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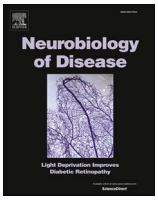

Diagnostic metabolomic profiling of Parkinson's disease biospecimens

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ABSTRACT

Background: Reliable and sensitive biomarkers are needed for enhancing and predicting Parkinson's disease (PD) diagnosis.
Objective: To investigate comprehensive metabolomic profiling of biochemicals in CSF and serum for determining diagnostic biomarkers of PD.
Methods: Fifty subjects, symptomatic with PD for ≥5 years, were matched to 50 healthy controls (HCs). We used ultrahigh-performance liquid chromatography linked to tandem mass spectrometry (UHPLC-MS/MS) for measuring relative concentrations of ≤1.5 kDalton biochemicals. A reference library created from authentic standards facilitated chemical identifications. Analytes underwent univariate analysis for PD association, with false discovery rate-adjusted *p*-value (≤0.05) determinations. Multivariate analysis (for identifying a panel of biochemicals discriminating PD from HCs) used several biostatistical methods, including logistic LASSO regression.
Results: Comparing PD and HCs, strong differentiation was achieved from CSF but not serum specimens. With univariate analysis, 21 CSF compounds exhibited significant differential concentrations. Logistic LASSO regression led to selection of 23 biochemicals (11 shared with those determined by the univariate analysis). The selected compounds, as a group, distinguished PD from HCs, with Area-Under-the-Receiver-Operating-Characteristic (ROC) curve of 0.897. With optimal cutoff, logistic LASSO achieved 100% sensitivity and 96% specificity (and positive and negative predictive values of 96% and 100%). Ten-fold cross-validation gave 84% sensitivity and 82% specificity (and 82% positive and 84% negative predictive values). From the logistic LASSO-chosen regression model, 2 polyamine metabolites (*N*-acetylcadaverine and *N*-acetylputrescine) were chosen and had the highest fold-changes in comparing PD to HCs. Another chosen biochemical, acisoga (*N*-(3-acetamidopropyl)pyrrolidine-2-one), also is a polyamine metabolism derivative.
Conclusions: UHPLC-MS/MS assays provided a metabolomic signature highly predictive of PD. These findings provide further evidence for involvement of polyamine pathways in the neurodegeneration of PD.

1. Introduction

The ongoing quest for diagnostic indicators of Parkinson's disease (PD) has explored a variety of options, including neuroimaging, biopsied tissues, gene sequencing, and biochemical measurements made in readily sampled biofluids (Farotti et al., 2017; Postuma and Berg, 2016; Kwon et al., 2022a, 2022b; Tönges et al., 2022). Some investigations for PD biomarkers have been based on targets known to have associations with this disorder, such as the dropout of striatal dopaminergic neurons and the intraneuronal accumulation of misfolded α-synuclein (αSyn). Unfortunately, the latter approaches have not yielded useful diagnostic biomarkers, particularly testing that would be useful in clinical trials for demonstrating disease modification (Mollenhauer et al., 2017), although recent refinements in α-synuclein seed amplification assays have shown increasing promise (Bellomo et al., 2022). There is a continuing challenge for novel disease indicators offering high sensitivity and specificity. Findings that would reliably distinguish persons with PD have the potential to aid in pre-clinical detection of this disorder and might offer new insights into the disease process.

Among the various 'omics' strategies for biomarker discovery in medical research, *metabolomics* has been one of the most productive (Caudle et al., 2010; Redenšek et al., 2018). The methodological

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advances underlying comprehensive (global) metabolomic analysis draw from technologies capable of separating and measuring hundreds of small-molecular weight (<1.5 kDalton) compounds (Evans et al., 2009; Guo et al., 2015). Metabolomics can characterize many of the chemically diverse compounds that comprise the body's biochemical milieu, or *metabolome*. The assay techniques utilized are generally chosen for their sensitivity to the minute concentrations of most metabolome constituents in biofluids. In the latest iterations of metabolomic assay platforms, automated instruments and highly standardized protocols offer improved control of inter-assay variability. Compounds that can be quantified arise from a wide range of metabolic origins and functions. With the state-of the-art assay methodology used in the current study and in our prior investigations (LeWitt et al., 2013, 2017), several hundred compounds can be distinguished and identified through informatics linked to spectral and chromatographic databases. Once metabolomic screening strategies have detected promising disease-specific biochemicals, subsequent analysis can be targeted to discern associated metabolic pathways (DeHaven et al., 2010; Xia et al., 2015) or other chemical characteristics underlying their origins (such as oxidative stress). Data analysis by univariate methods can seek out individual compounds serving as biomarkers while multivariate analysis may identify a panel of biochemicals offering prediction of diagnosis.

In this investigation, we used ultrahigh-performance liquid chromatography linked to tandem mass spectrometry in a search for a global metabolomic signature of PD. Our goal was to learn if this approach to nontargeted profiling might yield individual compounds or combinations of them as predictors of PD diagnosis in comparison to a matched group of healthy controls (HCs).

2. Materials and methods

2.1. Participants

The PD subjects whose specimens were used in this study were chosen from participants in the BioFIND study, a multicenter research initiative for creating biomarker resources from PD patients (ClinicalTrials.gov NCT01705327) (Kang et al., 2016; Goldman et al., 2018). The 8 BioFIND study sites utilized a uniform research protocol, and all consent forms were approved by institutional ethical standards committees on human experimentation. Subjects provided written informed consent before study participation. BioFIND specimens, collected between December 2012 and June 2015, were maintained frozen at −70 °C.

Characterization of PD (or, for HCs, their normal neurological status) was ascertained using United Kingdom PD Society Brain Bank clinical diagnostic criteria (Marsili et al., 2018). Beyond these criteria, selection of PD patients in the BioFIND study imposed additional diagnostic requirements, including the presence of three "classic" features of PD (bradykinesia, rigidity, and resting tremor) by either history or clinical examination. Moreover, BioFIND study requirements also mandated several other characteristics typical of PD, including unilateral onset, asymmetry of Parkinsonian signs, continuing responsiveness of Parkinsonism to levodopa therapy, and persistence of PD symptoms for at least 5 years. HCs were chosen at random from the BioFIND cohort to match for age and sex of PD subjects.

All PD medications were discontinued at least 12 h prior to collection of biospecimens. Once lumbar CSF and venous serum specimens were collected (using a standardized protocol), they were aliquoted into sealed, preservative-free storage tubes and immediately stored at −70 °C. Other details of the BioFIND study have been published (Kang et al., 2016; Goldman et al., 2018) and are available online (https://www.michaeljfox.org 〉 For Researchers 〉 BioFIND).

2.2. Assay methods

CSF and serum specimens were thawed just before assay. The sequential order of PD and HCs were randomized across platform runs. For quality control, several recovery standards were added prior to the extraction process. Methanol was added to samples, after which they were shaken for protein dissociation and release of small protein-bound or trapped molecules. Next, samples underwent centrifugation. The extract was dried and reconstituted in solvents compatible with 4 methods used to separate and identify hydrophilic, hydrophobic, and polar molecules (Guo et al., 2015).

The assays used separate reverse-phase Waters ACQUITY ultrahigh-performance liquid chromatography systems linked to a Thermo Scientific Q-Extractive high-resolution tandem mass spectrometry (MS) unit that was interfaced with a heated electrospray ionization source and Orbitrap mass analyzer (operated at 35,000 mass resolution). While the lower limit of detection and linear dynamic range varied by metabolite, a typical biochemical could be detected at or below nano-gm/ml concentrations and at a linear dynamic range comprising 8 orders of magnitude. The assay data output of all detected ions included normalized retention time/index, mass-to-charge ratio (m/z), and chromatographic data (including MS spectral data).

2.3. Data analysis

In this study, group sizes were planned to greatly exceed the minimal number (20 per group) needed for adequate statistical modeling of metabolomic data (Nyamundanda et al., 2013). Readouts of MS data were inserted into a relational database evaluated without binary large-object manipulation and using proprietary peak integration software. Next, the components were placed in a complex data structure. The chemical identifications arose from comparing each sample's ion features to entries in a spectral reference library of >4000 authentic chemical standards [DeHaven et al., 2010]. These characteristics included retention time, m/z, preferred adducts, in-source fragments, and associated tandem MS spectra of all detectable ions. Each of the structurally named metabolites reported here conform to the highest confidence level of identification set forth by the Metabolomics Standards Initiative (Sumner et al., 2017; Schrimpe-Rutledge et al., 2016). Chromatographic or MS peaks that could not be identified were not entered into the analysis. The curation of data included quality control measures for enhancing accuracy of chemical identifications and for removing system artifacts, mis-assignments, and background noise. For compounds with missing values (for example, due to thresholding of the MS data), data was imputed by use of minimum detection level. The data underwent $\log_2$ transformation prior to statistical analysis.

Because the BioFIND PD subjects had received levodopa as recently as 12 h prior to the collection of specimens, all identified compounds derived from levodopa metabolism (LeWitt et al., 1992) were eliminated from analysis because they might confound the differentiation of PD from HCs. Other xen\obiotics (compounds with known exogenous origins such as vitamins or dietary ingredients) also were dropped. However, caffeine and its catabolites were retained for analysis since prior studies have reported altered metabolism of this dietary compound in PD (Hatano et al., 2016).

To detect biochemicals of interest, we undertook several approaches for managing the relative concentrations of the hundreds of compounds arising from the metabolomic assays in CSF and serum. Data underwent a strategy of feature selection through unbiased univariate analysis, followed by multivariable modeling. Initially, each analyte was individually tested for possible association with PD using a two-sample *t*-test and calculation of false-discovery rate (FDR)-adjusted *p*-values (i.e., *q*-values) (Benjamini and Hochberg, 1995; Storey and Tibshirani, 2003). Next, relative concentrations of the measured compounds were analyzed to identify a multi-metabolite profile that best predicted PD diagnosis. Our analytic plan explored several methods: a) t-test followed by Support Vector Machine (SVM) (R package e1071); b) recursive feature selection followed by SVM (Guyon et al., 2002); c) *t*-test followed by Partial Least Squares (Boulesteix, 2004; R package: *plsgenomics*); and d)

multiple logistic regression with variable selection using Least Absolute Shrinkage and Selection Operator (LASSO) (Tibshirani, 1996; R package: glmnet). Ten-fold cross-validation was performed to yield an unbiased estimate of prediction error, so that the best model could be selected. After classifying the data using the four supervised learning models, we plotted their respective receiving operator characteristic (ROC) curves. For SVM, a linear kernel was used. The analysis was performed in a two-nested 10-fold cross-validation loops, an inner loop to selected optimal number of predictors and tuning parameters such as penalty parameter for LASSO, and an outer loop to measure the optimized model performance with estimation of ROC curves. The best model was selected based on comparison of ROC curves.

2.4. Metabolic pathway analysis

We also planned for interpreting the metabolomic data by investigation of known metabolic relationships between assayed biochemicals of interest, using available software designed for this purpose (Xia et al., 2015). This process involved the use of KEGG pathway maps (www.genome.jp/KEGG) and other sources of biochemical information for discerning over-representation of the detected biomarkers in canonical metabolic pathways. The procedure combines findings from a pathway enrichment analysis with a topology analysis (Ingenuity Pathway Analysis, Ingenuity Systems, Mountain View, California, USA).

3. Results

3.1. Participants

PD specimens came from twenty females and thirty males. Their mean age (±S.D.) at specimen collection was 69.5 (6.6) years. The median age was 69.0 years, and ages ranged from 57 to 84. All had Parkinsonism responsive to levodopa. For HCs, the specimens came from 29 females and 21 males. Their mean age (± S.D.) at specimen collection was 66.0 (6.9) years, and median age was 64.5, with ages ranging from 57 to 86.

3.2. Assay findings

In the serum samples, the assays detected 772 biochemicals that were structurally identified by referencing entries of authentic standards as maintained in the Metabolon spectral library (DeHaven et al., 2010). CSF assays yielded 353 identified biochemicals. Before analysis was conducted, compounds were eliminated from the analysis if they were derived from levodopa metabolism or xenobiotics, or if the data showed low variation. Following the data set revision, 706 serum and 317 CSF biochemicals remained.

3.3. Univariate and multivariate analysis of PD and HC data

Two-sample *t*-test and corresponding *p* and *q* values were calculated for relative concentrations of biochemicals measured in CSF and serum samples. However, after *p*-value adjustment, none of the serum compounds maintained statistical significance with the diagnosis of PD. For the serum data, we created a list of the top ten compounds showing the greatest associations to PD diagnosis [Table 1].

In the CSF data set, 21 compounds were differentially expressed in PD versus HCs and with FDR ≤0.05 [Fig. 1].

Next, we performed multivariate analysis of CSF data using the four strategies described above. The best prediction of PD diagnosis came from logistic LASSO regression, which identified 23 compounds. From these data, we created a composite index that was based on a linear combination of these biochemicals using the coefficients listed in Table 2.

The 23 CSF compounds and their overlap with compounds recognized by the univariate analyses as being differentially expressed in PD versus HCs (FDR ≤0.05) are listed in Fig. 2. We utilized a workflow of merging data from LASSO and univariate analysis similar to methods reported in an analysis of proteomics in neurodegenerative diseases (Gaetani et al., 2021).

Table 1
Univariate analysis of serum assay data listing the top ten of all the measured compounds that showed the greatest associations with PD diagnosis (but not showing statistical significance after multiple test correction).

Serum compounds chosen by univariate analysis	*p*-value	*q*-value
N-acetylcadaverine	0.0001	0.0903
taurocholenate sulfate	0.0006	0.2031
imidazole propionate	0.0012	0.2463
N-acetylisoleucine	0.0014	0.2463
phenylacetylglutamine	0.0026	0.3398
indolin-2-one	0.0031	0.3398
1-palmitoyl-GPI (16:0)	0.0034	0.3398
palmitoyl dihydrosphingomyelin (d18:0/16:0)	0.0041	0.3595
phenylacetylcarnitine	0.0053	0.3912
methylsuccinoylcarnitine	0.0061	0.3912

This data is expressed as fold-changes of mean relative concentrations (PD versus HCs), obtained from the univariate and multivariate models. For the group of compounds chosen by logistic LASSO regression, the area-under-the-ROC curve value was 0.897 [Fig. 3].

With optimal cutoff, this approach achieved 100% sensitivity and 96% specificity, and with positive and negative predictive values of 96% and 100%. Ten-fold cross-validation gave 84% sensitivity and 82% specificity, and 82% positive and 84% negative predictive values. The CSF biomarkers selected by LASSO regression were placed into a metabolic mapping enrichment analysis. While the results did not discern involvement of canonical metabolic pathways, three of the selected compounds (*N*-acetylputrescine, *N*-acetylcadaverine, and acioga) are catabolites of polyamine compounds, while L-ornithine serves as the initial substrate for synthesis of three polyamines: putrescine, spermidine, and spermine [Fig. 4].

As shown in Table 2, the two highest coefficients were for L-ornithine and *N*-acetylputrescine. *N*-acetylcadaverine, a polyamine metabolite which was also selected by LASSO regression, showed the highest fold-change in favor of PD [Fig. 1].

4. Discussion

There has been a longstanding need for easily sampled biochemicals whose measurements provide high sensitivity and specificity for the diagnosis of PD. So far, no individual substance has served this purpose and it is likely that diagnostic evidence for PD might require the combination of multiple substances to differentiate PD from healthy controls. This concept draws support from findings that PD manifests a systemic disturbance in mitochondrial function (Naren et al., 2022), involves multiple organs (Gelpi et al., 2014), and demonstrates aberrant activation of brain circuitry (Spetsieris et al., 2015). In the current investigation, we highlight a diverse group of biochemical alterations that together provide a distinctive PD profile in CSF. The multivariate analysis yielded a strong metabolomic signature for PD comprised of 23 compounds mostly unknown for participating in neurodegenerative processes. Consequently, some of the biochemicals we highlight may be pointing to novel mechanisms involved in PD pathogenesis. However, it is also possible that metabolomic profiles determined in this investigation may be nothing more than epiphenomena rather than a direct outcome of PD pathophysiology. Regardless of their biochemical origins, the compounds chosen for the multi-marker model might, if substantiated by replication in other sample sets, offer new insights for enhancing the diagnosis of PD. Although an online pathway enrichment analysis did not bring out over-representation of any specific metabolic pathway, we observed that four of the 23 compounds determined by the multivariate analysis are involved in polyamine metabolism. The

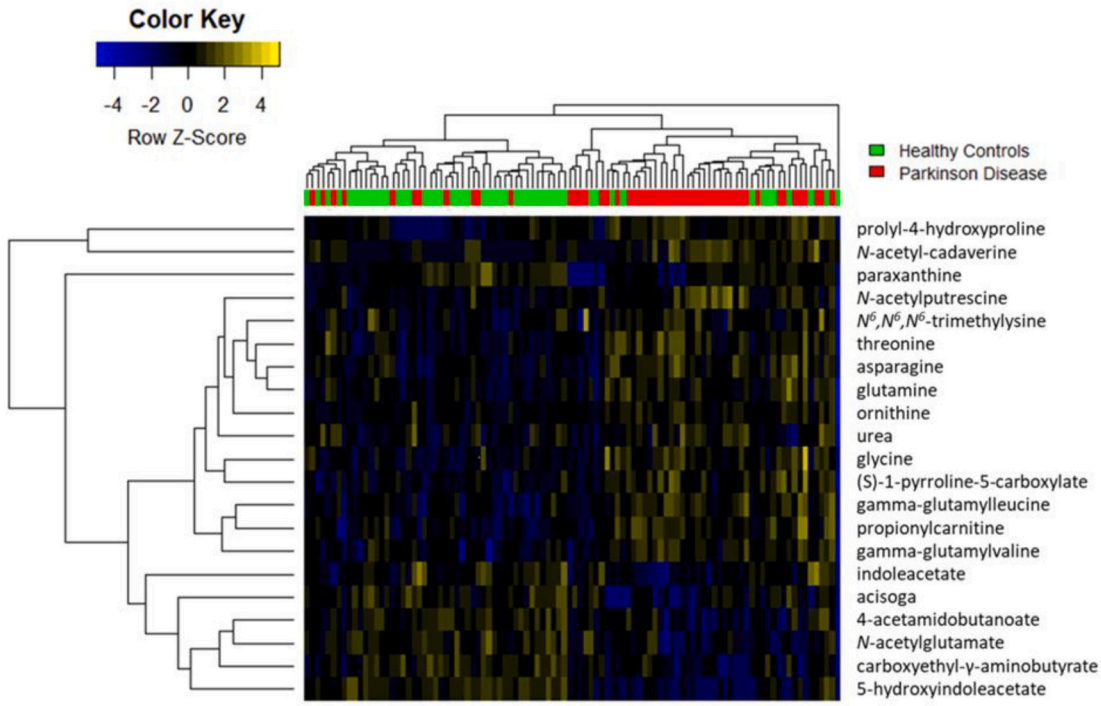

Fig. 1. Heat map of 21 CSF constituents that show significant difference between PD HC subjects, as determined by univariate analysis.

Table 2
Multivariate analysis of CSF constituents (identified by logistic LASSO regression) and their coefficients.

Coefficients	CSF compounds chosen by logistic LASSO regression
1.61	L-ornithine
1.14	*N*-acetylputrescine
−0.51	5-hydroxyindoleacetate
0.42	urea
−0.33	*N*-acetylglucosamine/*N*-acetylgalactosamine
−0.27	taurine
0.27	N^6,N^6,N^6-trimethyllysine
−0.23	paraxanthine
−0.21	ergothioneine
0.21	*N*-acetylcadaverine
−0.21	carboxyethyl-γ-aminobutyrate
−0.19	*N*-acetylglutamate
−0.18	succinate
−0.10	glycerophosphoglycerol
0.10	benzoate
−0.08	pantothenate
−0.08	ascorbate
−0.07	2-aminooctanoate
−0.07	indoleacetate
−0.06	2′-deoxyuridine
−0.05	*N*-acetylglucosaminylasparagine
−0.04	acisoga*
−0.04	*cis*-4-decenoyl carnitine

* Also known as *N*-(3-acetamidopropyl)pyrrolidine-2-one.

polyamines present in humans – cadaverine, spermine, spermidine, and putrescine - are small, positively-charged molecules distributed ubiquitously and engaged in multiple intracellular functions (Handa et al., 2018). Among these are regulation of gene transcription and translation, stabilization of polynucleotides, facilitation of cellular growth, interaction with multiple enzymatic activities, and antioxidant actions (Miller-Fleming et al., 2015; Pegg, 2016). Besides our study, another small-scale investigation in PD CSF also found altered polyamine metabolism (Paik et al., 2010). The authors reported that the ratio of putrescine and spermidine concentrations (± S.D.) for PD (2.80 ± 0.24) exceeded those measured in HCs (1.06 ± 0.19; $p < 0.001$). Compared to HCs, the concentration of N^8-acetylspermidine in PD was 52% greater ($p < 0.001$) and total polyamine content was increased by 69% ($p < 0.001$). Other reported investigations also found systemic evidence for altered polyamine metabolism in PD. For example, erythrocyte spermidine and spermine content was increased in PD compared to HCs in one report (Gomes-Trollin et al., 2002). In the latter study, putrescine concentration was diminished, although another investigation reported increased values (Betancourt et al., 2018). A third study (Roede et al., 2013) found the serum concentration of the polyamine metabolite N^8-acetylspermidine differentiated PD patients with a slower versus a more rapid progression of their Parkinsonism. Another investigation reported elevations of plasma N^8-acetylspermidine and *N*-acetylputrescine in PD versus controls and also found that N^1,N^8-diacetylspermidine plasma concentration correlated with severity of Parkinsonian motor features (Saiki et al., 2019).

Support for an association between altered polyamine metabolism and the pathogenesis of PD was offered by findings that activity of spermidine-spermine N^1-acetyl transferase-1 (SAT-1) is diminished in the PD brain (Lewandowski et al., 2010). SAT-1 is the major regulatory factor for catabolism and interconversion of intracellular polyamines (Pegg, 2008). Relationships between SAT-1 activity and α-synuclein (αSyn) anti-neuronal toxicity was investigated using genetically modified mice that manifested histological and neurochemical changes resembling those appearing in the PD brain (Lewandowski et al., 2010). In this animal model, experimentally increasing SAT-1 activity reduced the toxicity of αSyn, whereas inhibition of SAT-1 produced the opposite result. The same report described experiments with an αSyn-expressing yeast model that found exposure to certain polyamines enhanced lethality conferred by αSyn.

Our study joins an expanding group of metabolomic investigations that have been carried out with biospecimens from PD and HC subjects (Bogdanov et al., 2008; Michell et al., 2008; Johansen et al., 2009; LeWitt et al., 2013; Trapp et al., 2014; Hatano et al., 2016; Roede et al., 2013; Luan et al., 2015; Öhman and Forsgren, 2015; Kori et al., 2016; Wuolikainen et al., 2016; LeWitt et al., 2017; Han et al., 2017; Trezzi et al., 2017; Burté et al., 2017; Havelund et al., 2017; Nagesh Babu et al.,

	Fold Change*
MULTIVARIATE ANALYSIS	
benzoate	1.04
cis-4-decenoyl carnitine	1.03
N-acetylglucosamine/*N*-acetylgalactosamine	0.95
taurine	0.93
succinate	0.92
glycerophosphoglycerol	0.91
ascorbate	0.87
2-aminooctanoate	0.84
2'-deoxyuridine	0.81
pantothenate	0.76
N-acetylglucosaminylasparagine	0.75
ergothioneine	0.65
PRESENT IN BOTH ANALYSES	
N-acetyl-cadaverine	2.33
N-acetylputrescine	1.56
ornithine	1.37
urea	1.33
N^6,N^6,N^6-trimethyllysine	1.16
indoleacetate	0.75
N-acetylglutamate	0.71
carboxyethyl-γ-aminobutyrate	0.71
acisoga	0.68
5-hydroxyindoleacetate	0.52
paraxanthine	0.48
UNIVARIATE ANALYSIS	
prolyl-4-hydroxyproline	1.94
(S)-1-pyrroline-5-carboxylate	1.39
glycine	1.36
γ-glutamylvaline	1.35
propionylcarnitine	1.30
γ-glutamylleucine	1.29
threonine	1.27
asparagine	1.13
glutamine	1.07
4-acetamidobutanoate	0.81

Fig. 2. CSF compounds differentiating PD and HCs, as determined by univariate and multivariate (LASSO) analysis. Eleven compounds are found in both analyses. Numbers indicate fold-change of mean for each metabolite.

2013; Willkommen et al., 2018; Socha et al., 2019; Klatt et al., 2021; Kwon et al., 2022a, 2022b; Hwangbo et al., 2022; Chang et al., 2022; Meoni et al., 2022; Andújar et al., 2010). A recent comparison of these studies appeared in a recent systematic review summarizing the results of profiling PD biospecimens (Li et al., 2022). As would be expected in an emerging technology, assay and biostatistical methodologies varied among these studies (Redenšek et al., 2018; Schrimpe-Rutledge et al., 2016). Most of the prior reports differed in conclusions from the findings we report here. Though metabolomic profiling can be a powerful technology for biomarker discovery, a major caveat is the lack of standardization for collection of biospecimens and the rigor in diagnostic characterization of subjects. Biochemicals of interest may be present at concentrations below limits of instrument detection. With the assay platform we utilized, separations were achieved only for electrochemically charged compounds. Interpreting findings from metabolomic profiling need to recognize that, while some of the biochemicals in the analytical readout arise from endogenous metabolism, others are dietary or derived from the gut microbiome.

With concern for overfitting of the training set, we used ten-fold cross-validation to estimate training algorithm performance (which reduced average performance of the model to 84% sensitivity and 82% specificity). We recognize that validation of our findings requires replication in a different levodopa-treated patient population. Since this analysis provided positive identification for compounds of interest, subsequent investigations can enhance precision of measurements by utilizing targeted assays.

Metabolomic biomarker profiling in medicated PD patients is potentially confounded by the effects of altered dopamine turnover and other drug actions. In the analysis of our study, we excluded all compounds derived from administration of levodopa. Since the current study involved subjects symptomatic with PD for $\geq$5 or more years, it will be of interest to learn if patients at risk for PD or those experiencing symptoms for briefer periods might manifest a similar panel of biomarkers. Finally, any search for disease-specific biomarkers presents clinicians with the challenge for accurate diagnosis, which in most studies has been derived solely from clinical history information and neurological examination of subjects. One strength of our study was that, beyond meeting standard diagnostic clinical criteria, PD subjects also had years of typical levodopa-responsive Parkinsonian symptomatology. However, to validate that our exploratory findings constitute PD biomarkers, we plan to replicate our results with PD patients who have had diagnostic confirmation of decreased striatal dopamine neurotransmission by dopamine transporter neuroimaging.

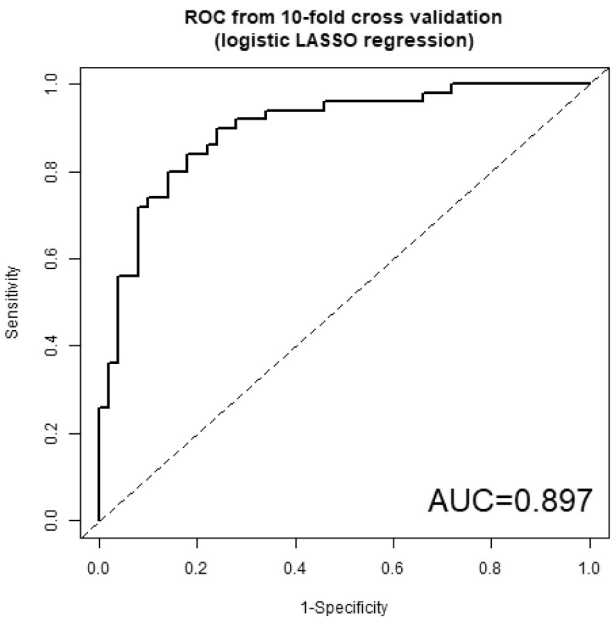

Fig. 3. Area-under-the-Receiver-Operator-Characteristic curve for logistic LASSO regression of CSF compounds for the prediction of PD diagnosis versus healthy controls.

5. Conclusions

In summary, we found that analysis of CSF specimens (but not serum) provided a distinctive metabolomic signature of PD with high sensitivity and specificity versus controls. Beyond findings of altered polyamine metabolism in PD, the 23-component biomarker panel provides additional targets for further inquiry into biochemicals potentially useful for enhancing diagnosis capabilities and investigation into the origins of this disease.

Data statement

The data presented in this report is available for review from the MJFFPR, which maintains a database of clinical and biomarker findings from supported studies and biospecimen repositories. For more information, contact Dr. LeWitt. or Fox Investigation for New Discovery of Biomarkers (BioFIND) database (http://biofind.ioni.usc.edu). For up-to-date information on the study, visit www.michaeljfox.org/biofind.

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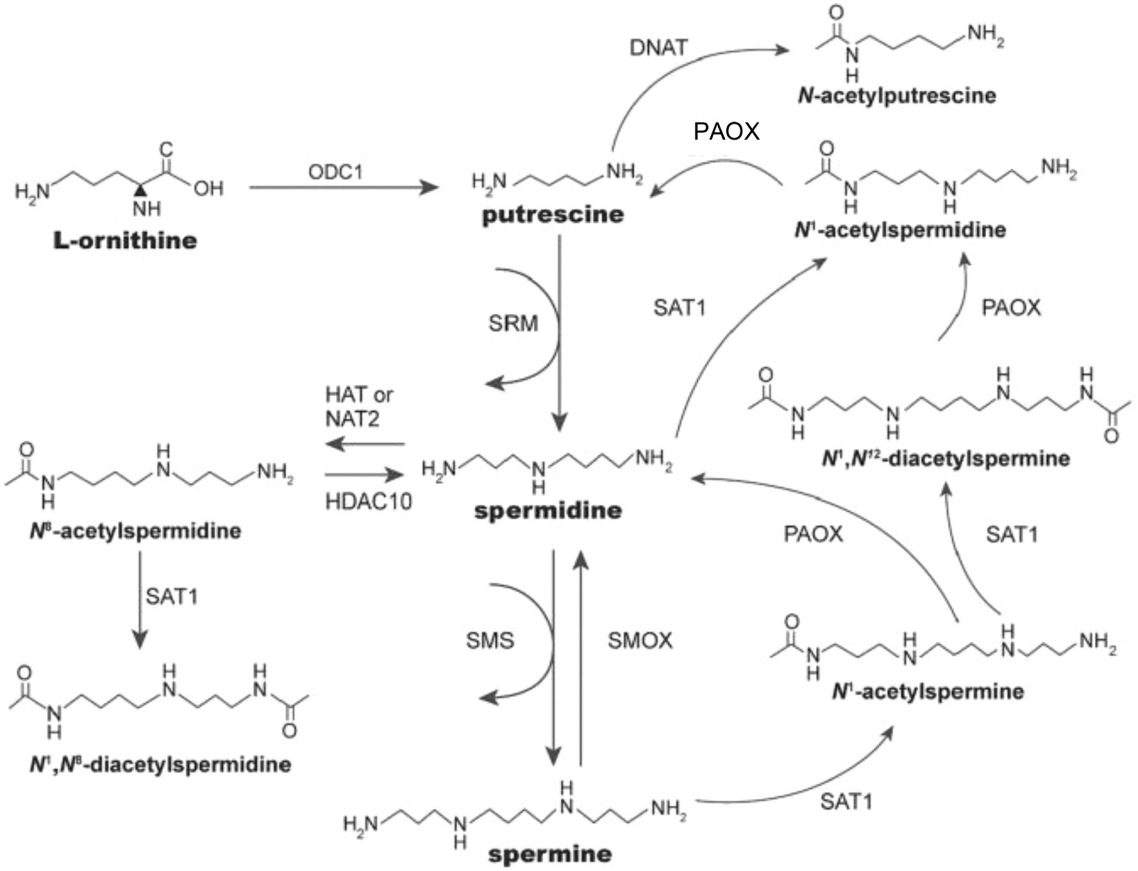

Fig. 4. Metabolism of polyamines derived from L-ornithine. Enzyme abbreviations are: diamine N^1-acetyltransferase (DNAT), histone acetyltransferase (HAT), histone deacetylase-10 (HDAC10), *N*-acetyltransferase-2 (NAT2), ornithine decarboxylase-1 (ODC1), N^1-acetylpolyamine oxidase (PAOX), spermidine/spermine N^1-acetyltransferase (SAT1), spermine oxidase (SMOX), spermine synthase (SPM), and spermidine synthase (SRM).

undertook this project. Data and biospecimens from BioFIND study used in the preparation of this research obtained from the Fox Investigation for New Discovery of Biomarkers (BioFIND) database (http://biofind.ioni.usc.edu). For up-to-date information on the study, visit www.michaeljfox.org/biofind. The BioFIND study is sponsored by The Michael J. Fox Foundation for Parkinson's Research (MJFFPR) with support from the National Institute for Neurological Disorders and Stroke, National Institutes of Health. Funding for this project came from a grant to PAL from the MJFFPR Biomarkers Initiative (which had no role in the writing of this report or its conclusions).

CRediT authorship contribution statement

Peter A. LeWitt: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Jia Li:** Data curation, Formal analysis, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Kuan-Han Wu:** Data curation, Formal analysis. **Mei Lu:** Data curation, Formal analysis, Methodology.

Declaration of Competing Interest

The authors report no conflicts of interest pertinent to this research report.

Data availability

Data will be made available on request.

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