Clinical and Biochemical Differences in Patients Having Parkinson Disease With vs Without GBA Mutations

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**IMPORTANCE** Biochemical abnormalities present in GBA (mut/wt) carriers may offer new pathogenetic insights to and potential therapeutic targets in Parkinson disease (PD).

**OBJECTIVE** To determine whether patients having PD with vs without GBA mutations differ in clinical phenotype or plasma protein expression.

**DESIGN AND SETTING** Case-control study of patients having PD with vs without GBA mutations. Clinical characteristics were compared between groups, and biochemical profiling of 40 plasma proteins was performed to identify proteins that differed in expression between groups.

**PARTICIPANTS** The discovery cohort included 20 patients having PD with GBA mutations. Clinical characteristics of GBA-associated PD cases were compared with those of 242 patients having PD in whom GBA mutations were excluded by full gene sequencing.

**MAIN OUTCOME MEASURES** Biochemical profiling was available for all 20 GBA-associated PD cases, as well as a subset (87 of 242) of the GBA-negative PD cases. The replication cohort included 19 patients having PD with GBA mutations and 41 patients having PD without GBA mutations.

**RESULTS** Compared with patients having PD without GBA mutations, patients having PD with GBA mutations were younger at disease onset ($P = .04$) and were more likely to demonstrate cognitive dysfunction ($P = .001$). In a multiple regression model that included age, sex, and assay batch as covariates, GBA mutation status was significantly associated with plasma levels of interleukin 8 ($P = .001$), monocyte chemotactic protein 1 ($P = .008$), and macrophage inflammatory protein 1α ($P = .005$). The association between interleukin 8 and GBA mutation status was replicated ($P = .03$) in a separate cohort of patients having PD with vs without GBA mutations.

**CONCLUSIONS AND RELEVANCE** Patients having PD with GBA mutations have earlier age at disease onset and are more likely to demonstrate cognitive dysfunction. Monocyte-associated inflammatory mediators may be elevated in patients having PD with GBA mutations.

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Patients Having PD With vs Without GBA Mutations

Parkinson disease (PD) is a neurodegenerative disorder characterized by clinical features of parkinsonism and the pathological finding of neuronal α-synuclein-positive Lewy bodies. A strong association has been established between PD and mutations in the GBA gene (OMIM 606463), encoding the lysosomal enzyme glucocerebrosidase. Pathogenetic mutations in both alleles of GBA (GBA [mut/mut]) cause the lysosomal storage disorder Gaucher disease. Carriers of a single mutant GBA allele (GBA [mut/wt]) are unaffected by Gaucher disease. However, recently, heterozygous GBA mutations have been demonstrated to be the most common single gene mutation associated with sporadic PD, occurring 5 times more often in patients with PD than in the general population.

While the underlying reason for the association between GBA mutations and PD is unknown, a direct mechanistic link between glucocerebrosidase and α-synuclein may exist because abnormal glucocerebrosidase activity has been shown to impair α-synuclein processing in vitro and in vivo. Accordingly, biochemical abnormalities present in GBA (mut/wt) carriers may offer new pathogenetic insights to and potential therapeutic targets in PD. In this study, we compared clinical characteristics and levels of 40 plasma proteins in a group of patients having PD with vs without GBA mutations.

Methods

Patient Cohorts

This was a case-control study of a group of patients having PD with GBA mutations (GBA [mut/wt] and GBA [mut/mut]) and a comparator group of patients having PD without GBA mutations (GBA [wt/wt]). Institutional review board approval at all involved institutions was obtained.

The discovery cohort included individuals evaluated at the University of Pennsylvania with a clinical diagnosis of PD based on United Kingdom Brain Bank criteria. For comparison of clinical characteristics, the discovery cohort consisted of 20 patients having PD with GBA mutations and 242 patients having PD in which GBA mutations were excluded (the mutation screening method is described in the next subsection). For comparison of biochemical characteristics, a convenience subset of these patients with PD was used, including 20 GBA-associated PD cases and a subset (87 of 242) of the GBA-negative PD cases. For the full discovery cohort and the subset of 107 patients (87 controls plus 20 mutation carriers) for whom biochemical profiling was performed, patients were recruited without bias for specific characteristics or prior knowledge of genetic status.

To replicate significant biochemical findings in an independent cohort, plasma samples were obtained from 60 patients with PD at the University of Washington, recruited as previously described. Among these, the replication cohort consisted of 17 GBA (mut/wt) cases, 2 GBA (mut/mut) cases, and 41 GBA (wt/wt) cases.

Genetic Screening for GBA Mutations

In the University of Pennsylvania discovery cohort of patients with PD, GBA mutations were identified by 1 of 2 approaches. For 254 patients with PD, long-range polymerase chain reaction amplification, followed by sequencing of all 11 exons of the GBA gene, was conducted as previously described (eMethods in Supplement). All patients having PD with GBA mutations identified in this manner were included as GBA mutation carriers; those without an identified mutation were included in the comparator nonmutation group. For an additional 231 patients with PD, screening was performed for only the 2 most common GBA mutations, N370S and L444P (eMethods in Supplement). All patients having PD with GBA mutations identified through the abbreviated screening technique were included as cases. Because other GBA mutations were not assessed in these 231 individuals, no persons from this group were included in the comparator group of patients having PD without GBA mutations.

In the University of Washington replication cohort of patients with PD, GBA mutation status was ascertained by long-range polymerase chain reaction amplification, followed by sequencing of all 11 exons of the GBA gene. Additional details are given in the eMethods in Supplement.

Clinical Characterization (Discovery Cohort)

Information obtained included demographics, age at the time of diagnosis of PD by a physician, and age at the time of PD symptom onset (reported by the patient), as well as modified Hoehn and Yahr (H&Y) staging of PD. Cognition was classified as normal cognition, mild cognitive impairment (MCI), or dementia as determined by consensus clinical determination (eMethods in Supplement).

Biochemical Characterization By Multiplex Immunoassay (Discovery Cohort)

Simultaneous screening of plasma samples from patients having PD with vs without GBA mutations was initially performed for a commercially available panel of 71 plasma proteins on a multiplex bead-based immunoassay (Rules-Based Medicine; Myriad RBM, Inc) as previously described, 40 of the 71 plasma analytes met quality control measures and were included in the analysis (eTable 1 in Supplement). The eMethods in Supplement describe the methods for sample handling and processing and quality control.

ELISA (Replication Cohort)

Enzyme-linked immunosorbent assay (ELISA) was used to replicate biochemical findings. Using standard manufacturer instructions for ELISA, monocyte chemotactic protein 1 (MCP1) (R&D Systems) and interleukin 8 (IL-8) (BD Biosciences) were measured. Further details are given in the eMethods in Supplement.

Statistical Analysis

Survival curve analyses using log-rank tests were used to compare age at disease onset and age at PD diagnosis for patients having PD with vs without GBA mutations. Binomial logistic regression was used to examine the effect of GBA status on cognition using consensus clinical determination of normal or abnormal cognition (MCI or dementia) as ordinal outcomes. For analyses pertaining to plasma protein levels, levels of 40 measured plasma proteins were compared in patients having PD with vs without GBA mutations by Mann-Whitney test (the cut-
off for initial screening was 2-tailed $P \leq .05$). Markers identified as nominally significant in this initial screening were then entered into a multivariate linear regression model designating sex, assay batch, and age at plasma draw as covariates.

In the replication cohort, candidate plasma protein levels were compared between patients having PD with vs without GBA mutations in univariate analysis and with a multivariate model adjusting for sex and age at plasma draw (no adjustment was needed for assay batch because all samples were assayed simultaneously). Significance was set at tailed $P \leq .05$ because expected directionality was known.

The association between levels of replicated plasma analytes and cognitive function (consensus clinical determination) was examined by linear regression with sex and age at consensus clinical determination as covariates. An association with motor impairment (modified H&Y stage) was assessed using a linear regression model that included sex and age at plasma draw as covariates.

All statistical analyses were performed using available software (R, version 2.14.0; http://cran.r-project.org/bin/windows/base/old/2.14.0/). R-scripts are available on request.

Table. Characteristics of Discovery Cohort by GBA Genotype

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>GBA (wt/wt)</th>
<th>GBA (mut/wt)</th>
<th>GBA (mut/mut)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No.</td>
<td>242</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>Male sex, No.</td>
<td>168</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Female sex, No.</td>
<td>74</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Modified Hoehn and Yahr stage, mean</td>
<td>2.7</td>
<td>2.3</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Results

Patients Having PD With GBA Mutations Demonstrate Earlier Age at Disease Onset and More Cognitive Impairment

Discovery cohort characteristics are given in the Table. The mean (SEM) age at onset of PD symptoms was younger in 20 patients having PD with GBA mutations (eTable 2 in Supplement) compared with 242 patients having PD without GBA mutations (59.0 [2.0] vs 63.1 [0.6] years; $P = .04$, log-rank test) (Figure 1A). The mean (SEM) age at PD diagnosis was also younger in patients having GBA mutations compared with those not having GBA mutations (59.4 [2.1] vs 64.7 [0.6] years; $P = .01$, log-rank test) (Figure 1B).

Cognitive performance differed between patients having PD with vs without GBA mutations. Specifically, 15 of 20 GBA mutation carriers (75.0%) had a consensus clinical determination of MCI or dementia compared with 105 of 242 mutation-negative patients (43.4%) with PD ($\chi^2 = 7.61, P = .02$). In a multivariate model adjusting for sex and age at consensus clinical determination, GBA mutation carriers were still more likely to show MCI or dementia (odds ratio, 7.65; 95% CI, 2.34-25.05; $P = .001$); this relationship persisted after adjusting for degree of motoric impairment (modified H&Y stage) and disease duration at consensus clinical determination (odds ratio, 9.95; 95% CI, 2.69-36.87; $P = .001$).

Patients Having PD With GBA Mutations Have Higher Plasma Levels of Monocyte-Associated Inflammatory Mediators

In univariate analyses, 6 plasma analytes were associated with the presence of GBA mutations, with higher analyte levels among mutation carriers (Figure 2). These included IL-8 ($P = .008$), MCP1 ($P = .004$), stem cell factor ($P = .02$), pulmonary and activation–regulated chemokine (PARC) ($P = .005$), and macrophage inflammatory protein 1α (MIP1α) ($P = .003$) and MIP1β ($P = .003$). Notably, 5 of these 6 plasma proteins (IL-8, MCP1, MIP1α, PARC, and stem cell factor) were elevated even in GBA (mut/wt) PD cases. Furthermore, plasma analytes differing between patients having PD with vs without GBA mutations in this initial analysis were highly enriched for monocyte-associated inflammatory mediators. Specifically, of 40 analytes screened, only 8 are monocyte-associated inflammatory mediators, but 5 of 6 plasma proteins differentiating GBA-associated PD (IL-8, MCP1, MIP1α, MIP1β, and PARC) fell within this group ($P < .001$, Fisher exact test).

Markers identified as significant in screening were entered into a multivariate model adjusting for sex, assay batch, and age at plasma draw. Interleukin 8, MCP1, and MIP1α, again enriched for monocyte-associated inflammatory mediators ($P = .006$, Fisher exact test), remained elevated in carriers of GBA mutations (Figure 2B).

We next sought to determine whether, in the multivariate model, these differences between GBA mutation carriers and GBA (wt/wt) PD cases were driven by the 3 GBA (mut/mut) PD cases. Confining our analysis to GBA (mut/wt) (n = 17) vs GBA (wt/wt) (n = 87) PD cases only, we found that increases in IL-8 ($P = .004$) and MCP1 ($P = .01$) persisted in GBA heterozygote mutation carriers, while the apparent increase in MIP1α levels was primarily due to very high levels in the 3 GBA (mut/mut) PD cases (Figure 2B).

GBA Mutation Carriers Demonstrate Increased Plasma Levels of IL-8 in an Independent Cohort of Patients With PD

To test the robustness of the finding of elevated IL-8 and MCP1 plasma levels among GBA (mut/wt) PD cases, we evaluated an independent replication cohort (19 patients having PD with GBA mutations vs 41 patients having PD without GBA mutations) using ELISA as an alternative measurement method. Despite differences in clinical characteristics between the discovery and replication cohorts (eTable 3 in Supplement), the correlation between GBA mutation status and IL-8 levels persisted ($P = .03$), with the same directionality, in a multivariate model adjusting for sex and age at plasma draw. Removing the 2 GBA (mut/mut) individuals and comparing only 17 GBA (mut/wt) with 41 GBA (wt/wt) PD cases, the increase in IL-8 levels in GBA (mut/wt) individuals re-
Elevated IL-8 LEVELS Are Associated With Poorer Cognition

For the cohort as a whole, higher IL-8 levels predicted worse cognitive function in a multivariate model that included age and sex as covariates ($\beta = .04, P = .01$). The results of subgroup analyses suggested that this relationship between higher IL-8 levels and consensus clinical determination of MCI or dementia is more prominent in the subgroup of GBA-associated PD cases ($\beta = .04, P = .06$) than among the patients having PD without GBA mutations ($P = .76$).

There was no association between IL-8 levels and modified H&Y stage in the cohort-wide analysis. Similarly, no association was observed between IL-8 levels and modified H&Y stage in the GBA-associated subgroup analysis.

### Discussion

Three key findings arose from our assessment of differences between patients having PD with vs without GBA mutations. Patients having PD with GBA mutations had a younger age at onset and were more likely to have clinical evidence of MCI or dementia. Biochemically, elevated plasma levels of several monocyte-associated inflammatory mediators were found in patients having PD with GBA mutations compared with those without GBA mutations. Elevated plasma levels of IL-8 among patients having PD with GBA mutations were then confirmed in an independent replication cohort.

Our findings agree with previous evidence that patients having PD with mutations in the GBA gene have a younger age at PD onset. Patients having PD who are heterozygous for a GBA mutation have also been reported to have more subjective cognitive dysfunction. In early-onset PD (at <50 years), cognitive impairment among GBA mutation carriers has been substantiated by neuropsychological test results, and otherwise asymptomatic GBA mutation carriers may also have impaired performance on cognitive tests compared with noncarriers. Consistent with these data, we found that GBA mutation carriers were more likely to have MCI or dementia in a large cohort of patients having PD with typical age at PD onset.

In this study, we also report on biochemical differences between patients having PD with vs without GBA mutations. Although it has long been known that patients with Gaucher disease, carrying 2 mutant copies of the GBA gene (GBA [mut/mut]), show elevated levels of several blood-based markers, heterozygous GBA mutation carriers have not been previously evaluated in a systematic way. In our screening analysis, we found elevated levels of MCP1, MIP1a, IL-8, and PARC, all previously shown to be increased in GBA (mut/mut) individuals, in the GBA (mut/wt) carriers with PD in our cohort as well. For most of these proteins, differences between patients having PD with vs without GBA mutations did not survive adjustment for potential confounders or replication efforts, suggesting that they are false-positive signals or that our study was underpowered to detect true differences. However, the cytokine IL-8 emerged from our screening of 40 plasma proteins as robustly elevated in GBA-associated PD, replicating in an independent cohort of patients assay by a different method.
It is worth noting the preponderance of monocyte lineage–associated inflammatory mediators found to be elevated in our GBA mutation carriers. In Gaucher disease, a state of systemic and (in the case of the neuronopathic form) central nervous system inflammation has long been recognized, attributed to glucocerebrosidase deposition in monocyte-lineage cells. Therefore, it is not entirely surprising that individuals with one mutated GBA allele should demonstrate signs of inflammation as well, although this has not been previously demonstrated.

In addition, an emerging literature increasingly suggests connections among PD, glucocerebrosidase, and inflammation relevant to our present findings. Specifically, a bidirectional loop has been demonstrated between glucocerebrosidase and α-synuclein: accumulated glucocerebrosidase leads to accumulation of insoluble α-synuclein amyloid fibrils, which block intracellular trafficking and further reduce glucocerebrosidase activity in neuronal lysosomes. Moreover, in neonatal rat glial cells, exposure to α-synuclein can induce the release of multiple inflammatory mediators (including MCP1 and MIP1α), with a potentially greater response for PD mutation-associated forms of α-synuclein. Finally, the introduction of an inflammatory stimulator into the substantia nigra of mice...
induces a neuroinflammatory response that seems to be dependent on α-synuclein because mice null for α-synuclein do not mount this response. Therefore, it is hypothetically possible that glucocerebrosidase, α-synuclein, and monocyte lineage-associated inflammation participate in a pathogenic cascade. If so, the fact that heterozygous GBA mutations may be sufficient to induce some degree of inflammation (as suggested by the present data) is relevant to understanding the predisposition of GBA mutation carriers, including GBA (mut/ wt) individuals, to develop PD at an earlier age and with more risk for cognitive dysfunction. Indeed, our finding that higher IL-8 levels correlate with poorer cognitive performance in the full cohort and in the GBA-associated PD cases is consistent with this interpretation.

Several limitations to our study warrant mention. We used a case-control design to compare PD with GBA mutations vs PD without GBA mutations. Therefore, our sample may not be representative of the general PD population. However, both clinical characterization and plasma profile screening were conducted blinded to GBA mutation status, so it is unlikely that the differences found were related to bias in mutation carriers selected. In addition, in 231 individuals, screening was performed for only the 2 most common GBA mutations (N370S and L444P). Therefore, it is possible that in this second group several more patients with PD carried rarer GBA mutations. However, we guarded against the possibility that those individuals undetected by our screening method had dramatically different clinical characteristics or plasma profiles from the ones analyzed in this study by covering both a severe GBA mutation (L444P) and a less severe GBA mutation (N370S) with our approach. Of note, for our comparison GBA (wt/wt) group, we only included individuals in whom GBA mutations had been excluded by complete sequencing.

While the number of GBA mutation-associated PD cases in this study is large, our study may not have had adequate statistical power to detect all differences. For instance, in the replication cohort, the difference in IL-8 values between PD with vs without GBA mutations achieved statistical significance when all 19 GBA mutation carriers were considered but fell to nonsignificance when the 2 GBA (mut/mut) individuals were excluded. Subsequent analyses randomly omitting 2 cases demonstrated that statistical significance was lost 30% of the time, suggesting that our study may have been underpowered to detect a true difference. Therefore, replication of our results in other cohorts or among larger numbers of patients would be a valuable addition to the data presented herein. Furthermore, regarding MCP1 measurements, ELISA and multiplex immunoassay values for duplicate plasma samples were poorly correlated, despite internal consistency within each technical platform, suggesting that technical limitations may have resulted in our failure to replicate higher MCP1 levels in the second cohort.

Finally, although we used an unbiased screening approach with a commercially available multiplex immunoassay to identify biochemical markers differentiating patients having PD with vs without GBA mutations, our 40-protein panel is certainly not comprehensive, and there may be many other plasma proteins that differentiate these groups. However, we note that, even within the 40 proteins evaluated here, a significant enrichment for monocyte-lineage inflammatory mediators was observed and may be biologically meaningful.

In conclusion, in this study, we extend previous findings that patients having PD with GBA mutations have distinctive features compared with patients having PD without GBA mutations. Clinically, these include an earlier age at onset and worse cognitive function. In addition, we show for the first time to date that patients having PD with GBA mutations, including heterozygous GBA mutations, may also have biochemical differences detectable in plasma, namely, elevated levels of monocyte-associated inflammatory mediators. Together, our findings suggest that GBA mutations, even in a heterozygous state, may be sufficient to cause some systemic inflammation, which may in turn contribute to PD pathogenesis.

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Figure 3. Comparison of Interleukin 8 (IL-8) and Monocyte Chemotactic Protein 1 (MCP1) Levels in Those With vs Without GBA Mutations in the Replication Cohort

<table>
<thead>
<tr>
<th>Plasma Analyte</th>
<th>GBA (mut/wt) Only</th>
<th>GBA (mut/wt) and GBA (mut/mut)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β Coefficient</td>
<td>P Value</td>
<td>β Coefficient</td>
</tr>
<tr>
<td>IL-8</td>
<td>1.3</td>
<td>.10</td>
</tr>
<tr>
<td>MCP1</td>
<td>-3.7</td>
<td>.32</td>
</tr>
</tbody>
</table>

The observation that GBA mutation carriers have higher levels of IL-8 was replicated. In contrast, no significant difference in MCP1 levels was observed. Raw values are shown in A, and age-adjusted and sex-adjusted regression models are summarized in B. *P < .05.

GBA (mut/wt) and GBA (mut/mut)
for Parkinson’s Disease Research, Perelman School of Medicine at the University of Pennsylvania, Philadelphia (Horn, Colcher, Hurtig, Lee, Van Deelin, Trojanowski, Chen-Plotkin); Institute on Aging, Perelman School of Medicine at the University of Pennsylvania, Philadelphia (Lee, Van Deelin, Trojanowski, Chen-Plotkin); Avid Radiopharmaceuticals, Philadelphia, Pennsylvania (Siderowf); Veterans Affairs Puget Sound Health Care System, University of Washington School of Medicine, Seattle (Yearout, Leverenz, Zabetian); Department of Neurology, University of Washington School of Medicine, Seattle (Yearout, Leverenz, Zabetian); Department of Psychiatry and Behavioral Sciences, University of Washington School of Medicine (Lee).

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REFERENCES


