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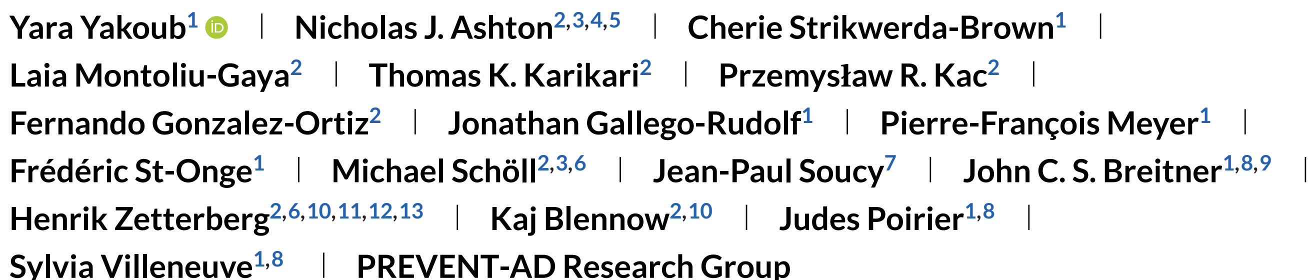
RESEARCH ARTICLE

Longitudinal blood biomarker trajectories in preclinical Alzheimer's disease



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Abstract

Introduction: Plasma biomarkers are altered years prior to Alzheimer's disease (AD) clinical onset.

Methods: We measured longitudinal changes in plasma amyloid-beta ($A\beta$)$_{42/40}$ ratio, pTau181, pTau231, neurofilament light chain (NfL), and glial fibrillary acidic protein (GFAP) in a cohort of older adults at risk of AD (n = 373 total, n = 229 with $A\beta$ and tau positron emission tomography [PET] scans) considering genetic and demographic factors as possible modifiers of these markers' progression.

Results: $A\beta_{42/40}$ ratio concentrations decreased, while NfL and GFAP values increased over the 4-year follow-up. Apolipoprotein E (*APOE*) ε4 carriers showed faster increase in plasma pTau181 than non-carriers. Older individuals showed a faster increase in

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plasma NfL, and females showed a faster increase in plasma GFAP values. In the PET subsample, individuals both Aβ-PET and tau-PET positive showed faster plasma pTau181 and GFAP increase compared to PET-negative individuals.

Discussion: Plasma markers can track biological change over time, with plasma pTau181 and GFAP markers showing longitudinal change in individuals with preclinical AD.

KEYWORDS
Alzheimer's disease, Aβ, biomarker, PET, plasma, pTau

Highlights

- Longitudinal increase of plasma pTau181 and glial fibrillary acidic protein (GFAP) can be measured in the preclinical phase of AD.
- Apolipoprotein E ε4 carriers experience faster increase in plasma pTau181 over time than non-carriers.
- Female sex showed accelerated increase in plasma GFAP over time compared to males.
- $A\beta_{42/40}$ and pTau231 values are already abnormal at baseline in individuals with both amyloid and tau PET burden.

1 | BACKGROUND

The clinical diagnosis of Alzheimer's disease (AD) dementia relies on clinical symptoms, while a definite diagnosis of AD can only be confirmed through *post mortem* examination.[1] Histopathological studies identified a series of non-demented cases in which individuals showed an accumulation of amyloid- and tau-related aggregates.[2,3] Recent advances in neuroimaging and fluid biomarkers allowed for in vivo identification of AD pathology.[1,4,5] As such, the National Institute on Aging/Alzheimer's Association (NIA–AA) defines AD as a biological construct that can be measured using amyloid and tau positron emission tomography (PET) or cerebrospinal fluid (CSF) biomarkers.[1] Based on this biological classification, cognitively unimpaired individuals with amyloid and tau (A+T+) are classified as having preclinical AD, individuals with only amyloid pathology (A+T–) are at risk of AD, and individuals with only tau (A–T+) or who are negative on both biomarkers (A–T–) are not on the AD continuum.[1,6] Neurodegeneration (N) measured using PET tracers for metabolic activity, magnetic resonance imaging (MRI), or CSF markers can be further used as an unspecific marker to stage disease severity. The cost and limited feasibility and availability of neuroimaging techniques, along with the invasive nature of lumbar puncture, have motivated the development of more cost-effective and minimally invasive tools to detect AD pathologies.

Among such developments, blood-based biomarkers offer versatility in targeting markers of amyloid-beta ($A\beta_{40}$, $A\beta_{42}$) and phosphorylated tau (pTau181, 231, 217) pathologies, as well as other features of AD pathology such as neuroaxonal damage (neurofilament light chain [NfL]) and reactive astrocytes (glial fibrillary acidic protein, [GFAP]).[7–10] These blood-based biomarkers are cost-effective and can be measured using a single assessment. Cross-sectional studies have shown their utility in the diagnostic evaluation of suspected AD and in identifying AD pathology in asymptomatic individuals.[11–14] However, the nature and implications of their dynamic changes over time are limited, especially in cognitively unimpaired persons at risk of AD.[15,16] Measurement of such changes in preclinical AD would have important implications in both research and preventive clinical trials. Our objectives were therefore to (1) assess the temporal trajectories of different AD blood biomarkers in cognitively unimpaired older adults with a first-degree family (mostly parental) history of AD dementia; (2) test the potential effects of genetic (apolipoprotein E [*APOE*]) and demographic (age, sex, and education) factors as modifiers of these trajectories; and (3) assess plasma biomarkers trajectories specifically in individuals having confirmed pathology (amyloid, or amyloid and tau) as demonstrated by PET.

2 | METHODS

2.1 | Participants

We included 373 participants from the Pre-symptomatic Evaluation of Experimental or Novel Treatments for Alzheimer's Disease (PREVENT-AD) cohort[17,18] from data release 6, of which 287 had more than one periodic evaluation. PREVENT-AD is an ongoing longitudinal

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observational study of cognitively normal older adults enrolled between 2011 and 2017, each with a self-reported parental history of AD or at least two siblings with AD dementia. Participants were cognitively unimpaired and 60+ years old at enrollment, or 55–59 if within 15 years of their youngest-affected relative's age at onset.[17] All participants underwent brief cognitive screening at study entry using the Clinical Dementia Rating (CDR) and Montreal Cognitive Assessment (MoCA) to assess normal cognition. In a few cases of ambiguous CDR (0.5) or MoCA (≤26), participants were evaluated by a certified neuropsychologist with a more extensive neuropsychological battery assessment. Furthermore, all participants underwent the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) at the baseline visit. Participants with scores below the expectation for their age and/or education were also evaluated by the neuropsychologist. They repeated the RBANS on a yearly basis, and performances that were lower than expected were reviewed in consensus group to assess the development of mild cognitive impairment (MCI). Information on *APOE* ε4 status, age, sex, and years of education were collected at entry into the program. Peripheral blood was drawn at baseline and up to four annual follow-up visits. All participants were cognitively unimpaired at the first blood visit. During the 4-year blood collection period, 12 individuals progressed to MCI. A subsample of 229 participants completed amyloid- and tau-PET scans within a range of 0 to 9 years [mean 5.52, SD 2.20] following initial blood collection. Written informed consent was obtained from all participants, and all research procedures were approved by the Institutional Review Board at McGill University.

RESEARCH IN CONTEXT

1. **Systematic review**: We reviewed the literature on Alzheimer's disease (AD) biofluid and neuroimaging using PubMed. A large body of evidence showed the promising value of plasma biomarkers in the disease's diagnosis. However, few studies evaluated the longitudinal trajectories of these biomarkers and the influence of genetic and demographic factors on these biomarkers' rates of change in preclinical AD.
2. **Interpretation**: We showed an increase in plasma pTau181 over time among *APOE* ε4 carriers compared to non-carriers in cognitively unimpaired older adults. Increasing age and female sex influence plasma NfL and GFAP rate of change over time. Additionally, we observed a faster increase in plasma pTau181 and GFAP among those with evidence of both pathologies on PET.
3. **Future directions**: Additional data with more diverse participants are needed to validate the longitudinal changes of plasma biomarkers and the impact of genetic and demographic factors before they are used in primary care and preventive trials.

2.2 | Plasma measures

Blood samples were available over a mean span of 2.25 (SD 1.56, range 1 to 4) years for the full cohort and 2.42 (SD 1.54, range 1 to 4) years for the PET sample. Plasma samples were taken on an annual basis. Additionally, 155 participants had a plasma sample available 3 months after their baseline measurement. Plasma pTau181 and pTau231 were analyzed using an in-house single-molecule array (Simoa) method developed at the University of Gothenburg.[11,12] NfL, GFAP, and $A\beta_{42/40}$ were analyzed using a commercial Simoa multiplex assay. Plasma samples were thawed, vortexed, and centrifuged (4000 × *g* for 10 min at RT), then analyzed by a HD-X analyzer using identical batches of reagents across the study. Three quality control plasma samples were added in duplicate to the test plates at the start and end of each run, resulting in an overall coefficient of variation of 4.9% to 12.5% across all the plasma marker measurements.

2.3 | PET acquisition and preprocessing

PET scans were performed at the McConnell Brain Imaging Centre of the Montreal Neurological Institute (Quebec, Canada). $A\beta$-PET images (^{18}F-NAV4694) were acquired 40 to 70 min after injection (dose injected ≈6 mCi). Tau-PET images (^{18}F-flortaucipir) were acquired 80 to 100 min after injection (dose injected ≈10 mCi). Frames of 5 min were acquired. An attenuation scan was also acquired. Images were reconstructed using a three-dimensional (3D) ordinary Poisson ordered subset expectation maximum ([OP-OSEM], 10 iterations, 16 subsets) algorithm. Images were also decay and motion corrected. Scatter correction was performed using a 3D scatter estimation method.[19] The MRI scan closest in time to the PET acquisition for each participant was chosen for the preprocessing of PET images. T1-weighted MRI images were parcellated into 34 bilateral regions of interest (ROIs) based on the Desikan-Killiany atlas using FreeSurfer version 5.3.[20] PET images were realigned, temporally averaged, and coregistered to the T1-weighted image, then masked to remove signal from the cerebrospinal fluid (CSF) and smoothed with a 6-mm^3 Gaussian kernel. Standardized uptake value ratios (SUVRs) were computed as the ratio of tracer uptake in the ROIs vs uptake in cerebellar gray matter for amyloid-PET scans or vs inferior cerebellar gray for tau-PET.[21,22] All PET scans were preprocessed using a standard pipeline (https://github.com/villeneuvelab/vlpp).

2.4 | Amyloid (A) and tau (T) uptake classification

Amyloid positivity relied on global neocortical amyloid-PET retention in the lateral and medial frontal, parietal, and lateral temporal regions (SUVR cut-off = 1.24; Centiloid cut-off = 18).[21,23] Tau-PET positivity

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was determined from entorhinal cortex flortaucipir binding[24] (SUVR cut-off = 1.25) exceeding 2 SDs from the mean of 11 healthy control individuals younger than 40 years. Using these thresholds (which differ from previous PREVENT-AD publications due to modifications of the scatter estimation method used during image reconstruction), 141 participants were classified as A−T−, 63 as A+T−, and 21 as A+T+. Four participants classified as A−T+ were excluded from the analyses. In a supplementary analysis, we classified our participants by amyloid-PET status only. Overall, 84 participants were classified as amyloid positive and 145 as amyloid negative.

2.5 | Statistical analyses

We compared the demographic characteristics of the full cohort and the PET subsample using Fisher''s tests and Kruskal–Wallis tests where appropriate.

In the full PREVENT-AD cohort analyses, we performed linear regression analyses using baseline plasma markers as the dependent variables and *APOE* ε4 status, demographic variables (age, sex, and years of education), and PET status as the independent variables to test for baseline difference in plasma biomarkers.

We then performed linear mixed-effects (LME) models in the full sample with random slopes and intercepts to assess the longitudinal rates of change in the plasma biomarkers $A\beta_{42/40}$ ratio, pTau181, pTau231, NfL, and GFAP [plasma biomarker ∼ time + (time|subject)]. To identify genetic (*APOE* ε4 status) or demographic (sex, age, and education) variables as potential modifiers of the plasma biomarkers' rate of change, we repeated the LME models with the previously cited variables as well as their interaction term. Each model was adjusted for age at baseline and sex variables as potential confounders. Thus, coefficients or interaction values of interest were, in all instances, adjusted for the remaining variables (e.g., [plasma biomarker ∼ time × *APOE* ε4 status + sex + age + (time|subject)]. In these models, plasma biomarkers were included as a dependent variable and the interaction was assessed between the variable of interest (e.g., *APOE* ε4 status) and time as the fixed effect. In the LME models, we entered years of education and age as continuous variables. The binary classification was used for visualization purposes only.

In the PET subsample analyses, we performed similar LME analyses to investigate plasma trajectories among individuals classified as A−T−, A+T−, and A+T+ (ignoring the A−T+ participants). Analyses in this PET subsample used the A−T− group as the reference group. Individual PET models included age at initial plasma collection, sex, and the time difference between the first plasma collection and PET visit. We also repeated the LME analyses in the PET subsample grouped based on amyloid-PET alone (A+ vs A−) for comparison with previous publications.

Two-sided p values ≤0.05 surviving multiple comparisons correction using false discovery rate (FDR) are considered significant and discussed. Extreme plasma values (> 12 median absolute deviations above the median[16]) were removed from the analyses (see supplementary material). The analyses were performed using R (version 4.1.2).

TABLE 1 Sample demographics

Measure	Full sample (n = 373)	PET subsample (n = 229)	p value
Baseline age, years	63.59 (5.13)	63.17 (4.58)	.61
Education, years	15.45 (3.40)	15.49 (3.25)	.65
MMSE score (/30)[a]	NA	28.71 (1.28)	NA
MoCA score (/30)	28.05 (1.55)	28.17 (1.52)	.93
Sex, F (%)	267 (71.58)	159 (69.43)	.64
APOE ε4 carriers, n (%)	144 (38.60)	92 (40.17)	.73
RBANS total score[b]	102.26 (10.58)	102.27 (10.11)	.97
Global amyloid SUVR	NA	1.29 (0.29)	NA
Tau entorhinal SUVR	NA	1.09 (0.14)	NA
Plasma $A\beta_{42/40}$	0.07 (0.01)	0.07 (0.01)	.90
Plasma pTau181 (pg/mL)[c]	6.63 (3.50)	6.78 (3.54)	.44
Plasma pTau231 (pg/mL)[d]	5.23 (2.87)	5.29 (2.85)	.75
Plasma GFAP (pg/mL)	95.31 (42.24)	95.24 (43.39)	.57
Plasma NfL (pg/mL)	15.25 (6.42)	15.57 (6.24)	.43

Data presented as mean (standard deviation), except for categorical variables where the count and percentage are presented. Fisher's exact or Kruskal–Wallis tests (where appropriate) were used to compare the demographic characteristics between the full cohort and the PET subsample. Age, RBANS total scores, and plasma data values are shown at baseline. MoCA scores were collected at entry into the program.
[a]MMSE scores were collected at PET visit and are only available for 154 participants.
[b]RBANS total score is standardized to an age range of 60-69.
[c]In the full sample, baseline pTau181 values were missing for 12 participants.
[d]pTau231 values were missing for 19 participants.
Abbreviations: APOE, apolipoprotein E; F, female; GFAP, glial fibrillary acidic protein; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; NA, not applicable; NfL, neurofilament light chain; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status; SUVR, standardized uptake value ratio.

3 | RESULTS

3.1 | Demographics

The characteristics of the full sample and the PET subsample are presented in Table 1. Overall, the study participants were 63.59 years old at baseline [range: 55.13 to 84.23] with 15.45 years of education on average [range: 7.00 to 29.00]; 71.58% were female, and 38.60% were *APOE* ε4 carriers.

3.2 | Baseline analyses

3.2.1 | Genetic and demographic factors association with baseline plasma measures

Comparing the baseline differences in plasma markers across *APOE* ε4 groups in the full sample, we found lower baseline plasma $A\beta_{42/40}$,

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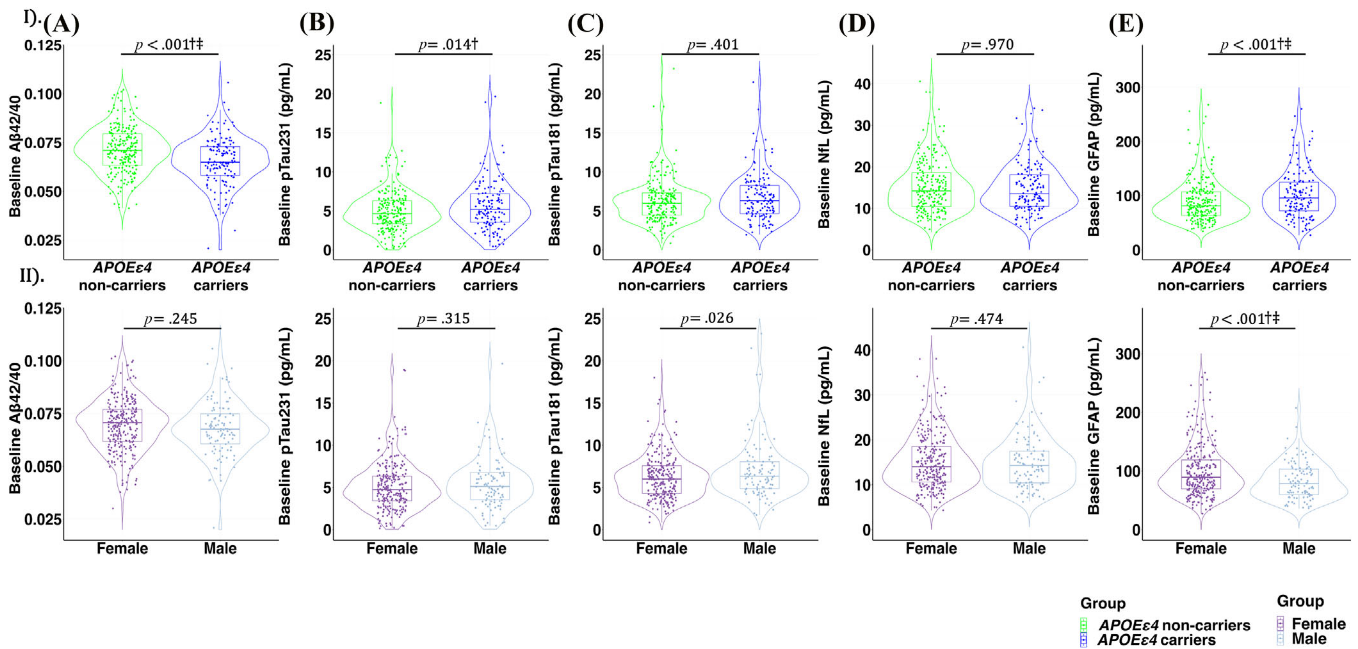

FIGURE 1 Distribution of baseline plasma markers across *APOE* and sex groups. Violin plots from linear regression analysis (I-II) showing baseline values of plasma markers $A\beta_{42/40}$, pTau231, pTau181, NfL, and GFAP across *APOE* and sex groups. (A) Plasma $A\beta_{42/40}$ ratio showed lower baseline values in *APOE* ε4 carriers vs *APOE* ε4 non-carriers; plasma $A\beta_{42/40}$ ratio showed no baseline differences between males and females. (B) Plasma pTau231 showed higher baseline values in *APOE* ε4 carriers vs *APOE* ε4 non-carriers; plasma pTau231 showed no baseline differences between males and females. (C, D) Plasma pTau181 and NfL showed no differences across *APOE* genotype or across sex. (E) Plasma GFAP showed higher baseline values in *APOE* ε4 carriers vs *APOE* ε4 non-carriers; plasma GFAP showed higher baseline values in females vs males. *Notes*: *APOE* models were adjusted for sex and age at baseline and sex models were adjusted for age at baseline. Uncorrected *p* values are presented; † = $p \leq .05$ surviving FDR adjustment (adjusted for the number of plasma markers = 5); ‡ = $p \leq .05$ surviving Bonferroni adjustment (adjusted for number of plasma markers = 5). Only findings that survived FDR adjustment are considered significant.

higher plasma pTau231, and higher GFAP values among *APOE* ε4 carriers compared with non-carriers ($\beta = -0.006$, $p < 0.001$, $R^2 = 0.067$, Figure 1IA; $\beta = 0.768$, $p = 0.014$, $R^2 = 0.023$, Figure 1IB; and $\beta = 16.886$, $p < 0.001$, $R^2 = 0.199$, Figure 1IE, respectively). We also observed higher baseline plasma GFAP levels in females ($\beta = 18.185$, $p < 0.001$, $R^2 = 0.164$, Figure 1IIE) than males. We found that older age was associated with higher NfL ($\beta = 0.445$, $p < 0.001$, $R^2 = 0.122$, Figure S1ID) and GFAP ($\beta = 3.037$, $p < 0.001$, $R^2 = 0.164$, Figure S1IE). We found no association between education and baseline plasma biomarkers (Figure S1II).

3.2.2 | Baseline plasma biomarkers differences across PET groups

The A+T+ group showed lower baseline values in plasma $A\beta_{42/40}$ ($\beta = -0.009$, $p = 0.002$, $R^2 = 0.051$, Figure 2A) compared to A−T− (reference group). The A+T+ group also had higher plasma pTau231 ($\beta = 1.632$, $p = 0.017$, $R^2 = 0.025$, Figure 2B) and NfL values ($\beta = 3.815$, $p = 0.006$, $R^2 = 0.105$, Figure 2D) compared to the A−T− group. Plasma GFAP concentrations were higher among individuals with A+T+ relative to both A−T− and A+T− groups ($\beta = 44.167$, $p < 0.001$; $\beta = 40.228$, $p < 0.001$, $R^2 = 0.195$, Figure 2E). When splitting the PET group only by amyloid status, we found lower plasma $A\beta_{42/40}$ ($\beta = -0.005$, $p = 0.002$, $R^2 = 0.044$, Figure S2A) and higher plasma GFAP in the A+ group compared to A− group ($\beta = 13.505$, $p = 0.016$, $R^2 = 0.133$, Figure S2E).

3.3 | Longitudinal analyses

3.3.1 | Longitudinal change of $A\beta_{42/40}$, pTau231, pTau181, NfL, and GFAP blood biomarkers in the full cohort

Longitudinal analyses in the full PREVENT-AD cohort showed a time-dependent decrease in plasma $A\beta_{42/40}$ values ($\beta = -0.0005$, $p = 0.001$, $R^2 = 0.020$) and an increase in plasma pTau181, NfL, and GFAP levels over time ($\beta = 0.008$, $p = 0.031$, $R^2 = 0.033$; $\beta = 0.042$, $p < 0.001$, $R^2 = 0.165$; $\beta = 0.342$, $p < 0.001$, $R^2 = 0.152$). No longitudinal rate of change was observed in plasma pTau231 in the full cohort.

3.3.2 | Genetic and demographic factors association with longitudinal plasma measures

We observed a faster increase in plasma pTau181 over time among *APOE* ε4 carriers compared with non-carriers ($\beta = 0.031$, $p < 0.001$, $R^2 = 0.146$, Figure 3C). No other group difference was observed. We

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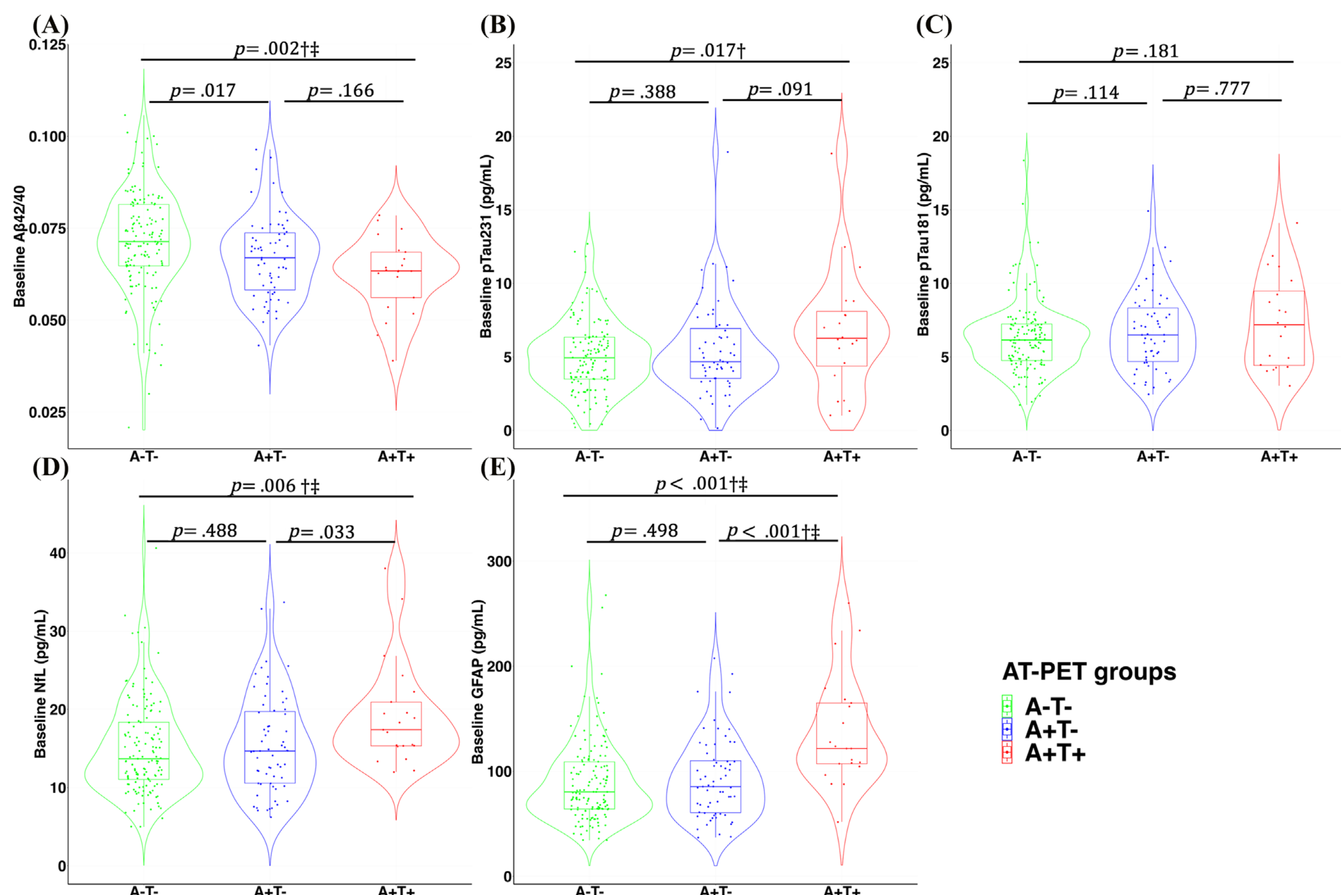

FIGURE 2 Distribution of baseline plasma markers stratified by pathological groups assessed by PET. Violin plots from linear regression analysis (A–E) showing baseline values of plasma markers $A\beta_{42/40}$, pTau231, pTau181, NfL, and GFAP across AT groups using PET scans. (A) Plasma $A\beta_{42/40}$ ratio showed lower baseline values in A+T+ when compared to A−T− reference group. (B) Plasma pTau231 showed higher baseline values in A+T+ vs A−T− group. (C) Plasma pTau181 showed no baseline differences in AT groups. (D) Plasma NfL showed higher baseline values in A+T+ group compared to A−T− group. (E) Plasma GFAP showed higher baseline values in A+T+ compared to both A+T− and A−T− groups. *Notes*: All models were adjusted for sex, age at baseline, and the time difference between initial blood collection and PET scans. Uncorrected *p* values are presented; † = $p \leq .05$ surviving FDR adjustment (adjusted for number of plasma markers = 5); ‡ = $p \leq .05$ surviving Bonferroni adjustment (adjusted for number of plasma markers = 5). Only findings that survived FDR adjustment are considered significant.

also investigated the potential role of demographic variables (age, education, and sex) on the plasma markers, and observed a faster increase in NfL levels over time with older age ($\beta = 0.001$, $p = 0.010$, $R^2 = 0.177$, Figure 4ID). Finally, we found an interaction between sex and time in plasma GFAP levels, suggesting that females showed faster increase in GFAP levels compared with males ($\beta = 0.321$, $p = 0.001$, $R^2 = 0.170$, Figure 4IIE). Years of education did not influence plasma markers' rates of change.

3.3.3 | Longitudinal plasma markers across PET groups

In the subsample of 229 individuals with PET scans we found a faster rate of change in plasma pTau181 and GFAP among those showing both amyloid and tau-PET (A+T+) pathology ($\beta = 0.047$, $p = 0.009$, $R^2 = 0.061$, Figure 5C; $\beta = 0.400$, $p = 0.020$, $R^2 = 0.203$, Figure 5E, respectively) when compared to those classified as negative on their PET biomarkers (i.e., the A−T− group). We found no time × group interaction in plasma $A\beta_{42/40}$, pTau231, or NfL markers. We repeated the analysis comparing longitudinal changes of plasma biomarkers across amyloid-PET groups only and found no slope differences between A+ and A− groups (Figure S3).

4 | DISCUSSION

We assessed temporal trajectories of pathological and neurodegenerative plasma biomarkers for AD and neurodegeneration in a longitudinal cohort of 373 cognitively unimpaired individuals with up to 4 years of plasma marker follow-up. We examined differences associated with *APOE* ε4 carrier status and with various demographic variables. In a subsample of 229 who had undergone ^{18}F-NAV4694 and ^{18}F-flortaucipir PET scans, we further examined the longitudinal differences between these markers in persons having various profiles of amyloid- and tau-PET positivity. Our overall intent was to show

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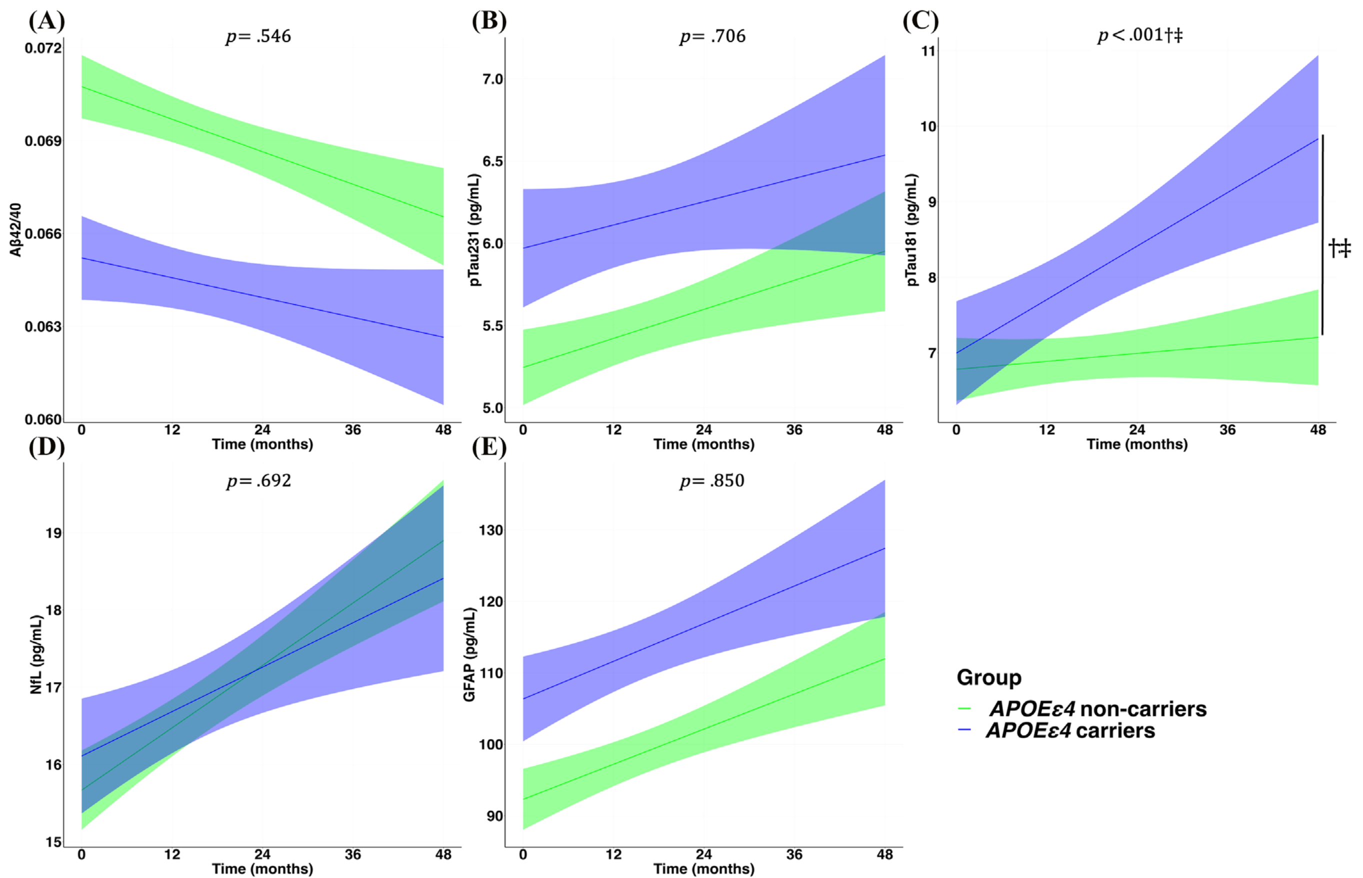

FIGURE 3 Longitudinal plasma trajectories stratified by *APOE* ε4 status. Linear mixed model analyses (A–E) showing slopes of plasma markers $A\beta_{42/40}$, pTau231, pTau181, NfL, and GFAP across *APOE* groups. *Notes*: The *x*-axis shows time from first plasma sample. Shaded areas represent 95% confidence intervals. All *p* values are from the interaction between time and *APOE* ε4 status as independent variables adjusted for sex and age at baseline. Uncorrected two-sided *p* values are presented; † = $p \leq .05$ surviving FDR adjustment (adjusted for number of plasma markers = 5); ‡ = $p \leq .05$ surviving Bonferroni adjustment (adjusted for number of plasma markers = 5). Only findings that survived FDR adjustment are considered significant.

differences, where apparent, in the dynamic trajectories of the plasma markers in the preclinical phase of AD and, thus, to suggest their utility for tracking disease progression in preventive trial applications targeting cognitively unimpaired individuals. Our most important results were faster longitudinal increase in plasma pTau181 and GFAP markers in those with both amyloid and tau pathologies on PET. Notably, these results were not found when only splitting participants by amyloid-PET status, suggesting that the presence of tau is driving these associations. Additionally, we observed a faster increase in plasma pTau181 across *APOE* ε4 carriers compared with non-carriers and accelerated increase in plasma GFAP levels in females compared to males. While no longitudinal changes were observed in plasma $A\beta_{42/40}$ and pTau231 in the full cohort, baseline $A\beta_{42/40}$ values were lower and pTau231 values were higher in individuals with amyloid and tau-PET positivity, suggesting that these markers might already have reached a plateau by the time individuals entered the preclinical phase of the disease.

Neuropathological studies have validated the use of amyloid and tau-PET imaging to stage disease progression in vivo. PET also has face validity in the tracking of pathological progression of the two specific proteins. Therefore, imaging biomarkers are now used increasingly in clinical care and therapeutic trials mostly among symptomatic patients. These markers are also valuable in identifying individuals for secondary preventive trials targeting individuals who are biomarker positive for AD.[25] Particularly in the latter application, however, the quantification and characterization of such markers in plasma would be less expensive and more practical. Among plasma biomarkers of interest, plasma levels of $A\beta_{42/40}$ ratio and pTau are thought to reflect the key pathological hallmarks of AD.[7,26,27] Plasma NfL, by contrast, is an apparent marker of axonal degeneration not specific to AD, but nonetheless potentially useful for staging of AD severity.[8,27,28] As well, plasma GFAP is a marker of glial activation that is not specific to AD but elevated in AD and other neurodegenerative diseases.[29–31] Using PET to classify individuals based on the NIA–AA criteria, we found that individuals with preclinical AD (A+T+ based on PET scans) showed faster longitudinal increase in plasma pTau181 and GFAP markers when compared to individuals free of significant AD pathology (A–T– based on PET scans). These results were not found when only splitting the groups by amyloid-PET status (A+ vs A–). These findings are similar to those in a recently published study by Ashton and colleagues, which found no difference between individuals with and without amyloid-PET binding

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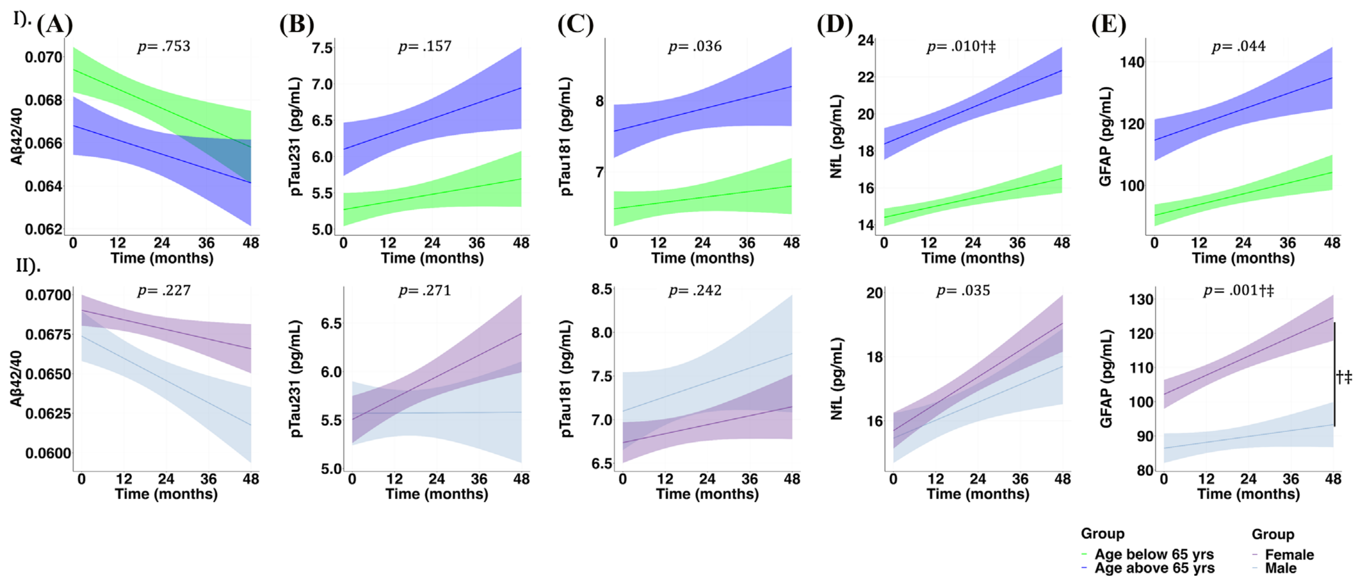

FIGURE 4 Longitudinal plasma trajectories' association with age and sex. Linear mixed model analyses (A–E) showing plasma markers A$\beta_{42/40}$, pTau231, pTau181, NfL, and GFAP slope association with age (I) and sex (II). *Notes*: The *x*-axis shows time from first plasma sample. Shaded areas represent 95% confidence intervals. (I) *p* values are from the interaction between time and age adjusted for sex (age was entered as a continuous variable; individuals were only grouped for representation purposes). (II) *p* values are from the interaction between time and sex adjusted for age at baseline. Uncorrected two-sided *p* values are presented; † = $p \leq .05$ surviving FDR adjustment (adjusted for number of plasma markers = 5); ‡ = $p \leq .05$ surviving Bonferroni adjustment (adjusted for number of plasma markers = 5). Only findings that survived FDR adjustment are considered significant.

when correcting for multiple comparisons.[32] In this last study, they only found group differences between A+ and A− when using pTau217, a promising new plasma marker that we unfortunately do not yet have access to.

By contrast, perhaps surprisingly, the A$\beta_{42/40}$ ratio showed no evidence of faster decrease in individuals having PET evidence of AD-related pathology (A+T+). However, we observed reduced baseline values of plasma A$\beta_{42/40}$ among A+T+ individuals. Higher baseline plasma levels in the A+T− group compared to the A−T− group, but no acceleration in increase over time, was also found with the pTau231 marker. These findings might indicate that plasma A$\beta_{42/40}$ and pTau231 have already reached a plateau in their acceleration rate by the time both pathologies are detected on PET scans.[32] CSF Aβ and tau biomarkers have been hypothesized to detect pathological changes earlier than PET biomarkers.[33,34] Similarly, plasma A$\beta_{42/40}$ ratio may capture early forms of Aβ, occurring prior to plaques,[26,35] and plasma pTau231 phospho-form may characterize the early stages of abnormal tau processing, occurring mainly before the formation of neurofibrillary tangles.[36,37] It is plausible, therefore, that plasma A$\beta_{42/40}$ ratio and pTau231 begin to change prior to PET positivity and that their rate of change slows when pathologies are detected on a PET scan. GFAP was also higher at baseline among individuals with preclinical AD (A+T+) as compared with A−T− reference group. These findings suggest that glial activation starts early in the disease process.[38,39]

Other observations include higher baseline GFAP values in females compared to males, which is similar to a previous study that reported higher plasma GFAP levels in cognitively unimpaired females.[31,40] Of interest, females also showed faster increase in plasma GFAP compared to males. In addition, we observed higher values in plasma NfL at older ages. The findings corroborate previous work that demonstrated an age-related increase in plasma NfL levels over time.[9,41] Finally, the A$\beta_{42/40}$ ratio values were lower, while pTau231 and GFAP values were higher at baseline in *APOE* ε4 carriers when compared to non-carriers, supported by earlier onset of AD in this genetically at-risk group. Faster plasma pTau181 increase among *APOE* ε4 carriers compared to non-carriers was also found. These last findings corroborate a previous study that suggested a significant role of the ε4 allele on the elevated levels of plasma pTau181 in both cross-sectional and longitudinal data prior to dementia onset.[42]

A principal strength of our study is its reliance on a unique enriched longitudinal observational cohort of cognitively unimpaired older adults with a family history of AD dementia, which is known to increase the risk of subsequent dementia by two- to fourfold.[43,44] Also, a subset of the cohort had PET assessments that allowed us to identify individuals with preclinical AD assessed by both amyloid and tau scans. All discussed results also survived a FDR correction, and most of them further survived a more stringent Bonferroni correction. A limitation of our study is that not all participants underwent PET imaging. Another weakness is the unavailability of plasma pTau217 measures, which is a promising marker showing high accuracy in detecting amyloid and tau PET pathology.[32] Furthermore, our Aβ_{42} and Aβ_{40} plasma values were analyzed using a Simoa platform, whereas mass spectrometry is considered the gold standard method in testing plasma Aβ.[45,46] The time between the plasma measurements and the PET scans also varied between individuals, with PET always performed after or at the

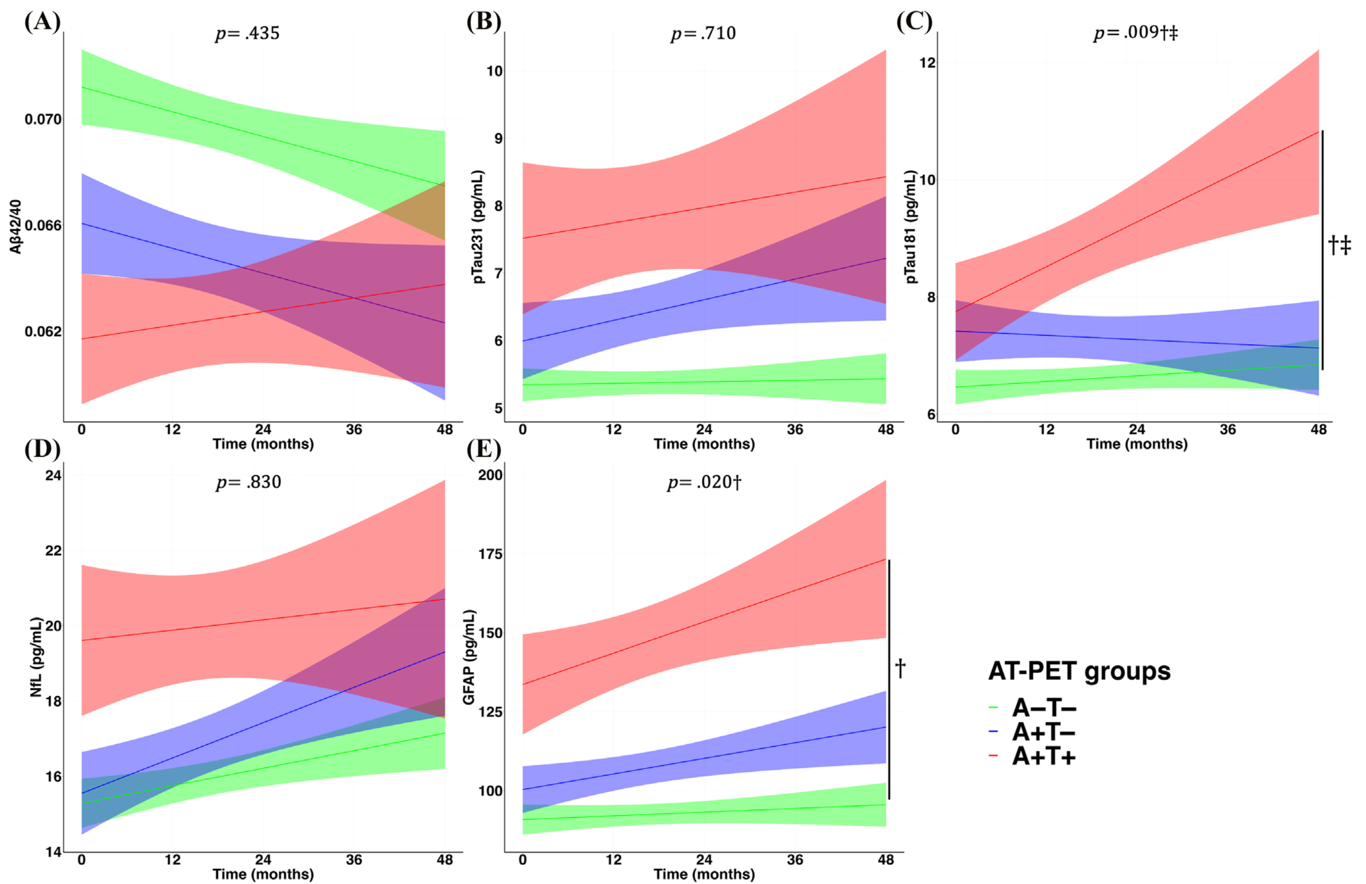

FIGURE 5 Longitudinal plasma trajectories stratified by pathological groups assessed by PET. Linear mixed models (A–E) showing plasma A$\beta_{42/40}$, pTau231, pTau181, NfL, and GFAP across the three PET groups classified as A−T−, A+T−, and A+T+. *Notes*: The *x*-axis shows time from first plasma sample. Shaded areas represent 95% confidence intervals. All *p* values are from the interaction between time and AT-PET groups as independent variables adjusted for sex, age at baseline, and the time difference between initial blood collection and PET scans. Uncorrected two-sided *p* values are presented; $\dagger = p \leq .05$ surviving FDR adjustment (adjusted for number of plasma markers = 5); $\ddagger = p \leq .05$ surviving Bonferroni adjustment (adjusted for number of plasma markers = 5). Only findings that survived FDR adjustment are considered significant.

last blood collection. Given the size and the asymptomatic nature of our cohort, we did not include neurodegeneration (N) as part of our A/T classification scheme. As in most such studies, some missing data and loss of follow-up make the results vulnerable to selection bias. While we infer linearity in our longitudinal data, it is possible that some biomarkers have more complex profiles that were not detected and that some relationships can only be detected over longer follow-up periods.[47] As well, most of our participants are Caucasian, leaving unanswered the generalizability of our results to other ethnic and racial groups. Replicating the results in a larger independent and more diverse dataset is required before making firm conclusions regarding the prognostic utility of these markers. Finally, we did not consider the role of comorbidities such as liver and kidney diseases when evaluating the performance of blood biomarkers. For instance, GFAP is expressed in the stellate cells of the liver in addition to the astrocytes of the central nervous system, which may impact the interpretation of our biomarker measurements.[48,49]

As we move toward precision medicine, diagnostic and prognostic biomarkers are evolving, with several reports evaluating the ability of these markers to distinguish AD from other causes of dementia, identify individuals with early stages of the disease, and predict the rate of cognitive decline.[50,51] Examining biomarkers' ability to monitor the disease progression helps to determine their potential utility in monitoring disease progression in response to therapy.[52] In the context of AD clinical trials, using blood biomarkers as screening tools or secondary outcomes requires examining and verifying the ability of these markers to monitor the disease state. Our study suggests an early decrease in A$\beta_{42/40}$ and an early increase in pTau231 and GFAP, earlier than other variants, including pTau181. We also found that pTau181 and GFAP increased over time among individuals with both amyloid and tau pathology on PET.

Taken together, we showed that different plasma markers have different dynamic trajectories over time across A/T profiles, with plasma pTau181 and GFAP showing an increased rate of change across individuals having both Aβ and tau pathologies. We also identified several demographic factors with the potential to influence plasma rate of change in cognitively unimpaired older adults at risk of AD dementia. Specifically, genetic status (*APOE* ε4 positivity), older age and female sex may have relevance for the longitudinal dynamic patterns of plasma levels of pTau181 and GFAP levels. The emergence

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of these findings among a high-risk but cognitively unimpaired cohort may have particular importance.

AUTHOR CONTRIBUTIONS

Yara Yakoub, Nicholas J. Ashton, Thomas K. Karikari, Michael Schöll, Judes Poirier, John C. S. Breitner, Henrik Zetterberg, Kaj Blennow, and Sylvia Villeneuve contributed to the study concept and design. Yara Yakoub, Nicholas J. Ashton, Thomas K. Karikari, Laia Montoliu-Gaya, Przemysław R. Kac, Fernando Gonzalez-Ortiz, and Jean-Paul Soucy contributed to data acquisition and analysis. Yara Yakoub, Cherie Strikwerda-Brown, Frédéric St-Onge, Jonathan Gallego-Rudolf, Pierre-François Meyer, and Sylvia Villeneuve drafted the manuscript and figures.

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CONFLICT OF INTEREST STATEMENT

H.Z. has served on scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Passage Bio, Pinteon Therapeutics, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a cofounder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). KB has served as a consultant, on advisory boards, or on data monitoring committees for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Ono Pharma, Pharmatrophix, Prothena, Roche Diagnostics, and Siemens Healthineers and is a cofounder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. Y.Y., N.J.A., T.K.K., M.S., J.P., J.C.S.B., L.M.G., P.R.K., F.G.O., J.P.S., C.S.B., F.S.O., J.R.G., P.F.M., and S.V. declare no conflicts of interest. Author disclosures are available in the supporting information.

DATA AVAILABILITY STATEMENT

Data used in the preparation of this manuscript were obtained from the Pre-symptomatic Evaluation of Experimental or Novel Treatments for Alzheimer's Disease (PREVENT-AD). Some of the data are publicly available (https://openpreventad.loris.ca and https://registeredpreventad.loris.ca), and the remaining data can be shared upon approval by the scientific committee at the Centre for Studies on Prevention of Alzheimer's Disease (StoP-AD) at the Douglas Mental Health University Institute. A complete listing of PREVENT-AD investigators can be found at https://preventad.loris.ca/acknowledgements/acknowledgements.php?date=2023-03-23.

CONSENT STATEMENT

Written informed consent was obtained from all participants, and all research procedures were approved by the Institutional Review Board at McGill University.

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