### **LETTER Open Access**



# DOPA-decarboxylase is elevated in CSF, but not plasma, in prodromal and de novo Parkinson's disease

Ellen Appleton<sup>1†</sup>, Shervin Khosousi<sup>1†</sup>, Michael Ta<sup>2,3,4</sup>, Michael Nalls<sup>2,3,4</sup>, Andrew B. Singleton<sup>2,4</sup>, Andrea Sturchio<sup>1,5</sup>, Ioanna Markaki<sup>1</sup>, Wojciech Paslawski<sup>1</sup>, Hirotaka Iwaki<sup>2,3,4</sup> and Per Svenningsson<sup>1,6\*</sup>

Parkinson's disease (PD) diagnosis is based solely on clinical presentation [\[1](#page-3-0)]. Therefore, diagnostic and prognostic biomarkers are needed. In recent years, seeding aggregation assays (SAA) have demonstrated the ability to discriminate PD from controls, showing also potential as a predictive marker [\[2](#page-3-1)]. Furthermore, the development of modern proteomic techniques such as the proximity extension assay (PEA) has enabled high throughput, highly sensitive multiplexing studies to become more prevalent. In the past year, several such studies identifed DOPA decarboxylase (DDC) as an analyte of particular interest, demonstrating consistent elevation in Lewy body diseases and promising diagnostic potential. Increased DDC has been found in the cerebrospinal fuid (CSF)  $[3-5]$  $[3-5]$  and plasma  $[4]$  $[4]$  of both PD  $[3-5]$  $[3-5]$  and dementia with Lewy bodies  $[4, 5]$  $[4, 5]$  $[4, 5]$  as well as atypical PD  $[3, 4]$  $[3, 4]$  $[3, 4]$  $[3, 4]$  $[3, 4]$ ,

† Ellen Appleton and Shervin Khosousi are shared authorship.

\*Correspondence:

per.svenningsson@ki.se

<sup>2</sup> Center for Alzheimer's and Related Dementias, National Institute On Aging and National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA

<sup>3</sup> DataTecnica LLC, Washington, DC, USA

4 Laboratory of Neurogenetics, National Institute On Aging, National Institutes of Health, Bethesda, MD, USA

<sup>5</sup> James J. and Joan A. Gardner Family Center for Parkinson's Disease and Movement Disorders, Department of Neurology, University of Cincinnati, Cincinnati, OH, USA

<sup>6</sup> Basic and Clinical Neuroscience, King's College London, London, UK

but not in non-parkinsonian neurodegenerative conditions such as Alzheimer's disease [\[5](#page-3-3)]. Intriguingly, CSF DDC is elevated in SAA-positive subjects, independent of Lewy body disease diagnosis [\[4](#page-3-4)].

This study aimed to evaluate CSF and plasma levels of DDC in prodromal, de novo, and treated PD patients and controls using PEA in three independent cohorts, the Parkinson's Progression Markers Initiative (PPMI) (de novo PD *n*=74 CSF, *n*=78 plasma; prodromal PD  $n=51$  CSF,  $n=62$  plasma; and control  $n=130$  CSF, *n*=130 plasma); Parkinson's Disease Biomarkers Program (PDBP) (PD *n*=84 CSF, *n*=84 plasma; and control *n*=54 CSF, *n*=54 plasma); and Biopark (PD *n*=120 [27 de novo] CSF, *n*=238 [26 de novo] plasma; and control *n*=69 CSF, *n*=50 plasma) (Additional fle [1:](#page-3-5) Table S1 and Supplementary Methods).

Diferential abundance analysis of 1463 proteins in the CSF revealed DDC to be the most signifcantly changed protein in PD (PDBP), prodromal PD and de novo PD (PPMI) compared to controls. Focused investigation of PD CSF samples from Biopark using the Olink "Metabolism" panel (91 proteins) similarly identifed DDC as the most increased analyte (Fig.  $1a-g$  $1a-g$ ). Further analysis revealed similar elevations of CSF DDC in both prodromal and de novo PD in the PPMI cohort (Fig. [1f](#page-1-0)).

Receiver operating characteristic (ROC) curves were constructed to assess the discriminatory capacity of CSF DDC (Fig. [1](#page-1-0)h, Table S2). DDC was able to discriminate PD from controls with area under the curve (AUC) of  $0.79-0.94$  in three cohorts. The highest AUC was in the PDBP cohort where most PD patients were on treatment,



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit [http://creativecommons.org/licenses/by/4.0/.](http://creativecommons.org/licenses/by/4.0/) The Creative Commons Public Domain Dedication waiver ([http://creativeco](http://creativecommons.org/publicdomain/zero/1.0/) [mmons.org/publicdomain/zero/1.0/](http://creativecommons.org/publicdomain/zero/1.0/)) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Per Svenningsson

<sup>&</sup>lt;sup>1</sup> Laboratory of Translational Neuropharmacology, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden



<span id="page-1-0"></span>**Fig. 1** CSF and plasma DDC levels in prodromal, de novo and treated PD. **a**–**d** Volcano plots showing diferential abundance of 1463 proteins in the CSF, corrected for age and sex. Axes display Log<sub>2</sub> fold change and Benjamini–Hochberg adjusted *P*-values. Plots show PD ( $n=84$ ) versus controls ( $n=54$ ) in the PDBP cohort (**a**), prodromal PD ( $n=51$ ) versus controls ( $n=130$ ) (**b**) and de novo PD ( $n=74$ ) versus controls ( $n=130$ ) (**c**) in the PPMI cohort, and PD (*n*=120) versus controls (*n*=69) (66 proteins) in the Biopark cohort (**d**). **e**–**g** Box-plots of age- and sex-adjusted CSF DDC levels in PD and controls from the PDBP cohort (independent *t*-test) (**e**), prodromal, de novo PD and control samples from the PPMI cohort (ANOVA, Tukey post-hoc test) (**f**), and de novo PD, PD and control samples from the Biopark cohort (ANOVA, Tukey post-hoc test) (**g**). **h** Receiver operating characteristic (ROC) curves for CSF DDC levels with area under the curve (AUC) for prodromal or PD vs controls in each cohort. **i**–**l** Volcano plots showing differential abundance of 1463 proteins in plasma, corrected for age and sex. Axes display Log<sub>2</sub> fold change and Benjamini– Hochberg adjusted *P*-values. Plots show PD (*n*=84) versus controls (*n*=54) in the PDBP cohort (**i**), prodromal PD (*n*=62) versus controls (*n*=130) (**j**) and de novo PD (*n*=78) versus controls (*n*=130) (**k**) in the PPMI cohort, and PD (*n*=238) versus controls (*n*=50) (71 proteins) in the Biopark cohort (**l**). **m**–**o** Box-plots of age- and sex-adjusted plasma DDC levels in PD and controls from the PDBP cohort (independent *t*-test) (**m**), prodromal, de novo PD and control samples from the PPMI cohort (ANOVA, Tukey post-hoc test) (**n**), and de novo PD, PD and control samples from the Biopark cohort (ANOVA, Tukey post-hoc test) (**o**). **p** ROC curves for plasma DDC levels with AUC for prodromal or PD vs controls in each cohort. \**P*<0.05, \*\*\*\**P*<0.0001

and the lowest in the de novo, unmedicated PD patients from PPMI and Biopark. Prodromal PD showed comparable results, with an AUC of 0.80.

As most controls and de novo PD patients in the PPMI cohort had data on SAA status available, we analysed its association with DDC levels in CSF. SAA positivity was associated with higher DDC levels in both PD and the few SAA<sup>+</sup> controls. Moreover, the few SAA<sup>-</sup> PD subjects had below-average levels of DDC in the PD group (Fig. S1).

The Olink analysis was repeated in plasma samples from the same three cohorts (Fig. [1](#page-1-0)i–o). As in CSF, DDC was the most signifcantly increased protein in PD plasma samples from the PDBP and Biopark cohorts, compared to controls. However, DDC was not altered in prodromal or de novo PD from the PPMI cohort (Fig. [1](#page-1-0)n). Additionally, separation of the Biopark PD samples into de novo and treated PD also demonstrated no signifcant plasma DDC increase in de novo PD vs controls (Fig. [1](#page-1-0)o).

Furthermore, ROC analysis showed plasma DDC levels to be capable of diferentiating between PD and controls in both the PDBP and the Biopark  $(AUC=0.87-0.88)$ cohorts. This was not replicated in de novo PD from PPMI and Biopark (AUC=0.50–0.58), or prodromal PD  $(AUC=0.60)$  (Fig. [1](#page-1-0)p, Table S2).

The three cohorts are heterogeneous in terms of PD stage and medication and further analyses revealed that disease duration and levodopa equivalent daily dose in the PDBP cohort were positively correlated with both CSF and plasma DDC levels (Fig. S2). The PPMI de novo PD cohort was followed over a period of four years, during which time many participants began PD treatment (Fig. S3). From this longitudinal dataset, we selected the latest time point for each patient (average disease duration 2.8 years for CSF, 2 years for plasma), in order to further explore the infuence of medication on DDC levels. As in the Biopark cohort, plasma DDC was signifcantly increased in treated PD samples only, whilst CSF DDC was increased in both treated and untreated PD (Fig. S3). The increase in plasma DDC appeared to be driven by levodopa/DDC-inhibitor treatment specifcally, as the same efect was not observed in those receiving only dopamine agonists, an efect mirrored in the PDBP cohort (Figs. S3 and S4).

After correcting for age and sex, a weak correlation between CSF DDC and Unifed Parkinson's Disease Rating Scale 3 (UPDRS-3) score (*R*=0.23, *P*=0.048) was found in de novo PD patients from the PPMI cohort, but it was not reproduced in other cohorts (Fig. S5). No other correlations were identifed in CSF and/or plasma.

By utilising PEA analysis in three independent cohorts, we have provided further evidence of the efficacy of DDC as a diagnostic PD biomarker. Of particular importance,

assessment of prodromal and de novo PD patients from the PPMI and Biopark cohorts demonstrated that CSF DDC has diagnostic capacity in early, untreated PD, and even before PD diagnosis.

The Biopark, PPMI, and PDBP cohorts are heterogeneous with regard to disease stage, disease duration, and PD treatment status, features which were shown to correlate with DDC levels. Levodopa had a small infuence on CSF DDC. For example, the highest AUC was in the PDBP cohort where most PD patients were on treatment, and the lowest were in the unmedicated, de novo PD patients from the PPMI and Biopark cohorts. Nevertheless, DDC is an excellent CSF biomarker across cohorts, independent of treatment.

We speculate that the increase of DDC in the CSF could be a compensatory mechanism as suggested in animal models [\[6\]](#page-3-6). Another possible reason could be the release of DDC from dying monoaminergic neurons. DDC has limited correlation with clinical features; in fact, only a mild correlation was found between DDC and UPDRS-3 total score, questioning its validity as a robust prognostic biomarker. Moreover, it has been shown that diferent DDC polymorphisms can afect the levodopa response, adding another layer of complexity for the clinical correlation [[7](#page-3-7)].

Whilst plasma DDC was very efective in discriminating between treated PD and controls, this did not extend to prodromal and de novo PD. As has been shown previously, plasma DDC levels are afected by levodopa/DDCinhibitor treatment [\[8\]](#page-3-8). Intriguingly, this efect was not seen in subjects treated with dopamine agonists. It would be interesting to investigate what other factors related to PD medication afect DDC levels, including the type and dose of DDC inhibitors, the efect of MAO/COMT inhibitors, and diferent dopamine agonists.

Our paper adds novelty to the recent findings  $[3-5]$  $[3-5]$  $[3-5]$  in which higher DDC levels have been found in subjects with Lewy body dementia and atypical parkinsonism, corroborating the importance of DDC in the diagnosis of parkinsonian syndromes.

A strength of this study is the large sample size and validation of the results using several cohorts. However, this could be strengthened further using orthogonal methods to corroborate DDC measurements.

In conclusion, our data show that CSF DDC has strong biomarker potential in PD. Since we found alterations even in the prodromal phase, DDC could be also an excellent biomarker for the identifcation of patients suitable for clinical trials.

#### **Abbreviations**



- SAA Seeding aggregation assay
- PEA Proximity extension assay



#### **Supplementary Information**

The online version contains supplementary material available at [https://doi.](https://doi.org/10.1186/s40035-024-00421-0) [org/10.1186/s40035-024-00421-0](https://doi.org/10.1186/s40035-024-00421-0).

<span id="page-3-5"></span>**Additional fle 1**: **Figure S1**. The relationship between SAA and DDC in the PPMI cohort. **Figure S2**. Spearman's rank correlation between CSF and plasma DDC levels with disease duration and LEDD in the PDBP cohort. **Figure S3**. PPMI cohort - last time point sampled. **Figure S4**. PD Treatment in the PDBP cohort. **Figure S5**. Spearman's rank correlation between CSF and plasma DDC levels with UPDRS 3 score in PD patients from each cohort. **Table S1**. Cohort distributions. **Table S2**. ROC analysis. Methods.

#### **Acknowledgements**

We thank all the subjects who participated in this study; and all clinical personnel aiding in assessing patients and collecting samples. The data of PPMI and PDBP participants were obtained through AMP-PD program's data release version 3. The PPMI investigators have not participated in reviewing the data analysis or content of the manuscript. For up-to-date information on the study, visit [www.ppmi-info.org.](http://www.ppmi-info.org) A full list of PDBP investigators can be found at [https://pdbp.ninds.nih.gov/policy.](https://pdbp.ninds.nih.gov/policy) The PDBP investigators have not participated in reviewing the data analysis or content of the manuscript. We would like to thank the Afnity Proteomics SciLifeLab Unit in Stockholm for conducting the proximity extension assay analyses on the Biopark samples.

#### **Authors' contributions**

PS and HI designed the study. Patient data were collected/collated by IM, HI, and PS. EA and SK analysed the data. Data were interpreted by all authors. EA and SK created the fgures and EA, SK, AS and PS wrote the manuscript. All authors edited and approved the manuscript.

#### **Funding**

The work was funded by Stockholm City Council, SciLife postdoctoral programme, Knut and Alice Wallenberg Foundation. This work was supported in part by the Intramural Research Program of the National Institute on Aging (NIA), and the Center for Alzheimer's and Related Dementias (CARD), within the Intramural Research Program of the National Institute on Aging and the National Institute of Neurological Disorders and Stroke (AG000546). PPMI is sponsored by The Michael J. Fox Foundation for Parkinson's Research and supported by a consortium of scientifc partners: [Funding: PPMI – a public–private partnership – is funded by the Michael J. Fox Foundation for Parkinson's Research and funding partners, including 4D Pharma, Abbvie, AcureX, Allergan, Amathus Therapeutics, Aligning Science Across Parkinson's, AskBio, Avid Radiopharmaceuticals, BIAL, Biogen, Biohaven, BioLegend, BlueRock Therapeutics, Bristol-Myers Squibb, Calico Labs, Celgene, Cerevel Therapeutics, Coave Therapeutics, DaCapo Brainscience, Denali, Edmond J. Safra Foundation, Eli Lilly, Gain Therapeutics, GE HealthCare, Genentech, GSK, Golub Capital, Handl Therapeutics, Insitro, Janssen Neuroscience, Lundbeck, Merck, Meso Scale Discovery, Mission Therapeutics, Neurocrine Biosciences, Pfzer, Piramal, Prevail Therapeutics, Roche, Sanof, Servier, Sun Pharma Advanced Research Company, Takeda, Teva, UCB, Vanqua Bio, Verily, Voyager Therapeutics, the Weston Family Foundation and Yumanity Therapeutics. The Parkinson's Disease Biomarker Program (PDBP) consortium is supported by the National Institute of Neurological Disorders and Stroke (NINDS) at the National Institutes of Health.]

#### **Availability of data and materials**

Data used in the preparation of this article were obtained [on October 31, 2022.] from the Parkinson's Progression Markers Initiative (PPMI) database ([www.ppmi-info.org/access-data-specimens/download-data\)](http://www.ppmi-info.org/access-data-specimens/download-data), RRID:SCR\_006431. The PDBP data were obtained from the DMR (data

management resources [\(https://pdbp.ninds.nih.gov/\)](https://pdbp.ninds.nih.gov/)) on May 27th 2021. The Biopark dataset analysed during the current study is available from the corresponding author on reasonable request.

#### **Declarations**

#### **Ethics approval and consent to participate**

All study individuals from the Stockholm-based cohort gave written consent to the storage of their samples for future use in studies; and the study has been approved by the Swedish Ethical Review Authority (dnr 2020–03684). The PD patients included were additionally part of the Stockholm BioPark cohort (dnr 2019–04967) and had been clinically assessed. The PPMI study was approved by the Institutional Review Boards of each PPMI site. Informed written consent was obtained from all subjects at each site.

#### **Consent for publication**

Not applicable.

#### **Competing interests**

H.I. and M.A.N.'s participation in this project was part of a competitive contract awarded to Data Tecnica LLC by the National Institutes of Health to support open science research. M.A.N. also currently serves on the scientifc advisory board at Clover Therapeutics and is an advisor and scientifc founder at Neuron23 Inc. A.S cofounded REGAIN Therapeutics and is co-inventor of the patent "Compositions and methods for treatment and/or prophylaxis of proteinopathies." All other authors report no conficts of interest.

## Received: 17 January 2024 Accepted: 9 May 2024

#### **References**

- <span id="page-3-0"></span>1. Bloem BR, Okun MS, Klein C. Parkinson's disease. Lancet. 2021;397(10291):2284–303.
- <span id="page-3-1"></span>2. Siderowf A, Concha-Marambio L, Lafontant DE, Farris CM, Ma Y, Urenia PA, et al. Assessment of heterogeneity among participants in the Parkinson's progression markers initiative cohort using α-synuclein seed amplifcation: a cross-sectional study. Lancet Neurol. 2023;22(5):407–17.
- <span id="page-3-2"></span>Paslawski W, Khosousi S, Hertz E, Markaki I, Boxer A, Svenningsson P. Large-scale proximity extension assay reveals CSF midkine and DOPA decarboxylase as supportive diagnostic biomarkers for Parkinson's disease. Transl Neurodegener. 2023;12(1):42.
- <span id="page-3-4"></span>4. Pereira JB, Kumar A, Hall S, Palmqvist S, Stomrud E, Bali D, et al. DOPA decarboxylase is an emerging biomarker for Parkinsonian disorders including preclinical Lewy body disease. Nat Aging. 2023;3(10):1201–9.
- <span id="page-3-3"></span>5. Del Campo M, Vermunt L, Peeters CFW, Sieben A, Hok AHYS, Lleó A, et al. CSF proteome profling reveals biomarkers to discriminate dementia with Lewy bodies from Alzheimer's disease. Nat Commun. 2023;14(1):5635.
- <span id="page-3-6"></span>6. Hadjiconstantinou M, Nef NH. Enhancing aromatic L-amino acid decarboxylase activity: implications for L-DOPA treatment in Parkinson's disease. CNS Neurosci Ther. 2008;14(4):340–51.
- <span id="page-3-7"></span>7. Li L, Lin H, Hua P, Yan L, Dong H, Li T, et al. Polymorphism of the Dopadecarboxylase gene modifes the motor response to Levodopa in Chinese patients with Parkinson's disease. Front Neurol. 2020;11:520934.
- <span id="page-3-8"></span>8. van Rumund A, Pavelka L, Esselink RAJ, Geurtz BPM, Wevers RA, Mollenhauer B, et al. Peripheral decarboxylase inhibitors paradoxically induce aromatic L-amino acid decarboxylase. NPJ Parkinsons Dis. 2021;7(1):29.