Survival and Dementia in *GBA*-Associated Parkinson's Disease: The Mutation Matters

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Objective: The objective of this work was to investigate survival, dementia, and genotype-phenotype correlations in patients with Parkinson's disease (PD) with and without mutations on the glucocerebrosidase gene (*GBA*).
Methods: We included 2,764 unrelated consecutive PD patients: 123 *GBA* carriers (67 mild-p.N370S and 56 severe mainly p.L444P) and 2,641 noncarriers. Brain perfusion and dopamine transporter imaging was analyzed, including dementia with Lewy Bodies (DLB) as an additional control group.
Results: Multivariable analysis adjusted by sex, age at onset, and disease duration attributed to *GBA* carriers a greater risk for dementia (hazard ratio [HR] = 3.16; $p < 0.001$) and death (HR = 1.85; $p = 0.002$) than noncarriers. When dementia was introduced in the model as a time-dependent covariate, the mortality risk remained greater in carriers (HR = 1.65; $p = 0.016$), suggesting that other clinical features are likely to contribute to reduced survival. At last examination, *GBA* carriers had worse motor symptoms, particularly nondopaminergic features. Carriers of severe mutations had greater risk for dementia compared to mild mutations ($p < 0.001$), but similar mortality risk. Consistent with clinical data, *GBA* carriers showed reduced posterior parietal and occipital cortical synaptic activity and nigrostriatal function than PD noncarriers. Neuroimaging features of carriers of mild mutations overlapped with PD noncarriers, whereas carriers of severe mutations were closer to DLB.
Interpretation: Survival is reduced in *GBA* carriers compared to noncarriers; this seems to be partially independent from the increased risk for early dementia. The risk for dementia is strongly modulated by type of mutation. In the clinical continuum between PD and DLB, patients with *GBA* mutations seem to localize midway, with carriers of severe mutations closer to DLB than to idiopathic PD.

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Parkinson's disease (PD) is a neurodegenerative and progressively disabling disease characterized by bradykinesia, tremor, and muscular rigidity.[1] One of the genes most commonly involved in PD is the glucocerebrosidase gene (*GBA*), encoding for the protein glucocerebrosidase (GCase), which is a lysosomal enzyme responsible for the conversion of glucocerebroside into glucose and ceramide (OMIM *606463). Heterozygous *GBA* mutations are the most common genetic factor associated with PD in all populations.[2,3] PD patients with *GBA* mutations have distinctive features compared to idiopathic PD, including earlier age at onset, greater cognitive decline, and a more-rapid progression of motor impairment.[4] In line with the more prominent progression of motor and nonmotor symptoms in *GBA* carriers, a reduced survival is likely to take place. Recently, an increased mortality risk has been suggested in *GBA* carriers, but this finding needs to be confirmed in larger populations.[5]

The two more common pathogenic *GBA* mutations in the PD population are the p.L444P and the p.N370S.[2,6] *GBA* mutations may induce a differential reduction of GCase activity, causing Gaucher disease (GD) when present on both alleles (http://www.ncbi.nlm.nih.gov/books/NBK1269/). GD is a lysosomal storage disorder with three clinical types: non-neuropathic (type I); acute neuropathic (type II); and chronic neuropathic (type III). Accordingly, *GBA* mutations have been categorized as mild or severe: mild mutations are those that cause GD type I, and severe mutations are those causing GD types II and III.[7] Concerning genotype-phenotype correlations, mutations causing a more-severe GD phenotype (eg, p.L444P) seem to be associated with an increased PD risk, earlier age at onset, and greater cognitive dysfunction compared to less severe mutations (eg, p.N370S).[8–10] However, the small sample size for each *GBA* subgroup in previous studies limited the generalization of such genotype-phenotype correlations so far. For similar reasons, no data are currently available on survival according to the type of *GBA* mutations.[5]

We previously investigated the frequency of major *GBA* mutations in a large cohort of 2,766 unrelated consecutive patients with clinical diagnosis of primary degenerative parkinsonism and 1,111 controls and demonstrated a 3-fold higher risk of developing dementia with Lewy bodies (DLB) than PD (relative risk, 21.9 vs 7.2),[6] consistent with a recent meta-analysis.[10] These data suggest that *GBA* dysfunction causes an aggressive phenotype, associated with early cognitive decline. In the present study, we investigated dementia and survival in a large cohort of Caucasian PD patients comparing *GBA* carriers and noncarriers using a retrospective longitudinal study design. In consideration of the very limited data on genotype-phenotype correlations, which are focused on the risk for PD and the age at disease onset,[9,10] we compared the effects of the type of *GBA* mutations on a wide spectrum of motor and nonmotor clinical features using a cross-sectional analysis. Finally, we investigated brain perfusion and nigrostriatal function in nested subgroups of *GBA* carriers compared to both PD noncarriers and DLB noncarriers.

Patients and Methods

Patient Population

We included 2,843 unrelated consecutive patients with PD, who contributed to the Parkinson Institute Biobank (www.parkinsonbiobank.com) from 2002 to 2013 regardless of family history, age at onset, or other clinical features. The clinical diagnosis of PD was established according to the UK Brain Bank criteria.[11,12] Disease duration at first examination was 4.9 ± 4.7 years, and average follow-up duration was 7.2 ± 5.1 years. Except for 25 patients originating mainly from other European countries, all patients were of Caucasian ethnicity and Italian origin.

Mutational Analysis

The mutational screening of *GBA* exons 9 and 10 (including the two major mutations, p.N370S and p.L444P) was performed as previously described.[6] We found 126 PD patients carrying one *GBA* mutation: a mild mutation (*GBA*-MM) was present in 70 patients (p.N370S, of which 3 were carriers of mutations in other PD genes, two *LRRK2* and one parkin), whereas a severe mutation (*GBA*-SM) was present in 56 individuals (54 p.L444P, 1 p.G377S, and 1 splicing mutation IVS10 + 1G>T). We excluded patients who were carriers of mutations in other PD-related genes (*LRRK2*, parkin, *PINK1*, and *SNCA*), including the 3 carriers of a *GBA* mutation mentioned above. We additionally excluded carriers of *GBA* variants of unknown function, such as p.E388K (6 subjects). We finally analyzed the remaining cohort of 2,764 PD patients, of whom 123 were *GBA* carriers and 2,641 were noncarriers.

Clinical Evaluation

All subjects were followed at the Parkinson Institute (Milan, Italy) by neurologists experienced in movement disorders and dementia. We reviewed clinical charts and retrospectively collected available clinical and demographic data of all 2,764 PD patients. All clinical data were independently reviewed by an additional experienced neurologist (R.C. or S.Te.). Patients were classified as "familial" if at least one relative among their first- or second-degree relatives had a diagnosis of PD. The remaining patients were classified as "sporadic." The age at which the patient noticed the first motor symptom was considered the age at onset. Clinical assessment was based on the last available Unified Parkinson Disease Rating Scale (UPDRS) from part I to part IV and the Hoehn and Yahr stage (H&Y). Distinct scores were calculated for dopaminergic and

nondopaminergic motor symptoms, according to the criteria proposed by Levy et al.[13]

Assessment of Dementia and Survival

Dementia was diagnosed according to DSM-IV criteria (American Psychiatric Association, 1994). Because not all patients underwent extensive neuropsychological assessment, we performed internal validation of the diagnosis of dementia using data on Mini–Mental State Examination (MMSE; pathological score < 24) from 1,034 patients by demonstrating a good agreement between the two methods (Cohen's $\kappa = 0.817$; 95% confidence interval [CI] = 0.780–0.855).[14,15] Accurate longitudinal assessment of dementia was not possible in 659 noncarriers, who were excluded from the analysis.

In patients lost to follow-up (no information available on death and no neurological examination at least from 2013 to 2015), we ascertained the vital status or the date of death by active follow-up (inquiries by telephone to participants or proxy respondents) during 2014 and 2015. If phone contact was not possible, information on survival was obtained through residential city council registries. In this way, survival information up to 2015 was collected for 2,685 of 2,764 patients (97%). In the 79 patients of whom no information was available, including 2 *GBA* carriers, we considered the patient as alive at the time of the last clinical neurological evaluation.

Image Acquisition and Analysis

A subgroup of 35 *GBA* carriers underwent perfusion single-photon emission computed tomography (SPECT; severe mutation, n = 20; mild mutation, n = 15), whereas 18 underwent dopamine transporter (DAT) imaging (severe mutation, n = 10; mild mutation, n = 8). As control groups, we used PD noncarriers and DLB noncarriers diagnosed according to established criteria,[16] matched for sex and disease duration to *GBA* carriers in a 1:1:1 ratio. PD noncarriers were also matched for age at SPECT to carriers, whereas this was not possible for the DLB group. Thirteen scans could not be retrieved for analysis (perfusion of 3 carriers and of 6 DLB; DAT of 4 DLB; Supplementary Table 1).

All brain SPECT scans were carried out at the IRCCS-Ospedale Maggiore in Milan and acquired by means of a dedicated triple detector gamma-camera (Prism 3000; Philips, Best, The Netherlands), equipped with ultra-high-resolution fan beam collimators. The interval between clinical assessment and SPECT scan was no longer than 9 months. All subjects were scanned after overnight withdrawal of dopaminergic medications. Brain perfusion SPECT scans were obtained 30 to 60 minutes after intravenous injection of 99m-Tc-ethyl cysteinate dimer bicisate (ECD), whereas DAT scans were performed 4 hours after intravenous administration of ^{123}I-2β-carbometoxy-3β-(4-iodophenyl)-N-(3-fluoropropyl) nortropane (^{123}I-FP-CIT; DaTscan; GE Healthcare, Little Chalfont, UK). Details of SPECT images acquisitions, reconstructions, and preprocessing have been described in detail elsewhere.[17,18] All SPECT images were preprocessed and analyzed in MATLAB R2010a (The MathWorks, Inc., Natick, MA) using Statistical Parametric Mapping 8 (SPM8; Wellcome Department of Imaging Neuroscience, London, UK).

After investigating DAT density in the entire striatum by a voxel-by-voxel approach, we additionally applied a striatal automated region of interest (ROI)-based approach: Specific-to-nondisplaceable binding ratios in the caudate nuclei and the putamina were computed using the second version of the Basal Ganglia software (BasGanV2; www.aimn.it), which allows automatic value extraction with partial volume effect correction.[19,20]

Ethics

The study was performed in agreement with the principles of the Declaration of Helsinki, and the local Ethics Committee was notified in compliance with Italian legislation on retrospective studies. Written informed consent was obtained from all subjects.

Statistical Analysis

Demographics and disease characteristics of the groups were compared using the *t* test for independent groups and chi-square test, as appropriate. Two sets of analyses were carried out.

LONGITUDINAL ANALYSIS. Two different outcomes were independently considered: dementia and death. Unadjusted analyses were performed using Kaplan-Meier methods, both on disease-duration-scale and age-scale time; log-rank tests for comparison of survival curves were performed. When age was used as time scale, left truncation was accounted for by starting analysis at the age of PD onset. Multivariable analyses were performed using time from PD onset to the outcome in study (dementia or death) or the last examination. Genotypes hazard ratio (HR) and 95% CIs of death and dementia were computed using Cox proportional hazard regression models. All Cox models were adjusted for age at onset and sex. A proportional hazard (PH) model was assessed by regression scaled Schoenfeld residuals against the log time. The model with death as outcome satisfied the PH. Age at onset was introduced as a time-varying covariate in the model with dementia as the outcome, given that it did not satisfy the PH assumption. Dementia was also considered as a time-dependent covariate in a Cox models considering death as outcome.

CROSS-SECTIONAL ANALYSIS. Regression models were performed to evaluate whether genotype was associated with the severity of motor symptoms at the last UPDRS assessment. Generalized regression models with identity or logit link (as appropriate) were performed and were adjusted by sex, age, and disease duration at UPDRS assessment. Analyses were performed using STATA software (v.14; StataCorp LP, College Station, TX).

NEUROIMAGING DATA ANALYSIS. After spatial and count normalization, significant differences in brain perfusion and DAT density in voxel-wise analyses were estimated as follows. A general linear model was employed to perform the appropriate univariate statistical tests in whole brain. Brain SPECTs were analyzed on a voxel-by-voxel basis using the one-way analysis of variance (ANOVA) design of SPM8. First, we performed a one-way ANOVA with the factors GBA-SM, GBA-MM, PD noncarriers, and DLB noncarriers (F-contrast = [–1 1 0 0; –1 0 1 0; 0

TABLE 1. Clinical and Demographic Characteristic and Comparison Between *GBA* Carrier With Noncarrier and Between Carriers of Severe Mutations With Carriers of Mild Mutations

Feature	Noncarriers (N = 2,641)	*GBA* Carriers (N = 123)	p^a	*GBA*-MM (N = 67)	*GBA*-SM (N = 56)	p^b
Male sex, N (%)	1,606 (60.8)	69 (56.1)	0.30	35 (52.2)	34 (60.7)	0.346
Positive family history, N (%)	446 (17.2)	38 (30.9)	**<0.001**	19 (28.4)	19 (33.9)	0.506
History of smoking, N (%)[c]	916 (38.1)	43 (39.1)	0.83	23 (39.7)	20 (38.5)	0.898
Age at onset, yr	57.4 (10.6)	52.4 (10.2)	**<0.001**	53.5 (10.2)	51.1 (10.1)	0.193
Early age at onset, N (%)[d]	673 (25.5)	53 (43.1)	**<0.001**	26 (38.8)	27 (48.2)	0.361
Age at last assessment, yr	69.4 (10.2)	64.3 (9.7)	**<0.001**	65.6 (9.6)	62.9 (9.8)	0.125
Disease duration at last assessment, yr	12.0 (6.6)	11.9 (6.3)	0.90	12.0 (6.9)	11.7 (6.5)	0.810
Advanced-stage therapy, N (%)[e]	221 (8.4)	20 (16)	**0.003**	13 (19.4)	7 (12.5)	0.302
Age at advanced-stage therapy, yr	61.23 (8.4)	58.9 (7.2)	0.23	58.5 (7.8)	59.7 (6.3)	0.720
Disease duration at advanced-stage therapy, yr	13.5 (4.7)	11.5 (4.0)	0.07	11.6 (4.6)	11.4 (3.1)	0.925

Data are reported as mean ± standard deviation, unless otherwise specified. Significant values ($p < 0.05$) are shown in **bold.**
[a]*GBA* carrier versus noncarrier.
[b]*GBA*-MM versus *GBA*-SM.
[c]Ever smoker. Incomplete or missing data in 251 subjects (238 noncarriers and 13 GBA carriers).
[d]Early age at onset ≤50 years.
[e]Advanced-stage therapy = deep brain stimulation, continuous apomorphine infusion, duodenal levodopa infusion.
GBA-MM = PD carriers of mild *GBA* mutations; *GBA*-SM = PD carriers of severe *GBA* mutations.

$-1\ 0\ 1]^T$) with nuisance covariate of age at SPECT. Then, we performed post-hoc analyses using a two-sample *t* test between groups with nuisance covariates of age and disease duration.[17] The significance threshold was set at the uncorrected $p < 0.001$ at peak level. Clusters of differences were regarded as significant if they survived at a threshold of $p < 0.05$, corrected for family-wise error at the cluster level. Only significant clusters containing at least 100 voxels were considered to be significant. The $p < 0.001$ height threshold at the voxel level was adopted to avoid type II errors attributable to overconservative thresholds.[21]

In region of interest (ROI)-based analyses, differences in semiquantitative FP-CIT binding values (contralateral putamen, ipsilateral putamen, contralateral caudate, and ipsilateral caudate) were investigated using a generalized linear model adjusted by age at SPECT. We subsequently performed post-hoc analyses by two-samples comparison, using $p < 0.05$ as a statistical threshold.

Results

GBA Carriers Versus Noncarriers

Of the 2,764 PD patients, 123 had a mutation in the *GBA* gene. *GBA* carriers had a 5-year earlier age at onset and more-frequent positive family history for PD (Table 1).

Dementia was diagnosed in 19.6% (388 of 1,982) of noncarriers and in 34.1% (42 of 123) of *GBA* carriers. Unadjusted analyses showed a greater frequency of dementia for carriers compared to noncarriers on both disease duration scale and age scale (Fig 1A,B). Multivariable analysis adjusted for sex, age at onset, and disease duration confirmed a 3-fold greater risk for dementia in carriers (Table 2, line A).

At survival analysis, we found that 597 subjects died, of whom 571 were noncarriers. Unadjusted analyses showed that death occurred at similar disease duration, but at an earlier age in *GBA* carriers compared to noncarriers (Fig 1C,D). However, multivariable analysis performed after adjusting for sex, age at onset, and disease duration showed a significantly increased risk of death in *GBA* carriers (Table 2, line B). Considering the greater risk for dementia in GBA carriers, to evaluate its role on survival we repeated the multivariable analysis including dementia as a time-dependent covariate. This analysis revealed that *GBA* mutations were associated with a greater mortality risk regardless of the presence of dementia (Table 2, line C). These findings suggested that, in addition to dementia, other clinical factors account for reduced survival.

We used cross-sectional analysis to investigate whether *GBA* carriers had a more-aggressive progression

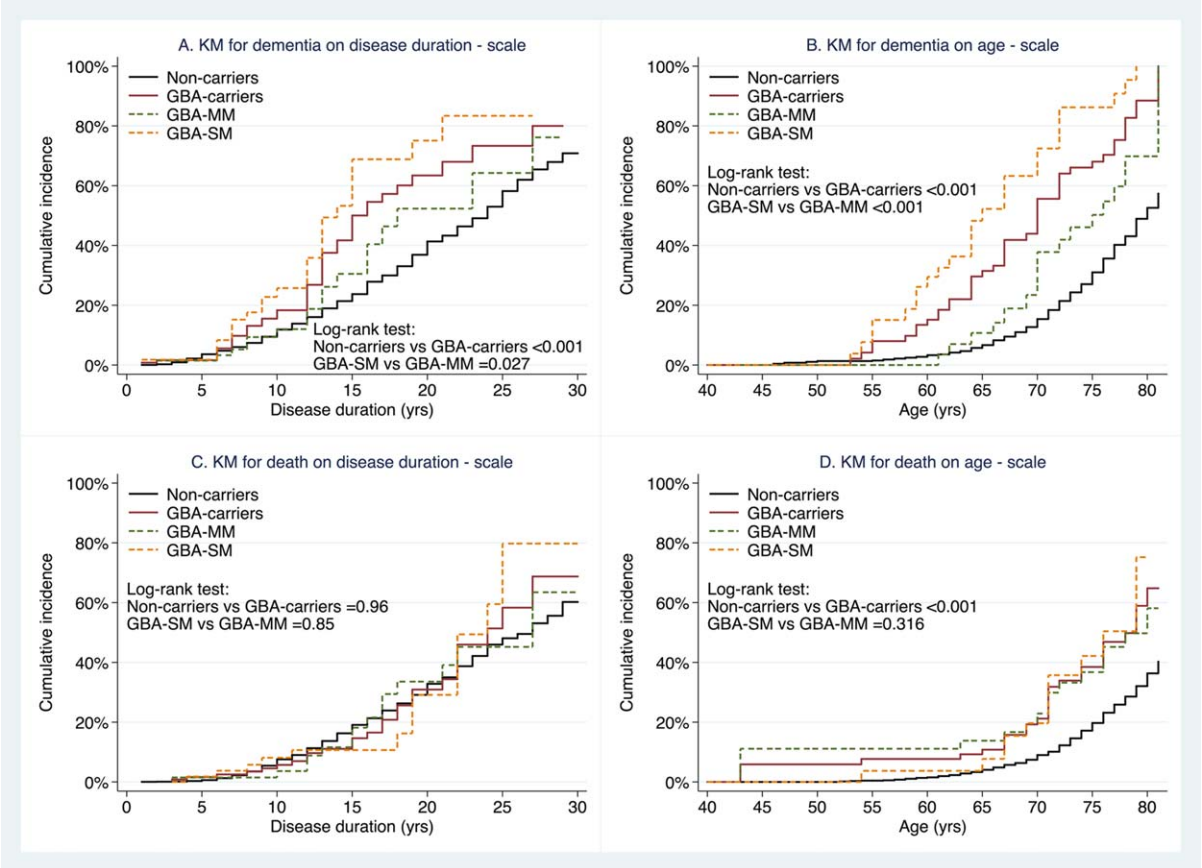

FIGURE 1: Unadjusted cumulative incidence for dementia (A,B) and for death (C,D) are illustrated using Kaplan-Meier methods, both on disease duration scale (A,C) and age scale (B,D). In each section, two analyses are reported: (1) *GBA* carriers versus PD noncarriers are reported in solid lines, noncarriers in black and *GBA* carriers in red, whereas (2) *GBA*-SM versus *GBA*-MM are reported in dot lines, *GBA*-SM in orange and *GBA*-MM in green. In each section, *p* values of long-rank comparisons between carriers versus noncarriers and between *GBA*-SM versus *GBA*-MM are reported. *GBA*-MM = PD carriers of mild *GBA* mutations; *GBA*-SM = PD carriers of severe *GBA* mutations; PD = Parkinson's disease.

of motor and/or nonmotor symptoms than noncarriers (Table 3). At the last follow-up, carriers scored significantly worse at UPDRS on part I (mentation), part II (activity of daily living), and part III (motor score), assessed both in the medication-ON and -OFF state. In particular, carriers showed more frequently nonmotor

TABLE 2. Cox Proportional Hazard Model for Death and Dementia on the Disease Duration Time Scale

	GBA Carriers vs Noncarriers		*GBA*-SM vs *GBA*-MM		*GBA*-SM vs Noncarriers		*GBA*-MM vs Noncarriers	
Outcome	**HR (95% CI)**	***p***	**HR (95% CI)**	***p***	**HR (95% CI)**	***p***	**HR (95% CI)**	***p***
A) Dementia	3.16 (2.3–4.4)	**<0.001**	2.89 (1.6–5.4)	**0.001**	5.62 (3.7–8.5)	**<0.001**	1.94 (1.2–3.2)	**0.008**
B) Death	1.85 (1.2–2.8)	**0.002**	1.25 (0.6–2.7)	0.568	2.10 (1.2–3.7)	**0.012**	1.68 (1.0–2.9)	0.056
C) Death	1.65 (1.1–2.5)	**0.016**	1.15 (0.5–2.5)	0.731	1.79 (1.0–3.2)	0.054	1.56 (0.9–2.7)	0.107

Significant values ($p < 0.05$) are shown in **bold.**

Line A: Cox regression model adjusted by sex and age at onset; age at onset was introduced as time dependent because it did not satisfy the proportional hazard.

Line B: Cox regression model adjusted by sex and age at onset.

Line C: Cox regression model adjusted by sex, age at onset, and dementia; dementia was introduced as a time-dependent covariate.

GBA-MM = PD carriers of mild *GBA* mutations; *GBA*-SM = PD carriers of severe *GBA* mutations; HR = hazard ratio; CI = confidence interval.

TABLE 3. Generalized Linear Model for Clinical Features at last UPDRS Adjusted by Sex, Age, and Disease Duration at Assessment

Feature	Noncarriers	*GBA* Carriers	OR (95% CI)	*p*
Age at assessment	68.9 (±10.3)	62.9 (±10.1)	—	**<0.001**
Disease duration at assessment	11.0 (±6.4)	9.9 (±5.9)	—	0.080
Part I: mentation	3.0 (±2.8)	3.3 (±2.7)	—	**0.001**
Intellectual impairment (%)	240/1,254 (19.1)	25/93 (26.9)	3.52 (2.0–6.1)	**<0.001**
Thought disorder (%)	213/1,254 (17)	18/93 (19.3)	1.76 (1.0–3.1)	**0.049**
Motivation/initiative (%)	251/1,253 (20)	23/93 (24.7)	2.33 (1.4–4.0)	**0.002**
Part II: activity daily living	13.6 (±7.6)	14.7 (±8.1)	—	**<0.001**
Dysphagia (%)	49/1,168 (4.2)	6/92 (6.5)	2.82 (1.1–7.1)	**0.027**
Falls (%)	154/1,209 (12.7)	10/92 (10.8)	1.30 (0.6–2.6)	0.474
Freezing (%)	332/1,210 (27.7)	28/92 (30.4)	1.82 (1.1–3.0)	**0.020**
Part III: motor score OFF[a]	30.4 (±13.7)	33.5 (±14.2)	—	**0.012**
Part III: motor score ON	23.9 (±13.6)	24.1 (±13.4)	—	**0.019**
Dopaminergic[b]	15.3 (±9.8)	14.5 (±9.4)	—	0.104
Nondopaminergic[b]	5.8 (±4.1)	5.5 (±4.0)	—	**0.002**
Speech (%)	603/2,257 (26.7)	33/106 (31.1)	1.99 (1.2–3.1)	**0.004**
Gait (%)	871/2,257(38.6)	39/106 (36.8)	1.58 (1.0–2.5)	**0.047**
Postural instability (%)	744/2,257 (33.0)	30/106 (28.3)	1.49 (0.9–2.4)	0.12
Part IV: dyskinesias (%)	655/1,099 (59.6)	47/83 (56.6)	1.02 (0.6–1.7)	0.92
Part IV: fluctuations (%)	726/1,068 (67.9)	48/82 (58.5)	0.68 (0.4–1.2)	0.16
Orthostatic hypotension (%)	89/840 (10.6)	12/65 (18.5)	2.61 (1.3–5.2)	**0.007**
H&Y stage ≥ 3 (%)	620/1,631 (38)	39/110 (35.5)	2.13 (1.32–3.5)	**0.002**

Significant values ($p < 0.05$) are shown in **bold.** In case of values reported as mean (±standard deviation), statistical analyses were performed using a generalized regression model with identity link adjusted by sex, age, and disease duration at assessment. In case of dichotomous values, we reported number of cases/total subjects (%), and ORs were evaluated using generalized linear regression model with logit link adjusted by sex, age, and disease duration at assessment. In the regression models, noncarriers were used as the reference group. Frequency was calculated on the total subjects where data were available. Patients who underwent deep brain stimulation had been excluded from the analysis.
Features were inferred from last UPDRS available, according to previously published criteria.[14]
[a]UPDRS motor score in OFF was available only for N = 1,266: 1,198 noncarriers and 68 GBA carriers.
[b]Items of UPDRS motor score were divided in dopaminergic (items 19, 20, 21, 22, 24, 25, 26, and 31) and nondopaminergic (items 18, 27, 28, 29, and 30) according to Levy et al.[13]
CI = confidence interval; H&Y = Hoehn and Yahr stage; OR = odds ratio; UPDRS = Unified Parkinson Disease Rating Scale.

symptoms (such as cognitive dysfunction, psychosis, and orthostatic hypotension) as well as non-levodopa-responsive motor symptoms (such as dysphagia and freezing of gait). The H&Y stage was also worst in carriers when analyzed adjusting for sex, age, and disease duration at assessment. When the motor score was stratified according to dopaminergic versus non-dopaminergic items,[13] only levodopa-non-responsive symptoms were significantly worse in carriers compared to noncarriers, whereas dopaminergic items and levodopa-related motor complications (On-Off fluctuations and dyskinesias) showed no differences (Table 3).

Severe Versus Mild GBA Mutations

Of the 123 carriers, a mild mutation (*GBA*-MM) was present in 67 patients (p.N370S), whereas a severe mutation (*GBA*-SM) was present in 56 individuals (54 p.L444P, 1 p.G377S, and 1 splicing mutation). We

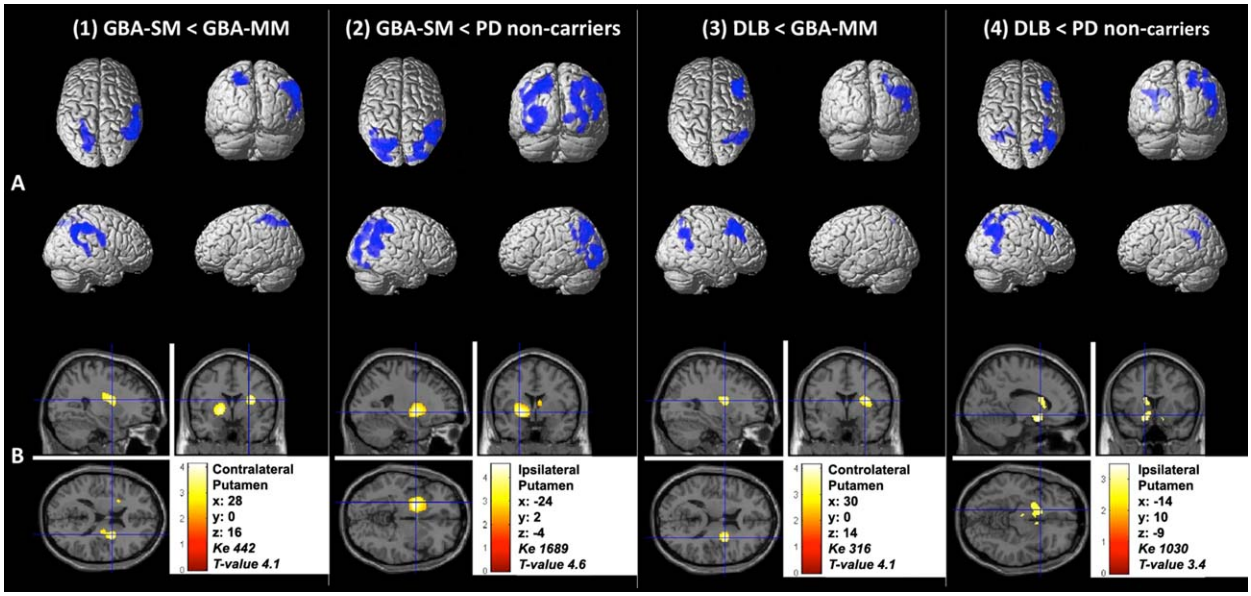

FIGURE 2: Statistical map of the two-sample *t* test performed among the four groups of patients (*GBA*-SM, *GBA*-MM, PD noncarriers, and DLB noncarriers) overlaid upon the average magnetic resonance imaging in stereotaxic space. Figures are displayed at a height threshold of T = 3.17. In the upper panel, brain regions with reduced cerebral blood flow are displayed. In the lower panel, voxel-wise comparison of FP-CIT binding maps are shown. The cluster showing the highest T value for each two-sample *t* test is reported along with the cluster size. All significant clusters and subclusters are described in detail in Supplementary Table S3A and S3B, respectively. Note that the comparison between *GBA*-MM versus PD noncarriers and *GBA*-SM and DLB noncarriers are not displayed because no clusters with significant difference were found at both brain perfusion and dopamine transporter single-photon emission computed tomography imaging analyses. DLB = dementia with Lewy bodies; FP-CIT = I-n-fluoropropyl-2b-carbomethoxy-3b-(4-iodophenyl) nortropane; *GBA*-MM = PD carriers of mild *GBA* mutations; *GBA*-SM = PD carriers of severe *GBA* mutations; PD = Parkinson's disease.

compared survival and clinical features of *GBA*-SM versus *GBA*-MM. We did not find significant differences in demographic features (Table 1). Although not statistically significant, the mean age at PD onset was about 2.5-year lower in *GBA*-SM than *GBA*-MM.

The main finding of genotype-phenotype analysis was the significantly greater risk for dementia in *GBA*-SM than *GBA*-MM. Overall, dementia was diagnosed in 25.4% of *GBA*-MM (17 of 67) and in 44.6% in *GBA*-SM (25 of 56). Unadjusted analysis showed a greater frequency of dementia in *GBA*-SM compared to *GBA*-MM on both disease duration scale and age scale (Fig 1A,B). Multivariable analysis adjusted for sex, age at onset, and disease duration confirmed a 3-fold greater risk for dementia in *GBA*-SM compared to *GBA*-MM (Table 2, line A). Compared to PD noncarriers, the risk for dementia was more than 5-fold in *GBA*-SM and approximately 2-fold in *GBA*-MM. Consistently, the frequency of patients with intellectual impairment was more than 2-fold in *GBA*-SM compared to *GBA*-MM according to UPDRS item-1 at the last follow-up (Supplementary Table 2).

Except for cognitive dysfunction, the direct comparison of nonmotor and motor clinical features between *GBA*-SM and *GBA*-MM at the last follow-up did not reveal other significant differences, possibly attributed to reduced statistical power (Supplementary Table 2). Therefore, we compared each *GBA* subgroup with PD noncarriers, adjusting for sex, age at onset, and disease duration. We found that a number of motor and nonmotor features that were similar between *GBA*-MM and PD noncarriers were instead significantly more impaired in *GBA*-SM than PD noncarriers (Supplementary Table 2, footnote c). Most notably, *GBA*-SM had more severe motor symptoms OFF-medication ($p = 0.011$) and H&Y ≥ 3 ($p = 0.043$), more frequent psychotic symptoms (odds ratio [OR], 2.38; 95% CI, 1.12–5.07; $p = 0.024$), apathy (OR, 3.95; 95% CI, 1.95–8.03; $p < 0.001$), and orthostatic hypotension (OR, 2.88; 95% CI, 1.11–7.42; $p = 0.029$).

Survival analyses did not show significant differences between the two *GBA* subgroups at both unadjusted analysis (Fig 1C,D) and multivariable analysis, regardless of the presence of dementia (Table 2, line B,C). However, it is worth mentioning that the risk of death was significantly greater in *GBA*-SM compared to noncarriers, whereas this difference did not reach statistical significance comparing *GBA*-MM to noncarriers ($p = 0.012$ vs $p = 0.056$, respectively; Table 2, line B). These data suggest a potential effect of the type of *GBA* mutation on survival that needs to be clarified by future studies on larger populations.

TABLE 4. Adjusted Means and *p* Values of Semiquantitative FP-CIT Binding Values According to Region of Interest–Based Analysis for Comparisons Among *GBA* Carriers Subdivided Into Carriers of Severe and Mild Mutations, PD Noncarriers, and DLB Noncarriers

	Mean (SE)[a]				*p*					
	PD (n = 18)	*GBA*-MM (n = 8)	*GBA*-SM (n = 18)	DLB (n = 14)	*GBA*-MM vs PD	*GBA*-SM vs PD	DLB vs PD	*GBA*-MM vs *GBA*-SM	*GBA*-MM vs DLB	*GBA*-SM vs DLB
Contralateral putamen	1.9 (0.1)	1.9 (0.2)	1.2 (0.2)	1.3 (0.2)	0.986	**0.002**	**0.005**	**0.008**	**0.014**	0.959
Ipsilateral putamen	2.3 (0.1)	2.0 (0.2)	1.6 (0.2)	1.7 (0.2)	0.119	**0.001**	**0.008**	0.127	0.297	0.665
Contralateral caudate	3.2 (0.2)	3.4 (0.3)	2.4 (0.3)	2.6 (0.3)	0.549	**0.010**	0.075	**0.008**	**0.042**	0.589
Ipsilateral caudate	3.6 (0.2)	3.4 (0.3)	2.9 (0.3)	3.1 (0.3)	0.556	**0.063**	0.172	0.299	0.489	0.759

The "contralateral" side was defined as the side opposite to the clinically most affected side. Significant values ($p<0.005$) are shown in **bold**. Patients were divided into four groups: PD noncarriers; *GBA*-MM, *GBA*-SM, and DLB noncarriers. Mean values were assessed by the model; age at single-photon emission computed tomography was set to 59.1 (mean age at SPECT).

[a]Binding values are given as mean (standard errors; SE) estimated using delta methods.

DLB = Dementia with Lewy bodies; FP-CIT = I-n-fluoropropyl-2b-carbomethoxy-3b-(4-iodophenyl) nortropane; *GBA*-MM = PD carriers of mild *GBA* mutations; *GBA*-SM = PD carriers of severe *GBA* mutations; PD = Parkinson's disease.

Neuroimaging

Age-adjusted analysis of perfusion SPECT showed synaptic activity differences in posterior parietal and occipital cortical areas bilaterally among PD *GBA* carriers, PD noncarriers, and DLB (Supplementary Table 3A). At post-hoc analyses, *GBA* carriers showed a significant decrease in synaptic activity in posterior parietal and occipital regions compared to PD noncarriers. *GBA*-SM showed a significant blood flow reduction in the bilateral parietal lobe than *GBA*-MM (Fig 2-A1). Similarly, *GBA*-SM showed a pronounced posterior parietal and occipital blood perfusion reduction than PD noncarriers (Fig 2-A2), whereas there was no significant difference comparing PD noncarriers and *GBA*-MM (Supplementary Table 3A). On the other hand, DLB showed reduced perfusion of the posterior parietal and occipital cortical areas and the dorsolateral prefrontal cortex compared to both *GBA*-MM and PD controls (Fig 2-A3,A4). We found a similar perfusion pattern between *GBA*-SM and DLB (Supplementary Table 3A). Taken as a whole, these data suggest a similar pattern of cortical involvement in *GBA*-MM and PD noncarriers and a different pattern in *GBA*-SM and DLB noncarriers. Additional analysis performed after excluding patients with dementia yielded similar results (Supplementary Tables 4 and 5).

Nigrostriatal terminal loss in the bilateral striatum significantly differed among *GBA* carriers, PD noncarriers, and DLB, mainly in the putamen. Carriers showed more-pronounced dopaminergic dysfunction than PD noncarriers, whereas the DLB group had the lowest binding values of the three groups (Table 4 and Supplementary Table 3B). *GBA*-SM had more pronounced nigrostriatal terminals reduction compared to *GBA*-MM, mainly in the contralateral striatum. Age-adjusted analysis showed similar DAT density between *GBA*-MM and PD noncarriers and between *GBA*-SM and DLB noncarriers (Figs 2-B1 and 3; Table 4 and Supplementary Table 3B). This pattern of distribution is particularly evident in the contralateral putamen (Fig 3A), in full consistence with perfusion imaging findings.

Discussion

We assessed survival and dementia in a large population of 2,764 unrelated PD patients who had been consecutively screened for major *GBA* mutations. Carriers of *GBA* mutations had a 3-fold increased risk for dementia and approximately 2-fold increased mortality risk. Reduced survival was not entirely attributed to increased progression to dementia, so that other variables are to be taken into account. Cross-sectional analysis of clinical features (UPDRS) and of neuroimaging (brain perfusion and DAT density) strengthen the hypothesis of a more-extensive brain synucleinopathy in *GBA* carriers, suggesting the involvement not only of neocortical areas (increasing the risk for dementia and psychosis), but also of subcortical regions and even the spinal cord (increasing the risk for orthostatic hypotension). Diseases with Lewy

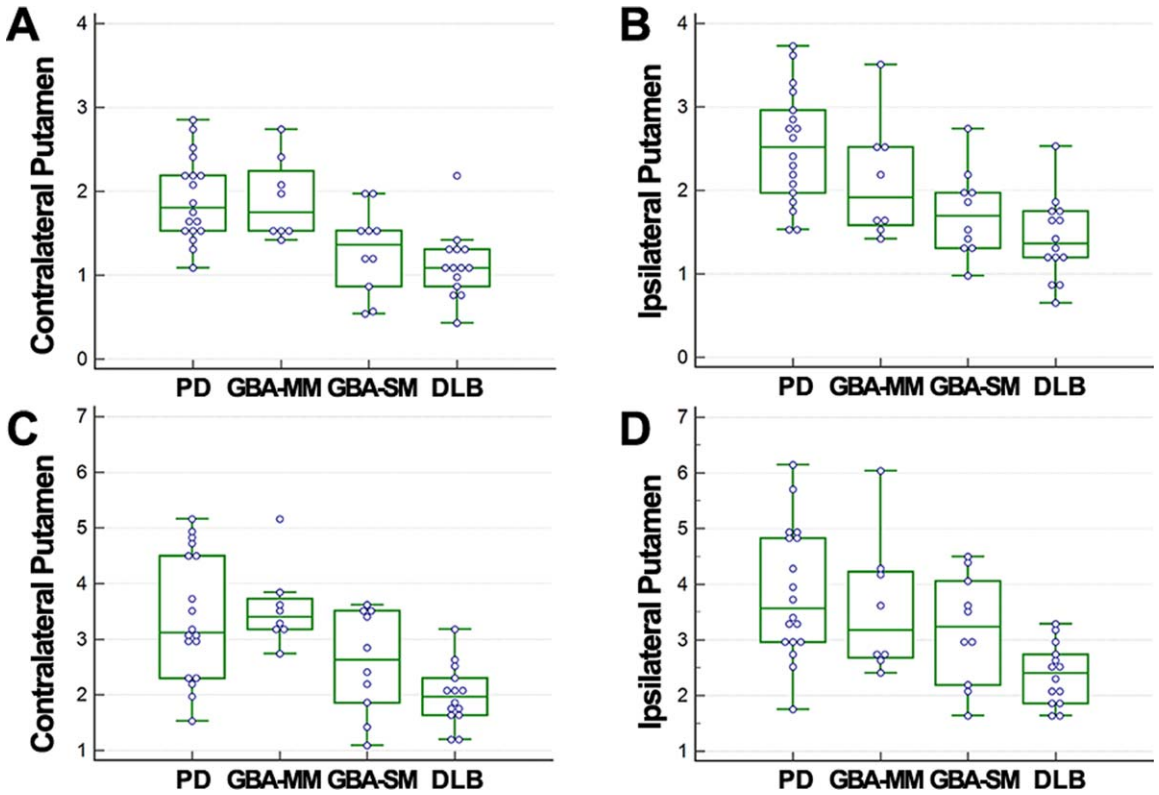

FIGURE 3: FP-CIT binding values of *GBA*-SM, *GBA*-MM, PD noncarriers, and DLB noncarriers shown as box-and-whisker plot. The central box represents the values from the lower to upper quartile (25th to 75th percentile), and the middle line represents the median. A line extends from the minimum to the maximum value, excluding outlier values (which are displayed as separate points). The "contralateral" side is defined as the side opposite to the clinically most affected side. DLB = dementia with Lewy bodies; FP-CIT = I-n-fluoropropyl-2b-carbomethoxy-3b-(4-iodophenyl) nortropane; *GBA*-MM = PD carriers of mild *GBA* mutations; *GBA*-SM = PD carriers of severe *GBA* mutations; PD = Parkinson's disease. [Color figure can be viewed in the online issue, which is available at www.annalsofneurology.org.]

bodies represent a clinicopathological continuum ranging from PD to PD dementia (PDD) and to DLB[16]; in this gradient, *GBA* carriers localize midway, with carriers of mild mutations closer to sporadic PD and carriers of severe mutations largely overlapping with DLB.

Dementia

Dementia is the major complication in PD course that greatly affects quality of life and survival.[22] Our multivariable analysis adjusted for sex, age at onset, and disease duration revealed a 3.2-fold greater risk for dementia in *GBA* carriers compared to noncarriers.

Considering that the prevalence of dementia in sporadic PD has been calculated to be 24% to 31%,[23] it is noteworthy that, in our cohort, the proportion of patients with dementia at 70 years of age was 56% in *GBA* carriers compared to 15% in noncarriers. The increased risk for dementia in *GBA*-associated parkinsonism (ie, DLB and PDD) is in agreement with previous studies.[24–31] However, our cohort of carriers is larger and allows genotype-phenotype correlations according to type of mutations. Among the main findings of our study, we underline the striking differential effect of the type of *GBA* mutation on the risk for dementia. Although an increase in cognitive decline could be expected, as reported for age at onset,[10] the effect of severe mutations toward dementia resulted surprisingly higher: carriers of severe mutations had a 2.9-fold greater risk for dementia than carriers of mild ones and 5.6-fold greater risk than PD noncarriers. Kaplan-Meier curves clearly show the important distinction between the two types of *GBA* mutation on both disease duration and age scale. At 70 years of age, 72% of GBA-SM and 38% of GBA-MM were affected by dementia. The differential effect of mild and severe mutations may well explain the remarkable clinical variability reported among patients with *GBA*-associated PD in terms of both cognitive dysfunction and motor disability,[9,25] suggesting a prognostic influence associated to the degree of GCase activity reduction. Our data further support the loss-of-function hypothesis of *GBA* mutations and parkinsonism[10] and have extremely important implications for prognosis and genetic counseling.

Survival

We found an increased mortality risk in *GBA* carriers compared to noncarriers. Kaplan-Meier curves show that

death occurred at similar disease duration, but at a significantly earlier age. Multivariable analysis showed that reduced survival is independent from the earlier age at onset and that, in addition to dementia, other specific mutation-related clinical features contribute to increase the mortality rate. The major predictors for mortality in idiopathic PD are chronological age, age at onset, sex, motor impairment, and dementia,[22,32] as well as dysphagia and orthostatic hypotension.[14] According to our UPDRS-based assessment at the last follow-up, *GBA* carriers showed a greater impairment in motor and nonmotor clinical features, including potential mortality risk factors such as dysphagia and orthostatic hypotension. No other survival analysis was previously published in PD *GBA* carriers. Brockmann et al followed 20 *GBA* carriers and 27 matched PD noncarriers for 3 years, suggesting a reduced survival in *GBA* carriers.[5] However, the generalization of these conclusions was limited by the small sample size: five carriers died during the 3-year observation period, whereas no death was recorded among noncarriers.

To our knowledge, this is the first study investigating the differential impact of the type of *GBA* mutation on survival. We found no significant difference in mortality rates between *GBA*-SM and *GBA*-MM. Most probably, larger cohorts are needed to detect it. Whereas *GBA*-SM showed a significantly increased mortality risk than noncarriers, this risk did not reach statistical significance for *GBA*-MM. In the future, survival studies should take *GBA* genotype into account, as previously reported for other genes.[33]

Neuroimaging

Imaging assessment of brain perfusion and nigrostriatal terminal density showed an overall more widespread and pronounced impairment in *GBA* carriers than PD noncarriers.

GBA carriers had reduced cortical activity in occipital and posterior parietal regions, including the cuneus/precuneus. These data are consistent with a previous study on resting-state cerebral blood flow in PD patients with homozygous or heterozygous *GBA* mutations.[28,34] This topographic pattern of cortical dysfunction is characteristic of diffuse Lewy body disease,[35] and it is consistent with clinicopathological studies showing a larger proportion of neocortical Lewy body pathology in *GBA* mutation carriers than in noncarriers.[9,34] Accordingly, the comparison between *GBA* carriers versus DLB noncarriers did not show any significant difference. *GBA* mutations involve hippocampal neurons projecting to lateral parietal cortical areas,[36] whose impairment well explains the reduced synaptic activity associated to hypoperfusion in these areas. Our findings are in line with neuropsychological profiles of *GBA* carriers, who performed more poorly than noncarriers on visuospatial and nonverbal (mainly visual) memory tasks associated to posterior cortical areas, whereas they did not differ in frontal-lobe executive functions and attention.[25]

Analysis of nigrostriatal function revealed that *GBA* carriers had lower striatal DAT density than PD noncarriers, supporting clinical evidence of worse UPDRS motor scores in carriers and consistent with evidences of lower DAT density in patients with diffuse Lewy body disease than PD.[37] Notably, *GBA*-SM had a more pronounced nigrostriatal damage than *GBA*-MM. Taken as a whole, the present imaging data are in line with clinical findings and suggest a more extensive and pronounced impairment in *GBA*-SM than *GBA*-MM: whereas the former have a pattern of reduction in cortical activity and dopamine terminal density similar to DLB, the latter findings are closer to PD noncarriers.

Limitations and Strengths

A limitation of our study is that we screened only exons 9 and 10 of the *GBA* gene missing rare mutations in other regions. Therefore, the carriers of these rare mutations were included in the PD noncarrier group. Considering the high cost of complete gene sequencing, we chose this screening strategy to analyze a large number of individuals. Given that mutations in exons 9 to 10 represent approximately 75% of *GBA* mutations in the Caucasian population,[38] we estimate that approximately 40 of the noncarriers should have an unknown *GBA* mutation. Considering that *GBA* carriers have a more severe phenotype, we expect that the addition of extra carriers would have strengthened the significance of our findings even further.

We performed a longitudinal assessment of survival and dementia, collecting data in the great majority of patients. However, because of the large study sample, we could not collect longitudinal data for other clinical milestones in PD progression (eg, psychosis, dysphagia, orthostatic hypotension, postural instability, and falls), thus precluding the use of these variables in a comprehensive multivariable longitudinal data analysis. Alternatively, we could have performed a comparison with matched noncarriers (123 carriers vs 123 noncarriers); in this case, all clinical milestones in PD progression could have been collected. However, this matched comparison would not be as informative and allow as much statistical power as analyzing the complete series of patients (123 vs 2,764). Finally, considering the earlier PD onset in *GBA* carriers, this matched analysis would have

introduced a selection bias and patients stratification errors in the control group.

The strengths of our study are mostly linked to the population characteristics and analysis. Our population is the largest ever studied in a single tertiary referral institute; all subjects were consecutively recruited in a single center, using strict enrollment and diagnostic criteria; all recruited patients resided in a defined geographical region (Italy); every patient was examined by movement disorders specialists applying a standardized, homogeneous, and comprehensive clinical assessment protocol. Also, given the large sample size, our study was powered to compare carriers of mild versus severe mutations.

Future Perspectives

The comparison between carriers of severe and mild GBA mutations is extremely important because severe mutations yield smaller enzymatic residual activity, and their association to a more severe phenotype suggests that the pathogenic mechanism is attributed to a loss of function and not to other possible gain-of-function effects.[39] We hypothesize that molecular pathways involving lysosomal enzymes may have a major role on the spreading of alpha-synuclein pathology throughout the central nervous system and contribute to explain individual differences in the progression of the disease along the continuum between PD and DLB. Finally, these results support the hypothesis that pharmacological chaperones able to increase GCase enzymatic activity, such as ambroxol, may be a possible therapy in *GBA*-associated PD.[40,41] This novel therapeutical approach might reduce progression to dementia and nigrostriatal terminal loss, substantially improving quality of life of PD patients. Indeed, given the relatively small difference in residual enzymatic activity between recombinant p.N370S and p.L444P mutant GCase (p.N370S = 32–38%; p.L444P = 13–24%),[42,43] the achievement of this result, at last a real neuroprotective therapy for a neurodegenerative disease, seems within reach.

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Author Contributions

R.C., S.Tu., E.C., and S.G. were responsible for conception and design of the study. R.C., S.Tu., G.M., C.S., S.Te., A.Z., M.G., C.B.M., N.M., G.Sa., M.Z., M.B., C.M., S.D., R.A., G.So., A.S., M.S., G.P., and S.G. were responsible for acquisition and analysis of data. R.C., S.Tu., G.M., E.C., and S.G. were responsible for drafting of the manuscript and figures.

Potential Conflicts of Interest

All authors have nothing to report.

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