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## Hypothetical Preclinical Alzheimer's Disease Groups and Longitudinal Cognitive Change

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### Abstract

**Importance and Objective**—Clinical trials testing treatments for Alzheimer's disease (AD) are increasingly focused on cognitively normal individuals in the preclinical phase of the disease. To optimize observing a treatment effect, such trials need to enroll cognitively normal individuals likely to show cognitive decline over the duration of the trial. The goal of the current study was to identify which group of cognitively normal individuals showed the greatest cognitive decline over time based on their cerebrospinal fluid (CSF) biomarker profile.

**Design, Setting, and Participants**—Cognitively normal participants (n=222, mean follow-up 11 years, follow-up range=0–18 years, mean baseline age=57 years, range=22–85 years) were classified into one of four hypothetical preclinical AD groups using baseline CSF levels of amyloid-beta and tau, or amyloid-beta and phosphorylated tau (p-tau): Stage 0 (high abeta/low

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Dr. Soldan reports no disclosures.

Dr. Pettigrew reports no disclosures.

Ms. Cai reports no disclosures.

Dr. Wang reports no disclosures.

Dr. Moghekar reports no disclosures.

Dr. O'Brien reports no disclosures.

Dr. Albert is an advisor to Eli Lilly.

**Author Contributions:**

Dr. Soldan took part in the design and conceptualization of the study, supervised the analysis of the data, participated in the interpretation of the analyses, drafted and revised the manuscript and takes overall responsibility for the data and the manuscript.

Dr. Pettigrew took part in the design and conceptualization of the study, participated in the interpretation of the data, and critically reviewed the manuscript.

Ms. Cai performed the statistical analyses, participated in the interpretation of the data, and critically reviewed the manuscript.

Dr. Wang supervised the statistical analyses, the interpretation of the data and critically reviewed the manuscript.

Dr. Moghekar supervised the analysis of the cerebrospinal fluid and critically reviewed the manuscript.

Dr. O'Brien supervised the analysis of the cerebrospinal fluid and critically reviewed the manuscript.

Dr. Albert took part in the design and conceptualization of the study, obtained funding, participated in the interpretation of the analyses, and critically reviewed the manuscript.

tau), Stage 1 (low abeta/low tau), Stage 2 (low abeta/high tau), and suspected non-AD pathology, SNAP (high abeta/high tau).

**Main Outcome Measure**—An a-priori cognitive composite score based on four tests previously shown to predict progression from normal cognition to symptom onset of mild cognitive impairment or dementia: Paired Associates immediate recall, Logical Memory delayed recall, Boston Naming, and Digit-Symbol Substitution. Linear mixed effects models were used to compare the cognitive composite scores across the four groups over time, adjusting for baseline age, gender, education and their interactions with time.

**Results**—Individuals in Stage 2 (low abeta and high tau (or p-tau)) showed lower baseline cognitive scores and greater decline in the cognitive composite score relative to the other three groups (all  $p < 0.001$ ). Subjects in Stage 0, 1, and SNAP did not differ from one another in cognitive performance at baseline or over time (11 years) and showed practice-related improvement in performance. APOE- $\epsilon 4$  genotype was not associated with baseline or rate of change in the cognitive score.

**Conclusions and Relevance**—These results suggest that in order to optimize observing a treatment effect, clinical trials enrolling cognitively normal individuals should selectively recruit participants with abnormal levels of both amyloid and tau (i.e., Stage 2), as this group would be expected to show the greatest cognitive decline over time if untreated.

Current evidence suggests that the neuropathological processes associated with Alzheimer's disease (AD) begin a decade or more before the emergence of obvious cognitive impairment.<sup>1</sup> This preclinical phase of the disease is currently the focus of clinical trials, as it is hypothesized that disease-modifying therapies are likely to be most successful when administered before the initial symptomatic phase, known as Mild Cognitive Impairment (MCI), in which there is substantial synaptic and neuronal damage<sup>2-4</sup>. The primary goal of the current study was to examine which cognitively normal individuals with evidence of AD pathology are most likely to demonstrate cognitive decline over time. This information would have important implications for determining subject selection criteria for clinical trials, since the rate of cognitive change over time must be sufficient to permit seeing a drug effect, if one is present.

We tested whether individuals with differing biomarker profiles show different cognitive trajectories over time, as would be predicted by the hypothetical staging model of preclinical AD laid out by the Preclinical AD Workgroup sponsored by the National Institute on Aging and Alzheimer's Association (NIA/AA).<sup>1</sup> This model proposes that the preclinical phase of AD can be subdivided into three successive stages. Stage 1 is characterized by amyloid pathology, but the absence of tau-related neurodegeneration. During Stage 2, both amyloid pathology and tau-related neurodegeneration are evident. Finally, during Stage 3, subtle cognitive decline becomes detectable in addition to amyloid and tau pathology. Individuals with normal measures of both amyloid and neurodegeneration are classified as Stage 0. Additionally, it has been proposed that individuals with evidence of neurodegeneration but normal levels of amyloid might be classified as having suspected non-Alzheimer pathology (SNAP).<sup>5</sup>

The subjects in this study were part of a longitudinal cohort of individuals with normal cognition when first assessed. We used baseline cerebrospinal fluid (CSF) measures of amyloid (abeta<sub>1-42</sub>), total tau, and phosphorylated tau (p-tau) to classify individuals into the hypothetical stages of preclinical AD and SNAP. These CSF biomarkers are particularly useful in addressing the goals of the study because they directly reflect the levels of abnormal brain proteins associated with the AD pathology, i.e., plaques and tangles.<sup>6, 7.</sup>

To our knowledge, only one prior study has examined the combined effects of CSF measures of amyloid and tau on cognitive change among individuals who were cognitively normal at baseline.<sup>8</sup> Vos et al. (2013) reported a greater decline on the Mini-Mental State Exam (MMSE) over an average of 3.9 years among individuals with evidence of both amyloid and tau pathology (Stage 2) compared to individuals classified as Stages 0, 1, and SNAP. The only other two studies to investigate the combined effects of amyloid and neuronal injury on cognitive change used imaging-based biomarkers, such as MRI, which do not provide a direct measure of tau-related neurofibrillary tangle pathology. These studies reported similar results as Vos et al. (2013), but the mean follow-up period was limited to 2–4 years.<sup>9, 10</sup>

The availability of CSF at baseline, when the subjects were cognitively normal, the extensive cognitive testing, and the unusually long duration of follow-up (mean=11 years) allowed us to examine several questions of particular relevance to clinical trials in preclinical AD. First, the present study used a cognitive composite score covering multiple domains of cognition as the outcome, allowing us to determine whether prior findings regarding the MMSE generalized to a broader range of cognitive domains and to tests likely to be more sensitive to subtle cognitive change. Second, most prior studies that have examined rates of cognitive change among cognitively normal individuals have been of short duration (mean follow-up 1–4 years),<sup>8-10</sup> have not included measures of tau pathology,<sup>11-14</sup> or did not examine possible interactions between amyloid and tau on the rate of change in cognition.<sup>15-20</sup> Third, it remains unclear whether the major genetic risk factor for AD, the apolipoprotein (APOE) ε4 genotype,<sup>21</sup> modulates the associations between amyloid, tau, p-tau and cognitive change. This may be highly relevant for the selection of subjects in clinical trials, since subgroups with differing rates of decline would make it more challenging to identify drug effects.

## Methods

### Study Design

The parent study from which these data are derived is known as the BIOCARD study, which was initiated at the National Institutes of Health (NIH) in 1995. By design, approximately 75% of the participants had a first degree relative with dementia of the Alzheimer type. The study was stopped in 2005 for administrative reasons and re-established at Johns Hopkins in 2009. While at the NIH, subjects were administered a comprehensive neuropsychological battery annually. MRI scans, cerebrospinal fluid, and blood specimens were obtained approximately every two years. See Figure 1 for a schematic representation of the study design.

## Selection of Participants

Recruitment was conducted by the staff of the Geriatric Psychiatry branch of the intramural program of the National Institute of Mental Health. At baseline, all participants completed a comprehensive evaluation at the NIH, consisting of a physical and neurological examination, an electrocardiogram, standard laboratory studies, and neuropsychological testing. Individuals were excluded from participation if they were cognitively impaired, or had significant medical problems such as severe cerebrovascular disease, epilepsy or alcohol or drug abuse. See eMethods-1 for details regarding the selection of participants.

A total of 349 individuals were initially enrolled in the study, after providing written informed consent. The analyses presented here are based on 222 participants of the 335 participants who provided baseline CSF (see eMethods-2 for reasons for exclusion of subjects from analyses).

## Clinical and Cognitive Assessment of Participants

A cognitive and clinical assessment and a consensus diagnosis were completed annually at the NIH and at Johns Hopkins (see Albert et al., 2014 for further details).<sup>22</sup> Each participant included in our analyses received a consensus diagnosis by the staff of the Johns Hopkins BIOCARD Clinical Core. Each case was handled in a similar manner: (1) clinical data pertaining to the medical, neurologic and psychiatric status of the subject were examined, (2) reports of changes in cognition by the subject and by collateral sources were reviewed, and (3) decline in cognitive performance, based on review of longitudinal testing from multiple domains, was established. We followed the diagnostic recommendations incorporated in the NIA/AA working group reports for the diagnosis of MCI<sup>23</sup> and dementia due to AD.<sup>24</sup> See eMethods-3 for additional details. The clinical diagnoses were blinded to CSF assessments.

The main outcome variable was an a-priori derived global cognitive composite score based on four individual measures that were identified previously to be the best combination of cognitive predictors of time to progress from normal cognition to clinical symptom onset.<sup>22</sup> These measures were the: (1) Logical Memory Delayed Recall (Story A) score of the Wechsler Memory Scale – Revised (WMS-R); (2) Verbal Paired Associates – Immediate subtest of the WMS-R; (3) Digit Symbol Test from the Wechsler Adult Intelligence Scale – Revised; (4) Boston Naming Test. These measures were administered annually at the NIH and are part of the annual neuropsychological battery at Johns Hopkins. To calculate the cognitive composite score, the individual measures were transformed to z-scores and then averaged, with the requirement that at least two of the four scores were present at a given time point. eFigure-1 shows a histogram of baseline scores.

## CSF Assessments

The CSF specimens were analyzed using the same protocol employed in the Alzheimer's Disease Neuroimaging Initiative. This protocol employed the xMAP-based AlzBio3 kit [Innogenetics] run on the Bioplex 200 system. Each subject had all samples (run in triplicate) analyzed on the same plate (see eMethods-4 and eFigure-2 for details regarding

the CSF assay and baseline biomarker frequency distributions; additional details have been published elsewhere<sup>25</sup>).

### APOE Genotyping and Coding

APOE genotype was established in all but one of the cohort participants (n=348). Genotypes were determined by restriction endonuclease digestion of polymerase chain reaction amplified genomic DNA (performed by Athena Diagnostics, Worcester, MA). APOE  $\epsilon 4$  carrier status was coded by an indicator variable, with  $\epsilon 4$  carriers coded as 1 if they had at least one  $\epsilon 4$  allele and non-carriers coded as 0. Analyses that included APOE carrier status excluded individuals with the  $\epsilon 2/\epsilon 4$  genotype because the  $\epsilon 4$  allele increases AD dementia risk,<sup>21</sup> whereas the  $\epsilon 2$  allele decreases AD dementia risk.<sup>26</sup>

### Statistical Methods

Based on the observation that about one-third of cognitively normal older adults have AD pathology in their brains, as indicated by amyloid imaging<sup>27-29</sup> and neuropathologic studies<sup>30-32</sup>, biomarker abnormality was defined as having CSF abeta<sub>1-42</sub> levels in the lower one-third of the distribution of participants (<374.5 pg/mL), or having tau (>74.9 pg/mL) or p-tau (>39.4 pg/mL) levels in the upper one-third of the distribution. The resulting proportion of individuals in the hypothetical preclinical AD groups (i.e., Stages 0, 1, and 2) was comparable to that reported in the literature.<sup>5</sup> The pattern of results was similar when using a median split (data not shown) or quintile split (supplementary eTable-1, eTable-2) to classify individuals into groups, suggesting robustness to cut-point variations.

The data were analyzed using general linear mixed regression models, including linear effects of time, to test if the rate of change in cognition differed across the groups. Two main analyses were performed, one using CSF abeta<sub>1-42</sub> and tau to classify individuals into the four groups, the other using CSF abeta<sub>1-42</sub> and p-tau for classification. Group status was coded using binary predictors (0 or 1) for each group. The following predictors were included in both models, treating Stage 0 as the implicit baseline: baseline age, gender, years of education, time, Stage 1 indicator, Stage 2 indicator, SNAP indicator, and the interaction (cross-product) of each predictor with time. In these models, the stage indicator $\times$ time interaction terms test if the rate of change in the cognitive composite score differs between Stage 0 and the other stages. The outcome variable in all analyses was the cognitive composite score (including baseline and all available follow-up scores, as defined above). Models were specified with a random intercept and slope.

To examine the role of APOE- $\epsilon 4$  genotype on cognitive change, both models were re-run, including the indicator for APOE- $\epsilon 4$ , and the APOE- $\epsilon 4$  genotype $\times$ time interaction term. Additionally, to test if the cognitive trajectories within a given stage differ by APOE- $\epsilon 4$  genotype, four mixed-effects models were run, one for each group, with the following predictors: baseline age, gender, education, APOE- $\epsilon 4$  indicator, time, baseline age $\times$ time interaction, and APOE- $\epsilon 4$  indicator $\times$ time interaction. (The gender $\times$ time and education $\times$ time interactions were not included because they were not significant in any previous analysis).

Differences in baseline characteristics of participants in Stage 0 compared to the other three groups were assessed using two-tailed t-tests or chi-square tests, with a significance level of  $p < .05$ , uncorrected for multiple comparisons. All data analyses used R, version 3.2.1.

## Results

Baseline characteristics for the BIOCARD cohort and for subjects in the analyses are shown in Table 1. Table 2 shows baseline characteristics separately for the four groups (Stage 0, 1, 2, and SNAP). The groups did not differ in education, gender, or MMSE at baseline. However, compared to Stage 0, individuals in Stage 2 were older, were more likely to be APOE- $\epsilon 4$  carriers, had lower baseline cognitive composite scores, and were more likely to progress to MCI or AD-dementia (Table 2, eTable-2). Individuals in Stage 0 had more follow-up cognitive testing than the other groups.

The results from the mixed effects models comparing the cognitive trajectories of individuals in Stage 0 to the other groups are shown in Table 3. The results were nearly identical whether CSF  $a\beta_{1-42}$  and tau or CSF  $a\beta_{1-42}$  and p-tau were used to define group membership. In both models, there was a main effect of time (reflecting practice-related improvement in cognitive performance over time), a main effect of age, and an age $\times$ time interaction (signifying lower cognitive performance and less improvement in performance over time with increasing age). Higher education was associated with better cognitive performance, but did not alter the rate of cognitive change over time.

Importantly, the main effects of Stage 1 and SNAP were not significant, nor were the interactions between Stage 1 $\times$ time and SNAP $\times$ time. This suggests that there was no difference in either the mean or the rate of change in the cognitive composite score over time between the Stage 0 group and the Stage 1 and SNAP groups. In contrast, the Stage 2 $\times$ time interactions were highly significant, indicating a more negative rate of change in cognition for the Stage 2 group compared to the Stage 0 group ( $p < 0.0001$ ). Mean cognitive performance was also lower in the Stage 2 group compared to the Stage 0 group. These results are presented graphically in Figure 2. Post-hoc mixed effects models directly comparing Stage 1 and SNAP to Stage 2 revealed a more negative rate of change in cognition for individuals in Stage 2 compared to Stage 1 (*estimate* = -0.074, *SE* = 0.018,  $p = 0.0002$  using p-tau and *estimate* = -0.068, *SE* = 0.019,  $p = 0.0001$  using tau) and compared to SNAP (*estimate* = -0.074, *SE* = 0.018,  $p = 0.0002$  for p-tau; and *estimate* = -0.06, *SE* = 0.017,  $p = 0.001$  using tau). Results were similar using the individual cognitive measures as outcomes.

The analyses were repeated including APOE- $\epsilon 4$  and the APOE- $\epsilon 4 \times$ time interaction term, but the results were unchanged and effects involving APOE- $\epsilon 4$  were non-significant (all  $p > 0.4$ ). Likewise, separate models for individuals in each stage showed no differences in the cognitive trajectories between APOE- $\epsilon 4$  carriers and non-carriers.

## Discussion

This study compared the cognitive trajectories of individuals with different CSF AD-biomarker profiles and normal cognition at baseline within the framework of four

hypothetical groupings related to preclinical AD.<sup>1, 5</sup> There was no difference in baseline cognitive performance or the rate of change in cognitive performance over an average of 11 years among individuals in Stage 1 (low levels of abeta) or SNAP (high levels of tau/p-tau) compared to those in Stage 0 (normal levels of both abeta and tau/p-tau). By comparison, individuals in Stage 2 (both low levels of abeta and high levels of tau/p-tau) showed lower cognitive performance at baseline and a more negative rate of change in cognition than the other three groups. Taken together, these results suggest that abnormal levels of both amyloid and tau are necessary for observing a marked decline in cognition among cognitively normal individuals.

These findings have important implications for the design of clinical trials aimed at individuals in the preclinical phase of AD. Our results suggest that in order to optimize observing a treatment effect, clinical trials enrolling cognitively normal individuals should selectively recruit participants with abnormal levels of both amyloid and tau (i.e., Stage 2), as this group would be expected to show the greatest cognitive decline over time if untreated. If participants are selected solely on the basis of their amyloid status (such as in the A4 study<sup>33</sup>), then the ability to observe a significant treatment effect on cognition might be greatly diminished because a large proportion of untreated participants (those with abnormal amyloid but normal tau levels) would not be expected to show meaningful cognitive decline over the relatively short time frame of a clinical trial. Our findings also suggest that while APOE-ε4 carriers may be more likely to be further along the AD trajectory and therefore have an earlier age of onset,<sup>12</sup> the cognitive trajectories do not differ by ε4 carrier status after accounting for CSF amyloid and tau/p-tau levels. Though we do not have data regarding the effectiveness of anti-amyloid drugs in reducing cognitive decline, our results suggest that to the extent that amyloid and tau pathology arise independently and cognitive decline simply depends on their co-occurrence,<sup>34-36</sup> anti-amyloid therapies may be effective in individuals with concurrent amyloid and tau pathology, and in those with amyloid pathology only who may subsequently develop tau pathology. However, if amyloid accumulation initiates a downstream cascade of tau-related neurodegeneration that becomes increasingly independent of amyloid itself,<sup>37</sup> then anti-amyloid agents may only be effective if administered prior to the onset of the neurodegenerative process.

The present results are consistent with prior short-term longitudinal studies reporting a disproportionately greater rate of cognitive decline for individuals classified as Stage 2 compared to Stages 0, 1, and SNAP using CSF biomarkers<sup>8</sup> or neuroimaging-based biomarkers<sup>9, 10</sup>. The study expands on prior findings in several ways. First, our cognitive outcome measure is clinically validated in the sense that it is based on neuropsychological tests previously shown to predict progression from normal cognition to MCI or dementia due to AD.<sup>22</sup> Both the baseline score and the rate of change in the measures that compose our composite score are associated with the time to onset of clinical symptoms, suggesting that these types of measures are useful for tracking AD progression in clinical trials. Second, our results demonstrate that the pattern of short-term cognitive trajectories observed previously remains stable over the course of a decade. Third, we found that although APOE-ε4 carriers were over-represented among individuals classified as Stage 2, APOE-ε4 genotype did not modify the rate of change in cognition. Taken together, these two findings suggest that the APOE-ε4 allele does not significantly alter the rate of AD progression, but is associated

with an earlier age of onset of AD.<sup>38, 39</sup> Fourth, higher education was associated with better cognitive performance, after accounting for baseline CSF levels, but did not modify the rate of change in cognition. This supports the view that education reduces the impact of AD neuropathology on cognition, but does not alter the rate of disease progression.<sup>40, 41</sup> Lastly, the current results point toward the utility of CSF biomarkers in identifying individuals at risk for cognitive decline at a significantly younger age (mean baseline age for Stage 2 = 63 years) than what has been reported by previous studies, which focused on individuals in their 70s at baseline.

Our study has several limitations. The participants are well educated, primarily Caucasian, primarily middle-aged at baseline, and the majority had a family history of dementia, so the results may not generalize to the population at large or to older cohorts. Additionally, the sample size may have been too small to detect differences by APOE-ε4 genotype. Future studies are necessary to determine if similar findings would be obtained using imaging-based biomarkers of amyloid and tau.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Dr. Soldan had full access to all the data in the study and takes full responsibility for the integrity of the data and the accuracy of the data analysis.

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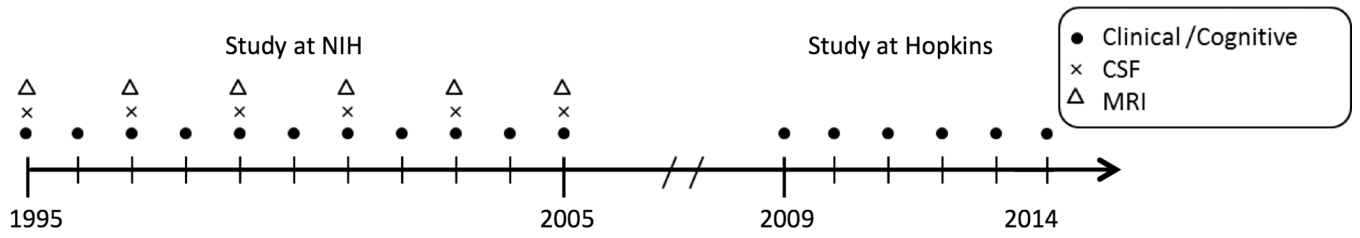


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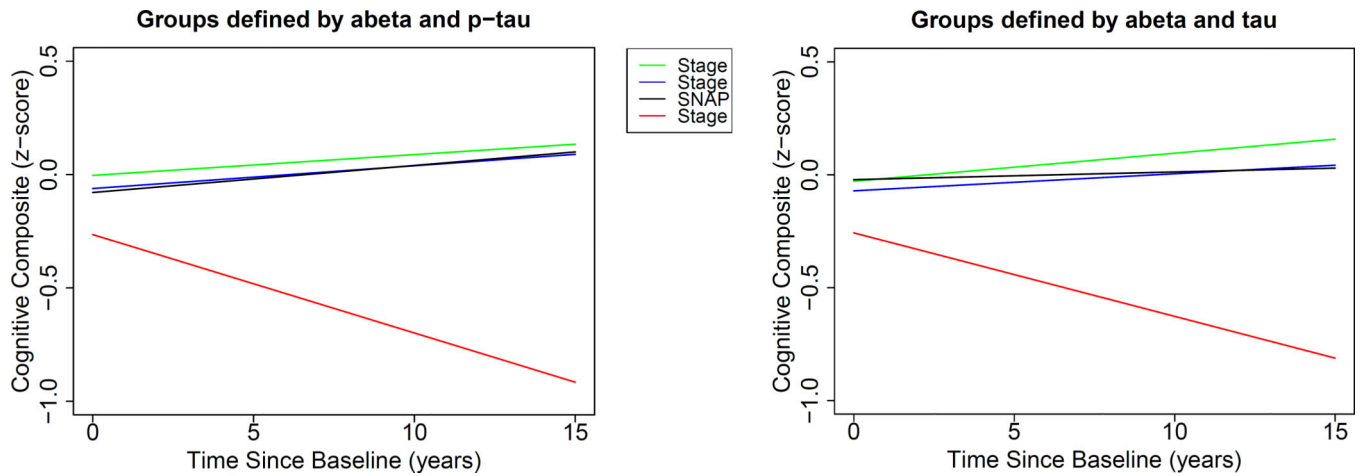
**Figure 1. Timeline showing the design of the BIOCARD study**  
Legend: Types of data collected each year for the BIOCARD study between 1995 and 2014.

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**Figure 2. Estimates of longitudinal cognitive change for the four hypothetical preclinical AD groups**

Legend: Estimates from linear mixed effects models predicting longitudinal cognitive composite scores over time among individuals classified into the four preclinical AD groups (Stage 0, 1, 2, and SNAP) using baseline CSF  $\text{abeta}_{1-42}$  and p-tau (left) or  $\text{abeta}_{1-42}$  and total tau (right) for classification. The estimates are adjusted for baseline age, gender, education, and their interactions with time. Stage 2 showed a greater decline and lower baseline scores than the other groups, which did not differ from one another (see Table 3).

**Table 1**

<i>Participant Characteristics at Baseline</i>	<b>Cohort as a whole</b>	<b>Subjects in analyses</b>
<b>Variable</b>	<b>(N = 349)</b>	<b>(N=222)</b>
Age, mean number of years (SD)	57.3 (10.4)	56.9 (10.1)
Age, range (min, max)	20.0 – 85.8	22.1 – 85.8
Follow-up time, mean number of years (SD)	10.9 (4.6)	11.0 (4.1)
Follow-up time, range (years)	0 – 18.7	0 – 18.3
Gender, females (%)	57.6%	59.9%
Ethnicity, Caucasians (%)	97.1%	97.3%
ApoE e4 carriers (%)	33.6%	32.9%
MMSE, mean score (SD)	29.5 (0.9)	29.5 (0.8)
Education, mean years (SD)	17.0 (2.4)	17.2 (2.3)
Education, range (years)	12 – 20	12 – 20
Paired Associates Immediate (SD)	20.2 (3.4)	20.1 (3.4)
Logical Memory Delayed (SD)	12.3 (4.0)	14.8 (4.1)
Boston Naming, % Correct (SD)	96.0 (5.3)	95.9 (5.3)
Digit Symbol Substitution (SD)	52.2 (11.7)	55.0 (12.6)
Cognitive Composite, mean (SD)	–0.10 (0.6)	–0.05 (0.6)

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**Table 2**

Baseline characteristics of participants in each of the four preclinical AD groups

Using baseline CSF abeta <sub>1-42</sub> and total tau to define group membership				
Baseline Participant Characteristics	Stage 0	Stage 1	Stage 2	SNAP
Variable	(N=102)	(N=46)	(N=28)	(n=46)
Age, mean number of years (SD)	54.8 (10.3)	56.8 (8.1)	63.6 (9.9) **	57.6 (9.9)
Follow-up time, mean years (SD)	12.0 (3.5)	10.0 (3.6) **	8.6 (5.2) **	11.2 (4.3)
Gender, females (%)	60.8%	58.7%	57.1%	60.9%
Ethnicity, Caucasians (%)	96.1%	97.8%	100.0% *	97.8%
ApoE ε4 carriers (%)	23.5%	34.8%	50.0% *	41.3% *
MMSE, mean score (SD)	29.6 (0.8)	29.4 (0.9)	29.6 (0.8)	29.5 (0.8)
Education, mean years (SD)	17.2 (2.4)	16.9 (2.4)	17.1 (2.1)	17.2 (2.2)
Paired Associates Immediate (SD)	20.3 (2.9)	20.0 (3.4)	20.3 (2.8)	20.7 (2.9)
Logical Memory Delayed (SD)	13.5 (3.8)	13.0 (3.9)	10.8 (4.5) *	12.8 (4.1)
Boston Naming, % Correct (SD)	96.1 (5.9)	96.4 (5.7)	94.8 (6.0)	95.1 (6.5)
Digit Symbol Substitution (SD)	54.5 (11.6)	52.2 (12.5)	47.9 (13.0) *	53.4 (11.9)
Cognitive Composite, mean (SD)	0.03 (0.6)	-0.06 (0.7)	-0.32 (0.6) *	-0.05 (0.6)
CSF abeta <sub>1-42</sub> in pg/mL (SD)	447.7 (51.7)	315.3 (41.2) **	250.8 (71.8) **	476.9 (57.2)
CSF p-tau <sub>181</sub> in pg/mL (SD)	30.7 (8.7)	26.6 (8.3)	60.5 (22.7) **	42.5 (13.5) **
CSF total tau in pg/mL (SD)	56.8 (11.1)	45.4 (13.3)	109.6 (31.7) **	95.8 (30.0) **
Using baseline CSF abeta <sub>1-42</sub> and p-tau <sub>181</sub> to define group membership				
Variable	(N=102)	(N=46)	(N=28)	(n=46)
Age, mean number of years (SD)	56.0 (9.9)	57.1 (8.5)	63.1 (9.6) **	55.1 (11.1)
Follow-up time, mean years (SD)	12.4 (3.9)	10.2 (3.9) **	8.3 (4.7) **	10.2 (3.1) **
Gender, females (%)	58.8%	54.3%	64.3%	65.2%
Ethnicity, Caucasians (%)	96.1%	97.8%	100.0% *	97.8%
ApoE ε4 carriers (%)	25.5%	34.8%	50.0% *	37.0%
MMSE, mean score (SD)	29.5 (0.8)	29.5 (0.9)	29.6 (0.7)	29.7 (0.7)
Education, mean years (SD)	17.3 (2.4)	17.1 (2.4)	16.8 (2.2)	17.0 (2.2)
Paired Associates Immediate (SD)	20.4 (2.9)	20.0 (3.5)	20.3 (2.5)	20.5 (2.9)
Logical Memory Delayed (SD)	13.3 (3.9)	13.0 (4.0)	10.8 (4.3) *	13.3 (4.0)
Boston Naming, % Correct (SD)	95.9 (6.3)	96.0 (6.2)	95.5 (5.3)	95.5 (5.7)
Digit Symbol Substitution (SD)	54.1 (11.9)	53.1 (12.9)	46.5 (11.8) *	54.4 (11.3)
Cognitive Composite, mean (SD)	0.00 (0.6)	-0.06 (0.7)	-0.32 (0.6) *	0.00 (0.6)
CSF abeta <sub>1-42</sub> in pg/mL (SD)	454.2 (57.2)	308.1 (51.8) **	262.7 (69.7) **	462.5 (50.0)
CSF p-tau <sub>181</sub> in pg/mL (SD)	28.6 (7.2)	25.6 (6.9)	62.2 (20.7) **	47.1 (9.8) **
CSF total tau in pg/mL (SD)	61.9 (20.3)	47.1 (16.7)	106.9 (34.5) **	84.7 (30.9) **

Significant differences between Stages 1, 2, and SNAP relative to Stage 0 are indicated by asterisks: \*  $p < 0.05$ ; \*\*  $p < 0.005$ .

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**Table 3**

Results of linear mixed effects models

Model Predictors	Groups defined by CSF abeta and p-tau			Groups defined by CSF abeta and total tau		
	Estimate	SE	p-value	Estimate	SE	p-value
Time	0.0905	0.0318	0.0050	0.0915	0.0324	0.0053
Baseline age	-0.0158	0.0043	0.0003	-0.0157	0.0043	0.0003
Gender (male)	-0.2590	0.0779	0.0010	-0.2508	0.0777	0.0015
Education	0.0466	0.0164	0.0051	0.0489	0.0164	0.0032
Stage 1	-0.0579	0.0977	0.5539	-0.0426	0.0987	0.6665
Stage 2	-0.2613	0.1211	0.0320	-0.2288	0.1214	0.0609
SNAP	-0.0755	0.0999	0.4511	0.0068	0.0980	0.9444
Baseline age × time	-0.0017	0.0004	<0.0001	-0.0018	0.0004	<0.0001
Gender × time	-0.0029	0.0068	0.6657	-0.0019	0.0068	0.7778
Education × time	0.0011	0.0014	0.4465	0.0014	0.0015	0.3536
Stage 1 × time	0.0009	0.0085	0.9179	-0.0048	0.0088	0.5825
Stage 2 × time	-0.0526	0.0116	<0.0001	-0.0494	0.0116	<0.0001
SNAP × time	0.0028	0.0087	0.7508	-0.0090	0.0084	0.2844

Note: Stage 0 was used as the implicit baseline in these models. Thus, estimates for Stage 1, 2, and SNAP and their interactions with time reflect differences relative to Stage 0.