RESEARCH ARTICLE

Cerebrospinal Fluid β-Glucocerebrosidase Activity Is Reduced in Parkinson's Disease Patients

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ABSTRACT: Background: Reduced β-glucocerebrosidase activity was observed in postmortem brains of both *GBA1* mutation carrier and noncarrier Parkinson's disease patients, suggesting that lower β-glucocerebrosidase activity is a key feature in the pathogenesis of PD. The objectives of this study were to confirm whether there is reduced β-glucocerebrosidase activity in the CSF of *GBA1* mutation carrier and noncarrier PD patients and verify if other lysosomal enzymes show altered activity in the CSF.

Methods: CSF β-glucocerebrosidase, cathepsin D, and β-hexosaminidase activities were measured in 79 PD and 61 healthy controls from the BioFIND cohort. The whole *GBA1* gene was sequenced.

Results: Enzyme activities were normalized according to CSF protein content (specific activity). β-glucocerebrosidase specific activity was significantly decreased in PD versus controls (-28%, $P < 0.001$). *GBA1* mutations were found in 10 of 79 PD patients (12.7%) and 3 of 61 controls (4.9%). *GBA1* mutation carrier PD patients showed significantly lower β-glucocerebrosidase specific activity versus noncarriers. β-glucocerebrosidase specific activity was also decreased in noncarrier PD patients versus controls (-25%, $P < 0.001$). Cathepsin D specific activity was lower in PD versus controls (-21%, $P < 0.001$). β-Hexosaminidase showed a similar trend. β-Glucocerebrosidase specific activity fairly discriminated PD from controls (area under the curve, 0.72; sensitivity, 0.67; specificity, 0.77). A combination of β-glucocerebrosidase, cathepsin D, and β-hexosaminidase improved diagnostic accuracy (area under the curve, 0.77; sensitivity, 0.71; specificity, 0.85). Lower β-glucocerebrosidase and β-hexosaminidase specific activities were associated with worse cognitive performance.

Conclusions: CSF β-glucocerebrosidase activity is reduced in PD patients independent of their *GBA1* mutation carrier status. Cathepsin D and β-hexosaminidase were also decreased. The possible link between altered CSF lysosomal enzyme activities and cognitive decline deserves further investigation. © 2017 International Parkinson and Movement Disorder Society

Key Words: Parkinson's disease; CSF biomarkers; lysosomal enzyme activity; β-glucocerebrosidase; *GBA1* gene

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Relevant conflict of interest/financial disclosures: Nothing to report.

Funding agencies: This study was funded by the Michael J. Fox Foundation for Parkinson's Research (project title: Lysosomal enzymes activity and *GBA1* genotyping in CSF of Parkinson's disease patients: a confirmatory study; grant 10204, 2015-2016).

Received: 11 May 2017; **Revised:** 20 June 2017; **Accepted:** 26 June 2017

Published online 26 August 2017 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.27136

Lysosomes play a pivotal role in the catabolic pathway of α-synuclein (α-syn). As a consequence, the aggregation of misfolded α-syn, which represents the main pathogenic factor for the development of Parkinson's disease (PD),[1] is favored by the impairment of lysosomal functionality.

The molecular mechanisms by which the altered lysosomal activity influences α-syn clearance are only partially known. A link between PD pathogenesis and lysosomal dysfunction has been proven by studies of the lysosomal enzyme β-glucocerebrosidase (GCase; EC = 3.2.1.45), a hydrolase involved in glyco

sphyngolipid catabolism. A large multicenter study showed that mutations in the GCase-encoding gene (*GBA1*) represent the most common genetic risk factor for PD. Approximately 7% of PD patients are *GBA1* mutation carriers (*GBA1*+), and *GBA1* mutation carriers are 5-fold more likely to develop PD compared with noncarriers (*GBA1*-).[2] Of interest, reduced GCase activity has been repeatedly documented in both *GBA1*+ and *GBA1*- postmortem PD brains,[3,4] suggesting that lower GCase activity is a key feature in the pathogenesis of PD. Accumulation of aggregated α-syn in cellular and animal models of GCase deficiency and in patients affected by Gaucher disease (GD) further supports this evidence.[3,5,6]

In the substantia nigra of PD brains, reduced levels of the lysosomal protease cathepsin D (CatD; EC = 3.4.23.5) were also found, particularly in neurons containing α-syn inclusions.[1] Aggregated α-syn was observed in different models of CatD deficiency.[7,8] In addition, β-hexosaminidase (β-Hex, EC = 3.2.1.52)-deficient mice have brain accumulation of α-syn,[9] suggesting a global deregulation of lysosomal homeostasis in PD.

The clear link between lysosomal dysfunction and PD pathogenesis, as well as the association between *GBA1* mutations and PD occurrence, led to investigation of the activities of lysosomal enzymes in the cerebrospinal fluid (CSF) of PD patients.[10,11] CSF GCase activity was reduced significantly in PD patients compared with neurological controls, and the combination of CSF GCase activity and oligomeric/total α-syn ratio satisfactorily discriminated PD patients from neurological controls (sensitivity, 0.82; specificity, 0.71).[11]

Here we investigated the activity of GCase, CatD, and β-Hex in the CSF of PD patients and healthy controls selected from the BioFIND (Fox Investigation for New Discovery of Biomarkers in Parkinson's Disease) cohort,[12] which includes patients with fully symptomatic forms of PD and healthy controls. The aims of this study were: (1) to confirm that CSF GCase activity is reduced both in *GBA1*+ and *GBA1*- PD patients, and (2) to assess the CSF activity of other lysosomal enzymes potentially involved in α-synhomeostasis as possible PD biomarkers.

Materials and Methods

Subjects

BioFIND is a cross-sectional, multicenter biomarker study in which moderate to advanced PD patients and healthy controls aged between 55 and 93 years are enrolled. Inclusion criteria are aimed at maximizing diagnostic specificity by selecting participants with clinically typical PD symptoms, and standardized protocols are used to minimize variability across sites in terms of clinical data and biospecimen collection. The study established a repository of clinical data, blood, DNA, RNA, CSF, saliva, and urine samples. Clinical data and biospecimens are available through the Michael J. Fox Foundation for Parkinson's Research (www.michaeljfox.org/biofind).

Enrolled PD patients met the United Kingdom PD Society Brain Bank clinical diagnostic criteria and showed all 3 classical motor signs of parkinsonism (bradykinesia, rigidity, and resting tremor). In these patients, disease duration ranged from 5 to 18 years. The cohort included subjects at all Hoehn and Yahr (H&Y) stages, independently of cognitive status as assessed by the Montreal Cognitive Assessment (MoCA) score. The control group was composed of healthy subjects without cognitive deficits (MoCA score $\geq$ 26).

Genomic DNA and CSF of 79 PD patients and 61 controls were requested and analyzed. To avoid significant effects on enzyme activities because of the length of storage, BioFIND samples were selected according to their length of storage (mean, 1.3 years; interquartile range, 0.9-1.7 years).

Lysosomal Enzyme Activity

The activities of the lysosomal enzymes GCase, β-Hex, and CatD were measured using fluorogenic substrates, according to previously published procedures.[13] All analyses were performed in a blinded fashion. For β-Hex activity, 10 μL of CSF was incubated with 40 μL of substrate solution for 10 minutes at 37°C. GCase activity was measured by incubating 20 μL of CSF with 40 μL of substrate solution in the presence of 0.2% taurodeoxicolic acid (TDC) for 3 hours. The reactions were stopped by adding ice-cold 0.2 M glycine-NaOH buffer (pH 10.4) to a final volume of 0.3 mL. The fluorescence of the liberated fluorophore (4-methylumbelliferone) was measured on a BMG Labtech FLUOstar OPTIMA fluorometer (excitation wavelength, 360 nm; emission wavelength, 446 nm).

CatD activity was determined using Mca-Gly-Lys-Pro-Ile-Leu-Phe-Phe-Arg-Leu-Lys(Dnp)-D-Arg-NH_2. CSF samples were incubated with the substrate for 1 hour at 40°C. The reactions were stopped by adding 5% (w/v) trichloroacetic acid to a final volume of 3 mL. The fluorescence was measured with a Jasco FP-750 spectrofluorometer (excitation wavelength, 328 nm; emission wavelength, 393 nm).

The protein concentration was determined using the Bradford method.

All measurements were performed in triplicate. For all assays the acceptance specification for within-run coefficient of variation was fixed at less than 10%.

DNA Sequencing

The genomic sequence of the *GBA1* gene (GenBank J03059.1) was analyzed by massive parallel paired-end sequencing using a MiSeq Illumina platform.

For each sample, the whole genomic *GBA1* sequence was PCR-amplified in 2 overlapped fragments of 2870 and 4492 bp using primers designed to selectively amplify the gene. Libraries were generated by processing the purified amplification products using a Nextera XT DNA sample preparation kit (Illumina) according to the manufacturer's recommendation. Equal volumes of normalized libraries (n = 50 subjects/run) were sequenced on an Illumina MiSeq using an Illumina MiSeq Reagent kit v2 (MS-102-2002) 300 cycles.

Paired-end reads were generated by sequencing forward and reverse strands of each target DNA fragment and mapping them to the reference human genome (hg19, Chr 1 155211205-155204618; amplicon 1, 155211205-155208234; amplicon 2, 155209110-155204618). MiSeq Control Software v2.3.0.3 was used to monitor the runs and quality control, whereas the secondary analysis including paired-end sequence alignment, duplicate removal, single-nucleotide variant (SNV) calling, and indel detection was performed using MiSeq reporter v2.4.60. Only samples with a sequencing quality control score $\geq$ 30 and with a minimal read depth of 200× were considered for variant analysis.

Annotation of SNV was performed with wANNOVAR (http://wannovar.usc.edu/). All exonic variants were confirmed by Sanger sequencing.

In addition, the possible presence of the RecΔ*55* allele (not detectable by next-generation sequencing) was ruled out by Sanger sequencing of exon 9.

Statistical Analysis

Statistical analyses were performed using R software version 3.1. Biomarker data were described by means and standard deviations. Normality of the distribution was checked by the Shapiro-Wilk test. Because of skewing in the distribution, the Mann-Whitney *U* test was initially used to compare biomarkers between the 2 diagnostic groups. Correlations were calculated using the Spearman rho (r). Diagnostic accuracy was later assessed by means of receiver operating characteristic (ROC) analysis. Cutoff values were calculated using sensitivity and specificity values that maximized the Youden index. A multivariate logistic regression approach was used to assess the diagnostic value of combinations of biomarkers. A backward elimination method was used for model selection by progressively eliminating predictors with the largest individual *P* value, one at a time, at each step in the process until only significant predictors remained. From these models, by using fitted probabilities we derived ROC curves, estimates of the area under the curves (AUC), sensitivity, and specificity. The De Long test was used to test for significant differences in AUC. A $P < 0.05$ was considered significant in all analyses.

TABLE 1. Demographics and clinical features in diagnostic groups

Variable	Controls[a]	PD[a]	*P*
No (M/F)	61 (25 of 36)	79 (50 of 29)	0.014
Age, years	65.3 ± 7.7	68.0 ± 6.5	0.036
Disease duration, years	—	7.7 ± 3.3	—
H&Y score	—	2.0 ± 0.6	—
MDS-UPDRS Part I	—	8.4 ± 4.0	—
MDS-UPDRS Part II	—	12.1 ± 6.7	—
MDS-UPDRS Part III		29.6 ± 14.3	
MDS-UPDRS total (I-III)	—	50.0 ± 20.5	—
MoCA	28.0 ± 1.3	26.9 ± 2.5	< 0.001
RBDSQ (questionnaire score ≥ 5)	7 (11.5%)	48 (60.8%)	< 0.001
Modified Schwab England ADL	—	85.1 ± 10.0	—
TD subtype	—	24 (30.4%)	—
PIGD subtype	—	46 (58.2%)	—
Intermediate subtypes	—	9 (11.4%)	—

Data are given as mean ± standard deviation or count (%).
[a]BioFIND cohort.[12]
PD, Parkinson's disease; H&Y, Hoehn and Yahr staging; MDS-UPDRS, Movement Disorders Society—Unified Parkinson's Disease Rating Scale Motor score; MoCA, Montreal Cognitive Assessment; RBDSQ, REM Sleep Behavior Disorder Screening Questionnaire; ADL, activities of daily living; TD, tremor dominant; PIGD, postural instability and gait disorders.

Results

Demographic and clinical data of the selected samples are listed in Table 1. These data reflect the features of the full cohort as described in Kang et al.[12]

CSF Lysosomal Enzyme Activities in PD Patients and Healthy Controls

In Supplementary Table 1 and Figure 1 CSF protein content, lysosomal enzyme activity, α-syn, and classical Alzheimer's disease (AD) biomarker level are reported. CSF protein concentration was significantly higher in the PD group than in the controls (+40%, $P < 0.001$). Therefore, to have comparable values, enzymatic activities were normalized according to the CSF total protein content (specific activity).

Consistent with previous studies,[10,11] CSF GCase activity was significantly decreased in the PD group compared with controls (-28%, $P < 0.001$). A significant reduction in CatD activity was also observed in PD patients (-21%, $P < 0.001$). β-Hex activity was moderately reduced (-9%, $P = 0.174$).

CSF GCase (-23%, $P < 0.001$), CatD (-20%, $P = 0.001$), and β-Hex activities (-23%, $P < 0.001$) were significantly reduced in male subjects, independent of Parkinson's disease diagnosis (Supplementary Table 2 and Supplementary Fig. 1).

Effect of Storage Time and Blood Contamination on Lysosomal Enzyme Activities

We evaluated the effects of longtime storage on enzyme stability. Although there was no correlation

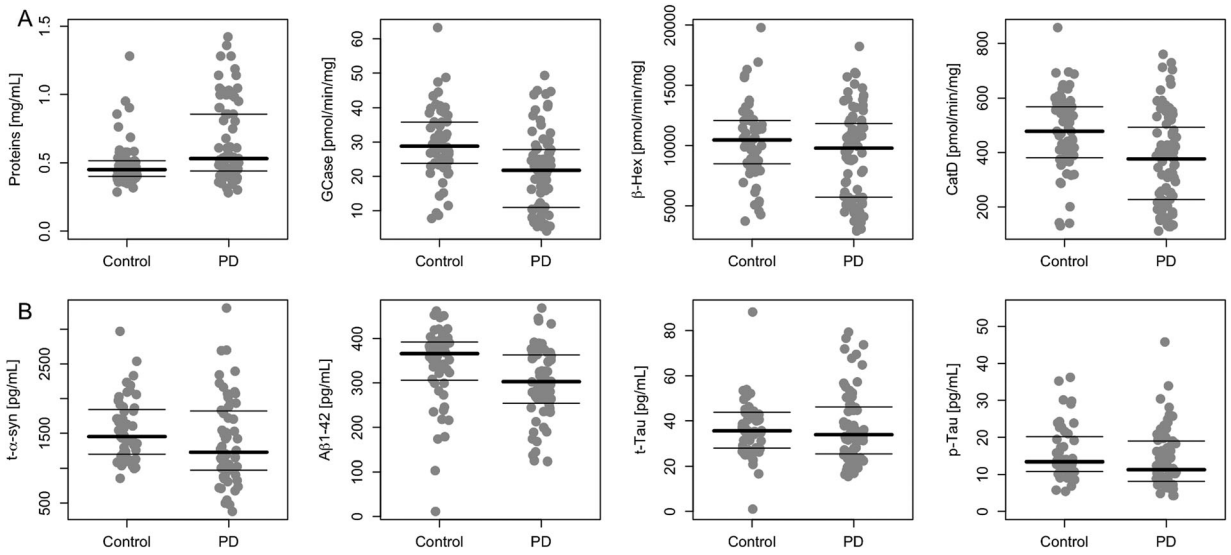

FIG. 1. Protein concentration, lysosomal enzyme activity, core AD biomarkers, and α-syn in CSF of PD patients and healthy controls. Protein concentration, GCase, β-Hex, and CatD activity measured in this study in the CSF of PD patients and healthy controls (A), and the levels of CSF Aβ1-42, t-Tau, p-Tau, and α-syn retrieved in the BioFIND database (B) are reported.

between storage length and β-Hex or CatD activity, GCase activity decreased to some extent (r = -0.17, P = 0.045). Nevertheless, after dividing storage time into quartiles, a significant decrease in GCase activity was observed only after 1.6 years (-23%, P = 0.039).

Based on the data contained in the BioFIND database, we also checked if blood contamination of CSF samples could have affected lysosomal enzyme activity. In the BioFIND database, blood contamination was defined in terms of hemoglobin (Hb) concentration. CSF samples were stratified into 3 Hb cutoffs: samples with none or small blood contamination (0-30 ng/mL Hb), samples with blood contamination (30-200 ng/mL Hb), and samples with high blood contamination (>200 ng/mL Hb). No difference was observed between PD and controls. Furthermore, in accordance with our previous findings,[13] CSF lysosomal enzyme activity remained unchanged across different levels of blood contamination (data not shown), confirming that blood contamination does not influence CSF lysosomal enzyme activity.

CSF α-Syn and AD Core Biomarkers in Diagnostic Groups

Values of CSF α-syn, t-Tau, p-Tau, and Aβ1-42, were retrieved from the BioFIND database.

CSF α-syn and Aβ1-42 were significantly decreased in the PD group compared with controls (-11%, P = 0.033, and -12%, P < 0.001, respectively; Fig. 1B).

Diagnostic Performance of CSF Parameters

The accuracy of CSF lysosomal enzymes in discriminating PD from controls was assessed by ROC analysis (Fig. 2). Diagnostic accuracy of GCase activity (AUC, 0.72; 95% CI, 0.63-0.80; sensitivity, 0.67; specificity, 0.77) and CatD activity (AUC, 0.68; 95% CI, 0.59-0.77; sensitivity, 0.61; specificity, 0.77) was suboptimal for discriminating PD from controls when these 2 enzymes were considered as single parameters.

ROC analysis of Aβ1-42 alone did not give better results than lysosomal enzyme activities (sensitivity, 0.63; specificity, 0.76). Also, α-syn as a single marker performed poorly in distinguishing PD patients from control subjects (sensitivity, 0.81; specificity, 0.42).

To assess the diagnostic value of combining the CSF biomarkers, we used fitted values of multivariate regression (Supplementary Table 3) as scores for

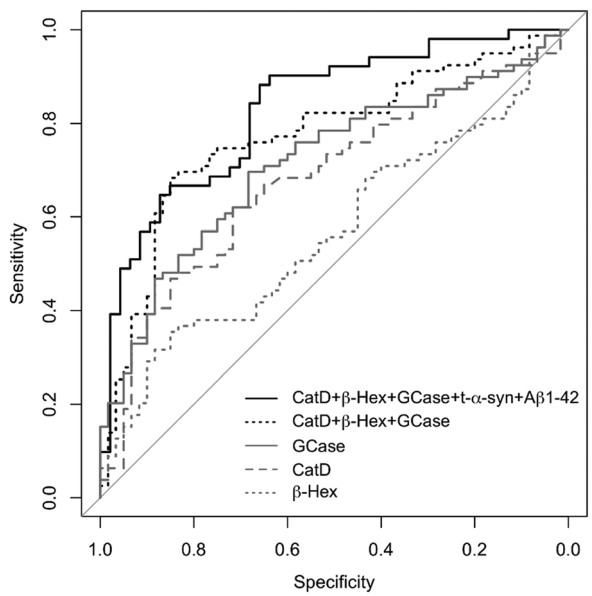

FIG. 2. Diagnostic value of CSF biomarkers, alone or in combination, in differentiating PD versus controls (ROC curves).

TABLE 2. Sequence variants identified within the coding region of *GBA1* gene

cDNA	Protein effect	Allele name	Reference	Controls	PD
c.274A>G	p.M92V	M53V	This study	1	0
c.84dupG	p.L29Afs*18	84GG	Beutler et al, 1991[43]	0	1
c.[703T>C;1483G>C]*	p.[S235P; A495P]	[S196P; A456P]	This study	0	1
c.882T>G	p.H294Q	H255Q	Stone et al, 2000[44]	0	1
c.887G>A	p.R296Q	R257Q	Beutler et al, 1994[45]	1	0
c.1093G>A	p.E365K	E326K	Eyal et al, 1991[46]	0	4
c.1223C>T	p.T408M	T369M	Beutler et al, 1996[47]	1	1
c.1226A>C	p.N409S	N370S	Tsuji et al, 1988[48]	0	1
c.1279G>A	p.E427K	E388K	Lesage et al, 2011[49]	0	1
			Total	3	10

Mutations are described considering nucleotide +1 the A of the first ATG translation initiation codon (http://www.hgvs.org/mutnomen/). Nucleotide numbers are derived from the *GBA1* cDNA (GenBank reference sequence NM_000157.1). Traditional protein mutation nomenclature does not start with the first ATG codon because of a processed leader sequence (reference sequence AAC63056.1). These mutations are presented without "p." in the mutation name.

classifying PD and control subjects in a ROC analysis (Supplementary Table 4, Fig. 2) and the De Long test to test for significant differences in AUC.

The combination of GCase and β-Hex activities showed a trend toward better diagnostic performance (AUC, 0.75; 95% CI, 0.67-0.83; sensitivity, 0.63; specificity, 0.83) compared with GCase or CatD alone. The panel of all the measured lysosomal enzyme activities led to even better diagnostic performance (AUC, 0.77; 95% CI, 0.69-0.85; sensitivity, 0.71; specificity, 0.85; Fig. 2). The inclusion of α-syn and Aβ1-42 in the model further increased the diagnostic accuracy (AUC, 0.83; 95% CI, 0.75-0.92; sensitivity, 0.84; specificity, 0.75; Fig. 2).

According to the De Long test, both the combination of lysosomal enzyme activities ($P = 0.042$) and the full panel with α-syn and Aβ1-42 ($P = 0.048$) were significantly more accurate with respect to GCase activity alone.

Correlation Between CSF Parameters

Protein concentration was positively associated with age ($r = 0.24$, $P = 0.004$).

A significant inverse correlation with age was documented in the whole sample for CSF GCase activity ($r = -0.31$, $P < 0.001$) and CatD activity ($r = -0.28$, $P = 0.001$).

In the PD group GCase activity correlated with β-Hex activity ($r = 0.79$, $P < 0.001$) and CatD activity ($r = 0.80$, $P < 0.001$). β-Hex activity correlated with CatD activity ($r = 0.84$, $P < 0.001$).

α-Syn correlated with Aβ1-42 ($r = 0.36$, $P = 0.009$), t-Tau ($r = 0.90$, $P < 0.001$), and p-Tau ($r = 0.49$, $P < 0.001$). T-Tau correlated with p-Tau ($r = 1.00$, $P < 0.001$) and Aβ1-42 ($r = 0.40$, $P < 0.001$).

Correlation With Clinical Parameters and Pharmacological Treatments

In the PD group the reduction in α-syn, GCase activity, and β-Hex activity was significantly associated with worse cognitive performance as measured by MoCA ($r = 0.26$, $P = 0.047$; $r = 0.22$, $P = 0.048$; and $r = 0.32$, $P = 0.004$, respectively).

When the PD group was stratified by H&Y score (H&Y < 2 and H&Y ≥ 2), a significant decrease in GCase activity (-22%, $P = 0.003$) and CatD activity (-15%, $P = 0.012$) was observed in the more advanced stages of disease.

No differences in lysosomal enzyme activities, CSF AD core biomarkers, or α-syn between PD subtypes (TD, PIGD, intermediate subtypes) were found.

No effect of antiparkinsonian drugs (levodopa, dopaminergic agents, and amantadine) was documented on the biomarker level, although a trend toward a reduction in GCase activity was observed in patients treated with levodopa (-27%, $P = 0.052$).

Relationship of Lysosomal Enzyme Activity With *GBA1* Genotype

The sequence of the *GBA1* gene was analyzed in the whole sample. Three controls (4.9%) and 10 PD patients (12.7%) had sequence variations within the coding region of *GBA1*; all of them led to amino acid changes (Table 2).

Variants 84GG, H255Q, R257Q, E326K, T369M, N370S, and E388K have been reported previously (www.hgmd.org), whereas the M53V variant and the complex allele (S196P; A456P) are novel.

GCase activity in the CSF was significantly lower in *GBA1+* subjects (-27%, $P = 0.042$) compared with *GBA1-* (Fig. 3). However, a significant reduction in GCase activity in PD patients was found even after excluding *GBA1+* subjects (-25%, $P < 0.001$), suggesting that the reduction in GCase activity in the CSF is independent of the presence of *GBA1* mutations (Fig. 3A). Of interest, in *GBA1-* PD patients, reduced GCase activity was still associated with worse cognitive performance ($r = 0.21$, $P = 0.021$). Furthermore, the significant reduction of GCase (-45%, $P = 0.004$)

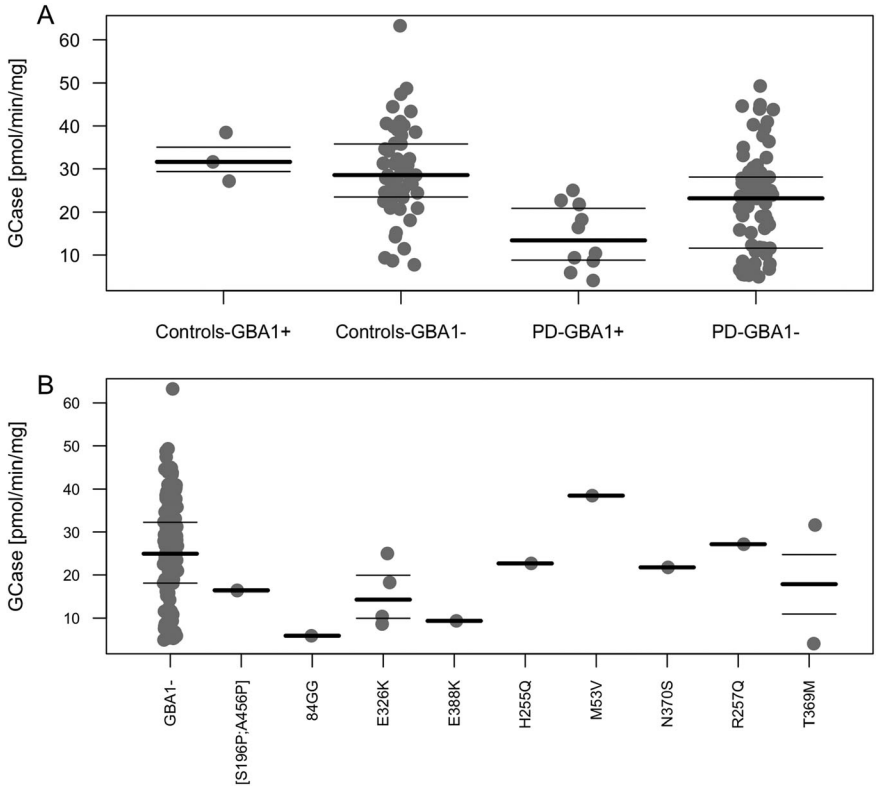

FIG. 3. (A) GCase specific activity in *GBA1* mutation carrier (controls-*GBA1*+) and noncarrier (controls-*GBA1*-) controls and in PD patients *GBA1*+ (PD-*GBA1*+) and *GBA1*- (PD-*GBA1*-). CSF GCase activity was lower in *GBA1*+ versus *GBA1*- (-27%, $P = 0.042$). In PD patients, GCase activity was significantly decreased in *GBA1*+ versus *GBA1*-. A significant reduction of GCase activity in PD patients was observed even after exclusion of *GBA1*+ subjects (-25%, $P < 0.001$). (B) GCase specific activity for single variant. For each variant the corresponding GCase activity was reported. Medians are presented with a line, whereas all the data points are reported in the strip chart.

and CatD activity (-40%, $P = 0.002$)in advanced stages of PD were observed even in *GBA1*- PD patients. Eight variants not previously reported or reported with a low frequency (minor allele frequency < 0.0078) have been found within the noncoding region of the *GBA1* gene in 9 PD patients and 5 controls. Most are deep intronic variants with unlikely clinical significance. The only exception is probably represented by the c.-203A>G variant, located within the 5′UTR. This variant has been described as a polymorphism resulting in reduced *GBA1* promoter activity. Although this change does not cause enough of a reduction in GCase activity to lead to GD, it may be considered a modifier of the disease phenotype.[14] The c.-203A>G variant was found in 2 PD patients and 1 healthy control; however, its possible role in PD remains unclear. Indeed, because of the small number of *GBA1*+ individuals identified in the study and their genetic heterogeneity, it was not possible to assess the impact of the single *GBA1* mutations on disease development and progression.

The impact of *GBA1* mutations on CSF CatD and β-Hex activities as well as on the levels of CSF α-syn, Aβ1-42, t-Tau, and p-Tau was also evaluated. No difference was found between *GBA1*+ and *GBA1*- PD patients (data not shown).

Discussion

These results showed a significant decrease in GCase activity in the CSF of PD patients in comparison with healthy controls, independent of their *GBA1* mutation carrier status. These findings confirm and reinforce our previous results.[10,11] Reduced GCase activity was also found in the CSF of patients affected by dementia with Lewy bodies, but not in patients affected by Alzheimer's disease or frontotemporal dementia,[15] indicating selective involvement of these enzymes in α-syn aggregation disorders.

The impact of *GBA1* mutations on CSF GCase activity was evaluated in the BioFIND cohort. *GBA1* mutations are the most important predisposing risk factor for PD, and *GBA1* mutation carrier status is the most common genetic risk factor for α-syn aggregation leading to α-syn aggregation disorders.[2] However, no detailed studies were performed to evaluate how the presence of *GBA1* mutations may affect CSF GCase activity and influence its use as a possible biomarker for PD. In our cohort, 12.7% of PD patients and 4.9% of healthy controls were *GBA1* mutation carriers, confirming the extensively reported association between *GBA1* mutations and PD. Seven variants were identified in our PD patients. Among them,

variants 84GG, R257Q, and N370S have been consistently shown to be pathogenic in patients affected by GD, whereas the pathogenic nature of E326K, T369M, and E388K variants is still controversial. However, all of them display reduced enzyme activity when expressed in vitro.[16-18] Finally, the complex allele (S196P; A456P) is likely to be pathogenic, because the presence of the S196P mutation alone has been described in patients affected by GD.[19] Furthermore, although the A456P variant has been identified as part of recombinant alleles, it has been shown to severely affect protein stability and activity when expressed as a single mutation in vitro.[20] It is worth noting that 8 of 10 PD patients are carriers of *GBA1* mutations that retain quite high residual activity when expressed in vitro. This suggests that development of PD does not correlate with residual activity of the mutated GCase.[16-18,21-23] Indeed, the E326K mutant, which retains 40% of wild-type activity, seems to be among the most prevalent alleles in PD patients and was the most frequent mutation in our cohort.[24,25]

Our results revealed that GCase activity was significantly reduced in the CSF of *GBA1* mutation carriers compared with noncarriers. Nevertheless, the overall decrease in GCase activity in PD patients was independent of *GBA1* mutation status. These data are in agreement with the decreased GCase activity consistently found in studies on postmortem brain tissue[3,4] from sporadic PD patients. Interestingly, in a work carried out in sporadic PD patients, a significant reduction in *GBA1* expression was documented in substantia nigra.[4] These data suggest that other mechanisms, beside *GBA1* mutations, may induce reduction in GCase activity in the central nervous system and are likely to be involved in the development and progression of PD. It might be possible that decreased GCase activity in the CSF reflects the reduction of *GBA1* expression taking place in the most affected regions of the PD brain. Another option is that the decrease in GCase activity is the consequence of altered saposin C (Sap C) functionality. Sap C is a cofactor that plays a protective role toward GCase.[26,27] Sap C deficit impairs GCase activity and causes a rare form of GD. It is known that GCase activity is inhibited by the interaction of membrane-bound α-syn,[28] and this inhibitory effect was abolished in the presence of Sap C.[26] It is worth noting that Sap C is generated through cathepsin D-dependent proteolytic cleavage of its precursor prosaposin.[29] Thus, it might be possible that reduced CatD activity would impair Sap C maturation and indirectly influence GCase activity.

Of interest, GCase, CatD, and β-Hex activities were significantly reduced in male subjects, independent of PD diagnosis. The more pronounced decrease in lysosomal enzyme activities observed in this group might be associated with the higher risk of developing PD[30] observed in male subjects. However, more in-depth studies are necessary to confirm this hypothesis.

In PD patients a significant association between reduced CSF GCase activity and worse cognitive performance was found. This association occurred even after exclusion from the analysis of *GBA1* mutation carriers. Recently, particular attention has been paid to the effects of *GBA1* mutations on cognitive decline. PD patients who carry *GBA1* mutations have more rapid cognitive decline compared with noncarriers.[31,32] Furthermore, genotype-phenotype analysis showed that mutations that cause the more severe neuropathic GD phenotype highly increase the risk of dementia compared with mutations that cause non-neuropathic GD.[33,34] In PD patients who carry neuropathic *GBA1* mutations, longitudinal cognitive decline was faster and more aggressive compared with PD patients who carried the N370S mutation or the *GBA1* risk variants,[34] indicating that GCase activity level influences cognitive performance and the rate of cognitive decline in PD patients.

Similarly to GCase, CatD activity was also significantly decreased in the CSF of PD patients versus healthy controls. CatD is a major lysosomal aspartyl protease[7] that is responsible for α-syn degradation and is capable of reducing α-syn toxicity in vitro.[35] Complete lack of CatD activity causes a congenital form of neuronal ceroidlipofuscinosis (NCL10).[36] However, it has been found that mutations that cause only a reduction in CatD activity lead to a mild form of NCL10 characterized by loss of motor function, brain atrophy, and progressive cognitive decline.[37] Nevertheless, little is known about the effects of CatD deficiency on PD onset and progression. Several studies described the increase in aggregated α-syn in CatD-deficient models.[1,7,8,38] In brain tissue of the α-syn-overexpressing mouse model of PD, the levels of the active mature form of CatD were significantly reduced,[39] confirming that CatD is particularly relevant for the development of PD. It has also been found that inhibition of CatD activity led to a buildup of potentially amyloidogenic protein fragments in brain slice cultures.[40] Thus, it might be possible that the decrease in CatD activity would be related, at least in part, to the alteration of CSF α-syn and Aβ1-42 levels observed in PD patients.

β-Hex activity was only moderately reduced in the CSF of PD patients versus healthy controls. Whether and how this enzyme is involved in PD pathogenesis is not clear. In β-Hex-deficient mice and in patients affected by either Sandhoff or Tay-Sachs disease, α-syn accumulation was found in different brain regions. These data led to the hypothesis that reduced β-Hex activity causes alteration in the cellular glycolipid pattern and contributes to α-syn accumulation.[9,41]

In PD patients CSF protein concentration was significantly higher compared with in healthy controls. The increase in the protein content of CSF in PD patients was already observed in previous studies.[42] It might be the result of progressive impairment of blood-brain barrier integrity during the course of disease.

Our results showed a high correlation among GCase, β-Hex, and CatD activities. We even found that the combination of the lysosomal enzyme activities resulted in a good diagnostic performance, which improved with the inclusion of Aβ1-42 and α-syn in the model. These data confirm the important role played by the lysosomal system in PD pathogenesis and demonstrate the usefulness of a CSF biomarker panel for PD diagnosis.

CSF GCase and CatD activities were significantly lower in the more advanced stages of PD (H&Y $\geq$ 2). If confirmed in a longitudinal setting, these findings would link the reduction in CSF GCase and CatD activities to clinical progression of PD symptoms.

In conclusion, this study represents confirmation, in a large cohort composed of patients and healthy controls enrolled in 8 centers, that CSF GCase activity is consistently decreased in PD patients independent of their *GBA1* mutation carrier status, further supporting the role of this enzyme as biomarker of PD. Also, other lysosomal enzymes involved in α-syn homeostasis, namely, CatD, were found to be altered in the CSF of PD patients and deserve further investigation. Finally, the association found between the decreased CSF activity of these enzymes and worse cognitive performance/disease progression highlights the need to carry out measurements in a longitudinal cohort composed of early, de novo PD patients. ■

Acknowledgments: Data and biospecimens used in the preparation of this article were obtained from the Fox Investigation for New Discovery of Biomarkers (BioFIND) database (http://biofind.loni.usc.edu/). For up-to-date information on the study, visit www.michaeljfox.org/biofind. BioFIND is sponsored by the Michael J. Fox Foundation for Parkinson's Research (MJFF) with support from the National Institute for Neurological Disorders and Stroke (NINDS).

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Supporting Data

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