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Research article

Mutations in glucocerebrosidase are a major genetic risk factor for Parkinson's disease and increase susceptibility to dementia in a Flanders-Belgian cohort

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HIGHLIGHTS

- *GBA* mutations are a strong genetic risk factor for Parkinson disease (PD).
- A heterozygous *GBA* mutation was identified in 4.5% of PD patients.
- More severe PD motor symptoms are observed in *GBA* mutation carriers.
- *GBA* mutation status is an independent predictor for dementia in PD patients.

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ABSTRACT

Objective: To investigate the frequency of glucocerebrosidase (*GBA*) mutations in a Flanders-Belgian Parkinson's disease (PD) patient cohort and to assess genotype-phenotype correlations.
Methods: We performed an in-depth sequencing of all coding exons of *GBA* in 266 clinically well-characterized PD patients and 536 healthy control individuals.
Results: We identified rare, heterozygous *GBA* mutations in 12 PD patients (4.5%) and in 2 healthy control individuals (0.37%), confirming the genetic association of *GBA* mutations with PD in the Flanders-Belgian population ($p < 0.001$). The patient carriers had a more severe Unified Parkinson's Disease Rating Scale (UPDRS) motor score than non-carriers. Also, *GBA* mutation status was a significant, independent predictor for the presence of dementia (OR = 12.43, 95% CI: 2.27–68.14. $p = 0.004$). Genetic association of PD with the common p.E326K and p.T369M variants in *GBA* was absent.
Conclusion: In our Flanders-Belgian cohort, carrier status of a heterozygous *GBA* mutation was a strong genetic risk factor for PD. The *GBA* mutation frequency of 4.5% is comparable to previously reported data in other European PD patient cohorts. Furthermore, our clinical data suggest a more severe motor phenotype and a strong predisposition to dementia in *GBA* mutation carriers.

1. Introduction

About 5–10% of Parkinson's disease (PD) patients suffer from an inherited monogenic form of the disease. Several genetic variants or copy number variations (CNVs) in autosomal dominant (*SNCA*, *LRRK2*, *VPS35*) or recessive (*PARK2*, *PINK1*, *DJ-1*) genes have a pathogenic role in the development of PD [3,16,23,26,32,33,36,37]. Furthermore, genetic variants in numerous PD genes (e.g. *SNCA*, *MAPT*, *LRRK2*), that are increasing susceptibility for sporadic PD, have been identified in candidate gene-based as well as in genome-wide association studies [18,21]. Heterozygous mutations in *GBA* have been associated with an increased risk for PD in the majority of the studies, and *GBA* mutation status is considered an important

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Table 1
Clinical features of the Flanders-Belgian PD cohort, the *GBA* mutation carriers and non-carriers.

	Total cohort N = 266	Mutation carriers N = 12	Non-carriers N = 254	p-value
Onset age (mean ± SD)	60.3 ± 11.4	58 ± 8.0	60.4 ± 11.5	0.241
Age (mean ± SD)	67.1 ± 10.4	64.1 ± 6.8	67.2 ± 10.4	0.151
M/F ratio	1.3	1.4	1.3	0.953
Disease duration (mean ± SD)	6.8 ± 5.7	6.1 ± 3.9	6.8 ± 5.6	0.941
Familial history (number and %)	82/266 (30.8%)	4/12 (33.3%)	78/254 (30.7%)	0.847
Resting tremor (number and %)	130/266 (48.9%)	4/12 (33.3%)	126/254 (49.6%)	0.270
UPDRS III	25.4 ± 13.7	34.5 ± 14.3	24.9 ± 13.5	0.039
HY stage	2.2 ± 0.9	2.7 ± 0.8	2.2 ± 0.9	0.045
Axial score	4.8 ± 4.1	7.1 ± 4.8	4.7 ± 4.1	0.091
Dementia (number and %)	26/266 (9.8%)	5/12 (41.7%)	21/254 (8.3%)	0.003

Note: SD: standard deviation, M/F ratio: male/female ratio; UPDRS III: Unified Parkinson's Disease Rating Scale part III (motor scale); HY stage: Hoehn and Yahr stage.

genetic risk factor for PD [17,22,31]. Several clinical studies have suggested that PD patients carrying a *GBA* mutation, display particular clinical features such as an earlier onset age and a higher risk to develop cognitive decline [13,15,17,29]. In addition, *GBA* mutations also increase the risk for dementia with Lewy bodies, indicating that these mutations probably have substantial influence on the clinical phenotype of Lewy body disorders [20].

In the present study, we investigated the contribution of genetic variants in all *GBA* coding exons to the genetic etiology of PD in a cohort of clinically well-characterized Belgian patients. Moreover, we attempted to identify particular clinical features in carriers of a *GBA* mutation.

2. Methods

2.1. Study population

The Flanders-Belgian cohort consisted of 266 PD patients (see Table 1) who were recruited in ambulatory neurology clinics or via cooperation with patient alliance groups. All patients were of Caucasian ethnicity, fulfilled the NINDS diagnostic criteria for PD and underwent a full neurological examination [14]. Motor symptoms were assessed with the Unified Parkinson Disease Rating Scale part III (UPDRS motor score) and Hoehn-and-Yahr stage (H&Y) in 'on' state. The axial score was calculated as previously reported (sum of UPDRS III items 18, 27, 28, 29 and 30) [28]. To detect probable PD with dementia (PDD) the Movement Disorder Society Task Force practical algorithm was used [9].

The control cohort consisted of 536 unrelated individuals of Caucasian ethnicity living throughout Flanders-Belgium. The average age at inclusion was 68.7 ± 10.5 years. The control individuals had no neurological or psychiatric familial antecedents and did not suffer from any organic disease involving the central nervous system. Moreover, all control individuals obtained a score over 25 points on the Mini-Mental State Examination [12].

Written informed consent was obtained from all patients and control individuals. The ethical committee of the Antwerp University Hospital and the University of Antwerp approved the current study.

2.2. Genetic analyses

We performed in-depth Sanger sequencing of PCR amplicons of the 11 exons of *GBA* and the intron-exon boundaries. Primer Express 2.0 (Applied Biosystems, Foster City, CA, USA) and Primer3 were used for primer design [27]. Primer sequences and PCR conditions are available upon request. We used the Big Dye Terminator Cycle sequencing kit (Applied Biosystems) and analyzed the sequences on an automated ABI3730 DNA analyzer (Applied Biosystems). Visual detection of nucleotide variants was performed with the in-house developed tool NovoSNP or with the Seqman (DNASTAR Inc, Madison, WI, USA) software package [34]. The pathogenic effect of two rare novel genetic variants was further assessed with the prediction software PMut, SNPs&Go and Polyphen2 [6,11].

2.3. Statistical analyses

Chi-square or Fisher's exact statistics were used to compare mutation frequencies between groups. Comparing means or distributions between groups was performed with an independent samples *t*-test or Mann-Whitney *U* test, depending on the presence of a normal distribution. Multivariate logistic regression was performed to assess the association of *GBA* mutation status and dementia. We used the following independent variables in a backward stepwise model (probability entry = 0.05 and probability removal = 0.10): UPDRS III motor score, axial score, H&Y stage and *GBA* mutation status. Significance levels were set at 0.05. Statistical analyses were performed using IBM SPSS Statistics version 23.

3. Results

A mean onset age of 60.3 ± 11.4 years and a mean age at examination of 67.1 ± 10.4 years were observed in the PD patient cohort (n = 266) (Table 1). The male to female ratio was 1.3. The mean disease duration was 6.8 ± 5.7 years with a mean UPDRS III motor score of 25.4 ± 13.7 and a median H&Y stage of 2 (range 1–5) in 'on' state. About one third (82/266, 30.8%) of the patients had at least one first- or second-degree family member suffering from PD. Probable PD dementia was diagnosed in 9.8% (26/266) of the PD patients.

Sequence analysis of *GBA* revealed 9 rare exonic and 23 rare intronic variants, and one recombinant variant (Supplementary Table 1). Rare coding *GBA* variants were present in 4.5% (12/266) of PD patients, respectively in 4.9% (4/82) of familial and in 4.3% (8/184) of sporadic PD patients. Most of the *GBA* mutations were observed in one or a few patients, and were non-synonymous, frameshift or recombinant exonic variants that were known to be pathogenic (p.D140H, p.Q256SfsX9, p.L324P, p.N370S, p.L444P and recNcil) [17,19,20]. Two common variants – p.E326K and p.T369M – were identified in patients and control individuals (Table 2); p.E326K was found in 12 PD patients (12/266, 4.5%) and 15 controls individuals (15/536, 2.8%) and, p.T369M in 3 PD patients (3/266, 1.1%) and 11 control individuals (11/536, 2.0%). In two control individuals, we also identified a known pathogenic *GBA* variant. A 74 year-old control person carried the p.N370S and a 54-year-old control person the p.L444P mutation. Furthermore, we identified two novel rare exonic variants in PD patients: p.G39R and p.H529R. The p.G39R variant is coding for the last amino acid residue of the glucocerebrosidase signal peptide and was absent in the control cohort. A pathogenic effect of the p.G39R mutation was predicted by Pmut ("pathological"), SNPs&Go ("disease-related") and PolyPhen2 ("possibly damaging"). The p.H529R variant was also absent in the