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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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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-naïve 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 processing¹⁷ 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-naïve 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 Initiative²⁴ and Kang²⁶).

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 ($\pm$ 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 $\pm$ 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 inpatient 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 inpatient 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

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

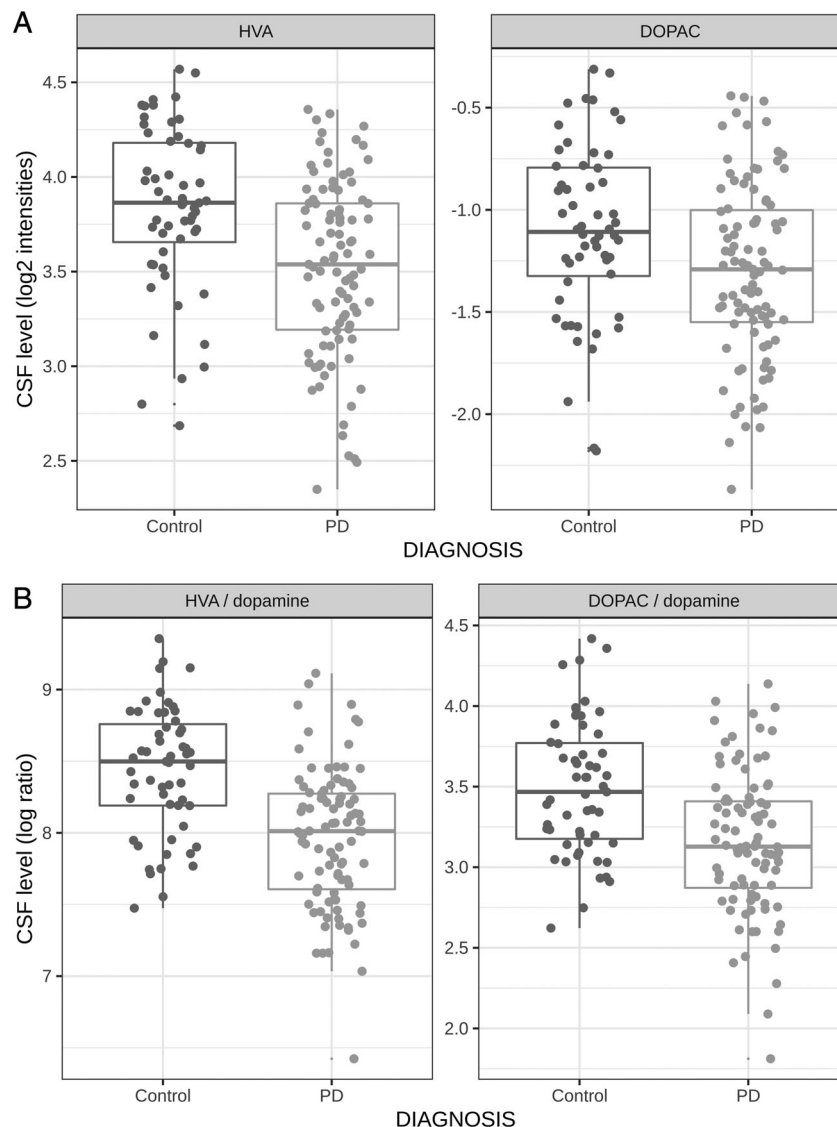


FIG. 1. Baseline levels of (A) homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) and (B) HVA/dopamine and DOPAC/dopamine in healthy control (HC; blue) and Parkinson's disease (PD; orange) Parkinson's Progressive Markers Initiative participants. CSF, cerebrospinal fluid. [Color figure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

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Supporting Data

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