

# Differences Between Plasma and Cerebrospinal Fluid Glial Fibrillary Acidic Protein Levels Across the Alzheimer Disease Continuum

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 Supplemental content

**IMPORTANCE** Glial fibrillary acidic protein (GFAP) is a marker of reactive astrogliosis that increases in the cerebrospinal fluid (CSF) and blood of individuals with Alzheimer disease (AD). However, it is not known whether there are differences in blood GFAP levels across the entire AD continuum and whether its performance is similar to that of CSF GFAP.

**OBJECTIVE** To evaluate plasma GFAP levels throughout the entire AD continuum, from preclinical AD to AD dementia, compared with CSF GFAP.

**DESIGN, SETTING, AND PARTICIPANTS** This observational, cross-sectional study collected data from July 29, 2014, to January 31, 2020, from 3 centers. The Translational Biomarkers in Aging and Dementia (TRIAD) cohort (Montreal, Canada) included individuals in the entire AD continuum. Results were confirmed in the Alzheimer's and Families (ALFA+) study (Barcelona, Spain), which included individuals with preclinical AD, and the BioCogBank Paris Lariboisière cohort (Paris, France), which included individuals with symptomatic AD.

**MAIN OUTCOMES AND MEASURES** Plasma and CSF GFAP levels measured with a Simoa assay were the main outcome. Other measurements included levels of CSF amyloid- $\beta$  42/40 (A $\beta$ 42/40), phosphorylated tau181 (p-tau181), neurofilament light (NfL), Chitinase-3-like protein 1 (YKL40), and soluble triggering receptor expressed on myeloid cells 2 (sTREM2) and levels of plasma p-tau181 and NfL. Results of amyloid positron emission tomography (PET) were available in TRIAD and ALFA+, and results of tau PET were available in TRIAD.

**RESULTS** A total of 300 TRIAD participants (177 women [59.0%]; mean [SD] age, 64.6 [17.6] years), 384 ALFA+ participants (234 women [60.9%]; mean [SD] age, 61.1 [4.7] years), and 187 BioCogBank Paris Lariboisière participants (116 women [62.0%]; mean [SD] age, 69.9 [9.2] years) were included. Plasma GFAP levels were significantly higher in individuals with preclinical AD in comparison with cognitively unimpaired (CU) A $\beta$ -negative individuals (TRIAD: A $\beta$ -negative mean [SD], 185.1 [93.5] pg/mL, A $\beta$ -positive mean [SD], 285.0 [142.6] pg/mL; ALFA+: A $\beta$ -negative mean [SD], 121.9 [42.4] pg/mL, A $\beta$ -positive mean [SD], 169.9 [78.5] pg/mL). Plasma GFAP levels were also higher among individuals in symptomatic stages of the AD continuum (TRIAD: CU A $\beta$ -positive mean [SD], 285.0 [142.6] pg/mL, mild cognitive impairment [MCI] A $\beta$ -positive mean [SD], 332.5 [153.6] pg/mL; AD mean [SD], 388.1 [152.8] pg/mL vs CU A $\beta$ -negative mean [SD], 185.1 [93.5] pg/mL; Paris: MCI A $\beta$ -positive, mean [SD], 368.6 [158.5] pg/mL; AD dementia, mean [SD], 376.4 [179.6] pg/mL vs CU A $\beta$ -negative mean [SD], 161.2 [67.1] pg/mL). Plasma GFAP magnitude changes were consistently higher than those of CSF GFAP. Plasma GFAP more accurately discriminated A $\beta$ -positive from A $\beta$ -negative individuals than CSF GFAP (area under the curve for plasma GFAP, 0.69-0.86; area under the curve for CSF GFAP, 0.59-0.76). Moreover, plasma GFAP levels were positively associated with tau pathology only among individuals with concomitant A $\beta$  pathology.

**CONCLUSIONS AND RELEVANCE** This study suggests that plasma GFAP is a sensitive biomarker for detecting and tracking reactive astrogliosis and A $\beta$  pathology even among individuals in the early stages of AD.

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**Group Information:** The members of the Translational Biomarkers in Aging and Dementia (TRIAD) study, Alzheimer's and Families (ALFA) study, and BioCogBank Paris Lariboisière cohort are listed in Supplement 2.

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The rapid advancements in the development of blood biomarkers to accurately detect Alzheimer disease (AD) point to a prompt application of these biomarkers in clinical routine and clinical trials. This application is especially true for individuals with preclinical AD, as scalable and less invasive biomarkers are needed to screen large populations of cognitively unimpaired (CU) individuals to test innovative interventions.

Currently, the most promising blood biomarkers for detecting AD are the phosphorylated tau species (p-tau)<sup>1-6</sup> and amyloid- $\beta$  42/40 (A $\beta$ 42/40) ratio.<sup>7-12</sup> However, it is still desirable to have more sensitive blood biomarkers for preclinical AD. Alzheimer disease pathology is associated with morphologic, molecular, and functional remodeling of astrocytes, a process termed *reactive astrogliosis*.<sup>13,14</sup> However, few astrocyte imaging and fluid biomarkers have been investigated.<sup>15</sup> Blood levels of glial fibrillary acidic protein (GFAP), a reactive astrogliosis biomarker, are higher in individuals with preclinical AD, constituting a promising candidate biomarker for this early stage of the disease.<sup>16</sup> A recent meta-analysis demonstrated that GFAP levels were consistently altered in the cerebrospinal fluid (CSF) of symptomatic patients with AD, but studies of blood GFAP present relatively high variability.<sup>17</sup>

It is not yet well known how plasma GFAP levels change across the overall AD continuum and whether GFAP concentrations in CSF and blood reflect the same pathologic processes because reactive astrocytes assume multiple states—the so-called astrocyte heterogeneity. Thus, our main aim was to evaluate the levels of plasma GFAP throughout the AD continuum and compare them with the levels of CSF GFAP, with particular attention to preclinical AD. We hypothesized that plasma GFAP levels are already higher early in the preclinical stage and further elevated in symptomatic stages.

## Methods

### Study Population

This cross-sectional study, which included participants from 3 cohorts, collected data from July 29, 2014, to January 31, 2020. The Translational Biomarkers in Aging and Dementia (TRIAD) cohort (Montreal, Canada)<sup>18</sup> comprised 300 individuals (177 women [59.0%]; mean [SD] age, 64.6 [17.6] years), including young CU adults, elderly CU adults, individuals with mild cognitive impairment (MCI), and patients with AD dementia. The ALFA+ cohort (Barcelona, Spain),<sup>19</sup> which is a nested study of the ALFA (for Alzheimer's and Families) study, included 384 middle-aged CU individuals (234 women [60.9%]; mean [SD] age, 61.1 [4.7] years) at elevated risk for AD. The BioCogBank Paris Lariboisière cohort (Paris, France)<sup>20</sup> included 166 patients with cognitive disorders from the Center of Cognitive Neurology, Lariboisière Hospital, as well as 21 CU individuals. In addition to clinical classification (CU, MCI, and dementia), participants were categorized according to A $\beta$  status (A $\beta$ -positive [A $\beta$ +] and A $\beta$ -negative [A $\beta$ -]), defined by results of A $\beta$  positron emission tomography (PET) in TRIAD and the CSF A $\beta$ 42/40 ratio in ALFA+ and Paris, if not otherwise specified. ALFA+ participants were also classified using the AT

### Key Points

**Question** What are the levels of plasma glial fibrillary acidic protein (GFAP) throughout the Alzheimer disease (AD) continuum, and how do they compare with the levels of cerebrospinal fluid (CSF) GFAP?

**Findings** In this cross-sectional study, plasma GFAP levels were elevated in the preclinical and symptomatic stages of AD, with levels higher than those of CSF GFAP. Plasma GFAP had a higher accuracy than CSF GFAP to discriminate between amyloid- $\beta$  (A $\beta$ )-positive and A $\beta$ -negative individuals, also at the preclinical stage.

**Meaning** This study suggests that plasma GFAP is a sensitive biomarker that significantly outperforms CSF GFAP in indicating A $\beta$  pathology in the early stages of AD.

(A $\beta$  and tau pathology) classification.<sup>21,22</sup> Participants with non-AD dementia (frontotemporal dementia [FTD] or dementia with Lewy bodies) from the TRIAD and Paris cohorts were included for supplementary analysis. All studies have been approved by their regional ethical committees (TRIAD: McGill University and Douglas Hospital Research Centre institutional review boards; ALFA+: Independent Ethics Committee “Parc de Salut Mar,” Barcelona; and Paris Cohort: Bichat Ethics Committee), and all participants provided written informed consent. Additional details of the 3 cohorts are reported in the eMethods in [Supplement 1](#).

### Fluid and Neuroimaging Biomarkers

Plasma and CSF samples from the 3 cohorts were independently analyzed at the Clinical Neurochemistry Laboratory, University of Gothenburg, Gothenburg, Sweden. Plasma and CSF GFAP levels were quantified for all cohorts on the Simoa HD-X (Quanterix) using the commercial single-plex assay (No. 102336). A comprehensive description of the fluid and neuroimaging biomarker measurements can be found in the eMethods in [Supplement 1](#).

### Statistical Analysis

We used linear regression models to assess the association between plasma or CSF GFAP levels and the other biomarkers. Similar models were applied to evaluate group differences and associations with age and sex; the Tukey honestly significant difference test was used for post hoc pairwise comparisons. Fold changes and the effect size of the differences (estimated with Cohen *d*) were calculated using A $\beta$ - CU (CU-) individuals (TRIAD and Paris) and A $\beta$ - and tau- (A-T-) individuals or A $\beta$ - individuals (ALFA+) as reference groups. All analyses were adjusted for age and sex if not otherwise specified. The Spearman rank test was used for correlations using raw biomarker values. Receiver operating curve (ROC) analyses provided the area under the curve (AUC) for A $\beta$  positivity or diagnostic groups. The “pROC” package in R, version 3.6.3 (R Group for Statistical Computing) was used to compare AUCs, and the false discovery rate was used to correct *P* values for multiple comparisons. Mediation analyses were performed with the R package “mediation.” All tests were 2-tailed, with a significance level

of  $\alpha = .05$ . All statistical analyses and figures were performed with R, version 3.6.3. Further details are provided in the eMethods in [Supplement 1](#).

## Results

### Participants' Characteristics and Correlations Between Biomarkers

Demographic and clinical data from the 3 studies are summarized in **Table 1** and eTable 1 in [Supplement 1](#). There was a positive association between age and both plasma and CSF GFAP levels in the 3 cohorts (TRIAD: plasma,  $\beta$  [SE] = 0.64 [0.13];  $P < .001$ ; CSF,  $\beta$  [SE] = 0.35 [0.15];  $P = .02$ ); ALFA+: plasma,  $\beta$  [SE] = 0.38 [0.048];  $P < .001$ ; CSF,  $\beta$  [SE] = 0.26 [0.049];  $P < .001$ ; and Paris: plasma,  $\beta$  [SE] = 0.26 [0.06];  $P < .001$ ; CSF,  $\beta$  [SE] = 0.32 [0.07];  $P < .001$ ), which can also be evidenced when comparing plasma or CSF GFAP mean levels between young CU participants and elderly CU- individuals (TRIAD: plasma, CU- mean [SD], 185.1 [93.5] pg/mL; young CU mean [SD], 95.1 [62.1] pg/mL;  $P = .001$ ; CSF, CU- mean [SD], 12 506 [5148] pg/mL; young CU mean [SD], 4134 [1483] pg/mL;  $P < .001$ ). Plasma GFAP levels were higher in CU women than in CU men (TRIAD: mean [SD], 161.0 [81.7] pg/mL in men vs 239.01 [123.84] pg/mL in women;  $P < .001$ ; ALFA+: mean [SD], 128.9 [59.7] pg/mL in men vs 145.6 [63.1] pg/mL in women;  $P < .001$ ) and were also higher specifically in CU- women compared with CU- men (TRIAD: mean [SD], 142.5 [63.2] pg/mL in men vs 209.1 [99.5] pg/mL in women;  $P < .001$ ; ALFA+: mean [SD], 117.0 [43.9] pg/mL in men vs 125.1 [41.2] pg/mL in women;  $P = .01$ ; and Paris cohort: mean [SD], 118.9 [34.6] pg/mL in men vs 179.34 [68.26] pg/mL in women;  $P = .03$ ). The same sex differences were also observed when all participants were included (adjusting for age and diagnosis, TRIAD: mean [SD], 224.7 [153.2] pg/mL in men vs 248.1 [146.1] pg/mL in women;  $P = .002$ ; Paris: mean [SD], 262.7 [138.4] pg/mL in men vs 326.7 [189.6] pg/mL in women;  $P < .001$ ). *APOE*  $\epsilon 4$  carriership (NCBI Gene ID: 348) was not associated with plasma or CSF GFAP levels in any of the cohorts when models accounted for  $A\beta$  status or clinical diagnosis.

There was a positive correlation between plasma and CSF GFAP levels in the 3 cohorts (eFigure 1 in [Supplement 1](#)). Spearman rank correlations between plasma and CSF GFAP levels and other biomarkers are presented in eFigure 2 in [Supplement 1](#).

### Plasma GFAP Levels Throughout the AD Continuum

In the TRIAD cohort, levels of plasma and CSF GFAP were higher across the AD continuum, namely, in  $A\beta+$  CU (CU+) individuals (ie, preclinical AD), individuals with  $A\beta+$  MCI (MCI+; ie, MCI due to AD), and individuals with AD dementia (**Figure 1A**). Compared with the CU- group, plasma GFAP levels were higher in the CU+ group (54% increase;  $P = .001$ ;  $d = 0.66$ ), in the MCI+ group (79% increase;  $P < .001$ ;  $d = 1.35$ ), and in the AD dementia group (107% increase;  $P < .001$ ;  $d = 2.10$ ). Patients with FTD had plasma GFAP levels as low as CU- individuals (eFigure 3A in [Supplement 1](#)). Levels of CSF GFAP were also higher in the AD continuum groups com-

pared with CU- individuals (**Figure 1B**), but the group differences were not significant after correction for multiple comparisons. The magnitude of the CSF GFAP changes was not as large as that of the plasma GFAP changes (the CSF GFAP level increases with CU- individuals as the reference group: CU+ individuals, 24% increase;  $P = .24$ ;  $d = 0.56$ ; individuals with MCI+, 35% increase;  $P = .06$ ;  $d = 0.82$ ; and individuals with AD dementia, 30% increase;  $P = .03$ ;  $d = 0.86$ ). Similar to plasma GFAP levels, patients with FTD had lower CSF GFAP levels than patients on the AD continuum (eFigure 3B in [Supplement 1](#)).

In ALFA+, we used the biomarker-based AT classification<sup>21,22</sup> to study 2 stages in preclinical AD:  $A\beta+$  but tau- (A+T-) and  $A\beta+$  and tau+ (A+T+) and compared it with the A-T- stage. Plasma GFAP levels were significantly higher in the A+T- group compared with the A-T- group (32% increase;  $P < .001$ ;  $d = 0.55$ ) (**Figure 1C**), whereas CSF GFAP levels were not (1% increase;  $P = .99$ ;  $d = 0.01$ ; **Figure 1D**). Both plasma and CSF GFAP were significantly higher in the A+T+ group compared with the A-T- group (plasma: 60% increase;  $P < .001$ ;  $d = 1.09$ ; CSF: 77% increase;  $P < .001$ ;  $d = 1.18$ ). Participants in the  $A\beta-$  and tau+ (A-T+ group) did not have higher plasma or CSF GFAP levels compared with the A-T- group. To further test whether plasma and CSF GFAP levels were increased in the earliest stage of the preclinical AD continuum, we analyzed a group of individuals with a low burden of  $A\beta$  pathology, namely, a positive CSF  $A\beta_{42/40}$  ratio but  $A\beta$  PET centiloids lower than 30<sup>23</sup> (eMethods in [Supplement 1](#)). We observed that plasma GFAP levels were significantly higher in this group compared with  $A\beta-$  participants (28% increase;  $P < .001$ ;  $d = 0.57$ ; eFigure 4A in [Supplement 1](#)) while CSF GFAP levels were not (8% increase;  $P = .37$ ;  $d = 0.16$ ; eFigure 4B in [Supplement 1](#)).

In the Paris cohort, plasma and CSF GFAP levels followed similar patterns to those described for TRIAD. Plasma GFAP levels were higher in individuals with MCI+ (128% increase;  $P < .001$ ;  $d = 1.40$ ) and in those with AD dementia (133% increase;  $P < .001$ ;  $d = 1.37$ ) compared with the CU- group, and no difference was found between the CU- group and non-AD group (**Figure 1E**). Levels of CSF GFAP were higher in individuals with MCI+ (72% increase;  $d = 0.44$ ) and AD dementia (89% increase;  $d = 0.64$ ) compared with CU- individuals, but differences were not statistically significant after correction for multiple comparisons (**Figure 1F**). Similar to TRIAD, patients with FTD and dementia with Lewy bodies had plasma and CSF GFAP levels comparable to CU- individuals (eFigure 3C and 3D in [Supplement 1](#)).

### Association of Plasma GFAP Levels With $A\beta$ Pathology and Discrimination of $A\beta$ Status

We evaluated the association of plasma and CSF GFAP levels with  $A\beta$  pathology as measured with CSF  $A\beta_{42/40}$  or  $A\beta$  PET. Because our aim was to study the AD continuum, for all subsequent analyses, we included only CU individuals, those with MCI, and those with AD dementia (for TRIAD and Paris cohorts). In the ALFA+ cohort, we excluded individuals with an A-T+ (non-AD pathologic change) biomarker profile. In TRIAD, both plasma and CSF GFAP levels were negatively associated with CSF  $A\beta_{42/40}$  (plasma GFAP,  $P < .001$ ;  $\eta_p^2 = 0.26$ ; CSF GFAP,  $P = .01$ ;  $\eta_p^2 = 0.11$ ; **Figure 2A** and **B**) and positively

Table 1. Demographic Characteristics and Biomarker Levels of the Study Cohorts by Clinical and Biomarker-Defined Groups<sup>a</sup>

Characteristic	TRIAD cohort (n = 300)				ALFA+ cohort (n = 384)				BioCogBank Paris Lariboisière cohort (n = 187)			
	Mean (SD)		P value		Mean (SD)		P value		Mean (SD)		P value	
	Young CU (n = 35)	CU- (n = 114)	CU+ (n = 42)	MCI+ (n = 39)	AD dementia (n = 45)	Non-AD (n = 25) <sup>b</sup>	CU- (n = 249) <sup>c</sup>	CU+ (n = 135) <sup>d</sup>	CU- (n = 21)	MCI+ (n = 42)	AD dementia (n = 76)	Non-AD (n = 48) <sup>e</sup>
Age, y	23.1 (1.8)	69.9 (9.4)	74.1 (7.7)	71.2 (7.7)	66.1 (9.7)	70.8 (11.0)	60.5 (4.5)	62.2 (4.9)	64.4 (9.5)	72.4 (7.9)	72.2 (8.4)	66.6 (9.7)
Female, No. (%)	22 (62.9)	73 (64.0)	29 (69.0)	21 (53.8)	21 (46.7)	11 (44.0)	153 (61.4)	81 (60.0)	14 (66.7)	26 (61.9)	47 (61.8)	29 (60.4)
Educational level, y	16.6 (1.5)	15.6 (3.9)	14.8 (3.2)	15.2 (3.2)	14.6 (3.6)	13.8 (3.9)	13.6 (3.5)	13.3 (3.6)	11.2 (1.6)	10.7 (1.8)	9.7 (2.0)	10.7 (1.9)
APOE ε4 carriers, No. (%)	8 (22.9)	29 (26.9)	12 (28.6)	23 (62.2)	24 (55.8)	5 (22.7)	106 (42.6)	103 (76.3)	6 (28.6)	24 (57.1)	49 (64.5)	7 (14.6)
MMSE score	30 (0)	29 (1.0)	29 (1.0)	28 (2.0)	19 (6.0)	27 (2.0)	29.1 (0.9)	29.1 (1.0)	27.4 (2.5)	23.5 (4.4)	19.3 (5.6)	24.6 (3.7)
Centiloids	-11.6 (6.6)	-3.12 (8.6)	52.5 (31.2)	91.1 (36.0)	91.8 (40.0)	1.10 (12.3)	-4.54 (6.6)	16.8 (21.1)	NA	NA	NA	NA
CSF biomarkers, pg/mL												
Aβ42/40	0.091 (0.006)	0.087 (0.017)	0.055 (0.015)	0.043 (0.010)	0.045 (0.011)	0.082 (0.026)	0.087 (0.009)	0.051 (0.012)	0.095 (0.007)	0.044 (0.009)	0.042 (0.009)	0.089 (0.012)
p-tau181	22.6 (7.1)	36.2 (14.4)	59.3 (35.2)	89.4 (34.6)	99.9 (55.8)	59.7 (63.5)	13.9 (4.2)	18.4 (7.2)	32.8 (8.6)	93.0 (46.9)	115.4 (59.3)	37.7 (16.4)
t-tau	195.3 (48.1)	311.0 (126.8)	396.4 (197.0)	539.4 (210.1)	659.6 (331.7)	448.4 (398.6)	174.8 (48.0)	222.6 (76.9)	243.1 (70.9)	587.6 (280.3)	732.6 (390.7)	305.6 (148.6)
NFL	184.6 (57.7)	1132.3 (1038.3)	862.5 (268.7)	1126.8 (257.7)	1646.2 (965.0)	1783.0 (1662.5)	76.3 (23.6)	89.2 (27.5)	889.3 (352.1)	1532 (643.4)	1695 (673.0)	1456 (121.4)
GFAP	4134 (1483)	12 506 (5148)	15 669 (6771)	17 114 (5890)	16 314 (8513)	14 074 (7497)	4090 (2018)	4859 (2333)	2423 (2194)	4189 (3313)	4601 (3759)	2872 (2356)
Plasma biomarkers, pg/mL												
NFL	6.5 (2.7)	22.1 (9.8)	27.9 (24.8)	25.7 (14.4)	33.6 (13.5)	28.6 (11.4)	9.8 (3.3)	11.6 (4.2)	13.1 (6.8)	24.2 (10.4)	24.4 (8.7)	21.2 (16.7)
p-tau181	7.9 (3.6)	9.9 (4.4)	14.8 (11.0)	18.1 (8.1)	24.1 (9.6)	11.8 (12.3)	8.8 (3.2)	11.0 (4.6)	3.0 (1.8)	11.5 (6.2)	12.8 (3.6)	9.5 (6.7)
GFAP	95.1 (62.1)	185.1 (93.5)	285.0 (142.6)	332.5 (153.6)	388.1 (152.8)	188.9 (105.9)	121.9 (42.4)	169.9 (78.5)	161.2 (67.1)	368.6 (158.5)	376.4 (179.6)	185.0 (96.0)

Abbreviations: Aβ, amyloid-β; AD, Alzheimer disease; ALFA, Alzheimer's and Families; CSF, cerebrospinal fluid; CU-, Aβ-negative cognitively unimpaired; CU+, Aβ-positive cognitively unimpaired; GFAP, glial fibrillary acidic protein; MCI+, Aβ-positive mild cognitive impairment; MMSE, Mini-Mental State Examination; NA, not available; NFL, neurofilament light chain; p-tau181, tau phosphorylated at threonine 181; t-tau, total tau; TRIAD, Translational Biomarkers in Aging and Dementia.

<sup>a</sup> Within each cohort, we used t test or 1-way analysis of variance to compare age, educational level, and MMSE between groups and Pearson  $\chi^2$  to compare sex and APOE ε4 frequencies between groups. Centiloids and fluid biomarker levels were compared with a 1-way analysis of covariance adjusted by age and sex and followed by false discovery rate multiple comparison correction. Aβ status for group definition was based on positron emission tomography visual result in the TRIAD cohort and on CSF Aβ42/40 for the ALFA+ and BioCogBank Paris Lariboisière cohorts.

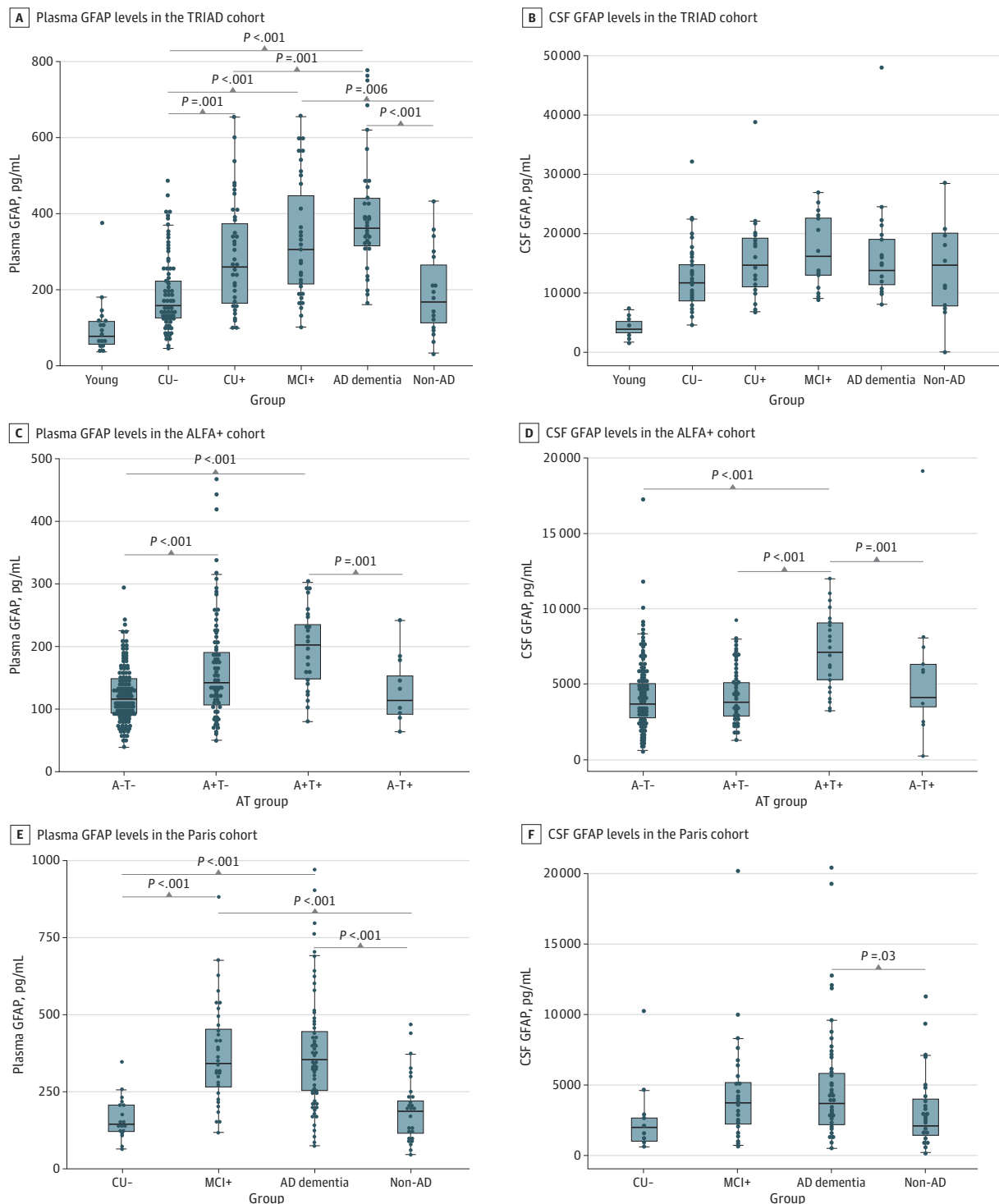
<sup>b</sup> Among the non-AD group, there were 21 individuals with MCI with a negative Aβ positron emission tomography visual result and 4 participants with a clinical diagnosis of AD dementia syndrome with a negative Aβ positron emission tomography visual result.

<sup>c</sup> A total of 68 of 248 participants (27.4%) had subjective cognitive decline.

<sup>d</sup> A total of 39 of 135 participants (28.9%) had subjective cognitive decline.

<sup>e</sup> In the non-AD group all participants had MCI with normal CSF Aβ42/40 levels.

Figure 1. Plasma and Cerebrospinal Fluid (CSF) Glial Fibrillary Acidic Protein (GFAP) Group Comparisons

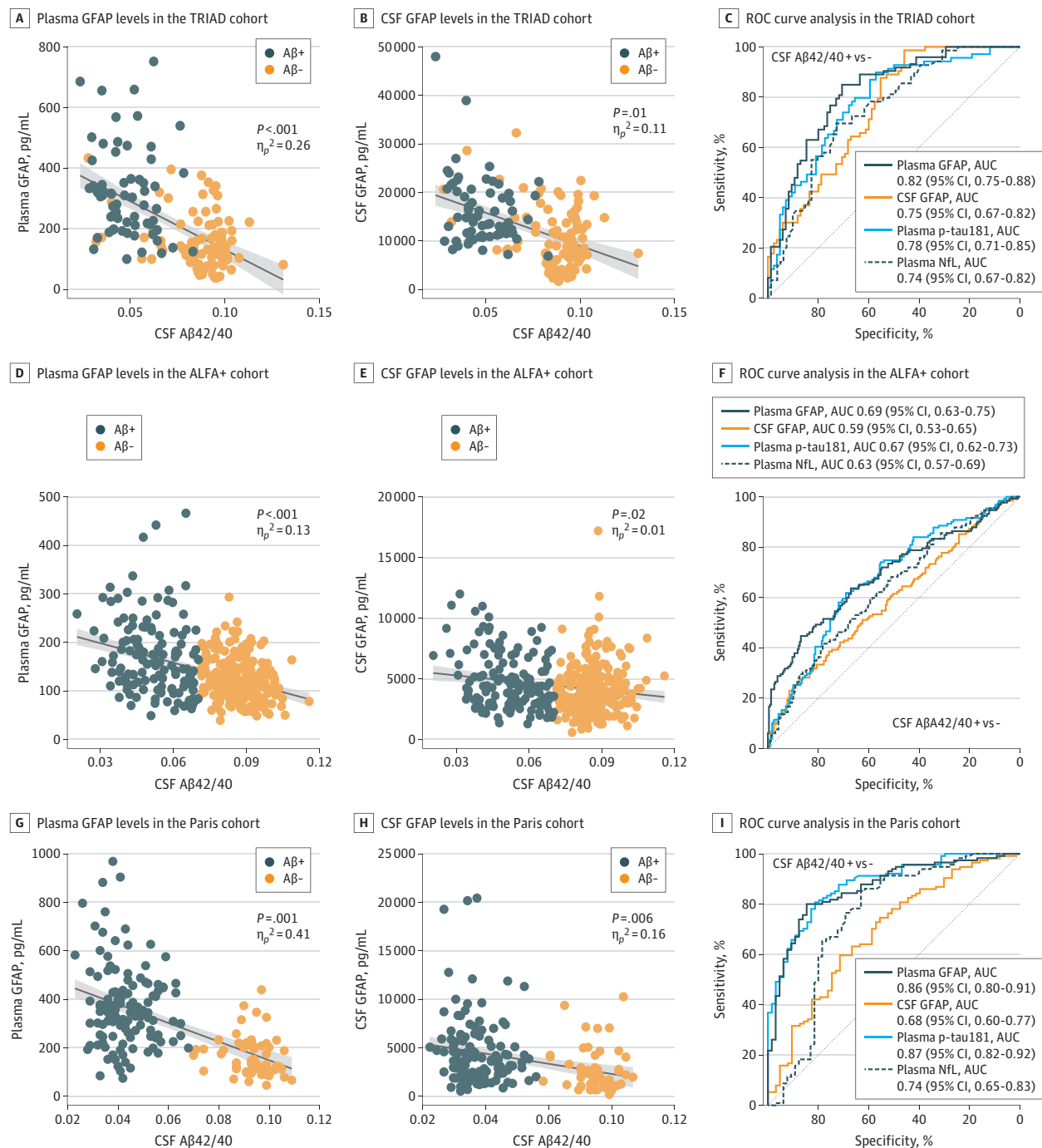


Box plots depict median (horizontal bar), IQR (hinges), and  $1.5 \times$  IQR (whiskers). Group comparisons were computed with a 1-way analysis of covariance adjusting for age and sex. The Tukey honestly significant difference test was used for post hoc pairwise comparisons in all cohorts. Fold changes are depicted for the Alzheimer disease (AD) continuum groups and were calculated using amyloid- $\beta$  ( $\text{A}\beta$ )-negative cognitively unimpaired (CU-) individuals (Translational Biomarkers in Aging and Dementia [TRIAD] and BioCogBank Paris Lariboisière [Paris] cohorts) or  $\text{A}\beta$ -negative and tau-negative (A-T-) individuals

(Alzheimer's and Families [ALFA+] cohort) as the reference group.  $\text{A}\beta$  status was defined by  $\text{A}\beta$  positron emission tomography in the TRIAD cohort and CSF  $\text{A}\beta_{42}/40$  ratio in the ALFA+ and Paris cohorts. The non-AD group included 21 individuals with  $\text{A}\beta$ -negative mild cognitive impairment (MCI), 4 individuals with  $\text{A}\beta$ -negative AD dementia syndrome in the TRIAD cohort, and 48 individuals with MCI- in the Paris cohort. A+T- indicates  $\text{A}\beta$ -positive and tau-negative; A+T+,  $\text{A}\beta$ -positive and tau-positive; A-T+,  $\text{A}\beta$ -negative and tau-positive; CU+,  $\text{A}\beta$ -positive cognitively unimpaired; MCI+,  $\text{A}\beta$ -positive MCI.



Figure 2. Associations of Plasma and Cerebrospinal Fluid Glial Fibrillary Acidic Protein Levels With Aβ Pathology and Discriminative Accuracy



Individuals are color coded by amyloid-β (Aβ) status (as defined by Aβ positron emission tomography in the Translational Biomarkers in Aging and Dementia [TRIAD] cohort and cerebrospinal fluid (CSF) Aβ42/40 ratio in the Alzheimer's and Families [ALFA+] and BioCogBank Paris Lariboisière [Paris] cohorts). Solid lines indicate the regression line and 95% CIs. P values were computed with linear models adjusted by age, sex, and clinical diagnosis (the latter only for the

TRIAD and Paris cohorts). Sizes of the associations between variables are shown by the partial  $\eta^2$  ( $\eta_p^2$ ). For comparative purposes, we also included plasma tau phosphorylated at threonine 181 (p-tau181) and plasma neurofilament light chain (NfL) in these analyses. AUC indicates area under the curve; GFAP, glial fibrillary acidic protein; ROC, receiver operating characteristic.

associated with Aβ PET (plasma GFAP,  $P < .001$ ;  $\eta_p^2 = 0.32$ ; CSF GFAP,  $P < .001$ ;  $\eta_p^2 = 0.10$ ; eFigure 5A and 5B in Supplement 1). The sizes of the associations of Aβ pathology (either

CSF Aβ42/40 or Aβ PET) with plasma GFAP levels were larger than those with CSF GFAP levels. We performed the same analyses within the CU individuals, and plasma GFAP levels

Table 2. ROC Curve Analyses to Discriminate A $\beta$ -Positive From A $\beta$ -Negative Individuals

Biomarker	A $\beta$ + vs A $\beta$ -, AUC (95% CI) <sup>a</sup>						
	CSF A $\beta$ 42/40			A $\beta$ PET			
	TRIAD cohort	ALFA+ cohort	BioCogBank Paris Lariboisière cohort	Visual result		Centiloid cutoff	
	TRIAD cohort	ALFA+ cohort		TRIAD cohort	ALFA+ cohort	TRIAD cohort	ALFA+ cohort
GFAP							
Plasma	0.82 (0.75-0.88)	0.69 (0.63-0.75)	0.86 (0.80-0.91)	0.85 (0.79-0.91)	0.75 (0.67-0.84)	0.83 (0.77-0.89)	0.82 (0.72-0.92)
CSF	0.75 (0.67-0.82) <sup>b</sup>	0.59 (0.53-0.65) <sup>c</sup>	0.68 (0.60-0.77) <sup>c</sup>	0.75 (0.69-0.82) <sup>c</sup>	0.68 (0.59-0.77)	0.75 (0.68-0.84) <sup>d</sup>	0.76 (0.64-0.87)
Other plasma biomarkers							
p-tau181	0.78 (0.71-0.85)	0.67 (0.62-0.73) <sup>e</sup>	0.87 (0.82-0.92) <sup>e</sup>	0.77 (0.70-0.85)	0.67 (0.58-0.76)	0.79 (0.71-0.86)	0.76 (0.67-0.86)
NfL	0.74 (0.67-0.82)	0.63 (0.57-0.69)	0.74 (0.65-0.83) <sup>c</sup>	0.67 (0.59-0.76) <sup>c</sup>	0.66 (0.58-0.75)	0.68 (0.59-0.76) <sup>c</sup>	0.73 (0.63-0.83)

Abbreviations: A $\beta$ , amyloid- $\beta$ ; ALFA, Alzheimer's and Families; AUC, area under the curve; CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; p-tau181, tau phosphorylated at threonine 181; PET, positron emission tomography; ROC, receiver operating characteristic; TRIAD, Translational Biomarkers in Aging and Dementia.

<sup>a</sup> ROC curve analyses to test whether plasma GFAP discriminates between A $\beta$ -positive (A $\beta$ +) and A $\beta$ -negative (A $\beta$ -) individuals, as defined by the CSF A $\beta$ 42/40 ratio, A $\beta$  PET visual result, or A $\beta$  PET using a cutoff of 24 (TRIAD) or 30 (ALFA) centiloids. We also included CSF GFAP, plasma p-tau181, and plasma NfL for comparison. AUC differences were tested using the DeLong test followed by false discovery rate multiple comparison correction.

<sup>b</sup>  $P = .06$  vs plasma GFAP (before correction for multiple comparisons).

<sup>c</sup>  $P < .05$  vs plasma GFAP.

<sup>d</sup>  $P = .03$  vs plasma GFAP (before correction for multiple comparisons).

<sup>e</sup>  $P < .05$  vs CSF GFAP.

were significantly associated with both A $\beta$  biomarkers (CSF A $\beta$ 42/40:  $P = .008$ ;  $\eta_p^2 = .07$ ; A $\beta$  PET:  $P < .001$ ;  $\eta_p^2 = .06$ ). In contrast, CSF GFAP levels were not significantly associated with CSF A $\beta$ 42/40 ( $P = .18$ ) or A $\beta$  PET ( $P = .07$ ) within the CU individuals.

In ALFA+, plasma GFAP levels were positively associated with A $\beta$  pathology as shown by a significant negative association with CSF A $\beta$ 42/40 in the whole sample ( $P < .001$ ;  $\eta_p^2 = 0.13$ ) but also in the CU- group ( $P = .002$ ;  $\eta_p^2 = 0.04$ ) and CU+ group ( $P = .03$ ;  $\eta_p^2 = 0.04$ ) (Figure 2D). Levels of CSF GFAP also showed a negative association with CSF A $\beta$ 42/40 in the whole sample ( $P = .02$ ;  $\eta_p^2 = 0.01$ ; Figure 2E) and in the CU+ group ( $P = .005$ ;  $\eta_p^2 = 0.06$ ). Conversely, a positive association between CSF GFAP levels and CSF A $\beta$ 42/40 was observed in CU- participants ( $P = .02$ ;  $\eta_p^2 = 0.02$ ). Both plasma and CSF GFAP levels were associated with A $\beta$  deposition as quantified by A $\beta$  PET (eFigure 5C and D in Supplement 1) in the whole sample (plasma GFAP,  $P < .001$ ;  $\eta_p^2 = 0.10$ ; CSF GFAP,  $P = .001$ ;  $\eta_p^2 = 0.04$ ).

The same analysis was repeated in the Paris cohort, and the size of the association of CSF A $\beta$ 42/40 with plasma GFAP levels (plasma,  $P < .001$ ;  $\eta_p^2 = 0.41$ ) was greater than that with CSF GFAP levels (CSF,  $P = .006$ ;  $\eta_p^2 = 0.16$ ; Figure 2G and H).

We next investigated how plasma and CSF GFAP levels discriminate A $\beta$  status using ROC analysis (Table 2 and Figure 2). A $\beta$  statuses were defined by CSF A $\beta$ 42/40, A $\beta$  PET visual read, or the A $\beta$  PET centiloids cutoffs used in each cohort (Table 2). In the entire TRIAD cohort, plasma GFAP as a biomarker accurately discriminated A $\beta$ + from A $\beta$ - individuals, with an AUC ranging from 0.82 to 0.85. In contrast, CSF GFAP as a biomarker had an AUC of 0.75. In CU individuals, plasma GFAP as a biomarker distinguished A $\beta$  status with an AUC of 0.75 to 0.79, whereas CSF GFAP as a biomarker had AUCs of 0.74 to 0.76. In ALFA+, plasma GFAP as a biomarker discriminated with an AUC of 0.69 to 0.82, while for CSF GFAP as a biomarker, AUCs were 0.59 to 0.76. In the Paris cohort, plasma GFAP as a

biomarker accurately differentiated CSF A $\beta$ 42/40 status with an AUC of 0.86, while CSF GFAP as a biomarker had an AUC of 0.68. In addition, ROCs were performed contrasting CU- individuals with those with MCI+, individuals with A $\beta$ - MCI (MCI-) with those with MCI+, and CU- individuals with those with AD (eTable 2 in Supplement 1). For comparison purposes, we also performed ROC analyses with plasma tau phosphorylated at threonine 181 (p-tau181) and neurofilament light chain (NfL), and none of them performed better than plasma GFAP.

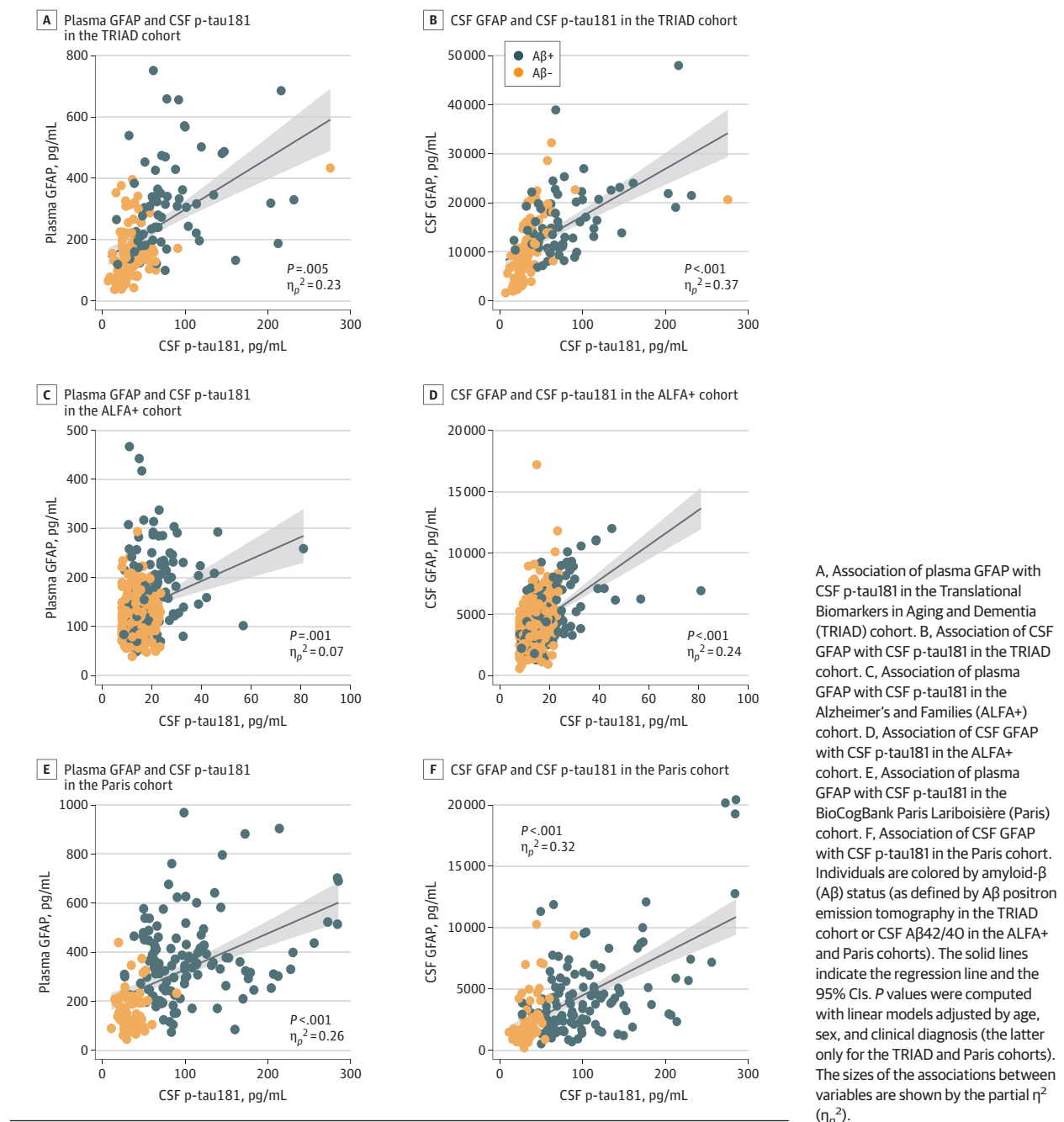
We also performed analyses comparing different combinations of plasma biomarkers (eTable 3 in Supplement 1). We found that adding plasma GFAP to any of the other plasma biomarkers (either p-tau181 or NfL) was associated with improved accuracy to discriminate A $\beta$  status (as measured by CSF A $\beta$ 42/40) in the 3 cohorts.

### Association of Plasma GFAP Levels With Tau Pathology Among Individuals With Concomitant A $\beta$ Pathology

We evaluated the associations between GFAP levels and tau biomarkers (CSF p-tau181 and tau PET). In TRIAD, higher plasma and CSF GFAP levels were associated with increased tau PET burden (plasma GFAP,  $P < .001$ ;  $\eta_p^2 = 0.29$ ; CSF GFAP,  $P = .005$ ;  $\eta_p^2 = 0.08$ ; eFigure 6A and B in Supplement 1). Both plasma and CSF GFAP levels were significantly associated with CSF p-tau181 levels in the 3 cohorts (Figure 3A-F).

We conducted a mediation analysis to assess whether the associations between GFAP levels and tau biomarkers were mediated by A $\beta$  status. Results in TRIAD indicated that the association of plasma GFAP levels with tau was mediated by A $\beta$  (eFigure 7A in Supplement 1), with a significant indirect association corresponding to 60% of the total association of tau with plasma GFAP levels. These findings were replicated using PET biomarkers (eFigure 7A in Supplement 1). A similar analysis was performed with CSF GFAP levels as the response variable, and tau had both a direct and an indirect association with CSF GFAP levels.

**Figure 3. Association of Plasma and Cerebrospinal Fluid (CSF) Glial Fibrillary Acidic Protein (GFAP) Levels With Tau Phosphorylated at Threonine 181 (p-tau181)**



Results were consistent across cohorts (eFigure 7B and C in Supplement 1). In the ALFA+ and Paris cohorts, the association of CSF p-tau181 with plasma GFAP levels was mediated by CSF  $A\beta_{42/40}$ , with a significant indirect association corresponding to 62% and 63% of the total association of CSF p-tau181 with plasma GFAP levels, respectively. Conversely, CSF p-tau181 did not show a significant indirect association with CSF GFAP levels, suggesting  $A\beta$ -independent effects.

### Association of CSF and Plasma GFAP Levels With Neuroinflammation

Finally, we explored how plasma and CSF GFAP levels are associated with other glial biomarkers. In TRIAD, levels of CSF GFAP, but not plasma GFAP, showed a positive association with CSF soluble triggering receptor expressed on myeloid cells 2 (sTREM2) and Chitinase-3-like protein 1 (YKL40) (TRIAD: plasma GFAP association with sTREM2,  $\beta$  [SE] = 0.11 [0.08];  $P = .17$ ; YKL40,  $\beta$  [SE] = 0.02 [0.06];  $P = .67$ ; CSF GFAP



association with sTREM2,  $\beta$  [SE] = 0.25 [0.09];  $P < .001$ ; YKL40,  $\beta$  [SE] = 0.32 [0.07];  $P < .001$  (eFigure 8A and B in Supplement 1). Similar results were observed in the ALFA+ and Paris cohorts (ALFA+: plasma GFAP association with sTREM2,  $\beta$  [SE] = 0.083 [0.086];  $P = .14$ ; YKL40,  $\beta$  [SE] = 0.075 [0.051];  $P = .14$ ; CSF GFAP association with sTREM2,  $\beta$  [SE] = 0.41 [0.048];  $P < .001$ ; YKL40,  $\beta$  [SE] = 0.40 [0.045];  $P < .001$ ; and Paris: plasma GFAP association with YKL40,  $\beta$  [SE] = 0.06 [0.09];  $P = .49$ ; CSF GFAP association with YKL40,  $\beta$  [SE] = 0.52 [0.12];  $P < .001$ ) (eFigure 8C-E in Supplement 1).

## Discussion

In this study, which includes 3 thoroughly characterized cohorts, we showed that plasma GFAP levels were significantly higher among individuals with preclinical AD and reached their higher levels at symptomatic stages of AD. The effect sizes of the increases of plasma GFAP levels were always larger than those of CSF GFAP levels. Therefore, plasma GFAP levels appear to be a superior biomarker tracking A $\beta$  pathology than its CSF counterpart. This finding is particularly evident for individuals with preclinical AD; plasma GFAP levels were significantly higher in CU+ individuals and significantly discriminated them from CU- individuals, whereas CSF did not.

Previous studies showed that plasma and serum GFAP levels are higher in those with symptomatic AD,<sup>9,24-27</sup> results that are in line with those reported for CSF GFAP levels.<sup>24,28-31</sup> However, less is known about plasma GFAP levels among individuals along the whole AD continuum and, particularly, in those with preclinical AD. A recent study demonstrated that plasma GFAP levels were higher in a group of 33 CU+ individuals compared with 63 CU- individuals (AUC = 0.795).<sup>16</sup> Preceding studies showed that plasma GFAP levels were associated with both clinical diagnosis and A $\beta$  status.<sup>25</sup> Another study revealed a quadratic (inverted U-shape) association between plasma GFAP levels and A $\beta$  deposition.<sup>26</sup> To our knowledge, no other studies investigated the whole AD continuum or included participants with preclinical AD, and no other studies compared plasma and CSF compartments in the same individuals.

We also analyzed the association of plasma GFAP levels with A $\beta$  pathology (either CSF A $\beta$ 42/40 ratio or A $\beta$  PET), and we found a positive association between plasma GFAP levels and A $\beta$  pathology in all cohorts and high rates of accuracy to discriminate A $\beta$ + from A $\beta$ - individuals (AUC = 0.82-0.86). It was also apparent when assessing the whole AD continuum that plasma GFAP levels were higher in individuals with a more advanced clinical diagnosis (CU+ less than MCI+, which was less than AD dementia). In contrast, CSF GFAP levels showed no significant difference across the AD continuum groups. Consistent with this finding, we observed a significant association between plasma GFAP levels and tau PET findings.

We included many individuals with preclinical AD: 42 in TRIAD and 135 in ALFA+. Plasma GFAP discriminated CU+ individuals from CU- individuals with an AUC of 0.75 to 0.79 in TRIAD, similar to the AUC of 0.795 previously described.<sup>16</sup> Furthermore, in ALFA+, we studied the earliest phase of preclinical AD. We assessed 104 individuals who were A+T- (ie, had

A $\beta$  pathology but not yet tau pathology) and 89 individuals with a low A $\beta$  burden (ie, they had decreased CSF A $\beta$ 42/40 but not yet a positive A $\beta$  PET result). Both groups had significantly higher plasma GFAP levels but not CSF GFAP levels, reinforcing the idea that plasma GFAP may be an early biomarker of AD pathologic changes. Levels of CSF GFAP only become significantly higher in the A+T+ group when there is biomarker evidence of both A $\beta$  and tau pathology. Data from cellular models indicate that astrocytes react to early preplaque-insoluble A $\beta$  oligomeric species.<sup>32</sup> Our results can be contextualized with findings using other fluid or imaging biomarkers of reactive astrogliosis. Studies using the PET tracer <sup>11</sup>C-deuterium-L-deprenyl ([<sup>11</sup>C]DED), which binds to monoamine oxidase-B, mainly expressed in reactive astrocytes, support fluctuations during the AD continuum in reactive astrocyte states. More specifically, [<sup>11</sup>C]DED binding in the frontal and parietal cortices is significantly increased in those with prodromal AD compared with CU individuals.<sup>33</sup> Early increases in [<sup>11</sup>C]DED binding have also been found in autosomal carriers of a dominant AD variation almost 30 years before the emergence of symptoms.<sup>34</sup> In a transgenic mouse model that overexpresses the human APP<sub>swe</sub> variation, increased [<sup>11</sup>C]DED binding precedes detectable A $\beta$  pathology.<sup>35</sup> Moreover, CSF YKL40, a biomarker of a subset of reactive astrocytes, is also elevated in those with preclinical AD.<sup>36,37</sup> Recently, a model of reactive astrogliosis in the AD continuum<sup>15</sup> has been proposed that would encompass early reactive astrocytes in the preclinical stage (supported by in vivo evidence of higher monoamine oxidase-B expression), followed by more widespread reactivity (supported by increases in CSF YKL40, GFAP, and S100b) and, finally, the end-stage reactive astrocytes, in which their physiological function may be lost. Our findings situate plasma GFAP levels as a marker of early reactive astrocytes.

Our results point to plasma GFAP as a possible biomarker specific for A $\beta$  pathology. First, plasma GFAP levels were not higher among individuals with non-AD neurodegenerative diseases in the TRIAD and Paris cohorts. Plasma GFAP levels were normal in those with FTD despite gliosis being a characteristic of FTD.<sup>38,39</sup> Second, in ALFA+, the A+T+ group did not have high plasma GFAP levels; this finding may suggest that plasma GFAP levels specifically reflect A $\beta$  pathology in preclinical stages, but a direct comparison with the preclinical stage of other neurodegenerative diseases should be performed. Third, the association between plasma GFAP levels and tau pathology was mediated by A $\beta$  pathology. These results are consistent with the increased expression of GFAP surrounding A $\beta$  plaques.<sup>40-43</sup> Although CSF GFAP levels were associated with other glial biomarkers (YKL40 and sTREM2), plasma GFAP levels were not. It is possible that CSF GFAP better reflects reactive astrocytes in response to neuroinflammatory changes, such as microglial activation, while plasma GFAP is more closely associated with reactive astrogliosis because of A $\beta$  burden. High levels of blood GFAP can be found in individuals with other neurodegenerative diseases,<sup>24,44,45</sup> but this finding occurs at the symptomatic, and thus advanced, stages of the disease. The increase in blood GFAP levels after acute brain conditions, such as subarachnoid hemorrhage and traumatic and hypoxic brain

injury, has been extensively documented,<sup>46-50</sup> but this increase may come through other mechanisms, such as a trauma-induced temporary opening of the blood-brain barrier. Based on these findings, it would seem that GFAP responds to acute neuronal injury; however, in a chronic neurodegenerative disease, and unlike NFL, plasma GFAP may principally (but not exclusively) reflect A $\beta$  pathology.

A unique feature of our study is that we measured both plasma and CSF GFAP levels in the same participants. This feature allowed us to draw one of the main conclusions of this study, namely, that differences in plasma GFAP levels are larger than those of CSF GFAP levels between the groups, and the effect sizes of the associations between plasma GFAP levels and biomarkers of A $\beta$  are greater than those of CSF GFAP levels. Moreover, the AUCs to discriminate A $\beta$  status are higher for plasma GFAP than CSF GFAP, especially when A $\beta$  pathology is defined by CSF A $\beta$ 42/40, suggesting an early increase of plasma GFAP levels. This result is surprising because neurologically associated blood biomarkers have usually been considered a proxy of the CSF biomarkers. A possible explanation of why plasma GFAP outperforms CSF GFAP would be the different clearance mechanisms into the biofluids. Astrocytes are part of the neurovascular unit and the blood-brain barrier, which is altered in individuals with AD.<sup>51</sup> Astrocytic end-feet cover brain capillaries, which may be a direct route for the release of GFAP from reactive astrocytes to the bloodstream.<sup>52</sup> It could be speculated that blood-brain barrier dysfunction facilitates the release of GFAP into the bloodstream; this may also explain the elevations of plasma GFAP in individuals with acute neurologic injuries. Astrocytes are also part of the glymphatic system, which is a highly organized system that clears the brain of insoluble proteins and metabolites by draining them into the venous system.<sup>53</sup> GFAP may also reach the bloodstream via the meningeal lymphatic system.<sup>54</sup> Finally, preanalytical and analytical factors that need to be further studied may also account for these differences. A previous study described that plasma GFAP is very stable to freeze-thaw cycles,<sup>55</sup> whereas CSF GFAP is far more sensitive over time.<sup>56</sup> The fact that plasma GFAP has a wider range of values than CSF GFAP may also be associated with the higher accuracy of the former.

It remains unanswered which plasma biomarker (GFAP, A $\beta$ 42/40, or forms of p-tau) is more accurately associated with A $\beta$  pathology in particular in the preclinical stage. A head-to-head comparison of these biomarkers in several independent cohorts is needed. However, GFAP is an additional tool that has shown consistent results across multiple cohorts and is easily detectable using commercially available immunoassays. Moreover, we show that adding plasma GFAP to models with other plasma biomarkers (p-tau181 and/or NFL) improves their accuracy. All of these biomarkers perform satisfactorily, but a combination of some will probably render the highest accuracy for A $\beta$  pathology. This is particularly true in preclinical AD, when the individual increases of these biomarkers may be statistically significant, but the effect sizes of these increases are not large.

### Limitations

This study has some limitations. It is a cross-sectional study, and findings need to be confirmed with longitudinal data. The 3 cohorts have differences in the design and goals, and not all of them had the same data available. Also, the definitions of A $\beta$  pathology differed between cohorts, which may limit comparability between them; however, the fact that the main results are validated in diverse studies confirms the robustness of our results. Finally, we did not include measurements of A $\beta$  in blood.

### Conclusions

Altogether, these results suggest that high plasma GFAP levels are found early in the AD continuum and become greater during disease progression, in parallel with clinical syndrome severity and markers of tau pathology. Our findings have important implications in facilitating the detection of AD, particularly in its preclinical stage. This earlier detection may accelerate primary and secondary prevention trials and the design of interventional studies at early stages of AD. Plasma GFAP, alone or in combination with other biomarkers, could be used to screen for A $\beta$ + individuals at any stage across the AD continuum.

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