

GBA-Related Parkinson's Disease: Dissection of Genotype–Phenotype Correlates in a Large Italian Cohort

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ABSTRACT: Background: Variants in *GBA* are the most common genetic risk factor for Parkinson's disease (PD). The impact of different variants on the PD clinical spectrum is still unclear.

Objectives: We determined the frequency of *GBA*-related PD in Italy and correlated *GBA* variants with motor and nonmotor features and their occurrence over time.

Methods: Sanger sequencing of the whole *GBA* gene was performed. Variants were classified as mild, severe, complex, and risk. β -glucocerebrosidase activity was measured. The Kaplan-Meier method and Cox proportional hazard regression models were performed.

Results: Among 874 patients with PD, 36 variants were detected in 14.3%, including 20.4% early onset. Patients with *GBA*-PD had earlier and more frequent occurrence of several nonmotor symptoms. Patients with severe and complex *GBA*-PD had the highest burden of symptoms and a higher risk of hallucinations and cognitive impairment. Complex *GBA*-PD had the lowest β -glucocerebrosidase activity.

Conclusions: *GBA*-PD is highly prevalent in Italy. Different types of mutations underlie distinct phenotypic profiles. © 2020 International Parkinson and Movement Disorder Society

Key Words: dementia; *GBA*; genotype–phenotype correlates; impulsive–compulsive behavior; Parkinson's disease

Heterozygous variants in the *GBA* gene, encoding the lysosomal enzyme β -glucocerebrosidase (GCase), are the most common genetic risk factor for Parkinson's disease (PD) worldwide.^{1–5}

Overall, 5% to 10% of patients with PD carry a heterozygous *GBA* variant, but such frequency varies widely among populations, from 10% to 31% in Ashkenazi Jewish to 3% to 12% in non-Jewish cohorts, and 2.8% to 4.5% in Italy.⁵⁻¹¹ However, most studies have only tested the most common *GBA* mutations, likely underestimating prevalence rates. To date, more than 500 pathogenic variants have been reported and classified as complex, severe, and mild based on the mutation type and residual GCase activity in patients with Gaucher disease (GD).^{5,12,13} Other variants not associated to GD recently emerged as risk factors for PD.¹⁴⁻¹⁷

GBA-PD is overall characterized by earlier onset, worse motor impairment, higher risk of cognitive decline and depression, more rapid progression, and decreased survival.^{3,7,10} Few studies have attempted to link variant severity with some clinical features,¹⁸⁻²¹ yet genotype-phenotype correlates have not been fully elucidated.

Here, we screened the whole *GBA* gene in a large cohort of Italian patients with PD and measured GCase activity in a subgroup. We performed a detailed comparison of motor and nonmotor features, and their occurrence over time, in patients with and without *GBA* mutations and, within the *GBA* group, among patients carrying variants of different severity.

Patients and Methods

We recruited 874 unrelated PD probands from 13 neurological tertiary centers spread in the Italian

territory. Approval was obtained by the Institutional Ethics Committee of the Coordinator Center (Protocol: 11799/08) and confirmed by the committees of each participating center. Written informed consent was obtained. The whole *GBA* coding region was sequenced, and variants were divided into 5 classes: mild (known to cause nonneuronopathic GD), severe (causing neuronopathic GD), risk (associated with higher PD risk, but not reported in GD), complex (2 or more variants in *cis* as the result of conversion, fusion, insertion of parts of *GBAP1* into *GBA*), and unknown. GCase activity was assessed in a subset of patients.

Methodological details regarding inclusion criteria, clinical assessment, molecular analysis, measurement of GCase enzymatic activity, and statistical analysis are reported in the Supplementary Material.

Results

Sequencing of the *GBA* gene was carried out in 874 patients with PD, and clinical data were obtained for 850 of them. Genetic variants were identified in 125 subjects (*GBA*-PD, 14.3%), including 36/176 (20.4%) early-onset PD (EOPD) and 89/674 (13.2%) late-onset PD cases. Details of all identified variants are presented in the Supplementary Results and Supplementary Tables 1 and 2.

Normalized GCase activity was calculated in 38 patients with *GBA*-PD, 27 nonmutated PD patients (NM-PD), 3 patients with GD, and 21 healthy controls.

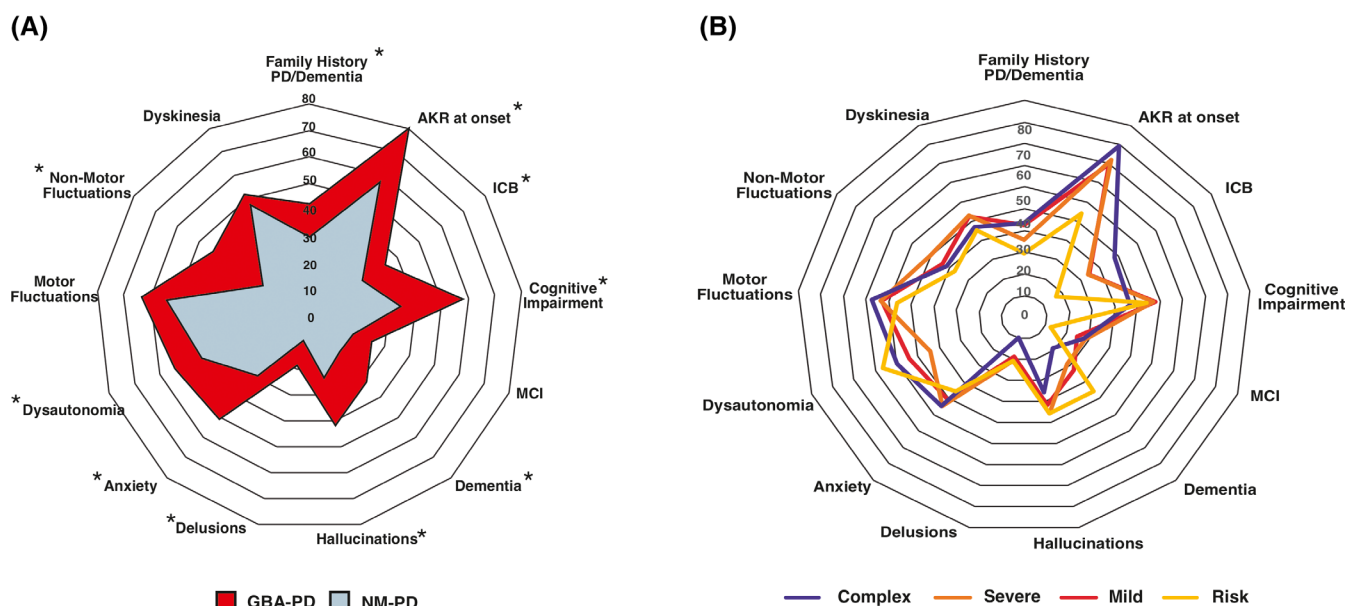


FIG. 1. Comparison of frequency of motor and nonmotor symptoms among different groups. **(A)** Frequency (%) of motor and nonmotor symptoms in *GBA*-PD versus NM-PD. **(B)** Frequency (%) of motor and nonmotor symptoms among *GBA*-PD subgroups carrying mild, complex, severe, and risk variants. Statistical comparisons are reported in Supplementary Table 5. *Significantly different comparisons (for details, see Supplementary Table 3). AKR, akinetic-rigid phenotype; *GBA*-PD, *GBA*-mutated PD; ICB, impulsive compulsive behavior; MCI, mild cognitive impairment; NM-PD, nonmutated PD; PD, Parkinson's disease.

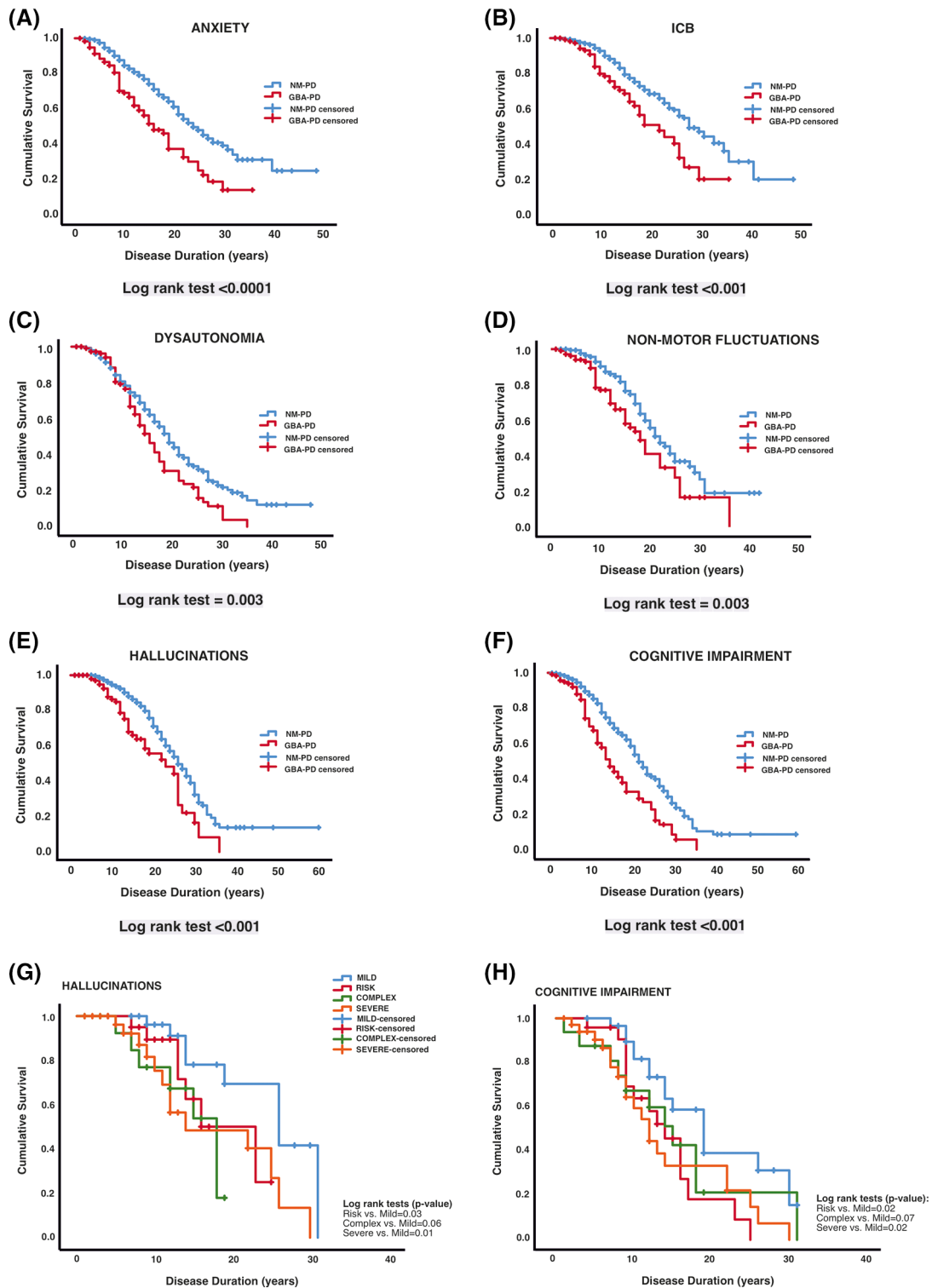


FIG. 2. Comparison of survival curves in GBA-PD versus NM-PD, and in carriers of distinct types of *GBA* variants. Kaplan-Meier method for disease-duration scale time and log-rank tests were used for comparison of survival curves in GBA-PD versus NM-PD (**A–F**) and in *GBA* carriers of mild, complex, severe, and risk variants (**G–H**). Log-rank tests showed that GBA-PD developed anxiety, ICB, dysautonomia, nonmotor fluctuations, hallucinations, delusions (not shown in figure; $P < 0.001$), and cognitive impairment significantly earlier than NM-PD. Log-rank tests also showed that patients with severe and risk *GBA* variants manifested hallucinations and cognitive impairment earlier than carriers of mild *GBA* variants. Patients with complex *GBA* variants had earlier hallucinations and cognitive impairment than carriers of mild *GBA* variants, albeit not significantly. GBA-PD, *GBA*-mutated PD; ICB, impulsive compulsive behavior; MCI, mild cognitive impairment; NM-PD, nonmutated PD; PD, Parkinson's disease.

The patients with GD had the lowest GCase activity, followed by GBA-PD and NM-PD ($P = 0.0001$). When GBA-PD patients were stratified by mutation type, GCase activity was significantly lower in carriers of complex variants compared with other categories (Supplementary Fig. 1).

Clinical features comparing GBA-PD and NM-PD groups are reported in Figure 1 and Supplementary Table 3. Compared with NM-PD the patients with GBA-PD showed a significantly younger age and more common akinetic-rigid phenotype at onset; more frequent family history for PD; and a higher burden of all nonmotor features, including anxiety, impulsive-compulsive behavior (ICB), dysautonomia, hallucinations, delusions, cognitive impairment, and nonmotor fluctuations. The frequency of motor complications (motor fluctuations and dyskinesia) was comparable in the 2 groups, albeit occurring earlier in patients with GBA-PD. Hallucinations also manifested earlier in the GBA-PD group, who was overall exposed to a lower total levodopa equivalent daily dose (LEDD) but a similar dose of Dag.

The patients with GBA-PD had a significantly higher risk of having a more advanced disease stage (Hoehn and Yahr >2), even after adjusting for age and gender. There was no difference in terms of risk of motor fluctuations and dyskinesia. This group also had a significantly higher risk of anxiety, ICB, dysautonomia, hallucinations, and cognitive impairment as well as nonmotor fluctuations, even after adjusting for gender, age, total LEDD, and LEDD Dag (Supplementary Tables 4 and 5). Log-rank tests showed that all these features also developed earlier in the GBA-PD group than in the NM-PD group (Fig. 2).

The patients with GBA-PD were further divided into 4 groups according to the type of *GBA* variant: mild (mGBA-PD), complex (cGBA-PD), severe (sGBA-PD), or risk alleles. No differences emerged among the groups in the frequency of motor and nonmotor symptoms except for ICB, delusions, and dementia (Supplementary Table 6).

In the Cox proportional hazard model, patients with mGBA-PD had a lower risk of dyskinesia than patients with sGBA-PD, even after adjusting for age, gender, and total and Dag LEDD. The patients with mGBA-PD also had a lower risk of hallucinations and cognitive impairment than the other 3 groups (not reaching statistical significance for comparison with cGBA-PD), which survived all adjustments. The risk of delusions was lower in patients with mGBA-PD compared with cGBA-PD and sGBA-PD groups, and survived all adjustments. There were no significant differences among *GBA* groups for anxiety, ICB, dysautonomia, and nonmotor fluctuations (Supplementary Table 7).

Log-rank tests showed that patients with mGBA-PD had a significantly later occurrence of cognitive

impairment and hallucinations (compared with sGBA-PD and risk GBA-PD groups; Fig. 2) and delusions (compared with sGBA-PD and cGBA-PD groups). There were no differences among groups with regard to the onset of anxiety ($P = 0.4$), ICB ($P = 0.3$), dysautonomia ($P = 0.3$), motor fluctuations ($P = 0.2$), nonmotor fluctuations ($P = 0.2$), and dyskinesia ($P = 0.1$).

Discussion

Here we report the first comprehensive analysis of the whole *GBA* gene in 874 Italian patients with PD. We detected *GBA* variants in 125 (14.3%) subjects, well above the 7% to 10% frequency reported in non-Ashkenazi Jewish populations.^{1,6,22-28}

Previous screenings in 2 other Italian PD cohorts disclosed a frequency of 3% to 5%.⁸⁻¹⁰ However, these studies focused either on detecting the 2 most common mutations (N370S and L444P) or on sequencing exons 9 and 10 only. Conversely, we detected 36 distinct variants, of which 3 novel. The N370S and L444P mutations were found in only 47%, and mutations in exons 9 and 10 in only 58% of the positive cases. These findings underlie the following 2 important concepts: (1) the frequency of *GBA*-related PD in Italy is among the highest worldwide among non-Ashkenazi Jewish populations and (2) a focused mutational screening cannot represent the method of choice at least in the Italian population.

Consistent with previous studies,^{16,29} the prevalence of *GBA* mutations among EOPD patients raised to 20.3%, suggesting that *GBA* screening in EOPD is as relevant for diagnostic purposes as testing the most common EOPD genes, such as *PARK2* and *PINK1* or even *LRRK2*.

We observed significant differences of normalized GCase values in the GBA-PD group versus both healthy controls and NM-PD groups, as reported.³⁰ When comparing enzymatic activity among different mutation classes, complex variants had the lowest activity, whereas risk variants had the highest. These data require replication in larger cohorts and correlation with clinical data given the variability at the individual level.

We confirm a significant association of *GBA* variants with earlier age at onset, positive family history for PD, and more rapid disease progression.^{5,7,10}

As a novel finding, we show a significant association of GBA-PD with akinetic-rigid onset and several nonmotor symptoms, such as anxiety, ICB, hallucinations, and dysautonomia, which also occurred earlier. This was paralleled by a significantly lower dopaminergic daily dose, supporting the neuropsychiatric and autonomic vulnerability of this group of patients. The

increased predisposition to develop ICB, previously reported in *PARK2*-associated PD,³¹ supports the view that ICB may represent a manifestation of PD rather than a pure drug-induced phenomenon. Dysautonomia also tended to occur earlier in patients with *GBA*. Indeed, worse autonomic and cognitive functions in *de novo* PD predict the development of ICB during the disease course,³² suggesting a close link of these symptoms as predictors of disease deterioration.

Although the clinical spectrum of *GBA*-PD seems well delineated, the variability of clinical features among mutation carriers is remarkable. This may be at least partly explained by the diverse impact of distinct *GBA* mutations. To date, only a few studies have attempted to delineate the phenotypic profile associated to specific mutation classes, showing earlier age at onset and a greater risk for dementia and other nonmotor symptoms in carriers of severe variants.^{10,18-21} In the present study, we further addressed this issue by dividing patients with *GBA*-PD into subgroups based on the variant type and attempted to profile the clinical features that recurred more frequently, or earlier in the disease course, within each subgroup. Severe *GBA* variants were characterized by younger onset and more severe progression as per the shorter time to develop balance disturbances and the higher risk of hallucinations and cognitive impairment. Subjects carrying complex variants had a similar phenotype, with a comparable risk of hallucinations and dementia, but also a higher frequency of delusions. Carriers of mild variants showed a milder phenotype, reaching postural instability after longer time, less frequent delusions, and later cognitive impairment. Finally, patients carrying a risk allele had the highest age at PD onset and were the only patients showing tremor-dominant phenotype at onset and later occurrence of nonmotor fluctuations. When considering ICB in the 4 subgroups, risk and mild variant carriers had, respectively, the lowest and highest frequency, likely determined by having the lowest and highest dose of Dag. This might reflect a lower vulnerability to late psychiatric complications (hallucinations and delusions) of mild variant carriers who could be treated with higher concentrations of Dag.

The main limitation of this study relates to its retrospective design. We tried to minimize this by employing the Kaplan-Meier method for the disease-duration scale time and log-rank tests for the comparison of survival curves. Moreover, we acknowledge the lack of objective outcome measures to assess symptom severity. Yet, given the heterogeneity of the cohort (including patients with widely variable disease duration and assessed in different pharmacological conditions), a comparison of scores would have provided unreliable results.

This study has a number of strengths. First, it provides a comprehensive assessment of motor and non-motor features in the same large cohort of subjects,

showing previously unreported associations. Second, it reports for the first time a comparison among all mutation classes, including complex alleles. Albeit these are considered similar to severe variants, our clinical and enzymatic data support the view that complex variants represent a distinct group. Finally, it is worth reporting the full picture of *GBA*-related PD in a different population from those described so far to allow meaningful epidemiological comparisons.

In conclusion, *GBA*-related PD has a high prevalence in the Italian population, also contributing to a significant proportion of EOPD cases. Our data expand the spectrum of nonmotor features associated to *GBA* and suggest that different types of mutations might underlie distinct phenotypic profiles. This evidence does not merely carry a clinical implication, but it is relevant in the attempt of developing disease-modifying strategies.³³ A fundamental research question now is whether *GBA* phenotypes related to distinct mutation types have a different rate of disease progression and survival in a prospective cohort. If so, stratification by mutation type will be mandatory when designing a clinical trial focusing on *GBA*-PD ■

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APPENDIX

Other collaborators of the ITA-GENE-PD Study Group are: Maria Concetta Altavista (RM), Marianna Amboni (NA), Gianluca Ardolino (MI), Alfredo Berardelli (RM), Filippo Cogiamanian (MI), Carlo Colosimo (TR), Danilo Costanti (TR), Giuseppe De Michele (NA), Carlo Di Bonaventura (RM), Giulia Di Lazzaro (RM), Vincenzo Di Lazzaro (RM), Antonio Emanuele Elia (MI), Roberto Erro (SA), Gina Ferrazzano (RM), Andrea Guerra (RM), Tamara Ialongo (RM), Maria Chiara Malaguti (TN), Marta Melis (CA), Elena Moro (Grenoble), Valentina Oppo (CA), Donatella Ottaviani (TN), Silvio Peluso (NA), Maria Luisa Quadri (Rotterdam), Luigi Michele Romito (MI), Marianna Sarchioto (London, TO), Tommaso Schirinzi (RM), Chiara Sorbera (ME), Alessandro Stefani (RM), Astrid Thomas (CH), Maria Luisa Valente (PV), and Giampiero Volpe (SA).

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

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GBA-related Parkinson disease: dissection of genotype-phenotype correlates in a large Italian cohort

Supplementary Material

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Supplementary Methods

Patients

The diagnosis of PD was based on the UK PD Society Brain Bank Clinical Diagnostic Criteria.¹ Age of onset of PD was considered the age at which the first motor symptoms manifested. Patients with onset ≤ 45 years of age were considered as early-onset PD (EOPD). Each patient was carefully assessed for motor and non-motor features, through clinical examination and specific interviews. Data were collected at the last available follow-up.

The assessed motor features included PD phenotype at onset (tremor-dominant or akinetic-rigid), presence of motor fluctuations and dyskinesias. Severity and progression of motor phenotype were investigated with the modified Hoehn and Yahr stage (H&Y, on medication) and the ratio H&Y/years of disease duration, respectively.

Assessment of autonomic symptoms included orthostatic hypotension, urge incontinence, erectile dysfunctions (in male), profuse sweating, and tachycardia. Presence of anxiety, depression, hallucinations, delusions, and impulsive-compulsive behaviours (ICB) were also evaluated through patients' and caregivers' interviews.

Mild Cognitive Impairment (MCI) and dementia were diagnosed in the clinical setting according to MDS PD-MCI Level I criteria.² Significant functional decline resulting from cognitive impairment was a primary feature in differentiating dementia from MCI.²

Cognitive impairment refers to the occurrence of either MCI or dementia.

Patients with dementia occurring before or within one year from the onset of motor symptoms were excluded.³ Information on dopaminergic medications was retrieved, and the levodopa equivalent doses, either for all drugs (LEDD) and for dopamine agonists only (LEDD Dag) were calculated.⁴

Molecular Analysis

The *GBA* gene (GenBank accession number NM_000157.3) was analyzed in 78 patients from Sardinia by whole exome sequencing and variants were confirmed by Sanger sequencing. Genetic data of these patients have been already reported.⁵ In the remaining 796, the 11 coding exons and flanking regions were analyzed by Sanger sequencing as reported.⁶ Identified variants were confirmed by repeating amplification and sequencing with alternative primers.

Novel variants were classified according to ACMG consensus recommendations using Varsome platform.^{7,8} Allele names refer to the processed protein (excluding the 39-residue signal peptide).

In all patients, pathogenic variants and rearrangements in the other major PD-related genes (*SNCA*, *LRRK2*, *PARK2*, *PINK1* and *DJ-1*) were previously excluded.

GCase enzymatic activity

GCase activity was analyzed from leucocytes (PBMCs) isolated from 5 ml EDTA-blood samples through Histopaque-1077 (Sigma) according to standard procedures. PBMCs were frozen upon isolation. To minimize experimental variability, GCase activity was always measured on first thawing, and the residual enzymatic activity was determined as reported. The substrate curve was constructed to the Lineweaver-Burk curve.⁹ To normalize GCase activity values using an independent lysosomal enzyme, acid maltase (AM) activity was measured by fluorometric assay with proper substrates.

Statistical analysis

Demographic and clinical data were compared using t-test, Mann-Whitney U test or chi-square test as appropriate and according to data distribution. For GCase activity, Kruskal-

Wallis test was employed to compare groups, followed by Mann-Whitney U test conditional on significant p-values.

Univariable analysis explored the association of clinical variables (anxiety, ICB, dysautonomia, hallucinations, delusions, cognitive impairment, motor fluctuations, non-motor fluctuations, dyskinesia) with the carrier status or with the variant type. Kaplan-Meier method for disease-duration-scale time and log-rank tests were used for comparison of survival curves. Genotype hazard ratio (HR) and 95% CI of each clinical variable were computed using Cox proportional hazard regression models and p values <0.05 were considered statistically significant. The multivariable Cox regression models for all outcomes of interest were adjusted for age, gender, total LEDD and LEDD Dag.

All analyses were performed with SPSS version 25.

Supplementary Results

Detailed genetic findings

Thirty-six distinct variants were identified, including fourteen severe, five complex, four mild and three risk alleles. For the remaining ten variants, the impact on GCase activity is still unknown. As expected, N370S and L444P, either isolated or as part of recombinant alleles, were the commonest variants with a cumulative frequency of 47.2% (N370S: 30/125, 24.0% and L444P: 29/125, 23.2%, respectively) within the GBA-PD group. A third variant, E326K, was also common (16/125, 12.8%). The remaining variants, detected in nearly half of mutated patients (51/125, 41.1%), were individually very rare, including three novel ones classified as pathogenic or likely pathogenic (Supplementary Tables 1 and 2).

Among the 125 GBA-PD subjects, 100 were heterozygous for single variants while in the remaining 25 more than one variant could be identified. Eight patients had biallelic variants, including seven homozygotes and one compound heterozygote. To exclude signs of GD, all patients were checked for hepatosplenomegaly and had normal blood count and protein electrophoresis. A subset of patients was also asked for history of spontaneous bone fractures and skeletal abnormalities, which were absent.

In two patients carrying, respectively, the N188K/W184R and the N370S/E326K variants, phase could not be assessed. The remaining fifteen individuals were heterozygous carriers of known complex alleles.

Supplementary Tables

Supplementary Table 1. Known *GBA* variants identified in the study.

Cases	Allele name	Amino acid change	Nucleotide change	Exon	Class of mutation	dbSNP
1	D24N	p.(Asp63Asn)	c.187G>A	3	severe	-
1	S107L	p.(Ser146Leu)	c.437C>T	4	severe	rs758447515
2	R120W	p.(Arg159Trp)	c.475C>T	5	severe	rs397515515
3	R131C	p.(Arg170Cys)	c.508C>T	5	severe	rs398123530
1	P182L	p.(Pro221Leu)	c.662C4T	6	severe	rs866075757
1†	N188S	p.(Asn227Ser)	c.680A>G	6	severe	rs364897
1	G202R	p.(Gly241Arg)	c.721G>A	6	severe	rs398123534
2	H255Q	p.(His294Gln)	c.882T>G	7	severe	rs367968666
1	D409H	p.(Asp448His)	c.1342G>C	9	severe	rs1064651
20	L444P	p.(Leu483Pro)	c.1448T>C	10	severe	rs421016
1	R463C	p.(Arg502Cys)	c.1504C>T	10	severe	rs80356771
1	W209Gfs*6	p.(Trp248Glyfs*6)	c.741delC	6	null [§]	-
1	R257*	p.(Arg296*)	c.886C>T	7	null [§]	rs1553217626
1†	R120W E388K	p.(Arg159Trp) p.(Glu427Lys)	c.475C>T c.1279G>A	5-9	severe risk	rs397515515/ rs149171124
1+1†	S196P G202R	p.(Ser235Pro) p.(Gly241Arg)	c.703T>C c.721G>A	6	complex	rs1064644 + rs398123534
4	H255Q D409H	p.(His294Gln) p.(Asp448His)	c.882T>G c.1342G>C	7-9	complex	rs367968666 + rs1064651
1	T369M D409H	p.(Thr408Met) p. (Asp448His)	c.1223C>T c.1342G>C	8-9	complex	rs75548401 + rs1064651
2	L444P A456P	p.(Leu483Pro) p.(Ala495Pro)	c.1448T>C c.1483G>C	10	complex	rs421016 + rs368060
7	L444P A456P V460V	p.(Leu483Pro) p.(Ala495Pro) p.(Val499Val)	c.1448T>C c.1483C>G c.1497G>C	10	complex	rs421016 + rs368060 + rs1135675
1	G46E	p.(Gly85Glu)	c.254G>A	3	mild	rs77829017
1†	G193R	p.(Gly232Arg)	c.694G>A	6	mild	NA

1	R329C	p.(Arg368Cys)	c.1102C4T	8	mild	rs374306700
27+2 [†]	N370S	p.(Asn409Ser)	c.1226A>G	9	mild	rs76763715
15	E326K	p.(Glu365Lys)	c.1093G>A	8	risk	rs2230288
6	T369M	p.(Thr408Met)	c.1223C>T	8	risk	rs75548401
1 [#]	N188K	p.(Asn227Lys)	c.681T>G	6	unknown	rs381418
	W184R	p.(Trp223Arg)	c.667T>C			rs61748906
1 [#]	N370S	p.(Asn409Ser)	c.1226A>G	9	unknown [‡]	rs76763715
	E326K	p.(Glu365Lys)	c.1093G>A	8		rs2230288
1 [†]	I161N	p.(Ile200Asn)	c.599T>A	6	unknown	rs77933015
1	K(-27)R	p.(Lys13Arg)	c.38A>G	2	unknown	rs150466109
1	M85V	p.(Met124Val)	c.370A>G	5	unknown	rs758455177
2	E326D	p.(Glu365Asp)	c.1095G>C	8	unknown	rs80317710
3	T369T	p.(Thr408=)	c.1224G>A	8	unknown	rs138498426
3	E388K	p.(Glu427Lys)	c.1279G>A	9	unknown	rs149171124
1	A456P	p.(Ala495Pro)	c.1483G>C	10	unknown	rs368060
1	V460L	p.(Val499Leu)	c.1495G>C	10	unknown	rs369068553

Known GBA variants identified in the screened population. [†]patients carrying biallelic mutations (homozygous or compound heterozygous, with phase available). NA: not available; Risk: variants considered risk factors for PD. [§]For statistical purposes, the W209Gfs*6 and R257* null variants have been included in the severe class. [#]Phase not available. [‡]since it was not possible to phase these two variants (one mild and one risk) and therefore to exclude the possibility that they would represent a complex allele, this patient has been included in the unknown group.

Supplementary Table 2. Novel *GBA* variants identified in the study.

Cases	Allele name	Aminoacidic change	Nucleotidic change	Exon	Polyphen	SIFT	Mutation Taster	Mutation Assessor	CADD	ACMG criteria	ACMG classification
1 ^s	T208S	p.(Thr247Ser)	c.740C>G	6	B	D	DC	M	22	PM1, PM2, PP2, PP3	LP
1	C342F	p.(Cys381Phe)	c.1142G>T	8	PD	D	DC	H	32	PM1, PM2, PM5, PP2, PP3	LP
2	M361Lfs*2	p.(Met400Leufs*2)	c.1197_1198insCTGTA	8	-	-	-	-	-	PVS1, PM1, PM2, PP1 [#]	P

Novel *GBA* variants identified in the screened population. ^spatients carrying biallelic mutations (homozygous or compound heterozygous, with phase available). [#]cosegregation in two affected siblings. B: Benign; D: deleterious; DC: disease-causing; H: high functional impact; M: medium functional impact; PD: probably damaging; LP: likely pathogenic; P: pathogenic. ACMG: American College of Medical Genetics and Genomics.⁷ Prediction databases include Polyphen (<http://genetics.bwh.harvard.edu/pph2/>), SIFT (www.sift.jcvi.org/), Mutation Taster (www.mutationtaster.org/), Mutation Assessor (<http://mutationassessor.org/r3/>) and CADD (<http://cadd.gs.washington.edu/>).

Supplementary Table 3. Demographic data and comparison of motor and non-motor features between GBA-PD and NM-PD cohorts.

Variable	GBA-PD	NM-PD	p
Male gender, % (M/tot)	52.8% (66/125)	61.7% (447/725)	0.0744
Age at onset (yrs), mean $\pm$ SD (tot)	53.5 $\pm$ 11.6 (124)	56.5 $\pm$ 11.5 (713)	0.007*
Age at last evaluation (yrs), mean $\pm$ SD (tot)	63.8 $\pm$ 11.6 (122)	67.4 $\pm$ 11.3 (686)	0.0010*
Disease duration (yrs), mean $\pm$ SD (tot)	12.5 $\pm$ 7.7 (122)	14.2 $\pm$ 8.2 (713)	0.0233*
EOPD, % (nr/tot)	29.0% (36/124)	19.6% (140/713)	0.0229*
Family history for PD, % (nr/tot)	42.4% (53/125)	30.0% (200/667)	0.0063*
Family history for dementia, % (nr/tot)	10.1% (11/98)	9.5% (31/357)	0.4284
AKR onset, % (nr/tot)	80.0% (84/105)	57.3% (275/480)	<0.0001*
H&Y at last evaluation, mean $\pm$ SD (tot)	2.7 $\pm$ 1.0 (121)	2.6 $\pm$ 0.9 (619)	0.2899
H&Y $\geq$ 2.5, % (nr/tot)	65.2% (77/118)	55.5% (343/618)	0.0499*
H&Y/disease duration (yrs), mean $\pm$ SD (tot)	0.4 $\pm$ 0.3 (121)	0.3 $\pm$ 0.3 (614)	0.0237*
Anxiety, % (nr/tot)	50.8% (60/118)	29.3% (183/624)	<0.0001*
Onset anxiety, (yrs), mean $\pm$ SD (tot)	1.5 $\pm$ 3.3 (50)	1.5 $\pm$ 4.7 (118)	0.8787
ICB, % (nr/tot)	34.7% (41/118)	24.3% (149/612)	0.0184*
Dysautonomia, % (nr/tot)	53.8% (63/117)	43.2% (267/618)	0.0338*
Hallucinations, % (nr/tot)	41.5% (49/118)	23.3% (149/640)	0.0001*
Onset hallucinations (yrs), mean $\pm$ SD (tot)	6.9 $\pm$ 6.9 (49)	9.2 $\pm$ 6.0 (92)	0.0127*
Delusions, % (nr/tot)	18.6% (22/118)	9.0% (57/634)	0.0004*
Onset delusions (yrs), mean $\pm$ SD (tot)	6.6 $\pm$ 6.7 (23)	8.5 $\pm$ 6.4 (45)	0.1448

MCI, % (nr/tot)	25.0% (30/120)	17.4% (110/632)	0.0553
Dementia, % (nr/tot)	32.5% (39/120)	17.1% (108/632)	0.0002*
Cognitive impairment, % (nr/tot)	57.5% (69/120)	34.5% (218/632)	<0.0001*
Motor fluctuations, % (nr/tot)	63.2% (72/114)	53.9% (234/434)	0.077
Onset motor fluctuations (yrs), mean $\pm$ SD (tot)	4.9 $\pm$ 3.1 (60)	6.3 $\pm$ 2.8 (159)	0.0010*
Non motor fluctuations, % (nr/tot)	43.5% (47/108)	21.2% (76/359)	<0.0001*
Onset non-motor fluctuations (yrs), mean $\pm$ SD (tot)	5.0 $\pm$ 3.9 (31)	6.6 $\pm$ 4.4 (54)	0.994
Dyskinesia, % (nr/tot)	52.2% (60/115)	47.5% (213/448)	0.3756
Onset dyskinesia (yrs), mean $\pm$ SD (tot)	5.6 $\pm$ 2.3 (44)	7.4 $\pm$ 4.1 (129)	0.0022*
LEDD, mean dose $\pm$ SD (tot)	604.2 $\pm$ 373.5 (114)	715.9 $\pm$ 411.7 (551)	0.0025*
LEDD D-ag, mean dose $\pm$ SD (tot)	265.0 $\pm$ 295.6 (100)	220.3 $\pm$ 220.5 (431)	0.3767

Significant differences (p value <0.05) are shown in bold and are marked with an asterix. (nr/tot): number of patients presenting the feature / total number of patients for whom the information was available; (tot): total number of patients for whom the information was available; yrs: years.

AKR: akinetic-rigid; LEDD: total Levodopa equivalent daily dose; D-ag: dopamine agonists; H&Y: Hoehn and Yahr Scale; ICB: impulsive compulsive behavior; MCI: mild cognitive impairment.

Supplementary Table 4. Cox Proportional Hazard Model for motor and non-motor symptoms on the disease duration-time scale (GBA-PD versus NM-PD)

	HR	95% CI	p
H&Y >2	1.455	(1.135-1.864)	0.003*
Anxiety	2.042	(1.525-2.736)	<0.0001*
ICB	1.899	(1.322-2.727)	0.001*
Dysautonomia	1.493	(1.134-1.965)	0.004*
Hallucination	2.347	(1.698-3.244)	<0.0001*
Delusions	2.557	(1.562-4.186)	<0.0001*
Cognitive impairment	1.998	(1.525-2.617)	<0.0001*
Motor fluctuations	1.115	(0.856-1.451)	0.42
Non-motor fluctuations	1.711	(1.189-2.462)	0.004*
Dyskinesia	1.105	(0.829-1.473)	0.497

Significant differences (p value <0.05) are shown in bold and are marked with an asterix.

CI= confidence interval; H&Y, Hoehn and Yahr Stage; HR = hazard ratio; ICB= impulsive-compulsive behaviours; NM = non mutated; PD = Parkinson's disease.

Supplementary Table 5. Cox Proportional Hazard Model for motor and non-motor symptoms on the disease duration-time scale adjusted for age, gender, LEDD and LEDD Dag

	aHR	95% CI	p
H&Y			
GBA-PD vs. NM-PD	1.3	(1.002-1.764)	0.048
Age	1.0	(0.978-0.998)	0.019
Gender	1.1	(0.880-1.408)	0.371
LEDD	1.0	(0.999-1)	0.003
LEDD Dag	1.0	(1-1)	0.896
Anxiety			
GBA-PD vs. NM-PD	1.7	(1.228-2.389)	0.002
Age	1.0	(0.964-0.988)	<0.0001
Gender	1.4	(1.018-1.873)	0.038
LEDD	1.0	(0.998-0.999)	<0.0001
LEDD Dag	1.0	(1-1.001)	0.006
ICB			
GBA-PD vs. NM-PD	1.6	(1.081-2.508)	0.02
Age	0.9	(0.929-0.963)	<0.0001
Gender	0.6	(0.398-0.903)	0.014
LEDD	1.0	(1-1)	0.935
LEDD Dag	1.0	(1-1.001)	0.253
Dysautonomia			
GBA-PD vs. NM-PD	1.4	(1.049-1.987)	0.024
Age	1.0	(0.979-1.002)	0.105
Gender	0.7	(0.561-0.987)	0.04
LEDD	1.0	(0.999-1)	<0.0001
LEDD Dag	1.0	(1-1.001)	0.021
Non-motor fluctuations			
GBA-PD vs. NM-PD	1.6	(1.041-2.396)	0.031
Age	1.0	(0.961-0.997)	0.026
Gender	1.6	(1.055-2.430)	0.027
LEDD	1.0	(0.999-1)	0.419
LEDD Dag	1.0	(1-1.001)	0.001

Hallucinations			
GBA-PD vs. NM-PD	2.7	(1.822-4.045)	<0.0001
Age	1.0	(0.985-1.017)	0.901
Gender	0.9	(0.620-1.310)	0.586
LEDD	1.0	(0.999-1)	0.086
LEDD Dag	1.0	(0.999-1)	0.198
Delusions			
GBA-PD vs. NM-PD	2.8	(1.503-5.166)	0.001
Age	1.0	(0.963-1.010)	0.248
Gender	1.0	(0.535-1.725)	0.894
LEDD	1.0	(0.999-1)	0.176
LEDD Dag	1.0	(0.999-1.001)	0.761
Cognitive impairment (MCI and Dementia)			
GBA-PD vs. NM-PD	2.4	(1.701-3.321)	<0.0001
Age	1.0	(0.988-1.014)	0.875
Gender	0.9	(0.630-1.179)	0.352
LEDD	1.0	(0.999-1)	0.047
LEDD Dag	1.0	(0.998-1)	0.01

H&Y, Hoehn and Yahr Stage; LEDD, total Levodopa equivalent daily dose; Dag dopamine agonists; ICB, impulsive compulsive behavior; MCI, mild cognitive impairment. GBA-PD: PD patients carrying GBA variants; NM-PD: PD patients negative at GBA testing.

Supplementary Table 6. Demographic data and comparison of motor and non-motor features among carriers of distinct *GBA* classes of variants

Variable	Complex (C)	Severe (S)	Mild (M)	Risk (R)	P-Value
Male gender, % (M/tot)	50.0% (8/16)	51.3% (20/39)	48.5% (15/32)	72.8% (17/24)	n.s.
Age at onset (yrs), mean $\pm$ SD (tot)	51.4 $\pm$ 11.6 (16)	51.3 $\pm$ 11.0 (38)	54.3 $\pm$ 11.1 (32)	57.0 $\pm$ 12.2 (24)	0.05 (S vs M+C+R)* 0.08 (R vs M+S=C)
Age at last evaluation (yrs), mean $\pm$ SD (tot)	59.7 $\pm$ 10.5 (16)	60.4 $\pm$ 11.5 (37)	66.4 $\pm$ 10.3 (32)	67.0 $\pm$ 11.9 (24)	0.0451 (S vs M)* 0.0402 (S vs R)*
Disease duration (yrs) , mean $\pm$ SD (tot)	12.3 $\pm$ 7.4 (16)	10.9 $\pm$ 8.2 (38)	14.6 $\pm$ 7.4 (32)	11.4 $\pm$ 5.6 (24)	0.0140 (S vs M)* 0.001 (M vs S+C+R)* 0.05 (S vs M+C+R)*
EOPD, % (nr/tot)	43.7% (7/16)	28.2% (11/39)	21.9% (7/32)	25.9% (6/24)	n.s.
Family history for PD, % (nr/tot)	62.5% (10/16)	35.9% (14/39)	43.7% (14/32)	29.2% (7/24)	0.05 (C vs M+S+R)*
AKR onset, % (nr/tot)	95.6% (15/16)	81.8% (27/33)	89.3 % (25/28)	54.2% (13/24)	0.0238 (M vs R)* 0.0202 (R vs M+S+C)*
H&Y at last evaluation, mean $\pm$ SD (tot)	2.4 $\pm$ 0.9 (16)	2.8 $\pm$ 1.1 (36)	2.6 $\pm$ 0.8 (32)	2.6 $\pm$ 1.1 (24)	n.s.
H&Y $\geq$ 2.5, % (nr/tot)	56.2% (9/16)	60.0% (21/35)	76.7% (23/30)	58.3% (14/24)	n.s.
H&Y / disease duration, mean $\pm$ SD (tot)	0.4 $\pm$ 0.2 (16)	0.5 $\pm$ 0.3 (36)	0.3 $\pm$ 0.3 (32)	0.4 $\pm$ 0.5 (24)	0.0027 (S vs M)* 0.0131 (S vs R)*

					0.0087 (M vs S+C+R)* 0.006 (S vs M+C+R)*
Anxiety, % (nr/tot)	53.8% (7/13)	54.3% (19/35)	54.8 % (17/31)	45.8% (11/24)	n.s.
Onset anxiety, (yrs), mean $\pm$ SD (tot)	3.0 $\pm$ 2.77 (7)	-0.67 $\pm$ 3.08 (12)	2.06 $\pm$ 2.41 (16)	2.4 $\pm$ 4.09 (10)	0.047 (S vs C)* 0.02 (S vs M+C+R)*
ICB, % (nr/tot)	46.7% (7/15)	34.3% (12/35)	48.4% (15/31)	16.7% (4/24)	0.0141 (M vs R)* 0.0468 (R vs M+S+C)*
Dysautonomia, % (nr/tot)	64.3% (9/16)	44.1% (15/34)	60.0% (18/30)	66.7% (16/24)	n.s
Hallucinations, % (nr/tot)	40.0% (6/15)	45.7% (16/35)	35.5% (11/31)	45.8% (11/24)	n.s.
Onset hallucinations, (yrs), mean $\pm$ SD (tot)	6.25 $\pm$ 4.86 (8)	4.63 $\pm$ 4.23 (19)	8.27 $\pm$ 8.16 (11)	6.37 $\pm$ 5.90 (8)	n.s.
Delusions, % (nr/tot)	33.3% (5/15)	20.6% (7/34)	9.7% (3/31)	20.8% (5/24)	0.0472 (M vs C)*
Onset delusions, (yrs), mean $\pm$ SD (tot)	6.0 $\pm$ 4.98 (6)	4.29 $\pm$ 3.30 (7)	3.4 $\pm$ 4.1 (5)	9.75 $\pm$ 5.85 (4)	n.s.
MCI, % (nr/tot)	18.7% (3/16)	28.6% (10/35)	28.1% (9/32)	12.5% (3/24)	n.s
Dementia, % (nr/tot)	50.0% (8/16)	28.6% (10/35)	18.7% (6/32)	45.8% (11/24)	0.0563 (M vs C)* 0.0591 (M vs R)*
Cognitive impairment, % (nr/tot)	68.7% (11/16)	57.1% (20/35)	46.9% (15/32)	54.2% (13/24)	n.s.
Motor fluctuations, % (nr/tot)	60.0% (9/15)	63.6 % (21/33)	67.7% (21/31)	56.5% (13/23)	n.s
Onset motor fluctuations (yrs) mean $\pm$ SD (tot)	5.9 $\pm$ 2.3 (7)	4.4 $\pm$ 2.7 (20)	5.7 $\pm$ 4.1 (18)	4.2 $\pm$ 2.1 (9)	n.s

Non motor fluctuations, % (nr/tot)	38.5% (5/13)	50.0% (14/30)	41.4% (12/29)	37.5% (9/24)	n.s.
Onset non motor fluctuations, (yrs), mean ± SD (tot)	3.5 ± 3.5 (2)	3.5 ± 2.7 (12)	5.1 ± 5.2 (10)	8.2 ± 2.6 (4)	0.0339 (R vs M) 0.0212 (R vs S)* 0.0091 (R-M+S+C)*
Dyskinesia, % (nr/tot)	42.9% (6/14)	52.9% (18/33)	47.1% (16/32)	45.4% (11/23)	n.s.
Onset dyskinesia (yrs), mean ± SD (tot)	6.5 ± 3.7 (4)	5.3 ± 1.4 (15)	5.8 ± 3.1 (13)	6.0 ± 2.1 (6)	n.s.
LEDD, mean dose ± SD (tot)	629.0 ± 319.1 (16)	632.7 ± 424.6 (30)	606.1 ± 383.6 (31)	563.6 ± 362.3 (24)	n.s.
LEDD Dag, mean dose ± SD (tot)	141.7 ± 156.1 (8)	287.9 ± 268.3 (28)	444.9 ± 386.4 (29)	123.4 ± 135.6 (20)	0.0023 (C vs M)* 0.0002 (M vs R)* 0.0181 (S vs R)* 0.0017 (M vs S+C+R)* 0.0264 (C vs M+S+R)* 0.0201 (R vs M+S+C)*

*Significant differences (p value <0.05) obtained from pairwise comparisons are shown in bold (type of comparison in brackets). (nr/tot): number of patients presenting the feature / total number of patients for whom the information was available; (tot): total number of patients for whom the information was available; yrs: years.

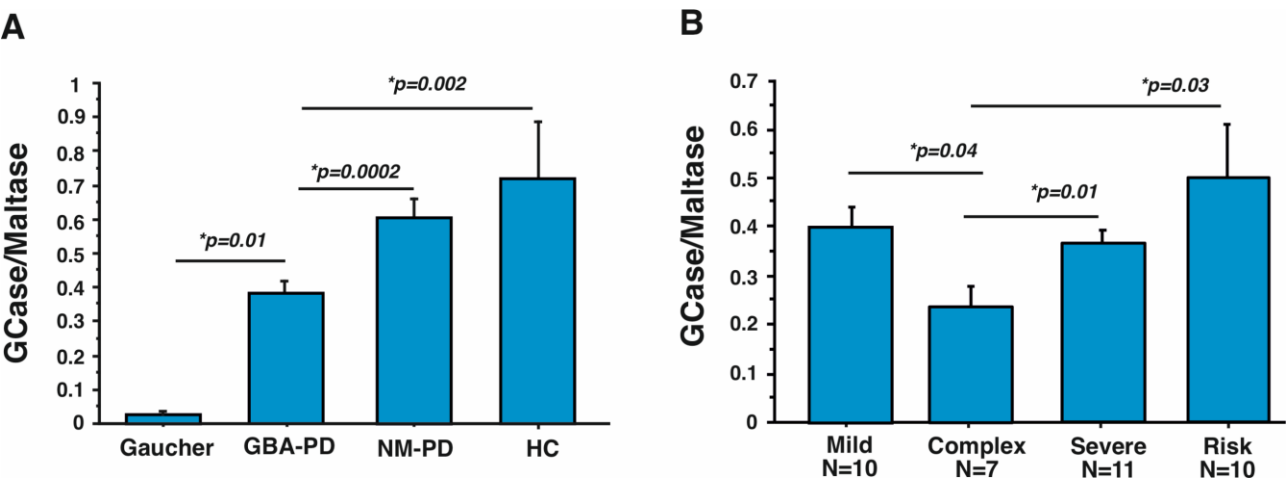
AKR: akinetic-rigid; LEDD: total Levodopa equivalent daily dose; D-ag:dopamine agonists; H&Y: Hoehn and Yahr Scale; ICB: impulsive compulsive behavior; MCI: mild cognitive impairment

Supplementary Table 7. Cox Proportional Hazard Model for cognitive impairment, hallucination, delusion, psychosis on the disease duration-time scale

Cognitive impairment (MCI and Dementia)				
<i>Univariable</i>			<i>Multivariable</i>	
	HR (95% CI)	p	aHR (95% CI)	p
GBA Mild vs. Risk	2.2 (1.0, 4.5)	0.04	2.3 (0.8, 6.1)	0.10
GBA Mild vs Complex	1.9 (0.9, 4.3)	0.08	3.2 (1.2, 8.2)	0.02
GBA Mild vs. Severe	2.2 (1.1, 4.4)	0.02	2.9 (1.3, 6.6)	0.01
Age			1.0 (0.9, 1.0)	0.25
Gender			1.2 (0.7, 2.3)	0.46
LEDD			0.9 (0.9, 0.9)	<0.001
LEDD Dag			1 (0.9, 1.0)	0.86
Hallucinations				
<i>Univariable</i>			<i>Multivariable</i>	
	HR (95% CI)	p	aHR (95% CI)	p
GBA Mild vs. Risk	2.5 (1.0, 6.1)	0.04	3.3 (0.9, 10.9)	0.05
GBA Mild vs Complex	2.6 (0.9, 7.0)	0.06	3.4 (1.0, 11.5)	0.05
GBA Mild vs. Severe	2.6 (1.2, 5.8)	0.02	4.2 (1.6, 11.2)	0.004
Age			0.9 (0.9, 1.0)	0.77
Gender			1.6 (0.7, 3.5)	0.22
LEDD			0.9 (0.9, 1)	0.04
LEDD Dag			1.0 (0.9, 1.0)	0.44
Delusions				
<i>Univariable</i>			<i>Multivariable</i>	
	HR (95% CI)	p	aHR (95% CI)	p
GBA Mild vs. Risk	3.4 (0.8, 14.6)	0.09	3.0 (0.5, 19.1)	0.24
GBA Mild vs Complex	5.7 (1.3, 24.5)	0.02	6.1 (1.1, 32.4)	0.04
GBA Mild vs. Severe	3.6 (0.9, 13.8)	0.07	2.9 (0.6, 14.8)	0.18
Age			1.0 (0.9, 1.0)	0.96
Gender			1.8 (0.6, 5.7)	0.27
LEDD			0.9 (0.9, 1)	0.03
LEDD Dag			1 (0.9, 1.0)	0.68

Significant values ($p < 0.05$) are shown in bold. HR: unadjusted Hazard Ratio extracted from Cox-Prop Hazard Model; aHR: adjusted HR extracted from Cox-Prop Hazard Model; LEDD, total Levodopa equivalent daily dose; Dag, dopamine agonists; MCI, mild cognitive impairment.

Supplementary Figure 1



Comparison of normalized GCase/Acid Maltase (AM) activity rate evaluated in PBMCs from healthy controls (HC), PD patient without and with GBA mutations (NM-PD and GBA-PD), and GD patients (GD) (A). GCase/AM activity rate was also compared among the four subclasses of GBA-PD patients (B).

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