Cytokines and Gaucher Biomarkers in Glucocerebrosidase Carriers with and Without Parkinson Disease

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ABSTRACT: Background: Homozygous and compound heterozygous variants in glucocerebrosidase (*GBA*) can cause Gaucher disease (GD), whereas heterozygous variants increase the risk of developing Parkinson's disease (PD). GD patients display altered peripheral immune proteins. However, it is unknown if these are altered in *GBA* carriers with PD.
Objectives: To determine whether plasma cytokines and immune biomarkers associated with GD are also altered in *GBA* carriers with or without PD.
Methods: Inflammatory cytokines and established GD biomarkers, ferritin, CD162, CCL18, and chitotriosidase (28 biomarkers) were measured in *GBA* pathogenic variant carriers with (n = 135) and without (n = 83) PD, and non-carriers with (n = 75) and without PD (n = 77).
Results: PD patients with biallelic pathogenic variants in *GBA* had elevated plasma levels of ferritin, CCL18, and MIP1α. These biomarkers were not elevated in heterozygous *GBA* carriers.
Conclusion: GD plasma biomarkers are not promising candidates for stratifying the risk for PD in carriers of heterozygous *GBA* pathogenic variants. © 2021 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society

Key Words: Parkinson's disease; cytokine; monocyte; inflammation; Gaucher disease; glucocerebrosidase

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Homozygous and compound heterozygous mutations in *GBA*, which encodes the enzyme β-glucocerebrosidase (GCase), cause the lysosomal storage disorder Gaucher disease (GD).[1] GD patients, as well as heterozygous carriers of pathogenic variants, are at risk for Parkinson's disease (PD).[2-5] The penetrance of pathogenic *GBA* variants for PD is estimated at 10%–30%, indicating that the majority of mutation carriers will never develop PD.[6-9] Thus, biomarkers that can inform which *GBA* variant carriers are more likely to develop PD are required. Moreover, a number of strategies targeting the GCase pathway are currently being explored as potential therapeutics for PD,[10-12] so there is much interest in finding biomarkers that can identify efficacious trial compounds.

The link between pathogenic *GBA* variants and PD highlights the potential role of the immune system in PD. Cells of the reticuloendothelial system (eg, macrophages), are particularly affected in GD. Accumulation of lipids (primarily glucocerebroside) in macrophages results in macrophage dysfunction and increased systemic inflammation in GD patients[13] and in preclinical models.[14,15] Indeed, a number of monocyte activation markers have been validated as biomarkers for monitoring patient responses to GCase enzyme replacement therapy or substrate reduction therapy, the standard treatments for GD. These include CD163,[16] chitotriosidase (CHIT1),[17] CCL18,[18] and IL-1β.[19] Importantly, monocyte dysfunction and elevated peripheral inflammation have also been reported in PD patients.[20-22]

In the present study we used plasma from a cohort of *GBA* pathogenic variant carriers with and without PD, as well as PD patients without *GBA* mutations, and matched controls to determine whether cytokines

and/or peripheral immune biomarkers associated with GD, were also elevated in carriers of *GBA* pathogenic variants associated with PD.

Materials and Methods

Samples were obtained from participants of the Spot study at Columbia University Irving Medical Center (CUIMC) and the Icahn School of Medicine at Mount Sinai (ISMMS). Details of patient recruitment and assessment are provided in the supplementary methods. All clinical study procedures were approved by the Columbia University IRB (and ISMMS IRB, if collected at Mount Sinai, NY), and all participants signed informed consent. All samples were shipped to Sydney, Australia on dry ice. All biomarker studies were approved by the University of Sydney Human Research Ethics Committee (2017/076 and 2017/857).

Multiplex Cytokine ELISAs

Bio-Rad Bio-Plex Pro Human Cytokine 27-plex assays (#M500KCAF0Y, 1:4 plasma dilution) were used to measure cytokines and chemokines with details provided in the supplementary methods.

CCL18, CD163, and Ferritin ELISA Assays

Human PARC (CCL18, #EHCCL18, 1:500 plasma dilution), CD163 (#EHCD163, 1:50 plasma dilution), and ferritin (#EHFTL, 1:20 plasma dilution) were measured by enzyme-linked immunosorbent assay (ELISA) (all Thermo Scientific) with details provided in the supplementary methods.

Chitotriosidase Activity Assay

CHIT1 activity was measured using a BioVision Chitotriosidase Activity Assay Kit (Fluorometric, #K512-100, 1:4 dilution) with details provided in the supplementary methods.

Statistical Analysis

Statistical analyses were performed using SPSS v25 Statistics software[23] unless otherwise specified. The mean of each replicate sample was determined for each marker and those below the level of detection were given a notional value of 0. Principal component analysis was used to determine clustering of the measured plasma protein profiles. Multivariate analysis of covariance (MANCOVA) was performed on $\log_{10}$ +1 transformed variables and unless otherwise indicated included age, sex, and PD status as covariates. An overall significant effect for MANCOVA analysis was accepted at $P < 0.05$ using Wilks' Lambda test. Significant MANCOVA effects were followed with pairwise comparison post hoc tests using the estimated marginal means. A Bonferroni corrected $P < 0.002$ (0.05/28 variables) was applied where appropriate to correct for multiple testing. To detect differences between groups in clinical or demographic variables, one-way ANOVA with least-significant difference multiple comparison post hoc tests was used. Spearman's correlations were performed to identify any associations between plasma proteins and clinical data. Graphs were made with Prism (v8.00 GraphPad Software). More specific analysis details are provided in the supplementary methods.

Results

Demographic Comparisons

The demographics and phenotype of *GBA* carriers with PD (*GBA*+/PD+, n = 135), *GBA* carriers without PD (*GBA*+/PD−, n = 83), non-carriers with PD (*GBA*−/PD+, n = 75), and non-carriers without PD (*GBA*−/PD−, n = 77) are presented in Table 1. A breakdown of the mutation types is provided in Table S1. The groups were matched by age and sex, and the PD groups were also similar in age at onset, disease duration, levodopa equivalent daily dose, and Unified Parkinson's Disease Rating Scale Part III (UPDRS-III) scores. The *GBA*+/PD+ group had a significantly lower Montreal Cognitive Assessment (MoCA) score than all other groups ($P = 0.001$), indicating greater cognitive dysfunction in this group. Principal component analysis was then used to determine any clustering of the measured plasma proteins across the entire cohort. This did not indicate any clear separation between groups; however, three high inflammatory individuals clearly separated from the cohort majority (Fig. 1A) and were removed from the analysis (see supplementary methods). The absolute values for the 28 proteins that could robustly be detected in plasma for the remaining participants are shown in Table S2.

Increased Ferritin, MIP1α, and CCL18 in Biallelic *GBA* Pathogenic Variant Carriers

Six subjects in the study carried biallelic *GBA* pathogenic variants, four of which also had a diagnosis of GD, of whom two were receiving enzyme replacement therapy. As anticipated, multivariate analysis covarying for age and sex indicated that biallelic mutations had a significant overall effect on the plasma protein profile compared to no *GBA* mutation carriers ($P = 0.002$). Post hoc analysis indicated that ferritin ($P = 0.002$, Fig. 1B), CCL18 ($P = 0.001$, Fig. 1C), and MIP1α ($P < 0.001$, Fig. 1D) were significantly increased in the biallelic group. Chitotriosidase activity was also included in the multivariate analysis and was not increased in the biallelic group (Fig. S1).

TABLE 1. Demographic and clinical characteristic data. Participants were grouped by the presence or absence of either Parkinson's disease or a *GBA* mutation

Parameter	*GBA*−/PD−	*GBA*+/PD−	*GBA*−/PD+	*GBA*+/PD+
n	77	83	75	135
Age (yr)	62.6 ± 1.2	62 ± 1.2	62.4 ± 1.2	64.4 ± 0.9
Sex (M/F)	40/37	32/51	37/38	84/51
AAO	–	–	57.5 ± 1.3	58.8 ± 0.9
MoCA	26.8 ± 0.3	26.5 ± 0.3	26.2 ± 0.4	25.1 ± 0.4[ab]
Education (yr)	16.7 ± 0.3	17.6 ± 0.4	17 ± 0.4	17 ± 0.3
UPDRS-III	1 ± 0.2	1.5 ± 0.3	17.3 ± 1.2[a]	18.8 ± 1[a]
LEDD	–	–	413.8 ± 46.2	446.1 ± 33.4

Values are presented as mean ± SEM. Data were analyzed by one-way ANOVA with a least-significant difference post hoc test except sex, which was analyzed by Kruskal–Wallis followed by Mann–Whitney *U*.
[a]$P < 0.05$ compared to the control group.
[b]$P < 0.05$ compared to the PD group without a *GBA* mutation.
Abbreviations: PD, Parkinson's disease; M, male; F, female; AAO, age at clinical onset; MoCA, Montreal Cognitive Assessment; UPDRS-III, Unified Parkinson's Disease Rating Scale Part III; LEDD, levodopa equivalent daily dosage.

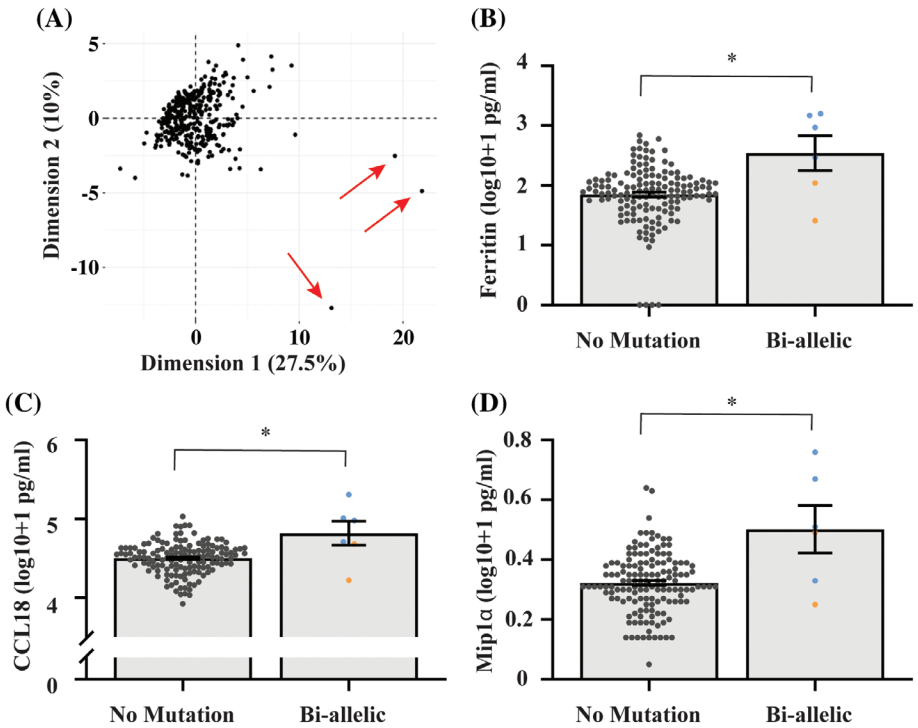

FIG. 1. Increased plasma proteins in biallelic GBA mutation carriers. (**A**) Principal component analysis was performed to determine clustering of the measured 28 plasma proteins (n = 371). Three participants clearly separated from the cohort (indicated with red arrows) due to high expression of multiple inflammatory cytokines and these participants were excluded from downstream analysis. MANCOVA analysis covarying for age and sex was used to compare the 28 measured plasma proteins between biallelic *GBA* mutation carriers (n = 6) and subjects with no *GBA* mutation (n = 138). Ferritin (**B**), CCL18 (**C**), and MIP1α (**D**) were significantly higher in biallelic *GBA* mutation carriers compared to those with no mutation. Homozygous *GBA* N370S carriers are blue and compound heterozygous *GBA* carriers are orange. Graphs show individual values and also the mean ± SE. *$P \leq 0.002$.

No Change in Inflammatory Plasma Biomarkers in Heterozygous *GBA* Pathogenic Variants Carriers

To determine any differences in the levels of the 27 measured plasma proteins and chitotriosidase activity in heterozygous carriers of *GBA* mutations, two-factor MANCOVA analysis using transformed data and covarying for age sex was performed. The results showed that neither PD ($P = 0.097$) nor *GBA* mutation status ($P = 0.157$) were significantly associated with the plasma levels of the measured proteins. The statistical analysis also revealed that there was no interactive or additive effect between *GBA* mutation status and PD status on the plasma proteins ($P = 0.670$). Removing

the eight *GBA* mutation cases which also had a *LRRK2* mutation from the analysis did not alter the results (all $P > 0.05$). The same analysis was performed using only heterozygous N370S mutation carriers, with the same result of no significant difference due to either PD ($P = 0.108$) nor N370S mutation status ($P = 0.272$).

Correlations Between Inflammatory Plasma Markers and Clinical Variables

Spearman's correlation analysis was performed to determine whether any of the measured plasma proteins correlated to the clinical scores for motor severity and cognitive dysfunction. The UPDRS III score significantly positively correlated to IL4, IL8, MCP1, TNFα, and MIP1α, while the MoCA score significantly negatively correlated to IL17RA, CXCL10, MIP1α, and CCL18, and significantly positively correlated to PDGF (Table S3).

Discussion

In the current study we measured 28 peripheral immune proteins across a cohort of 371 individuals. We found that biallelic *GBA* mutation carriers, which included four homozygous N370S carriers clinically diagnosed with both PD and GD, had significantly higher plasma levels of ferritin, CCL18, and MIP1α compared to those with no *GBA* mutation. Hyperferritinemia is commonly observed in GD patients and indicates immune dysregulation,[24-26] and treatment of GD can be effective for decreasing or normalizing ferritin.[25] Likewise, CCL18 released from macrophages is markedly elevated in GD patient plasma,[27] and is useful for monitoring disease severity and response to treatment.[28] In contrast, these proteins were not increased in heterozygous *GBA* mutation carriers. That neither ferritin, MIP1α, nor CCL18 were increased in heterozygous N370S carriers suggests that the increased plasma levels of these proteins observed in biallelic carriers was due to the presence of GD rather than PD.

Elevated plasma levels of the inflammatory chemokine MIP1α (also known as CCL3) have also been documented in GD patients,[29] and MIP1α was another protein increased in biallelic *GBA* mutation carriers in the current study. Intriguingly, MIP1α was also strongly correlated with the UPDRS III PD severity scores. A recent study in a large PD cohort indicated that higher plasma levels of MIP1α were associated with faster PD disease progression,[30] and stimulation of peripheral immune cells from PD patients with the inflammatory agonist lipopolysaccharide (LPS) resulted in higher secretion of MIP1α, that again associated with disease severity.[31] Moreover, LPS stimulation of peripheral PD immune cells also results in higher secretion of MCP1, IL8, TNFα, CCL5, and IL-1β,[31] with MCP1, IL8, and TNFα also correlating with disease severity in the current study. A number of reports have demonstrated an underlying inflammatory phenotype in PD patients; however, the extent of any inflammation certainly varies across studies.[32] Inflammatory medication use, recent illness, or the presence of other comorbid inflammatory diseases can contribute to variability in peripheral cytokine measures and were not recorded in the current study. Furthermore, it is possible that measurement of cytokines in other biofluids (eg, CSF) may provide additional information. Importantly, associations between inflammatory cytokines/chemokines and disease severity measures were independent of *GBA* pathogenic variant type or presence, and thus more likely a feature of PD in general.

The major strengths of this study are the relatively large number of *GBA* pathogenic variant carriers and the blinding of the laboratory that measured the cytokines. Our study did not include GD patients without PD however, and thus it could not be determined if the above biomarkers can distinguish between GD patients with and without PD. Also, among all genotypes, only the N370S group was sufficiently large enough to compare carriers with and without PD. Lastly, we did not genotype for *CHIT1* mutations, which would affect chitotriosidase plasma levels.[33] Future studies exploring the potential role of plasma chitotriosidase as a PD biomarker should stratify analyses by *CHIT1* genotype.

In summary, these results, based on a large number of *GBA* pathogenic variant carriers, indicate that plasma cytokines and biomarkers used to monitor the severity of GD, are not promising candidates for stratifying the risk of developing PD for carriers of heterozygous *GBA* pathogenic variants.

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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.