Changes in A β biomarkers and associations with APOE genotype in two longitudinal cohorts

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ABSTRACT

Apolipoprotein E (APOE) genotype influences onset age of Alzheimer's disease but effects on disease progression are less clear. We investigated amyloid- β ($A\beta$) levels and change in relationship to APOE genotype, using two different measures of $A\beta$ in two different longitudinal cohorts. $A\beta$ accumulation was measured using PET imaging and ^{11}C -Pittsburgh compound-B (PiB) in 113 Baltimore Longitudinal Study of Aging (BLSA) participants (mean age 77.3 years; 107 normal, 6 cognitively impaired) and CSF $A\beta_{1-42}$ assays in 207 BIOCARD study participants (mean age 62 years; 195 normal, 12 cognitively impaired). Participants in both cohorts had up to 7 serial assessments (mean 2.3-2.4). PET-PiB retention increased and CSF $A\beta_{1-42}$ declined longitudinally. APOE $\epsilon 4$ was significantly associated with higher PET-PiB retention and lower CSF $A\beta_{1-42}$, independent of age and sex, but APOE genotype did not significantly affect $A\beta$ change over time. APOE $\epsilon 4$ carriers may be further along in the disease process, consistent with earlier brain $A\beta$ deposition and providing a biological basis for APOE genotype effects on onset age of Alzheimer's disease.

Keywords: PET amyloid imaging, CSF $A\beta_{1-42}$, biomarkers, Apolipoprotein E genotype, longitudinal

1. Introduction:

Epidemiologic studies have shown that the Apolipoprotein E (APOE) $\epsilon 4$ risk allele is associated with a lower age of onset of Alzheimer's disease (AD) in a dose-dependent manner, such that two $\epsilon 4$ alleles are associated with the earliest onset age.[1] However, studies of the effect of APOE $\epsilon 4$ genotype on rate of disease progression have shown inconsistent associations.[2-4]

One method to elucidate the mechanism by which APOE genotype affects disease progression is to study the relationship between APOE genotype and amyloid-beta ($A\beta$) accumulation. Recent studies have confirmed that $A\beta$ pathology, indexing AD-associated plaques, likely begins more than ten to fifteen years before the diagnosis of clinical dementia.[5] The development of tools for *in vivo* evaluation of $A\beta$ biomarkers allows measurement of disease state and progression, including preclinical staging of AD in cognitively normal individuals.[6] These tools permit investigation of $A\beta$ deposition over time, as well as associations between $A\beta$ burden and APOE genotype. Positron emission tomography (PET) imaging studies of $A\beta$ consistently demonstrate increases over time in individuals with more than minimal $A\beta$ at baseline imaging, where "more than minimal" refers to a threshold for detection of elevated $A\beta$. [7-9] Although a number of studies have demonstrated an association between the APOE $\epsilon 4$ risk allele and $A\beta$ burden on imaging, even in non-demented individuals,[9-12] the relatively brief history of PET radiotracers for *in vivo* $A\beta$ imaging has limited the availability of longitudinal measurements for investigation of rates of change.[13] Similarly, lower levels of CSF $A\beta$ have been associated with increased risk of progression from MCI to AD.[14] However, longitudinal studies of CSF $A\beta$ in cognitively normal individuals at risk of progression are limited, primarily due to the long follow-up times that are required.[15] APOE $\epsilon 4$ carriers

have lower CSF levels of A β [16,17] but few studies have characterized the longitudinal trajectories of CSF A β in relation to APOE genotype.

We investigated longitudinal changes in biomarkers of A β deposition and their associations with APOE genotype using data from two prospective cohorts. Using data from the neuroimaging substudy of the Baltimore Longitudinal Study of Aging (BLSA), we used PET and ^{11}C Pittsburgh compound-B (PET-PiB) to investigate longitudinal changes in A β deposition and the relation of baseline A β levels and A β changes with APOE genotype in 113 older adults. Using complementary data from the BIOCARD study, we investigated serial changes in CSF A β_{1-42} for 207 participants in BIOCARD, and the relation of CSF A β_{1-42} to APOE genotype. In both cohorts, participants were evaluated at 1 to 2 year intervals and had up to 7 serial A β assessments. We first confirm prior observations that PET-PiB retention increases over time, especially in individuals with more than minimal A β burden at baseline,[7] and show significant decreases in CSF A β_{1-42} over time. Next, we show that APOE genotype affects level of A β , with higher PET-PiB retention and lower CSF A β with age in $\epsilon 4$ carriers compared with non-carriers. However, APOE genotype does not significantly influence rates of A β change over time, measured by PET-PiB or CSF.

2. Materials and Methods

2.1. Subjects: Research protocols were approved by the local institutional review boards, and written informed consent was obtained from all participants.

2.1.1. BLSA: The sample included 113 participants (63 men, 50 women) from the neuroimaging substudy of the BLSA. The BLSA neuroimaging substudy was initiated in 1994 and included annual magnetic resonance imaging (MRI) scans of brain structure and PET scans

of regional cerebral blood flow through 2003.[18] At enrollment, participants were free of central nervous system disease (dementia, stroke, bipolar illness, epilepsy), severe cardiac disease (myocardial infarction, coronary artery disease requiring angioplasty or coronary artery bypass surgery), severe pulmonary disease, or metastatic cancer. Since 2003, participants older than 80 years have continued to receive annual assessments, while those between the ages of 60 and 79 years are assessed every 2 years, and those whose age is less than age 60 years are assessed every 4 years. Beginning in 2005, participants received amyloid PET scans. At baseline PET-PiB, 3 of the 113 participants were no longer normal and had a consensus diagnosis[19,20] of mild cognitive impairment (MCI), two had diagnoses of AD, and one was Impaired-Not MCI. Diagnoses of dementia and Alzheimer's disease (AD) were based on DSM-III-R[21] and the NINCDS-ADRDA criteria[22], respectively. Mean (SD) age at baseline ^{11}C -PiB imaging was 77.3 (8.1); participants were studied at 1 to 2- year intervals with up to 7 (mean 2.3 SD 1.6) scans over up to 8 years since 2005. The sample overlaps those reported previously.[7,11] Characteristics of the present sample are detailed in Table 1.

2.1.2. BIOCARD: The BIOCARD Study enrolled and followed a cohort of 349 cognitively normal individuals who were primarily in middle age. By design the majority had a first-degree relative with AD (approximately 75%). Neuropsychological assessments were conducted annually and MRI scans and CSF and blood specimens were obtained approximately every 2 years. The study was initiated at the NIH in 1995, and was stopped in 2005 for administrative reasons. In 2009, investigators at the Johns Hopkins School of Medicine were funded to reestablish the cohort, continue the annual clinical and cognitive assessments, collect blood, and evaluate the previously acquired MRI scans and CSF and blood specimens.[15]

At baseline, all participants completed a comprehensive evaluation at the Clinical Center of the NIH. This evaluation consisted of a physical and neurologic examination, an ECG, standard laboratory studies, and neuropsychological testing. Individuals were excluded from participation if they were cognitively impaired, as determined by cognitive testing, or had significant medical, psychiatric, or neurologic disorders. Since the study has been conducted at Johns Hopkins, the clinical evaluation has included the following: a physical and neurologic examination, assessments of medication use, behavioral and mood assessments, family history of dementia, history of symptom onset, and a Clinical Dementia Rating, based on a semi-structured interview.[23,24] The clinical assessments given at the NIH covered similar domains. Consensus diagnosis of cognitive status was based on clinical data, reports of changes in cognition by the subject and by collateral sources, and decline in cognitive performance on objective tests. Clinical diagnoses were made blinded to the results of CSF analyses.

To increase comparability with the BLSA sample, the BIOCARD data included in the current analysis was restricted to CSF samples collected at age 55 years and older ($N = 207$), yielding a mean age (SD) of 62 (6.4) with up to 7 serial assessments (mean 2.4, SD 1.5). Sample characteristics are presented in Table 1.

2.2. *PET Imaging and Image Processing:* Dynamic ^{11}C -PiB PET studies were performed in 3D mode on a GE Advance scanner. Participants were fitted with a thermoplastic head-mask to minimize motion during scanning. The PET scanning started immediately after IV bolus injection of a mean (SD) 14.7 (0.8) mCi of ^{11}C -PiB. PET data were acquired according to the following protocol for the duration of the frames: 4×0.25, 8×0.5, 9×1, 2×3, and 10×5 min (70 min total, 33 frames). Dynamic images were reconstructed using filtered back-projection with a

ramp filter (image size = 128 x 128, pixel size = 2 x 2 mm, slice thickness = 4.25 mm), yielding a spatial resolution of approximately 4.5 mm FWHM at the center of the field of view.

The Statistical Parametric Mapping PET template was affinely registered onto the 20-minute mean PET-PiB image for each PET scan study separately, and the resulting transformations were used to map the AAL atlas labels[25] accordingly on each PET-PiB distribution volume ratio (DVR) image. Quantification of DVR was performed using the AAL cerebellar gray matter reference regions that have been mapped to the native space of each PET image and a simplified reference tissue model.[26] Mean cortical DVR (cDVR) was calculated as the average of DVR values in superior, middle and inferior frontal and orbitofrontal, superior parietal, supramarginal and angular gyrus regions, precuneus, superior, middle and inferior occipital, superior, middle and inferior temporal, anterior, middle and posterior cingulate regions.

A two-class Gaussian mixture model was fitted to baseline mean cDVR data. The cDVR value corresponding to the intersection of the probability density functions of the two classes was used to define the separation between minimal and higher than minimal levels of PiB retention.

2.3. CSF measurement: Detailed procedures for acquisition and measurement of CSF A β_{1-42} are described in Moghekar et al.[15] Briefly, CSF was collected from 307 participants at baseline. Of these, 199 had CSF collected on more than one visit during the time the study was conducted at the NIH. The CSF specimens were analyzed using the same protocol used in the Alzheimer's Disease Neuroimaging Initiative. This protocol used the xMAP-based AlzBio3 kit (Innogenetics, Ghent, Belgium) run on the Bioplex 200 system. The kit contains monoclonal antibodies specific for A β_{1-42} (4D7A3), total tau (t-tau) (AT120), and p-tau181p (AT270), each

chemically bonded to unique sets of color-coded beads, and analyte-specific detector antibodies (HT7, 3D6). Calibration curves were produced for each biomarker using aqueous buffered solutions that contained the combination of 3 biomarkers at concentrations ranging from 25 to 1,555 pg/mL for recombinant tau, 54 to 1,799 pg/mL for synthetic Ab1–42, and 15 to 258 pg/mL for a synthetic tau peptide phosphorylated at the threonine 181 position (i.e., the p-tau181p standard). All assays were run in triplicate. Each subject had all their samples analyzed on the same plate, and the same batch of plates (same lot number) was used for analyzing CSF from the whole cohort. An external high volume CSF Standard was used across all plates to ensure A β values were within +/- 5% of previously determined mean concentration.

2.4. APOE Genotype. APOE genotype in both cohorts was determined by standard procedures.[27] APOE ϵ 4 carrier status was coded by creating an indicator variable, with APOE ϵ 4 carriers coded as 1, if they had at least one ϵ 4 allele, and non-carriers coded as 0.

2.5. Statistical Analysis: Linear mixed models were used in all analyses (SAS 9.3, Cary, NC).

To estimate annual rates of change, mean cDVR or CSF A β _{1–42} were used as the dependent variables and follow-up time from baseline in years (interval) as the main predictor. Other covariates included were: baseline age (defined as age at initial PET-PiB or first CSF A β _{1–42} from age 55), sex, and their interactions with time interval. Random effects were intercept and coefficient of interval, and unstructured covariance structure was assumed for random effects. To investigate the effects of APOE genotype on baseline levels and change in A β , we added genotype (APOE ϵ 4 carrier vs. non-carrier) and its interactions with baseline age and interval to the model. Baseline age was mean centered, sex was coded as -0.5 as female and 0.5 as male. BLSA participants missing APOE genotype data were not included in analyses of the effects of APOE on A β .

3. Results:

3.1. Effects of Baseline Age and Longitudinal Change in A β .

3.1.1. PET-PiB Imaging in BLSA: PET-PiB cDVR showed significant cross-sectional elevations with advancing age ($p = 0.002$). Longitudinal findings revealed overall increases in PET-PiB cDVR over time in the whole sample, after adjusting for baseline age and sex, of 0.86% per year ($p < 0.0001$). Individuals with more than minimal PET-PiB retention at baseline (cDVR of 1.15 or greater based on a two-class Gaussian mixture model) showed increases over time of approximately 2% per year ($p < 0.0001$). In contrast, individuals with minimal PiB retention at baseline (cDVR less than 1.15) tended to remain stable over time (0.40% per year). Longitudinal trajectories of amyloid deposition are shown for the entire sample in Figure 1a. Notably, with the longer follow-up intervals in the present report compared with our earlier observations,[7] we now observe the beginning of an upward trajectory in some of the oldest-old individuals with initially low baseline PiB retention (see Figure 1a).

3.1.2. CSF A β in BIOCARD: Similar results were obtained from linear mixed effects regression of serial CSF A β_{1-42} levels in the BIOCARD sample (Figure 1b). Overall rates of CSF declines over time were -5.51 pg/ml per year ($p = 0.005$). BIOCARD CSF data were not analyzed as a function of baseline CSF A β_{1-42} because Gaussian mixture models did not yield a clear cutpoint for grouping.

3.2. *APOE* genotype as a modifier of $A\beta$.

3.2.1. *APOE* Genotype and PET-PiB $A\beta$: Mixed model results investigating effects of *APOE* genotype on level of $A\beta$ burden at baseline PET-PiB imaging and change in $A\beta$ over time are shown in Table 2, and individual trajectories over time as a function of *APOE* $\epsilon 4$ carrier status are shown in Figure 2a. *APOE* $\epsilon 4$ carriers compared with non-carriers had significantly higher $A\beta$ burden overall ($p = 0.001$). There was a significant age by *APOE* genotype interaction ($p < 0.05$), indicating greater $A\beta$ elevations with age for $\epsilon 4$ carriers compared with non-carriers (Figure 3a). However, the effect of *APOE* genotype on rate of increase in PiB retention over time did not reach significance ($p = 0.12$). Rates of change in $A\beta$ deposition were similar for men and women, and the trend toward greater rates of $A\beta$ increase in older individuals did not reach significance ($p = 0.082$). Separate analyses of three individual regions of interest, precuneus, lateral temporal and inferior frontal regions, showed significant effects of *APOE* genotype on levels of $A\beta$ ($p < 0.002$) but not on rates of change in $A\beta$ ($p > 0.05$). Sensitivity analyses excluding non-white individuals, as well as data points subsequent to diagnosis of AD or MCI, yielded similar findings. Additional sensitivity analysis adding education as a covariate showed no significant main effect of education or significant interactions between *APOE* genotype and education on $A\beta$.

3.2.2. *APOE* genotype and CSF $A\beta_{1-42}$. Analogous results were observed in analyses investigating the effect of *APOE* genotype on overall level of CSF $A\beta$, as well as change over time, in the BIOCARD sample (Table 2, Figure 2b and 3b). *APOE* $\epsilon 4$ carriers had significantly lower overall levels of CSF $A\beta$ compared with non-carriers ($p = 0.0001$). There was a trend toward an age by *APOE* interaction ($p = 0.076$), indicating a greater reduction in CSF $A\beta$ with age in *APOE* $\epsilon 4$ carriers compared with non-carriers. Consistent with the BLSA PET-PiB

assessment of A β burden, the effect of APOE genotype on rate of change in CSF A β did not reach significance ($p = 0.355$). Sensitivity analysis restricted to individuals who remained free of cognitive impairment showed similar results, with the age by APOE interaction reaching significance in the restricted sample ($p = 0.01$). Sensitivity analyses adding education and its interaction with APOE showed no significant effects of these terms on A β .

4. Discussion:

We investigated longitudinal rates of change in two biomarkers of A β and associations of APOE genotype with overall levels and changes over time in these biomarkers. Our samples included a large number of participants and up to 7 serial assessments, with 3 or more measurements in more than one-third of the samples. Analysis of PET-PiB measures of A β in BLSA and CSF A β_{1-42} in BIOCARD yielded remarkably similar findings despite the use of different biomarkers and two distinct longitudinal cohorts. Our results demonstrated significant increases in PET-PiB retention and declines in CSF A β_{1-42} over time. Further, we confirmed previously reported associations between APOE genotype and A β levels,[5,11,12,28] with APOE $\epsilon 4$ carriers showing higher PET-PiB retention and lower CSF A β_{1-42} compared with non-carriers. We extended these findings by demonstrating an interaction between age and APOE genotype that indicated more pronounced associations of A β with age in APOE $\epsilon 4$ carriers than non-carriers, in part driven by greater likelihood of elevated A β with age in APOE $\epsilon 4$ carriers. In contrast to the effects of APOE genotype on A β biomarker levels, APOE genotype did not significantly modify rates of change in A β over time. Together, these findings suggest that APOE $\epsilon 4$ carriers are further along in the disease process, but that effects of APOE genotype on disease progression are less pronounced.

In this expanded sample and extended longitudinal follow-up of BLSA participants, we confirm and extend our initial observation[7] that PET-PiB imaging of *in vivo* amyloid burden is sensitive to even small increases in A β deposition over time. We found an overall increase in PET-PiB cDVR of 0.86% per year. Similarly, we found complementary longitudinal decreases in CSF A β_{1-42} of 5.51 pg/ml per year in BIOCARD, a distinct longitudinal cohort with a younger mean age. Further, in the PET-PiB BLSA cohort, we investigated whether baseline PET-PiB retention was associated with longitudinal increases in A β . Consistent with our initial report in a smaller sample[7] and findings from the Australian Imaging, Biomarkers and Lifestyle study[29], longitudinal increases in PiB retention were most pronounced in individuals with more than minimal baseline A β , approaching 2% per year in our subgroup of A β accumulators. In addition, Figure 1 shows that the initial stages of amyloid accumulation are detectable with longitudinal follow-up, even in some older individuals with low baseline PET-PiB retention. Thus, amyloid deposition can be initiated at very old ages despite stable levels in the preceding years, confirming that a negative A β scan at old ages does not guarantee prolonged absence of neuropathology. The distribution of CSF₁₋₄₂ values and the younger ages of the BIOCARD cohort did not allow similar analysis of association with baseline level of A β and observations at older ages, but the data presented in Figure 1b suggest that CSF measures of A β may begin changing and plateau earlier than those associated with PET-PiB. Prospective studies using both biomarkers in the same sample are necessary to test this hypothesis.

Our second goal was to confirm the reported effect of APOE genotype on A β burden and to investigate potential effects of APOE genotype on rates of increase in A β . Although a prior PET-PiB study suggested that APOE ϵ 4 carriers showed higher rates of A β deposition, this finding did not hold after age adjustment.[9] Our results confirm the association between APOE

$\epsilon 4$ carrier status and higher $A\beta$ burden for both PET-PiB and CSF measures, but neither biomarker of $A\beta$ burden showed significant effects of APOE genotype on change in $A\beta$ over time.

While epidemiological studies indicate that APOE $\epsilon 4$ carriers compared with non-carriers have earlier onset age of AD in a dose-dependent manner,[1] the mechanisms explaining this observation are not clear. Our findings confirm cross-sectional observations[11,12] that $A\beta$ levels are higher in APOE $\epsilon 4$ carriers vs. non-carriers at any given age during the preclinical phase, consistent with a younger onset of brain $A\beta$ deposition and a later disease stage in APOE $\epsilon 4$ carriers compared with non-carriers. Combined with similar rates of $A\beta$ progression in $\epsilon 4$ carriers and non-carriers for both PET-PiB and CSF $A\beta$ and the lag between initiation of $A\beta$ deposition and appearance of clinical symptoms,[5] the earlier initiation of $A\beta$ deposition may explain, in part, the earlier age of AD onset in APOE $\epsilon 4$ carriers. These findings may provide a biological substrate for the shift in the age of onset distribution of AD observed in epidemiological studies and may explain the less consistent associations of APOE genotype with disease progression. This interpretation is supported by recent findings from the BIOCARD study[30] showing that baseline scores on 9 cognitive tests and APOE $\epsilon 4$ status were independently associated with time to onset of clinical symptoms, whereas APOE $\epsilon 4$ status was associated with rates of cognitive change in only 2 measures.

Our study has several strengths including the use of two different biomarkers of $A\beta$ burden in two distinct well-characterized longitudinal cohorts. Limitations of our study include the relatively high level of education which characterizes both cohorts and may limit generalizability to individuals with lower educational levels. In addition, there were only a limited number of individuals homozygous for the APOE $\epsilon 4$ allele, and we were unable to investigate $\epsilon 4$ dose

effects. Further, the relatively recent development of amyloid imaging and the gap in obtaining additional CSF samples in BIOCARD resulted in limited follow-up intervals to date in these ongoing studies. It is possible that with additional follow-up and larger samples, APOE $\epsilon 4$ carriers may show significantly higher rates of change in A β than non-carriers, but the current data suggest that the magnitude of any potential effect on rate of change in A β is less than the effect on overall level of A β burden.

5. Conclusion:

Overall, our results in two longitudinal cohorts show that PET and CSF biomarkers of brain amyloid burden reliably detect even modest levels of A β and change in A β deposition even in asymptomatic individuals. Moreover, APOE genotype modulates level but does not significantly affect change in A β though it is possible that more subtle effects of the $\epsilon 4$ risk allele on within-individual rates of A β deposition will be evident in larger samples with longer follow-up intervals. In conclusion, the ability to track A β deposition over time with both PET imaging and CSF biomarkers provides tools for investigation of the evolution of AD neuropathology in relation to onset of clinical symptoms and to guide patient selection and therapeutic monitoring in clinical trials.

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Figure Captions

Figure 1. Observed values of $A\beta$ over time for (a) PET-PiB mean cortical DVR (cDVR) and (b) CSF $A\beta_{1-42}$. Within-individual changes are shown by connecting lines. Individuals who are cognitively normal at baseline evaluation are shown in black, mild cognitive impairment (MCI) and Alzheimer disease in red, and “cognitively impaired, not MCI” in blue. Threshold for detection of elevated PiB retention is shown as a gray line in (a).

Figure 2. Observed values of $A\beta$ over time stratified by APOE $\epsilon 4$ carrier status for (a) PET-PiB mean cortical DVR (cDVR) and (b) CSF $A\beta_{1-42}$. Within-individual changes are shown by connecting lines. APOE $\epsilon 4$ negative individuals are shown in black and APOE $\epsilon 4$ positive in red. Threshold for detection of elevated PiB retention is shown as a gray line in (a).

Figure 3. Cross-sectional effect of age on (a) PET-PiB mean cortical DVR (cDVR) and (b) CSF $A\beta_{1-42}$, showing stronger association of age with $A\beta$ for APOE $\epsilon 4$ carriers compared with non-carriers. This interaction reached significance for PET-PiB ($p = 0.047$) and was at trend level for CSF $A\beta_{1-42}$ ($p = 0.076$).

Table 1. SAMPLE CHARACTERISTICS

	BLSA	BIOCARD_{≥55}
N	113	207
Age at Baseline		
mean (SD)	77.3 (8.1)	62 (6.4)
range	56 to 93	55 to 86
Female, No. (%)	50 (44%)	116 (56.0%)
White, No. (%)	92 (81%)	202 (97.6%)
Education (yrs)		
mean (SD)	16.8 (2.1)	17.2 (2.3)
Range	12 to 20	12 to 20
No. Assessments		
mean (SD)	2.3 (1.6)	2.4 (1.5)
Range	1 to 7	1 to 7
N with 1	51	78
N with 2	21	44
N with 3	20	38
N with 4 or more	21	47

**Follow-up interval (yrs
from baseline)**

mean (SD)	2.2 (2.4)	2.4 (2.6)
Range	0 to 7	0 to 9.1
APOE, No. (%)		
e4-	76 (67%)	126 (61%)
e4+	33 (29%)	81 (39%)
Missing	4 (4%)	0 (0%)
MMSE at baseline	28.7 (1.7)	29.5 (0.8)
Diagnosis at baseline		
Normal	107	195
MCI	3	4
Impaired, Not MCI	1	7
Dementia	2	1

Table 2. Mixed Model Results Examining Effects of APOE Genotype on A β Biomarkers

Effect	BLSA			BIOCARD		
	Estimate	Standard	Pr > t	Estimate	Standard	Pr > t
		Error			Error	
Intercept	1.125	0.011	<.0001	418.99	8.55	<0.0001
Sex	0.04	0.023	0.088	-12.76	13.46	0.344
Age	0.006	0.001	0.0002	-3.06	1.20	0.012
APOE ϵ 4 genotype	0.082	0.025	0.001	-54.84	13.72	0.0001
Age * APOE ϵ 4	0.007	0.003	0.047	-3.77	2.11	0.076
Interval	0.009	0.001	<0.0001	-5.51	1.90	0.005
Age* interval	0.0004	0.0002	0.082	0.13	0.21	0.550
Sex*interval	0.0009	0.003	0.754	2.34	2.90	0.423
APOE ϵ 4*interval	0.005	0.003	0.120	-2.72	2.92	0.355

Significant results are in bold.

References

- [1] Khachaturian AS, Corcoran CD, Mayer LS, Zandi PP, Breitner JC, Cache County Study I. Apolipoprotein E epsilon4 count affects age at onset of Alzheimer disease, but not lifetime susceptibility: The Cache County Study. *Arch Gen Psychiatry*. May 2004;61(5):518-524.
- [2] Slioter AJ, Houwing-Duistermaat JJ, van Harskamp F, et al. Apolipoprotein E genotype and progression of Alzheimer's disease: the Rotterdam Study. *J Neurol*. Apr 1999;246(4):304-308.
- [3] Bracco L, Piccini C, Baccini M, et al. Pattern and progression of cognitive decline in Alzheimer's disease: role of premorbid intelligence and ApoE genotype. *Dement Geriatr Cogn Disord*. 2007;24(6):483-491.
- [4] Martins CA, Oulhaj A, de Jager CA, Williams JH. APOE alleles predict the rate of cognitive decline in Alzheimer disease: a nonlinear model. *Neurology*. Dec 27 2005;65(12):1888-1893.
- [5] Rowe CC, Ellis KA, Rimajova M, et al. Amyloid imaging results from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging. *Neurobiology of aging*. Aug 2010;31(8):1275-1283.
- [6] Sperling RA, Aisen PS, Beckett LA, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. May 2011;7(3):280-292.
- [7] Sojkova J, Zhou Y, An Y, et al. Longitudinal patterns of beta-amyloid deposition in nondemented older adults. *Arch Neurol*. May 2011;68(5):644-649.

- [8] Villemagne VL, Pike KE, Chetelat G, et al. Longitudinal assessment of Abeta and cognition in aging and Alzheimer disease. *Annals of neurology*. Jan 2011;69(1):181-192.
- [9] Vlassenko AG, Mintun MA, Xiong C, et al. Amyloid-beta plaque growth in cognitively normal adults: longitudinal [11C]Pittsburgh compound B data. *Ann Neurol*. Nov 2011;70(5):857-861.
- [10] Reiman EM, Chen K, Liu X, et al. Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America*. Apr 21 2009;106(16):6820-6825.
- [11] Thambisetty M, Tripaldi R, Riddoch-Contreras J, et al. Proteome-based plasma markers of brain amyloid-beta deposition in non-demented older individuals. *J Alzheimers Dis*. 2010;22(4):1099-1109.
- [12] Fleisher AS, Chen K, Liu X, et al. Apolipoprotein E epsilon4 and age effects on florbetapir positron emission tomography in healthy aging and Alzheimer disease. *Neurobiol Aging*. Jan 2013;34(1):1-12.
- [13] Fouquet M, Besson FL, Gonneaud J, La Joie R, Chetelat G. Imaging brain effects of APOE4 in cognitively normal individuals across the lifespan. *Neuropsychology review*. Sep 2014;24(3):290-299.
- [14] Mattsson N, Zetterberg H, Hansson O, et al. CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. *JAMA*. Jul 22 2009;302(4):385-393.
- [15] Moghekar A, Li S, Lu Y, et al. CSF biomarker changes precede symptom onset of mild cognitive impairment. *Neurology*. Nov 12 2013;81(20):1753-1758.

- [16] Peskind ER, Li G, Shofer J, et al. Age and apolipoprotein E*4 allele effects on cerebrospinal fluid beta-amyloid 42 in adults with normal cognition. *Arch Neurol*. Jul 2006;63(7):936-939.
- [17] Morris JC, Roe CM, Xiong C, et al. APOE predicts amyloid-beta but not tau Alzheimer pathology in cognitively normal aging. *Annals of neurology*. 67(1):122-131.
- [18] Resnick SM, Goldszal AF, Davatzikos C, et al. One-year age changes in MRI brain volumes in older adults. *Cerebral cortex*. May 2000;10(5):464-472.
- [19] Driscoll I, Resnick SM, Troncoso JC, An Y, O'Brien R, Zonderman AB. Impact of Alzheimer's pathology on cognitive trajectories in nondemented elderly. *Annals of neurology*. Dec 2006;60(6):688-695.
- [20] Kawas C, Gray S, Brookmeyer R, Fozard J, Zonderman A. Age-specific incidence rates of Alzheimer's disease: the Baltimore Longitudinal Study of Aging. *Neurology*. Jun 13 2000;54(11):2072-2077.
- [21] *Diagnostic and Statistical Manual of Mental Disorders: Fourth Edition, DSM-IV*. Washington, D.C.: American Psychiatric Association; 1994.
- [22] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*. Jul 1984;34(7):939-944.
- [23] Morris JC. The clinical dementia rating (CDR): Current version and scoring rules. *Neurology*. 1993;43:2412-2414.

- [24] Daly E, Zaitchik D, Copeland M, Schmahmann J, Gunther J, Albert M. Predicting conversion to Alzheimer disease using standardized clinical information. *Arch Neurol*. May 2000;57(5):675-680.
- [25] Tzourio-Mazoyer N, Landeau B, Papathanassiou D, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage*. Jan 2002;15(1):273-289.
- [26] Zhou Y, Resnick SM, Ye W, et al. Using a reference tissue model with spatial constraint to quantify [11C]Pittsburgh compound B PET for early diagnosis of Alzheimer's disease. *NeuroImage*. Jun 2007;36(2):298-312.
- [27] Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res*. Mar 1990;31(3):545-548.
- [28] Johnson KA, Sperling RA, Gidyczin CM, et al. Florbetapir (F18-AV-45) PET to assess amyloid burden in Alzheimer's disease dementia, mild cognitive impairment, and normal aging. *Alzheimers Dement*. Oct 2013;9(5 Suppl):S72-83.
- [29] Villemagne VL, Burnham S, Bourgeat P, et al. Amyloid beta deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. *The Lancet. Neurology*. Apr 2013;12(4):357-367.
- [30] Albert M, Soldan A, Gottesman R, et al. Cognitive changes preceding clinical symptom onset of mild cognitive impairment and relationship to ApoE genotype. *Curr Alzheimer Res*. 2014;11(8):773-84.

Figure 1

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Figure 2

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Figure 3

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