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Reference:

Crosiers David, Verstraeten Aline, Wauters Eline, Engelborghs Sebastiaan, Peeters Karin, Mattheijssens Maria, De Deyn Peter Paul, Theuns Jessie, Van Broeckhoven Christine, Cras Patrick.- Mutations in glucocerebrosidase are a major genetic risk factor for Parkinsons disease and increase susceptibility to dementia in a Flanders-Belgian cohort
Neuroscience letters - ISSN 0304-3940 - 629(2016), p. 160-164
Full text (Publisher's DOI): <http://dx.doi.org/doi:10.1016/J.NEULET.2016.07.008>
To cite this reference: <http://hdl.handle.net/10067/1351010151162165141>

Accepted Manuscript

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PII: S0304-3940(16)30494-3
DOI: <http://dx.doi.org/doi:10.1016/j.neulet.2016.07.008>
Reference: NSL 32163

To appear in: *Neuroscience Letters*

Received date: 10-5-2016
Revised date: 4-7-2016
Accepted date: 6-7-2016

Please cite this article as: David Crosiers, Aline Verstraeten, Eline Wauters, Sebastiaan Engelborghs, Karin Peeters, Maria Mattheijssens, Peter P. De Deyn, Jessie Theuns, Christine Van Broeckhoven, Patrick Cras, Mutations in glucocerebrosidase are a major genetic risk factor for Parkinson's disease and increase susceptibility to dementia in a Flanders-Belgian cohort, *Neuroscience Letters* <http://dx.doi.org/10.1016/j.neulet.2016.07.008>

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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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Word/character count:

Abstract: 1309 characters and spaces

Body text: 1872 words

Tables: 2

References: 37

Supplementary data files: 1

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.

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 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.

Keywords:

Parkinson's disease, glucocerebrosidase, *GBA*, genotype-phenotype correlation

INTRODUCTION

About 5 to 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 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.

METHODS

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.

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].

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.

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 years-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 control cohort and a pathogenic effect of this variant was predicted by Pmut (“pathological”), SNPs&Go (“disease-related”) and Polyphen2 (“possibly damaging”).

We calculated a strong genetic association between carrier status of a rare exonic *GBA* variant and PD (OR=12.61; 95%; CI=2.66-82.21; $p<0.001$). The p.E326K and p.T369M mutations were not associated with PD in our patient/control cohorts. The mean onset age in p.E326K and p.T369M patient carriers was 52.6 ± 12.6 years (range: 33-79 years). The clinical features of PD patient carriers of a rare *GBA* variant were compared to those of the PD patient non-carriers. The mean onset age in rare *GBA* variant carriers was 58 ± 8 years (range: 49-75 years). Onset age, age at inclusion and disease duration were not significantly different between carriers and non-carriers. Incidence of affected first- or second-degree family members was similar among carriers and non-carriers. However, the UPDRS III ‘on’ motor score was significantly higher in carriers (34.5 ± 14.31 vs. 24.95 ± 13.54 , $p=0.039$), suggesting more severe motor and axial symptoms in the *GBA* mutation carriers. Also, the H&Y stage showed a significantly more pronounced severity in *GBA* mutation carriers ($p=0.045$). Furthermore, frequency of dementia was significantly higher in carriers as compared to non-carriers ($p=0.002$). In a multivariate logistic regression analysis, we confirmed that *GBA* mutation status (OR= 12.43, 95% CI: 2.27-68.14, $p=0.004$) is an independent predictor of dementia in our study population.

DISCUSSION

Pathogenic homozygous or compound heterozygous loss-of-function *GBA* mutations in Gaucher's disease lead to deficiency of the lysosomal beta-glucocerebrosidase enzyme and subsequent lysosomal accumulation of glycolipid substrates [30]. Conversely, pathogenic heterozygous *GBA* mutations are considered the most important genetic risk factor for PD. Moreover, pathogenic *GBA* mutations are more common in PD patients than pathogenic mutations in the most prevalent, autosomal dominant, causal PD genes (*LRRK2* and *SNCA*) [31]. However, the penetrance of *GBA* mutations for PD at age 80 is rather low and was estimated at approximately 30 % [2]. The pathology in PD patients, carrying a *GBA* mutation, is similar to idiopathic patients [22]. There is some evidence for more widespread Lewy body pathology, but this finding has not been confirmed by other neuropathological studies [7, 25].

In this study, we reported the first in-depth sequencing study of the *GBA* coding sequences in a PD patient cohort with Flanders-Belgian ancestry. The *GBA* mutation frequency of 4.5 % that we observed, is in accordance with mutation frequencies reported in other European, non-Jewish PD populations [1, 4, 17, 31]. In addition to known pathogenic *GBA* mutations, we identified two novel missense mutations (p.G39R and p.H529R), each occurring in a single PD patient. Both *GBA* variants were absent in the control cohort. *In silico* prediction, using three different software programs, indicated that both amino acid substitutions were likely pathogenic. Previously, we reported another novel *GBA* mutation in another PD patient, the frameshift mutation p.Q256SfsX9 which leads to a premature stop codon [19]. Pathogenic mutations that were observed in multiple patients were p.N370S (in 3 patients and 1 control) and p.L444P (in 3

patients and 1 control). We calculated a 12-fold increase in risk for individuals carrying a pathogenic rare coding variant or recombinant allele in the Flanders-Belgian cohorts. The common genetic variants, p.E326K and p.T369M, were not associated, though other studies had reported significant association of the p.E326K variant with PD in a single association study, as well as in a meta-analysis of several genome-wide association studies [10, 24].

GBA mutation carriers in our study cohort displayed more severe motor symptoms, as illustrated by a higher UPDRS III motor score and a worse H&Y stage. These findings could not be explained by a difference in disease duration, as mean disease duration was similar in the carrier and non-carrier groups. Nor did we observe a significant earlier disease onset age in mutation carriers. However, onset age in mutation carriers was between 1.7 to 6.0 years earlier in previous studies with larger sample sizes [8]. Evidence for a more rapid progression of motor symptoms in *GBA* mutation carriers was reported in some studies, but further longitudinal studies are needed to confirm this observation [5, 35]. Also in our study, *GBA* mutation status was an independent predictor for dementia in our PD patient cohort, which is in accordance with other reports [29, 31, 35].

Potential limitations of our study include the small number of *GBA* mutation carriers and the rather low prevalence of PD patients with dementia, which might have biased association data. The patients were mainly recruited in a tertiary referral medical center and through collaboration with patient alliance groups. This patient recruitment strategy could possibly account for selection bias, leading to an enrichment of the study population with familial PD patients. Differences in ethnic background can jeopardize comparison of our *GBA*

mutation analysis with previous studies. Mutations in *GBA* have for instance been shown to be far more prevalent in PD patients of Ashkenazi-Jewish ancestry [31].

In conclusion, mutations in *GBA* appear to be a common genetic risk factor for PD in the Flanders-Belgian population. Our data are in line with previous reports and suggest the presence of a more severe motor phenotype and a predisposition to the development of dementia in PD patients carrying a *GBA* mutation. The identification of PD patients with more severe disease progression is crucial to select patients for future clinical trials with disease-modifying treatments.

ACKNOWLEDGEMENTS

The authors acknowledge the contribution of Dr. Barbara Pickut to the clinical studies and of the technicians Ellen Corsmit and Ellen Elinck to the genetic studies, and to the personnel of the VIB Genetic Service Facility (<http://www.vibgeneticservicefacility.be>) for the Sanger sequencing. The research was in part funded by the Belgian Science Policy Office Interuniversity Attraction Poles (IAP) program, the Flemish Government initiated Methusalem Excellence program, the Alzheimer Research Foundation (SAO/FRA), the Research Foundation Flanders (FWO), the Agency for Innovation by Science and Technology Flanders (IWT), and the University of Antwerp Research Fund; Belgium. The IWT provided a PhD fellowship to A.V. and E.W.

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

	Total cohort	Mutation carriers	Non-carriers	p-value
	N = 266	N = 12	N = 254	
Onset age (mean $\pm$ SD)	60.3 $\pm$ 11.4	58 $\pm$ 8.0	60.4 $\pm$ 11.5	0.241
Age (mean $\pm$ SD)	67.1 $\pm$ 10.4	64.1 $\pm$ 6.8	67.2 $\pm$ 10.4	0.151
M/F ratio	1.3	1.4	1.3	0.953
Disease duration (mean $\pm$ SD)	6.8 $\pm$ 5.7	6.1 $\pm$ 3.9	6.8 $\pm$ 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 $\pm$ 13.7	34.5 $\pm$ 14.3	24.9 $\pm$ 13.5	0.039
HY stage	2.2 $\pm$ 0.9	2.7 $\pm$ 0.8	2.2 $\pm$ 0.9	0.045
Axial score	4.8 $\pm$ 4.1	7.1 $\pm$ 4.8	4.7 $\pm$ 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

Table 2: Rare coding GBA variants in PD patients and controls

Exonic	predicted cDNA ^a	predicted protein ^b	traditional nomenclature ^c	PD patients (n) (MAF)	Controls (n) (MAF)
Ex2+88G>C	c.115G>C	p.G39R	p.G-1R	1/266 (0.0019)	0/536 (0)
Ex5+81G>C	c.535G>C	p.D179H	p.D140H ^d	1/266 (0.0019)	0/536 (0)
Ex7+122delC	c.833delC	p.Q295SfsX9	p.Q256SfsX9	1/266 (0.0019)	0/536 (0)
Ex8+89T>C	c.1088T>C	p.L363P	p.L324P	1/266 (0.0019)	0/536 (0)
Ex8+94G>A	c.1093G>A	p.E365K	p.E326K	12/266 (0.0293)	15/536 (0.0145)
Ex8+224C>T	c.1223C>T	p.T408M	p.T369M	3/266 (0.0058)	11/536 (0.0116)
Ex9+2A>C	c.1226A>C	p.N409S	p.N370S	3/266 (0.0058)	1/536 (0.001)
Ex10+60T>C	c.1448T>C	p.L483P	p.L444P	3/266 (0.0058)	1/536 (0.001)
RecNcil	c.1448T>C	p.L483P	p.L444P	1/266 (0.0019)	0/536 (0)
	c.1483G>C	p.A495S	p.A456S		
	c.1497G>C	p.V499V	p.V460V		
Ex11+81A>G	c.1753A>G	p.H529R	p.H490R	1/266 (0.0019)	0/536 (0)

Note: MAF: minor allele frequency; ^a cDNA numbering relative to reference sequence NM_000157.3 and starting at first nucleotide of translation initiation codon; ^b Protein numbering according to reference sequence NP_000148.2; ^c Traditional protein nomenclature omits the first 39 amino acid residues; ^d The p.D140H and p.E326K mutations occur together in one same patient.