Original Investigation

Association of Cerebrospinal Fluid β-Amyloid 1-42, T-tau, P-tau$_{181}$, and α-Synuclein Levels With Clinical Features of Drug-Naive Patients With Early Parkinson Disease

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IMPORTANCE We observed a significant correlation between cerebrospinal fluid (CSF) levels of tau proteins and α-synuclein, but not β-amyloid 1-42 (Aβ1-42), and lower concentration of CSF biomarkers, as compared with healthy controls, in a cohort of entirely untreated patients with Parkinson disease (PD) at the earliest stage of the disease studied so far.

OBJECTIVE To evaluate the baseline characteristics and relationship to clinical features of CSF biomarkers (Aβ1-42, total tau [T-tau], tau phosphorylated at threonine 181 [P-tau$_{181}$], and α-synuclein) in drug-naive patients with early PD and demographically matched healthy controls enrolled in the Parkinson's Progression Markers Initiative (PPMI) study.

DESIGN, SETTING, AND PARTICIPANTS Cross-sectional study of the initial 102 research volunteers (63 patients with PD and 39 healthy controls) of the PPMI cohort.

MAIN OUTCOMES AND MEASURES The CSF biomarkers were measured by INNO-BIA AlzBio3 immunoassay (Aβ1-42, T-tau, and P-tau$_{181}$; Innogenetics Inc) or by enzyme-linked immunosorbent assay (α-synuclein). Clinical features including diagnosis, demographic characteristics, motor, neuropsychiatric, and cognitive assessments, and DaTscan were systematically assessed according to the PPMI study protocol.

RESULTS Slightly, but significantly, lower levels of Aβ1-42, T-tau, P-tau$_{181}$, α-synuclein, and T-tau/Aβ1-42 were seen in subjects with PD compared with healthy controls but with a marked overlap between groups. Using multivariate regression analysis, we found that lower Aβ1-42 and P-tau$_{181}$ levels were associated with PD diagnosis and that decreased CSF T-tau and α-synuclein were associated with increased motor severity. Notably, when we classified patients with PD by their motor phenotypes, lower CSF Aβ1-42 and P-tau$_{181}$ concentrations were associated with the postural instability–gait disturbance–dominant phenotype but not with the tremor-dominant or intermediate phenotype. Finally, we found a significant correlation of the levels of α-synuclein with the levels of T-tau and P-tau$_{181}$.

CONCLUSIONS AND RELEVANCE In this first report of CSF biomarkers in PPMI study subjects, we found that measures of CSF Aβ1-42, T-tau, P-tau$_{181}$, and α-synuclein have prognostic and diagnostic potential in early-stage PD. Further investigations using the entire PPMI cohort will test the predictive performance of CSF biomarkers for PD progression.

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The Parkinson's Progression Markers Initiative (PPMI) was designed to identify Parkinson disease (PD) progression biomarkers and to define subsets of patients with PD by their clinical and biomarker signatures.[1,2] The PPMI study is a 5-year observational, international, multicenter longitudinal study of drug-naive patients with early-stage idiopathic PD (n = 400) and healthy controls (HCs; n = 200) recruited from 24 selected clinical sites (18 US sites, 5 European sites, and 1 Australian site) with expertise in PD research. These clinical sites were closely assessed by the PPMI steering committee as highly qualified centers to ensure standardization of data acquisition and biospecimen collection. Within the aims of the PPMI study, one of particular interest is to discover biomarkers that identify PD subgroups whose disease progression rates are likely to differ. To meet the objectives of the PPMI study, we evaluated the baseline characteristics of cerebrospinal fluid (CSF) biomarkers (β-amyloid 1-42 [Aβ1-42], total tau [T-tau], tau phosphorylated at threonine 181 [P-tau$_{181}$], and α-synuclein [α-syn]) in patients with PD and HCs and the relationship between CSF biomarkers and clinical features of PD in the initial 102 subjects enrolled at 15 clinical sites. The implementation of standardized procedures including preanalytical and analytical steps involved in the acquisition of CSF, aliquoting, storage at −80°C, and assessment of hemolysis by measurement of hemoglobin (Hb) in each collected sample has been important to the study.

Among several subtypes of PD defined on the basis of clinical characteristics as well as underlying neuropathology,[3-6] cognitive impairment in PD, which is a common nonmotor comorbidity, progresses to overt dementia in approximately 80% of patients with PD, with wide variations in duration from onset of PD to the emergence of dementia onset.[7-10] For several reasons, including the increased cost of care and the higher mortality rate in PD with dementia compared with nondemented patients with PD,[11,12] early prediction of dementia is critical to the clinical management of patients with PD as well as to stratifying patients at highest risk for dementia in clinical trials of disease-modifying therapies that could slow dementia onset and progression. A motor phenotype dominated by postural instability-gait disturbance (PIGD) has been associated with more rapid cognitive decline and/or more functional disability in patients with PD compared with the tremor-dominant (TD) PD phenotype.[3,13-16]

Cerebrospinal fluid measurements of Aβ1-42, T-tau, and P-tau$_{181}$ are widely recognized as sensitive and specific assays for the early diagnostic distinction of patients with Alzheimer disease (AD) from cognitively normal individuals, for predicting the progression from mild cognitive impairment to AD, and for discriminating AD- vs non-AD-type neurodegenerative diseases.[17-23] Several studies have reported that CSF Aβ1-42 levels in patients with PD with or without dementia are lower than in HCs[24-29] and recent data show that CSF levels of T-tau or P-tau$_{181}$ in patients with PD are significantly lower than those in HCs,[28-30] but other data do not confirm such differences.[24-27,31] The reasons for such discrepancy may include but not are limited to methodologic variables including CSF processing, the biomarker assays used, and the diversity in criteria for elderly controls and in the stage of PD across the studies. Interestingly, a recent prospective cohort study with 2 years of longitudinal follow-up evaluation suggested that reduced concentration of CSF Aβ1-42 (≤192 pg/mL), but not T-tau or P-tau$_{181}$, is an independent predictor of cognitive decline in patients with PD.[32] Cerebrospinal fluid α-syn concentrations may be reduced in PD and related disorders compared with healthy subjects.[29,31,33] However, to our knowledge, studies evaluating the association of these CSF biomarkers measured using validated and standardized methods in study subjects with clinical features of patients with very early-stage PD enrolled at multinational qualified clinical sites are very limited. Therefore, the simultaneous measurement of Aβ1-42, T-tau, P-tau$_{181}$, and α-syn in CSF of PPMI study subjects may provide diagnostic value and/or biological insight for progression of disease in patients with early-stage PD. Moreover, although previous studies have revealed an association between α-syn and tau or Aβ1-42 in vitro and in transgenic animals,[34-36] there is no report to our knowledge describing the relationship between AD-related biomarkers and α-syn in human antemortem CSF samples from patients with PD and HCs. We hypothesized that measurement of Aβ1-42, T-tau, P-tau$_{181}$, and α-syn in CSF can differentiate patients with early-stage PD from demographically matched HCs and can reflect the heterogeneity of clinical features of PD. To test our hypothesis, we report the CSF profile of Aβ1-42, T-tau, P-tau$_{181}$, and α-syn in the initial 102 PPMI subjects and assess whether specific clinical features of PD are associated with distinct biomarker signatures for Aβ1-42, T-tau, P-tau$_{181}$, and α-syn in untreated patients with PD at the earliest stage of the disease studied so far.

Methods

Participants

The PPMI study is an ongoing international multicenter study involving PD centers in Europe, Australia, and the United States as described in detail elsewhere[2] and at the PPMI website (http://www.ppmi-info.org/study-design/). The study was approved by the institutional review board of all participating sites. Written informed consent was obtained from all participants before inclusion in the study. All subjects were comprehensively assessed at the screening and baseline (BL) visits for clinical (motor, neuropsychological, and cognitive) characteristics by the site investigators. A diagnosis of PD in all patients was made less than 2 years before the screening visit, and only patients with a Hoehn and Yahr stage of I or II and a dopamine transporter deficit on DaTscan imaging were enrolled, while demographically comparable cognitively normal HCs free of a current or active neurological disorder and with no detectable dopamine transporter deficit evidence of PD also were recruited into the PPMI study as controls. Subjects in this study have regularly scheduled assessments to collect clinical data and to participate in biomarker studies, including acquisition of CSF. Included in this 5-year longitudinal PPMI study is the analysis of CSF Aβ1-42, T-tau, P-tau$_{181}$, and α-syn. Herein, we report our analyses of these CSF biomarkers in the initial 102 subjects (63 subjects with PD, 39 HCs) to test our hypothesis. Subjects without evidence of dopamine transporter deficit on DaTscan who showed parkinsonian symptoms and provided CSF at the BL visit (n = 4) were

excluded from the PD group, and their data were also excluded from further statistical analyses in this report. Demographic information and clinical characteristics, including diagnosis, disease severity based on the Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale ratings and the Hoehn and Yahr stage, and results of neuropsychological and cognitive function tests, were downloaded from the PPMI database according to guidelines for data access and use. For this study, we classified PD as manifesting the TD (TD-PD), PIGD (PIGD-PD), or intermediate (IND-PD) phenotype in a modification of the method previously described (eAppendix in Supplement).[3]

CSF Sample Collection and Handling

Baseline CSF collection was performed at each study site as described in the PPMI biologics manual (http://www.ppmi-info.org/). Briefly, CSF was collected into siliconized polypropylene tubes, and the first 1 to 2 mL of CSF was sent to the site's local laboratory for routine testing for cell count, total protein level, and glucose level. An additional 15 to 20 mL of CSF was transferred into 15-mL conical polypropylene tubes at room temperature, mixed gently, centrifuged at 2000*g* for 10 minutes at room temperature, and transferred into 1.5-mL precooled siliconized polypropylene aliquot tubes followed by immediate freezing on dry ice. The frozen aliquots of CSF were shipped overnight to the PPMI Biorepository Core laboratories on dry ice and then thawed, aliquoted into 0.5-mL siliconized polypropylene tubes, refrozen once, and stored at −80°C. Thus, each PPMI CSF aliquot will have undergone 2 freeze-thaw cycles. Since previous investigations have shown stability of CSF biomarkers for at least 2 freeze-thaw cycles, freeze-thaw is not expected to be a contributor to the total variance associated with each biomarker measurement.[37,38] Coded frozen aliquots from the first 102 BL CSF samples were transferred to the University of Pennsylvania and to Covance for the studies described here.

Analysis of CSF Biomarkers

Measurements of Aβ1-42, T-tau, and P-tau$_{181}$ were taken in each of 102 CSF aliquots at the University of Pennsylvania using the multiplex Luminex xMAP platform (Luminex Corp) with research-use-only Fujirebio-Innogenetics INNO-BIA AlzBio3 immunoassay kit-based reagents (Innogenetics Inc) of a single lot as described previously.[17,39] All standards, aqueous controls, and CSF samples (including 2 CSF pools for quality control, 75 µL of each) were analyzed in duplicate in each run as described.[17,39] A result was defined as the arithmetic mean of the calculated concentration of duplicates. Cerebrospinal fluid α-syn was analyzed at Covance using a commercially available enzyme-linked immunosorbent assay kit (Covance) that was developed and optimized from an assay previously described.[31,40] Briefly, 200 µL/well of diluted α-syn standards (range, 6.1-1500 pg/mL) using reconstituted stock and diluted duplicate CSF samples (200 µL/well) were added to the capture antibody-coated plate after washing the plate 4 times. After overnight incubation of the plate at 2°C to 8°C with shaking, 50 µL/well of biotinylated detector antibody was added followed by incubation for 2 hours at room temperature. Diluted streptavidin horseradish peroxidase was added, and the plate was incubated at room temperature for an additional 1 hour. After washing the plate 4 times, a mixture of 2 different chemiluminescent substrates was added and end-point luminescence was read with a luminometer (Synergy 2; BioTek). The concentration of α-syn was measured using standard curves with 4-parameter curve fitting. There is no cross-reactivity of the antibodies used in this enzyme-linked immunosorbent assay with β-syn or γ-syn.[31,40] Cerebrospinal fluid Hb was analyzed at Covance using an enyzme-linked immunosorbent assay method with reagents obtained from Bethyl Laboratories according to the manufacturer's instruction. This was done to evaluate the quality of CSF collection, to use as an index of the degree of blood contamination, and to control for the possible effect of hemolysis on the CSF α-syn level.[33] After completion of these CSF biomarker analyses, the code for each of the subjects was opened.

Statistical Analysis

Statistical analysis was performed using SAS version 9.3 (SAS Institute, Inc) and GraphPad Prism version 5.0 (GraphPad Software, Inc) statistical software. Mann-Whitney *U* test was used to assess differences between 2 groups, and Kruskal-Wallis test with Dunn correction was used for multiple comparisons for 3 or more groups. All patient data included in this study were simultaneously downloaded by 2 laboratories (University of Pennsylvania and University of Iowa), which agreed on statistical analyses performed independently. The correlations were evaluated using linear regression analysis (Pearson correlation). The analyses were also done using an analysis of covariance model to check our results after controlling for possible confounding variables, ie, age, sex, and education. To explore the association between biomarkers and clinical variables, we used multivariate logistic regression (MLGR) or multivariate linear regression models and adjusted for confounding factors (age, sex, and education). The diagnostic utility of each biomarker (sensitivity and specificity) was determined by receiver operating characteristic curve analysis using cut points giving the highest Youden index, [sensitivity + specificity] − 1.[41] Differences in percentage of subjects in each group were evaluated by χ^2 test. Values with $P < .05$ were regarded as statistically significant.

Results

Analytical Performance of CSF Biomarkers Measurement

The analytical performance of the Luminex xMAP platform and AlzBio3 immunoassay research-use-only reagents for AD CSF biomarkers in PPMI subjects was comparable to that reported in our earlier studies.[17,39] The mean percentage coefficients of variation across 4 runs of Aβ1-42, T-tau, and P-tau$_{181}$ measurement were 3.8%, 5.6%, and 4.4% for aqueous controls and 7.5%, 6.4%, and 3.0% for 2 CSF pools, respectively. The analytical performance of α-syn measurement by enzyme-linked immunosorbent assay showed that the mean percentage coefficient of variation for duplicates of α-syn measurements was 6.0% for 102 CSF samples (range, 0.8%-13.2%), and the mean percentage coefficient of variation of 8 calibrators through 11 runs was 7.9% (range, 6.9%-9.8%).

Table 1. Demographic Information and Cerebrospinal Fluid Hemoglobin Levels

Characteristic	HCs (n = 39)	PD (n = 63)	*P* Value
Age, mean (SD) [95% CI], y	58 (13) [54-63]	62 (10) [59-64]	.24[a]
Female/male, No. (% male)	18/21 (53.8)	24/39 (61.9)	.42[b]
Education, mean (SD) [95% CI], y	16.8 (2.4) [16.0-17.6]	16.4 (2.5) [15.8-17.0]	.15[a]
Age at diagnosis, mean (SD) [95% CI], y	...	61.1 (10.0) [58.6-63.7]	...
Disease duration, median (range), y	...	0.4 (0.0-2.6)	...
Subjects with CSF Hb >200 ng/mL, No.	6	18	.13[b]
CSF total protein, mean (SD) [95% CI], mg/dL	40 (12) [36-43]	46 (21) [40-51]	.32[a]

Abbreviations: CSF, cerebrospinal fluid; Hb, hemoglobin; HCs, healthy controls; PD, Parkinson disease; ellipses, not applicable.

[a] Based on Mann-Whitney *U* test.

[b] Based on χ^2 test.

Table 2. Comparison of Clinical Outcomes Between Healthy Controls and Patients With Parkinson Disease

Outcome	Mean (SD)		*P* Value[a]
	HCs (n = 39)	PD (n = 63)	
Hoehn and Yahr stage	0.03 (0.16)	1.65 (0.51)	<.001
MDS-UPDRS part III motor score	1.6 (2.7)	22.6 (7.6)	<.001
Tremor score	0.05 (0.13)	0.53 (0.32)	<.001
PIGD score	0.01 (0.04)	0.24 (0.26)	<.001
UPSIT score	35.1 (3.4)	21.9 (8.1)	<.001
Striatal binding ratios for PR/PL/CR/CL, mean	1.38/1.39/2.06/2.05	0.62/0.64/1.35/1.34	<.001
MoCA, mean (SD) [95% CI]	28.4 (1.0) [28.0-28.7]	27.2 (2.0) [26.7-27.7]	.005
Semantic fluency	53.8 (12.1)	49.5 (10.6)	.06
WMS-III LNS test score	12.1 (2.8)	11.0 (2.0)	.05
SDMT score	48.6 (11.2)	41.9 (8.9)	.005
HVLT-R score			
Total recall	9.0 (1.6)	8.2 (1.5)	.008
Delayed recall	9.9 (2.3)	8.3 (2.3)	<.001

Abbreviations: CL, left caudate; CR, right caudate; HCs, healthy controls; HVLT-R, Hopkins Verbal Learning Test-Revised; MDS-UPDRS, Movement Disorder Society–sponsored revision of the Unified Parkinson's Disease Rating Scale; MoCA, Montreal Cognitive Assessment; PD, Parkinson disease; PIGD, postural instability–gait disturbance; PL, left putamen; PR, right putamen; SDMT, Symbol Digit Modalities Test; UPSIT, University of Pennsylvania Smell Identification Test; WMS-III LNS, Wechsler Memory Scale third edition Letter-Number Sequencing.

[a] Assessed by Mann-Whitney *U* test.

Demographic Information and Effects of Age, Sex, and CSF Hb

There were no significant differences in mean age, sex distribution, educational level, and median CSF Hb concentration between HCs and patients with PD (**Table 1**). The median duration from diagnosis of PD to BL was 0.4 years, and the Hoehn and Yahr stage of the patients was limited to I or II at BL.

Previous studies demonstrated that contamination of blood in CSF could have an influence on the level of some proteins, including α-syn and Aβ1-42.[29,33] Therefore, we evaluated the relationship between the CSF biomarkers and CSF Hb levels. No association was observed between levels of CSF Hb and T-tau, P-tau$_{181}$, or Aβ1-42, consistent with earlier reports that there is no effect of added blood on measured concentrations of these biomarkers.[33,42] The CSF α-syn level was modestly increased by CSF Hb contamination at concentrations higher than 1000 ng/mL. A trend toward increasing values of α-syn was observed at very high CSF Hb concentrations (Pearson r = 0.3975; P < .001) (eFigure in Supplement).

Comparison of Clinical Characteristics Between HCs and Patients With PD

As described in detail elsewhere,[1,2] various neuropsychological and cognitive function tests were performed in the PPMI subjects, including the following: Montreal Cognitive Assessment, Wechsler Memory Scale third edition Letter-Number Sequencing test for verbal working memory, Hopkins Verbal Learning Test-Revised, Symbol Digit Modalities Test for screening of cognitive impairment, semantic fluency test to detect cognitive decline, University of Pennsylvania Smell Identification Test for olfactory function, 15-item Geriatric Depression Scale for depression, and State-Trait Anxiety Inventory to measure emotional state anxiety. As expected, almost all measured test scores of cognitive function in patients with PD were significantly different from those of the HC group (**Table 2**). In addition to evidence of decreased cognitive function, mean University of Pennsylvania Smell Identification Test scores in patients with PD were significantly lower than those in HCs. However, mean 15-item Geriatric Depression Scale and State-Trait Anxiety Inventory scores were not significantly different between the 2 groups (data not shown).

Comparison of CSF Biomarkers Between HCs and Patients With PD

Comparisons of CSF biomarker concentrations between patients with PD and HCs are summarized in **Table 3**. Because we observed a small but significant correlation between CSF Hb and α-syn levels in 102 subjects, we stratified subjects by their CSF Hb levels. We observed that CSF α-syn levels in patients with PD were significantly lower than those in HCs (P = .01), and this was still observed after exclusion of subjects with CSF Hb levels higher than 200 ng/mL (P = .02) or 500 ng/mL (P = .007) (**Figure 1**). Levels of Aβ1-42, P-tau$_{181}$, T-tau, and T-tau/Aβ1-42 were significantly lower in the PD group as compared with HCs (**Figure 2**),

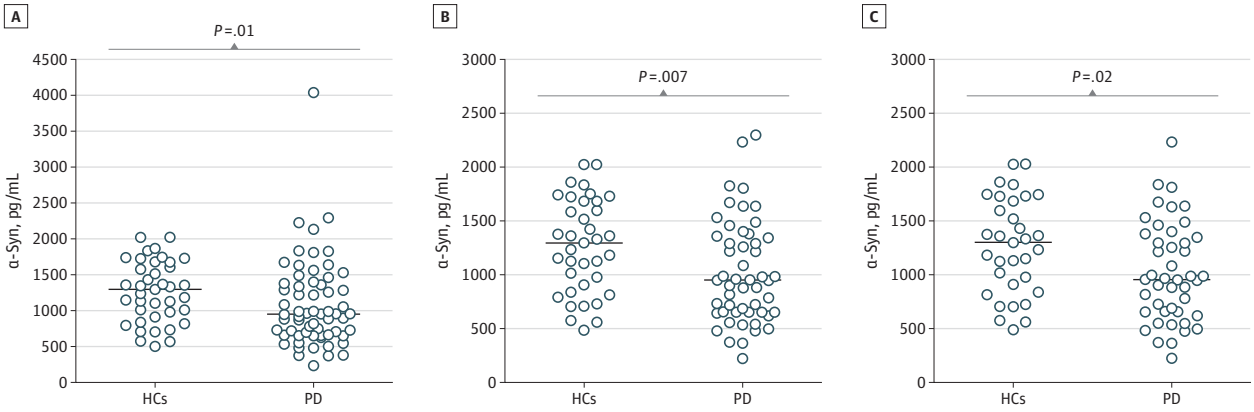
Table 3. Comparison of Cerebrospinal Fluid Biomarker Levels Between Healthy Controls and Patients With Parkinson Disease

Biomarker	Mean (SD) [95% CI] HCs (n = 39)	PD (n = 63)	*P* Value[a]
Aβ1-42, pg/mL	242.8 (49.95) [226.7-259.0]	228.7 (45.63) [217.2-240.2]	.047
T-tau, pg/mL	53.9 (19.33) [47.6-60.1]	46.1 (24.71) [39.8-52.3]	.03
P-tau$_{181}$, pg/mL	24.9 (8.45) [22.2-27.6]	21.0 (7.83) [19.0-23.0]	.009
T-tau/Aβ1-42 ratio	0.240 (0.141) [0.195-0.286]	0.215 (0.157) [0.176-0.255]	.045
P-tau$_{181}$/Aβ1-42 ratio	0.113 (0.075) [0.089-0.138]	0.099 (0.063) [0.084-0.115]	.15
P-tau$_{181}$/T-tau ratio	0.491 (0.160) [0.439-0.543]	0.543 (0.263) [0.477-0.609]	.68
α-Syn, pg/mL	1264 (425.7) [1126-1403]	1082 (611.1) [928-1235]	.01

Abbreviations: Aβ1-42, β-amyloid 1-42; α-Syn, α-synuclein; HCs, healthy controls; PD, Parkinson disease; P-tau$_{181}$, tau phosphorylated at threonine 181; T-tau, total tau.

[a] Assessed by Mann-Whitney *U* test.

Figure 1. Scatterplots of Cerebrospinal Fluid α-Synuclein Concentrations in Healthy Controls and Patients With Parkinson Disease



Each group was differentiated according to their cerebrospinal fluid hemoglobin concentration to total subjects (A), subjects with a cerebrospinal fluid hemoglobin concentration less than 500 ng/mL (B), and subjects with a cerebrospinal fluid hemoglobin concentration less than 200 ng/mL (C). *P* values were assessed by Mann-Whitney *U* test. α-Syn indicates α-synuclein; HCs, healthy controls; and PD, Parkinson disease.

although significant overlap in levels of CSF biomarkers between patients with PD and HCs was observed.

Correlation Between α-Syn and Tau Proteins in CSF

As shown in **Figure 3**, CSF α-syn levels strongly correlated with measures of CSF T-tau in both patients with PD (Pearson $r = 0.7899$; $P < .001$) and HCs (Pearson $r = 0.6863$; $P < .001$). The correlation between levels of CSF α-syn and P-tau$_{181}$ also was significant in both patients with PD (Pearson $r = 0.5481$; $P < .001$) and HCs (Pearson $r = 0.3173$; $P = .049$), but the correlations were weaker than for CSF T-tau. There was no correlation between measures of CSF Aβ1-42 and α-syn in either group ($r = 0.1553$ for patients with PD and $r = -0.0450$ for HCs).

Association of CSF Biomarkers With PD Diagnosis and Motor Severity of PD

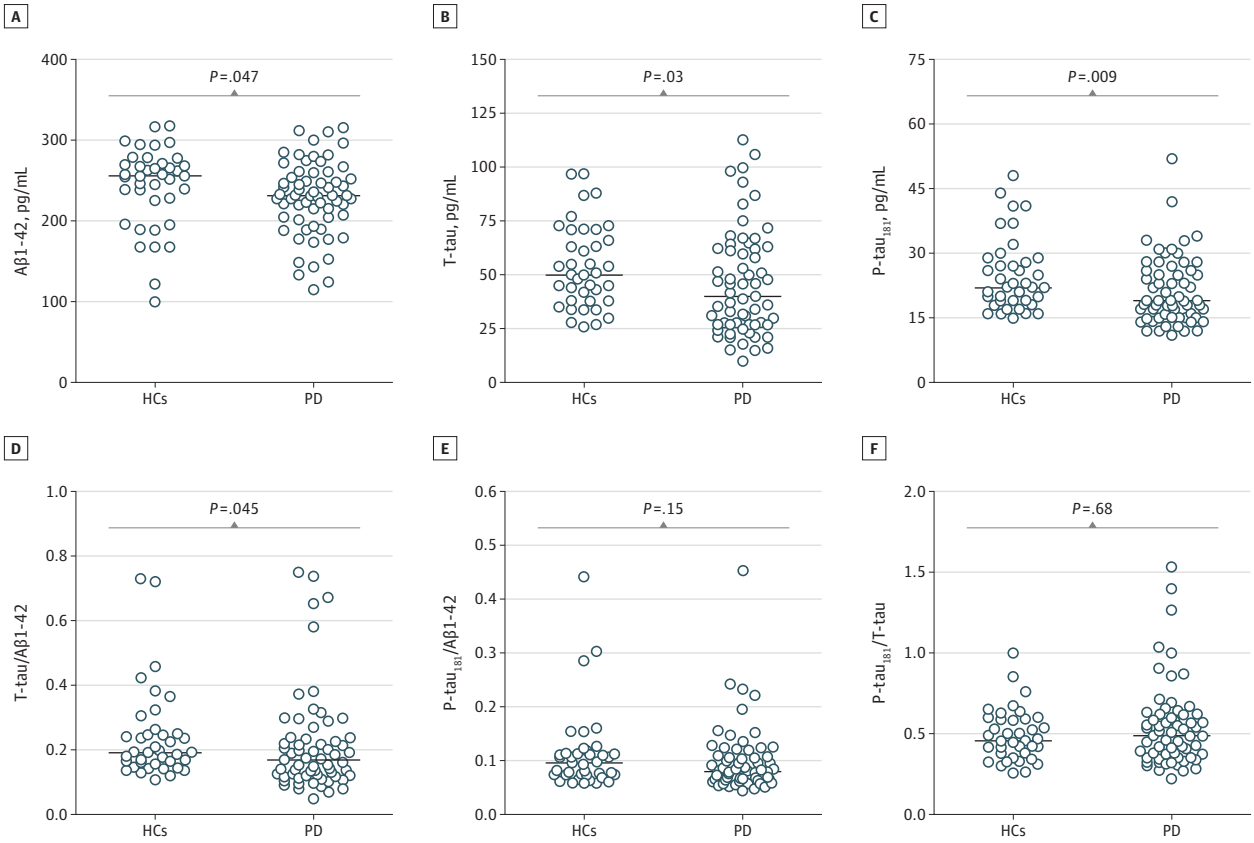
As part of the process of development of an MLGR model for the association of CSF biomarkers with PD diagnosis, we first performed bivariate analysis of each CSF biomarker and PD clinical features along with adjustment for confounders (age, sex, and education). This step revealed that CSF T-tau ($P = .02$), P-tau$_{181}$ ($P = .005$), and α-syn ($P = .04$) were significantly associated with PD diagnosis. However, when the stepwise selection method was used to generate the final MLGR model, including all CSF biomarkers with adjustment for confounders, only lower levels of CSF Aβ1-42 (odds ratio = 1.010; 95% CI, 1.001-1.020; $P = .04$) and P-tau$_{181}$ (odds ratio = 1.102; 95% CI, 1.038-1.170; $P = .002$) were significantly associated with PD diagnosis. When we used receiver operating characteristic curve analyses to calculate sensitivity and specificity to differentiate patients with PD from HCs, the area under the receiver operating characteristic curve for each CSF biomarker for PD diagnosis was relatively low, consistent with a previous study (data not shown).[29]

Using multivariate linear regression analysis with adjustment for confounders to explore the association of CSF biomarkers with motor severity of patients with PD, we found that α-syn (β = 0.00679 [SE = 0.00247]; $P = .008$) was significantly associated with the Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale part III motor score and T-tau was marginally associated (β = −0.14771 [SE = 0.07111]; $P = .04$). The CSF α-syn level was marginally associated with Hoehn and Yahr stage (β = −0.00021269 [SE = 0.0001044]; $P = .046$). However, no CSF biomarkers were significantly associated with cognitive function test scores.

Association of CSF Biomarkers With PIGD Motor Phenotype

Based on previous studies showing that the progression of cognitive dysfunction may be predictive of poorer prognosis in

Figure 2. Scatterplots of Alzheimer Disease Cerebrospinal Fluid Biomarkers and Their Ratios in Healthy Controls and Patients With Parkinson Disease



Scatterplots of β-amyloid 1-42 (Aβ1-42) (A), total tau (T-tau) (B), and tau phosphorylated at threonine 181 (P-tau$_{181}$) (C) and ratios of T-tau/Aβ1-42 (D), P-tau$_{181}$/Aβ1-42 (E), and P-tau$_{181}$/T-tau (F) are shown. *P* values were assessed by Mann-Whitney *U* test. HCs indicates healthy controls; PD, Parkinson disease.

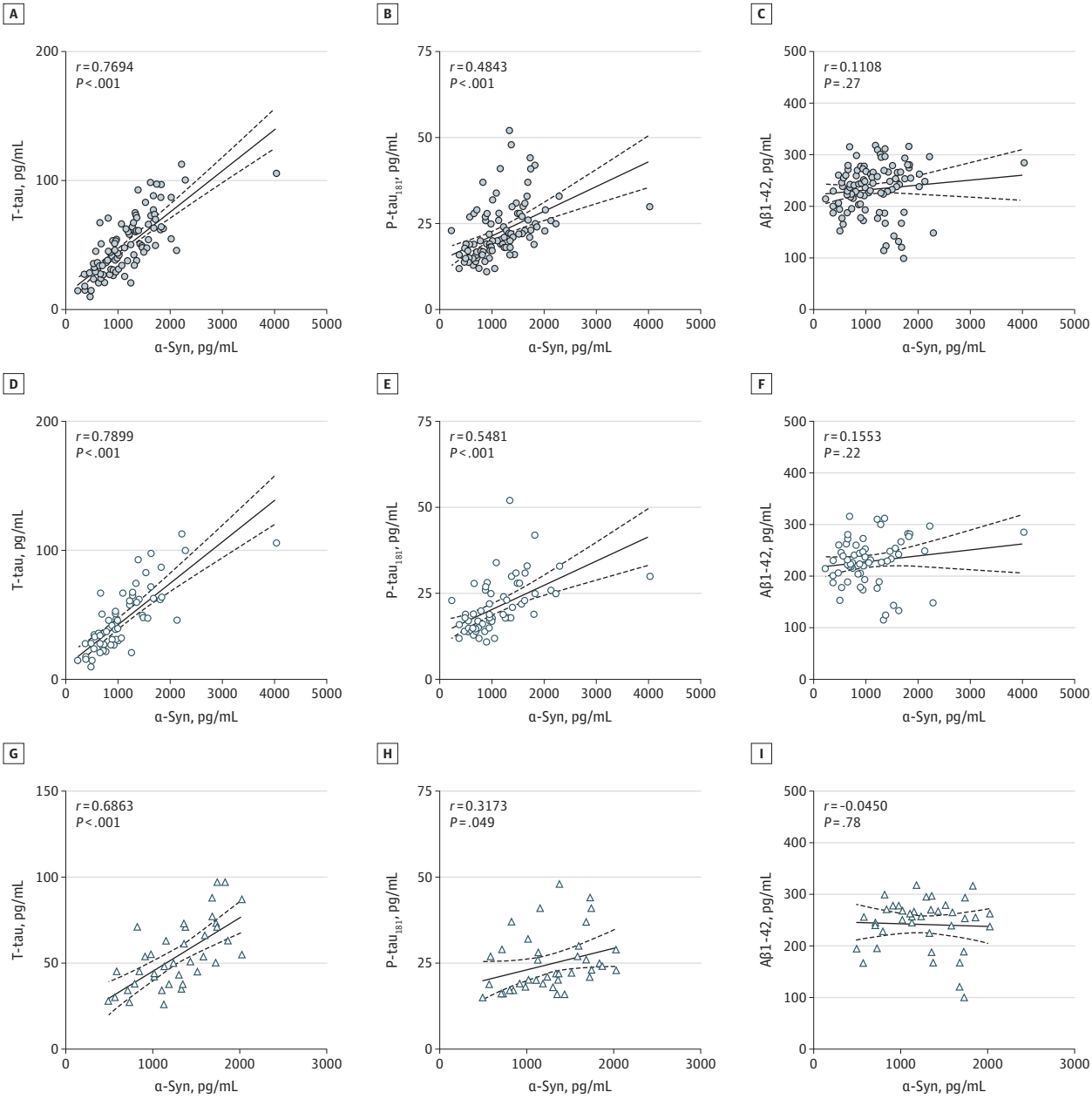
patients with PD who have PIGD-PD rather than TD-PD symptoms,[3,13-16] we compared CSF biomarkers between TD-PD and PIGD-PD. Of the 63 patients with PD, 14 were classified as having PIGD-PD, 43 as having TD-PD, and 6 as indeterminate (IND-PD) (**Table 4**). There were no significant differences for patients with PIGD-PD vs those with TD-PD and those with IND-PD in age (mean [SD], 60 [9.2] vs 63 [9.6] and 55 [13.8] years, respectively), age at diagnosis (mean [SD], 60 [9.4] vs 63 [9.5] and 54 [13.5] years, respectively), education (mean [SD], 16.6 [3.4] vs 16.4 [2.2] and 16.2 [1.6] years, respectively), and sex distribution (male to female ratio, 7:7 vs 29:14 and 3:3, respectively). When we compared the CSF biomarkers studied here between patients with PIGD-PD and those with TD-PD, CSF Aβ1-42 and P-tau$_{181}$ levels in those with PIGD-PD were significantly lower than in those classified as having TD-PD (Table 4). Moreover, CSF α-syn levels in the subjects with PIGD-PD were lower than those in the TD-PD group, albeit with only marginal significance (*P* = .06), but this reached statistical significance when patients with CSF Hb levels greater than 200 ng/mL were excluded (*P* = .03). Interestingly, when we compared levels of CSF biomarkers among patients with TD-PD, PIGD-PD, and IND-PD and HCs, levels of all CSF biomarkers (not including T-tau/Aβ1-42, P-tau$_{181}$/Aβ1-42, and P-tau$_{181}$/T-tau ratios) in those with PIGD-PD were significantly lower than in HCs, while those in patients with TD-PD or IND-PD were comparable to those in HCs (Kruskal-Wallis test with correction for multiple comparisons using Dunn test). In addition, we found that lower levels of CSF Aβ1-42 and P-tau$_{181}$ were significantly associated with the PIGD motor phenotype, with odds ratios of 1.023 (95% CI, 1.006-1.040; *P* = .008) and 1.124 (95% CI, 1.014-1.247; *P* = .03), respectively, using MLGR analysis with adjustment for confounders.

Discussion

To minimize the preanalytical and analytical variability in CSF biomarker measurement and to assure the quality of this study, we performed the research described here using highly standardized procedures (eg, use of siliconized polypropylene CSF aliquot tubes, centralized analyses of CSF biomarkers, and assessment of blood contamination by CSF Hb measurement). The quality of CSF biomarker measurements in this study is supported by the observed consistency (low run-to-run percentage coefficient of variation) for the CSF biomarker levels and control for the possible influence of hemolysis on α-syn values.

This study is the first cross-sectional report on CSF biomarkers (Aβ1-42, T-tau, P-tau$_{181}$, and α-syn) in PPMI subjects. We found several characteristics of CSF biomarkers related to

Figure 3. Correlation of Cerebrospinal Fluid α-Synuclein Levels With Total Tau, Tau Phosphorylated at Threonine 181, and β-Amyloid 1-42



Correlations of cerebrospinal fluid α-synuclein (α-syn) levels with total tau (T-tau) (A), tau phosphorylated at threonine 181 (P-tau$_{181}$) (B), and β-amyloid 1-42 (Aβ1-42) (C) in all 102 subjects, with T-tau (D), P-tau$_{181}$ (E), and Aβ1-42 (F) in 63 patients with Parkinson disease, and with T-tau (G), P-tau$_{181}$ (H), and Aβ1-42 (I) in 39 healthy controls are shown. Solid lines indicate linear regression; dotted lines, 95% CIs. *P* values were assessed by Pearson correlation.

clinical features of early-stage PD in this study. First, the levels of CSF Aβ1-42, T-tau, P-tau$_{181}$, T-tau/Aβ1-42, and α-syn in patients with PD were significantly lower than those in demographically matched HCs, and CSF Aβ1-42 and P-tau$_{181}$ levels differentiated patients with PD from HCs in our MLGR model. Second, the observed PD-associated lower levels of CSF Aβ1-42, T-tau, P-tau$_{181}$, and α-syn may be driven primarily by the PIGD-PD subgroup since these biomarkers were lower in the patients with PIGD-PD vs HCs but did not differ comparing HCs and patients with TD-PD or IND-PD. Third, CSF T-tau and α-syn concentrations were significantly associated with severity of motor dysfunction in PD. Finally, we found that CSF α-syn levels had a strong correlation with the levels of CSF tau proteins (T-tau and P-tau$_{181}$), particularly in patients with PD.

In contrast to AD, we observed lower levels of tau proteins in CSF of patients with PD as compared with HCs, which is consistent with the results of some[28,29] but not all prior studies.[24-27] To our knowledge, the PPMI cohort is the first cohort of entirely untreated subjects with PD to demonstrate a reduction in tau proteins compared with HCs. Similar to AD, albeit with dif-

Table 4. Comparison of Cerebrospinal Fluid Biomarkers Between Patients With Parkinson Disease Who Have the Tremor-Dominant vs Postural Instability–Gait Disturbance Motor Phenotype

Biomarker	Mean (SD)		*P* Value for Mann-Whitney *U* Test[a]	Mean (SD)		*P* Value for Kruskal-Wallis Test[b]	Significance (PIGD-PD vs HC, After Dunn Test)[c]
	PIGD-PD (n = 14)	TD-PD (n = 43)		HCs (n = 39)	IND-PD (n = 6)		
Aβ1-42, pg/mL	211.4 (45.0)	236.2 (46.8)	.03	242.8 (50.0)	215.5 (25.0)	.02	Yes, .01 < *P* < .05
T-tau, pg/mL	39.3 (28.27)	50.3 (24.01)	.05	53.9 (19.33)	31.2 (9.97)	.007	Yes, .01 < *P* < .05
P-tau$_{181}$, pg/mL	18.0 (6.74)	22.5 (8.17)	.04	24.9 (8.45)	17.7 (4.97)	.005	Yes, .001 < *P* < .01
α-Syn, pg/mL							
Total subjects	892.8 (542.4)	1185 (649.6)	.06	1264 (425.7)	782.6 (150.1)	.008	Yes, .01 < *P* < .05
Subjects with CSF Hb <200 ng/mL	766.3 (446.3)	1122 (451.8)	.03	1267 (443.5)	775.9 (184.8)	.01	Yes, .001 < *P* < .01
T-tau/Aβ1-42 ratio	0.211 (0.213)	0.225 (0.145)	.11	0.240 (0.141)	0.151 (0.072)	.03	No
P-tau/Aβ1-42 ratio	0.093 (0.059)	0.104 (0.068)	.22	0.113 (0.075)	0.083 (0.026)	.25	No
P-tau/T-tau ratio	0.617 (0.398)	0.513 (0.217)	.76	0.491 (0.160)	0.588 (0.164)	.61	No

Abbreviations: Aβ1-42, β-amyloid 1-42; α-Syn, α-synuclein; CSF, cerebrospinal fluid; Hb, hemoglobin; HCs, healthy controls; IND, intermediate; PD, Parkinson disease; PIGD, postural instability–gait disturbance; P-tau$_{181}$, tau phosphorylated at threonine 181; TD, tremor-dominant; T-tau, total tau.

[a] Assessed by Mann-Whitney *U* test of comparison between PIGD-PD and TD-PD.

[b] If the comparison of biomarker level among 4 groups by nonparametric analysis of variance (Kruskal-Wallis test) resulted in *P* < .05, it was followed by the Dunn corrected multiple-comparisons test.

[c] Significance refers to the comparison of PIGD-PD vs HCs after correction with the Dunn multiple-comparisons test.

ferent magnitudes, our study, like several previous studies, detected lower levels of CSF Aβ1-42 in patients with PD compared with HCs,[24-29] and lower levels of CSF Aβ1-42 have been reported to be associated with rapid decline of cognitive function in PD.[32] One interpretation for lower concentration of CSF tau proteins in our patients with early PD is that the interaction between tau proteins and other proteins including α-syn may limit the release of tau proteins into CSF. It is interesting to note that lower CSF concentrations are observed in other neurodegenerative disorders as shown for frontotemporal lobar degeneration,[21,43] suggesting that the observed lowering of these biomarkers that traditionally reflect neurodegeneration is caused by an as yet unknown pathophysiological mechanism. In connection with our finding of a strong correlation between CSF tau proteins (T-tau and P-tau$_{181}$) and α-syn, particularly in patients with PD (Figure 3), previous studies using animal models or postmortem brain report that CNS α-syn pathology is accompanied by increased levels of hyperphosphorylated tau proteins.[44-47] Indeed, tau-positive tangles and α-syn–positive Lewy bodies may colocalize in the same neuron in AD brains,[48] while both cross seed the fibrillization of each other.[35] Moreover, a recent genome-wide association study demonstrated significant genetic association with PD of the genes *MAPT* and *SNCA* encoding tau and α-syn, respectively.[49] Thus, taken together, these findings are consistent with the notion that the deposition of α-syn in the PD brain may cause the decrease of α-syn in CSF of patients with PD and also may inhibit the release of tau proteins into CSF by unknown molecular mechanisms. Our finding of a significant correlation between T-tau and α-syn in HCs could result from the same putative mechanism suggested for the situation in subjects with PD. However, there is little direct evidence that α-syn interacts with tau proteins at the molecular level in the brain of patients with PD,[46,47,50] although evidence for this has been reported in transgenic mouse models of PD-like α-syn pathology.[35] The results of our study suggest an interaction between tau and α-syn in the brain of cognitively normal elderly subjects based on the result of significant correlation between these 2 biomarkers observed in the HC group. We believe that this result does not contradict the hypothetical pathophysiological relationship between α-syn and tau since Lewy body pathology is frequently found in elderly subjects with normal cognitive function,[51] and thus the CSF α-syn correlation with tau proteins' concentration in HCs is consistent with the observed pathology in elderly cognitively normal subjects. Further evidence for direct and/or indirect physiological and pathological interaction between α-syn and tau proteins in human brain will be needed to explain the simultaneous decrease of CSF tau proteins and α-syn in subjects with PD as compared with HCs.

Consistent with previous reports,[29,31,33] the diagnostic utility of CSF α-syn or Aβ1-42, T-tau, or P-tau$_{181}$ to differentiate patients with PD from HCs is low. Although the levels of CSF Aβ1-42 and P-tau$_{181}$ are significantly associated with PD diagnosis, neither biomarker had an area under the receiver operating characteristic curve greater than 0.8 for PD diagnosis. The combination of other biomarkers including genetic markers and plasma or CSF measures of DJ-1, for example, will also likely be necessary to improve the diagnostic utility of the CSF biomarkers studied here.[29,33]

Intriguingly, we found that several CSF proteins measured here may be promising biomarkers for a specific motor endophenotype within PD—the PIGD-PD motor subtype. Specifically, lower CSF Aβ1-42, T-tau, P-tau$_{181}$, and α-syn concentrations were found in the individuals with the PIGD-PD motor subtype, and in an MLGR model, CSF Aβ1-42 and P-tau$_{181}$ levels were significantly associated with PIGD-PD. Consistent with these results was the recent report of lower CSF Aβ1-42 level in the PIGD phenotype of newly diagnosed and untreated patients with PD as compared with the TD phenotype or HCs, although the motor phenotype was assessed by the Unified Parkinson's Disease Rating Scale instead of the Movement Disorder Society–sponsored revision of the Unified Parkinson's Disease Rating Scale that we used in this study.[52] The significance of this finding is that the PIGD-PD motor subtype has been reported

to progress more quickly,[3,13,16] along both motor and nonmotor trajectories. Albeit preliminary, our findings are particularly important as our patients are in early stages of PD and many subjects have not fully "declared" themselves as having either PIGD-PD or TD-PD. Since the data suggest that these CSF protein patterns may be able to differentiate between the various motor subtypes even in early stages of PD, they may turn out to be promising biomarkers of differential trajectories of PD progression. However, we recognize that further studies in the PPMI cohort with a large number of subjects with each motor phenotype are needed to adequately address this question.

Several limitations of our study should be noted. This is a cross-sectional study using data from the initial 102 subjects from the PPMI study cohort, thereby limiting our ability to determine the relationships between the CSF biomarkers studied here and disease progression. In addition, we did not evaluate genetic factors or other biomarker candidates, including DJ-1. However, these limitations will be resolved in future PPMI analyses.

In conclusion, based on this first report of our analyses of CSF Aβ1-42, T-tau, P-tau$_{181}$, and α-syn biomarkers in the first 102 members of the PPMI cohort, our results demonstrate that the levels of AD-related CSF biomarkers (Aβ1-42, T-tau, and P-tau$_{181}$) and CSF α-syn in drug-naive patients with early-stage PD are lower than those in demographically similar HCs. Furthermore, CSF Aβ1-42 and P-tau$_{181}$ are significant predictors of PD vs HCs and T-tau and α-syn are associated with severity of motor dysfunction in early PD. Although further study using the full PPMI data set is necessary to validate the current results, patients with PD who have lower CSF Aβ1-42, P-tau$_{181}$, and α-syn levels are more likely to have the PIGD-dominant motor phenotype, which has been associated with more rapid disease progression. These results provide evidence for the potential value of these CSF biomarkers for the diagnosis and assessment of heterogeneous disease progression in early-stage PD and suggest biomarker strategies for the possible recognition of prodromal PD similar to what is being pursued with AD biomarkers.[22]

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