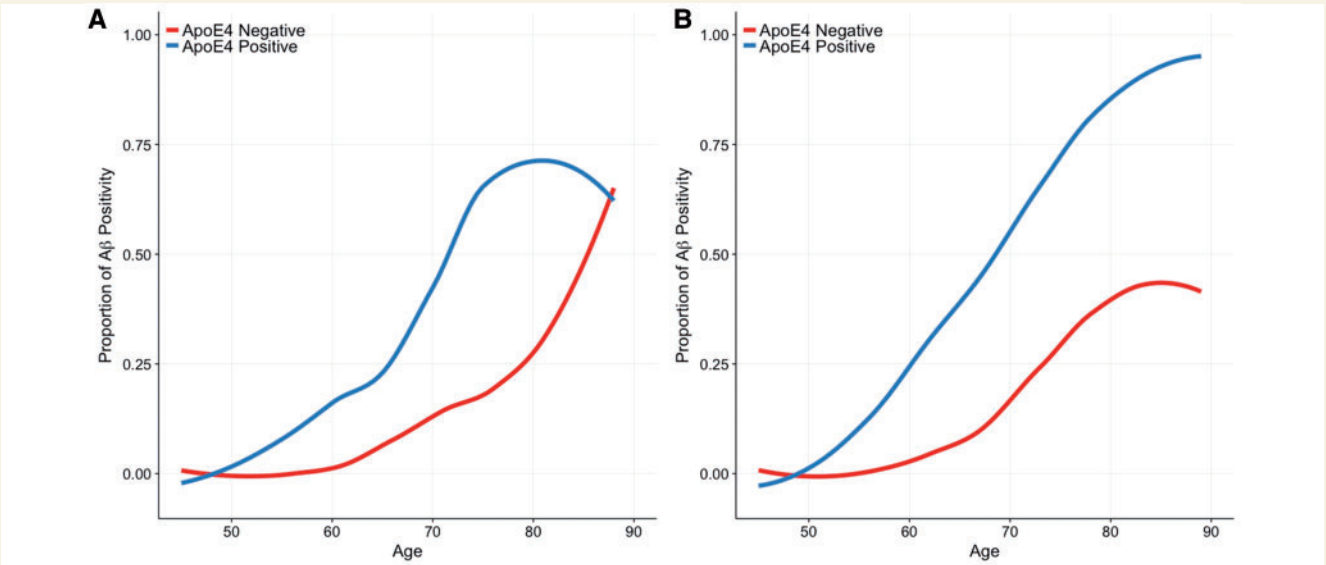

Figure 5 Characterization of abnormal levels of amyloid-β PET across the lifespan. The proportion of *APOE* ε4 carriers and non-carriers who are amyloid-β PET positive is depicted as function of age in (**A**) non-demented individuals only and (**B**) the combined sample of non-demented and demented individuals.

on amyloid-β, the neurodegeneration seen in both the *APOE* ε4 carriers and non-carriers was proportional to baseline levels of amyloid-β. This suggests that when considering populations matched for clinical severity or amyloid-β pathology, the ε4 genotype may have minimal effects on atrophy.

There are some limitations of our study. Our results reflect the detectable genotype differences given our sample. Larger samples with greater power would likely detect differences even earlier or detect effects in additional regions. Our results also focus on cognitively normal individuals. The effects of *APOE* genotype likely vary in demented cohorts. The earliest ages at which we can detect *APOE* ε4 carriers and non-carriers diverging are limited by the ages represented in our cohort, which begins at age 45. Including even younger ages could reveal that the influence of the ε4

allele begins even earlier in the lifespan. Prior work also indicates that the ε4 allele has a dose effect (Corder *et al.*, 1993), with homozygotes having greater risk of developing Alzheimer's disease. Even in our larger population with longitudinal MRI only 23 individuals were homozygous for the ε4 allele. This limits our ability to accurately model the interaction of ε4 dosage across the studied age ranges. Results from our exploratory analyses in the precuneus and hippocampus do suggest that ε4 homozygotes have greater and earlier amyloid-β accumulation and atrophy. However, our samples size is very modest. Future work integrating multiple cohorts would be able to better estimate how ε4 homozygosity or the ε2 alleles modify biomarker trajectories starting in middle age. Our cohort consisted of adults with longitudinal imaging biomarker data who were cognitively normal at baseline. The influence of the ε4 allele may be different as cognition declines. This study focused only on PIB PET and MRI. Evaluation of longitudinal changes of other imaging biomarkers of neurodegeneration, such as FDG and tau PET would be of interest as these may better predict cognition. Finally, our work represents the influence of the *APOE* ε4 allele in only one cohort of middle-aged and older adults. Replication of our results by other groups in an independent cohort would strengthen the interpretation of our findings.

In summary, the present study provides evidence that *APOE* ε4 is linked to increased rates of change of amyloid-β accumulation detectable in late middle-aged cognitively normal adults. In addition to *APOE* ε4 carriers having earlier amyloid-β accumulation, in comparison to non-carriers, they also have faster rates of accumulation as a function of baseline disease status. Through using a regional approach, the present study provides the first evidence that the temporal evolution of *APOE* ε4-related amyloid-β accumulation varies regionally. Finally, this study shows that cognitively normal *APOE* ε4 carriers show increased rates of volumetric change in medial temporal lobe structures as early as 60 years of age, in comparison to non-carriers.

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Conflicts of interest

D.M.H. co-founded and is on the scientific advisory board of C2N Diagnostics. He consults for Genentech, AbbVie, Eli Lilly, Proclara, Glaxosmithkline, and Denali. Washington University receives research grants to the lab of D.M.H. from C2N Diagnostics, Eli Lilly, AbbVie, and Denali and to the laboratories of L.S.B. and J.C.M. from Eli Lilly and Avid Radiopharmaceuticals (a wholly owned subsidiary of Eli Lilly). All other authors report no conflicts.

Supplementary material

Supplementary material is available at *Brain* online.

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