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Higher Urine bis(monacylglycerol) phosphate (BMP) levels in *LRRK2* G2019S mutation carriers: implications for therapeutic development

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Abstract

Objective: To study alterations in urinary phospholipids as biomarkers of *LRRK2* mutations and Parkinson's disease (PD) status/phenotypes.

Background: *LRRK2* mutations are a common cause of dominantly inherited PD. Previous studies showed decreases in urine levels of di-docohexaenoyl (22:6) bis(monacylglycerol) phosphate (di-22:6-BMP) in *LRRK2* knockout mice and in non-human primates treated with LRRK2 kinase inhibitors. We hypothesized that urine levels of BMP isoforms will be higher in individuals with PD-causing gain-of-kinase function mutation, *LRRK2* G2019S.

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

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Results: In each cohort, four BMP isoforms (di-18:1-BMP, di-22:6-BMP, 2,2'-di-22:6-BMP, and 2,2'-di-18:1-BMP) were significantly higher (2.5–4.3 fold) in G2019S carriers compared to non-carriers. Interestingly, 2,2' di-18:1-BMP levels were marginally higher in *LRRK2* carriers with PD than those without PD (p=0.045). Moreover, increased 2,2' and total di-22:6-BMP were associated with worse cognitive status assessed by Montreal Cognitive Assessment (MoCA; (p=0.0033, 0.0144, respectively).

Conclusions: The observed association of BMP isoforms with *LRRK2* G2019S mutation, PD status among G2019S carriers and correlation with cognitive decline suggest the potential use of urinary BMP isoforms as biomarkers for clinical trials of LRRK2-targeted therapies.

INTRODUCTION:

Mutations in the gene encoding Leucine-rich repeat kinase 2 (LRRK2) are among the most common genetic causes of Parkinson's disease (PD).[1] While 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 (AD) highlights the need for biomarker-informed patient enrichment strategies for successful clinical development of disease-modifying therapies. For LRRK2 kinase inhibitors, three 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 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. Lastly, 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 (Di Maio et al., 2018[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 due to alterations in the metabolic pathways of LRRK2 are required. A reliable biomarker of LRRK2 activity would also be beneficial to confirm target engagement and monitor 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 (multi-vesicular bodies) and lysosomes, where they contribute to the multi-vesicular/lamellar morphology of the endo-lysosomal network.[6, 7] Recent studies have identified several GTPases as bonafide substrates of LRRK2 and in conjunction with other evidence, have implicated a role of LRRK2 in endolysosomal vesicular trafficking and

lysosomal functions.[8] Di-docosahexaenoyl(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* knock out 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 two 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 of 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 three 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 an MJFF-funded LRRK2 biomarker project from March 2016 – 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

Quantitative Ultra Performance Liquid Chromatography Tandem Mass Spectrometry (UPLC-MS/MS) analyses: A multiplexed UPLC-MS/MS method was used to simultaneously quantitate (semi-quantitate) 54 species of urinary BMPs, phosphotidyl choline (PC), phosphotidylethanolamine (PE), and phosphotidyl inositol (PI), and globotriacylceramides (Gb3s) with various fatty acid chains (Supplement Tables 1–3). The analyses were conducted by Nextcea, Inc. (Woburn, MA) as previously described.[11] Standard curves were prepared from related standards using a class-based approach (i.e., 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. Concentrations of urine BMPs, phospholipids and Gb3s were quantitated using a SCIEX TripleTOF 6600 UPLC-MS/MS System. Injections were made using a Shimadzu Nexera XR UPLC system (Shimadzu Scientific Instruments, Japan). A SCIEX TripleTOF 6600 mass spectrometer equipped with an IonDrive Turbo V source was used in negative electrospray ionization (ESI) mode for detection (SCIEX, Framingham, MA). 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 in 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 three geometrical isoforms (2, 2'-, 2, 3'-, and 3, 3'- BMP), which may influence their functional properties.[12, 13] Targeted UPLC-MS/MS using multiple reaction monitoring was used to absolutely quantitate total di-22:6-BMP (the sum of its three 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 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 variable and the Kruskal-Wallis test for continuous variables to compare the four 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 two cohorts, we combined the data from both cohorts and compared the BMP analytes across the four groups in the merged database.

Lastly, in the CUIMC cohort where 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 four groups were balanced for all demographic variables. The broad profiling of LCC cohort urine for 54 distinct analytes are 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-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 four 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 to those without PD, although only the 2,2’-di-18:1-BMP isoform reached marginal statistical significance (p=0.0459).

BMP and PD phenotypes

Lastly, 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 negatively associated with creatinine-normalized 2,2'and total di-22:6-BMP isoforms, both 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 non-carriers, independent of PD status and sex in two 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 to 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 (PG) 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 (e.g., glucocerebrosidase) and activator proteins (saposins) at the interface of the lysosomal inner membranes.[6] Alternations 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 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 to non-carriers. In contrast, mice with germ-line deletion of *Lrrk2* and cynomolgus monkeys treated with three 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 upon withdrawal from treatment with a LRRK2 kinase inhibitor.[9] Since 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 indicates 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 to 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 the urine. One candidate LRRK2 Rab GTPase substrate contributing to the observed increases in urinary BMP is Rab35, since it has been shown to regulate 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 due to 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 clinical trials with the patients most likely to respond to LRRK2 inhibitors (i.e., those with higher LRRK2 kinase activity). Our data indicate the potential utility of urinary di-22:6-BMP and di-18:1-BMP as a 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 to those without PD, and that among PD patients higher di-22:6-BMP levels predicted worse cognitive performance, even after adjustment for covariates. These data raise the question 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 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 (e.g., 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 (PPMI) 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 since 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 where LRRK2 is natively expressed. Future preclinical studies assessing urinary BMP levels in combination with markers of brain LRRK2 kinase inhibition (e.g., 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 due to 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 two independent cohorts and quantitative mass spectrometric assays we provide 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 since *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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Healy DG, Falchi M, O'Sullivan SS, et al. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. Lancet Neurol 2008;7:583–590. [PubMed: 18539534]

2. Zimprich A, Biskup S, Leitner P, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron 2004;44:601–607. [PubMed: 15541309]
3. West AB. Ten years and counting: moving leucine-rich repeat kinase 2 inhibitors to the clinic. Movement disorders : official journal of the Movement Disorder Society 2015;30:180–189. [PubMed: 25448543]
4. Di Maio R, Hoffman EK, Rocha EM, et al. LRRK2 activation in idiopathic Parkinson's disease. Science translational medicine 2018;10.
5. Roosen DA, Cookson MR. LRRK2 at the interface of autophagosomes, endosomes and lysosomes. Molecular neurodegeneration 2016;11:73. [PubMed: 27927216]
6. Gallala HD, Sandhoff K. Biological Function of the Cellular Lipid BMP-BMP as a Key Activator for Cholesterol Sorting and Membrane Digestion. Neurochemical research 2011;36:1594–1600. [PubMed: 21136156]
7. Schmitz G, Muller G. Structure and function of lamellar bodies, lipid-protein complexes involved in storage and secretion of cellular lipids. Journal of lipid research 1991;32:1539–1570. [PubMed: 1797938]
8. Steger M, Tonelli F, Ito G, et al. Phosphoproteomics reveals that Parkinson's disease kinase LRRK2 regulates a subset of Rab GTPases. Elife 2016;5.
9. Fuji RN, Flagella M, Baca M, et al. Effect of selective LRRK2 kinase inhibition on nonhuman primate lung. Science translational medicine 2015;7:273ra215.
10. Alcalay RN, Levy OA, Waters CC, et al. Glucocerebrosidase activity in Parkinson's disease with and without GBA mutations. Brain : a journal of neurology 2015.
11. Liu N, Tengstrand EA, Chourb L, Hsieh FY. Di-22:6-bis(monoacylglycerol)phosphate: A clinical biomarker of drug-induced phospholipidosis for drug development and safety assessment. Toxicology and applied pharmacology 2014;279:467–476. [PubMed: 24967688]
12. Amidon B, Schmitt JD, Thuren T, King L, Waite M. Biosynthetic Conversion of Phosphatidylglycerol to Sn-1-Sn-1' Bis(Monoacylglycerol) Phosphate in a Macrophage-Like Cell-Line. Biochemistry 1995;34:5554–5560. [PubMed: 7727416]
13. Thornburg T, Miller C, Thuren T, King L, Waite M. Glycerol reorientation during the conversion of phosphatidylglycerol to bis(monoacylglycerol)phosphate in macrophage-like RAW 264.7 cells. The Journal of biological chemistry 1991;266:6834–6840. [PubMed: 2016299]
14. Meikle PJ, Duplock S, Blacklock D, et al. Effect of lysosomal storage on bis(monoacylglycero)phosphate. The Biochemical journal 2008;411:71–78. [PubMed: 18052935]
15. Alcalay RN, Mallett V, Vanderperre B, et al. SMPD1 mutations, activity, and alpha-synuclein accumulation in Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society 2019.
16. Blanc L, Vidal M. New insights into the function of Rab GTPases in the context of exosomal secretion. Small GTPases 2018;9:95–106. [PubMed: 28135905]
17. Hsu C, Morohashi Y, Yoshimura S, et al. Regulation of exosome secretion by Rab35 and its GTPase-activating proteins TBC1D10A-C. The Journal of cell biology 2010;189:223–232. [PubMed: 20404108]

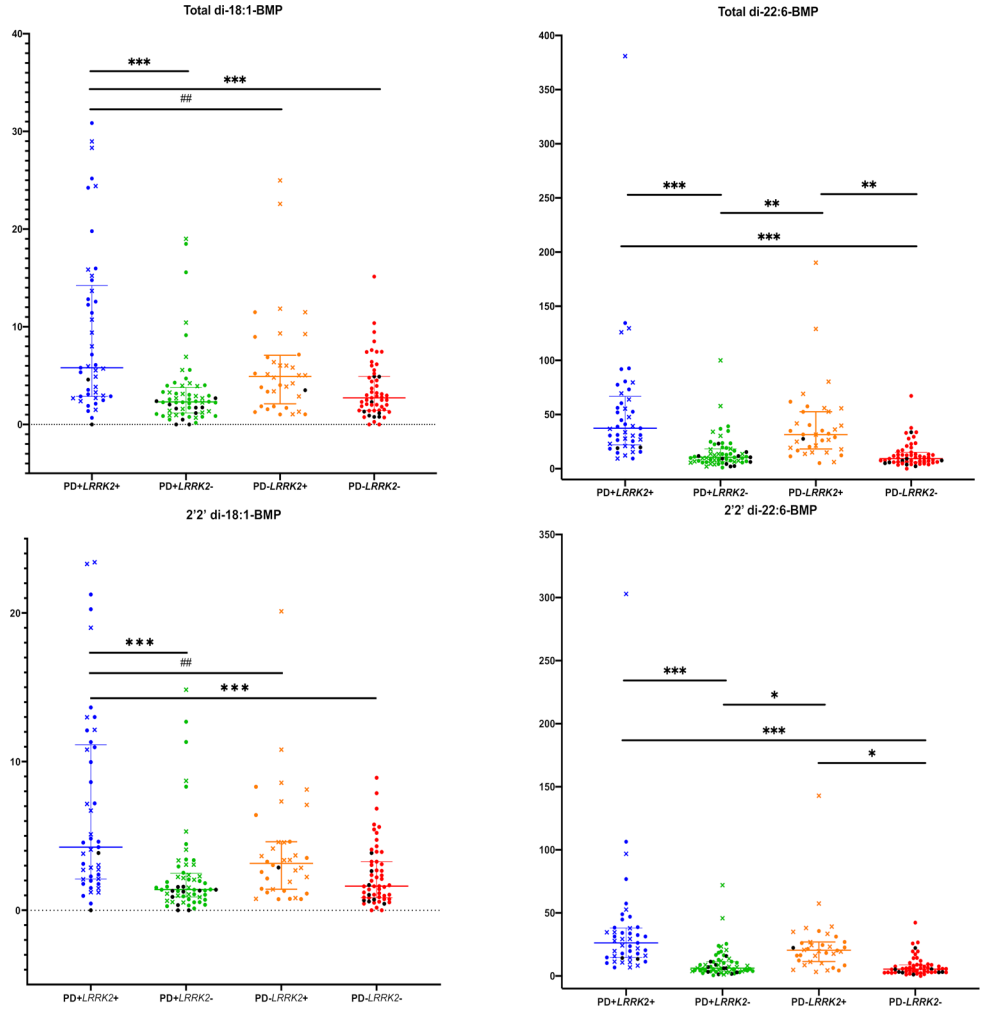

Figure 1.

Levels of BMP isoforms in the combined LCC and CUIMC data-sets. Panels A-D depict data for a specific BMP isoform measured in the four 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 pair-wise comparisons to identify groups differing from each other. *p<0.05, **p<0.005, ***p<0.0001; #p<0.025, ##p<0.01

Table 1:

Demographics and urine BMP concentration among LCC participants

	PD+*LRRK2*+ (n=20)	PD+*LRRK2*− (n=20)	PD-*LRRK2*+ (n=20)	PD-*LRRK2*−(n=20)	p-value
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[1]
Disease duration	11.4 (5.9)	11.9 (6.2)	n/a	n/a	0.6643[1]
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*
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*
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*
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*
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 and (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).

*Indicates statistically significant difference; note that it is driven primarily by the *LRRK2* genotype. Chi-square test was used for the categorical variable, sex.

[1]The p-values were calculated using the Wilcoxon-Rank Sum test for continuous variables, Age of onset and Disease duration to compare the two Parkinson's disease groups with each other.

Table 2:

Demographics and urine BMP concentration among CUIMC participants

	PD+*LRRK2*+ (n=25)	PD+*LRRK2*− (n=40)	PD−*LRRK2*+ (n=16)	PD−*LRRK2*− (n=35)	p-value
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[1]
Disease duration	11 (1–26)	7 (0–18)	n/a	n/a	0.0026[1]
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*
MoCA mean, (SD)	26.5 (4.7)	26.9 (1.6)	28.7 (1.1)	27.5 (2.1)	0.0080*
LEDD	547 (313.3)	415 (375.7)	n/a	n/a	0.0971[1]
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*
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*
2,2’ di-18:1-BMP, median (range))	4.56 (0–21.3)	1.36 (0–12.69)	2.36 (0.75–8.31)	1.15 (0–6.84)	<0.0001*
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*
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 and (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).

*Indicates statistically significant difference; note that it is driven primarily by the *LRRK2* genotype. Chi-square test was used for the categorical variable, sex.

[1]The p-values were calculated using the Wilcoxon-Rank Sum test for continuous variables, Age of onset and Disease duration to compare the two 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-value**	**p-value[1]**
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*	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*	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*	0.0459*
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*	0.0871

BMP values shown are median and (range) of creatinine-normalized isoforms (ng/mg creatinine). The p-values were calculated using the Kruskal-Wallis test.

*Indicates statistically significant difference; note that it is driven primarily by the *LRRK2* genotype.

[1]The p-values were calculated using the Wilcoxon-Rank Sum test to compare *LRRK2*+PD+ to *LRRK2*+PD−.

Table 4.

Assessing MoCA and UPDRS as predictors of BMP levels in PD Patients (N=65)

	Model 1		Model 2	
MoCA	**Beta (SE)**	**p-value**	**Beta (SE)**	**p-value**
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*	−1.76 (0.86)	0.0461*
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*	−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

*Indicates statistically significant differences.