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Ser(P)-1292 LRRK2 in Urinary Exosomes is Elevated in Idiopathic Parkinson Disease

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Abstract

Background—Mutations in *Leucine-rich repeat kinase 2 (LRRK2)* enhance levels of autophosphorylated LRRK2 protein and are the most common known cause of inherited Parkinson disease (PD). *LRRK2* has been further implicated in susceptibility to idiopathic PD in genetic association studies.

Objective—To compare autophosphorylated Ser(P)-1292 LRRK2 levels from biobanked urine samples with clinical data in PD and controls.

Methods—Ser(P)-1292 LRRK2 levels were measured from urine exosome fractions from 79 PD and 79 neurologically healthy controls enrolled into the Parkinson Disease Biomarker Program at the University of Alabama at Birmingham.

Results—Ser(P)-1292 LRRK2 levels were higher in males than females ($p < 0.0001$) and elevated in PD compared to controls ($p = 0.0014$). Ser(P)-1292 LRRK2 levels were higher in PD cases with worse cognition and correlated with poor performance in MoCA ($r = -0.2679$ [$-0.4628, -0.0482$]), MDS-UPDRS subscales 1 and 2 ($r = 0.2239$ [$0.0014, 0.4252$], 0.3404 [$0.1276, 0.5233$] respectively), Epworth Sleepiness Scale ($r = 0.3215$ [$0.1066, 0.5077$]), and Modified Schwab and England Activities of Daily Living Scales ($r = -0.4455$ [$-0.6078, -0.2475$]). Ser(P)-1292 LRRK2 levels predicted those with worse cognitive impairment in PD patients with some success ($c = 0.73$).

Conclusions—Urinary exosome Ser(P)-1292 LRRK2 levels are elevated in idiopathic PD and correlated with the severity of cognitive impairment and difficulty in accomplishing activities of daily living. These results implicate biochemical changes in LRRK2 in idiopathic PD.

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*PDBP consortium consists of multiple scientists that contributed to study design and are listed at <http://pdbp.ninds.nih.gov/projects-we-support>

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Keywords

Biomarker; PARK8; Microvesicles; Cognitive decline

Introduction

Genetic studies identified mutations in the *leucine-rich repeat kinase 2 (LRRK2)* gene as the most common known cause of autosomal-dominant PD^{1–5}. The *LRRK2* gene is also linked to idiopathic PD (i.e., cases without *LRRK2* or other known genetic mutations) through genome-wide association studies⁶. LRRK2 protein includes two enzymatic domains proven to have GTPase and protein kinase activity. The kinase domain that harbors the most common LRRK2 mutation, G2019S, can direct intrinsic phosphorylation (i.e., autophosphorylation) in and nearby the GTPase domain^{7, 8}. Pathogenic *LRRK2* mutations including the R1441C, Y1699C, and G2019S mutation increase autophosphorylated LRRK2 protein levels in model systems^{8–11}. One of the most abundant sites of autophosphorylation is the Ser-1292 residue nearby the GTPase domain⁸. Treatment with LRRK2 kinase inhibitors diminish levels of Ser(P)-1292 LRRK2 in model systems^{8, 12}.

Our previous work demonstrated that LRRK2 protein can be purified from urinary exosomes in biobanked samples¹³. Recently we discovered that medicated PD patients with the G2019S-*LRRK2* mutation show a ~5-fold elevation of autophosphorylated Ser(P)-1292 LRRK2 protein in urinary exosomes compared to non-carrier controls and medicated PD patients without the G2019S-*LRRK2* mutation¹¹. LRRK2 autophosphorylation has not been previously evaluated in a larger series of idiopathic PD cases. Here, exosome Ser(P)-1292 LRRK2 phosphorylation is measured in urine from both idiopathic PD cases and controls. Among PD cases, we assess the association between autophosphorylated LRRK2 levels and clinical motor and non-motor disease severity scales.

Methods

Study Subjects

Urine was obtained from study patients (81 diagnosed with PD [41 males] and 81 neurologically healthy controls [40 males]) enrolled at the University of Alabama at Birmingham (UAB) Movement Disorder Clinic. Subjects were selected from the UAB cohort for this study based on frequency matching of age and gender between cases and controls. Inclusion criteria for cases were a diagnosis of PD based on the United Kingdom Parkinson's Disease Society Brain Bank Criteria. Inclusion criteria for healthy controls were ages of 35–85 years, lack of PD in first-degree blood relatives, and a lack of positive responses on more than three items in the PD Screening Questionnaire¹⁵. Exclusion criteria for all patients included atypical features indicative of progressive supranuclear palsy, multiple system atrophy, corticobasal degeneration, cerebellar signs, supranuclear gaze palsy, apraxia, disabling autonomic failure, neuroleptic treatment at time of onset of parkinsonism, active treatment with a neuroleptic at time of study entry, history of repeated strokes with stepwise progression of parkinsonism, history of repeated head injury, history of definite encephalitis, dementia, known severe anemia, kidney disease as determined by

measurement of serum creatinine and/or medical records, urinary tract or bladder infection, or a serious comorbidity that would interfere with participation in the study. None of the participants carried a *LRRK2* mutation (e.g., G2019S, R1441C/G, Y1699C) as determined with the NeuroX array following enrollment as described¹⁶. This study was approved by the UAB Institutional Review Board, and all subjects signed informed consents. All samples were processed by investigators blinded to PD status, and final curated datasets were achieved before associating clinical characteristics to sample values.

Clinical Evaluations

Demographics, PD characteristics, medications, medical history and family history of PD were collected. Levodopa equivalent daily dose was calculated as described¹⁷. Subjects were evaluated between 08:00 and 12:00 and PD patients were taking their usual medications. Motor assessment was conducted with the Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS). Cognition was screened by the Montreal cognitive assessment (MoCA). Non-motor symptoms and performance were assessed by the Epworth sleepiness scale (Epworth SS), Parkinson's Disease Questionnaire-39 (PDQ-39), and modified Schwab and England v1.0 Activities of Daily Living (Mod. S&E ADL v1.0) scale. All assessments were conducted by a single rater (R.G.C.) throughout the duration of the study.

Sample Collection and Handling

Samples were collected between January 2013 and December 2014 in a cross-sectional study as part of the Parkinson's Disease Biomarker Program¹⁴ (<https://pdpb.ninds.nih.gov>). Urine samples were procured during the subject visit along with blood samples. Within two hours of collection, urine was aliquoted into 50 mL polypropylene tubes and stored at -80°C for future processing and exosome isolation.

Exosome Isolation

Exosomes were isolated as previously described¹¹. Briefly, 50 mL polypropylene tubes were quick-thawed at 42°C at 100 RPM in a water bath. Urine was then centrifuged at $10,000 \times g$ for 30 min in Beckman JA-14 rotors and the supernatant then centrifuged at $100,000 \times g$ in Beckman UltraClear tubes for 2 hours in a Beckman SW-32 Ti rotor. The resultant exosome pellet was washed in a saline-buffered solution, resuspended into lysis buffer, and stored at -80°C for further analysis. Supplementary Figure S1 includes a cryo-electron micrograph of these isolated urinary exosomes following saline-buffered solution wash prior to exosomes lysis.

Immunoblotting and Quantitation

Samples were analyzed onto TGX gels (7.5%) SDS-PAGE, and transferred onto PVDF membrane (Millipore Immobilon-FL PVDF). Antibodies were applied to membranes (anti-TSG101 [Abcam poly rabbit], N241 (anti-LRRK2) [Antibodies Inc], and MJFR-19 (anti-pS1292-LRRK2) [Abcam]). Membranes probed with TSG101 and N241 were blocked using Odyssey blocking buffer (LiCOR) for 1 hour at room temperature and then incubated overnight in primary antibody diluted in blocking buffer at 4°C on a shaker (1:2500 TSG101,

1:2000 N241, both $\sim 1 \mu\text{g mL}^{-1}$ in solution). Membranes were washed 3×5 min in $1 \times$ tris-buffered tween solution and then incubated overnight with secondary antibodies. LiCOR secondary antibodies were used at 1:20,000 dilutions and consisted of Goat-anti mouse or anti rabbit IRDye 800CW and IRDye 680LT.

Blots were developed using a LiCOR Odyssey Scanner. Signals were recorded with LiCOR Image Studio. An exosomal pellet and a corresponding exosome protein marker (TSG101 protein) were detected in all specimens with the exception of two control subjects and two PD subjects where no protein could be measured for any of the markers. These four subjects were excluded from the study.

Exosome LRRK2 was therefore measured in 79 healthy controls and 79 PD patients (Table 1). Expanded immunoblots utilizing Ser(P)-1292 LRRK2, total LRRK2, and TSG101 antibodies in this study are included in Supplementary Figure S1. Total LRRK2 and Ser(P)-1292 LRRK2 signal are within a range of detection where a linear relationship existed between signal and target concentration (Supplementary Figure S1 C–D). This allows for minimal technical variation with this assay. Representative western blot images used to calculate Ser(P)-1292 LRRK2, total LRRK2, and TSG101 levels in the cohort study are presented in Supplementary Figure S2. No correlation was identified between the time in the freezer for the urine and observed values for any of the exosome proteins including Ser(P)-1292 LRRK2 levels ($r = -0.0473$ [$-0.2035, 0.1111$]).

Statistical Analysis

For continuous variables we compared controls and PD patients using Student's t-test or Mann-Whitney U test when assumptions of a t-test may not be met. Fisher's exact test was used to test the association between disease status and categorical variables. For assessing correlations, Pearson product-moment coefficients were calculated. As clinical measures of disease severity are highly correlated we report exact p -values as recommended by the Uniform Requirements for Manuscripts Submitted to Biomedical Journals¹⁸. In addition, we also report adjusted p values to account for potential false discovery using the Benjamini and Hochberg method¹⁹. Ser(P)-1292 LRRK2 levels were $\log_{10}$ -transformed to better meet model assumptions and were included in an ANCOVA model adjusted for gender and total LRRK2 levels as model covariates (see Supplemental Table S2 for details on the gender relationship). PD cases alone, controls alone, and PD and controls combined, were stratified into quartiles based on Ser(P)-1292 LRRK2 levels and differences in clinical measures of disease severity between quartiles were compared with Mann-Whitney U test. All statistical analyses and graphs were generated in JMP Pro 10 (SAS Institute Inc., Cary, NC, USA) or GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). Significance was ascribed to p -value < 0.05 , and all significance levels presented are two-tailed.

Results

Characteristics of study participants where exosome proteins could be measured (79 cases and 79 controls) are summarized in Table 1. Demographic characteristics were similar between cases and controls. MoCA scores as low as 17 (with a median of 26) were recorded in the healthy control population. In this cohort, no significant differences were observed in

frequencies of alcohol consumption, smoking, benzodiazepine use, antidepressant use, non-steroidal anti-inflammatory use, or vitamin and supplement use, between cases and controls. In evaluation of exosome preparations from both cases and controls, TSG101 levels were similar in concentration (Mann Whitney U test, $p = 0.2472$), and in both males and females (Mann Whitney U test, $p = 0.3048$). These results indicate that exosome concentrations were similar between groups. In several control subjects, we evaluated Ser(P)-1292 LRRK2 levels in urinary exosomes over the course of several weeks as well as in afternoon versus morning collections of urine, and we found good intra-individual stability for Ser(P)-1292 LRRK2 levels in these subjects (Supplementary Figure S1 E–F).

Unadjusted Ser(P)-1292 LRRK2 levels in urinary exosomes were elevated in PD versus controls in the complete cohort (Figure 1, $p = 0.0014$). Gender affected Ser(P)-1292 LRRK2 levels (~3-fold higher median levels in males versus females, Supplementary Table S2). Ser(P)-1292 LRRK2 levels were elevated in PD females compared to female controls (~7.5-fold higher median values in PD, $p = 0.0028$, Mann-Whitney U). In contrast, there was a less-significant trend toward elevated Ser(P)-1292 LRRK2 levels in male PD cases compared to male controls (~1.5-fold higher median values in PD, $p = 0.0822$, Mann-Whitney U). We found no correlation between Ser(P)-1292 LRRK2 levels and patient age ($r = 0.0473$, $p = 0.5587$) or disease duration among those with PD ($r = -0.0172$, $p = 0.8813$). Levels of total LRRK2 protein positively correlated with Ser(P)-1292 LRRK2 levels ($r = 0.44$, $p < 0.0001$). When adjusting for the effects of gender and total LRRK2 levels, Ser(P)-1292 LRRK2 levels remained significantly elevated in the entire PD cohort compared to the entire control cohort ($p = 0.0286$).

The Ser(P)-1292 LRRK2 to total LRRK2 ratio in urinary exosomes were calculated and also elevated in PD versus controls (~1.5-fold higher median values in PD, $p = 0.0074$). Gender affected the ratio value as well (~1.6-fold higher median levels in males versus females). The ratio of phospho- to total LRRK2 was also elevated in PD females compared to female controls (~2.9-fold higher median values in PD, $p = 0.008$, Mann-Whitney U). Similar to raw Ser(P)-1292 LRRK2 levels, there was an elevated ratio in male PD cases compared to male controls but this was not significant (~1.6-fold higher median values in PD, $p = 0.1907$, Mann-Whitney U).

Table 2 summarizes correlations between Ser(P)-1292 LRRK2 levels and clinical measures of PD. Non-motor aspects of PD captured with MDS-UPDRS part II, and Epworth SS were significantly and positively correlated with Ser(P)-1292 LRRK2 levels after adjustment for false discovery (Table 2). Significant associations between Ser(P)-1292 LRRK2 levels and Modified S&E v1.0 were also identified. There was no association between Ser(P)-1292 LRRK2 levels and levodopa equivalent daily dose ($r = 0.1512$, $p = 0.3933$).

Patients with PD in the upper quartile of Ser(P)-1292 LRRK2 levels had worse performance in the Modified S&E v1.0 and Epworth SS scores than those in the lower quartile Ser(P)-1292 LRRK2 group, as well as a trending difference in MoCA scores (Table 3). However, worse performance in the MoCA was also detected in control subjects that had the highest levels of Ser(P)-1292 LRRK2 (Table 3).

Among PD patients, Ser(P)-1292 LRRK2 levels predicted poor MoCA and S&E scores with some success (Figure 2). Ser(P)-1292 LRRK2 levels predicted the lower quartile of poor MoCA performance with a ROC area under the curve c-statistic of 0.731 (Figure 2B). Ser(P)-1292 LRRK2 levels were likewise elevated in PD patients with lower S&E scores ($p = 0.0061$, Figure 2C). Ser(P)-1292 LRRK2 levels predicted the lower quartile of poorer S&E scores with a ROC area under the curve of 0.788 (Figure 2D). However, performance on MoCA did not predict S&E scores in our cohort ($r = 0.0737$ [$-0.1514, 0.2914$], $p = 0.5216$). Only half of the PD cases in the lower quartile of S&E scores were also in the lower quartile of MoCA scores. Thus Ser(P)-1292 LRRK2 levels appear to predict several aspects of PD disease severity.

Discussion

This is the first study assessing Ser(P)-1292 LRRK2 levels in a large group of idiopathic PD cases and controls. To evaluate Ser(P)-1292 LRRK2 protein in idiopathic PD patients, we prioritized the collection of urine as a non-invasively derived biofluid in a typical medicated PD population. In a cross-sectional cohort of idiopathic PD patients enrolled in the Parkinson's Disease Biomarker Program at the University of Alabama at Birmingham, we found exosome Ser(P)-1292 LRRK2 levels were significantly elevated in PD and evidence of correlation with multiple non-motor measures of PD including MoCA, MDS-UPDRS Part I and II, and Epworth SS, as well as global measures of successful daily living (Mod. S&E v1.0 and PDQ-39).

We did not detect significant associations between Ser(P)-1292 LRRK2 and motor impairment despite demonstration in larger studies that motor impairment scores do worsen over time in PD patients. In our cohort, patients were on medication at the time of clinical evaluation and sample donation, and our power to separate mild from moderate motor impairment was efficiently masked by dopaminergic therapies. For example, in this cohort of 79 PD cases, we had a poor correlation of MDS-UPDRS III scores and disease duration ($r = -0.0216$, $p = 0.8521$). Ser(P)-1292 LRRK2 levels may not successfully predict motor impairment in PD, or Ser(P)-1292 LRRK2 may be useful to predict motor impairment in non-medicated PD cases. Future studies are required to test these hypotheses.

Given a possible correlation between Ser(P)-1292 LRRK2 and MoCA performance, it would be of interest to determine if Ser(P)-1292 LRRK2 levels correlate with measures of symptom severity in patients diagnosed with Diffuse Lewy Body disease. Levels of exosome Ser(P)-1292 LRRK2 may generally predict cognitive impairment associated with α -synuclein. Or, Ser(P)-1292 LRRK2 levels may not be specific for predicting cognitive decline in α -synuclein linked disease. Specificity for Ser(P)-1292 LRRK2 as a biomarker for cognitive impairment in PD versus other causes of impairment like Alzheimer disease can be determined in future studies.

We found no evidence of association between LEDD levels and Ser(P)-1292 LRRK2 levels (or any other reported medication) that would alter the relationship between Ser(P)-1292 LRRK2 and non-motor measures of PD severity. Recent observations in G2019S-*LRRK2* mutation carriers demonstrated that Ser(P)-1292 LRRK2 was relatively insensitive to

medication because levels of Ser(P)-1292 were not different between medicated groups and non-medicated groups compared to G2019S-*LRRK2* mutation carriers¹¹. However, future studies to evaluate Ser(P)-1292 *LRRK2* in *de novo* groups of PD patients that are not yet medicated as well as longitudinal series would be beneficial to better understand the relationship of Ser(P)-1292 *LRRK2* and measures of disease progression and severity.

LRRK2 has been genetically linked to neurodegenerative disease, and we have recently demonstrated that the G2019S-*LRRK2* mutation elevates urinary exosome Ser(P)-1292 *LRRK2* levels ~5-fold above controls¹¹. Here, we have identified some PD cases, as well as a few controls, that show comparable levels of Ser(P)-1292 *LRRK2* as G2019S-*LRRK2* mutation carriers with PD. Overall idiopathic PD with the highest levels of Ser(P)-1292 *LRRK2* also demonstrated worse performance on clinical assessments of daily living impairment and cognitive performance.

The biological source of urinary Ser(P)-1292 *LRRK2* in our exosome preparations has not been determined which is a limitation to interpretation of our results. *LRRK2* is highly expressed in cells of the innate immune system and it has recently been demonstrated that inflammatory stimuli such as lipopolysaccharide can induce increased levels of *LRRK2* autophosphorylation and exosomal release²⁰. A possible explanation for increased Ser(P)-1292 *LRRK2* levels in PD patients with poorer scores on assessments of cognitive performance and activities of daily living may be increased inflammation in the patients with aggressive progression of disease. Elevations in several cytokines have been observed in the CSF of PD patients as well as increased plasma C-reactive protein levels with correlations to PD severity^{21–24}. Multivariate modeling for most of these inflammation markers also observed significant gender effects, potentially in line with observations from this study.

In PD, there are currently no validated biochemical biomarkers for independently assessing PD diagnosis or prognosis, or measuring disease severity²⁵. In Alzheimer disease (AD), CSF levels of A β ₄₂, in concert with total tau and phosphorylated tau levels, may have value in identifying prodromal AD²⁶. However, levels of CSF A β ₄₂ and tau stabilize when a patient has clinically manifested AD and thus fail to inform on disease progression. In PD, CSF levels of A β ₄₂, total tau, phospho-tau, and α -synuclein have been proposed to have prognostic and diagnostic value in early-stage PD²⁷. However, each study reported major limitations for practical diagnostic or prognostic utility of α -synuclein, tau, or phospho-tau in PD^{28–32}. Here, we observed that Ser(P)-1292 *LRRK2* levels associated with several features of PD. As few viable biomarkers for PD exist, particularly for non-motor features³³, Ser(P)-1292 *LRRK2* may be a promising candidate for further exploration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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All named authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. KBF, RNA, DGS, and ABW contributed to experimental design and analysis of the data. KBF and NL performed statistical analysis. KBF and ABW wrote the manuscript. KBF performed the experiments. RGC was the study coordinator and ABR assisted in collecting biosamples and data curation.

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Figure 1. Elevated Ser(P)-1292 LRRK2 levels in PD

Urinary exosomes were isolated from 79 healthy and 79 PD patients and quantities of Ser(P)-1292 LRRK2 were determined relative to an exosome reference pool (defined as 1.0). Unadjusted values for Ser(P)-1292-LRRK2 are shown, ** $p < 0.01$, Mann-Whitney U-test.

Figure 2. Ser(P)-1292 LRRK2 changes as stratified by patient Montreal Cognitive Assessment and Modified Schwab and England v1.0 Score

(A) Exosomal Ser(P)-1292 LRRK2 levels progressively increase with declining MoCA score in PD cases. Levels are significantly different across quartiles ($p = 0.05$, Kruskal-Wallis) (B) Receiver Operating Characteristic (ROC) Curve for Ser(P)-1292 LRRK2 prediction of the lower quartile of MoCA performance. Risk prediction is success is 0.731 with a sensitivity of 60% and specificity of 89% at the optimum threshold. (C) Ser(P)-1292 LRRK2 levels increase with declining S&E scores. Levels are significantly different across quartiles ($p = 0.0061$, Kruskal-Wallis). (D) ROC Curve analysis of Ser(P)-1292 LRRK2 levels distinguishing lower S&E quartile. Area under the ROC curve is 0.788 with a sensitivity of 60% and specificity of 90% at optimum threshold.

Table 1

Demographics and clinical characterization of PD and control subjects

Descriptive Title	Control (n = 79) [*]	PD (n = 79) [*]	<i>p</i> ^a
Gender, Male (Female)	40 (39)	41 (38)	n.s. ^b
Age (years), Median (Range)	64 (36–77)	65 (37–79)	n.s. ^c
Caucasian, Number (%)	76 (96.2)	77 (97.5)	n.s. ^b
Creatinine (mg dL ⁻¹), Mean (S.D.)	0.96 (0.234)	0.93 (0.179)	0.333 ^d
Weight (kg), Mean (S.D.)	82.7 (22.29)	80.0 (17.86)	0.407 ^d
Height (cm), Median (Range)	170 (155–193)	173 (150–193)	n.s. ^c
Age at Diagnosis, Median (Range)	n/a	57 (28–76)	n/a
MDS-UPDRS, Median (Range)			
Part I	4 (0–16)	12 (1–28)	<0.0001^c
Part II	0 (0–13)	12 (1–33)	<0.0001^c
Part III	0 (0–31)	25 (2–52)	<0.0001^c
Part IV	0 (0–0)	0 (0–12)	<0.0001^c
Modified S&E v1.0, Median (Range)	100 (80–100)	90 (40–100)	<0.0001^c
MoCA, Median (Range)	26 (17–30)	25 (12–30)	0.0004^c
Education (%), High school, College, Masters, PhD	12.7, 62.0, 20.3, 5.1	21.5, 60.8, 8.9, 8.9	0.1039 ^e
Dementia Family History [1°, 2°], %	26.6	22.8	n.s. ^b
PDQ-39, Median (Range)			
Mobility	0 (0–32.5)	20 (0–92.5)	<0.0001^c
Activities of Daily Living	0 (0–50)	25 (0–79.2)	<0.0001^c
Emotional Well Being	8.3 (0–41.7)	16.7 (0–83.3)	<0.0001^c
Stigma	0 (0–18.75)	12.5 (0–100)	<0.0001^c
Social Support	0 (0–33.3)	0 (0–75)	0.0063^c
Cognition	6.25 (0–50)	25 (0–81.3)	<0.0001^c
Communication	0 (0–41.7)	16.7 (0–100)	<0.0001^c
Bodily Discomfort	8.3 (0–83.3)	25 (0–91.7)	<0.0001^c
Total	4.4 (0–30.1)	19.8 (0.3–76.9)	<0.0001^c
Epworth SS, Median (Range)	6 (0–16)	10 (0–24)	<0.0001^c
LEDD, Median (Range)	n/a	700 (0–2500)	n/a

^{*} 2 healthy controls and 2 PD subjects were excluded from this analysis as their urine was determined to be too dilute for measurement of TSG101 (see Methods).

^a *p* values > 0.5 are indicated as n.s. (not significant). *p* values < 0.05 are boldface.

^b Determined by use of Fisher's two-tailed exact test.

^c Determined by use of Mann-Whitney U two-tailed test.

^dDetermined by use of students two-tailed t-test.

^eDetermined by use of chi-square test.

PD = Parkinson disease; UPDRS = Unified Parkinson's Disease Rating Scale; Modified S&E v1.0 = Modified Schwab and England v1.0 Activities of Daily Living Scale; MoCA = The Montreal Cognitive Assessment; PDQ-39 = Parkinson's Disease Questionnaire-39; Epworth SS = Epworth Sleepiness Scale; LEDD = L-Dopa Equivalent Daily Dosage

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Table 2

Correlations between Ser(P)-1292 LRRK2 levels and clinical features in PD cases

Clinical Measure	Correlation Coefficient	95% CI	<i>p</i> *	<i>p</i> **
<u>Motor Assessments</u>				
MDS-UPDRS				
UPDRS Part III	0.0918	[-0.1334, 0.3081]	0.4240	0.4543
<u>Non-Motor Assessments</u>				
MDS-UPDRS				
UPDRS Part I	0.2239	[0.0014, 0.4252]	0.0488	0.0813
UPDRS Part II	0.3404	[0.1276, 0.5233]	0.0023	0.0173
MoCA	-0.2679	[-0.4628, -0.0482]	0.0177	0.0664
Epworth SS	0.3215	[0.1066, 0.5077]	0.0041	0.0205
<u>Global Assessments</u>				
Modified S&E v1.0	-0.4455	[-0.6078, -0.2475]	<0.0001	<0.0015
PDQ-39				
Mobility	0.2457	[0.0245, 0.4440]	0.0301	0.0813
Activities of Daily Living	0.2242	[0.0018, 0.4255]	0.0484	0.0813
Emotional Well-Being	0.2343	[0.0124, 0.4342]	0.0390	0.0813
Stigma	0.1171	[-0.1082, 0.3310]	0.3071	0.3768
Social Support	0.2044	[-0.0190, 0.4083]	0.0727	0.1091
Cognition	0.1827	[-0.0415, 0.3894]	0.1094	0.1492
Communication	0.1125	[-0.1128, 0.3269]	0.3266	0.3768
Bodily Discomfort	0.0728	[-0.1522, 0.2906]	0.5266	0.5266
Total	0.2250	[0.0026, 0.4262]	0.0477	0.0813

* *p* values < 0.05 are indicated in bold; determined by Pearson product moment correlation

** *p* values represent those adjusted using the false discovery rate method by Benjamini & Hochberg (1995).

PD = Parkinson disease; UPDRS = Unified Parkinson's Disease Rating Scale; Modified S&E v1.0 = Modified Schwab and England v1.0 Activities of Daily Living Scale; MoCA = The Montreal Cognitive Assessment; PDQ-39 = Parkinson's Disease Questionnaire-39; Epworth SS = Epworth Sleepiness Scale; LEDD = L-Dopa Equivalent Daily Dosage

Table 3

Selected clinical features stratified by Ser(P)-1292 LRRK2 levels

Descriptive Title	PD		Controls		Combined PD & Controls		
	Low-1292 (n = 20)	High-1292 (n = 19)	p	Low1292 (n = 19)	High-1292 (n = 19)	p	High-1292 (n = 39)
MoCA	25.5 (16–30)	24 (17–29)	0.0583	27 (20–30)	25 (20–29)	0.0459	25 (17–29)
S&E v1.0	90 (60–100)	80 (40–90)	0.0143	100 (90–100)	100 (90–100)	n.s.	90 (40–100)
Epworth SS	9.5 (0–17)	11 (5–22)	0.0434	7 (0–13)	4 (0–12)	n.s.	9 (3–22)

* *p* values determined by Mann-Whitney U two-tailed test. *p* values > 0.5 are indicated as n.s. *p* values < 0.05 are boldface

Low-1292 and High-1292 represent subjects within the lower or higher quadrant of Ser(P)-1292 LRRK2 levels for that group.

Summary statistics represent median (range). MoCA = The Montreal Cognitive Assessment; Modified S&E v1.0 = Modified Schwab and England v1.0 Activities of Daily Living Scale; Epworth SS = Epworth Sleepiness Scale