Evolution of prodromal parkinsonian features in a cohort of GBA mutation positive individuals; a 6-year longitudinal study.

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ABSTRACT

**Objectives**—GBA1 mutations are a frequent risk factor for Parkinson disease (PD). Aim of this study is to evaluate clinical features in a group of GBA1 mutation positive individuals over a 6-year follow-up.

**Methods**—This is a longitudinal study on a cohort of GBA1 positive carriers. We enrolled 31 Gaucher Disease type 1 patients (GD), 29 GBA1 heterozygous carriers (Het GBA group) and 30 controls (HC) at baseline and followed them for 6-years. We assessed baseline motor and non-motor signs of PD in all subjects using clinical questionnaires and scales (reduced UMSARS, MoCA, UPSIT, RBDsq, MDS-UPDRS III and BDI). We repeated these at the 6-year follow-up alongside venous blood sampling for measurement of glucocerebrosidase enzymatic activity (GCase). We explored whether the GCase activity level was altered in leukocytes of these subjects and how it was related to development of PD.

**Results:** We observed a significant worsening in UMSARS, RBDsq, MDS-UPDRS III and BDI scores at the 6-year follow-up compared to baseline in both the GD and Het GBA groups. Intergroup comparisons showed that GD subjects had significantly worse scores in UPSIT, UMSARS, MoCA and MDS-UPDRS III than HC, while Het GBA displayed worse outcomes in UPSIT and MDS-UPDRS III compared to HC. In GBA1 mutation positive individuals (Het GBA and GD), an UPSIT score of 23 at baseline was correlated with worse outcome at 6 years in UPSIT, MoCA, MDS-UPDRS III and BDI.

**Conclusion:** in this 6-year long longitudinal study, GBA1 mutation positive subjects showed a worsening in motor and non-motor prodromal PD features.
INTRODUCTION

Significant advances have been made in recent years in understanding the genetic contribution to Parkinson disease (PD). Glucocerebrosidase 1 (GBA1) mutations are considered the most important risk factor for PD[1]. GBA1 encodes for the lysosomal enzyme glucocerebrosidase (GCase) and biallelic mutations in this gene cause Gaucher disease (GD)[2]. The observation that type 1 GD patients exhibit a high incidence of PD was made more than 20 years ago [3,4]. Studies have reported a significantly higher frequency of heterozygous GBA1 mutations in PD patients, suggesting a causative role of GBA1 in the pathogenesis of the disease[5–9]. Additionally, numerous genome wide association studies confirmed the strong association of GBA1 with PD, identifying an Odds Ratio higher than 5 for developing PD [1,10–13]. Recent reports have also shown that decreased GCase activity in the brain of sporadic PD are linked with increased a-synuclein levels[14,15], suggesting a role for GCase in the pathogenesis of ‘idiopathic’ PD.

The identification of the GBA1 link has therapeutic implications, as enzyme replacement and substrate reduction therapies are regularly employed to treat GD [16] and it is possible that the same strategies could be used to slow PD progression. Furthermore, numerous studies are investigating compounds that directly interact with GCase to treat GBA1 mutated PD patients [17]. Only about 30% of individuals with a GBA1 mutation develop PD [18] and so a better risk stratification is still needed to identify those at greater risk and to select participants for clinical trials for disease modifying therapy. In 2012, our group published a study on the assessment of a cohort of neurologically asymptomatic GBA1 mutation carriers and showed that cognitive, olfactory and parkinsonian motor signs were worse in not only homozygous, but also in heterozygous GBA1 mutation carriers compared to controls[19]. A 2-year follow up study confirmed these findings and their progression[20]. A separate paper will report the evolution of prodromal features over 4-5 years. The identification of clinical and biochemical markers is therefore important to phenotype GBA1 mutation positive individuals potentially at risk of developing PD.

In this study, we assessed our cohort focusing on how parkinsonian clinical markers evolved over a 6-year observation period and whether baseline findings were able to predict a worsening of these signs over
time. We also explored whether the GCase activity level is altered in peripheral blood leukocytes of these subjects and how it is related to PD development.

MATERIALS AND METHODS

Participants

This is a longitudinal study over 6 years involving type 1 GD patients, heterozygous GBA1 mutation carriers (Het GBA) and genetically unrelated controls (HC) that started in 2010[19]. The GD patients were recruited from the Lysosomal Storage Disorder Unit at the Royal Free London NHS Foundation Trust. Potential Het GBA subjects were identified among the family members of each GD patient. At baseline, HC were matched to GD patients and Het GBA for age, sex, and ethnicity. Controls had no neurological disease or systemic disease that could impair motor function. Demographic participant characteristics at baseline and 2 years-timepoint are reported in Supplementary Table 1. Exclusion criteria were the presence of neurological signs or cognitive impairment at baseline. All the participants from the original cohort[19] were invited to take part in the 6-year follow-up assessment. From the 90 subjects at baseline, 63 completed the follow-up. The main reasons for dropout (14 HC and 13 Het GBA subjects, respectively) were death (5 HC and 4 Het GBA), failure to contact (3 HC and 2 Het GBA) and withdrawal of consent (6 HC and 7 Het GBA). The protocol was approved by the North-West London Research Ethics Committee (reference number 10/H0720/21) and all subjects gave written consent before being enrolled in the study.

Follow-up evaluation

We examined all subjects with a structured clinical evaluation that included: motor and cognitive performance, olfactory function, REM sleep behavior disorder (RBD), autonomic dysfunction and depression. Motor performances were assessed by the Movement Disorders Society Unified Parkinson’s Disease Rating Scale motor subscale (MDS-UPDRS part III)[21], olfactory function by the University of Pennsylvania Smell Identification Test (UPSIT), cognitive function by the Montreal Cognitive assessment (MoCA), RBD with the RBD Questionnaire (RBDsq), depression by the Beck’s Depression Inventory version II.
(BDI) and autonomic dysfunction by a subscale of the Unified Multiple System Atrophy Rating Scale (UMSARS) that included questions 9, 10, 11 and 12 of the original scale. In accordance with the references listed below, the following cut-offs were used: MoCA score less than 26 for cognitive impairment[22], UPSIT score less than or equal to 23 for severe microsmia [23,24], BDI greater than 9 for mild depression [25], RBDsq greater than 4 for RBD[26]. For the sub-items of the MDS-UPDRS part III we used a cut-off of greater or equal to 1. Two unblinded evaluators (MA and MT) independently examined all the subjects in order to avoid possible bias on the clinical scores.

**Genotyping and GCase activity assay**

All participants were genotyped in order to confirm their GBA1 mutation status using Sanger sequencing of the GBA1 gene[27]. At the 6-year follow-up, all subjects underwent a blood test to measure the GCase activity in peripheral blood leucocytes according to commonly used methods [28,29]. The final result is reported as nmol of substrate per hour per microgram of proteins. In GD participants that were taking enzyme replacement therapy blood samples were collected just prior to the infusion (infusions were carried out every 2 weeks), in order to have a better sense of the residual enzymatic activity in each participant [30].

**Statistical Analysis**

Statistical analysis was performed using “Stata” version v.13.0 (StataCorp, Texas). For age and years of education, differences between groups were analyzed using ANOVA (analyses of variance) with groups as the independent factors. For sex, difference between groups was tested with the Chi-square test. To assess within groups differences (“baseline” vs “6 years follow-up”) in each group separately, Wilcoxon matched-pairs signed-rank test for paired samples was used, with a false discovery rate of 5% (Benjamini-Hochberg procedure)[31]. For this analysis, only participants that completed the 6 years follow-up were included. The longitudinal clinical data over 6 years were analysed using a linear mixed effect model with groups as independent variable and age, sex and years of education (when applicable) as covariates. The p-values refer to the fixed coefficients for each group compared to the others. A false discovery rate of 5% was set.
(Benjamini-Hochberg procedure). For this analysis, all participants were included, even if they dropped out before the 6-years timepoint.

In an exploratory analysis, we investigated whether baseline scores may be a predictor for worsening of motor and non-motor performances over time. Among all clinical markers at baseline, participants with severe microsmia (UPSIT <=23)[23,24] showed a trend toward deterioration of motor and non-motor symptoms. We used a two-sample Fisherman-Pitman randomization test to determine whether this trend was statistically significant. A false discovery rate of 5% was set (Benjamini-Hochberg procedure)[31].

Statistical analysis for GCase activity was performed by Kruskal-Wallis test followed by Dunn's Pairwise test for group comparison. The relationship between GCase activity and clinical parameters was performed by linear regression for UPSIT score and censored linear regression for MoCA, RBDsq, reduced UMSARS, MDS-UPDRS III and BDI to avoid ceiling and bottom effects. To check for possible differences at baseline between dropouts and non-dropouts, we used the Mann-Whitney two-sample test.

RESULTS

Demographic characteristics of subjects that completed follow up are reported in Table 1. No significant differences in gender, age and years of education were found between groups (all p > 0.05). 63 participants completed the 6-year follow-up: 31 type I GD patients, 16 Het GBA carriers and 16 HC. After re-genotyping, we identified 1 new GD patient that was previously classified as Het GBA. No GBA1 mutation was identified in HC subjects in exons 1 to 11 of the GBA1 gene. The most frequent GBA1 mutation in the Het GBA group was N370S (8/16; 50 %), followed by L444P and V394L (both 2/16; 12.5%). For GD patients, N370S/L444P was the most common genotype (7/31; 22.6%), followed by N370S/N370S (3/31; 9.68%). Among the GD patients, 25 (81%) were receiving enzyme replacement therapy and 2 (7%) were receiving substrate reduction therapy.
### TAB.1 - Demographic characteristics of the 3 groups of subjects at 6-years

<table>
<thead>
<tr>
<th></th>
<th>Tot.</th>
<th>HC</th>
<th>Het GBA</th>
<th>GD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. subjects</td>
<td>63</td>
<td>16</td>
<td>16</td>
<td>31</td>
<td>-</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>28/ 35</td>
<td>8 / 8</td>
<td>7 / 9</td>
<td>13/ 18</td>
<td>0.95</td>
</tr>
<tr>
<td>Age at baseline</td>
<td>55.13 ± 10.97</td>
<td>56.5 ± 11.</td>
<td>59 ± 9.02</td>
<td>52.39 ± 11.13</td>
<td>0.12</td>
</tr>
<tr>
<td>Years of education</td>
<td>14.04 ± 3.31</td>
<td>15.62 ± 3.51</td>
<td>13.12 ± 3.07</td>
<td>13.69 ± 3.14</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. Group comparison performed with one-way ANOVA for age and years of education, Fisher exact test was used for sex variable.

After 6 years of observation, 1 GD subject, age range 45-50 at baseline (real age not disclosed to protect the privacy of the subject), developed PD according to clinical diagnostic criteria[32]. At baseline this subject did not show any neurological signs and all the evaluated clinical parameters were within normal range except for UPSIT score (20). At 6-year evaluation, subject showed resting tremor, bradykinesia and cognitive impairment with the following clinical scores: MOCA 19, UPSIT 9, BDI 36, MDS-UPDRS III 63 (off state), UMSARS 7, RBDsq 3.

In the GD group, 5 subjects (16.1%) developed cognitive impairment, 4 subjects (12.9%) developed hyposmia, 8 subjects (25.8%) reported depression symptoms, 4 subjects (18.2%) had RBD disorders, 4 subjects (12.9%) developed global bradykinesia, 2 subjects (6.5%) developed upper limbs resting tremor, 8 subjects (25.8 %) developed abnormal posture, 6 subjects (19.4 %) developed postural instability, 10 subjects (32.3 %) developed action tremor of hands, and 4 subjects (12.5 %) developed postural tremor of hands. In accordance with the same cut-offs, in the Het GBA group, 1 subject (6.3 %) developed cognitive impairment, 3 subjects (19%) developed hyposmia, 1 subject (6.3%) developed depression symptoms, 2 subjects (13.3%) developed RBD disorders, 3 subjects (19 %) showed abnormal posture, 4 subjects (25%) developed postural instability, 1 subject (6.3 %) developed action and postural tremor of hands. In the HC group, 2 subjects (12.5 %) developed cognitive impairment, 1 subject (6.3 %) developed hyposmia, 1 subject (8.3%) developed RBD disorder, 3 subjects (19 %) showed slightly stooped posture, 1 subject (6.3%) slightly postural instability, 2 subjects (12.5 %) developed action tremor of hands and 3 subjects (18.9 %) developed postural tremor of hands (Data are summarised in Table 2). Baseline clinical scores of
participants that dropped out at follow-up are reported in Supplementary Table 2. Of the subjects that showed parkinsonian features at baseline and at 2-years follow-up [19,20], 3 GD patients and 1 Het GBA subject remained clinically stable, 1 GD patient showed a worsening of parkinsonian motor features that were not yet sufficient for a diagnosis of PD and 1 Het GBA was lost at follow-up.

Table. 2 – Description of progression of clinical features after 6 years follow-up in the 3 groups of subjects

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>Het GBA</th>
<th>GD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive impairment (MoCA &lt; 26)</td>
<td>2 (12.5%)</td>
<td>1 (6.3%)</td>
<td>5 (16.1%)</td>
</tr>
<tr>
<td>Hyposmia (Upsit cut-off &lt;= 23)</td>
<td>1 (6.3%)</td>
<td>3 (19%)</td>
<td>4 (12.9%)</td>
</tr>
<tr>
<td>Depression (BDI cut-off ≥ 10)</td>
<td>-</td>
<td>1 (6.3%)</td>
<td>8 (25.8%)</td>
</tr>
<tr>
<td>RBD disorders (RBDsq ≥ 5)</td>
<td>1 (8.3%)</td>
<td>2 (13.3%)</td>
<td>4 (18.2%)</td>
</tr>
<tr>
<td>Global bradykinesia (MDS-UPDRS part III subscore &gt;= 1)</td>
<td>-</td>
<td>-</td>
<td>4 (12.9%)</td>
</tr>
<tr>
<td>Resting tremor (MDS-UPDRS part III subscore &gt;= 1)</td>
<td>-</td>
<td>-</td>
<td>2 (6.5%)</td>
</tr>
<tr>
<td>Abnormal posture (MDS-UPDRS part III subscore &gt;= 1)</td>
<td>3 (18.9%)</td>
<td>3 (19%)</td>
<td>8 (25.8%)</td>
</tr>
<tr>
<td>Postural instability (MDS-UPDRS part III subscore &gt;= 1)</td>
<td>1 (6.3%)</td>
<td>4 (25%)</td>
<td>6 (19.4%)</td>
</tr>
<tr>
<td>Action tremor (MDS-UPDRS part III subscore &gt;= 1)</td>
<td>2 (12.5%)</td>
<td>1 (6.3%)</td>
<td>10 (32.3%)</td>
</tr>
<tr>
<td>Postural tremor (MDS-UPDRS part III subscore &gt;= 1)</td>
<td>3 (18.9%)</td>
<td>1 (6.3%)</td>
<td>4 (12.9%)</td>
</tr>
<tr>
<td>Clinically defined PD</td>
<td>-</td>
<td>-</td>
<td>1 (3.2%)</td>
</tr>
</tbody>
</table>

Data are presented as number of cases and percentage of all subjects within group. Cut-off references are reported in the Methods section.
Evolution of clinical markers over 6 years

Means and standard errors of the clinical scales for participants that completed the 6-year follow-up are reported in table 3 and figure 1. At 6 years, both the GD and Het GBA groups displayed a significant deterioration in the reduced UMSARS (p < 0.001 and =0.001 respectively), RBDsq (p <0.001 and <0.001 respectively), MDS-UPDRS III (p<0.001 and =0.010 respectively) and BDI (p =0.001 and =0.007 respectively) compared to baseline. Neither the GD nor Het GBA groups as a whole showed any significant worsening in the MoCA and UPSIT scores. The HC group displayed a significant worsening in UPSIT (p =0.013), UMSARS (p =0.004), BDI (0.031) compared to baseline, while the MoCA score showed a significant improvement (p =0.019).

To compare longitudinal data between the three groups, we ran a mixed effect linear regression model. In this analysis, the GD group had significantly worse scores than HC in UPSIT (p 0.001), reduced UMSARS (p 0.019), MoCA (p 0.006) and MDS-UPDRS III (p 0.001), while the Het GBA group displayed worse outcomes than HC in UPSIT (p <0.001) and MDS-UPDRS III (p =0.001). We observed the same trend of worse performance of GD and Het GBA compared to HC in RBDsq and BDI scores, although it did not reach statistical significance (Figure 1). Main findings are summarized in Table 3. Parameter estimates for the longitudinal models are reported in Supplementary Table 3.
### TAB. 3 – Evolution of clinical markers over 6 years and comparison between groups

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>Het GBA</th>
<th>GD</th>
<th>P (between a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P1</td>
</tr>
<tr>
<td><strong>UPSIT</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.013*</td>
</tr>
<tr>
<td>Baseline</td>
<td>35.15 (± 0.50)</td>
<td>31.00 (± 1.38)</td>
<td>32.39 (± 0.91)</td>
<td></td>
</tr>
<tr>
<td>Follow-up</td>
<td>31.54 (± 1.17)</td>
<td>30.29 (± 1.25)</td>
<td>31.00 (± 1.37)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>13</td>
<td>14</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>P (within a)</td>
<td>0.004*</td>
<td>0.001*</td>
<td>&lt;0.001*</td>
<td>0.019</td>
</tr>
<tr>
<td><strong>UMSARS reduced</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0</td>
<td>0.25 (± 0.14)</td>
<td>0.39 (± 0.14)</td>
<td></td>
</tr>
<tr>
<td>Follow-up</td>
<td>1.21 (± 0.37)</td>
<td>2.13 (± 0.44)</td>
<td>1.90 (± 0.34)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>14</td>
<td>16</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>P (within a)</td>
<td>0.117</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>0.183</td>
</tr>
<tr>
<td><strong>MoCA</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.019*</td>
</tr>
<tr>
<td>Baseline</td>
<td>26.56 (± 0.50)</td>
<td>25.81 (± 0.7)</td>
<td>26.40 (± 0.40)</td>
<td></td>
</tr>
<tr>
<td>Follow-up</td>
<td>28.06 (± 0.42)</td>
<td>27.19 (± 0.46)</td>
<td>26.83 (± 0.52)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>16</td>
<td>16</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>P (within a)</td>
<td>0.048</td>
<td>0.010*</td>
<td>&lt;0.001*</td>
<td>0.001*</td>
</tr>
<tr>
<td><strong>MDS-UPDRS III</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.44 (± 0.27)</td>
<td>0.94 (± 0.46)</td>
<td>2.81 (± 1.08)</td>
<td></td>
</tr>
<tr>
<td>Follow-up</td>
<td>2.06 (± 0.77)</td>
<td>3.31 (± 1.05)</td>
<td>7.52 (± 2.40)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>16</td>
<td>16</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>P (within a)</td>
<td>0.048</td>
<td>0.010*</td>
<td>&lt;0.001*</td>
<td>0.001*</td>
</tr>
<tr>
<td><strong>BDI</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.031*</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.00</td>
<td>0.00</td>
<td>2.12 (± 1.28)</td>
<td></td>
</tr>
<tr>
<td>Follow-up</td>
<td>3.00 (± 1.48)</td>
<td>5.67 (± 1.19)</td>
<td>10.00 (± 2.16)</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>7</td>
<td>9</td>
<td>26</td>
<td></td>
</tr>
</tbody>
</table>

Data are reported as means (± standard errors).

a Wilcoxon matched-pairs signed-rank test comparing baseline and 6-year follow-up scores within each group separately. A false discovery rate of 5% was set using the Benjamini-Hochberg procedure.

b Linear mixed effect model comparing the scores of the 3 groups over time. A false discovery rate of 5% was set using the Benjamini-Hochberg procedure. P1 = GD vs HC, P2 = Het GBA vs HC, P3 = GD vs Het GBA

* Significant result
UPSIT at baseline predicts a greater deterioration in the clinical scores at 6 years in GBA1 positive individuals

Among the Het GBA and GD groups (pooled together), a baseline UPSIT score of less than or equal to 23 was associated with a subsequent greater deterioration in UPSIT (-5.0 points vs -0.7 points, p=0.02), MoCA (-2.0 points vs +1.5 points, p=0.02), MDS-UPDRS III (10.0 points vs 1.9 points, p=0.01) and BDI (17.0 points vs 4.6 points, p=0.01). Results are reported in table 4 and figure 2. No difference was observed for RBDsq and reduced UMSARS scores. None of the HC subjects had a baseline UPSIT score less than or equal to 23 so no comparison with HC was performed (Table 4, Figure 2).

**TAB. 4 – UPSIT at baseline and 6-year change in clinical markers in pooled GBA Het and GD groups**

<table>
<thead>
<tr>
<th></th>
<th>UPSIT &gt; 23</th>
<th>UPSIT &lt;= 23</th>
<th>p-value (FP test) a</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPSIT</td>
<td>-0.7 ± 0.5 (33)</td>
<td>-5.0 ± 3.1 (4)</td>
<td>0.020 *</td>
</tr>
<tr>
<td>UMSARS</td>
<td>1.4 ± 0.2 (40)</td>
<td>2.8 ± 1.0 (4)</td>
<td>0.080</td>
</tr>
<tr>
<td>RBDsq</td>
<td>2.6 ± 0.5 (32)</td>
<td>1.7 ± 0.3 (3)</td>
<td>0.727</td>
</tr>
<tr>
<td>MoCA</td>
<td>1.5 ± 0.4 (40)</td>
<td>-2 ± 1.7 (3)</td>
<td>0.022 *</td>
</tr>
<tr>
<td>MDS-UPDRS III</td>
<td>1.9 ± 0.8 (40)</td>
<td>10.0 ± 5.3 (4)</td>
<td>0.010 *</td>
</tr>
<tr>
<td>BDI</td>
<td>4.6 ± 1.3 (29)</td>
<td>17 ± 6.1 (3)</td>
<td>0.012 *</td>
</tr>
</tbody>
</table>

Difference between 6-year follow up and baseline measurements. Data are reported as mean ± standard error (number of subject).

a Nonparametric two-sample Fisher-Pitman permutation test. The statistic used is the difference in means of two samples. A false discovery rate of 5% was set using the Benjamini-Hochberg procedure.

* Significant result.

**GCase activity in GBA1 mutation carriers’ leucocytes**

We collected blood samples from 56 subjects (14 HC, 16 GBA Het, 26 GD). GCase activity was significantly lower in both GD and Het GBA subjects compared to controls. The GD group also displayed a significantly lower GCase activity compared to the Het GBA group. Results are shown in Figure 3. No significant correlation between clinical parameters and GCase activity was observed between the three groups.
DISCUSSION

Although GBA1 mutations are recognised genetic risk factors for developing PD, both in the biallelic and in heterozygote carrier states [1,33], the underlying mechanisms that determine penetrance are incompletely understood. Hyposmia, cognitive dysfunction, autonomic dysfunction, RBD, and depression are recognised prodromal features of PD and they are prevalent among GBA1 positive individuals [17,34].

In this study, we assessed GD, Het GBA carriers and a group of GBA1 mutation-negative controls for the presence of non-motor features and parkinsonian motor signs that could determine an increased risk of developing PD. We analyzed these individuals at baseline and after 6 years in order to evaluate the clinical progression of these features and therefore to identify potential clinical early markers of neurodegeneration.

The main finding of our study is a clear biological effect of GBA1 mutations: Het GBA carriers and GD patients showed progressive worsening of scores in most of the clinical markers evaluated over the 6 years compared to HC. Moreover, at 6 years, some Het GBA carriers and the majority of GD subjects developed non-motor and motor symptoms and 1 GD subject developed PD. The major deterioration observed was for olfactory function, motor scores and cognitive functions.

After 6 years of follow-up, the HC group additionally showed a deterioration in some of the parameters (UPSIT, reduced UMSARS and BDI). This is particularly evident for olfaction, where the HC group had the largest difference of the three groups from baseline. However, both GD and Het GBA showed consistently worse scores compared to HC over the 6 years and the statistical analysis shows that this difference is significant for UPSIT, MDS-UPDRS III and MoCA.

MoCA results require particular mention, as the HC group showed a statistically significant improvement (not reduction) at 6-years, the same slight improvement has been shown in the other groups although they did not reach the significance. The lack of efficacy in detecting a cognitive decline is probably attributable to a “training effect” as participants repeated the same test multiple times, and this may have limited our evaluation.
These findings are in line with the previous papers published by our group [19,20] and support the hypothesis that GBA1 mutations have a significant effect on the development of early clinical markers of neurodegeneration. In particular, the six year follow-up of our study confirms the deterioration of motor and non-motor features that were found in GBA1 positive individuals in the previous publications[19,20]. Over the 6-year follow-up, only 1 GD patient developed clinically definite PD associated with cognitive impairment.

Previous studies tried to quantify the risk of developing PD in GBA1 mutation carriers and they estimated this risk at 5-30% by the age of 80 [35]. Given that the mean age at recruitment in our study was relatively low (<65 years), this finding is not unexpected. Moreover, all subjects were recruited without any neurological symptom at baseline. This likely introduced a selection bias, excluding individuals with a higher risk of developing PD and thus reducing the numbers of converters. Nonetheless, some of the GBA1 mutation positive individuals showed significant motor and non-motor features at six years which, although not diagnostic for PD so far, might be suggestive of a progression toward a neurodegenerative disorder, such as PD or Lewy Body Dementia[36].

As an exploratory analysis, we sought to define clinical characteristics at baseline that could predict a worse outcome at 6 years. We found that a low baseline UPSIT score (severe microsmia) was a strong predictor for greater deterioration in motor and non-motor markers. Olfactory dysfunction is considered an early ‘pre-clinical’ sign of PD in the general population. However, by assessing this prodromal feature (low UPSIT score) in a population that has a genetic predisposition to PD, we can amplify its strength in predicting the development of the disease. Of course, given the small numbers on which this particular analysis was carried out, it is mandatory to confirm our results on a bigger sample.

At 6 years, we also analysed the GCase activity level in peripheral blood leukocytes of our cohort. As expected, mean GCase enzymatic activity in GD was significantly lower compared to other groups while it was intermediate in Het GBA carriers. GCase is a lysosomal enzyme that hydrolyzes glucosylceramide to glucose and ceramide. Reduced GCase enzymatic activity is reported in patients with PD and Lewy body dementia in brain autopsy studies [37] CSF, [38,39] leucocytes [40] and monocytes[41] suggesting a lower GCase enzymatic activity in peripheral cells may be a potential early marker of PD[40,42]. Accordingly, we
explored whether clinical markers at 6 years correlated with GCase enzymatic activity among GBA Het and GD. No significant association was found between GCase activity and the clinical scales (UPSIT, reduced UMSARS, MDS-UPDRS III, MOCA, RBDsq and BDI). Alcalay et al[40] tested the association between GCase enzymatic activity and PD severity and reported that in patients with idiopathic PD, higher GCase enzymatic activity was associated with longer disease duration and a milder disease course. No data are available on the association between GCase activity and parkinsonian clinical features in GBA Het carriers and further studies are needed to define this aspect.

A limitation of our study is the lack of longitudinal measurement of GCase activity. It would be interesting to assess potential changes in GCase activity over time and correlate to disease progression. Furthermore, we acknowledge that the small sample size, as a consequence of the drop-out rate after 6 years, constitutes a limitation of this study and calls for validation of our results in an independent cohort. It is also possible that the participants that dropped out at follow-up were the most clinically impaired. This could introduce an attrition bias, where the GBA1 positive individuals with the higher risk of developing PD were lost at follow-up before the development of motor symptoms. Moreover, since the dropouts were all within the Het GBA and HC groups, a surveillance bias might explain, at least partially, the higher incidence of non-motor symptoms in the GD group compared to the other groups. In this regard, we did not find any significant difference in the baseline clinical scores of dropout participants (supplementary table 2). The longitudinal model we used also partially overcomes this problem by taking into account incomplete assessment from dropout participants, although this required treating as continuous variables that should ideally be treated as categorical, in particular the RBDsq. Finally, we defined the presence of RBD by using only the RBDsq. While this is considered a good screening tool, polysomnography is required to confirm the diagnosis of RBD. Nonetheless, our study includes a significant period of observation (6 years) in a unique cohort of GBA1 positive individuals carefully evaluated for the presence of prodromal features over time. We were able to confirm the biological effect of GBA1 mutations in determining clinical features suggestive of PD and we identified hyposmia as a predictive factor for deterioration in this population.
Figures titles and legends

**FIG. 1 – Evolution of clinical markers over 6 years**

The graphs show the mean scores at baseline and at the 6-year follow-up for HC, Het GBA and GD subjects.

HC = healthy controls Het GBA = heterozygotes GBA1 mutation carriers GD = Gaucher disease patients

* a Het GBA statistically different from HC

b GD statistically different from HC

*6-year score significantly different from baseline score

**FIG. 2 – UPSIT at baseline as predictor of worse clinical outcome at 6 years**

The graphs show the mean difference between 6-year and baseline scores for GBA1 positive subjects (Het GBA and GD), stratified for baseline UPSIT (cutoff <=23). A p-value <0.05 is considered significant.

* Significant difference

**FIG. 3 – GCase activity level in leucocytes in the 3 groups**

Box plots of GCase activity for the 3 groups. Group comparison performed with Kruskal-Wallis followed by post-hoc analysis with Dunn’s Pairwise Comparison test. After Bonferroni correction, a p-value of less than 0.017 was considered significant. HC range: 7.7 - 17.47; Het GBA range: 4.01 - 12.76; GD range: 0.26 - 4.48. HC vs GBA Het p< 0.0001; HC vs GD p< 0.0001; GBA Het vs GD p< 0.0001

*Significant difference.
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