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Plasma biomarkers predict amyloid conversion and cognitive decline in cognitively unimpaired adults

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Abstract

Background: Alzheimer's disease (AD) has a long preclinical phase. Development of disease-targeting therapies which may act in the early disease stage is dependent on the identification of at-risk individuals. We investigated whether plasma biomarkers phosphorylated-tau-217 (p-tau217), amyloid- β -42/40 ($A\beta$ -42/40), neurofilament light (NfL) and glial fibrillary acidic protein (GFAP) associated with increasing amyloid burden and cognitive decline in cognitively unimpaired older adults.

Methods: The cohort consisted of 205 monozygotic twins (complete pairs=101) aged ≥ 60 years at baseline. Twenty-seven participants (13.3%) were amyloid positive ($A\beta$ +) at baseline and the number of $A\beta$ + participants doubled to 37 (25.9%) at follow-up. Longitudinal measurements of plasma biomarkers, cognition (memory, language, executive function, attention), and amyloid status (assessed by $A\beta$ -PET; baseline and 4 year follow-up) were available (mean total follow-up time = 6.28 years (SD=0.83)). Receiver Operating Characteristic models assessed whether baseline biomarker concentrations and changes in biomarker concentrations could predict conversion from amyloid negative ($A\beta$ -) to $A\beta$ +. Linear mixed models were used to investigate associations between baseline biomarker concentrations and changes in biomarker concentrations with cognitive decline. A twin-difference design was used to examine whether observed associations reflected progressive pathology, or were confounded by shared genetic and environmental factors.

Results: Baseline p-tau217 (AUC=0.68[0.56–0.81]) and p-tau217 change over time (AUC=0.79[0.70–0.88]) predicted conversion from PET $A\beta$ - to $A\beta$ +. Adding demographic measures (age, sex, education) did not improve predictions. Using the twin-difference design, the association between p-tau217 change over time and follow-up $A\beta$ + remained significant, consistent with p-tau217 change reflecting progressive AD-related pathology. Baseline p-tau217 associated with decline in memory (β [SE]=−0.072[0.021]) and language

(β [SE]=−0.066[0.020]). Change in p-tau217 associated with decline in memory (β [SE]=−0.131[0.022]) and language (β [SE]=−0.066[0.024]). Baseline GFAP (β [SE]=−0.062[0.021]) and change in GFAP (β [SE]=−0.070[0.027]) associated with memory decline. Baseline NfL associated with attention decline (β [SE]=−0.094[0.025]). A β -42/40 did not associate with cognitive changes. Using the twin-difference design, the association between change in p-tau217 and memory decline remained marginally significant ($p=0.016$; $FDRp=0.055$).

Conclusions: Associations of plasma biomarkers, particularly p-tau217, with future AD pathology and cognitive decline, even in cognitively unimpaired individuals, suggest potential utility for identifying at-risk individuals and disease progression monitoring, enabling early intervention.

Key words: Pre-clinical stage; plasma biomarkers; p-tau217; amyloid PET; cognition; cognitive decline; disease progression; cognitively unimpaired; twin study; twin design

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia worldwide (1). It is a progressive disease with a long preclinical phase of up to 20 years (2-5), during which pathological changes start to accumulate in the brain while clinical symptoms cannot yet be detected. Although AD is not yet curable, novel medications targeting amyloid- β buildup in the brain have shown promising results in slowing the progression of the disease (6-9). These medications have been approved for treatment of AD in the United States and Europe in specific patient subsets (10, 11). They cannot, however, reverse damage that has already occurred. Given the long preclinical phase of AD, it becomes relevant to detect AD pathology in the earliest stages of the disease, so that at-risk individuals may be reliably identified and included in clinical trials for development of disease-modifying treatment.

Plasma biomarkers are a cost-effective and accessible tool for detecting AD pathological changes (12). One such marker is phosphorylated-tau (p-tau), which has multiple isoforms that can be detected in blood, including p-tau181, p-tau217, and p-tau231 (13). P-tau217 concentrations have shown good accuracy to detect A β pathology (14-17), even in cognitively unimpaired individuals (18, 19). Likewise, the amyloid β 42/40 (A β -42/40) ratio has been shown to accurately detect A β pathology even in the early stages of AD (20-22). Other non-AD specific biomarkers have shown value in the diagnosis and staging of AD, such as neurofilament light chain (NfL), a broad marker of neurodegeneration (20, 23), and glial fibrillary acidic protein (GFAP), a marker of astrocytic activation and neuroinflammation (20, 24).

Beyond their cross-sectional applications, changes in AD plasma biomarkers over time have also been investigated for their potential to predict the biological progression of AD. Both cross-sectional concentrations and longitudinal changes in concentrations of p-tau217, p-tau181, A β -42/40, NfL and GFAP have been found to associate with longitudinal increases in A β burden in cognitively unimpaired individuals (20, 25). P-tau217 and A β -42/40 trajectories were further

101 able to predict conversion from A β - to A β + in a mixed cohort of cognitively unimpaired and
102 impaired individuals over 4 years (26). Cross-sectional concentrations of p-tau217, A β -42/40,
103 NfL and GFAP, as well as changes in these biomarker concentrations over time, have also been
104 reported to predict conversion to MCI or AD-dementia in individuals with subjective cognitive
105 decline (SCD) (27-29).

106 Apart from changes in AD status, plasma biomarkers have been found to associate with changes
107 in cognition. A broad body of research in individuals with MCI and AD-dementia has shown
108 that cross-sectional measures of plasma p-tau217, p-tau181, A β 42/40, NfL, and GFAP are
109 associated with future cognitive decline (23, 26, 30-33). Longitudinal changes in these plasma
110 biomarkers have also been associated with concurrent changes in cognition (32, 33). The same
111 baseline and longitudinal associations have been reported in a limited number of studies
112 involving individuals with SCD (27) and in mixed cohorts across the AD continuum (26, 34).

113 Although previous studies have shown promising results, they were either conducted in
114 individuals with SCD, who represent a distinct population at a higher risk of progressing to
115 dementia (35); participants further along the AD-continuum (e.g. MCI or AD-dementia); or in
116 mixed cohorts of cognitively impaired and unimpaired individuals. To date, no study has
117 investigated the potential of these plasma biomarkers for predicting future onset of amyloid
118 pathology in a cohort of purely cognitively unimpaired individuals. Similarly, studies
119 examining associations between plasma biomarkers and cognition have either been conducted
120 in individuals with SCD, MCI, or AD-dementia (23, 27, 30, 31) or in mixed populations across
121 the AD-continuum (26). Studies in cognitively unimpaired populations have either included
122 individuals with already measurable AD-pathology or relied on cognitive tests covering specific
123 cognitive domains, such as the Mini Mental State Exam and the modified Preclinical Alzheimer
124 Cognitive Composite (32, 34). These screening tools are limited in detecting subtle cognitive
125 changes (36, 37), and AD affects multiple cognitive domains (38). As of yet, no study has

assessed associations between plasma biomarkers and multiple cognitive domains in cognitively unimpaired individuals and how these associations differ between individuals with early AD pathology and those free of pathology.

Additionally, previous studies have reported substantial genetic contributions to both the lifetime risk of AD (39) and variation in plasma AD biomarker concentrations (40-42). It however remains unknown how the genetic influences on AD risk and on plasma biomarker concentrations relate to each other. For example, shared upstream processes, including genetic factors, may independently influence both AD pathology and plasma biomarker concentrations, resulting in associations between AD pathology and plasma biomarker concentrations that do not necessarily reflect a direct pathological relationship. This could potentially confound the interpretation of observed associations between plasma biomarkers and AD pathology.

The current study had two main aims. First, to determine whether baseline concentrations and longitudinal changes in plasma p-tau217, A β -42/40, NfL, and GFAP can predict future onset of amyloid pathology in a cognitively unimpaired population. Second, to examine whether these plasma biomarkers are associated with early cognitive changes across memory, attention, executive functioning, and language domains. Additionally, a twin-difference design in monozygotic twins was used to assess whether associations between plasma biomarkers, amyloid status and cognition are confounded by shared genetic and environmental confounding.

2. Materials and methods

2.1. Cohort description

The cohort comprised 205 cognitively healthy monozygotic twins, all aged 60 or older at baseline, included from the longitudinal Amsterdam sub-study of the European Medical Information Framework for Alzheimer's Disease (EMIF-AD PreclinAD) (43, 44). Twin

zygosity was confirmed through DNA analysis. Participants were included if they were cognitively unimpaired at baseline, as defined by a global Clinical Dementia Rating score of 0, a score of 0 on the memory subdomain, and a delayed recall score greater than -1.5 standard deviations from demographic-adjusted normative data based on the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) 10-word list. Exclusion criteria included any neurological, systemic, or psychiatric disorder that could lead to cognitive impairments. Participants were included from 2014 to 2016. They were followed longitudinally for an average of 6.28 (SD = 0.83) years, with follow-up assessments conducted approximately every two years, totaling to three follow-up points (mean time to follow-up 1 = 1.94 years (SD = 0.26), mean time to follow-up 2 = 4.33 years (SD = 0.42), mean time to follow-up 3 = 6.59 years (SD = 0.31)). Reasons for dropping out included indicating a lack of interest in continuing the study or death. Blood collection and neurocognitive testing were performed at baseline and each follow-up point, while an A β [18F]flutemetamol-PET (A β PET) scan was performed at baseline and at second follow-up (4 years).

2.2. Data collection

2.2.1. Blood collection and plasma biomarker measurements

Blood was collected at baseline and each follow-up point in EDTA acid plasma tubes after 2 hours of fasting. Samples were centrifuged at 1300–2000xg for 10 min before being aliquoted and stored for long-term storage at -80°C. Plasma biomarker concentrations were all measured on the Simoa HD-X. Prior to measurement, samples were shortly thawed at room temperature in front of a cold-air fan and subsequently centrifuged at 10,000xg for 10 min. Concentrations of A β -42, A β -40, NfL, and GFAP were determined using the Simoa® Neurology 4-Plex E Advantage kit (Quanterix). These concentrations were obtained over four runs and samples were measured in singular according to manufacturer's instructions. Inter-assay coefficients of

variation (CVs) for the quality controls were 7.3% for A β 42, 5.0 for A β 40, 5.5% for NfL, and 5.0% for GFAP. P-tau217 concentrations were determined using the Simoa© ACC pTau217 Assay (Janssen). These concentrations were obtained over six runs and samples were measured in duplicate and according to manufacturer's instructions. The inter-assay CV for the quality controls was 5.6%, and the intra-assay CV for the samples was 7.1%.

2.2.2. Amyloid- β status assessment

An A β PET scan was performed at baseline and at follow-up 2 (4.33 years (SD = 0.42) later). PET imaging was conducted following the protocol outlined by Konijnenberg et al. (43) using a Philips Ingenuity Time-of-Flight PET–MRI scanner at the Department of Radiology & Nuclear Medicine, Amsterdam University Medical Center. During the baseline and follow-up visit, twins underwent dynamic dual-time window [18F]flutemetamol PET scans. Participants were injected intravenously with 185 MBq ($\pm$ 10%) of [18F]flutemetamol and scanned dynamically for 0 to 30 minutes. They were then scanned again 90 to 110 minutes after the injection. Prior to each part of the PET scan, a dedicated MR sequence (atMR) was performed to correct for attenuation in the PET images. To minimize movement artifacts during scanning, participants' heads were positioned using laser guidance and monitored throughout the scan to maintain a stable head position. The static part of these scans (90 to 110 minutes) were visually evaluated by three trained readers following General Electric Healthcare guidelines to classify participants as A β ⁺ or A β ⁻. In cases of disagreement, the consensus rating of two readers was utilized.

2.2.3. Cognitive testing

Cognitive functioning was assessed using a standardized neuropsychological test battery covering memory, attention, executive functions, and language domains at baseline and all of

the three follow-up points. Memory was evaluated with the Dutch version of the Rey Auditory Verbal Learning Test (RAVLT) (45), which included total immediate and delayed recall, the three- and twenty-minute recall of the Rey Complex Figure Test (RCFT) (46), and the total score for the FNAME-names and occupations test (47). Attention was measured using the Trail Making Test (TMT) Part A (48), the Digit Symbol Substitution test (49), and the forward condition of the Digit Span task (49). Executive function was assessed using the Trail Making Test (TMT) Part B, adjusted for TMT Part A (48), the backward condition of the Digit Span task (49), and the Dutch Controlled Oral Word Association Task, in which participants generated words starting with the letters D, A, and T (50). Language was evaluated using the Category Fluency Animal task (51) and the Graded Naming Test (52).

2.3. Statistical analysis

2.3.1. Predicting A β status at baseline and over time

The first set of analyses focused on the relationship between plasma biomarkers and A β status. Only data from baseline to the 4-year follow-up was used, as this was when the follow-up A β PET scan was performed.

First, we tested whether baseline biomarker concentrations could discriminate between A β - and A β + individuals at baseline. Receiver Operating Characteristic (ROC) models were fit, with individual biomarker concentrations as predictors and A β status (A β -/A β +) as the outcome. The area under the ROC curve (AUC) of each model was compared using the DeLong test. Resulting p-values were then corrected for multiple testing using the False Discovery Rate (FDR) correction. Once the best performing biomarker was identified, a linear model was fit with this biomarker as predictor, as well as additional linear models where age, sex (biological), or education (in years) were added. An ANOVA was then used to test whether these demographic covariates improved the prediction of the plasma biomarker only model. If

multiple covariates were found to improve the model, then an additional model was fit including all significant covariates and compared to the single covariate model.

Secondly, we tested whether baseline biomarker concentrations could predict conversion from A β - to A β + over 4 years. For this step, only participants who were A β - at baseline were included (n = 125). The same approach was followed as for the first analyses, replacing the outcome from baseline A β status to follow-up A β status. Similarly to the first analyses, we additionally tested whether the inclusion of age, sex, and/or education further improved model performance. Thirdly, we determined whether changes in plasma biomarker concentrations over time could predict with conversion from A β - to A β +. Only participants who were A β - at baseline were included. Linear mixed-effects models (LMMs) with random intercepts and slopes for time at the individual level were used to estimate individual changes in biomarker concentrations. These individual change estimates were then used as predictors of conversion from A β - to A β +, using the same analytical framework as in the first and second analyses. The added value of age, sex, and/or education to the best performing model was also assessed. As an additional step, the best baseline biomarker and change in biomarker concentration over time for predicting follow-up amyloid status were combined into a model, and this model was compared to the best baseline biomarker model and the best biomarker change over time model using an ANOVA.

Finally, we tested whether the observed associations were a true reflection of pathophysiology, or if they were influenced by a common genetic liability and/or shared environmental confounding. To do so, we applied a twin-difference design using generalized estimating equations. Mean family biomarker concentrations (mean concentration per biomarker across twin 1 and twin 2) and family biomarker concentrations difference (difference between each twin biomarker concentration and the mean family biomarker concentration) were included as predictors of PET amyloid- β status at 4 year follow-up, as described previously (22). Hence,

the biomarker concentration difference is corrected for all that is shared between the twins (age, sex, genome, shared environmental history). A significant association between the biomarker concentration difference between twin 1 and twin 2 and amyloid- β status indicates that the association between the biomarker and the outcome is minimally affected by genetic and shared environmental confounding.

2.3.2. Association between AD plasma biomarkers and cognition over time

In the second set of analyses, we focused on the relationship between plasma biomarkers and cognitive change over time, including participants up to follow-up 3 (mean follow-up time = 6.28, SD = 0.83). Neuropsychological test scores were standardized by calculating z-scores, using the baseline mean and standard deviation from the Amsterdam EMIF-AD PreclinAD cohort, to allow different tests within the same domain to be combined into a composite score. Composite scores were obtained by averaging the z-scores of the tests within that domain. To ensure that lower scores consistently reflected poorer performance across all domains, z-scores for the TMT were inverted by multiplying them by -1. Composite scores could only be calculated if data were available for at least two-thirds of the relevant tasks. Consequently, composite scores could not be calculated for two participants at baseline, three at follow-up 1, and one participant at both follow-up 2 and follow-up 3.

To examine the relationship between plasma biomarkers and cognitive performance, we applied LMMs. These models included subject-specific random intercepts and slopes, as well as a random effect for family to account for clustering within twin pairs. Age, sex, and years of education were included as covariates. First, we ran separate models for each biomarker and each cognitive composite score to test whether baseline plasma biomarker concentrations were associated with baseline cognition and change over time. We additionally evaluated whether longitudinal changes in plasma biomarker concentrations were associated with cognitive

change. For this, individual biomarker slopes were derived using linear models (see 3.1) and subsequently included in the LMMs.

For significant results from the initial cognition analysis, two additional analyses were conducted. First, we examined whether the relationship between plasma biomarkers and cognitive change over time differed by amyloid- β status, specifically stratifying the cohort in three groups: participants who were A β - at baseline and 4-year follow-up (stable negative), participants who were A β + at baseline and 4-year follow-up (stable positive), and participants who were A β - at baseline but A β + at follow-up (converters). This involved models with an interaction term between baseline plasma biomarker concentrations or their slopes, time, and PET amyloid- β status. These models included the same random effects and covariates as the initial analyses. Additionally, we applied a twin-difference design to control for genetic and shared environmental confounding in the biomarker-cognition association. For this, LMM's were used to assess whether within-pair differences in plasma biomarker concentrations or their concentration change over time were associated with within-pair differences in cognitive change. These analyses were adjusted for years of education, as this may differ between twins of the same pair, and included family-specific random intercepts and slopes to correct for repeated measures.

3. Results

3.1. Cohort demographic

The baseline cohort consisted of 205 participants, of whom 120 (58.5%) were female. The mean age was 71 years, and the average years of education was 11 years. The sample consisted of 101 twin pairs and 3 individuals for whom data was available for only one twin. Twenty-seven (13%) participants had a positive A β PET scan at baseline. This number increased to 37 (26%) out of the 152 twins which participated in follow-up 2, consisting of 16 participants already

positive at baseline and 21 participants who were negative at baseline and had converted to a positive status over 4 years. The A β ⁺ group at baseline had a significantly higher age ($p = 0.006$), higher p-tau217 concentrations ($p < 0.001$), lower A β -42/40 ratio ($p < 0.001$), higher GFAP concentrations ($p < 0.001$), and lower memory composite scores ($p = 0.003$) (Table S1). No significant differences were found between the groups in terms of years of education, proportion of female participants, NfL concentrations, or language, attention, and executive function composite scores ($p \geq 0.06$ for all). Demographic information per timepoint is reported in Table 1.

3.2. Association between plasma biomarkers and A β status

3.2.1. Cross-sectional association between plasma biomarker concentration and A β status

We first tested whether individual baseline biomarker concentrations could discriminate between A β ⁻ and A β ⁺ individuals. The best-performing individual biomarker was p-tau217 (AUC: 0.89, 95% CI = 0.80 – 0.97), followed by GFAP (AUC = 0.80, 95% CI = 0.71 – 0.90), A β -42/40 (AUC = 0.75, CI 95% = 0.66 – 0.83), and NfL (AUC = 0.61, 95% CI = 0.50 – 0.73) (Table S2, Figure 1A). P-tau217 outperformed both NfL (FDR-corrected p-value ($FDRp$) < 0.001) and A β -42/40 ($FDRp = 0.04$), but not GFAP ($FDRp = 0.13$), while GFAP outperformed NfL only ($FDRp = 0.001$). The performance of p-tau217 was not improved by the addition of either age, sex, or education ($FDRp \geq 0.28$ for all) (Table S3).

3.2.2. Association between baseline biomarker concentrations and conversion from A β ⁻ to A β ⁺

We then tested whether individual baseline biomarker concentrations could identify participants who had converted from A β - to A β + during the 4-year follow-up period. The only significant predictor of A β status conversion was p-tau217 (AUC = 0.68, 95% CI = 0.56 – 0.81) (Table S2, Figure 1B). None of the biomarkers outperformed each other ($FDRp \geq 0.11$ for all). Adding age, sex, or education to the p-tau217 model did not further improve the performance of p-tau217 only ($FDRp \geq 0.37$ for all) (Table S3).

3.2.3. Association between biomarker concentration change over time and conversion from A β - to A β +

We further tested whether individual changes in biomarker concentrations over time could predict conversion from A β - to A β + at 4-year follow-up. The best performing individual biomarker and only significant predictor of A β conversion was longitudinal p-tau217 (AUC = 0.79, 95% CI = 0.70 – 0.88) (Table S2, Figure 1C). Longitudinal p-tau217 outperformed both the NfL and GFAP models ($FDRp \leq 0.004$ for both) but not the A β -42/40 model ($FDRp = 0.14$). The prediction of p-tau217 change over time was not improved by the addition of age, sex, or education to the model ($FDRp \geq 0.08$ for all) (Table S3). Finally, we combined baseline p-tau217 concentrations and p-tau217 concentration change over time into a model to predict conversion from A β - to A β + at follow-up. This combined model outperformed the model with p-tau217 change over time only (F-statistic = 5.84; $FDRp = 0.02$).

3.2.4. Associations between plasma biomarkers and amyloid status, accounting for underlying genetic and environmental liability

Finally, we used a twin-difference design to determine whether the association between biomarkers and amyloid status observed in the previous steps are reflective of AD pathology or confounded by genetic or shared environment influences. While in the overall cohort, higher

baseline p-tau217 associated with a higher risk of being A β ⁺ at follow-up, within twin pairs this association was not significant (β [SE] = 0.22 [0.23], p = 0.37, Table 2) . This suggest that the observed association may be partially confounded by shared genetic or environmental factors influencing both AD pathology and p-tau217 concentrations, rather than reflecting a direct association between p-tau217 concentrations and AD pathology. For biomarker change over time, in the overall cohort we found that a steeper change in p-tau217 over time was associated with a higher risk of being A β ⁺ at follow-up. This association remained significant within twin pairs (β [SE] = 0.72 [0.26], p = 0.004), indicating that even when accounting for age, sex, genetics and a substantial part of shared environment history, a steeper increase in p-tau217 concentrations over time is significantly associated with a greater risk of converting from A β ⁻ to A β ⁺ over time. This suggests that the association between p-tau217 change and A β status conversion is unlikely to be confounded by genetics or shared environmental factors.

3.3. Association between plasma biomarkers and cognition

3.3.1 Association between baseline biomarker concentration and cognition

We first examined whether baseline plasma biomarker concentrations were associated with cognition at baseline and cognitive changes over time. We observed that higher baseline p-tau217 concentrations were associated with lower memory scores at baseline (β [SE] = -0.145 [0.050], $FDRp$ = 0.005) and a steeper decline in memory over time (β [SE] = -0.072 [0.021], $FDRp$ = 0.002). Higher baseline GFAP concentrations were also associated with lower memory scores at baseline (β [SE] = -0.121 [0.057], $FDRp$ = 0.040) and a steeper decline in memory over time (β [SE] = -0.062 [0.021], $FDRp$ = 0.005). Additionally, higher baseline p-tau217 concentrations were associated with a faster decline in language functioning over time (β [SE] = -0.066 [0.020], $FDRp$ = 0.006). Finally, baseline NfL concentrations were associated with deterioration in attention over time (β [SE] = -0.094 [0.025], $FDRp$ < 0.001). Baseline A β ⁻

42/40 was not associated with any cognitive domain scores ($FDRp \geq 0.22$ for all). None of the baseline biomarker concentrations were associated with executive functioning scores ($FDRp \geq 0.30$ for all) (Table S4, Figure 2).

3.3.2 Associations between biomarker concentration change over time and cognition over time

Next, we examined whether longitudinal changes in plasma biomarker concentrations were associated with changes in cognition. Both p-tau217 (β [SE] = -0.131 [0.022], $FDRp < 0.001$) and GFAP change over time (β [SE] = -0.070 [0.027], $FDRp = 0.013$) were associated with a steeper decline in memory functioning over time. Additionally, p-tau217 changes were associated with a faster decline in language functioning over time (β [SE] = -0.066 [0.024], $FDRp = 0.011$). Changes in A β -42/40 and NfL over time were not associated with change in performance for any cognitive domain ($FDRp \geq 0.15$ for all). None of the changes in biomarker concentrations over time were associated with change in executive function ($FDRp \geq 0.30$ for all) or attention performance ($FDRp \geq 0.40$ for all) (Table S4 and Figure 3).

3.3.3 Associations between biomarker concentration and cognition over time, stratified by A β -status

A significant three-way interaction was observed between baseline p-tau217 concentrations, time, and PET A β status on memory (β [SE] = -0.065 [0.025], $p = 0.010$, $FDRp = 0.021$), indicating that the association between baseline p-tau217 concentrations and cognitive change over time differed across the three PET A β status groups (Table S5). Pairwise group comparisons showed that stable A β - individuals differed significantly from stable A β + individuals in their association between baseline p-tau217 concentration and longitudinal memory functioning (β [SE] = -0.125 [0.052], $p = 0.018$, $FDRp = 0.039$). Specifically, elevated

baseline p-tau217 concentrations in stable A β ⁺ individuals were more strongly associated with a faster decline in memory over time compared to stable A β ⁻ negative individuals. No significant differences were observed in the association between baseline p-tau217 concentrations and longitudinal memory functioning in comparisons between converters and stable A β ⁻ individuals or stable A β ⁺ individuals (Table S6). For the other baseline plasma biomarker concentrations and for all plasma biomarker slopes, no significant three-way interaction with time and PET A β status on cognition across all cognitive domains was found ($FDRp \geq 0.061$ for all) (Table S5 and Figure 4).

3.3.4 Associations between plasma biomarkers and cognition, accounting for underlying genetic and environmental liability

Using the twin-difference design, no significant associations were found between within twin-pair differences in baseline plasma markers and within twin-pair differences in cognitive decline over time. Regarding changes in plasma biomarkers over time, within twin-pair differences in the slope of p-tau217 showed a marginally significant association with within twin-pair differences in memory change over time (β [SE] = -0.099 [0.041], $p = 0.016$, $FDRp = 0.055$) (Table 3 and Figure S1).

4. Discussion

In this study, we show that in cognitively unimpaired individuals both baseline p-tau217 concentrations and change in p-tau217 concentrations over time are moderate predictors of future AD pathology, while A β -42/40, NfL and GFAP are not associated with future AD pathology. Importantly, the association between plasma p-tau217 concentration change over time and future amyloid status was not confounded by shared genetic and environmental factors, suggesting a pathology-driven temporal relation between this plasma biomarker and developing AD pathology. We further observed that both baseline p-tau217 and change in p-tau217 concentrations over time associated with decline in memory and language functioning. In addition, both baseline GFAP and change in GFAP over time were associated with memory decline, while baseline NfL was associated with decline in attention. When stratifying by A β status, we further observed that the association between baseline p-tau217 and longitudinal memory decline was strongest in individuals who were A β -positive at baseline, consistent with an interaction effect indicating that this association is more pronounced in the presence of amyloid pathology. On the other hand, associations of NfL and GFAP with cognitive decline were independent of AD pathology, suggesting these biomarkers are likely most useful as predictors of general, rather than AD-specific, cognitive decline. The association between changes in p-tau217 concentrations over time and memory decline was minimally influenced by (genetic) confounding. Taken together, our results suggest that longitudinal changes in p-tau217 may directly reflect biological progression of AD, highlighting the potential of this biomarker not only for AD diagnosis but also for assessing future AD risk and monitoring biological and clinical disease progression.

A recent study in a mixed cohort of cognitively unimpaired, MCI, and AD-dementia individuals reported that higher p-tau217 concentrations were associated with a 2-fold increased risk of becoming A β ⁺ over 10 years (26). Our study demonstrates that this association also exists in a

cohort of cognitively unimpaired individuals only. Yakoub et al. additionally report that change in A β -42/40 and GFAP over time associate with risk of conversion to amyloid positivity in a mixed cohort of cognitively unimpaired and cognitively impaired individuals, a result we did not replicate (26). We hypothesize that changes in plasma A β -42/40 and GFAP may only be detectable further along the AD-continuum; A β -42/40, because of the overall low fold-change in plasma (53), and GFAP because increases in concentrations are most pronounced in more advanced disease stages (54).

We further observed that both p-tau217 and GFAP were associated with longitudinal decline in memory and language. Our findings of associations between p-tau217, GFAP, and cognitive decline are in line with a recent study in a cohort of participants with SCD by Trieu et al (27), which reported associations of p-tau217 and GFAP with memory, language, attention, and executive functioning, as well as global cognition. Trieu et al. further reported associations between A β -42/40 and global cognition and language, as well as between NfL and global cognition, language, and executive function, none of which were found in the current study. However, people with SCD differ from the general population without cognitive complaints, as they are at a higher risk of progressing to dementia, which may explain the differences in our results (35). Yakoub et al (26) additionally reported association between p-tau217 and GFAP and the Alzheimer's Disease Assessment Scale-Cognitive Subscale 13 (ADAS13), a composite measure sensitive to AD-related cognitive changes (55). They found fairly consistent associations between NfL and ADAS13 scores, while in the current study NfL was only associated with attention. However, because they used a composite measure rather than separate cognitive domains, it is unclear which components drove the association with NfL. Their inclusion of cognitively impaired individuals might additionally be partially responsible for this association. Importantly, by assessing distinct cognitive domains rather than relying on global

cognitive scores alone, we provided a more nuanced characterization of early cognitive changes associated with plasma biomarkers.

Leveraging the uniqueness of our longitudinal cohort of monozygotic twins, we were further able to investigate whether the associations observed between plasma biomarkers and AD outcomes were reflective of present and developing pathology, or if they were (partially) confounded by shared genetic or environmental factors. Applying a twin-difference design, we determined that the relationship between longitudinal changes in p-tau217 and subsequent AD and memory decline was not confounded by unmeasured genetic and environmental factors. This indicates that p-tau217 is not merely a proxy of shared genetic liability, but may reflect processes more directly involved in biological disease progression. All other associations between the plasma biomarkers (baseline and change over time) and AD pathology and cognition were, however, partly confounded, though the direction of the associations between baseline p-tau217 and GFAP with memory, as well as between baseline p-tau217 and conversion from A β ⁻ to A β ⁺, was consistent with the main analyses. Since the sample size for the twin-difference design was relatively small, as only complete twin pairs with full data could be included and each pair was treated as a single unit, these findings may have been limited by reduced statistical power.

Our study has several strengths. In particular, the cohort consists of cognitively unimpaired older adults with longitudinal measurements and well-characterized physical and cognitive health. Moreover, the twin-difference design of monozygotic twins enabled unique twin-based analyses to account for shared genetic and environmental confounding. The relatively healthy nature of the cohort is also an advantage, as it enables the investigation of the earliest stages of disease development and progression prior to overt clinical impairment.

5. Limitations

The relatively healthy nature of the cohort may have increased susceptibility to practice effects from repeated cognitive testing, which is seen in individuals without amyloid pathology (56). This practice effect may have masked subtle declines in cognition. The healthy nature of our cohort also resulted in limited variability in disease progression, with only a small proportion of participants converting from $A\beta^-$ to $A\beta^+$. As additional limitations, we point to the overall sample size which was relatively small, and the $A\beta$ PET scans only being performed at baseline and 4-year follow-up. This precluded exact determination of the timing of conversion from $A\beta^-$ to $A\beta^+$ within the four-year interval. Future studies in larger and more diverse cohorts, with a longer follow-up time, are warranted to better capture the temporal dynamics of $A\beta$ conversion. The fact that such associations were already observable within a relatively small and healthy cohort over a limited time frame suggests that the predictive value of these biomarkers may be even more pronounced in larger cohorts with extended follow-up periods.

6. Conclusions

In conclusion, our findings expand on previous works by demonstrating that in addition to its diagnostic and prognostic utility, p-tau₂₁₇ may serve as a predictive marker of future AD pathology and AD-related cognitive decline, even in cognitively healthy individuals currently free of AD pathology. Future research should aim to identify additional factors which may enhance the predictive utility of these biomarkers, for example by incorporating known risk factors for AD, such as family history, genetics, comorbidities (obesity, traumatic brain injuries) and life style factors (alcohol consumption, smoking) (57). Further work is also needed to elucidate the biological mechanisms linking plasma p-tau₂₁₇ dynamics to early amyloid accumulation and cognitive decline. Accurate risk biomarkers would enable identification of individuals at risk of developing AD prior to biological disease onset, facilitating preventive interventions.

516

517 **List of abbreviations**

518 A β 42/40: Amyloid beta1-42/1-40

519 AD: Alzheimer's disease

520 FDR: False discovery rate

521 GFAP: Glial fibrillary acidic protein

522 LMM: Linear mixed models

523 MCI: Mild cognitive impairment

524 NfL: Neurofilament light

525 PET: Positron emission tomography

526 p-tau: Phosphorylated-tau

527 SCD: Subjective cognitive decline

528

Declarations

Ethics approval and consent to participate

The Medical Ethics Review Committee of the Amsterdam University Medical Center approved the study. Research was performed according to the principles of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act and codes on ‘good use’ of clinical data and biological samples as developed by the Dutch Federation of Medical Scientific Societies. All participants gave written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

Data will be made available to researchers who provide a methodologically sound research proposal. Data sharing requests for such research proposals should be directed to ntr.datamanagement.fgb@vu.nl (see https://tweelingenregister.vu.nl/information_for_researchers/working-with-ntr-data for NTR data sharing procedures). To gain access, data requestors will need to sign a data access agreement.

Competing interests

E.d.G. is chair of the executive board of the VUmc research BV. P.S. has received consultancy fees (paid to the university) from AC Immune, Alzheon, Brainstorm Cell and ImmunoBrain Checkpoint. Within his university affiliation, he is PI of studies with AC Immune, FUJIFilm/Toyama, IONIS, UCB, Novartis, Novo Nordisk and Vivoryon. He is also an employee of Life Sciences Partners Amsterdam. L.E.C. has received research support from GE Healthcare (paid to institution). C.E.T. has a collaboration contract with ADx Neurosciences, Quanterix and Eli Lilly and performed contract research or received grants from AC-Immune, Axon

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Authors' contributions

RZR was involved in the measurements of biomarkers, performed all analyses pertaining to conversion from A β - to A β +, and was a main participant in the writing of the manuscript. EMD performed all analyses pertaining to cognitive decline and was a main participant in the writing of the manuscript. CET and AdB conceptualized the study and provided specialized input. AdB was a main participant in the writing of the manuscript. EdG and PJV contributed to cohort design and statistical analyses. IMWV supervised blood sample processing and biomarker analyses. JT, SMvdL, and MtK contributed to EMIF data collection. LC contributed to amyloid PET imaging processing and visual assessment. EdG, IMWV, JT, SMvdL, MtK, LC, PJV, CET and AdB all reviewed the manuscript critically.

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Table 1. Demographic information of the cohort at baseline and follow-ups.				
	<i>Baseline</i> (n = 205)	<i>Follow-up 1</i> (n = 192)	<i>Follow-up 2</i> (n = 152)	<i>Follow-up 3</i> (n = 131)
Aβ+ participants at baseline who were lost to follow-up (% of total drop-outs per FU)	—	2 (15.38%)	6 (15%)	7 (31.82%)
Age Mean, SD	70.7 (7.86)	72.18 (7.4)	73.1 (6.83)	74.6 (5.7)
Sex Female, %	120 (58.54%)	110 (57.29%)	84 (55.26%)	73 (55.73%)
Year of Education Mean, SD	11.39 (2.60)	11.49 (2.62)	11.8 (2.64)	11.8 (2.69)
Aβ status Aβ+, %	27 (13.3%)	—	37 (25.9%)	—
p-tau217 (pg/mL) Median, IQR	0.037 [0.026 – 0.049]	0.037 [0.028 – 0.052]	0.037 [0.029 – 0.054]	0.040 [0.027 – 0.061]
Aβ-42/40 Median, IQR	0.062 [0.054 – 0.068]	0.061 [0.053 – 0.067]	0.061 [0.054 – 0.067]	0.061 [0.053 – 0.065]
NfL (pg/mL) Median, IQR	14.5 [11.1 – 19.77]	15.2 [11.3 – 19.6]	16.0 [12.9 – 19.5]	17.6 [13.7 – 23.0]
GFAP (pg/mL) Median, IQR	97.26 [73.22 – 139.32]	104 [72.6 – 143]	104 [78.1 – 148]	117 [87.5 – 157]
Memory composite score (Mean, SD)	-0.03 (0.77)	-0.01 (0.80)	0.25 (0.85)	0.09 (0.89)
Language composite score (mean, SD)	0.003 (0.81)	0.04 (0.81)	0.19 (0.82)	0.01 (0.88)
Attention composite score (mean, SD)	-0.008 (0.73)	-0.07 (0.74)	0.10 (0.68)	0.01 (0.96)
Executive function composite score (mean, SD)	-0.005 (0.67)	-0.095 (0.69)	0.05 (0.81)	-0.13 (0.73)

Aβ = amyloid beta. Aβ-42/40 = amyloid beta 42/40 ratio. GFAP = glial fibrillary acidic protein. IQR = interquartile range. NfL = neurofilament light. P-tau217 = phosphorylated tau-217. SD = standard deviation. Time between each follow-up is about 2 years.

Table 2. Association between within twin-pair difference in plasma biomarkers and in Aβ status over time.			
Model	β (SE)	p	FDR p
Baseline biomarker concentration prediction of conversion from Aβ- to Aβ+ at follow-up within twin pair			
p-tau217	0.22 (0.23)	0.37	NA
Biomarker concentration change over time prediction of conversion from Aβ- to Aβ+ at follow-up			
p-tau217	0.72 (0.26)	0.004	NA

771 FDR-corrected p-values are not computed, as only one test was performed per condition.

772 A β = amyloid beta. A β -42/40 = amyloid beta 42/40 ratio. AUC = area under the curve. GFAP = glial fibrillary acidic protein. NfL =
773 neurofilament light. P-tau217 = phosphorylated tau-217. P = p-value. FDR p = FDR-corrected p-value.

774

Table 3. Association between within twin pair difference in plasma biomarkers and in cognitive change over time.									
	Memory			Language			Attention		
Model	β (SE)	p	FDRp	β (SE)	p	FDRp	β (SE)	p	FDRp
Baseline biomarker concentration association with cognitive change									
p-tau217	-0.059 (0.038)	0.112	0.304	-0.019 (0.038)	0.611	0.743	–	–	–
NfL	–	–	–	–	–	–	0.012 (0.025)	0.615	0.918
GFAP	-0.013 (0.029)	0.648	0.740	–	–	–	–	–	–
Biomarker concentration change association with cognitive change									
p-tau217	-0.099 (0.041)	0.016	0.055 [±]	0.010 (0.042)	0.815	0.868	–	–	–
GFAP	-0.036 (0.043)	0.408	0.916		–	–	–	–	–

775 [±] $p < 0.05$, but $FDRp$ non-significant ; * $FDRp < 0.05$; ** $FDRp < 0.01$; *** $FDRp < 0.001$

776 β = beta coefficient. GFAP = glial fibrillary acidic protein. NfL = neurofilament light. P-tau217 = phosphorylated tau-217. P = p-value. $FDRp$ =
777 FDR-corrected p-value. SE = standard error. Change in cognition was evaluated over a period of minimum 2 years and maximum 6 years.

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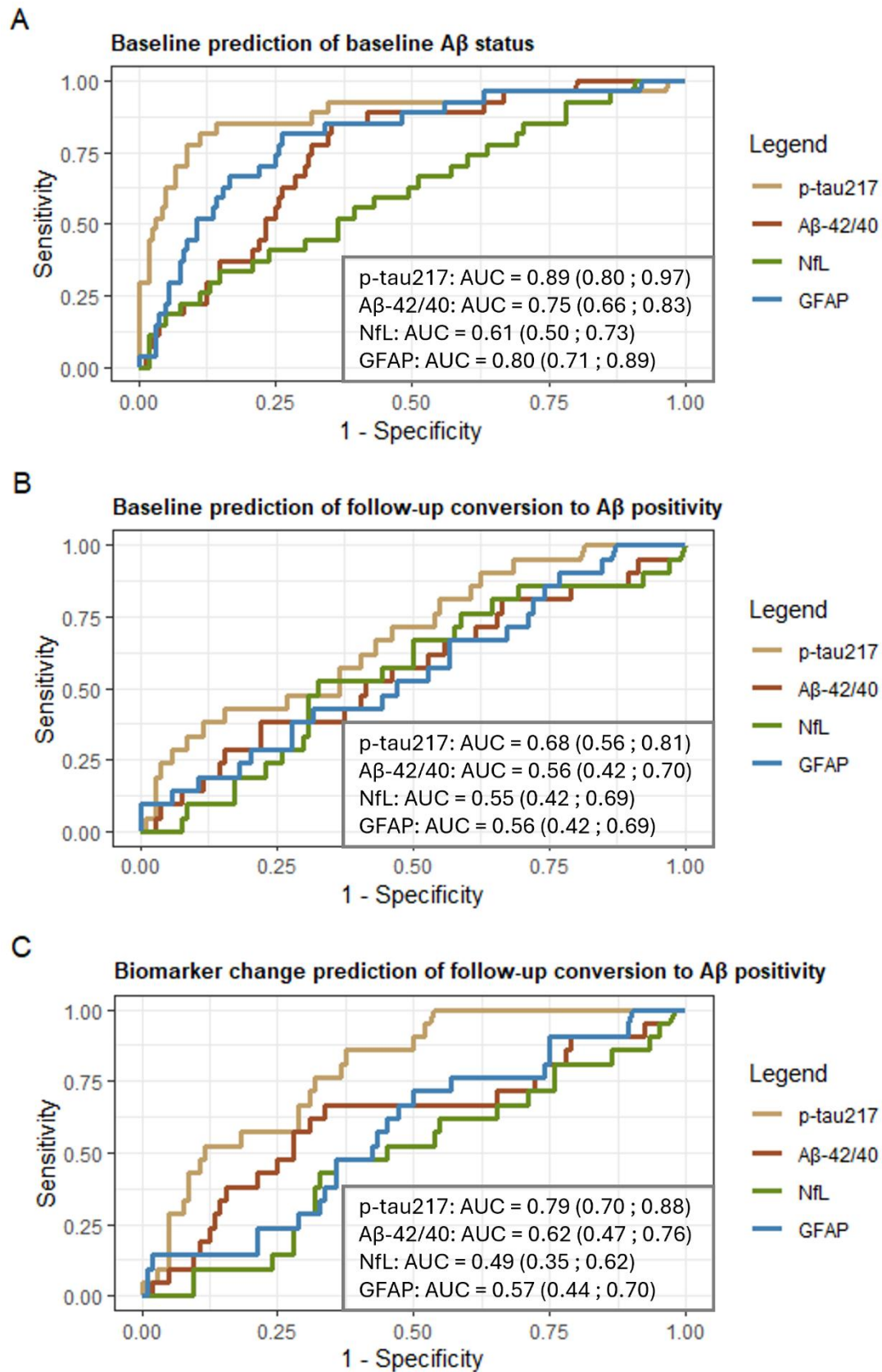


Figure 1. Performance of baseline and change in biomarker concentrations for discriminating between A β - and A β +. A = Performance of individual baseline biomarkers

783 for discriminating between baseline A β - and A β +. B = Performance of individual baseline
784 biomarkers for predicting conversion to A β at follow-up. C = Performance of individual
785 change in biomarker over time for predicting conversion to A β at follow-up. A β = Amyloid-
786 β . GFAP = Glial fibrillary acidic protein. NfL = Neurofilament light chain. P-tau217 =
787 phosphorylated-tau 217.
788

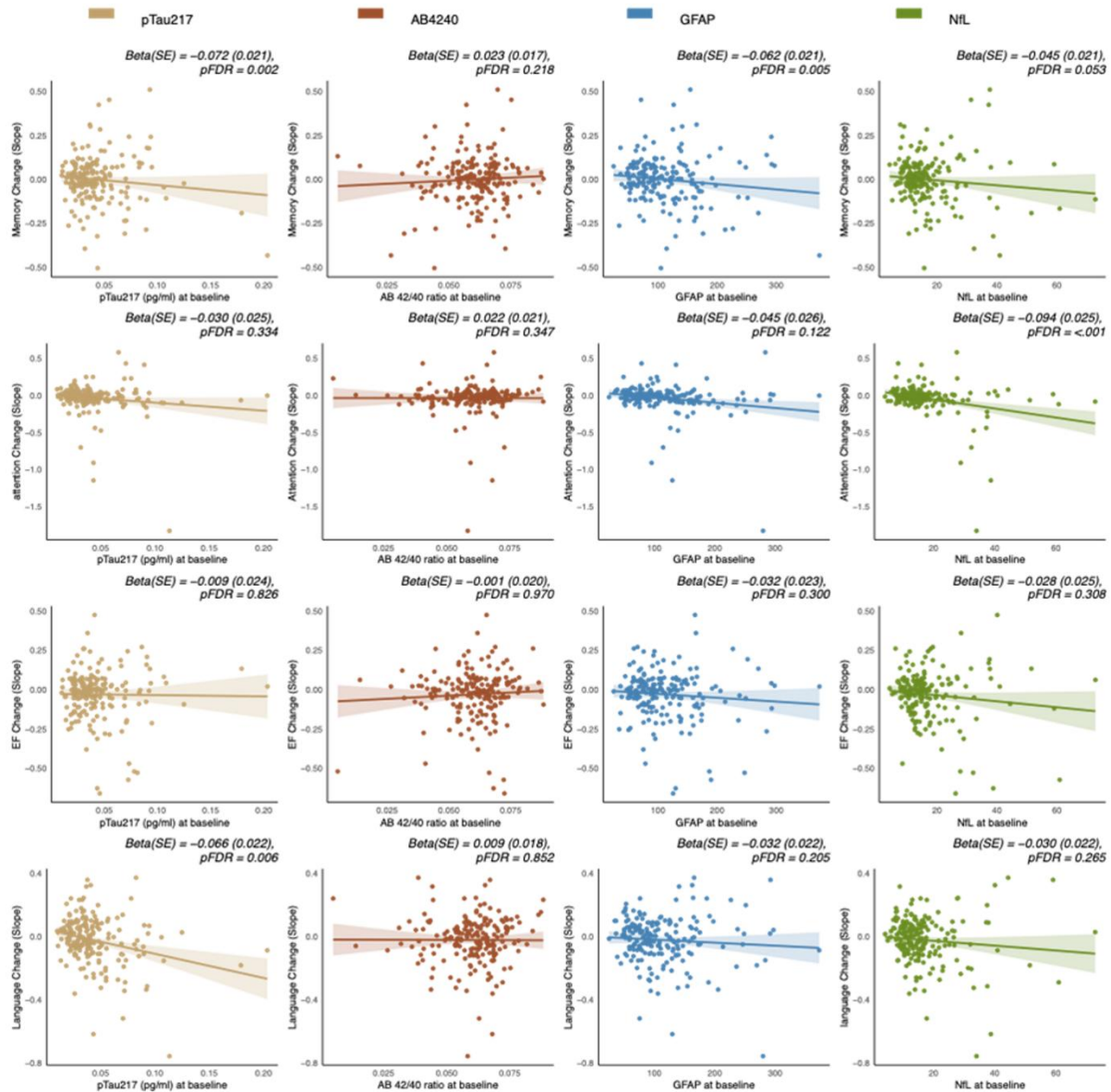


Figure 2. Relations between baseline plasma biomarkers and cognitive change over time.

Baseline biomarker concentrations are on the X axis, while individual cognitive slopes (representing the rate of cognitive change over time) are on the Y axis. The results displayed above each scatter plot are derived from a linear mixed-effects model testing the interaction between baseline plasma biomarker concentrations and time on cognitive composite scores. Models were adjusted for age, sex, and education. Abbreviations: A β , amyloid beta; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; pTau, 217-phosphorylated-tau.

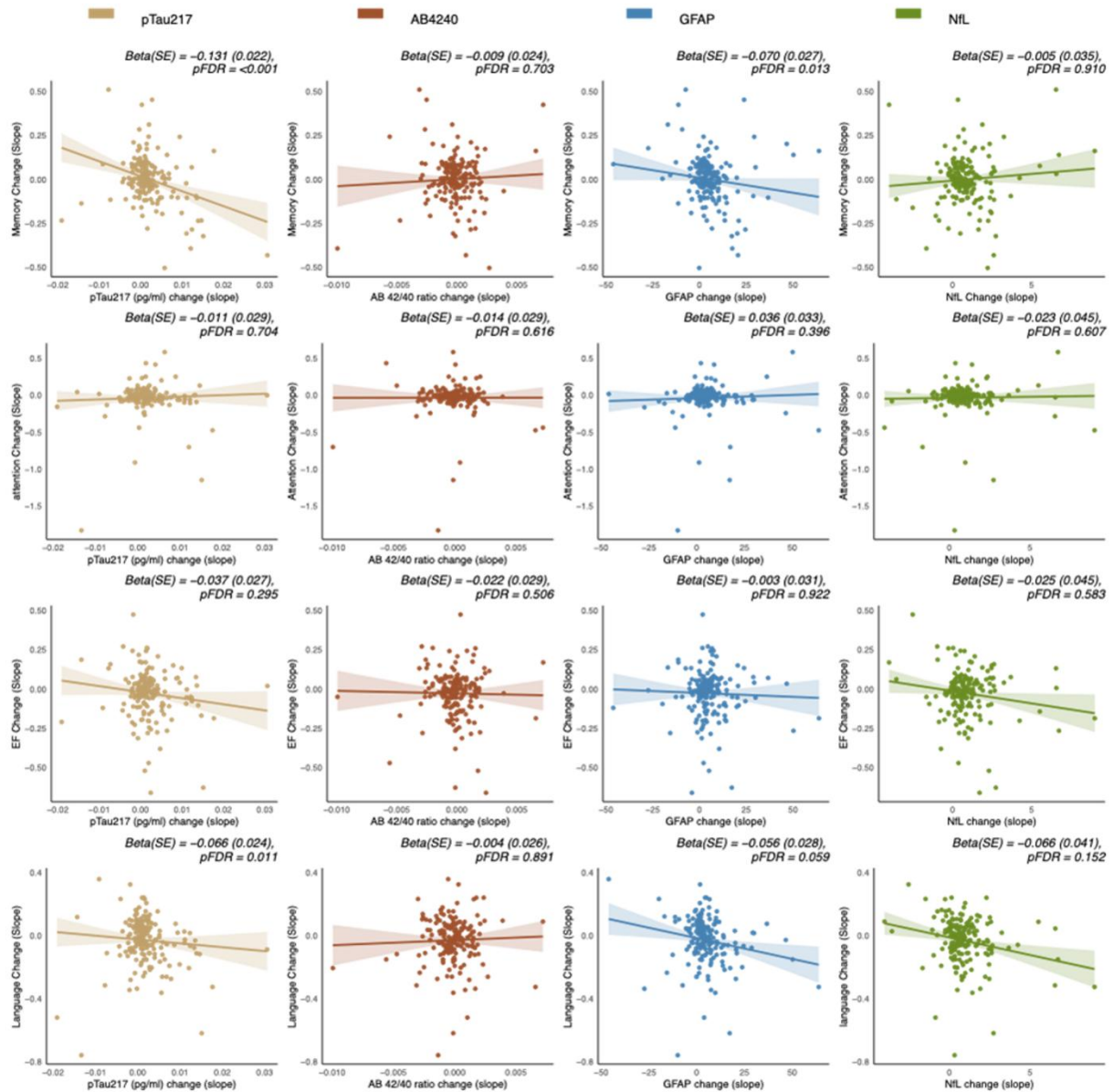
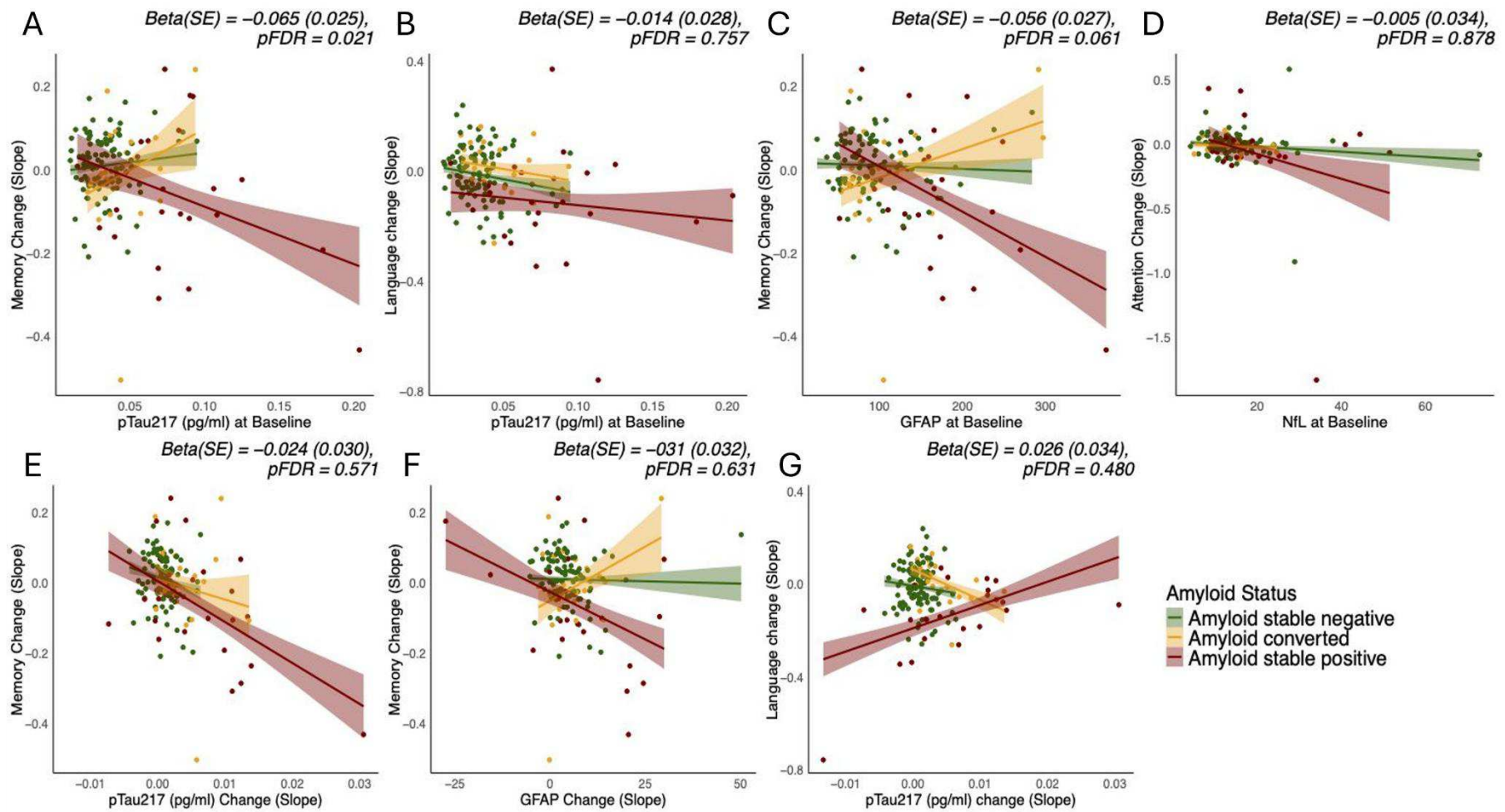


Figure 3. Relations between plasma biomarker change and cognitive change over time.

Individual biomarker slopes (representing the rate of concentration change over time) are on the X axis, while individual cognitive slopes (representing the rate of cognitive change over time) are on the Y axis. The results displayed above each scatter plot are derived from a linear mixed-effects model testing the interaction between the rate of plasma biomarker concentrations change over time and time on cognitive composite scores. Models were adjusted for age, sex, and education. Abbreviations: A β , amyloid beta; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; pTau, 217-phosphorylated-tau.



806

807 **Figure 4. Associations between baseline and change in biomarker concentrations and cognitive change stratified by Aβ status.** Baseline
 808 biomarker concentrations (A, B, C, D) or individual biomarker slopes (representing the rate of concentration change over time; E, F, G) are on

809 the X axis, while individual cognitive slopes (representing the rate of cognitive change over time) are on the Y axis. The results displayed above
810 each scatter plot are derived from a linear mixed-effects model testing the interaction between baseline plasma biomarker concentrations (A, B,
811 C, D) or rate of biomarker change over time (E, F, G), time, and amyloid- β PET status on cognitive composite scores. Models were adjusted for
812 age, sex, and education. Subjects are stratified into stable negatives ($A\beta^-$ at baseline and follow-up, in green), stable positives ($A\beta^+$ at baseline
813 and follow-up, in red) and converters ($A\beta^-$ at baseline and $A\beta^+$ at follow-up, in yellow). Abbreviations: $A\beta$, amyloid beta; GFAP, glial fibrillary
814 acidic protein; NfL, neurofilament light chain; pTau, 217-phosphorylated-tau.

815

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementsLongitudinalplasmabiomarkersinEMIF.docx](#)