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Incident cognitive impairment: longitudinal changes in molecular, structural and cognitive biomarkers

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Longer periods are needed to examine how biomarker changes occur relative to incident sporadic cognitive impairment. We evaluated molecular (CSF and imaging), structural, and cognitive biomarkers to predict incident cognitive impairment and examined longitudinal biomarker changes before and after symptomatic onset. Data from participants who were cognitively normal, underwent amyloid imaging using Pittsburgh compound B and/or CSF studies, and at least two clinical assessments were used. Stepwise Cox proportional hazards models tested associations of molecular (Pittsburgh compound B; CSF amyloid-β_{42}, tau, ptau$_{181}$, tau/amyloid-β_{42}, ptau$_{181}$/amyloid-β_{42}), structural (normalized hippocampal volume, normalized whole brain volume), and cognitive (Animal Naming, Trail Making A, Trail Making B, Selective Reminding Test - Free Recall) biomarkers with time to Clinical Dementia Rating (CDR) > 0. Cognitively normal participants ($n = 664$), aged 42 to 90 years (mean ± standard deviation = 71.4 ± 9.2) were followed for up to 16.9 years (mean ± standard deviation = 6.2 ± 3.5 years). Of these, 145 (21.8%) participants developed a CDR > 0. At time of incident cognitive impairment, molecular, structural, and cognitive markers were abnormal for CDR > 0 compared to CDR = 0. Linear mixed models indicated rates of change in molecular biomarkers were similar for CDR = 0 and CDR > 0, suggesting that the separation in values between CDR = 0 and CDR > 0 must have occurred prior to the observation period. Rate of decline for structural and cognitive biomarkers was faster for CDR > 0 compared to CDR = 0 ($P < 0.0001$). Structural and cognitive biomarkers for CDR > 0 diverged from CDR 0 at 9 and 12 years before incident cognitive impairment, respectively. Within those who developed CDR > 0, a natural separation occurred for Pittsburgh compound B values. In particular, CDR > 0 who had at least one *APOE* ε4 allele had higher, and more rapid increase in Pittsburgh compound B, while *APOE* ε2 was observed to have slower increases in Pittsburgh compound B. Of molecular biomarker-positive participants followed for at least 10 years ($n = 16$–23), ~70% remained CDR = 0 over the follow-up period. In conclusion, conversion from cognitively normal to CDR > 0 is characterized by not only the magnitude of molecular biomarkers but also rate of change in cognitive and structural biomarkers. Findings support theoretical models of biomarker changes seen during transition to cognitive impairment using longitudinal data and provide a potential time for changes seen during this transition. These findings support the use of molecular biomarkers for trial inclusion and cognitive/structural biomarkers for evaluating trial outcomes. Finally, results support a potential role for *APOE* ε in modulating amyloid accumulation in CDR > 0 with *APOE* ε4 being deleterious and *APOE* ε2 protective.

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Abbreviations: CDR = Clinical Dementia Rating; PIB = Pittsburgh compound B; SRTFREE = Selective Reminding Test – Free Recall

Introduction

Hypothesized models detailing molecular, structural, and cognitive changes before and after the onset of symptomatic cognitive impairment have been proposed (Sperling *et al.*, 2011; Jack *et al.*, 2013, 2015, 2018; Dubois *et al.*, 2016). Development and refinement of these models has relied greatly on findings generated by cross-sectional studies, including examination of estimated age at symptomatic onset among persons with mutations causing autosomal dominant Alzheimer's disease (Bateman *et al.*, 2012). Within autosomal dominant Alzheimer's disease studies, the estimated years to onset has allowed for evaluating changes in biomarkers in relation to a known event. Results from these studies suggest that asymptomatic mutation carriers in autosomal dominant Alzheimer's disease families have molecular biomarker changes 15–20 years prior to estimated year of onset and changes in brain structural and cognitive symptoms 8–10 years prior to estimated year of onset (Bateman *et al.*, 2012; Fagan *et al.*, 2014).

More recent studies have demonstrated that biomarker abnormalities may be present during preclinical stages of sporadic Alzheimer's disease (Stomrud *et al.*, 2015; Sutphen *et al.*, 2015; Toledo *et al.*, 2015; Fletcher *et al.*, 2016; Dumurgier *et al.*, 2017; Insel *et al.*, 2017). However, since there is no 'estimated years to onset' for sporadic Alzheimer's disease, it has been difficult to elucidate when changes will occur as long follow-up periods are required. Relatively few longitudinal studies have been performed, and hypothesized models for sporadic Alzheimer's disease have not defined specific time intervals. The few longitudinal studies that have been performed using a limited number of biomarkers suggest that the asymptomatic period for persons with preclinical Alzheimer's disease may last for at least a decade (Buchhave *et al.*, 2012; Roe *et al.*, 2013; Stomrud *et al.*, 2015).

A gap therefore exists with longitudinal studies examining multiple biomarkers (molecular, structural, and cognitive), such that longer periods are needed to examine how biomarker changes occur relative to incident sporadic cognitive impairment. In this study, we first examined molecular, structural, and cognitive biomarkers to predict incident cognitive impairment up to 16.9 years after initial evaluation. Symptomatic cognitive impairment was operationalized by a Clinical Dementia Rating (CDR) > 0 and encompassed both mild cognitive impairment and mild dementia. Our main question of interest, the magnitude and rate of change in biomarkers for persons who did, and did not, develop incident symptomatic cognitive impairment was then examined.

Materials and methods

Participant selection

Data were used from participants enrolled in longitudinal studies at the Knight Alzheimer's Disease Research Center at Washington University in Saint Louis, USA, who were cognitively normal at their initial visit, underwent PET using Pittsburgh compound B (PIB) imaging and/or had CSF collected within 1 year of the baseline clinical assessment, and had at least one additional clinical assessment after their baseline visit. All procedures were reviewed and approved by the Washington University in St. Louis Human Research Protection Office and informed consent was obtained according to the Declaration of Helsinki from all participants.

Clinical assessment

Participants in Knight Alzheimer's Disease Research Center longitudinal studies have annual clinical and psychometric assessments. During these assessments, cognitive normality was determined using the CDR (Morris, 1993). Experienced clinicians derive the CDR by integrating information obtained from interviews with the participant and separately with a collateral source who knows the participant well. These interviews rely on the principle of intra-individual change in cognitive and functional abilities, where the individual serves as his or her own control. The CDR is obtained via a standard scoring algorithm based on scores in six domains and indicates whether the participant has dementia, and if so, the severity of dementia (CDR 0 = cognitively normal, CDR 0.5 = very mild, CDR 1 = mild, CDR 2 = moderate, and CDR 3 = severe dementia) (Morris, 1993). Summation of the scores from the six domains results in the CDR Sum of Boxes, a continuous

measure of cognitive impairment, which is often used as an outcome in clinical trials (Williams *et al.*, 2009).

Design

We evaluated the ability of structural MRI and cognitive biomarkers together with the molecular biomarkers to predict time to a first diagnosis of cognitive impairment and evaluated the number of years that participants with 'positive' molecular biomarkers could remain cognitively normal. For participants who developed cognitive impairment over the follow-up period, changes in biomarkers before and after the date of first diagnosis of cognitive impairment were examined and compared to each other, and to biomarker changes for persons who remained cognitively normal. We also examined associations between *APOE* ε genotype and changes in molecular biomarkers across time.

Molecular biomarkers

Amyloid imaging

PIB imaging was used to determine brain amyloid burden (Klunk, 2011). Dynamic scans were used. Regional target-to-reference intensity ratio—standard uptake ratio—was estimated using 30 to 60 min post-injection as the time window for PIB and using the cerebellum cortex as the default reference region. Global amyloid-β burden was estimated using a set of regions of interest known to be sensitive to amyloid-β deposition (Su *et al.*, 2013). PIB positivity was defined as having a standardized uptake value ratio (SUVR) $\geqslant$ 1.31 (Vlassenko *et al.*, 2016). Partial volume correction was not applied.

CSF biomarkers

CSF analytes (Fagan *et al.*, 2006) [amyloid-β_{42}, tau and ptau$_{181}$; Innotest, Fujirebio (formerly Innogenetics)] were measured using sensitive and quantitative enzyme-linked immunosorbent assays (ELISA). CSF was obtained using a 22-gauge Sprotte spinal needle to draw 20–30 ml of CSF at 8:00 am following an overnight fast. CSF samples were gently inverted and centrifuged at low speed to avoid possible gradient effects and frozen at −84°C after aliquoting into polypropylene tubes. Biomarker assays included a common reference standard, within-plate sample randomization and standardized protocol adherence. Samples were reanalysed if coefficients of variability exceed 25% [per Alzheimer's Disease Neuroimaging Initiative (ADNI) criteria]; if there were 'edge artefacts'; or if the pooled common CSF sample yielded widely discrepant values.

We examined the CSF variables of amyloid-β_{42}, tau, ptau$_{181}$, and the ratios of tau/amyloid-β_{42} and ptau$_{181}$/amyloid-β_{42}. In dichotomizing the CSF biomarkers, previously published cut-offs (Vos *et al.*, 2013) were used for tau (339 pg/ml) and ptau$_{181}$ (67 pg/ml). Because of concerns about upward drift in Innotest immunoassay amyloid-β_{42} values over the years (Schindler *et al.*, 2018), positive and negative values of amyloid-β_{42} were assigned using assay- and date-specific cut-offs recommended by the Knight Alzheimer's Disease Research Center Biomarker Core based on the work of Schindler *et al.* (2018). Using receiver operating curve (ROC) analyses, we determined the CSF amyloid-β_{42} cut-off that best distinguishes between amyloid PET positive and amyloid PET negative cases as a function of amyloid-β_{42} assay time period and assay type. Then, CSF amyloid-β_{42} levels were dichotomized as positive (if lower than the cut-off) or negative (if higher than the cut-off) according to the assay date and type (for more specific information, see Schindler *et al.*, 2018). Adjusted values of amyloid-β_{42}, tau/amyloid-β_{42}, and ptau$_{181}$/amyloid-β_{42} were constructed by calculating studentized residuals based on amyloid-β_{42} lot numbers and dates. Because cut-offs for the adjusted ratio variables are not yet available, we examined the frequency distributions of each variable and operationally defined the highest 30% of values as positive based on Alzheimer's disease biomarker measurement and autopsy studies suggesting that roughly 30% of cognitively normal persons have preclinical Alzheimer's disease (Price *et al.*, 2009; Morris *et al.*, 2010; Jansen *et al.*, 2015).

Structural imaging

Scans were acquired using Siemens BioGraph mMR PET-MR 3T and Siemens TIM Trio 3 T MRI scanners.

To transition our cohort from the Siemens TIM Trio 3 T MRI to the Siemens Biograph 3 T molecular magnetic resonance (mMR), we performed direct correlations in a subset of our participants. Sixty-nine participants with a mean age of 65.9 years (CDR 0–0.5) received both the Trio and mMR MRI within 2 weeks; 67 participants were cognitively normal (CDR 0); two participants had a diagnosis of mild symptomatic Alzheimer's disease (CDR 0.5). FreeSurfer v5.1 was used to segment the brain into various regions of interest for quantitative analysis.

For the left hippocampal volume as measured by Trio and the PET MRI, the estimated concordance correlation coefficient (CCC) on the raw data is 0.83 with a 95% confidence interval (CI) from 0.73 to 0.89, and after the standardization, the estimated CCC is 0.83 with a 95% CI from 0.74 to 0.90. For the right hippocampus volume as measured by Trio and the PET MRI, the estimated CCC on the raw data is 0.79 with a 95% CI from 0.67 to 0.87, and after the standardization, the estimated CCC is still 0.79 with a 95% CI from 0.67 to 0.87. Because of the two potential outliers in the scatter plot of the data on hippocampal volumes, we also performed rank-based CCC on these measures, the rank-based CCC for left hippocampal volume is 0.92 with a 95% CI from 0.86 to 0.95, and the rank-based CCC for right hippocampal volume is 0.91 with a 95% CI from 0.86 to 0.95, again both indicating excellent rank-based reproducibility of measuring hippocampal volumes. These findings are within the reported test-retest reliability range for repeat MRI visits on the same scanner (Han *et al.*, 2006).

All MRI sessions were processed through the FreeSurfer image analysis suite using Dell PowerEdge 1950 servers with Intel Xeon processors running CentOS 5.5 Linux. FreeSurfer 5.3 (http://surfer.nmr.mgh.harvard.edu/) analyses involved cortical reconstruction and volumetric segmentation of T_1-weighted images. The technical details of these procedures have been described previously (Dale *et al.*, 1999; Fischl *et al.*, 1999*a*, 2002). The cross-sectional processing pipeline included motion correction and segmentation of the subcortical white matter and deep grey matter volumetric structures on a T_1-weighted image (Fischl *et al.*, 2002), intensity normalized, registered to a spherical atlas, which used individual cortical folding patterns to match cortical geometry across

participants (Fischl *et al.*, 1999*b*), and parcellated into units based on gyral and sulcal structure (Desikan *et al.*, 2006).

The structural biomarkers examined included: normalized whole brain volume and normalized hippocampal volume. Normalization was accomplished by computing the mean intracranial volume (ICV) for the current sample, performing a regression analysis using ICV as the independent variable and the raw volume as the dependent variable to obtain the b-weight, and then applied using the following equation: normalized volume = raw volume – [b-weight * (ICV for individual participant – mean sample ICV)] (Mathalon *et al.*, 1993).

Psychometric battery

Independent of the CDR assessment, a psychometric test battery was administered to participants, typically within a few weeks of the CDR assessment. Psychometric tests common to all Knight Alzheimer's Disease Research Center longitudinal protocols include Animal Naming (Goodglass and Kaplan, 1983), Trail Making A test (Armitage, 1946), Trail Making B test (Armitage, 1946), the Selective Reminding Test containing the Free Recall (SRTFREE) and Cued subtests (Grober *et al.*, 1988), and the Mini-Mental State Examination (Folstein *et al.*, 1975). Because baseline clinical and neuropsychometric assessments followed the National Alzheimer's Disease Coordinating Center Uniform Data Set protocols (Morris *et al.*, 2006; Beekly *et al.*, 2007; Weintraub *et al.*, 2009), data were available regarding behavioural changes, medications, and health history.

APOE ε genotyping

Briefly, all DNA samples underwent stringent quality control before genotyping with the Illumina 610 or the Omniexpress chip (Cruchaga *et al.*, 2012). Complete information regarding *APOE* ε genotyping is available using previously described methods (Cruchaga *et al.*, 2012).

Statistical analyses

Portions of the data were collected and managed using research electronic data capture (REDCap) tools (Harris *et al.*, 2009). For all analyses, SAS statistical software version 9.4 (SAS Institute Inc.) was used, alpha = 0.05 was taken to indicate statistical significance, and all tests were two-tailed.

Biomarker prediction of incident CDR > 0

Six stepwise Cox proportional hazards models tested the association of each of the molecular biomarkers (PIB, adjusted CSF amyloid-β_{42}, CSF tau, CSF ptau$_{181}$, adjusted CSF tau/amyloid-β_{42}, adjusted CSF ptau$_{181}$/amyloid-β_{42}) with time to incident CDR > 0. These models included terms for demographic [age, education, gender, race, number of *APOE* ε4 allele (0, 1, or 2) copies], psychometric (Animal Naming, Trail Making A, Trail Making B, SRTFREE, Selective Reminding Test – Cued Recall), and structural imaging (normalized hippocampal volume, normalized whole brain volume) measures for stepwise selection. Mini-Mental State Examination and CDR Sum of Boxes scores were not included as candidate variables because of extreme ceiling and floor effects. Because stepwise selection was used, data from participants with non-missing data on all candidate variables were used. Normalized hippocampal volume and normalized whole brain volume data from the MRI visit closest to the baseline clinical assessment were used. The significance level for entry and retention of each term was set at $P = 0.05$. Data from participants who did not become symptomatic over the follow-up period were censored at the date of last clinical assessment. We repeated the Cox proportional hazards models after first forcing age, gender, and education into the model and then allowed the stepwise selection method to select from the remaining variables those that met entry and retention criteria. Kaplan-Meyer survival curves and bubble plots were used to graphically illustrate survival findings.

Time remaining cognitively normal despite biomarker abnormality

For each molecular biomarker, we examined and reported the number and percentage of biomarker-positive individuals who remained cognitively normal among those followed at least 10 years. As exploratory analyses, we also examined differences in demographics and baseline cognitive scores for those who did and did not remain cognitively normal.

Comparison of biomarker changes across time for persons who did and did not develop incident CDR > 0

To examine longitudinal changes in biomarkers before and after CDR > 0 onset, spaghetti plots for individual biomarkers were constructed showing changes in that biomarker for persons who developed CDR > 0. A smoothed Loess curve illustrating biomarker changes before and after the first clinical assessment with CDR > 0 was fitted to the data. To compare biomarker changes in individuals who remained CDR 0 during an earlier and later period in their follow-up, we randomly chose an arbitrary clinical assessment date analogous to the date of incident CDR > 0 in the affected group. This allowed us to compare biomarker changes at an earlier and later period of follow-up within the same individual who remained CDR 0 (Fig. 1). A smoothed curve was then fitted to the data as described above. Linear mixed models were used to test whether there were differences between the slopes of biomarker change across time for the groups.

Associations between *APOE* ε genotype and molecular biomarker cut-offs

Spaghetti plots illustrated changes in adjusted amyloid-β_{42}, tau, and ptau$_{181}$ values with time as they related to *APOE* ε genotype. Logistic regression was used to examine *APOE* ε genotypes as they relate to molecular biomarker cut-off values.

Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Results

Data from $n = 664$ participants, ranging in age from 42 to 90 years at baseline (mean ± standard deviation = 67.6 ± 9.6 years), were available. Participants were followed for up to

Figure 1 Assignment of arbitrary clinical assessment (ACA) date. Only persons with a CDR of 0 at first assessment were included in analyses, so no participants could have developed CDR > 0 at that assessment (**A**). Participant A had a first CDR > 0 at Assessment 5, and Participant C at Assessment 3 (**A**). Biomarker behaviour before and/or after first assessment with a CDR > 0 was examined for Participants A and C, who developed cognitive impairment (**B**). For participants remaining CDR 0 across the follow-up period, all assessments following the first were candidates for ACA (**B**). An ACA was randomly assigned to one of the candidates (**C**). For candidates remaining CDR 0, biomarker behaviour before and/or after the assigned ACA was examined (**D**). Since Participant E only had one assessment after the first, Assessment 2 was assigned as his/her ACA (**D**).

16.9 years with a mean follow-up period of 6.2 ± 3.5 years. During follow-up, 145 (21.8%) participants became CDR > 0 and 519 remained cognitively normal. Table 1 presents the demographic characteristics of the sample.

Biomarker prediction of incident CDR > 0

Figure 2 shows the survival curves for the unadjusted association between each of the molecular biomarkers and time to incident CDR > 0 for the 664 participants. Because non-missing data on all 13 candidate variables for stepwise selection were required, Cox proportional hazards analyses consisted of smaller subsamples ($n = 286$ for PIB and $n = 302$ for CSF). Forty-two participants in the PIB, and 39 in the CSF analyses developed CDR > 0. Molecular (PIB, CSF amyloid-β_{42}, tau, tau/amyloid-β_{42}, ptau/amyloid-β_{42}), psychometric (SRTFREE), and structural (normalized hippocampal volume) biomarkers combined to predict first time to cognitive impairment in the models (Table 2). Trail Making B also independently contributed to prediction of CDR > 0 in the model testing PIB ($P = 0.024$). Age did not independently predict CDR > 0 once normalized hippocampal volume was included in the models. Supplementary Table 1 shows the results when age, gender and education were first forced into the models, and then the stepwise selection procedure was used to choose among the remaining candidate variables for model entry.

Because normalized hippocampal volume was a consistent predictor in all models, bubble plots were used to graphically illustrate relationships between each of the molecular biomarkers and normalized hippocampal volume with regards to follow-up time, and CDR > 0 (Supplementary Fig. 1). As illustrated in those plots, none

Table 1 Baseline demographics ($n = 664$)

	Developed CDR > 0 ($n = 145$)		Remained CDR 0 ($n = 519$)			Total	
	n/Mean	%/SD	*n*/Mean	%/SD	*P*-value	*n*/Mean	%/SD
Age, years	73.9	7.6	65.9	9.3	<0.0001	67.6	9.6
Female, *n*	83	57.2%	307	59.2%	0.680	390	58.7%
Minority race, *n*	13	9.0%	65	12.5%	0.024	78	11.8%
APOE, *n*	176	33.9%	52	35.9%	0.721		
$\varepsilon2\varepsilon2$	2	1.4%	3	0.6%		5	0.8%
$\varepsilon2\varepsilon3$	15	10.3%	62	12.0%		77	11.6%
$\varepsilon2\varepsilon4$	3	2.1%	19	3.7%		22	3.3%
$\varepsilon3\varepsilon3$	76	52.4%	278	53.6%		354	53.3%
$\varepsilon3\varepsilon4$	43	29.7%	133	25.6%		176	26.5%
$\varepsilon4\varepsilon4$	6	4.1%	24	4.6%		30	4.5%
Education, years	15.7	3.2	16.0	2.6	0.175	15.9	2.7
PIB, SUVR	1.4	0.4	1.2	0.2	<0.0001	1.2	0.3
Adjusted CSF $A\beta_{42}$	−0.36	.95	0.10	0.95	<0.0001	0.0	1.0
CSF tau, pg/ml	371.0	206.0	276.3	151.9	<0.0001	295.7	168.7
CSF $ptau_{181}$, pg/ml	64.2	30.3	53.7	25.2	<0.001	55.9	26.6
Adjusted tau/$A\beta_{42}$	0.62	1.48	−0.16	0.77	<0.0001	0.0	1.0
Adjusted $ptau_{181}$/$A\beta_{42}$	0.56	1.40	−0.14	0.82	<0.0001	0.0	1.0
SRTFREE	26.9	6.2	31.3	5.6	<0.0001	30.4	6.0
Animal Naming	19.0	5.4	22.0	5.6	<0.0001	21.3	5.6
Trail Making A	36.8	15.2	31.6	12.3	<0.001	32.8	13.1
Trail Making B	96.4	41.1	75.9	31.0	<0.0001	80.4	34.5
Normalized hippocampal volume, ml	6.8	1.0	7.7	0.9	<0.0001	7.6	1.0
Normalized whole brain volume, ml	963.7	63.2	1025.4	65.6	<0.0001	10 164.4	68.7
Follow-up time, years	7.4	3.6	5.9	3.4	<0.0001	6.2	3.5

$A\beta$ = amyloid-β; SD = standard deviation.

of the participants with a normalized hippocampal volume >8673 mm^3 became CDR > 0 regardless of molecular biomarker values and length of follow-up. Similarly, lower SRTFREE baseline scores were associated with reduced time to symptomatic onset, such that only 1 of 34 persons (2.9%) with SRTFREE scores of 40 or above became CDR > 0 (data not shown).

Time remaining cognitively normal despite biomarker abnormality

Among participants followed for at least 10 years, the number and per cent of participants who were biomarker positive at baseline and who remained cognitively normal over the follow-up period were as follows: 15/18 (83.3%) for CSF amyloid-β_{42}, 16/23 (69.6%) for CSF tau, 11/16 (68.8%) for CSF $ptau_{181}$, 12/17 (70.6%) for CSF tau/amyloid-β_{42}, and 13/17 (76.5%) for CSF $ptau_{181}$/amyloid-β_{42}. Only three participants with positive PIB values were followed for at least 10 years, and all three remained cognitively normal during this time. One participant with positive CSF biomarkers for tau, $ptau_{181}$, CSF tau/amyloid-β_{42} and CSF $ptau_{181}$/amyloid-β_{42} was followed for 15 years and remained cognitively normal. However, this participant had a normal baseline CSF amyloid-β_{42} value, and so may have not converted because he or she had a disease other than Alzheimer's disease, such as suspected non-Alzheimer's disease pathophysiology (SNAP) (Jack *et al.*, 2016). Exploratory analyses indicated that persons who were resilient to underlying Alzheimer's disease pathology tended to be younger and to have better performance on the Trail Making tests (Supplementary Table 2).

Comparison of biomarker changes across time for persons who did and did not develop incident CDR > 0

The length of biomarker data available for observation differed by biomarker and was dependent on how long each biomarker had been included in Knight Alzheimer's Disease Research Center protocols. Including all biomarkers, data were collected from 8 May 1985 to 16 December 2016, a total of over 31 years. Specific information regarding when each biomarker was collected, and how much data were available per participant, is presented in Supplementary Table 3. As shown in Fig. 3, mixed linear models indicated that the rate of change of the molecular biomarkers, other than CSF amyloid-β_{42} ($P = 0.034$), did not differ for those who did and did not develop CDR > 0 ($P > 0.094$). Of note, Fig. 3A appears to show a downward curvature after the onset of CDR > 0 for that group. This is likely to be an artefact of the smaller number of data points following CDR

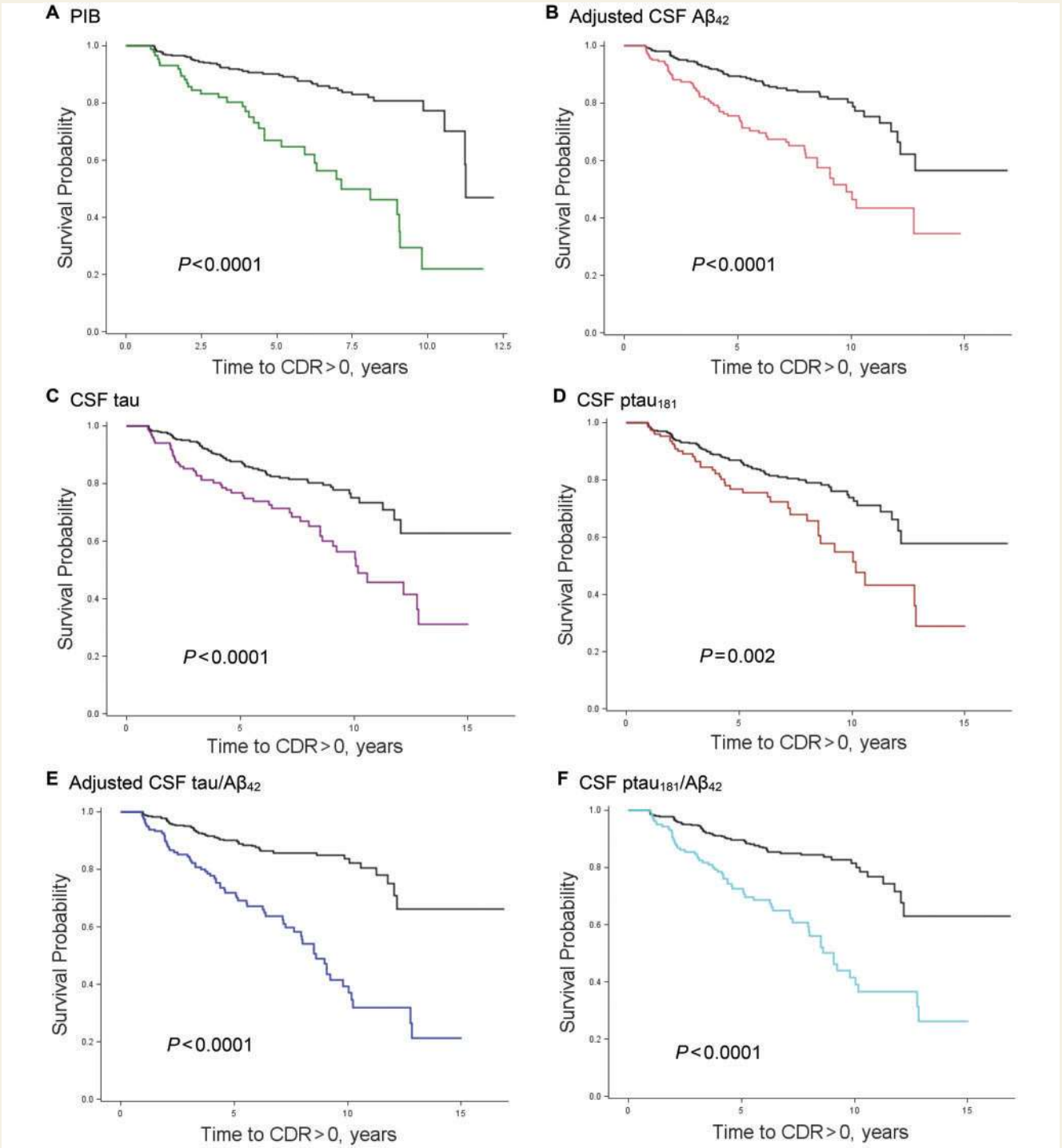

Figure 2 Kaplan-Meier survival curves for molecular biomarkers. Time to first CDR > 0 for participants with abnormal (coloured line) and normal (black line) values of PIB (**A**), and CSF amyloid-β_{42} (**B**), tau (**C**), $ptau_{181}$ (**D**), tau/amyloid-β_{42} (**E**), and $ptau_{181}$/amyloid-β_{42} (**F**). Aβ = amyloid-β.

0.5 onset (see Fig. 4 for an illustration of how many data are available before and after CDR > 0 onset). However, highly significant group differences in rate of change were found for both cognitive biomarkers (Fig. 3G–L) and a structural measure—normalized hippocampal volume (Fig. 3M) ($P < 0.0001$). Group differences in the slope for the other structural measure—normalized whole brain volume—were marginally significant ($P = 0.054$). The overall magnitude of biomarker values, as reflected in the *y*-intercept (time 0), was more abnormal for the group that developed CDR > 0 for every biomarker examined compared to the group that remained cognitively normal ($P < 0.0001$). Supplementary Fig. 4 shows the Loess curves along with lines representing the linear fits.

Table 2 Results of Cox proportional hazards models

	P-value	HR	L95%CI	U95%CI	*P*-value	HR	L95%CI	U95%CI
	Pittsburgh Compound B				**Adjusted CSF amyloid-β_{42}**			
Biomarker	0.004	3.28	1.46	7.36	0.014	0.65	0.47	0.92
SRTFREE	<0.0001	0.88	0.83	0.93	0.001	0.91	0.86	0.96
Normalized hippocampal volume, ml	0.004	0.59	0.41	0.84	<0.0001	0.41	0.29	0.59
Trail Making B	0.024	1.01	1.001	1.02	–	–	–	–
	CSF tau				**CSF ptau$_{181}$**			
Biomarker	0.010	1.002	1.000	1.003	–	–	–	–
SRTFREE	0.002	0.92	0.87	0.97	0.002	0.91	0.86	0.97
Normalized hippocampal volume, ml	<0.0001	0.40	0.28	0.57	<0.0001	0.40	0.28	0.58
Trail Making B	–	–	–	–	–	–	–	–
	Adjusted CSF tau/amyloid-β_{42}				**Adjusted CSF ptau$_{181}$/amyloid-β_{42}**			
Biomarker	0.000	1.54	1.22	1.96	0.003	1.44	1.13	1.83
SRTFREE	0.006	0.94	0.87	0.98	0.007	0.92	0.87	0.98
Normalized hippocampal volume, ml	<0.0001	0.39	0.27	0.57	<0.0001	0.38	0.26	0.55
Trail Making B	–	–	–	–	–	–	–	–

HR = hazard ratio; L95%CI = lower 95% confidence interval; U95%CI = upper 95% confidence interval.

Associations between *APOE* ε genotype and molecular biomarker values

Figure 4A and B shows that among those who developed CDR > 0, there was a clear separation of participants according to the amount and rate of amyloid accumulation. Individuals with high initial PIB values showed increased accumulation over the follow-up period, whereas those with low initial values generally did not show increased accumulation of fibrillar amyloid with time. For ease of discussion, and based on where the separation of the data occurred, individuals with PIB values above the PIB-positive cut-off of 1.31 SUVR (Vlassenko *et al.*, 2016) were assigned to an 'accumulator' group, and those with PIB values below this cut-off were assigned to a 'non-accumulator' group. However, as can be seen in Fig. 4, any cut-off value within the approximate range of 1.3–1.4 SUVR could have been used. Linear mixed models confirmed that the rate of change in PIB values across the study period differed significantly for the accumulators and non-accumulators ($P = 0.044$).

Further, as shown in Fig. 4A, the majority of accumulators had one or more *APOE* ε4 alleles whereas the majority of non-accumulators did not. Figure 4B shows the same data, only now highlighting *APOE* ε2 individuals. *APOE* ε2 has a protective effect on amyloid accumulation, such that almost all participants with an *APOE* ε2 allele were non-accumulators. Only one participant in the accumulator group had an *APOE* ε2 allele, but that person also had an *APOE* ε4 allele. Logistic regression analyses confirmed the graphic information, indicating that being an accumulator was highly associated with having an *APOE* ε4 allele [odds ratio (OR) = 9.73, 95% CI = 2.87–32.94, $P < 0.001$], and that accumulators were less likely to have an *APOE* ε2 allele (OR = 0.10, 95% CI = 0.01–0.90, $P = 0.040$), and were older (OR = 1.07, 95% CI = 1.004–1.15, $P = 0.038$) than non-accumulators.

Among participants who remained cognitively normal (Fig. 4C and D), there was no clear separation into accumulator and non-accumulator groups. To explore the proportion of CDR 0 participants who showed this rapid increase in PIB, analogous to the CDR > 0 accumulators, we first found the 25th percentile of the slopes of the CDR > 0 accumulators. We then operationally defined an 'accumulator' in the CDR 0 group as persons with slopes above that 25th percentile. Of the 208 persons who remained cognitively normal and had at least two PIB measurements, 64 (30.8%) could be considered to be accumulators. In the CDR 0 group, *APOE* ε genotype was again associated with PIB behaviour across time. The majority of cognitively normal persons with high and rising PIB values had an *APOE* ε4 allele (Fig. 4C), whereas most persons with *APOE* ε2 values had low, stable values of PIB across the follow-up period (Fig. 4D). All cognitively normal persons with *APOE* ε2 who showed high and rising PIB values also had an *APOE* ε4 allele (Fig. 4D).

T-tests indicated that among those who developed CDR > 0, PIB accumulators were found to have lower mean values of adjusted CSF amyloid-β_{42}, higher values of CSF ptau$_{181}$, smaller normalized whole brain volume, and similar cognition (Supplementary Table 4).

In contrast to the PIB results, CSF biomarkers did not show a clear pattern of separation observed for participants who developed CDR > 0. The association between *APOE* ε genotype and CSF biomarkers was also different from those observed for PIB accumulators and non-accumulators (Fig. 5 and Supplementary Figs 2 and 3). Here, many individuals who became CDR > 0 and who were *APOE* ε4 had CSF

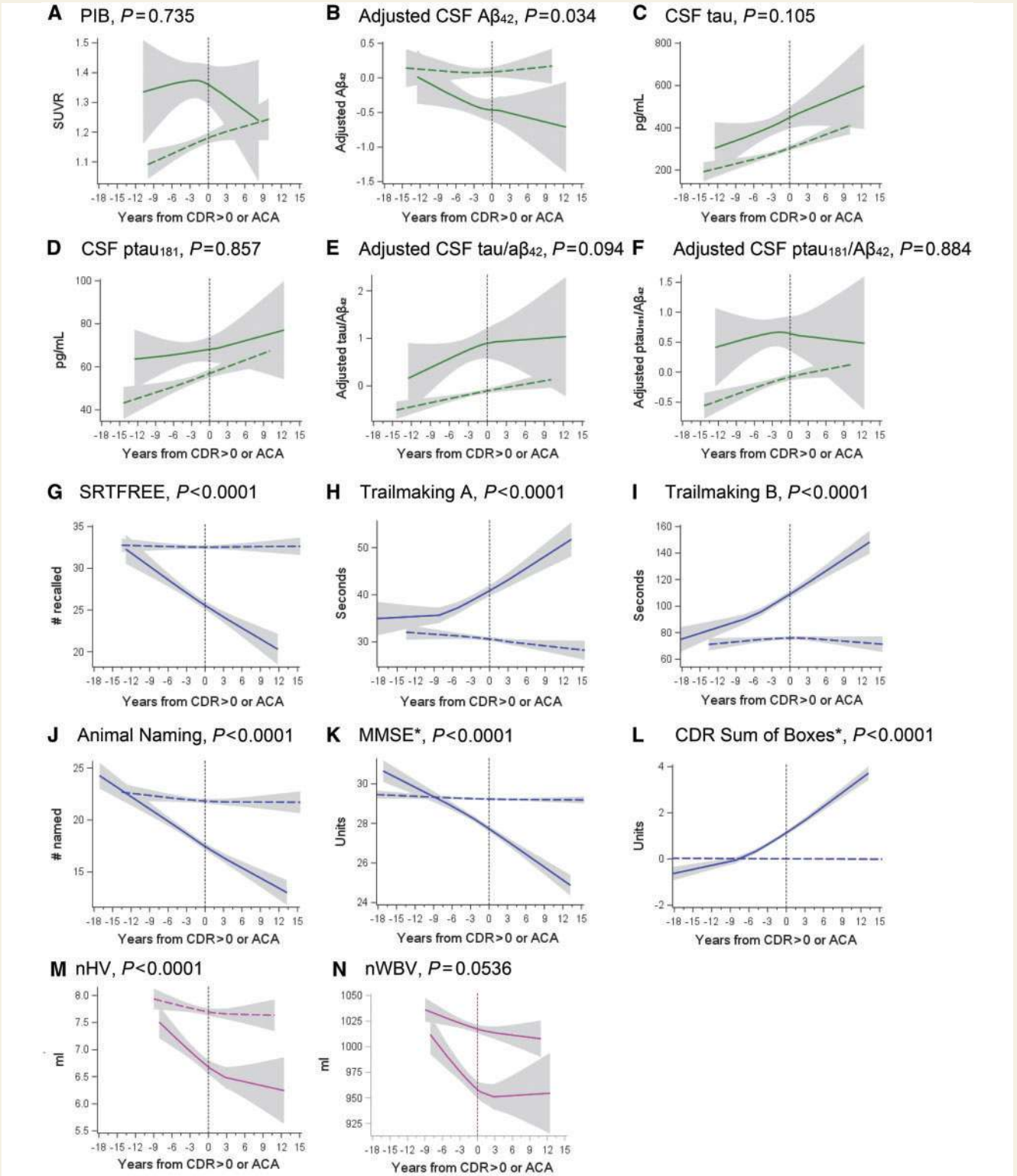

Figure 3 Biomarker changes for persons who did and did not develop CDR > 0. Comparison of selected biomarker, structural, and clinical changes for persons who did and did not develop CDR > 0. Solid lines represent those who developed dementia and dotted lines indicate persons who remained cognitively normal. *P*-values indicate whether there was a significant difference in the mean slope of each group. Aβ = amyloid-β; ACA = arbitrary clinical assessment; MMSE = Mini-Mental State Examination; nHV = normalized hippocampal volume; nWBV = normalized whole brain volume. *No persons had MMSE scores >30, nor CDR Sum of Boxes scores < 0. Extension of lines across the *x*-axis for these measures is an artefact of the curve-fitting process.

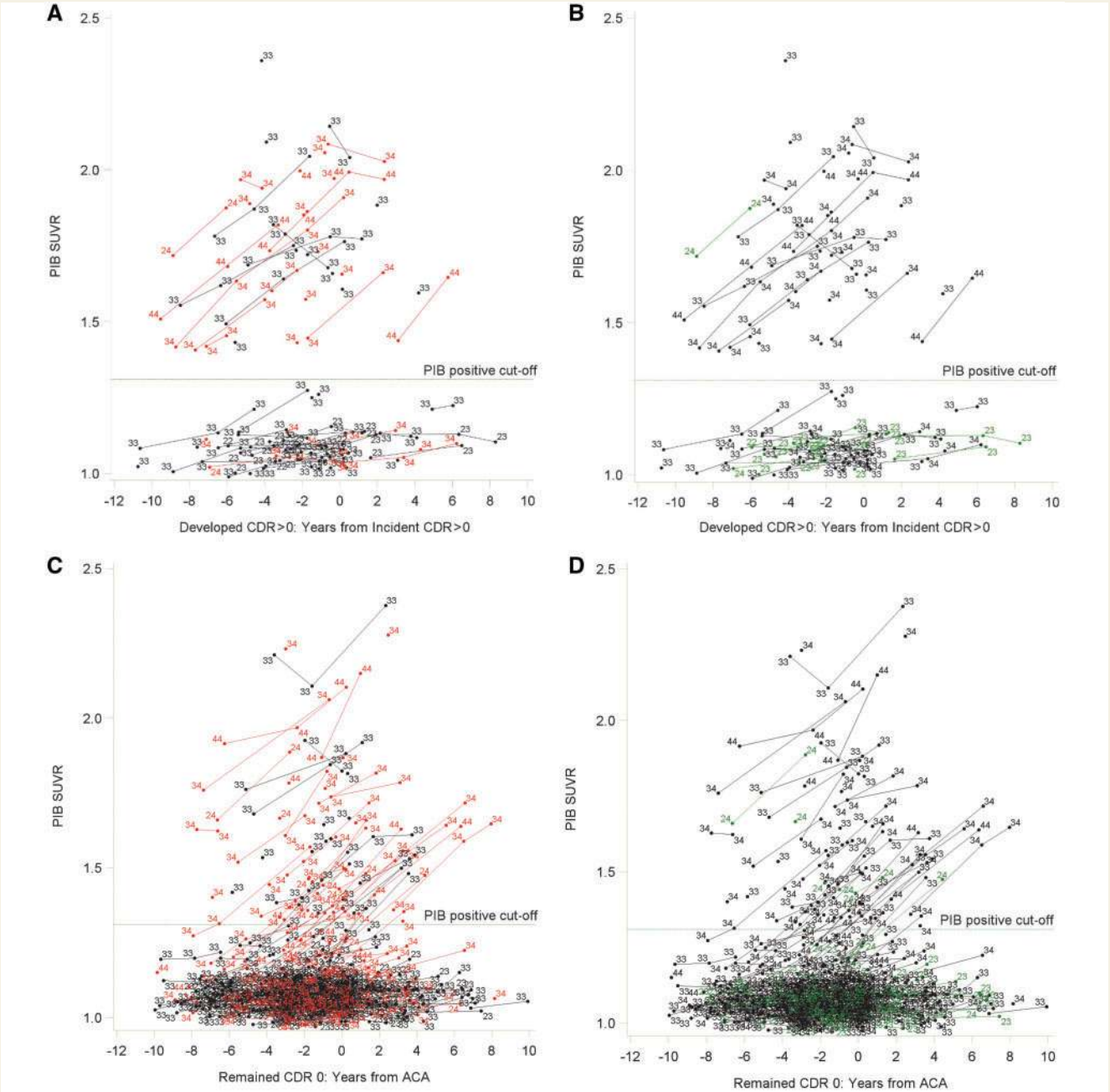

Figure 4 Spaghetti plots for PIB accumulators and non-accumulators. Spaghetti plots for PIB accumulators and non-accumulators who developed CDR > 0 showing the relationship between having at least one *APOE* ε4 allele (in red) (**A**), having at least one *APOE* ε2 allele (in green) (**B**), and magnitude and changes in PIB SUVR values with time. Also shown are spaghetti plots illustrating relationships between having at least one *APOE* ε4 allele (**C**), having at least one *APOE* ε2 allele (**D**), and magnitude and changes in PIB SUVR values with time for individuals who remained cognitively normal over the follow-up period. Data points are labelled with the specific *APOE* ε genotype for that individual. ACA = arbitrary clinical assessment; *APOE* ε4 = at least one *APOE* ε4 allele; *APOE* ε2 = at least one *APOE* ε2 allele; SUVR = standard uptake value ratio.

amyloid-β_{42}, tau, and ptau$_{181}$ values in the normal range, and a few CDR > 0 and who were *APOE* ε2 had CSF biomarker values in the abnormal range. However, logistic regression indicated that generally, *APOE* ε genotypes were associated with lower CSF amyloid-β_{42} values for persons who developed CDR > 0 [*APOE* ε4: OR (95% CI) = 7.68 (2.17–27.26), $P = 0.002$; *APOE* ε2: OR (95% CI) = 0.14 (0.03–0.81), $P = 0.028$] and those who did not [*APOE* ε4: OR (95% CI) = 3.17 (2.04–4.94), $P < 0.0001$; *APOE* ε2: OR (95% CI) = 0.53 (0.27–1.02), $P = 0.055$]. No significant relationships were found between *APOE* ε and tau and ptau$_{181}$ values ($P > 0.096$).

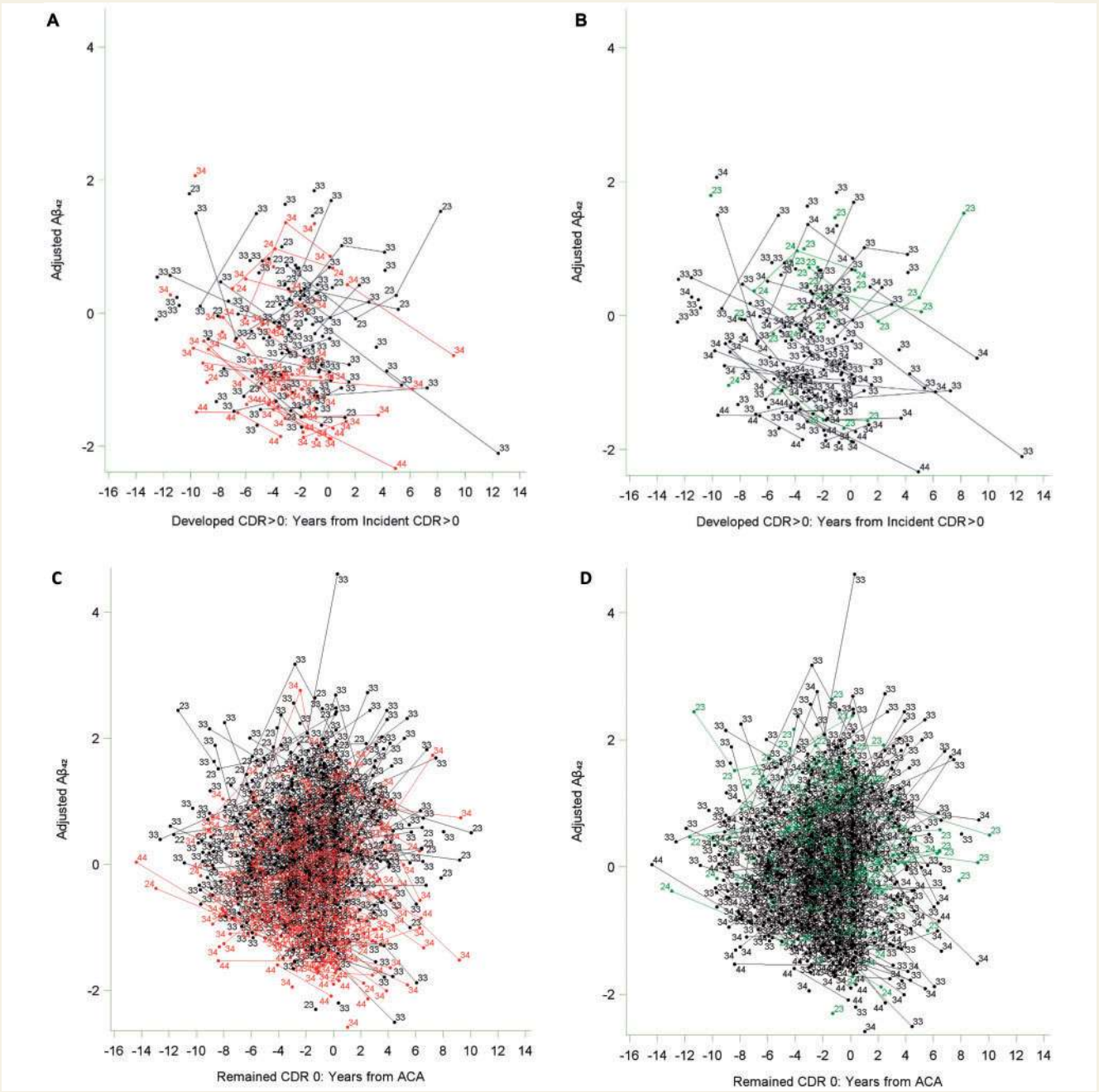

Figure 5 Spaghetti plots for adjusted CSF amyloid-β_{42}. Spaghetti plots for CSF adjusted amyloid-β_{42} showing the relationship between having at least one *APOE* ε4 allele (in red), having at least one *APOE* ε2 allele (in green), and magnitude and changes in CSF amyloid-β_{42} with time for participants who did (**A** and **B**) and did not (**C** and **D**) develop CDR > 0. Data points are labelled with the specific *APOE* ε genotype for that individual. Because appropriate cut-off values for amyloid-β_{42} depended on date and lot number, no reference line is presented. Aβ = amyloid-β; ACA = arbitrary clinical assessment.

Discussion

We explored how molecular, psychometric, and structural biomarkers predict time to CDR > 0, and how they change close to the time of CDR > 0 onset. Results from our first set of analyses indicate that molecular (amyloid imaging and CSF), cognitive (free recall), and structural (normalized hippocampal volume) biomarkers independently contribute to prediction of the onset of symptomatic sporadic Alzheimer's disease. Although past work has demonstrated that these biomarkers individually are predictive of incident dementia (Grober *et al.*, 2000; Fagan *et al.*, 2007; Morris *et al.*, 2009; Wolz *et al.*, 2011), our results indicate that given a wide variety of biomarkers to choose from, these three biomarker types provide different, non-redundant information and combine to predict onset of CDR > 0.

As expected from previous longitudinal studies (Fagan *et al.*, 2007; Morris *et al.*, 2009; Stomrud *et al.*, 2015; Baker *et al.*, 2017; Donohue *et al.*, 2017; Dumurgier *et al.*, 2017), all molecular biomarkers, with the exception of CSF $ptau_{181}$, predicted time to onset of incident cognitive impairment. However, around 70% of cognitively normal persons with abnormal Alzheimer's disease molecular biomarkers remained cognitively normal for at least the next 10 years. Potential reasons why participants with abnormal molecular biomarkers may remain cognitively normal include greater cognitive reserve (Stern, 2012) and fewer other risk factors for dementia (e.g. head trauma, diabetes, hypertension, cerebrovascular disease). Although based on small numbers of participants, our results suggest that persons resilient to the effects of underlying Alzheimer's disease pathology may be younger and have better scores on the Trail Making tests. One participant remained cognitively normal 15 years after positive tau and $ptau_{181}$ measurements. However, because this participant had a normal CSF amyloid-β_{42} value, he or she may have had a non-Alzheimer's disease disorder, such as SNAP (Jack *et al.*, 2016).

Better structural (as measured by larger normalized hippocampal volume) and cognitive performance (higher SRTFREE scores) values at baseline were associated with later onset of CDR > 0. Persons with normalized hippocampal volume > 8673 mm^3 did not become CDR > 0 regardless of length of follow-up or baseline molecular biomarker values. Although these results are consistent with the brain reserve hypothesis (Stern, 2014), which would suggest that large hippocampal volumes at baseline may protect against the development of incident symptomatic Alzheimer's disease, it is possible that smaller hippocampi resulted from a cumulative neurodegenerative process. As seen in Fig. 3M, participants who subsequently developed CDR > 0 had similar hippocampal volumes as those who remained CDR = 0 at 10 or more years prior to symptomatic onset. Some of the observed decreases that may start to develop at 10 years before clinical onset of symptoms may reflect tau-mediated changes within the hippocampus; however, further studies using tau PET are needed. Likewise, only one (2.9%) participant with a SRTFREE score above 39 developed CDR > 0 regardless of molecular biomarker values and length of follow-up. Of note, neither structural (normalized hippocampal volume) nor free recall performance (SRTFREE) are considered when calculating CDR, yet they are often performed in the clinical evaluation of patients.

Others have reported that smaller normalized hippocampal volumes are associated with ageing (Knopman *et al.*, 2016). Both normalized hippocampal volume ($r = -0.563$) and whole brain volume ($r = -0.666$) were highly correlated with age ($P \leqslant 0.0001$), and with each other ($r = 0.700$, $P < 0.0001$) in our sample, indicating that they share variance. Variables that are highly related can be thought of as proxies of each other, and therefore, once normalized hippocampal volume was included in stepwise models, age contributed little additional independent predictive information, and so failed to enter the models predicting time to incident CDR > 0. Indeed, when age was first forced into each model before stepwise selection of other variables, age was not significant in the CSF tau and $ptau_{181}$ models upon stepwise entry of normalized hippocampal volume.

In our second, and main, set of analyses examining the behaviour of molecular biomarkers surrounding transition to CDR > 0, we found no differences in the rate of change for participants who did and did not develop cognitive impairment (with the exception of CSF amyloid-β_{42}). However, individuals who developed CDR > 0 had abnormal intercept values for each of the biomarkers. Assuming that participants had similar levels of molecular biomarkers early in life, the separation between the groups, reflected in the magnitudes of the intercepts, must have occurred years prior to our observation period.

However, the assumption that participants had similar levels of molecular biomarkers at an earlier time point may not hold true for all molecular biomarkers. Studies in autosomal dominant Alzheimer's disease have suggested that initial amyloid-β_{42} levels at younger ages before symptom onset are higher among mutation carriers compared to mutation non-carriers but that there are no differences in CSF tau and $ptau_{181}$ values (Bateman *et al.*, 2012; Reiman *et al.*, 2012). Later, amyloid-β_{42} values decrease for mutation carriers compared to non-carriers (Bateman *et al.*, 2012). These studies have also demonstrated overproduction of CSF amyloid-β_{42} in mutation carriers compared to controls *in vivo* (Potter *et al.*, 2013). Results have been interpreted as consistent with a model of autosomal dominant Alzheimer's disease development whereby increased, abnormal amyloid-β_{42} production occurs, followed by a reduction in CSF amyloid-β_{42} levels as amyloid-β is sequestered into amyloid plaques (Potter *et al.*, 2013). It has been suggested that a similar process occurs in sporadic Alzheimer's disease (Selkoe and Hardy, 2016). Thus, if changes in CSF amyloid-β_{42} occur first in the pathological process, such that amyloid-β_{42} levels are higher initially (i.e. before the period of observation in this study) in persons who will develop Alzheimer's disease, but levels of other biomarkers are similar, as suggested by autosomal dominant Alzheimer's disease research, our results do not imply that changes in CSF amyloid-β_{42} occur before those of other molecular biomarkers. CSF amyloid-β_{42} data collected many years before onset of cognitive impairment are required to address this possibility. Further, CSF amyloid-β_{42} values are thought to reflect an ongoing pathological state, rather than accumulation of neuropathological load (Jack *et al.*, 2018). Finally, the *P*-value indicating a difference in slope of CSF amyloid-β_{42} for participants who did and did not progress was relatively large ($P = 0.034$) compared to the *P*-values indicating slope differences for normalized hippocampal volume and the cognitive biomarkers, suggesting that replication of these results is necessary.

Persons who developed CDR > 0 had significantly greater decline in structural (normalized hippocampal volume) and cognitive (SRTFREE, Trail Making A and B) values compared to those who remained cognitively normal. Overall these results complement existing hypothesized models and suggest that molecular biomarker changes occur prior to structural or cognitive markers in the Alzheimer's disease pathological process (Jack *et al.*, 2013).

These results have implications for clinical trials in preclinical Alzheimer's disease if replicated in other cohorts. Research into disease-modifying drug treatment relies on assessing change in the slope of decline as an outcome to demonstrate efficacy (Aisen, 2015). Therefore, desirable outcomes for clinical trials in preclinical Alzheimer's disease are those that show clear, abnormal decline among persons who will develop symptomatic Alzheimer's disease compared to those who will not, and where the decline occurs relatively close to the time of symptomatic onset.

Because changes in molecular biomarkers, other than CSF amyloid-β_{42}, were similar for those who did and did not develop CDR > 0 around the time of onset or arbitrary clinical assessment, little to no change in trajectory would be expected for these measures regardless of the efficacy of the treatment. Instead, our results support the idea that changes in cognition should be considered in preclinical Alzheimer's disease populations (Henley *et al.*, 2015). Separation between the Mini-Mental State Examination and CDR Sum of Boxes mean scores occurs at around 8 years prior to symptomatic onset in our sample (Fig. 3K and L). The separation between the groups who did and did not develop symptomatic Alzheimer's disease occurred even earlier, around 12 years, on the other cognitive tests examined here: SRTFREE, Trail Making A and B, and Animal Naming (Fig. 3G–J). These tests do not have ceiling effects among cognitively normal persons, unlike the Mini-Mental State Examination. Our results also suggest that structural change in normalized hippocampal volume (Fig. 3M) shows dramatic decline prior to symptomatic onset and may also be a suitable choice to investigate the effects of disease-modifying treatments. On the other hand, the molecular biomarkers that show overall separation in magnitude between those who do and do not go on to develop Alzheimer's disease, but show little slope differences across the groups, may be most useful in screening for inclusion in clinical trials rather than as outcomes.

The finding of slope differences in one measure of amyloid, CSF amyloid-β_{42}, but not in another, PIB, may be associated with differences in what each measure represents. It is possible that CSF amyloid-β_{42} may reflect what is occurring at the time of study, while PIB may reflect not only changes at the time of scan, but also total accumulation up to that point.

A surprising finding was the stark division of PIB values among persons who developed CDR > 0. Among these participants (Fig. 4A and 4B), PIB longitudinal behaviour separated into two distinct groups. One group had high (above the 1.31 SUVR criterion for PIB positivity, Vlassenko *et al.*, 2016) and rising values for PIB as symptomatic onset approached, the other group had initial PIB values below the cut-off and maintained PIB negativity across the observation period, despite developing CDR > 0. No other molecular biomarkers examined here, including CSF amyloid-β_{42} showed this same pattern of separation (Fig. 4 and Supplementary Figs 2 and 3). These results are consistent with work by others suggesting that there are PIB 'accumulators' and 'non-accumulators' (Villain *et al.*, 2012). Accumulators who developed CDR > 0 tended to have more abnormal CSF amyloid-β_{42} and ptau$_{181}$ biomarker values, but similar hippocampal volumes and cognitive scores (Supplementary Table 4). Considered in the context of the recently published National Institute on Aging – Alzheimer Association guidelines (Jack *et al.*, 2018), these results suggest that accumulators may be further along on the Alzheimer's disease pathological continuum compared to non-accumulators, or that non-accumulators may have developed a CDR > 0 due, at least in part, to factors that may independently increase the risk of Alzheimer's disease (e.g. vascular factors, lower cognitive reserve), or their cognitive impairment might be non-Alzheimer's disease in nature (e.g. Lewy body dementia, vascular dementia).

In contrast, among those who remained cognitively normal, there was no clear separation point of PIB behaviour into two groups. As would be expected, some people showed increasing PIB levels with time, rising from the group with low, stable values, suggesting that these people may be transitioning to CDR > 0 due to Alzheimer's disease, whereas others show little increase in PIB levels across the follow-up period (Fig. 4A and B). Based on the slopes of accumulators in the CDR > 0 group, we found that 30.8% of those who remained CDR 0 could be considered accumulators suggesting that these individuals may be transitioning to preclinical Alzheimer's disease.

Analyses indicated that having an *APOE* ε4 allele is strongly associated with being a PIB accumulator, and that having an *APOE* ε2 allele is associated with being a non-accumulator. No clear separation into accumulator and non-accumulator groups was shown for CSF molecular biomarkers. CSF amyloid-β_{42}, but not tau or ptau$_{181}$, was also linked to *APOE* ε genotype, consistent with previous cross-sectional findings (Morris *et al.*, 2010).

In addition to the possibility, mentioned earlier, that cognitive impairment may be due to a condition other than Alzheimer's disease, our research has other limitations. We used a sample of research volunteers willing to take part in cognitive, imaging, and molecular biomarker assessments. Therefore, the degree to which the results of this study will generalize to the larger population is unknown. The amount of time during which particular biomarkers were collected varied over the course of data collection, such that more information was available for some biomarkers (e.g. CDR Sum of Boxes) than for others (e.g. PIB). Greater statistical power and stability of findings is

yielded by biomarkers with more data available. Both the confidence intervals and number of participants contributing data (Supplementary Fig. 5) indicate that for all biomarkers, greatest confidence in the stability of findings occurs approximately 9 years before, and 6 years after, incident cognitive impairment. As noted earlier, hippocampal volume is related to age. Hippocampal volume is also susceptible to TDP-43 pathology (Josephs *et al.*, 2017) and hippocampal sclerosis (Leverenz *et al.*, 2002); therefore, the extent to which hippocampal atrophy is due to Alzheimer's disease or another pathology in this study is unknown. As noted earlier, CSF amyloid-β_{42} values in our cohort have exhibited upward drift over the years (Schindler *et al.*, 2018) although we attempted to control for drift statistically by adjusting values by lot number and date. As in previous work, we examined time to first CDR > 0 (Fagan *et al.*, 2007; Roe *et al.*, 2013). However, it is possible that persons who developed CDR > 0 may receive a CDR 0 on a subsequent assessment, but in our experience, often these individuals will eventually progress to Alzheimer's disease. The analysis of those individuals who oscillate is outside the realm of the current study and will be investigated in future manuscripts. Also, the high number of participants with SNAP in this cohort limits the ability to make conclusions regarding Alzheimer's disease biomarkers. Given these limitations, replication of our results in additional samples is needed.

Despite these limitations, these results generally support the pathological sequence of biomarker events proposed in current theoretical models of Alzheimer's disease and help to provide specific time points as to when biomarker changes begin to occur in the sequence of Alzheimer's disease development.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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