4. Gao X, Schwarzschild MA, O'Reilly EJ, Wang H, Ascherio A. Restless legs syndrome and Parkinson's disease in men. Mov Disord 2010;25(15):2654–2657.
5. Wong JC, Li Y, Schwarzschild MA, Ascherio A, Gao X. Restless legs syndrome: an early clinical feature of Parkinson disease in men. Sleep 2014;37(2):369–372.
6. Szatmari S Jr, Bereczki D, Fornadi K, Kalantar-Zadeh K, Kovesdy CP, Molnar MZ. Association of restless legs syndrome with incident Parkinson's disease. Sleep 2017;40(2):zsw065.
7. Jiménez-Jiménez FJ, Alonso-Navarro H, García-Martín E, Agúndez JA. Neurochemical features of idiopathic restless legs syndrome. Sleep Med Rev 2019;45:70–87.
8. Ryu JH, Lee MS, Baik JS. Sonographic abnormalities in idiopathic restless legs syndrome (RLS) and RLS in Parkinson's disease. Parkinsonism Relat Disord 2011;17(3):201–203.
9. Alberts J, Adler CH, Saling M, Stelmach G. Prehension patterns in restless legs syndrome patients. Parkinsonism Relat Disord 2001;7(2):143–148.
10. Ferini-Strambi L, Carli G, Casoni F, Galbiati A. Restless legs syndrome and Parkinson disease: a causal relationship between the two disorders? Front Neurol 2018;9:551.
11. Kia DA, Noyce AJ, White J, et al. Mendelian randomization study shows no causal relationship between circulating urate levels and Parkinson's disease. Ann Neurol 2018;84(2):191–199.
12. Schormair B, Zhao C, Bell S, et al. Identification of novel risk loci for restless legs syndrome in genome-wide association studies in individuals of European ancestry: a meta-analysis. Lancet Neurol 2017;16(11):898–907.
13. Nalls MA, Blauwendraat C, Vallerga CL, et al. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. Lancet Neurol 2019;18(12):1091–1102.
14. Burgess S. Sample size and power calculations in Mendelian randomization with a single instrumental variable and a binary outcome. Int J Epidemiol 2014;43(3):922–929.
15. Burgess S, Thompson SG, Collaboration CCG. Avoiding bias from weak instruments in Mendelian randomization studies. Int J Epidemiol 2011;40(3):755–764.
16. Bowden J, Holmes MV. Meta-analysis and Mendelian randomization: a review. Res Synth Methods 2019;10(4):486–496.
17. Verbanck M, Chen C-y, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet 2018;50(5):693–698.
18. Machiela MJ, Chanock SJ. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. Bioinformatics 2015;31(21):3555–3557.
19. Bulik-Sullivan BK, Loh P-R, Finucane HK, et al. LD score regression distinguishes confounding from polygenicity in genome-wide association studies. Nat Genet 2015;47(3):291–295.
20. Bulik-Sullivan B, Finucane HK, Anttila V, et al. An atlas of genetic correlations across human diseases and traits. Nat Genet 2015;47(11):1236.
21. Mohtashami S, He Q, Ruskey JA, et al. TOX3 variants are involved in restless legs syndrome and Parkinson's disease with opposite effects. J Mol Neurosci 2018;64(3):341–345.
22. Pittock SJ, Parrett T, Adler CH, Parisi JE, Dickson DW, Ahlskog JE. Neuropathology of primary restless leg syndrome: absence of specific τ-and α-synuclein pathology. Mov Disord 2004;19(6):695–699.
23. Connor JR, Wang X-S, Allen RP, et al. Altered dopaminergic profile in the putamen and substantia nigra in restless leg syndrome. Brain 2009;132(9):2403–2412.
24. Connor JR, Boyer P, Menzies S, et al. Neuropathological examination suggests impaired brain iron acquisition in restless legs syndrome. Neurology 2003;61(3):304–309.
25. Connor JR, Wang X, Patton S, et al. Decreased transferrin receptor expression by neuromelanin cells in restless legs syndrome. Neurology 2004;62(9):1563–1567.
26. Gan-Or Z, Alcalay RN, Bar-Shira A, et al. Genetic markers of restless legs syndrome in Parkinson disease. Parkinsonism Relat Disord 2015;21(6):582–585.
27. Vilariño-Güell C, Soto A, Young J, et al. Susceptibility genes for restless legs syndrome are not associated with Parkinson disease. Neurology 2008;71(3):222–223.
28. Adel S, Djarmati A, Kabakci K, et al. Co-occurrence of restless legs syndrome and Parkin mutations in two families. Mov Disord 2006;21(2):258–263.
29. Tan EK, Yew K, Chua E, et al. PINK1 mutations in sporadic early-onset Parkinson's disease. Mov Disord 2006;21(6):789–793.
30. Lahut S, Vadasz D, Depboylu C, et al. The PD-associated alpha-synuclein promoter Rep1 allele 2 shows diminished frequency in restless legs syndrome. Neurogenetics 2014;15(3):189–192.
31. Di Angelantonio E, Thompson SG, Kaptoge S, et al. Efficiency and safety of varying the frequency of whole blood donation (INTERVAL): a randomised trial of 45 000 donors. Lancet 2017;390(10110):2360–2371.

Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Longitudinal Analysis of Multiple Neurotransmitter Metabolites in Cerebrospinal Fluid in Early Parkinson's Disease

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Relevant conflicts of interest/Financial disclosures: T.K., J.S.-P., A.S., S.D., K.I.T., and G.P. are employees of F. Hoffmann–La Roche Ltd. and respective affiliates. T.G. is a former employee of Metanomics Health GmbH. J.D. is a former employee and received consultancy fees from F. Hoffmann–La Roche Ltd. This does not alter our adherence to policies on sharing data and materials.

Full financial disclosures and author roles may be found in the online version of this article.

Received: 15 September 2020; **Revised:** 15 March 2021; **Accepted:** 16 March 2021

Published online 4 May 2021 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.28608

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ABSTRACT: Background: Cerebrospinal fluid (CSF) levels of monoamine metabolites may represent biomarkers of Parkinson's disease (PD).
Objective: The aim of this study was quantification of multiple metabolites in CSF from PD versus healthy control subjects (HCs), including longitudinal analysis.
Methods: Absolute levels of multiple monoamine metabolites in CSF were quantified by liquid chromatography coupled with tandem mass spectrometry from 161 individuals with early PD and 115 HCs from the Parkinson's Progression Marker Initiative and de novo PD (DeNoPA) studies.
Results: Baseline levels of homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were lower in individuals with PD compared with HCs. HVA levels correlated with Movement Disorder Society Unified Parkinson's Disease Rating Scale total scores ($P < 0.01$). Both HVA/dopamine and DOPAC/dopamine levels correlated with caudate nucleus and raw DOPAC with putamen dopamine transporter single-photon emission computed tomography uptake ratios ($P < 0.01$). No metabolite changed over 2 years in drug-naive individuals, but some changed on starting levodopa treatment.
Conclusions: HVA and DOPAC CSF levels mirrored nigrostriatal pathway damage, confirming the central role of dopaminergic degeneration in early PD. © 2021 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society

Key Words: monoamine metabolites; catecholamine; neurotransmitter; biomarker; Parkinson's disease; CSF; homovanillic acid

Parkinson's disease (PD) is characterized by progressive loss of dopaminergic neurons in the substantia nigra pars compacta,[1] but also depletion of other neurotransmitters, such as serotonin in the striatum and noradrenaline in the hypothalamus and frontal cortex.[2,3] Cerebrospinal fluid (CSF) represents the most proximal source of molecular biomarkers for these deficiencies.[4] Although quantification of CSF protein biomarkers improves early diagnosis in Alzheimer's disease,[5] no analogous protein biomarkers for PD diagnosis exist. Neurotransmitter metabolites represent a potential proxy to PD-specific neurodegeneration and may serve as promising biomarkers of disease severity and its progression.

Several studies investigating dopamine metabolites in PD found consistent signatures, in particular, decreased levels of the main dopamine metabolite homovanillic acid (HVA).[6-10] However, their utility for monitoring disease progression has been questioned, mainly because of the results of the DATATOP (deprenyl and tocopherol antioxidative therapy of parkinsonism) study in which repeated CSF measurements of dopamine metabolites by gas chromatography–mass spectrometry yielded variable results. Despite efforts to standardize CSF collection, processing, and measurement,[11,12] potential confounding factors on catecholamine levels remain (eg, diurnal changes, total CSF volume) and may impede the reliable quantification.[13-16]

Although high-performance liquid chromatography with electrochemical detection (HPLC-ECD) and gas chromatography–mass spectrometry were previously

considered gold standards for analyzing dopamine and its metabolites,[7,11] LC–MS/MS (liquid chromatography coupled with tandem mass spectrometry) has evolved during the last two decades with comparable sensitivity to HPLC-ECD and greatly improved selectivity.[17,18] LC–MS/MS reduces the complexity of preanalytical processing[17] and is now considered the gold standard for quantitative analytics. This enables simultaneous analyses of metabolites of the dopaminergic (eg, 3,4-dihydroxyphenylalanine [DOPA], dopamine, 3,4-dihydroxyphenylacetic acid [DOPAC]), noradrenergic (eg, 3,4-dihydroxyphenylglycol, 4-hydroxy-3-methoxyphenylglycol) and serotonergic (eg, 5-hydroxy-3-indoleacetic acid [5-HIAA]) pathways in biofluids, including CSF.[19]

We for the first time applied LC–MS/MS to measure multiple monoamine metabolite concentrations in human CSF samples from the single-center de novo PD (DeNoPa)-cohort,[20,21] including longitudinal analysis in the multicenter Parkinson's Progressive Markers Initiative (PPMI)[22-24] study, to assess their utility as biomarkers of both PD severity and its progression.

Materials and Methods

Study Participants and CSF Sampling Procedure

DeNoPa Cohort

CSF baseline samples from 49 age- and sex-matched healthy control subjects (HC) and 62 drug-naive PD participants were analyzed from the DeNoPa study.[20] CSF samples were collected and processed as previously described.[25]

PPMI Cohort

Baseline and 1-year CSF samples from 56 HCs and 95 age-, sex-, body mass index (BMI)-, and total CSF volume-matched participants with dopamine transporter single-photon emission computed tomography (DaT-SPECT)-confirmed PD were analyzed (https://www.ppmi-info.org/study-design/). Fifty-four individuals with PD remained unmedicated at the 1-year visit, while 39 individuals with PD had started L-dopa medication. Two-year follow-up CSF samples were available from all 56 HCs and 39 individuals with PD, all of whom were on L-dopa medication. Clinical and medication data were retrieved from the PPMI data portal (https://www.ppmi-info.org/access-data-specimens/download-data/). CSF samples were collected and processed following standardized procedures (https://www.ppmi-info.org/study-design/) (see also The Parkinson's Progression Marker Initiative[24] and Kang[26]).

A comparison of CSF sampling procedures for DeNoPa and PPMI is provided in Table S4. Demographics and clinical characteristics for DeNoPa and PPMI are provided in Table S1. Both studies were approved by the ethics committees: in Frankfurt (Hessen, Germany) for DeNoPa and the Institutional Review Board of all participating sites for PPMI. Written informed consent was obtained from all participants before inclusion in the study.

Metabolite Quantification

Absolute metabolite quantification was performed at Metanomics Health GmbH, Germany. CSF samples were subjected to ultracentrifugation and dansyl chloride derivatization prior to solid-phase extraction and LC–MS/MS analysis: data were normalized against internal standards and quantified using calibration standards as previously described.[17,27] The metabolite panels that were analyzed, including their limit of detection, are provided in Table S2. Technical robustness of the analytical method was confirmed in a subset of seven CSF randomly selected blinded samples from the DeNoPa study (see Table S3). Ratios were derived for analyses of metabolite levels normalized by the concentration of the respective neurotransmitter. Stringent procedures to minimize time between thawing and monoamine metabolite analysis were consistently applied for all samples.

Statistical Analysis

All statistical analyses were performed using *R* and are described in detail in the Supporting Data.

Results

Demographic and Clinical Data in the DeNoPa and PPMI Cohorts

Groups did not differ with respect to mean age (± standard deviation) (HC: 65.6 ± 6.6, PD: 64.1 ± 9.4, F = 1.2, $P = 0.28$) or sex distribution (HCs [male/female]: 30/19, PD: 42/20, $\chi^2 = 0.51$, $P = 0.47$) in the DeNoPa study. Groups differed with respect to Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS UPDRS) Part III (HC: 0.35 ± 1.03, PD: 19.4 ± 9.9, F = 386, $P < 0.001$) and total scores (HC: 3.1 ± 2.8, PD: 29.8 ± 15.6, F = 290, $P < 0.001$).

Groups had comparable baseline ages (mean ± standard deviation) (HC: 62.7 ± 10.7, PD: 62.4 ± 9.8, F = 0.21, $P = 0.65$), sex distributions (HCs [male/female]: 40/16, PD: 64/31, $\chi^2 = 0.27$, $P = 0.6$), BMI (HC: 26.8 ± 5.2, PD: 26.7 ± 4.2, F = 0.06, $P = 0.95$), and total CSF volume (HC: 17.8 ± 3.1, PD: 16.9 ± 2.9, F = 1.9, $P = 0.15$) but differed with respect to MDS UPDRS Part III (HC: 1.2 ± 2.0, PD: 21.1 ± 8.5, F = 346, $P < 0.001$) and total scores (HC: 4.7 ± 4.0, PD: 33.4 ± 13.5, F = 318, $P < 0.001$) in the PPMI study (see Table S1).

Quantifiable Metabolites

Overall, 8 of 17 metabolites could be quantified in the DeNoPa and 12 of 17 in the PPMI samples, and they were considered in further analyses. The upper levels of detection were reached in some PPMI PD samples for 3-methoxytyrosine (25%) and DOPA (13%).

CSF Monoamine Metabolite Levels at Baseline

Given that dopamine levels in the DeNoPa cohort were mostly below the limit of detection for multiple samples, only nonratio metabolite and neurotransmitter levels were analyzed. Four metabolites differed between DeNoPa PD and HC groups: HVA (estimate = -0.41 ± 0.10, $P < 0.0001$, effect size = 0.13), 5-HIAA (estimate = -0.32 ± 0.10, $P = 0.002$, effect size = 0.08), 4-hydroxy-3-methoxyphenylglycol (estimate = -0.12 ± 0.04, $P = 0.008$, effect size = 0.05), and DOPAC (estimate = -0.25 ± 0.09, $P = 0.06$, effect size = 0.04) (see Table S5).

The DeNoPa findings were partially confirmed in the PPMI samples, in which HVA and DOPAC raw and normalized levels differed between HC and PD groups (HVA: estimate = -0.33 ± 0.07, $P < 0.0001$, effect size = 0.15; DOPAC: estimate = 0.2 ± 0.07, $P = 0.01$, effect size = 0.06) (see Table 1 and Fig. 1; ROC curves are provided in Fig. S1).

In the PPMI cohort, dopamine could be reliably quantified in >97% of the samples, which was supported by test–retest analysis for a subset of samples (see Table S3) and allowed analyses of metabolite ratios. PD CSF levels of HVA correlated with MDS UPDRS total scores ($r = -0.26$, $P < 0.01$). Both HVA/dopamine and DOPAC/dopamine correlated with DaT-SPECT uptake ratios of the mean caudate (both ratios: $r = 0.28$; $P < 0.01$) and ipsilateral caudate nucleus (both ratios: $r = 0.29$, $P < 0.01$), while raw DOPAC levels correlated with ipsilateral and mean putamen DaT-SPECT uptake ratios ($r = 0.27$ and $r = 0.28$, respectively, both $P < 0.01$; see Fig. S2).

Long-Term Within-Subject Stability

Within-subject signal stability in longitudinal analyses was assessed by calculating the intraclass correlation coefficient (ICC) for the PPMI HCs at baseline, year 1, and year 2 test values. ICCs ranged from 0.19 (for histamine) to 0.74 (for HIAA), with a median of 0.69 (see Table 1).

Change of Catecholamine Metabolite Levels over Time

No raw or normalized metabolite level changed significantly over 1 year in unmedicated PPMI PD patients (all $P > 0.05$). Dopaminergic medication affected the levels of DOPA, methoxytyrosine, dopamine, and their respective metabolite ratios (see Fig. S3).

Discussion

This study measured absolute quantities of multiple monoamine metabolites in longitudinal CSF samples from individuals with early PD in the presence and absence of dopaminergic medication.

Various cross-sectional studies on CSF monoamine metabolites in individuals with PD have been performed.[6-10,28-30] However, longitudinal analyses were lacking because the large multicenter DATATOP trial reported no difference in CSF HVA and DOPAC in early PD and during disease progression.[7,11] Longitudinal analyses suffered from high intrapatient variability.[11] We hypothesized that multiple factors, such as preanalytical sample processing, site-to-site variability,[31] and misdiagnoses in PD,[32] may have affected the results. Also, the complex analytical method applied may add to the observed variability.[12] This study aimed to address these factors with a robust single-center recruitment (DeNoPa cohort), DaT-SPECT confirmation of diagnoses in most DeNoPa and all PPMI subjects, and clinical follow-up and LC–MS/MS method for absolute quantification of metabolites.[17,19]

CSF levels of DOPAC and HVA, the end product of dopamine metabolism, were reduced in early PD, confirming previous cross-sectional studies.[8-10,28,29] Correlations observed for dopaminergic metabolites with MDS UPDRS total scores and DaT-SPECT uptake values support that nigrostriatal neurodegeneration is relevant to early PD and that deficiencies are reflected in CSF.

CSF procedures applied in both studies relied on consensus guidelines and are not necessarily optimized for a given metabolite.[33] Thus, absolute values obtained in this study may be affected by ex vivo changes and should be interpreted accordingly. Despite this limitation, the comparably low intrapatient signal variability for a subset of metabolites in longitudinal HC samples is encouraging and supports the future use of this assay in longitudinal studies.

The utility of CSF neurotransmitter metabolite levels to identify prodromal PD or differentiate PD from atypical parkinsonian syndromes remain open questions. Encouraging results from a small prospective cohort study support analysis of CSF monoamine metabolites in prodromal cohorts to identify people who will develop clinical PD.[34] Given the proximity of this biomarker panel to the underlying disease pathology, as supported by the present DaT-SPECT results, it may also identify PD subtypes with diverging neurotransmitter systems deficiencies. Although the present longitudinal data span a relatively short time frame,

TABLE 1. *CSF levels for monoamine metabolites in the PPMI cohort*

	HC (n = 56)			PD (n = 95)		Difference PD-HC				
Variable	**Mean ± SD**	**<LOD**	**ICC**	**Mean ± SD**	**<LOD**	**Estimate**	***P* Value**	**AUC**	**Specificity**	**Sensitivity**
3-Methoxytyrosine	1.33 ± 0.28	0.00	0.58	2.79 ± 2.11	0.00	−0.02	0.64	0.54	0.00	1.00
DOPAC	−1.10 ± 0.41	0.00	0.74	−0.99 ± 0.77	0.00	−0.20	**0.011**	0.63	0.23	0.89
DOPA	−0.31 ± 0.25	0.00	0.63	0.59 ± 1.42	0.00	−0.01	0.85	0.53	0.00	1.00
DOPEG	0.49 ± 0.32	0.00	0.70	0.50 ± 0.37	0.00	0.04	0.50	0.53	0.00	1.00
HMPG	2.07 ± 0.23	0.00	0.47	2.00 ± 0.25	0.00	−0.04	0.51	0.57	0.00	1.00
4-Hydroxy-3-methoxymandelic acid	−0.14 ± 0.34	0.00	0.69	−0.13 ± 0.33	1.31	−0.02	0.84	0.54	0.00	1.00
5-HIAA	3.71 ± 0.45	0.00	0.74	3.53 ± 0.45	0.00	−0.12	0.076	0.59	0.07	0.99
Dopamine	−4.58 ± 0.41	2.38	0.61	−4.19 ± 0.61	2.62	0.15	0.072	0.61	0.13	0.96
Histamine	−2.56 ± 0.44	18.45	0.19	−2.32 ± 0.95	24.02	0.25	0.077	0.58	0.02	0.93
HVA	3.85 ± 0.42	0.00	0.71	3.74 ± 0.60	0.00	−0.33	**<0.0001**	0.71	0.43	0.87
Noradrenaline (norepinephrine)	−1.52 ± 0.46	0.00	0.69	−1.47 ± 0.44	0.00	0.08	0.48	0.56	0.00	1.00
Normetanephrine	−2.28 ± 0.43	0.00	0.71	−2.27 ± 0.43	0.00	0.02	0.76	0.47	1.00	0.00
(DOPEG + NM + HMPG + NA)/dopamine	7.22 ± 0.39	0.02	n/a	6.78 ± 0.56	0.03	−0.17	**0.013**	0.64	0.24	0.93
DOPAC/dopamine	3.49 ± 0.43	0.02	n/a	3.22 ± 0.55	0.03	−0.35	**<0.0001**	0.71	0.38	0.84
Dopamine/DOPA	−4.28 ± 0.41	0.02	n/a	−4.82 ± 1.10	0.03	0.16	0.086	0.62	0.07	0.96
DOPEG/noradrenaline	2.01 ± 0.42	0.00	n/a	1.97 ± 0.38	0.00	−0.05	0.45	0.55	0.00	1.00
HMPG/noradrenaline	3.59 ± 0.40	0.00	n/a	3.47 ± 0.38	0.00	−0.13	0.082	0.61	0.07	0.95
HVA/dopamine	8.44 ± 0.45	0.02	n/a	7.95 ± 0.50	0.03	−0.48	**<0.0001**	0.77	0.47	0.85
Noradrenaline/DOPA	−1.21 ± 0.43	0.00	n/a	−2.06 ± 1.46	0.00	0.09	0.17	0.58	0.02	0.99
Normetanephrine/noradrenaline	−0.76 ± 0.23	0.00	n/a	−0.79 ± 0.28	0.00	−0.07	0.31	0.55	0.00	1.00

Monoamine metabolite levels in PPMI HC and PD CSF samples: levels (log2 transformed), percentages <LOD, ICCs to assess stability between baseline, 1-year follow-up, and 2-year follow-up in the PPMI HC group, HC-PD group differences with corresponding AUC and receiver operating characteristics curves, and specificity and sensitivity. Boldface reflects significant case–control differences after multiple testing correction.

CSF, cerebrospinal fluid; PPMI, Parkinson's Progressive Markers Initiative; HC, healthy control; PD, Parkinson's disease; SD, standard deviation; LOD, below the limit of detection; ICC, intraclass correlation coefficient; AUC, area under the curve; DOPAC, 3,4-dihydroxyphenylacetic acid; DOPA, 3,4-dihydroxyphenylalanine; DOPEG; 3,4-dihydroxyphenylglycol; HMPG, 4-hydroxy-3-methoxyphenylglycol; 5-HIAA, 5-hydroxy-3-indoleacetic acid; HVA, homovanillic acid; n/a, not applicable.

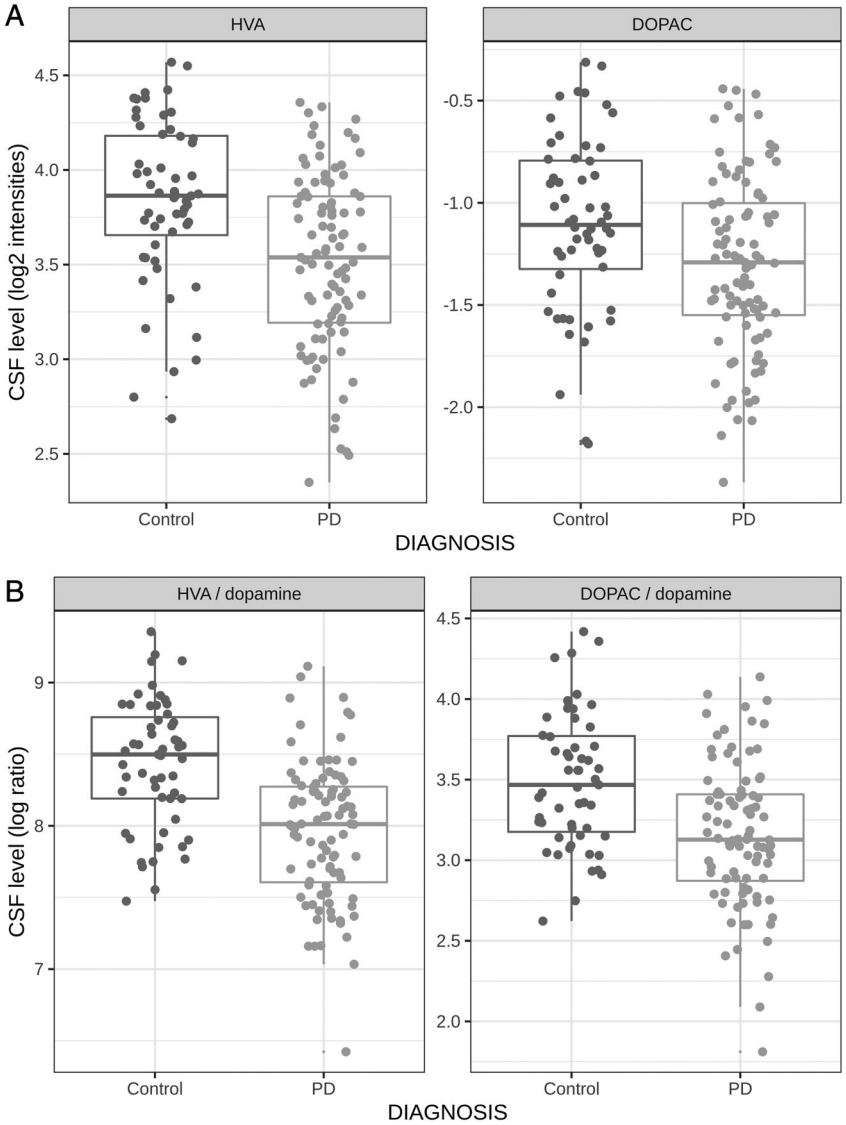

FIG. 1. Baseline levels of (**A**) homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) and (**B**) HVA/dopamine and DOPAC/dopamine in healthy control (HC; blue) and Parkinson's disease (PD; orange) Parkinson's Progressive Markers Initiative participants. CSF, cerebrospinal fluid. [Color figure can be viewed at wileyonlinelibrary.com]

clinical follow-up of the PPMI continued since our analysis was performed, and additional information on clinical scales and for various biomarker modalities is available, including their progression with time. We encourage researchers to use our data, which are accessible for downloading, to further deepen our understanding of PD pathophysiology and its progression.

Acknowledgments: This work was supported by The Michael J. Fox Foundation for Parkinson's Research, Abbott, Avid Radiopharmaceuticals, Biogen Idec, Covance, Elan, Eli Lilly and Co, F. Hoffmann–LaRoche Ltd., GE Healthcare, Genentech, Glaxo Smith Kline, Merck and Co., Pfizer Inc., and UCB Pharma SA. The DeNoPa study was supported by unrestricted research grants from the Paracelsus-Elena-Klinik, Kassel, Germany, and unrestricted research grants from TEVA Pharma/Lundbeck, Parkinson Fonds Deutschland, The Michael J. Fox Foundation for Parkinson's research, and Deutsche Parkinson Vereinigung. The monoamine metabolite analysis was entirely funded by F. Hoffmann–La Roche. This funder provided support in the form of salaries for authors (T.K., K.I.T., J.S.-P., A.S., C.C., J.D., S.D., G.P.) but did not have any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. PPMI is sponsored by The Michael J. Fox Foundation for Parkinson's Research (MJFF) and is cofunded by MJFF, AbbVie, Avid Radiopharmaceuticals, Biogen Idec, Bristol-Myers Squibb, Covance, Eli Lilly & Co., F. Hoffman-La Roche, Ltd., GE Healthcare, Genentech, GlaxoSmithKline, Lundbeck, Merck, MesoScale, Piramal, Pfizer, and UCB. The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication. The authors thank The Michael J. Fox Foundation, their PPMI colleagues, and the individuals who participated in this study.

References

1. Kordower JH, Olanow CW, Dodiya HB, Chu Y, Beach TG, Adler CH, et al. Disease duration and the integrity of the nigrostriatal system in Parkinson's disease. Brain 2013;136(8):2419–2431.
2. Ehringer H, Hornykiewicz O. Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their

behavior in diseases of the extrapyramidal system. Klin Wochenschr 1960;38:1236–1239.

3. Buddhala C, Loftin SK, Kuley BM, Cairns NJ, Campbell MC, Perlmutter JS, et al. Dopaminergic, serotonergic, and noradrenergic deficits in Parkinson disease. Ann Clin Transl Neurol 2015;2(10): 949–959.
4. Parnetti L, Castrioto A, Chiasserini D, Persichetti E, Tambasco N, El-Agnaf O, et al. Cerebrospinal fluid biomarkers in Parkinson disease. Nat Rev Neurol 2013;9(3):131–140.
5. Zetterberg H, Lautner R, Skillbäck T, Rosén C, Shahim P, Mattsson N, et al. CSF in Alzheimer's disease. Adv Clin Chem 2014;65:143–172.
6. Goldstein DS, Holmes C, Bentho O, Sato T, Moak J, Sharabi Y, et al. Biomarkers to detect central dopamine deficiency and distinguish Parkinson disease from multiple system atrophy. Parkinsonism Relat Disord 2008;14(8):600–607.
7. LeWitt PA, Galloway MP, Matson W, Milbury P, McDermott M, Srivastava DK, et al. Markers of dopamine metabolism in Parkinson's disease. The Parkinson Study Group. Neurology 1992; 42(11):2111–2117.
8. LeWitt P, Schultz L, Auinger P, Lu M. Parkinson study group DATATOP investigators. CSF xanthine, homovanillic acid, and their ratio as biomarkers of Parkinson's disease. Brain Res 2011;1408:88–97.
9. Chia L-G, Cheng F-C, Kuo J-S. Monoamines and their metabolites in plasma and lumbar cerebrospinal fluid of Chinese patients with Parkinson's disease. J Neurol Sci 1993;116(2):125–134.
10. Herbert MK, Kuiperij HB, Bloem BR, Verbeek MM. Levels of HVA, 5-HIAA, and MHPG in the CSF of vascular parkinsonism compared to Parkinson's disease and controls. J Neurol 2013;260 (12):3129–3133.
11. Cerebrospinal fluid homovanillic acid in the DATATOP study on Parkinson's disease. Parkinson Study Group. Arch Neurol 1995;52 (3):237–245.
12. Hildebrand J, Bourgeois F, Buyse M, Przedborski S, Goldman S. Reproducibility of monoamine metabolite measurements in human cerebrospinal fluid. Acta Neurol Scand 1990;81(5):427–430.
13. Degrell I, Nagy E. Concentration gradients for HVA, 5-HIAA, ascorbic acid, and uric acid in cerebrospinal fluid. Biol Psychiatry 1990;27(8):891–896.
14. Hartikainen P, Soininen H, Reinikainen KJ, Sirviö J, Soikkeli R, Riekkinen PJ. Neurotransmitter markers in the cerebrospinal fluid of normal subjects. Effects of aging and other confounding factors. J Neural Transm Gen Sect 1991;84(1–2):103–117.
15. Haijes HA, Willemse EAJ, Gerrits J, van der Flier WM, Teunissen CE, Verhoeven-Duif NM, et al. Assessing the pre-analytical stability of small-molecule metabolites in cerebrospinal fluid using direct-infusion metabolomics. Metabolites 2019;9(10):236.
16. Noga MJ, Zielman R, van Dongen RM, Bos S, Harms A, Terwindt GM, et al. Strategies to assess and optimize stability of endogenous amines during cerebrospinal fluid sampling. Metabolomics 2018;14(4):44.
17. Yamada H, Yamahara A, Yasuda S, Abe M, Oguri K, Fukushima S, et al. Dansyl chloride derivatization of methamphetamine: a method with advantages for screening and analysis of methamphetamine in urine. J Anal Toxicol 2002;26(1):17–22.
18. Kamlage B, Maldonado SG, Bethan B, Peter E, Schmitz O, Liebenberg V, et al. Quality markers addressing preanalytical variations of blood and plasma processing identified by broad and targeted metabolite profiling. Clin Chem 2014;60(2):399–412.
19. Czech C, Berndt P, Busch K, Schmitz O, Wiemer J, Most V, et al. Metabolite profiling of Alzheimer's disease cerebrospinal fluid. PloS One 2012;7(2):e31501.
20. Mollenhauer B, Trautmann E, Sixel-Döring F, Wicke T, Ebentheuer J, Schaumburg M, et al. Nonmotor and diagnostic findings in subjects with de novo Parkinson disease of the DeNoPa cohort. Neurology 2013;81(14):1226–1234.
21. Mollenhauer B, Zimmermann J, Sixel-Döring F, Focke NK, Wicke T, Ebentheuer J, et al. Monitoring of 30 marker candidates in early Parkinson disease as progression markers. Neurology 2016;87(2):168–177.
22. Kang J-H, Irwin DJ, Chen-Plotkin AS, Siderowf A, Caspell C, Coffey CS, et al. Association of cerebrospinal fluid β-amyloid 1-42, T-tau, P-tau181, and α-synuclein levels with clinical features of drug-naive patients with early Parkinson disease. JAMA Neurol 2013;70(10):1277–1287.
23. Marek K, Jennings D, Lasch S, Siderowf A, Tanner C, Simuni T, et al. The Parkinson progression marker initiative (PPMI). Prog Neurobiol 2011;95(4):629–635.
24. The Parkinson's Progression Marker Initiative, Kang J-H, Mollenhauer B, Coffey CS, Toledo JB, Weintraub D, et al. CSF biomarkers associated with disease heterogeneity in early Parkinson's disease: the Parkinson's progression markers initiative study. Acta Neuropathol (Berl) 2016;131(6):935–949.
25. Mollenhauer B, El-Agnaf OMA, Marcus K, Trenkwalder C, Schlossmacher MG. Quantification of α-synuclein in cerebrospinal fluid as a biomarker candidate: review of the literature and considerations for future studies. Biomark Med 2010;4(5):683–699.
26. Kang J-H. Cerebrospinal fluid amyloid β1-42, tau, and alpha-Synuclein predict the heterogeneous progression of cognitive dysfunction in Parkinson's disease. J Mov Disord 2016;9(2):89–96.
27. Walk TB, Dostler M. Massenspektrometrisches verfahren zur analyse von substanzgemischen [Internet]. WO2003073464A1, 2003 [Cited 2021 Feb 28]. https://patents.google.com/patent/WO2003073464A1/en/und
28. Eldrup E, Mogensen P, Jacobsen J, Pakkenberg H, Christensen NJ. CSF and plasma concentrations of free norepinephrine, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), 3,4-dihydroxyphenylalanine (DOPA), and epinephrine in Parkinson's disease. Acta Neurol Scand 1995;92(2):116–121.
29. Stefani A, Pierantozzi M, Olivola E, Galati S, Cerroni R, D'Angelo V, et al. Homovanillic acid in CSF of mild stage Parkinson's disease patients correlates with motor impairment. Neurochem Int 2017;105:58–63.
30. Goldstein DS, Holmes C, Sharabi Y. Cerebrospinal fluid biomarkers of central catecholamine deficiency in Parkinson's disease and other synucleinopathies. Brain J Neurol 2012;135(Pt 6):1900–1913.
31. Lewczuk P, Beck G, Esselmann H, Bruckmoser R, Zimmermann R, Fiszer M, et al. Effect of sample collection tubes on cerebrospinal fluid concentrations of tau proteins and amyloid beta peptides. Clin Chem 2006;52(2):332–334.
32. Adler CH, Beach TG, Hentz JG, Shill HA, Caviness JN, Driver-Dunckley E, et al. Low clinical diagnostic accuracy of early vs advanced Parkinson disease: clinicopathologic study. Neurology 2014;83(5):406–412.
33. del Campo M, Mollenhauer B, Bertolotto A, Engelborghs S, Hampel H, Simonsen AH, et al. Recommendations to standardize preanalytical confounding factors in Alzheimer's and Parkinson's disease cerebrospinal fluid biomarkers: an update. Biomark Med 2012;6(4):419–430.
34. Goldstein DS, Holmes C, Lopez GJ, Wu T, Sharabi Y. Cerebrospinal fluid biomarkers of central dopamine deficiency predict Parkinson's disease. Parkinsonism Relat Disord 2018;50:108–112.

Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.