

# Glucocerebrosidase mutations influence the natural history of Parkinson's disease in a community-based incident cohort

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Carriers of mutations in the glucocerebrosidase gene (*GBA*) are at increased risk of developing Parkinson's disease. The frequency of *GBA* mutations in unselected Parkinson's disease populations has not been established. Furthermore, no previous studies have investigated the influence of *GBA* mutations on the natural history of Parkinson's disease using prospective follow-up. We studied DNA from 262 cases who had been recruited at diagnosis into one of two independent community-based incidence studies of Parkinson's disease. In 121 cases, longitudinal data regarding progression of motor disability and cognitive function were derived from follow-up assessments conducted every 18 months for a median of 71 months. Sequencing of the *GBA* was performed after two-stage polymerase chain reaction amplification. The carrier frequency of genetic variants in *GBA* was determined. Baseline demographic and clinical variables were compared between cases who were either *GBA* mutation carriers, polymorphism carriers or wild-type homozygotes. Cox regression analysis was used to model progression to major motor (Hoehn and Yahr stage 3), and cognitive (dementia) end-points in cases followed longitudinally. We show that in a representative, unselected UK Parkinson's disease population, *GBA* mutations are present at a frequency of 3.5%. This is higher than the prevalence of other genetic mutations currently associated with Parkinson's disease and indicates that *GBA* mutations make an important contribution to Parkinson's disease encountered in the community setting. Baseline clinical characteristics did not differ significantly between cases with and without *GBA* sequence variants. However, the hazard ratio for progression both to dementia (5.7,  $P = 0.003$ ) and Hoehn and Yahr stage 3 (4.2,  $P = 0.003$ ) were significantly greater in *GBA* mutation carriers. We also show that carriers of polymorphisms in *GBA* which are not generally considered to increase Parkinson's disease risk are at significantly increased risk of progression to Hoehn and Yahr stage 3 (3.2,  $P = 0.004$ ). Our results indicate that genetic variation in *GBA* has an important impact on the natural history of Parkinson's disease. To our knowledge, this is the first time a genetic locus has been shown to influence motor progression in Parkinson's disease. If confirmed in further studies,

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this may indicate that *GBA* mutation status could be used as a prognostic marker in Parkinson's disease. Elucidation of the molecular mechanisms that underlie this effect will further our understanding of the pathogenesis of the disease and may in turn suggest novel therapeutic strategies.

**Keywords:** genetic risk; models; Parkinson's disease; natural history; cognitive deficits

**Abbreviation:** CamPalGN = Cambridgeshire Incidence of Parkinson's disease from General Practitioner to Neurologist

## Introduction

Parkinson's disease is a common, progressive neurodegenerative disorder of unknown aetiology. The core features of Parkinson's disease, namely bradykinesia, rigidity, resting tremor and postural instability, are also features of a number of other rarer disorders. In this respect, parkinsonism is occasionally a feature of Gaucher's disease, a recessive disorder caused by mutations in the gene glucocerebrosidase (*GBA*) encoding the lysosomal enzyme glucocerebrosidase (Neudorfer *et al.*, 1996; Tayebi *et al.*, 2003; Goker-Alpan *et al.*, 2004; Capablo *et al.*, 2008). At post-mortem, patients with Gaucher's disease and parkinsonism have been found to have several of the neuropathological features of Parkinson's disease, including alpha-synuclein positive Lewy bodies in the cortex and brainstem (Wong *et al.*, 2004). Furthermore, Parkinson's disease is frequently seen in the otherwise-healthy relatives of patients with Gaucher's disease (Goker-Alpan *et al.*, 2004), and such observations have led to the hypothesis that *GBA* mutations in the heterozygous state might constitute a genetic risk factor for sporadic Parkinson's disease.

It is now established that *GBA* mutations are over-represented in cases of both familial (Nichols *et al.*, 2009) and sporadic Parkinson's disease. Indeed, a recent international multicentre collaborative study identified one of two specific *GBA* mutations in 3% of >5000 patients with Parkinson's disease (15% in the sub-cohort of Ashkenazi Jewish origin), and reported an odds ratio for any *GBA* mutation of 5.43 for cases versus control subjects (Sidransky *et al.*, 2009). In a UK-based study, Neumann *et al.* (2009) found a corresponding odds ratio of 3.7, indicating that *GBA* mutations are found in British subjects with Parkinson's disease at a higher frequency than any other known Parkinson's disease-associated gene.

Furthermore, there is some evidence to suggest that *GBA* mutations may contribute to the heterogeneity of Parkinson's disease. Compared with idiopathic Parkinson's disease, patients carrying *GBA* mutations have been reported to have an earlier age at onset, more symmetrical clinical signs and an increased incidence of neuropsychiatric disturbance (Goker-Alpan *et al.*, 2004; Brockmann *et al.*, 2011). This may relate to the earlier pathological involvement of neocortical areas by Lewy body pathology that has been reported in patients with *GBA* mutations (Neumann *et al.*, 2009).

Hitherto, Parkinson's disease cohorts screened for *GBA* mutations have typically been drawn from hospital-based clinics or post-mortem series. The patients are therefore unlikely to be representative of Parkinson's disease as it is encountered in primary or

secondary care, being enriched for younger patients, those with a positive family history or those with atypical or unusual presentations. Although Toft *et al.* (2006) recruited patients from primary care in a Norwegian population, they only screened for two common *GBA* mutations. Systematic *GBA* sequencing in unselected cases with Parkinson's disease in a community-based cohort has not previously been undertaken, but is of obvious importance if we are to determine whether *GBA* mutations make a practically important contribution to the incidence and heterogeneity of Parkinson's disease, as it is most commonly encountered in clinical practice.

Our aim in this study was therefore to screen a large sample of population-representative incident cases of Parkinson's disease for mutations in *GBA* to estimate their frequency and importance in unselected patients. We were also interested in studying the influence of these mutations on the natural history of the disease in that part of our incident cohort that has now been followed for several years from the point of their original diagnosis.

## Materials and methods

### Patients

Genomic DNA from 262 patients was analysed. Patients were participants in either of two separate, community-based epidemiological studies of incident Parkinson's disease conducted in the county of Cambridgeshire. In all, 122 patients were participants in the Cambridgeshire Incidence of Parkinson's disease from General Practitioner to Neurologist (CamPalGN) Study (Foltynie *et al.*, 2004) and have been followed prospectively since recruitment in 2000–02 with assessments every 18 months. A further 140 patients were recruited to a novel incidence study, PICNICS (Parkinsonism: Incidence, Cognition and Non-motor heterogeneity in Cambridgeshire), between 2008 and 2010 (Evans, in preparation). Both studies recruited incident (newly diagnosed) Parkinson's disease cases directly from the community using multiple methods of case ascertainment designed to be as inclusive as possible (for description, see Foltynie *et al.*, 2004). Patients in both cohorts underwent a comprehensive assessment comprising clinical history, motor disability rating using the Unified Parkinson's Disease Rating Scale, Hoehn and Yahr scale, the Beck Depression Inventory and evaluation of cognitive function using the Mini-Mental State Examination and tests derived from the CANTAB® battery (Cambridge Cognition). A positive family history was defined as one or more first or second degree relatives with a history compatible with Parkinson's disease.

The diagnosis of Parkinson's disease was made according to UK Parkinson's Disease Society Brain Bank Criteria (Hughes *et al.*, 1992). Subjects in both cohorts were followed up at 18-month

intervals, and only those consistently meeting diagnostic criteria were included. For subjects in the CamPaiGN cohort, we used two additional outcome measures, namely the time to reach Hoehn–Yahr stage 3 (onset of postural instability) and the time to onset of dementia, diagnosed according to DSM-IV criteria in patients with a Mini-Mental State Examination score of  $\leq 24$ . We have previously shown these to be pertinent milestones of progression in Parkinson's disease (Evans *et al.*, 2011). Data from the Unified Parkinson's Disease Rating Scale were used to define the dominant Parkinson's disease motor phenotype at baseline as either tremor dominant, postural instability/gait disorder dominant or intermediate, according to a widely used algorithm (Zettsky *et al.*, 1985).

Cases were pre-screened for the common *LRRK2* G2019S mutation to reduce the likelihood of inadvertent inclusion of other Mendelian forms of Parkinson's disease, although this has been shown to be uncommon in our study population (Williams-Gray *et al.*, 2006). Subjects had previously been genotyped at the *MAPT* (microtubule-associated protein tau) locus rs9468, discriminating the H1 versus H2 haplotype, which has been previously shown to influence dementia risk in Parkinson's disease (Goris *et al.*, 2007).

All patients provided written informed consent. Both studies were approved by local research ethics committees.

## Molecular genetic analysis of *GBA* mutations

Amplification of *GBA* is complicated by the existence of a homologous, non-processed pseudogene (*GBAP*) 16-kb downstream of the functional gene. To counter this, we used separate PCR reactions initially to amplify 150-ng genomic DNA in three fragments, as previously described by Stone *et al.* (2000). Following this initial phase, PCR products were visualized on a 0.8% agarose gel with ethidium bromide to confirm successful amplification. Clean-up of the PCR product was performed using ExoSAP-IT® (Affymetrix Inc.) as a precursor to exon-by-exon sequencing PCR. PCR conditions and primers are supplied in the Supplementary material. Further clean-up was performed using Illustra Autoscreen plates (GE healthcare). Sequence chromatograms were analysed using ChromasLite and SeqScape v2.1.1. Reference complementary DNA sequences for *GBA* were taken from GenBank (NM 000157). All identified mutations were confirmed by resequencing.

We adopted the conventional nomenclature for *GBA* alleles, referring to the processed protein and excluding the 39-residue signal peptide. The control data published by Neumann *et al.* (2009) in 257 British Caucasian control subjects were used as a reference population. The demographics of this group were well-matched with our Parkinson's disease study population.

## Designation of mutant versus variant alleles

Mutations in *GBA* were defined as those previously reported to be consistently and unequivocally identified as pathogenic in Gaucher's disease, and associated with Parkinson's disease in the heterozygotic state (Beutler *et al.*, 2005; Neumann *et al.*, 2009; Lesage *et al.*, 2011). These included the common *L444P* and *N370S* mutations. The majority of these Parkinson's disease-associated mutations are located in exons 8–10 inclusive. In addition, we identified other exonic sequence variants (hereafter referred to as polymorphisms) in *GBA* which, although linked with Gaucher's disease when occurring in conjunction with other *GBA* mutations, have a less clearly defined association with

Parkinson's disease risk when occurring in the heterozygotic state. These include the polymorphisms E326K (exon 8), T369M (exon 8) and E388K (exon 9) (Horowitz *et al.*, 2011; Lesage *et al.*, 2011; Pankratz *et al.*, 2012). Patients harbouring these polymorphisms were analysed separately from those with pathogenic *GBA* mutations.

## Statistical analysis

Ninety-five per cent confidence intervals (95% CI) for allele frequencies were calculated using Mid-P exact tests based on a binomial distribution. Baseline demographic and clinical variables were compared between patients with Parkinson's disease who were either *GBA* mutation carriers, *GBA* polymorphism carriers or wild-type homozygotes using one-way ANOVA and likelihood ratios for categorical variables.

The influence of *GBA* variant carrier status on the natural history of Parkinson's disease history was investigated in the CamPaiGN cohort ( $n = 121$ ) using Kaplan–Meier analysis. Defining time of Parkinson's disease diagnosis as  $t = 0$ , survival time to the diagnosis of dementia and to the onset of Hoehn and Yahr stage 3 were evaluated. Patients were assessed every 18 months, and when either milestone was reached, time of onset was taken to be the mid-point of the preceding inter-assessment interval. Multivariate Cox regression analysis was then performed with *GBA* variant carrier status and age as covariates, with the latter selected, as it is known to influence progression in Parkinson's disease (Evans *et al.*, 2011).

Corrected *P*-values of  $< 0.05$  were considered significant. Statistical analysis was performed using SPSS v 16.0 and Openepi.

## Results

### *GBA* mutation analysis

Following three rounds of gene sequencing, we were unable to obtain complete sequencing reads for all patients across all exons, likely reflecting suboptimal DNA integrity. For exons 8–11, containing the majority of Parkinson's disease-associated mutations, complete reads were achieved for 994 of a total 1048 exons (95%). For exons 1–7, we were able to obtain clear reads across designated *GBA* mutation loci in all cases except for three individuals in whom sequencing failed across all exons. These patients were excluded from subsequent analysis. Of the 259 patients included, two originated from South Asia, with the remainder being British Caucasian. No patients were aware of Ashkenazi Jewish ancestry when directly questioned, and none had a family history of Gaucher's disease.

*GBA* mutations were identified in 9/259 patients, corresponding to a carrier frequency of 3.5% (1.7–6.3%). These mutations included *L444P* ( $n = 3$ ), *N370S* ( $n = 3$ ), *N462K* ( $n = 1$ ), *R463C* ( $n = 1$ ) and *R257Q* ( $n = 1$ ). In addition, *GBA* polymorphisms were found in a further 15 patients: *E326K* ( $n = 8$ ); *T369M* ( $n = 5$ ); *E388K* ( $n = 1$ ); and *L119L* ( $n = 1$ ). No complex or recombinant alleles were identified.

Demographic details for patients stratified according to their *GBA* variant carrier status are shown in Table 1. Patients with mutations and polymorphisms in *GBA* showed a trend towards younger age at disease onset (wild-type 67.7 versus any *GBA* variant 64.4), but this did not reach statistical significance. Both

**Table 1** Baseline demographic and clinical variables of patients included in the analysis

	All patients	CamPaiGN	Wild-type	Polymorphism carriers	Mutation carriers	P-value <sup>a</sup>
<i>n</i>	259	121	235	15	9	
Sex (male:female)	157:102	68:53	140:95	12:3	4:5	0.28
Age at onset (years)	67.4 (10.4)	67.0 (11.2)	67.7 (10.5)	62.7 (9.1)	67.1 (6.7)	0.19
Positive FH (%)	21 (8.1)	14 (11.6)	17 (7.3)	2 (13.3)	2 (22.2)	0.23
Baseline UPDRS-3	26.1 (11.4)	25.5 (11.7)	25.7 (11.9)	25.9 (10.2)	28.1 (8.6)	0.21
Baseline HYS	1.8 (0.7)	1.9 (0.7)	1.8 (0.7)	1.9 (0.7)	1.8 (0.7)	0.80
Baseline MMSE	28.3 (1.8)	27.8 (2.0)	28.3 (1.8)	28.3 (2.0)	28.5 (1.3)	0.96
Baseline BDI	6.9 (5.1)	7.3 (5.5)	6.9 (5.2)	8.7 (4.0)	6.9 (9.6)	0.10
Baseline motor phenotype (TD/PIGD/Int)	84/71/104	40/32/49	76/64/95	5/3/7	3/4/2	0.73

Group means (standard deviation) are shown. The 'all patients' column shows data for the population as a whole the 'CamPaiGN' column shows data and for the subset of patients in the CamPaiGN cohort who were included in the survival analysis. Columns 4–6 show data for subjects separated by *GBA*-mutation status.

<sup>a</sup> Group-wise comparisons performed using one-way ANOVA, or likelihood ratios for categorical variables.

BDI = Beck Depression Inventory; FH = Family History; HYS = Hoehn–Yahr stage; Int = intermediate; MMSE = Mini-Mental State Examination; PD = Parkinson's disease; PI GD = postural instability/gait disorder; TD = tremor-dominant.

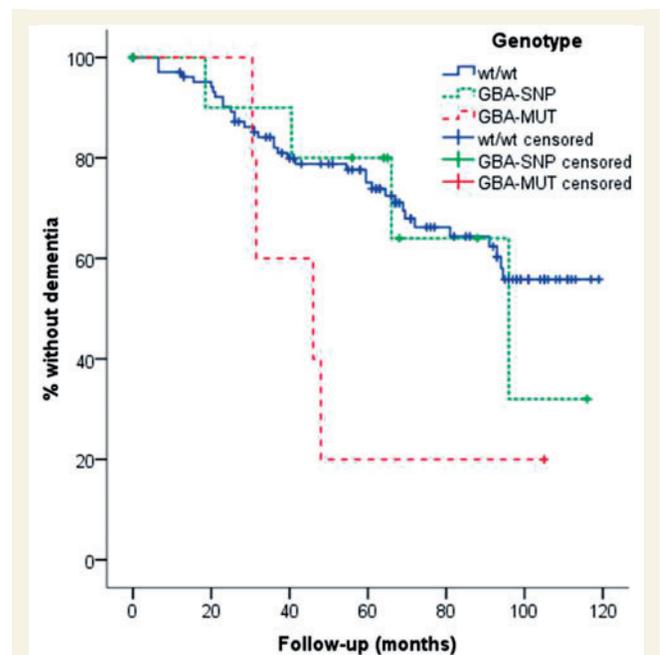
patients with *GBA* mutations and polymorphisms were more likely to have a positive family history of Parkinson's disease. Baseline motor characteristics, Mini-Mental State Examination performance and scores on a commonly used index of depression were similar across all groups. Similarly, there were no differences in the Parkinson's disease motor phenotype between groups at baseline.

## Survival and natural history analysis

Complete sequencing reads were achieved in 121 patients in the CamPaiGN cohort, who were thereafter included in the survival analysis. This included four patients with *GBA* mutations (*L444P*, *n* = 1; *N370S*, *n* = 1; and *N462K*, *n* = 1) and 11 with polymorphisms (*T369M*, *n* = 5; *E326K*, *n* = 5; and *L119L*, *n* = 1). Median follow-up time was 70.5 months (range 12–119 months), and a total of 729 person-years follow-up data were analysed.

For patients with *GBA* mutations, the projected median time to dementia was 46.0 months (95% CI 14.9–77.1), with the corresponding figure for patients with polymorphisms being 96 months (95% CI 51.4–140.6). In contrast, fewer than half of wild-type subjects developed dementia over their median follow-up period of 82 months. The corresponding median time to progression to Hoehn and Yahr stage 3 was 23.5 months (range 0–57.9 months) for patients with Parkinson's disease-associated *GBA* mutations, 32.0 months (range 23.0–41.0 months) for patients with polymorphisms and 49.0 months (range 31.0–67.0 months) for wild-type patients. The corresponding Kaplan–Meier plots trichotomized by *GBA* status are depicted in Figs 1 and 2.

The results of the multivariate Cox regression model are shown in Table 2. *GBA* status was a significant predictor of progression both to dementia and to Hoehn and Yahr stage 3. Subgroup analysis showed that patients carrying *GBA* polymorphisms also progressed more rapidly to Hoehn and Yahr stage 3 than wild-type patients. In addition, there was a trend towards an increased risk of dementia in this group, which reached statistical significance when controlled for the effect of *MAPT* genotype (Table 3).



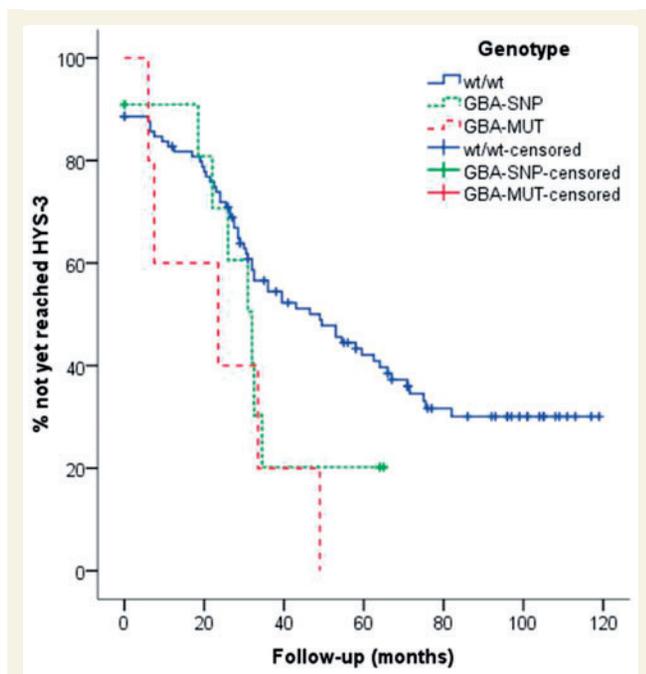
**Figure 1** Kaplan–Meier plot of progression to dementia as a function of *GBA* variant carrier status. Cumulative dementia-free survival in months from diagnosis is shown. Sample size: wild-type patients (wt/wt) patients, *n* = 106; patients with *GBA* mutations (*GBA*-MUT), *n* = 4; patients with polymorphisms (*GBA*-SNP), *n* = 11.

## Discussion

We have shown that in our unselected, community-based Parkinson's disease population, *GBA* mutations are present at a frequency of 3.5%. Given a corresponding frequency in a UK non-Parkinson's disease control population of 1.2%, this equates to an odds ratio (OR) of 2.9. This figure is in line with estimates produced by Neumann *et al.* (2009) (4.2% prevalence, OR 3.7), Lesage *et al.* (2011) (6.7% prevalence) and the multicentre pooled

analysis reported by Sidransky *et al.* (2009) (OR 5.43), from studies conducted in tertiary care settings. Our figure is higher than that reported by Toft *et al.* (2006) (2.3% prevalence) in the only previous study performed in an unselected Parkinson's disease population, although this study used only a limited screen for L444P and N370S mutations, it is highly probable that it underestimated the true mutation frequency.

Our result adds weight to the hypothesis that *GBA* mutations are found at a higher frequency than other known Parkinson's disease-associated genetic mutations. This study is also the first to sequence *GBA* in an unselected Parkinson's disease cohort. Although patients with *GBA* mutations were more likely to have a positive family history, the majority (7/9, 78%) of individuals had sporadic, ostensibly idiopathic Parkinson's disease. This is in



**Figure 2** Kaplan–Meier plot of progression to Hoehn–Yahr stage 3 (HYS-3) as a function of *GBA* variant carrier status. Cumulative survival to Hoehn and Yahr stage 3 in months from diagnosis is shown. Sample size: wild-type patients (wt/wt),  $n = 106$ ; patients with *GBA* mutations (*GBA*-MUT),  $n = 4$ ; patients with polymorphisms (*GBA*-SNP),  $n = 11$ .

accordance with the equivalent figure from the study of Neumann *et al.* (2009) (29/33, 88%). Although recognized in familial disease (Nichols *et al.* 2009), the majority of *GBA* mutations are seen in otherwise isolated Parkinson's disease cases. This is consistent with reduced penetrance of *GBA* mutations as a causative factor in Parkinson's disease. Variable penetrance in certain pathogenic *GBA* mutations has previously been well described in Gaucher's disease (Beutler *et al.*, 2005). Notwithstanding this, the prevalence of mutations in our own study population is, for example, considerably higher than that which we have previously reported for the common *LRRK2* mutation, G2019S (0.4%) (Williams-Gray *et al.*, 2006). Based on our analysis, *GBA* is confirmed as an important risk allele for Parkinson's disease in unselected clinical populations (i.e. those identified in primary and secondary, rather than tertiary, care settings).

At the point of diagnosis, patients with Parkinson's disease-associated *GBA* mutations appear clinically indistinguishable from idiopathic disease. However, in a subset of patients followed longitudinally over a period of up to 9.9 years, we have shown that *GBA* carrier status has a significant impact on the natural history of Parkinson's disease, influencing the time of progression to two key milestones in the disease course.

In Parkinson's disease patients with *GBA* mutations, the risk of progression to dementia is more than five times that of wild-type patients. Although a relationship between *GBA* mutations and accelerated cognitive decline in Parkinson's Disease has previously been suggested, this has been based either on retrospective case

**Table 3** Results of a Cox regression model evaluating progression to dementia in Parkinson's disease incorporating *MAPT* haplotype (H1/H1 versus H2-carrier) as a co-factor in addition to age and *GBA* mutation status

Baseline variable	Relative risk dementia (95% CI)	P-value
Age at diagnosis	1.13 (1.08–1.18)	<0.001
<i>GBA</i> genotype		0.011
Wild-type versus <i>GBA</i> -MUT	4.60 (1.33–15.89)	0.016
Wild-type versus <i>GBA</i> -SNP	3.33 (1.11–10.00)	0.032
<i>MAPT</i> genotype (H1/H1 versus H2-carrier)	3.09 (1.39–6.87)	0.006

Sample size: wild-type patients,  $n = 106$ ; patients with *GBA* mutations (*GBA*-MUT),  $n = 4$ ; patients with polymorphisms (*GBA*-SNP),  $n = 11$ .

**Table 2** Results of the Cox regression analysis of *GBA* mutation status and age as predictor variables for progression to dementia and Hoehn–Yahr stage 3

Baseline variable	Progression to dementia		Progression to Hoehn and Yahr stage 3	
	Relative risk (95% CI)	P-value	Relative risk (95% CI)	P-value
Age at diagnosis	1.13 (1.08–1.18)	<0.001	1.08 (1.05–1.12)	<0.001
<i>GBA</i> genotype		0.006		0.001
Wild-type versus <i>GBA</i> -MUT	5.45 (1.81–16.40)	0.003	4.15 (1.61–10.71)	0.003
Wild-type versus <i>GBA</i> -SNP	2.28 (0.78–6.61)	0.131	3.24 (1.47–7.15)	0.004

For the non-categorical covariate, age, relative risk represents change in risk per unit change in predictor variable. Sample sizes: wild-type patients,  $n = 106$ ; patients with *GBA* mutations (*GBA*-MUT),  $n = 4$ ; and patients with polymorphisms (*GBA*-SNP),  $n = 11$ .

note review (Neumann *et al.*, 2009; Seto-Salvia *et al.*, 2012), or on a single cross-sectional assessment (Brockmann *et al.*, 2011). Our study is the first to quantify this effect using prospective follow-up and statistically robust survival analysis. Unlike the effect of polymorphisms in the gene encoding *MAPT*, hitherto the only gene proven to influence dementia risk in Parkinson's disease (Goris *et al.*, 2007), this effect of *GBA* is not synergistic with the effect of increasing age. Based on this observation, we hypothesize that *GBA* mutations may be particularly implicated in patients with Parkinson's disease with the premature onset of cognitive impairment. We would qualify this by stressing that these conclusions are based on follow-up of a small cohort of mutation carriers ( $n = 4$ ).

*GBA* variants also influence the evolution of Parkinson's disease motor impairment. Compared with wild-type patients with Parkinson's disease, those with *GBA* mutations showed a 4-fold increase in the risk of progression to Hoehn and Yahr stage 3, an end-point associated with impaired quality of life (Goetz *et al.*, 2004; Evans *et al.*, 2011). Importantly, patients with *GBA* polymorphisms also showed a significantly increased rate of progression in comparison with wild-type patients, even though the identified *GBA* polymorphisms (E328K, E388K, T369M and L119L) are not in themselves considered risk alleles for Parkinson's disease. This is the first time that a genetic polymorphism has been shown to influence motor progression in Parkinson's disease and, once again allowing for the limited size of the cohort under study, might indicate that these variants are functionally important.

The strengths of our study are that by recruiting subjects directly from the community, the problems of selection bias inherent in studies performed in the tertiary care setting are avoided. Following patients longitudinally minimizes the risk of erroneous inclusion of non-Parkinson's disease cases. Specifically, none of the patients included in the analysis reached dementia criteria within 1 year of the onset of parkinsonism; so by definition, cases of dementia with Lewy bodies have not been included.

Potential criticisms are that our study was neither designed nor powered to detect novel pathogenic mutations, and that we did not include a control group. As we used published denominator data from an otherwise well-matched UK control population data, we anticipate that the effect of the latter on our calculated OR would be negligible, and that this figure is therefore representative. As previously discussed, our conclusions regarding the influence of *GBA* mutations on Parkinson's disease natural history are based on data from a small number of subjects and need to be confirmed in independent Parkinson's disease cohorts.

Consistent with previously reported prevalence figures, despite sequencing the entire *GBA*, we identified only one mutation (R257Q) located outside exons 8–11 inclusive. In the larger study of Lesage *et al.* (2011), pathogenic mutations in exons 1–7 were identified in 15/1130 unrelated patients with Parkinson's disease, corresponding to only 15.0% of patients with *GBA* mutations. The corresponding figures from the study of Neumann *et al.* (2009) were 4/790 and 12.1%, respectively. Given that these two studies were performed in the tertiary setting, it should be anticipated that the prevalence of these rare *GBA* mutations in the community setting to be lower still, and

our findings are thus consistent with this hypothesis (1/259, 11.1%). Theoretically, it might be possible to use limited sequencing of exons 8–11 as a screening measure for *GBA* mutations in Parkinson's disease, which would represent a considerable cost saving over whole gene sequencing. However, it is premature to suggest that such a strategy may be useful in clinical practice without further investigation.

The mechanism of the association between *GBA* mutations and Parkinson's disease has yet to be elucidated. The pathogenesis appears distinct from that of Gaucher's disease, where deficiency of glucocerebrosidase leads to accumulation of substrate, primarily in cells of the reticuloendothelial system. *In vitro* models of alpha-synuclein aggregation have provided evidence for co-localization of this protein with mutant glucocerebrosidase (Goker-Alpan *et al.*, 2010). It has been proposed that a gain of function mechanism operates in patients with Parkinson's disease-associated *GBA* mutations, whereby mutant glucocerebrosidase promotes alpha-synuclein aggregation, accelerating Lewy body formation and neuronal loss *in vivo* (Westbroek *et al.*, 2011).

An alternative theory is that in the heterozygotic state *GBA* mutations are associated with abnormalities in the cellular processes involved in the degradation of misfolded proteins performed by the endoplasmic reticulum. Endoplasmic reticulum-associated degradation typically involves the poly-ubiquitination of proteins allowing them to be directed to the proteasome. A link between dysfunctional endoplasmic reticulum-associated degradation and Parkinson's disease is supported by the observation that mutations in the gene encoding parkin, a ubiquitin ligase, causes a Mendelian form of Parkinson's disease (Beasley *et al.*, 2007) and that *in vitro* mutant glucocerebrosidase acts as a substrate for parkin-mediated endoplasmic reticulum-associated degradation (Ron *et al.*, 2010). Horowitz *et al.* (2011) have shown that a similar effect is seen with the E326K glucocerebrosidase variant, which demonstrates ~80% of normal activity, a level intermediate between wild-type and mutant (Horowitz *et al.*, 2011). It is plausible that an inverse relationship exists between functional glucocerebrosidase activity and the extent to which a mutant or variant glucocerebrosidase is targeted for endoplasmic reticulum-associated degradation (Ron *et al.*, 2010), and subtle degrees of endoplasmic reticulum-associated degradation dysregulation could represent a mechanism for our observation that *GBA* sequence variants influence the clinical phenotype of Parkinson's disease whilst not functioning in themselves as Parkinson's disease risk alleles.

Our conclusions are based on the analysis of a comparatively small number of patients and require validation in larger cohorts. In particular, our natural history analysis included only 4 mutation carriers and 11 single nucleotide polymorphism carriers. It was not feasible to perform subgroup analysis of individual mutations and single nucleotide polymorphisms in the present study, and follow-up studies could explore the hypothesis that different *GBA* mutations have a differential effect on phenotype, as has been suggested (Gan-Or *et al.*, 2009).

One clinically pertinent question that arises as a consequence of this work is whether screening unselected patients with Parkinson's disease for *GBA* mutations should become part of the routine diagnostic work-up in clinical practice. As the costs of whole

exome and/or whole genome screening continue to fall, this is likely to become a realistic proposition in the near future. The potential influence of *GBA* mutations on Parkinson's disease natural history is of obvious relevance to our efforts to learn more about the basis of heterogeneity in Parkinson's disease, including factors that may influence prognosis in the disorder. Whilst it is premature to suggest that specific therapy based on *GBA* mutation status might be feasible, efforts to understand the contribution of mutant glucocerebrosidase to the pathogenesis of Parkinson's disease continue and are supported by clinical studies of this type. Large-scale screening would have implications for genetic counselling, although, given the low penetrance of *GBA* mutations, we would not view the screening of unaffected relatives of patients with Parkinson's disease-associated mutations as either necessary or desirable.

## Conclusion

*GBA* mutations account for a modest but important proportion of Parkinson's disease in an unselected, UK population-based cohort implying that unlike other rare forms of monogenic Parkinson's disease, patients with *GBA* mutations might reasonably be encountered by the non-specialist clinician. Furthermore, both mutations and polymorphisms in *GBA* adversely affect the natural history of the disease and, although follow-up studies are required, the concept that the gene might prove useful as a prognostic marker in Parkinson's disease is an exciting one. Elucidating the molecular mechanisms that are responsible for these effects will contribute to a broader understanding of the pathogenesis in Parkinson's disease, and may in turn suggest new disease-modifying therapies for the disorder.

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## Supplementary material

Supplementary material is available at *Brain* online.

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