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

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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, Kruskall-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](https://www-ncbi-nlm-nih-gov.operapadrepio.clas.cineca.it/SNP/snp_ref.cgi?rs=398123530) |
| 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  W184R | p.(Asn227Lys)  p.(Trp223Arg) | c.681T>G  c.667T>C | 6 | unknown | rs381418  rs61748906 |
| 1# | N370S  E326K | p.(Asn409Ser)  p.(Glu365Lys) | c.1226A>G  c.1093G>A | 9  8 | unknownǂ | rs76763715  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 carring 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. ǂsince 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§ | 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. §patients carring 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/](http://www.sift.jcvi.org/)), Mutation Taster ([www.mutationtaster.org/](http://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 ± SD (tot)** | 53.5 ± 11.6 (124) | 56.5 ± 11.5 (713) | **0.007\*** |
| **Age at last evaluation (yrs), mean ± SD (tot)** | 63.8 ± 11.6 (122) | 67.4 ± 11.3 (686) | **0.0010\*** |
| **Disease duration (yrs), mean ± SD (tot)** | 12.5 ± 7.7 (122) | 14.2 ± 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 ± SD (tot)** | 2.7 ± 1.0 (121) | 2.6 ± 0.9 (619) | 0.2899 |
| **H&Y ≥ 2.5, % (nr/tot)** | 65.2% (77/118) | 55.5% (343/618) | **0.0499\*** |
| **H&Y/disease duration (yrs), mean ± SD (tot)** | 0.4 ± 0.3 (121) | 0.3 ± 0.3 (614) | **0.0237\*** |
| **Anxiety, % (nr/tot)** | 50.8% (60/118) | 29.3% (183/624) | **<0.0001\*** |
| **Onset anxiety, (yrs), mean ± SD (tot)** | 1.5 ± 3.3 (50) | 1.5 ± 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 ± SD (tot)** | 6.9 ± 6.9 (49) | 9.2 ± 6.0 (92) | **0.0127\*** |
| **Delusions, % (nr/tot)** | 18.6% (22/118) | 9.0% (57/634) | **0.0004\*** |
| **Onset delusions (yrs), mean ± SD (tot)** | 6.6 ± 6.7 (23) | 8.5 ± 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 ± SD (tot)** | 4.9 ± 3.1 (60) | 6.3 ± 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 ± SD (tot)** | 5.0 ± 3.9 (31) | 6.6 ± 4.4 (54) | 0.994 |
| **Dyskinesia, % (nr/tot)** | 52.2% (60/115) | 47.5% (213/448) | 0.3756 |
| **Onset dyskinesia (yrs), mean ± SD (tot)** | 5.6 ± 2.3 (44) | 7.4 ± 4.1 (129) | **0.0022\*** |
| **LEDD, mean dose ± SD (tot)** | 604.2 ± 373.5 (114) | 715.9 ± 411.7 (551) | **0.0025\*** |
| **LEDD D-ag, mean dose ± SD (tot)** | 265.0 ± 295.6 (100) | 220.3 ± 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 equilavent 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 ± SD (tot)** | 51.4 ± 11.6 (16) | 51.3 ± 11.0 (38) | 54.3 ± 11.1 (32) | 57.0 ± 12.2 (24) | **0.05 (S vs M+C+R)\***  0.08 (R vs M+S=C) |
| **Age at last evaluation (yrs), mean ± SD (tot)** | 59.7 ± 10.5 (16) | 60.4 ± 11.5 (37) | 66.4 ± 10.3 (32) | 67.0 ± 11.9 (24) | **0.0451 (S vs M)\***  **0.0402 (S vs R)\*** |
| **Disease duration (yrs) , mean ± SD (tot)** | 12.3 ± 7.4 (16) | 10.9 ± 8.2 (38) | 14.6 ± 7.4 (32) | 11.4 ± 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 ± SD (tot)** | 2.4 ± 0.9 (16) | 2.8 ± 1.1 (36) | 2.6 ± 0.8 (32) | 2.6 ± 1.1 (24) | n.s. |
| **H&Y ≥ 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 ± SD (tot)** | 0.4 ± 0.2 (16) | 0.5 ± 0.3 (36) | 0.3 ± 0.3 (32) | 0.4 ± 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 ± SD (tot)** | 3.0 ± 2.77 (7) | -0.67 ± 3.08 (12) | 2.06 ± 2.41 (16) | 2.4 ± 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 ± SD (tot)** | 6.25 ± 4.86 (8) | 4.63 ± 4.23 (19) | 8.27 ± 8.16 (11) | 6.37 ± 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 ± SD (tot)** | 6.0 ± 4.98 (6) | 4.29 ± 3.30 (7) | 3.4 ± 4.1 (5) | 9.75 ± 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 ± SD (tot)** | 5.9 ± 2.3 (7) | 4.4 ± 2.7 (20) | 5.7 ± 4.1 (18) | 4.2 ± 2.1 (9) | n.s |
| **Non motor fluctuations, % (nr/tot)** | 38.5% (5/13) | 50.0% (14ì5/30) | 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 equilavent 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, delution, psychosis on the disease duration-time scale

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Cognitive impairment (MCI and Dementia)** | | | | |
| ***Univariable*** |  |  | ***Multivariable*** |  |
|  | 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** | | | | |
| ***Univariable*** |  |  | ***Multivariable*** |  |
|  | 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** | | | | |
| ***Univariable*** |  |  | ***Multivariable*** |  |
|  | 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 heathy 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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