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Anti-Correlated Cerebrospinal Fluid Biomarker Trajectories in Preclinical Alzheimer's Disease

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Abstract.
Background: The earliest stage of preclinical Alzheimer's disease (AD) is defined by low levels of cerebrospinal fluid (CSF) amyloid-β ($A\beta_{42}$). However, covariance in longitudinal dynamic change of $A\beta_{42}$ and tau in incipient preclinical AD is poorly understood.
Objective: To examine dynamic interrelationships between $A\beta_{42}$ and tau in preclinical AD.
Methods: We followed 47 cognitively intact participants (CI) with available CSF data over four years in ADNI. Based on longitudinal $A\beta_{42}$ levels in CSF, CI were classified into three groups: 1) $A\beta_{42}$ stable with normal levels of $A\beta_{42}$ over time ($n = 15$); 2) $A\beta_{42}$ declining with normal $A\beta_{42}$ levels at baseline but showing decline over time ($n = 14$); and 3) $A\beta_{42}$ levels consistently abnormal ($n = 18$).
Results: In the $A\beta_{42}$ declining group, suggestive of incipient preclinical AD, CSF phosphorylated tau (p-tau) showed a similar longitudinal pattern of increasing abnormality over time ($p = 0.0001$). Correlation between longitudinal slopes of $A\beta_{42}$ and p-tau confirmed that both trajectories were anti-correlated (rho = –0.60; $p = 0.02$). Regression analysis showed that $A\beta_{42}$ slope (decreasing $A\beta_{42}$) predicted p-tau slope (increasing p-tau) ($R^2 = 0.47$, $p = 0.03$). Atrophy in the hippocampus was predicted by the interaction of $A\beta_{42}$ and p-tau slopes ($p < 0.0001$) only in this incipient preclinical AD group. In all groups combined, memory decline was predicted by p-tau.
Conclusions: The evolution of $A\beta_{42}$ and p-tau CSF biomarkers in CI subjects follows an anti-correlated trajectory, i.e., as $A\beta_{42}$ declined, p-tau increased, and thus was suggestive of strong temporal coincidence. Rapid pathogenic cross-talk between $A\beta_{42}$ and p-tau thus may be evident in very early stages of preclinical AD.

Keywords: $A\beta_{42}$, brain atrophy, cerebrospinal fluid, cognition, p-tau, preclinical AD

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INTRODUCTION

Preclinical Alzheimer's disease (AD) has been defined according to the progressive appearance of pathophysiological specific stages triggered by amyloid-β (Aβ), i.e., decreasing $A\beta_{42}$ in cerebrospinal fluid (CSF), followed by brain injury neurodegeneration, i.e., abnormal levels of tau protein and cortical and hippocampal atrophy, which ultimately results in subtle cognitive decline, though not yet so severe as to meet criteria for mild cognitive impairment (MCI) [1]. However, accumulating evidence has challenged this temporally ordered model of the evolution of the underlying pathophysiological processes that lead to AD. In the context of autosomal dominant early-onset AD, cross-sectional data inspection showed evidence of CSF total tau abnormalities more or less coinciding with CSF $A\beta_{42}$ abnormalities suggestive of increased amyloid aggregation [2, 3]. More recently, using probabilistic event modeling in a mixed group of cognitively intact (CI), MCI, and AD subjects, Young et al. showed that CSF tau may become abnormal before amyloidosis [4].

Similarly, several lines of evidence suggest that tau abnormalities in medial temporal lobe and limbic regions without amyloidosis may be age-associated (i.e., a so-called PART syndrome) [5]. Findings that a group of CI subjects had CSF tau but not amyloid abnormalities were consistent with this view [6]. Indeed, tau pathology in the form of abnormally phosphorylated protein has been documented to start in early young adulthood and even childhood [7, 8]. It has also been recently suggested that the initial appearance of brain injury biomarkers (in cognitively normal subjects) may not be dependent on β-amyloidosis. Based on their observations of a subgroup of CI people showing evidence of widespread neuronal injury (glucose hypometabolism and hippocampal atrophy) in absence of amyloid aggregation (PIB- positron emission tomography (PET)) [9, 10], it was hypothesized that this group had a suspected non-AD pathophysiology (SNAP).

In this study we sought to expand and refine the recent study of Mattsson et al. [11]. They identified three temporal patterns of CSF amyloid burden in CI subjects in ADNI: 1) subjects with normal levels of amyloid at baseline that remained longitudinally stable (hereafter termed $A\beta_{42}$ stable normal); 2) subjects with normal levels of amyloid at baseline consistently declining longitudinally (termed $A\beta_{42}$ declining); and 3) subjects with abnormal amyloid levels at baseline that consistently remained abnormal over time (termed $A\beta_{42}$ abnormal). Here we extend this work by carefully examining CSF p-tau trajectories in these $A\beta_{42}$-classified groups and the attendant interplay between these biomarkers.

MATERIALS AND METHODS

Participants

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), PET, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. For up-to-date information, see http://www.adni-info.org.

Only CI participants at study entry were included in the analyses. Inclusion criteria are described elsewhere [12]. Briefly, participants were between 55–90 (inclusive) years old, had a Clinical Dementia Rating (CDR) [13] score of 0, Mini-Mental State Examination (MMSE) [14] scores between 24 and 30 (inclusive), normal memory function documented by scoring at specific cutoffs on the Logical Memory II subscale (delayed Paragraph Recall) from the Wechsler Memory Scaled – Revised subscale [15], no memory complaints aside from those common to other normal participants of that age range, non-depressed, non MCI, non-demented, absence of significant impairment in other cognitive domains, and preserved activities of daily living. All participants signed written informed consent for participation in ADNI as approved by the institutional board at each participating center.

Cerebrospinal fluid measures

CSF was acquired by lumbar puncture in the morning after overnight fast as described in the ADNI procedures manual (http://adni.loni.usc.edu). A 15–20 mL CSF sample was collected at each of the participant centers and stored at ADNI Biomarker Core laboratory at the University of Pennsylvania at –80°C pending biochemical analysis. Samples were shipped overnight to the ADNI Biomarker Core at the University of Pennsylvania on dry ice. The 1 to 42 amino acid form of amyloid-β peptide ($A\beta_{42}$),

tau, and tau phosphorylated at the threonine181site (p-tau) were quantified in CSF. The specific monoclonal antibodies used were 4D7A3 for $A\beta_{42}$, AT120 for total tau (t-tau), and AT270 for p-tau. $A\beta_{42}$, t-tau, and p-tau181p were measured using a multiplex platform (xMAP; Luminex Corporation) with Innogenetics (INNO-BIA AlzBio3; Ghent, Belgium; for research use-only reagents) immunoassay kit-based reagents. As described in Toledo et al. [16], fresh and never before thawed 0.5 mL aliquots for each subject's set of longitudinal time points were analyzed on the same 96-well plate in the same analytical run to minimize run-to-run and reagent kit lot sources of variation. We utilized log-transformed values for analyses of $A\beta_{42}$, t-tau, and p-tau181p from ADNI UPENN batch 4 in ADNI. Using proposed cut-off values for ADNI [17], we used the same classification followed by Mattsson et al. [11]. Briefly, we first fit linear mixed models with follow-up as single predictor to derive intercepts and slopes for each CSF measure. Then we subdivided the CI sample into: 1) $A\beta_{42}$ stable normal group, that had estimated intercepts above the pathological cut-off value for $A\beta_{42}$ ($A\beta_{42} > 192$ pg/mL) and estimated slopes less than the median among all $A\beta_{42}$-normal individuals; 2) $A\beta_{42}$ declining group, that had intercepts above the pathological cut-off value for $A\beta_{42}$ ($A\beta_{42} > 192$ pg/mL) and slopes above the median (negative slopes) among all $A\beta_{42}$-normal individuals; and 3) $A\beta_{42}$ abnormal group, that had intercepts in the pathological range ($A\beta_{42} \leq 192$). We also included four subjects crossing the cut-off to $A\beta_{42}$ abnormality. We included these subjects to avoid "investigator biases" in selecting the cases, and to obtain a raw representation of the population (Table 1 depicts a description of the groups). Also see the Supplementary Material for a detailed description of individuals excluded from study.

Brain atrophy measures

Scans were obtained from 1.5 Tesla scanners at different sites involved in ADNI with minor variations in the MRI protocol based on the specific configuration of each scanner. Volumetric measures of the whole brain, cerebral white matter, and hippocampus, as well as cortical thickness measures of entorhinal, inferior temporal, middle temporal, parahippocampal, posterior cingulate, and precuneus were extracted combining both hemispheres. These measures were selected because they represent brain areas that show atrophy in AD, and where $A\beta_{42}$ and tau seem to have greater impact [8, 18–20]. Computations for brain atrophy measures were performed using Freesurfer (http://surfer.nmr.mgh.harvard.edu/). We excluded full MRI scans for subjects whose cortical reconstruction failed in any of the brain regions examined.

Cognitive measures

Cognitive measures included immediate and delayed episodic verbal memory of the logical memory test of the Wechsler Memory Scale [15], immediate and delayed recall of the Auditory Verbal Learning Test [21], working memory as measured by digit span, and speed of processing as measured by digit symbol substitution test [22].

Statistical analysis

Statistical comparisons between demographic variables were performed through ANOVA tests adjusted for multiple comparisons, and Chi square tests for continuous and dichotomous variables respectively. Between group comparisons of CSF measures at baseline were performed using ANCOVA models with *post-hoc* comparisons and Bonferroni correction. Linear mixed models (SAS 9.3. PROC MIXED) were fitted to inspect longitudinal CSF trajectories within each group ($A\beta_{42}$ stable normal, $A\beta_{42}$ declining, and $A\beta_{42}$ abnormal), and between groups (Group x Time factor). In these mixed models, the covariance pattern was set as heterogeneous autoregressive structure, time (follow-ups) was included as a repeated factor, and subject was the random factor. In order to measure relationship between longitudinal trajectories of the different CSF biomarkers, correlation between the slopes within $A\beta_{42}$ groups was computed using Spearman's rank correlation coefficients. Stepwise regression models, based on maximizing R square, were also fit to inspect the contribution of the variation in p-tau that might be attributed to $A\beta_{42}$ variation ($A\beta_{42}$ at baseline was forced into these models), and second to inspect independent and interactive contributions of $A\beta_{42}$ and p-tau on brain atrophy and cognition in the $A\beta_{42}$ declining group. In these models, $A\beta_{42}$ at baseline, $A\beta_{42}$ longitudinal slope, p-tau longitudinal slope, and the interaction between $A\beta_{42}$ and p-tau longitudinal slopes were the predictor variables, and each of the atrophy and cognitive variables (in the form of longitudinal slopes) were the outcome variables. Significance

level for entry in the models was set up at Bonferroni corrected significance level of $p = 0.006$ for brain morphometry measures, and $p = 0.008$ for cognitive measures. Age, gender, and educations (in years) were forced into all the analyses. Effect sizes (ES) were computed according to Cohen's formula.

RESULTS

Participant characteristics

Study demographics are summarized in Table 1. Forty-seven CI had valid CSF measures at baseline (46 at first year, 25 at second year, 31 at third year, and 13 at fourth year). The APOE gene distribution differed between groups by Chi square test ($\chi^2 = 16.16$; $p = 0.003$). The $A\beta_{42}$ abnormal group had higher frequency of the APOE E4 allele compared to $A\beta_{42}$ stable normal group (Table 1). Interestingly, within $A\beta_{42}$ stable normal group, none of the participants was APOE E4 carrier while 6 (40%) were carriers of the neuroprotective APOE E2 variant (Table 1 and Supplementary Table 1). Demographic characteristics were similar across groups. A description of how missing data were handled can also be found in the Supplementary Material plus a comparison between missing individuals at follow-up and non-missing individuals at follow-up on demographic and clinical stating variables (Supplementary Table 2).

Baseline CSF findings

As expected, the $A\beta_{42}$ abnormal group showed lower levels of $A\beta_{42}$ compared to the $A\beta_{42}$ stable normal and $A\beta_{42}$ declining groups (Table 2). P-tau mean was also found to be higher in the $A\beta_{42}$ abnormal group compared to both $A\beta_{42}$ stable normal and $A\beta_{42}$ declining groups and exceeding the established cutoff to be considered as pathological ($p\text{-}tau_{181p} > 23$ pg/mL) [17]. Therefore, both $A\beta_{42}$ and p-tau mean levels were above the threshold of pathology at baseline in the group considered as suggestive of preclinical AD. Total tau mean levels were also higher in the $A\beta_{42}$ abnormal group but without exceeding the cutoff value of total tau namely 93 pg/mL. Total tau also did not differ between the three groups. Correlation analyses showed that levels of $A\beta_{42}$ and p-tau were not correlated at baseline in any of the groups (all p values > 0.54), but they were correlated when considering the whole CI sample (rho = –0.35/ $p = 0.01$).

Longitudinal CSF findings

$A\beta_{42}$

Mixed models fitted within each group revealed that the $A\beta_{42}$ declining group showed a significant longitudinal decline of $A\beta_{42}$ ($F_{4,29} = 10.28/p < 0.0001$), whereas the $A\beta_{42}$ stable normal ($F_{4,31} = 0.29/p = 0.88$) and the $A\beta_{42}$ abnormal ($F_{4,40} = 1.40/p = 0.25$) did not (Fig. 1A). In the whole CI subject sample, $A\beta_{42}$ longitudinal decline was found to show a trend to significance ($F_{4,108} = 2.26/p = 0.07$). In a secondary analysis in which group was a between subject factor in the model, the Group x Time interaction was also significant ($F_{8,100} = 2.98/p = 0.005$). ANCOVA models comparing longitudinal slopes between the three groups corroborated this. As described above, we computed individual slopes for longitudinal trajectories of the CSF biomarkers. Table 2 shows the means, standard deviations, and the range of these slopes for each of the groups. Longitudinal $A\beta_{42}$ slope was significantly higher (more declining) in the $A\beta_{42}$ declining group as compared to $A\beta_{42}$ stable normal, and showed a trend to significance compared to $A\beta_{42}$ abnormal group.

P-tau and t-tau

Mixed models fitted within each group revealed that the $A\beta_{42}$ declining group showed

Table 1
Demographic characteristics and APOE status

	$A\beta_{42}$ Stable Normal ($n = 15$)	$A\beta_{42}$ Declining ($n = 14$)	$A\beta_{42}$ Abnormal ($n = 18$)	Statistical Test
Age, Mean (SD)	76.07 (6.88)	77.86 (3.86)	75.89 (3.76)	$F_{2,44} = 0.71/p = 0.50$
Range	62 – 90	72 – 84	71 – 85	
Gender M/F	10/5	8/6	9/9	$\chi^2 = 0.93/p = 0.63$
Education, Mean (SD)	16.33 (2.58)	16.14 (2.71)	16.00 (3.50)	$F_{2,44} = 0.05/p = 0.95$
Range	12 – 20	13 – 20	6 – 20	
APOE Status	E2 carriers = 6	E2 carriers = 2	E2 carriers = 0	$X = 16.16/p = 0.003$
	E3/E3 = 9	E3/E3 = 10	E3/E3 = 10	$A\beta_{42}$ Stable versus $A\beta_{42}$ Declining $\chi^2 = 4.02/p = 0.13$
	E4 carriers = 0	E4 = 2	E4 carriers = 8	$A\beta_{42}$ Stable versus $A\beta_{42}$ Abnormal $\chi^2 = 13.89/p = 0.001$
				$A\beta_{42}$ Declining versus $A\beta_{42}$ Abnormal $\chi^2 = 5.18/p = 0.07$

Table 2
CSF Measurements at baseline and longitudinal trajectory (in the form of slope) at 4 years of follow-up (including age, gender and education as covariates)

	$A\beta_{42}$ Stable Normal ($n=15$)	$A\beta_{42}$ Declining ($n=14$)	$A\beta_{42}$ Abnormal ($n=18$)	Statistical Test
$A\beta_{42}$ Baseline, Mean (SD) Range	268.93 (30.33) 214 – 324	258.93 (31.08) 198 – 305	157.44 (24.26) 111 – 190	$F_{2,41} = 81.95/p<0.0001$ $A\beta_{42}$ Stable versus $A\beta_{42}$ Declining $p = 1.00$ $A\beta_{42}$ Stable versus $A\beta_{42}$ Abnormal $p < 0.0001$ $A\beta_{42}$ Declining versus $A\beta_{42}$ Abnormal $p < 0.0001$
$A\beta_{42}$ Slope	2.20 (5.50) –3 – 16	–10.36 (9.71) –24 – 10	–0.65 (9.34) –9 – 34	$F_{2,41} = 5.01/p = 0.01$ $A\beta_{42}$ Stable versus $A\beta_{42}$ Declining $p = 0.01$ $A\beta_{42}$ Stable versus $A\beta_{42}$ Abnormal $p = 1.00$ $A\beta_{42}$ Declining versus $A\beta_{42}$ Abnormal $p = 0.08$
P-tau Baseline, Mean (SD) Range	20.73 (7.74) 13 – 41	21.07 (7.61) 13 – 38	31.78 (13.90) 9 – 54	$F_{2,41} = 5.10/p = 0.01$ $A\beta_{42}$ Stable versus $A\beta_{42}$ Declining $p = 1.00$ $A\beta_{42}$ Stable versus $A\beta_{42}$ Abnormal $p = 0.03$ $A\beta_{42}$ Declining versus $A\beta_{42}$ Abnormal $p = 0.03$
P-tau Slope	0.16 (3.55) –9 – 8	2.01 (2.63) –2 – 8	2.91 (3.12) –3 – 11	$F_{2,41} = 3.42/p = 0.04$ $A\beta_{42}$ Stable versus $A\beta_{42}$ Declining $p = 0.16$ $A\beta_{42}$ Stable versus $A\beta_{42}$ Abnormal $p = 0.05$ $A\beta_{42}$ Declining versus $A\beta_{42}$ Abnormal $p = 1.0$
T-tau Baseline, Mean (SD) Range	58.53 (18.40) 30 – 94	72.79 (23.47) 45 – 122	84.94 (36.69) 14 – 179	F2,41 = 1.99/$p = 0.15$
T-tau Slope	2.06 (3.72) –3 – 10.4	3.07 (4.57) –1.7 – 15.5	2.21 (5.41) –4.5 – 12.5	F2,41 = 0.23/$p = 0.80$

Baseline data are presented in the form of raw CSF pg/mL for clarity purposes, although statistical tests were performed using log-transformed values.

a significant longitudinal increase of p-tau ($F_{4,29} = 8.31/p = 0.0001$), whereas the $A\beta_{42}$ stable normal ($F_{4,32} = 1.98/p = 0.12$) and the $A\beta_{42}$ abnormal ($F_{4,40} = 2.10/p = 0.10$) did not (Fig. 1B). In the whole CI sample p-tau longitudinal increase was significant ($F_{4,109} = 5.71/p = 0.0003$). However, mixed model with $A\beta_{42}$ groups as between subjects factor was not significant for the Group x Time interaction term ($F_{8,101} = 0.83/p = 0.58$). Finally, effect size analyses revealed an incremental pattern on p-tau levels in the $A\beta_{42}$ declining group between each of the follow-ups and baseline, going from small (0.20) to large (1.30) (Fig. 2B). The $A\beta_{42}$ abnormal group also showed a similar increase in p-tau levels (small to large: 0.20–1.10) (Fig. 2C), whereas the $A\beta_{42}$ stable group levels tended toward a small longitudinal decrease initially, but a considerable increase at last follow-up (ES = 0.90) (Fig. 2A). This suggests that p-tau levels increased at each follow-up compared to baseline in all three groups, but this increase was largest in the $A\beta_{42}$ declining group.

$A\beta_{42}$ and p-tau relationships

Figure 3 shows mean slopes of $A\beta_{42}$ and p-tau by group. As can be seen in Figure 3B, $A\beta_{42}$ and p-tau showed parallel trajectories in the $A\beta_{42}$ declining group. Individual trajectories for each subject in this $A\beta_{42}$ declining group are also shown in Figure 4. In the $A\beta_{42}$ abnormal group, while $A\beta_{42}$ remained stable, p-tau showed a considerable decline (Fig. 3C). In the $A\beta_{42}$ normal stable group, both $A\beta_{42}$ and p-tau remained stable through follow-up (Fig. 3A). Correlation coefficients between longitudinal p-tau and $A\beta_{42}$ slopes were as follows: rho = –0.60 ($p = 0.02$) for the $A\beta_{42}$ declining group, rho = –0.45 ($p = 0.09$) for the $A\beta_{42}$ stable normal group and rho = –0.16, ($p = 0.51$) for the $A\beta_{42}$ abnormal group (Fig. 5). These correlation coefficients indicated that $A\beta_{42}$ and p-tau trajectories were anti-correlated in the $A\beta_{42}$ declining group. A regression analysis to ascertain the contribution of the variation of p-tau slope predicted by $A\beta_{42}$ slope and $A\beta_{42}$ baseline (forced into the model) corroborated this; in the $A\beta_{42}$ declining group, p-tau slope was predicted by $A\beta_{42}$ slope ($F_{2,11} = 4.94$, $p = 0.03$, $R^2 = 0.47$; $A\beta_{42}$ slope parameter estimate $b = –1.26$, $t = –2.79$, $p = 0.02$, Variance Inflation Factor (VIF) = 1.04), whereas it was not predictive in the $A\beta_{42}$ stable normal group ($F_{2,12} = 2.47$, $p = 0.13$) and the $A\beta_{42}$ abnormal group ($F_{2,15} = 0.66$, $p = 0.53$). Total tau was not predicted by $A\beta_{42}$ slope in any of the groups.

Impact of $A\beta_{42}$ and p-tau on brain atrophy and cognition

Atrophy rates in several regions were predicted by both $A\beta_{42}$ and p-tau slopes and their interaction.

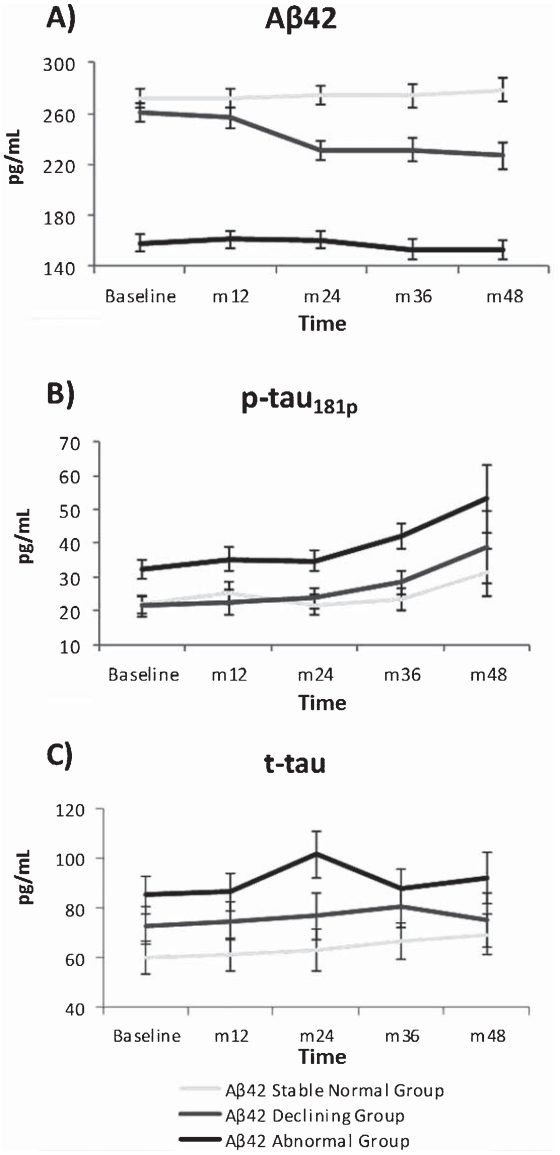

Fig. 1. Longitudinal trajectories of CSF biomarkers. Figure represents least squares (LS) means and standard errors for the three CSF biomarkers at each one of the 5 follow-ups: Baseline, 12, 24, 36, and 48 months. A) $A\beta_{42}$ levels for $A\beta_{42}$ normal stable (light grey), $A\beta_{42}$ declining (dark grey), and $A\beta_{42}$ abnormal (black) groups; B) p-tau_{181p} levels for $A\beta_{42}$ normal stable (light grey), $A\beta_{42}$ declining (dark grey), and $A\beta_{42}$ abnormal (black) groups; C) t-tau levels for $A\beta_{42}$ normal stable (light grey), $A\beta_{42}$ declining (dark grey), and $A\beta_{42}$ abnormal (black) groups.

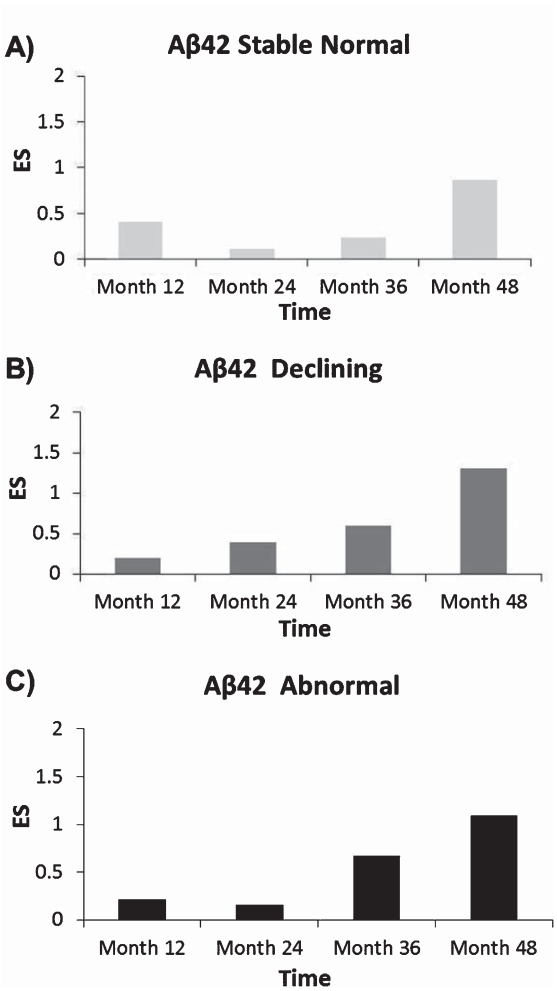

Fig. 2. Effect sizes of the longitudinal change in p-tau by group. Figure represents effect sizes for p-tau at each follow-up (12, 24, 36, and 48 months) compared to baseline: A) $A\beta_{42}$ stable normal group; B) $A\beta_{42}$ declining group; and C) $A\beta_{42}$ abnormal group.

Specifically in the $A\beta_{42}$ declining group, showing incipient preclinical AD, atrophy in the hippocampus was influenced by p-tau slope, $A\beta_{42}$ at baseline, and the $A\beta_{42}$ slope x p-tau slope interaction; atrophy in the precuneus was also predicted by $A\beta_{42}$ at baseline in this group (Table 3). Thickness measures of the medial temporal lobe (i.e., middle temporal and inferior temporal) were also associated to $A\beta_{42}$ and p-tau slopes but only when setting a more liberal level of significance criteria for entry ($p = 0.10$ instead of $p = 0.05$). Atrophy of the $A\beta_{42}$ normal stable and $A\beta_{42}$ abnormal groups was not predicted by CSF biomarkers. The same held true for the whole sample.

Regarding cognitive decline, the effect of CSF biomarkers were only observed in the whole CI sample: 1) logical memory immediate and delayed decline were found to be predicted by p-tau slope (Model for delayed memory $F_{4,42} = 3.20$, $p = 0.02$, $R^2 = 0.23$; Model for immediate memory $F_{4,42} = 2.62$, $p = 0.05$, $R^2 = 0.20$); and 2) digit symbol

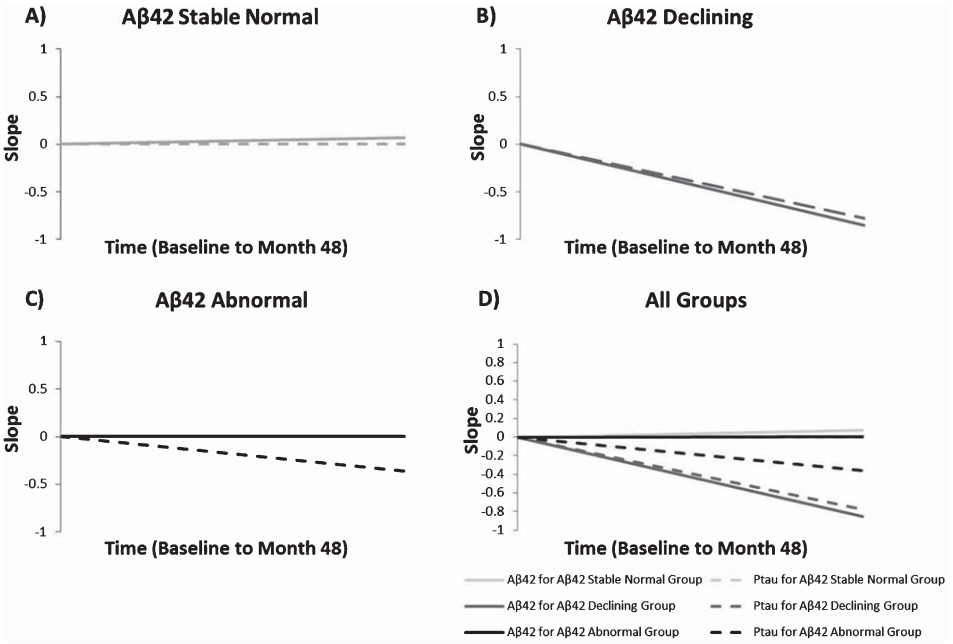

Fig. 3. Longitudinal trajectories of $A\beta_{42}$ and p-tau by group. Figure represents intercepts and slopes (transformed to z scores) for each of the groups. In these figures, p-tau levels have been transformed to negative values in order to be comparable to $A\beta_{42}$ levels. A) In the $A\beta_{42}$ stable normal group, $A\beta_{42}$ and p-tau were longitudinally stable; B) In the $A\beta_{42}$ declining group, both $A\beta_{42}$ and p-tau showed an anti-correlated trajectory; C) In the $A\beta_{42}$ abnormal group, while $A\beta_{42}$ remained abnormally stable, p-tau showed decline; D) all groups are plotted together for comparative purposes between groups.

processing was predicted by the interaction between the slopes of $A\beta_{42}$ and p-tau plus p-tau slope (Model $F_{5,41} = 2.51$, $p = 0.04$, $R^2 = 0.23$). Within group analyses showed that none of the models reached the Bonferroni corrected level of significance. Tables for regression models on cognitive measures are in supplement (Supplementary Table 3). Despite non-significance, R^2 for the full models were quite large (e.g., approximately in the 0.50 to 0.75 range for $A\beta_{42}$ declining group).

We also provide longitudinal changes of clinical staging variables (CDR and MMSE) in Supplementary Table 4. While longitudinal decline on CDR did not reach statistical significance for any of the groups, MMSE scores showed a significant decline in the $A\beta_{42}$ abnormal group ($p = 0.04$), and a trend for significance in the $A\beta_{42}$ declining group ($p = 0.07$).

DISCUSSION

In a subgroup of clinically CI individuals who demonstrated declining CSF $A\beta_{42}$ levels, p-tau levels in CSF demonstrated coincident increases, suggesting that slopes were tightly coupled. Importantly, at least four subjects in the declining subgroup of the ten for whom data were available, eventually demonstrated abnormal amyloid levels ("amyloid positivity"), i.e., they progressed to preclinical AD. Covarying with $A\beta_{42}$ decline was a p-tau increase, which began at normal levels and ended at abnormal levels. $A\beta_{42}$ slope accounted for 47% of the variance in p-tau slope increases. These findings suggest that both CSF biomarkers are changing at similar rates in individuals who are converting or may be converting from CI to preclinical AD status. Of course, correlations do not equate with causality and it thus might be possible that some underlying disease processes account for both amyloid and p-tau changes independently.

In this $A\beta_{42}$ declining group, p-tau and $A\beta_{42}$ interactions also had a significant effect on hippocampus atrophy. This suggests that neurodegenerative processes involving cell death or neuronal loss in medial temporal lobe were commencing and required interactions between $A\beta_{42}$ and p-tau. Additionally, baseline $A\beta_{42}$ level had an impact on precuneus thickness, as has been reported previously [23–25].

The $A\beta_{42}$ stable normal group demonstrated increases in p-tau. However the slope was considerably more gradual than in the $A\beta_{42}$ declining group. Thus, such increases may be age associated [26].

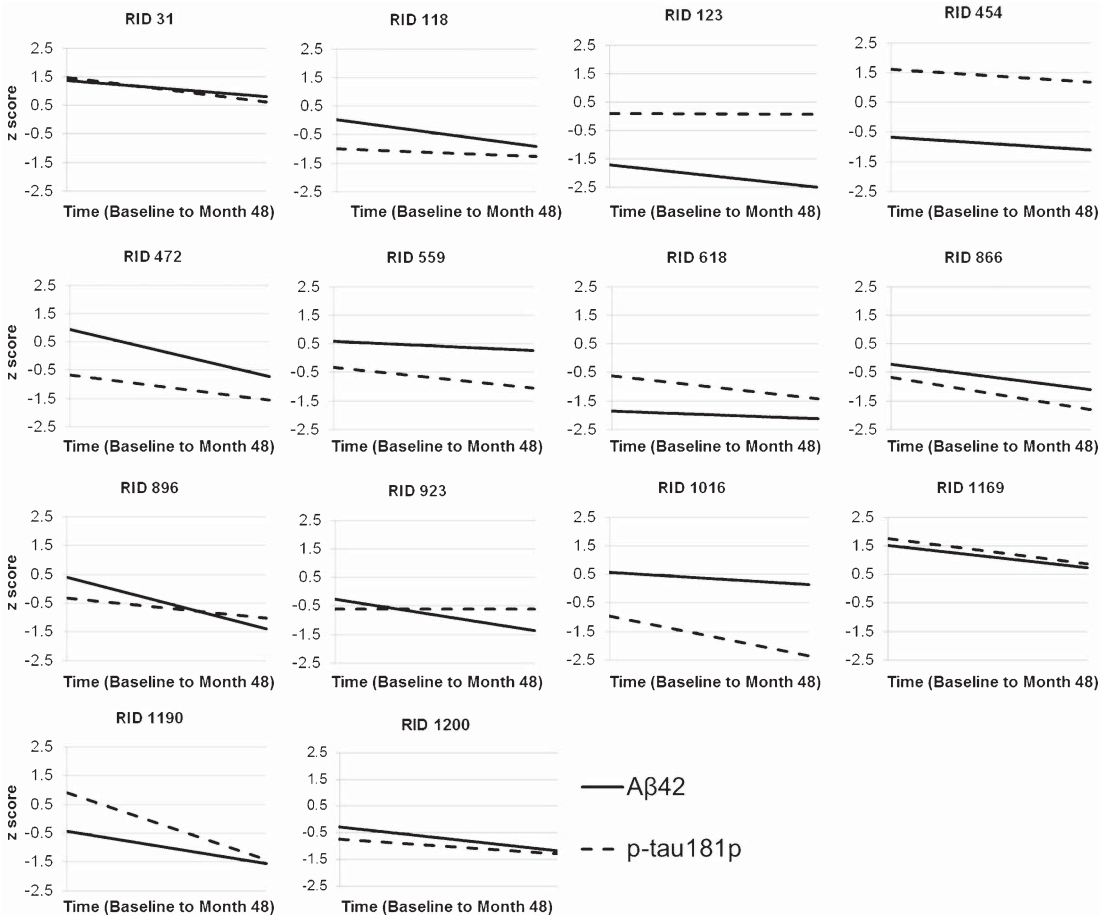

Fig. 4. Individual longitudinal trajectories for $A\beta_{42}$ and p-tau for each subject of the $A\beta_{42}$ declining group. Figure shows $A\beta_{42}$ and p-tau intercepts and slopes (transformed to z scores) for each of individuals examined in the $A\beta_{42}$ declining group. In these figures, p-tau levels have been transformed to negative values in order to be comparable to $A\beta_{42}$ levels. RID, roster identification number in ADNI.

Based on the high proportion of individuals carrying the neuroprotective APOE E2 variant in this stable group, it is unlikely that a significant number will advance to AD. The $A\beta_{42}$ abnormal group demonstrated a steep increase in p-tau, while $A\beta_{42}$ levels remained relatively stable but abnormal. In this case it may be argued that while $A\beta_{42}$ levels have reached an asymptote, p-tau related neurodegeneration may be ongoing and accelerated [27]. As APOE E4 carriers were disproportionately observed in this $A\beta_{42}$ abnormal group, it is possible that this group was more advanced in terms of AD-related neurobiological progression because of E4-driven earlier age of onset.

With respect to cognition, our findings in the whole group of CI suggest that immediate and delayed memory and speed of processing changes were predicted by increases in p-tau, supporting the already reported finding that p-tau may be more directly associated with behavioral memory performance than $A\beta_{42}$ [28]. However, we must acknowledge that overall cognition was inconsistently related to CSF changes in our study sample. This should be considered into the context of the very subtle cognitive impairment usually seen in CI individuals, and the possible lack of sensitivity of the traditional neuropsychological tests employed in detecting those subtle variations in cognition. Last, it is plausible that CSF effects on cognition may be mediated by brain atrophy and thus not directly related to $A\beta_{42}$ or p-tau variations per se.

Total tau did not demonstrate the same type of changes that p-tau showed in our study. T-tau seems to be a general marker of damage to cortical axons, whereas p-tau appears to be more characteristic of AD [29]. In the same line, the $_{42}$ amino acid form of the Aβ peptide ($A\beta_{42}$) is particularly considered the driving force in the AD disease process as it is the

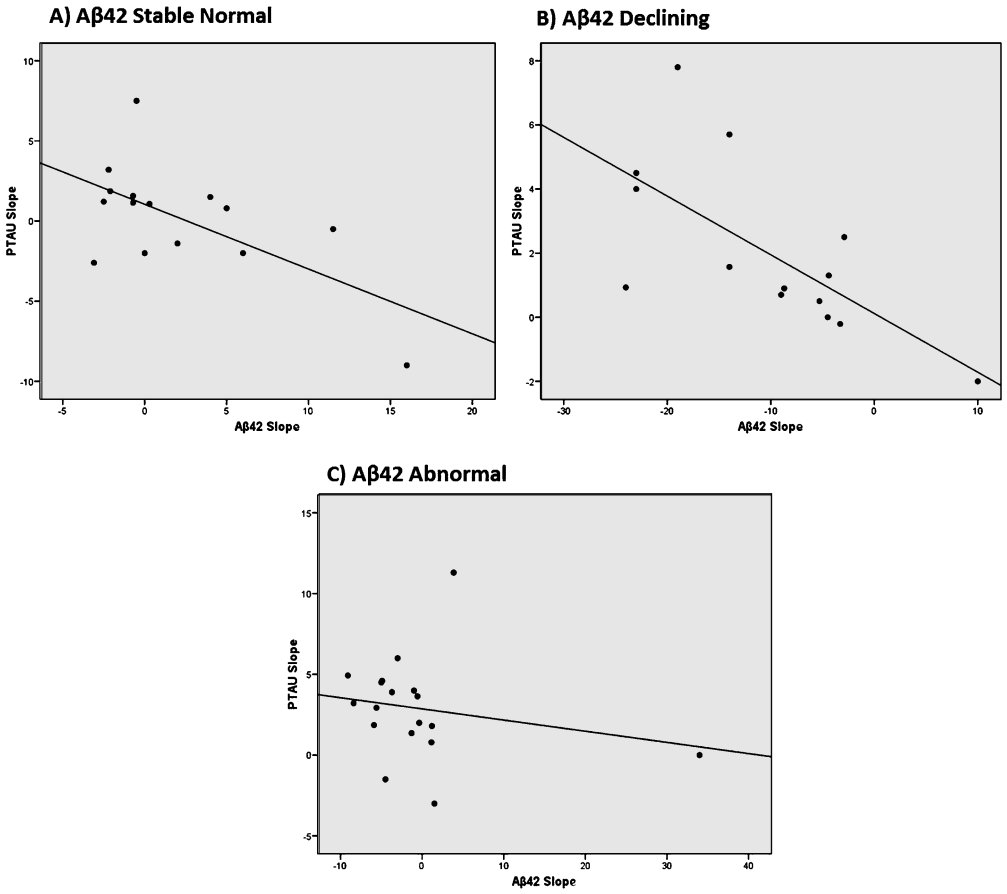

Fig. 5. Correlation between $A\beta_{42}$ and p-tau slopes by group. Figure shows scatter plots between the longitudinal slopes of $A\beta_{42}$ and p-tau for each of the groups in log-transformed values. A) $A\beta_{42}$ stable normal group correlation coefficient rho = –0.45 ($p = 0.09$); B) $A\beta_{42}$ declining group correlation coefficient rho = –0.60 ($p = 0.02$); and C) $A\beta_{42}$ abnormal group correlation coefficient rho = –0.16, ($p = 0.51$).

Table 3
Atrophy rates in the $A\beta_{42}$ declining group

	DF	Standardized Estimate	*t*	*p*	R2	VIF
Hippocampus Volume: Model F6, 7 = 22.05 $p = 0.0003$, $R^2 = 0.95$ (Covariates Age, Gender and Education $R^2 = 0.29$)						
$A\beta_{42}$ x p-tau Slopes	1	1.89	9.02	<0.0001	0.33	6.12
p-tau Slope	1	1.47	6.69	0.0003	0.15	6.74
$A\beta_{42}$ Baseline	1	–0.65	–5.06	0.001	0.18	2.29
Precuneus Thickness: Model F4, 8 = 14.74 $p = 0.0009$, $R^2 = 0.88$ (Covariates Age, Gender and Education $R^2 = 0.38$)						
$A\beta_{42}$ Baseline	1	–0.82	–5.80	0.0004	0.50	1.33

most abundant species of $A\beta_{42}$ in amyloid plaques [30]. Therefore, reduction of CSF $A\beta_{42}$ seems to be a consequence of the aggregation of this $A\beta_{42}$ isoform into plaques [31].

Several caveats to our study must be acknowledged. First, the sample sizes for each $A\beta_{42}$ derived subgroup were small. However, lumbar puncture is considered an invasive procedure and less widely available, factors that limit enrollment in CSF studies. Second, longitudinal follow-up may be considered as short (4 years of follow-up). Future studies should confirm our conclusions with longer follow-up. We have reviewed the literature to ascertain sample size and missing data issues in longitudinal CSF studies in aging and preclinical AD (See Supplementary Table 5). Briefly, three studies [11, 16, 32] in the

same database had same rates of missing data, and one very recent study (Baltimore Aging) did have a slightly greater sample: 20/21 individuals with three or more follow-ups, as opposed to 31 individuals at three years of follow-up and 13 at four years in our study [33]. Thus, our study is the second largest with longitudinal CSF data in aging and preclinical AD. Furthermore, we also detail in the Supplementary Material the procedure we followed for handling missing data. Additionally, and as with every study of this type, we do not know the slope of $A\beta_{42}$ or p-tau before baseline. Third, the age range of our sample (from 62 to 90 years) may have some role in our findings, though it was a forced covariate in our regressions.

For the purposes of this study, we assumed that $A\beta_{42}$ can initiate neurodegeneration of the Alzheimer-type in older individuals. Support for this view comes from early onset familial autosomal dominant mutations in PS1, PS2, and APP, all of which directly induce amyloid misprocessing. Nevertheless, and as noted in the introduction, we are aware of so called SNAP cases in which neurodegeneration may be independent from or precede amyloid abnormalities [9, 10], that tau aggregation may occur in the absence of amyloidosis in so-called PART [5, 34], and that early tau changes may be characteristic of admixed CI, MCI, and AD subpopulations. More broadly, it is important to appreciate that for the specific purposes of this study, we began our work from a premise in which amyloid changes precede tau changes. However, this premise itself is debatable [35, 36]. Whether one accepts it, rejects it, or is agnostic as we are, there are interesting, and in our view draw unexpected implications that go beyond biomarker staging. Thus, and as described, we have shown tight temporal coupling between p-tau and $A\beta_{42}$. However, recent *in vivo* tau imaging in CI controls has indicated that fibrillary tau is generally restricted to medial temporal lobe/inferior temporal regions, while $A\beta_{42}$ aggregates reside primarily in neocortex [25]. This in turn suggests that there is little spatial overlap between the two. In our view, determining the extent and nature of rapid, pathological molecular cross-talk between aberrantly phosphorylated tau and presumptively toxic $A\beta_{42}$ species will be an increasingly important area of investigation. Indeed, several studies have underscored the importance of the interaction between $A\beta_{42}$ and tau to accelerate neurofibrillary disease and induce neurodegeneration [27, 37]. Additionally, both *in vitro* and preclinical work in transgenic mouse models of tau aggregation also suggests that $A\beta_{42}$ may accelerate tau proto-aggregation or aggregation [38–40].

The primacy of amyloid in initiating neurodegenerative events, while arguable, continues to have traction in research studies and clinical trials. Notions about: 1) the amyloid cascade [41]; 2) amyloid as an initiating event in AD (as in Jack and colleagues model of staging); and 3) use of amyloid to define so-called preclinical AD including in A4 study [42] hold much sway in the field. Moreover, several recent meta-analyses of $A\beta_{42}$ in the preclinical and prodromal stages of AD (both in *JAMA* 2015) highlight its continuing relevance for the field. Indeed, multiple clinical trials target only amyloid [43, 44]. We view our data as a potential corrective, in that it provides anomalous data demonstrating that coincident with consistent declines in CSF $A\beta_{42}$ in a subsample of healthy individuals, increases in p-tau were observed.

In sum, $A\beta_{42}$ and p-tau show anti-correlated longitudinal trajectories in CI subjects converting to preclinical AD, and both factors impact hippocampal atrophy. Both $A\beta_{42}$ and p-tau and their interaction are of obvious importance in understanding the development of neuropathological processes leading to the manifestation of preclinical AD, and therefore both proteins may be important targets for clinical prevention trials in individuals at an incipient state of AD.

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SUPPLEMENTARY MATERIAL

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