RESEARCH ARTICLE

Higher Urine bis(Monoacylglycerol)Phosphate Levels in LRRK2 G2019S Mutation Carriers: Implications for Therapeutic Development

Roy N. Alcalay, MD, MS,[1*] Frank Hsieh, PhD,[2] Elizabeth Tengstrand, MS,[2] Shalini Padmanabhan, PhD,[3] Marco Baptista, PhD,[3] Caitlin Kehoe, MA,[1] Sushma Narayan, MS,[1] Amelia K. Boehme, PhD,[1] and Kalpana Merchant, PhD[4*]

[1]*Columbia University, Department of Neurology, New York, New York, USA*
[2]*Nextcea, 500 West Cummings Park, Suite 4550, Woburn, Massachusetts, USA*
[3]*Michael J. Fox Foundation for Parkinson's Research, New York, New York, USA*
[4]*TransThera Consulting Co, Portland, Oregon, USA*

ABSTRACT: Background: *LRRK2* mutations are a common cause of dominantly inherited PD. Previous studies showed decreases in urine levels of didocohexaenoyl (22:6) bis(monoacylglycerol)phosphate in *LRRK2*-knockout mice and in non-human primates treated with LRRK2 kinase inhibitors. We hypothesized that urine levels of bis(monoacylglycerol)phosphate isoforms will be higher in individuals with a PD-causing gain-of-kinase function mutation, *LRRK2* G2019S. The objective of this study was to investigate alterations in urinary phospholipids as biomarkers of *LRRK2* mutations and Parkinson's disease status/phenotypes.

Methods: Ultra-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) was used to assess 54 bioactive phospholipids in urine from the *LRRK2* Cohort Consortium (n = 80). To confirm and extend the findings, urine from an independent *LRRK2* cohort from Columbia University Irving Medical Center (n = 116) was used. Both cohorts were composed of *LRRK2* G2019S carriers and non-carriers with and without PD.

Results: In each cohort, 4 bis(monoacylglycerol)phosphate isoforms (di-18:1-bis[monoacylglycerol]phosphate, didocohexaenoyl [22:6] bis[monoacylglycerol] phosphate, 2,2′-di-22:6-bis[monoacylglycerol]phosphate, and 2,2′-di-18:1-bis[monoacylglycerol]phosphate) were significantly higher (2.5- to 4.3-fold) in G2019S carriers compared with non-carriers. Interestingly, 2,2′-di-18:1-bis(monoacylglycerol)phosphate levels were marginally higher in *LRRK2* carriers with PD than in those without PD ($P = 0.045$). Moreover, increased 2,2′ and total di-22:6-bis(monoacylglycerol) phosphate were associated with worse cognitive status assessed by the Montreal Cognitive Assessment ($P = 0.0033$ and 0.0144, respectively).

Conclusions: The observed association of bis(monoacylglycerol)phosphate isoforms with *LRRK2* G2019S mutation, PD status among G2019S carriers, and correlation with cognitive decline suggest the potential use of urinary bis(monoacylglycerol)phosphate isoforms as biomarkers for clinical trials of *LRRK2*-targeted therapies. © 2019 The Authors. *Movement Disorders* published by Wiley Periodicals, Inc. on behalf of International Parkinson and Movement Disorder Society.

*__Correspondence to:__ Kalpana Merchant, PhD, TransThera Consulting Co., 3907 NW Lewis Lane, Portland, OR 97229; E-mail: merchant.transthera@gmail.com; or Roy Alcalay, MD, MS, Columbia University, Dept of Neurology, 710 West 168th St., New York, NY 10025; E-mail: rna2104@cumc.columbia.edu

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Mutations in the gene encoding leucine-rich repeat kinase 2 (*LRRK2*) are among the most common genetic causes of Parkinson's disease (PD).[1] Although the association between *LRRK2* gene mutations and PD was initially reported in 2004,[2] the mechanism by which these mutations cause PD is not entirely clear. However, several lines of evidence indicate that increased LRRK2 kinase activity is important in the pathogenesis of PD.[3] Therefore, *LRRK2* kinase inhibitors are highly pursued as a therapeutic approach for PD.[3]

Experience from failed therapeutic trials of PD and Alzheimer's disease highlights the need for biomarker-informed patient enrichment strategies for successful clinical development of disease-modifying therapies. For *LRRK2* kinase inhibitors, 3 potential strategies may be considered. The first would be to assess the slowing of disease progression in *LRRK2* pathogenic mutation carriers with PD (PD+*LRRK2*+). Although attractive, a major hurdle of this approach is access to a sufficient number of patients for the entirety of the drug approval path. Second is to study whether disease onset may be prevented in non-manifesting carriers of *LRRK2* pathogenic mutations without motor deficits of PD (PD-*LRRK2*+). This approach could be medically most beneficial, but the length of the trial duration and regulatory path for drug approval present major challenges currently. Last, idiopathic PD without *LRRK2* mutations (PD+*LRRK2*-) offers an attractive treatment group to circumvent the challenges mentioned above. Scientifically, this group could also be rationalized on the basis of the potential role of *LRRK2* in idiopathic PD pathogenesis[4] (reviewed by Roosen and Cookson, 2016[5]). However, idiopathic PD represents highly heterogeneous pathogenic mechanisms. Therefore, biomarkers that can help enrich the trial with those most likely to benefit from alterations in the metabolic pathways of *LRRK2* are required. A reliable biomarker of *LRRK2* activity would also be beneficial for confirming target engagement and monitoring treatment compliance of *LRRK2* kinase inhibitors.

Several lines of evidence indicate that bis(monoacylglycerol)phosphate (BMP) isoforms are candidate biomarkers of *LRRK2* activity. BMPs are localized within the inner membranes of late endosomes (multivesicular bodies) and lysosomes, where they contribute to the multivesicular/lamellar morphology of the endolysosomal network.[6,7] Recent studies have identified several GTPases as bona fide substrates of *LRRK2* and in conjunction with other evidence have implicated a role of *LRRK2* in endolysosomal vesicular trafficking and lysosomal functions.[8] Didocosahexaenoyl(22:6)-BMP (di-22:6-BMP), a specific species of BMP, is decreased in the urine of *LRRK2*-knockout mice and in non-human primates treated with chemically distinct *LRRK2* kinase inhibitors (PFE-360, MLi-2, and GNE-7915),[9] directly implicating its utility as a biomarker of *LRRK2* activity. However, changes in BMP levels have not been examined in humans with *LRRK2* mutations.

The G2019S mutation in *LRRK2* has been demonstrated to increase *LRRK2* kinase activity in various model systems.[3] Thus, we hypothesized that in contrast to the decreased levels of di-22:6-BMP in *LRRK2*-knockout and non-human primates treated with *LRRK2* kinase inhibitors, G2019S mutation carriers will have elevated levels of urinary di-22:6-BMP and other BMP species. In addition, we aimed to evaluate whether PD status and disease severity indicated by the Unified Parkinson's Disease Rating Scale (UPDRS) and the Montreal Cognitive Assessment (MoCA) are associated with BMP levels in *LRRK2* G2019S carriers and non-carriers.

Methods

Clinical Cohorts

Urinary BMPs were measured in 2 independent cross-sectional cohorts. Both studies were approved by local institutional review boards, and all participants signed informed consents. The first cohort included bio-banked urine samples from the Michael J. Fox Foundation (MJFF) *LRRK2* Cohort Consortium (LCC). Urine samples were analyzed from 80 participants who were frequency-matched by sex, disease duration, and age at onset: 20 PD patients with *LRRK2* G2019S mutation (PD+*LRRK2*+), 20 idiopathic PD without *LRRK2* G2019S or other *LRRK2* pathogenic mutations (PD +*LRRK2*-), 20 non-manifesting carriers of *LRRK2* G2019S mutation (PD-*LRRK2*+), and 20 healthy individuals without *LRRK2* pathogenic mutations (PD-*LRRK2*). Disease severity scales were not available for these participants. In this cohort, in addition to quantitative assessment of di-22:6-BMP levels, we conducted preliminary analysis of 54 distinct bioactive phospholipids from 3 classes: BMPs, cell membrane phospholipids (phosphotidylinositol [PI], phosphotidylethanolamine [PE], and phosphotidylcholine [PC]), and globotriaosylceramides (Gb3s; implicated in Fabry's disease).

To confirm and extend the findings from the LCC cohort, urine from a second cohort recruited at Columbia University Irving Medical Center (CUIMC) was tested. Participants in the CUIMC cohort donated urine under a MJFF-funded *LRRK2* biomarker project from March 2016 to April 2017. They underwent clinical evaluation for motor severity using the UPDRS part III (UPDRS-III), and their cognitive functioning was assessed using the MoCA. Genotyping for *LRRK2* G2019S and *GBA* mutations was conducted as previously described.[10] Urine was analyzed from 25 PD patients with *LRRK2* G2019S mutation (PD+*LRRK2*+), 40 idiopathic PD without *LRRK2* pathogenic mutations (PD+*LRRK2*-), 16 non-manifesting carriers of *LRRK2* G2019S mutation (PD-*LRRK2*+), and 35 healthy individuals without *LRRK2* pathogenic mutations (PD-

LRRK2-). There was no participant overlap between the LCC and CUIMC cohorts. In this cohort, UPLC-MS/MS analysis was focused only on di-oleoyl-BMP (di-18:1-BMP), di-22:6-BMP, and their 2,2′ isoforms.

Broad Profiling of Bioactive Phospholipids in LCC Urine

UPLC-MS/MS analyses

A multiplexed UPLC-MS/MS method was used to simultaneously quantitate (semiquantitate) 54 species of urinary BMPs, PC, PE, and PI, and Gb3s with various fatty acid chains (Supplementary Tables 1–3). The analyses were conducted by Nextcea, Inc. (Woburn, MA) as previously described using a SCIEX TripleTOF 6600 mass spectrometer equipped with an IonDrive Turbo V source (SCIEXm Framingham, MA).[11] Standard curves were prepared from related standards using a class-based approach (ie, di-22:6-BMP, di-myristoyl-PC [di-14:0-PC], di-14:0-PE, di-octanoyl-PI [di-8:0-PI], and Gb3 d18:1/16:0, respectively). Internal standards were used for each analyte reported. Injections were made using a Shimadzu Nexera XR UPLC system (Shimadzu Scientific Instruments, Japan). A SCIEX TripleTOF 6600 mass spectrometer was used for the detection of analytes. The instruments were controlled by AnalystTF 1.7 software.

Calibration and data processing

The intensities of the analytes and internal standards were determined by integration of extracted ion peak areas using AnalystTF 1.7 and MultiQuant 3.0 software. Calibration curves were prepared by plotting the peak area ratios for each analyte to internal standard versus concentration. The model for the calibration curve was linear with ($1/x^2$) weighting. Measured concentrations of urine lipids (ng/mL) were divided by the concentration of urine creatinine and reported as ng/mg creatinine.

Measurement of Urinary 2,2′-di-22:6-BMP, Total di-22:6-BMP, 2,2′-di-18:1-BMP, and Total di-18:1-BMP

BMPs can exist in 3 geometrical isoforms (2,2′-, 2,3′-, and 3,3′-BMP), which may influence their functional properties.[12,13] Targeted high resolution UPLC-MS/MS was used to accurately quantitate total di-22:6-BMP (the sum of its 3 isoforms) and its distinct 2,2′-isoform (2,2′-di-22:6-BMP). Total di-18:1-BMP and 2,2′-di-18:1-BMP were measured as well. Quantitation was performed by Nextcea, Inc. (Woburn, MA) using authentic di-22:6-BMP and di-18:1-BMP reference standards. Di-14:0-BMP was employed as an internal standard. Urinary BMPs were extracted by liquid-liquid extraction and measured by UPLC-MS/MS as described above.

Creatinine measurement

Concentrations of urinary creatinine were measured by a colorimetric assay (method of Jaffé) with Parameter Creatinine Assay test reagents (R&D Systems, Minneapolis, MN) using a BioTek ELx800 absorbance microplate reader with Gen5 Microplate Reader and Imager Software 2.09 (Fisher Scientific, Hampton, NH).

Statistical Analyses

We used descriptive statistics to report findings from both *LRRK2* cohorts. In both cohorts we used chi-square for categorical variables and the Kruskal-Wallis test for continuous variables to compare the 4 groups (PD+*LRRK2*+, PD+*LRRK2*-, PD-*LRRK2*+, and PD-*LRRK2*-). In the LCC cohort, we present the concentration of 54 analytes in supplementary files. For both cohorts, we used Kruskal-Wallis to compare creatinine-normalized BMP levels across the groups. Given that BMP levels in each of the groups were comparable between the 2 cohorts, we combined the data from both cohorts and compared the BMP analytes across the 4 groups in the merged database.

Last, in the CUIMC cohort in which UPDRS, MoCA, and L-dopa-equivalent daily dose (LEDD) were available, we tested the association between disease severity markers (predictors) and BMP isoforms (outcome) in linear regression models among PD patients. We first ran unadjusted models and then adjusted for covariates. When testing for the association between cognitive performance (by MoCA), we adjusted for age, sex, disease duration, education (years), and *LRRK2* mutation status. When we tested the association between motor functioning (UPDRS-III) and BMPs, we adjusted for age, sex, duration of disease, LEDD, and *LRRK2* mutation status.

Results

Analyses of Biospecimens From the LCC Donors

The demographics and *LRRK2* genotype status of LCC participants are described in Table 1. As shown, the 4 groups were balanced for all demographic variables. The broad profiling of LCC cohort urine for 54 distinct analytes is reported in Supplemental Tables 1–3. These data showed that all BMP species measured and PI levels (but not Gb3 isoforms) were higher in *LRRK2* G2019S mutation carriers independent of PD status and sex. A correlational analyses indicated that total di-18:1-BMP and total di-22:6-BMP most strongly discriminated the *LRRK2* mutation carriers from non-carriers (data not shown). Hence, all subsequent studies focused on analyzing total and 2,2′ isoforms of di-18:1 BMP and di-22:6 BMP. Creatinine-

TABLE 1. Demographics and urine BMP concentrations among LCC participants

	PD+*LRRK2*+ (n = 20)	PD+*LRRK2*- (n = 20)	PD-*LRRK2*+ (n = 20)	PD-*LRRK2*- (n = 20)	*P*
Age, mean (SD)	69.5 (9.9)	70.7 (9.0)	68 (12.4)	71 (11.3)	0.7622
Age at onset, mean (SD)	58.2 (9.8)	58.8 (11.3)	n/a	n/a	0.9245[b]
Disease duration	11.4 (5.9)	11.9 (6.2)	n/a	n/a	0.6643[b]
Sex (female/male)	10/10	10/10	10/10	10/10	1
Total di-18:1-BMP, median (range)	5.84 (1.50–28.97)	2.80 (0.69–19.01)	5.49 (1.05–24.98)	4.79 (1.43–15.14)	0.0084[a]
Total di-22:6-BMP, median (range)	35.25 (9.37–380.92)	11.28 (2.06–100.01)	33.80 (6.13–190.25)	11.5 (3.97–67.20)	<0.0001[a]
2,2′-di-18:1-BMP, median (range)	3.95 (1.20–23.42)	2.21 (0.53–14.84)	3.68 (0.770–20.12)	3.27 (0.54–8.92)	0.0407[a]
2,2′-di-22:6 BMP, median (range)	23.04 (6.81–302.84)	7.21 (1.24–72.02)	20.19 (3.29–142.91)	6.11 (1.10–42.26)	<0.0001[a]
Creatinine, median (range)	1.10 (0.12–3.14)	1.10 (0.07–3.19)	1.07 (0.29–2.08)	0.91 (0.36–2.58)	0.7658

BMP values shown are median (range) of creatinine-normalized isoforms (ng/mg creatinine). The *P* values were calculated using the Kruskal-Wallis test for continuous variables (all variables other than sex, age of onset, and disease duration).
[a]Statistically significant difference. Note that it is driven primarily by the *LRRK2* genotype. The chi-square test was used for the categorical variable, sex.
[b]*P* values were calculated using the Wilcoxon rank sum test for continuous variables, Age at onset and disease duration were used to compare the 2 Parkinson's disease groups with each other.

normalized BMP isoforms were significantly elevated in *LRRK2* G2019S mutation carriers independent of PD status and sex. BMP levels in urine of idiopathic PD patients (PD+*LRRK2*-) did not differ from healthy controls (PD-*LRRK2*-). Note that urinary creatinine levels were consistent across the 4 groups. As a result, normalization of phospholipids to creatinine had no effect on the overall BMP concentration differences.

Analyses of Biospecimens From the CUIMC Participants

The demographics, disease characteristics, and BMP concentrations of the CUIMC participants are described in Table 2. Similar to the LCC cohort, BMP isoforms were significantly elevated in urine from *LRRK2* G2019S mutation carriers, and there were no significant differences in BMP levels in *LRRK2* non-carriers with or without PD. Twenty-two participants in this cohort also carried a *GBA1* mutation, including 4 who carried both a *GBA* mutation and the *LRRK2* G2019S mutation.

Analyses of BMP Isoforms in the Combined Cohorts

Table 3 and Figure 1(A-D) demonstrate BMP analytes by *LRRK2* genotype and PD status in the combined cohorts. Interestingly, the normalized levels of di-18:1-BMP, di-22:6-BMP, and their 2,2′ isoforms were ~20%–30% higher in the *LRRK2* G2019S mutation carriers with PD when compared with those without PD, although only the 2,2′-di-18:1-BMP isoform reached marginal statistical significance ($P = 0.0459$).

BMP and PD Phenotypes

Last, in the CUIMC cohort, we tested the association between creatinine-normalized urine BMP levels and PD severity scales, UPDRS, and MoCA either unadjusted (model 1) or adjusted (model 2). MoCA score was

TABLE 2. Demographics and urine BMP concentrations among CUIMC participants

	PD+*LRRK2*+ (n = 25)	PD+*LRRK2*- (n = 40)	PD-*LRRK2*+ (n = 16)	*PD-LRRK2*- (n = 35)	*P*
Age, mean (SD)	69.4 (8.5)	65.1 (9.3)	56.8 (12.7)	67.4 (10.4)	0.0053
Age at onset, mean (SD)	56.9 (11.3)	57.9 (10.9)	n/a	n/a	0.4298[b]
Disease duration	11 (1–26)	7 (0–18)	n/a	n/a	0.0026[b]
Sex (% female)	10 (40%)	16 (40%)	8 (50%)	17 (48.6%)	0.8127
UPDRS-III, mean (SD)	22.1 (10.3)	16.9 (9.6)	0.81 (1.04)	1.08 (1.5)	<0.0001[a]
MoCA, mean (SD)	26.5 (4.7)	26.9 (1.6)	28.7 (1.1)	27.5 (2.1)	0.0080[a]
LEDD	547 (313.3)	415 (375.7)	n/a	n/a	0.097[b]
Total di-18:1-BMP, median (range)	5.81 (0–30.8)	2.26 (0–18.5)	3.43 (1.03–11.49)	2.30 (0–8.5)	0.0007[a]
Total di-22:6-BMP, median (range)	38.9 (9.36–134.5)	10.5 (1.13–39.1)	30.3 (5.16–61.9)	8.29 (0–33.7)	<0.0001[a]
2,2′-di-18:1-BMP, median (range)	4.56 (0–21.3)	1.36 (0–12.69)	2.36 (0.75–.31)	1.15 (0–6.84)	<0.0001[a]
2,2′-di-22:6 BMP, median (range)	31.3 (6.73–106.42)	5.72 (0.59–25.5)	20.84 (4.35–35.62)	5.29 (0–25.8)	<0.0001[a]
Creatinine, median (range)	0.87 (0.34)	0.89 (0.59)	0.72 (0.44)	0.68 (0.56)	0.0984

BMP values shown are median (range) of creatinine-normalized isoforms (ng/mg creatinine). The *P* values were calculated using the Kruskal-Wallis test for continuous variables (all variables other than sex, age at onset, and disease duration).
[a]Statistically significant difference. Note that it is driven primarily by the *LRRK2* genotype. The chi-square test was used for the categorical variable, sex.
[b]*P* values were calculated using the Wilcoxon rank sum test for continuous variables, Age at onset and disease duration were used to compare the 2 Parkinson's disease groups with each other.

TABLE 3. Median BMP analyte levels in the combined cohort

	PD+*LRRK2*+ (n = 45)	PD+*LRRK2*- (n = 60)	PD-*LRRK2*+ (n = 36)	PD-*LRRK2*- (n = 55)	*P*	*P*[b]
Total di-18:1-BMP	5.81 (0–30.85)	2.31 (0–19.1)	4.91 (1.03–24.9)	1.63 (0–8)	<0.0001[a]	0.1061
Total di-22:6-BMP	37.4 (9.36–380.92)	10.5 (1.13–100.01)	31.5 (5.16–190.3)	9.23 (0–67)	0.0004[a]	0.1802
2,2′-di-18:1-BMP	4.25 (0–23.42)	1.39 (0–14.84)	3.15 (0.75–20.12)	1.62 (0–8.92)	<0.0001[a]	0.0459[a]
2,2′-di-22:6-BMP	26.2 (6.73–302.8)	6.15 (0.59–72.02)	20.5 (3.29–142.9)	5.38 (0–42.26)	<0.0001[a]	0.0871

BMP values shown are median (range) of creatinine-normalized isoforms (ng/mg creatinine). The *P* values were calculated using the Kruskal-Wallis test.
[a]Statistically significant difference. Note that it is driven primarily by the *LRRK2* genotype.
[b]*P* values were calculated using the Wilcoxon rank sum test to compare *LRRK2*+PD+ to *LRRK2*+PD-.

negatively associated with creatinine-normalized 2,2′ and total di-22:6-BMP isoforms, in both models (Table 4). For example, for every point increase in the MoCA score the total di-22:6-BMP decreases by 1.76 (adjusting for age, sex, disease duration, education years, and *LRRK2* mutation status).

Discussion

In the current study we demonstrated higher urine BMP isoform concentrations in carriers of *LRRK2* G2019S mutation than in non-carriers, independent of PD status and sex in 2 independent cohorts. Furthermore, in the combined cohort analyses, a small but statistically significant increase in 2,2′-di-18:1 BMP isoform was seen in PD+*LRRK2*+ when compared with PD-*LRRK2*+. Finally, among PD patients, higher di-22:6-BMP (total levels and 2,2′ isoform levels) were associated with worse cognitive performance on the MoCA suggesting that BMP isoforms may be biomarkers of pathophysiology of PD. These data indicate the potential of urinary BMP isoforms as biomarkers of *LRRK2* biology, PD status, and symptoms.

BMP is a structural isomer of phosphatidylglycerol found in most tissues and in different cell types. BMP is negatively charged at the acidic pH of lysosomes, and these charges are central to its role in the degradation of lipids and membranes in the lysosome by facilitating the adhesion of the soluble positively charged hydrolases (eg, glucocerebrosidase) and activator proteins (saposins) at the interface of the lysosomal inner membranes.[6] Alterations in BMP levels have been linked to lysosomal dysfunction. Both di-18:1-BMP and di-22:6-BMP are elevated in patients with Niemann-Pick disease,[11,14] a lysosomal disorder caused by diminished acid sphingomyelinase levels. Interestingly, variants in the gene encoding sphingomyelinase are also risk factors for PD.[15]

In this study, urinary di-18:1-BMP and di-22:6-BMP and their 2,2′ isoforms were elevated in *LRRK2* G2019S mutation carriers (with and without PD), compared with non-carriers. In contrast, mice with germline deletion of *LRRK2* and cynomolgus monkeys treated with 3 distinct *LRRK2* kinase inhibitors show decreases in urinary di-22:6-BMP levels.[9] Moreover, urinary di-22:6-BMP levels were restored to normal concentrations on withdrawal from treatment with a *LRRK2* kinase inhibitor.[9] Because the G2019S mutation increases *LRRK2* kinase activity 2-3X,[3] our data could suggest that the elevation in urinary di-22:6-BMP levels may reflect the kinase activity of *LRRK2*. However, the animal studies cited above also showed lysosomal alterations in both rodents and non-human primates. Furthermore, elevated BMP levels in Niemann-Pick C patients indicate that changes in BMP levels may not be specific to *LRRK2* activity, but may also represent lysosomal dysfunction. Therefore, additional studies are required to determine whether urinary BMP levels reflect *LRRK2* kinase activity and/or endolysosomal deficits. For example, studying alterations in BMP levels in *GBA* mutation carriers may provide critical insights into upstream mechanisms of regulation of urinary BMP levels in humans.

What is a possible molecular mechanism underlying the observed increases in urine from *LRRK2* G2019S carriers? Recent studies have demonstrated that a subset of Rab GTPases is phosphorylated by *LRRK2* kinase activity.[8] Rab GTPases modulate the maturation/formation of MVBs, vesicular trafficking, and exocytosis of MVB-derived vesicles (exosomes) at the plasma membrane.[16] A consequence of higher kinase activity of G2019S *LRRK2* will be increased phosphorylation of its Rab GTPase substrates and thereby their accumulation in membranes as shown by Steger et al.[8] We propose that this shift in membrane-bound Rabs may affect the biogenesis, motility, or extracellular release of exosomes, resulting in increased concentrations of BMPs in urine. One candidate *LRRK2* Rab GTPase substrate contributing to the observed increases in urinary BMP is Rab35, because it has been shown to regulate the endocytic/recycling pathway and secretion.[17]

As *LRRK2* kinase inhibitors move forward into the clinic, better strategies are needed to enrich clinical trials with those most likely to benefit because of elevated *LRRK2* activity and evaluate pharmacodynamic effects of the drug. One obvious patient population is those carrying kinase-activating mutations of *LRRK2*, such as G2019S. However, in light of recent data indicating that *LRRK2* may also play a role in the pathogenesis of iPD,[4] a biomarker to identify iPD patients with increased *LRRK2* kinase activity is required to enrich

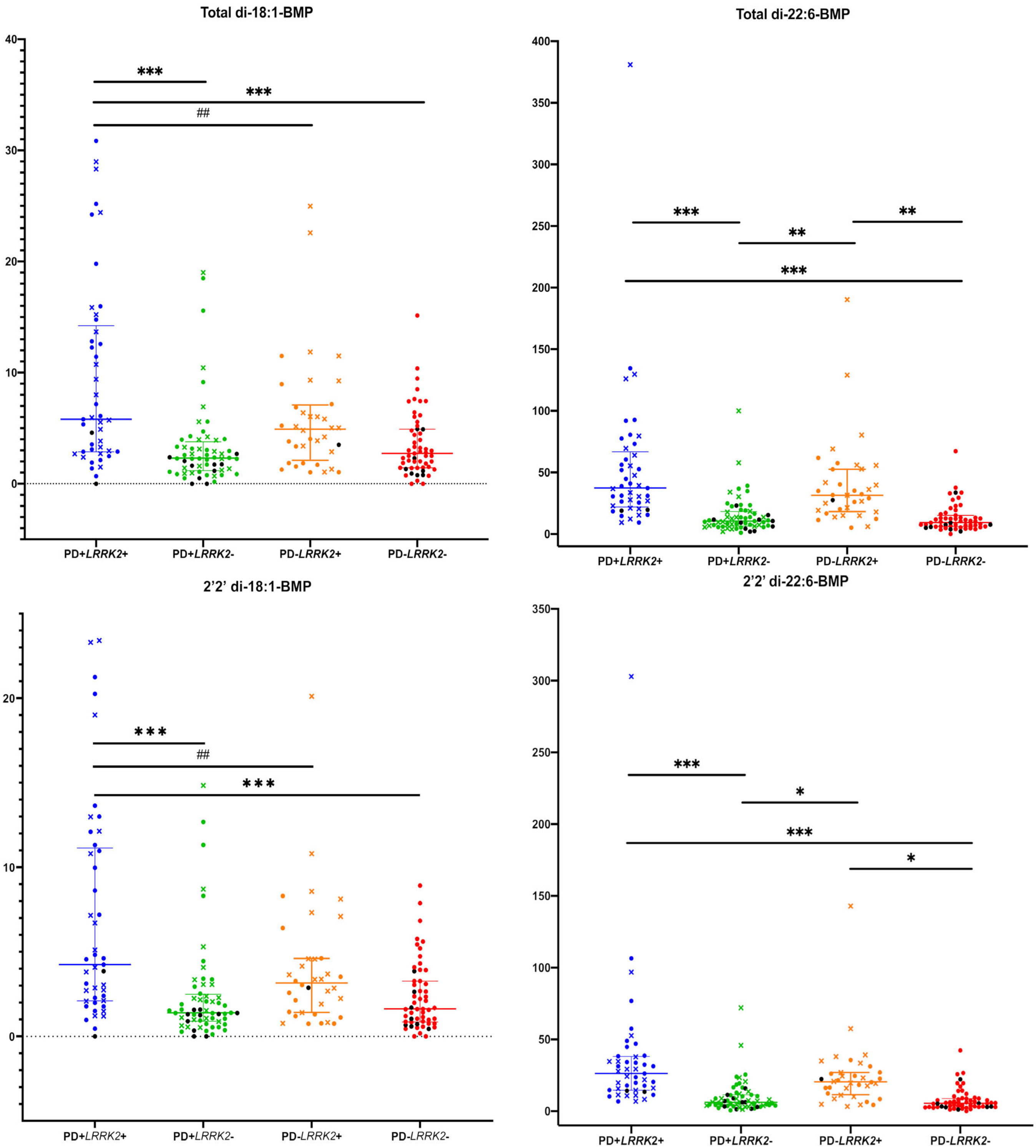

FIG. 1. Levels of BMP isoforms in the combined LCC and CUIMC data sets. (A–D) Data for a specific BMP isoform measured in the 4 groups indicated on the *X* axis. Circular symbols indicate the measurement was from the CUIMC cohort, and X symbols indicate the measurement was from the LCC cohort. Any symbol in black is a *GBA* mutation/variant carrier. Wilcoxon rank sum test was used for pairwise comparisons to identify groups differing from each other. $^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.0001$; $^{\#\#}P < 0.01$.

clinical trials with the patients most likely to respond to *LRRK2* inhibitors (ie, those with higher *LRRK2* kinase activity). Our data indicate the potential utility of urinary di-22:6-BMP and di-18:1-BMP as non-invasive, quantitative, and relatively facile measures to monitor *LRRK2* kinase activity. A preliminary but intriguing observation of the present study was that BMP levels were higher in *LRRK2* mutation carriers with PD compared with those without PD and that among PD patients higher di-22:6-BMP levels predicted worse

TABLE 4. Assessing MoCA and UPDRS as predictors of BMP levels in PD patients (n = 65)

	Model 1		Model 2	
	Beta (SE)	*P*	Beta (SE)	*P*
MoCA				
Normalized total di-18:1-BMP	0.185 (0.26)	0.4875	0.34 (0.27)	0.2226
Normalized total di-22:6-BMP	−2.48 (0.98)	0.0144[a]	−1.76 (0.86)	0.0461[a]
Normalized 2,2′-di-18:1-BMP	0.121 (0.19)	0.5246	0.26 (0.19)	0.1900
Normalized 2,2′-di-22:6-BMP	−2.14 (0.70)	0.0033[a]	−1.60 (0.61)	0.0104
UPDRS-III				
Normalized total di-18:1-BMP	0.14 (0.08)	0.0878	0.07 (0.09)	0.4416
Normalized total di-22:6-BMP	0.40 (0.32)	0.2129	−0.13 (0.27)	0.6396
Normalized 2,2′-di-18:1-BMP	0.09 (0.06)	0.1079	0.04 (0.06)	0.5265
Normalized 2,2′-di-22:6-BMP	0.29 (0.23)	0.2222	−0.12 (0.20)	0.5424

Model 1, unadjusted; model 2, adjusted for covariates as indicated below.
MoCA, adjusted for age, sex, disease duration, education years, and *LRRK2* status.
UPDRS-III, adjusted for age, sex, disease duration, LEDD, and *LRRK2* status.
[a]Statistically significant differences.

cognitive performance, even after adjustment for covariates. These data raise the question of whether urine BMP levels may also offer a marker of disease state and progression. Of note, *LRRK2* carriers without PD were not screened for non-motor symptoms or for dopamine deficiency on dopamine transporter (DAT) scans. It is critical to assess whether urinary BMP levels are associated with preclinical signs of PD such as abnormal DaT scans or non-motor symptoms (eg, hyposmia). Studies are underway to monitor BMP isoform levels in *LRRK2*-PD and iPD patients at baseline and longitudinally in the deeply phenotyped cohort of Parkinson's Progression Markers Initiative to directly assess the possibility of urinary BMP as a state and/or progression marker of PD.

Urinary BMP levels also appear to be a viable pharmacodynamic biomarker of *LRRK2* kinase inhibitors. Indeed, the non-human primate studies discussed above[9] support this contention because these studies demonstrated decreases in urinary di-22:6-BMP levels following treatment with 3 distinct *LRRK2* kinase inhibitors and its restoration to normal levels following treatment cessation.[9] At this point, the tissue source of BMP in the urine remains unclear. BMP-enriched exosomes may be secreted from a variety of cells and tissue types in which *LRRK2* is natively expressed. Future preclinical studies assessing urinary BMP levels in combination with markers of brain *LRRK2* kinase inhibition (eg, pSer1292-*LRRK2*, p-Rabs) and detailed pharmacokinetics in plasma, brain, and cerebrospinal fluid are needed to determine whether urinary BMP levels may be extrapolated to brain *LRRK2* inhibition. It is noteworthy that urine contains brain-derived biomarkers such as neurofilament proteins and brain-specific Rab GTPases, possibly because of the excretion of brain-generated exosomes in the urine. Thus, the possibility that BMP levels in the urine reflect altered endolysosomal function in the brain also needs to be further investigated.

In conclusion, using 2 independent cohorts and quantitative mass spectrometric assays we have provided robust evidence that specific BMP species in urine are associated with G2019S mutations in the *LRRK2* gene. The biology of BMP suggests that the alterations in urine BMP levels may indicate deficits in endolysosomal function. An exciting preliminary finding is that BMP levels are also state markers because *LRRK2* carriers with PD had higher BMP levels than those without PD. Moreover, increased total di-18:1-BMP and total di-22:6-BMP were associated with worse cognitive performance by MoCA. Thus, the results described here open avenues for future studies to establish the utility of urinary BMP as a biomarker to monitor disease progression and as a pharmacodynamic biomarker to demonstrate *LRRK2* kinase modulation by drug candidates.

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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.