

The impact of *GBA1* variants on long-term clinical progression and mortality in incident Parkinson's disease

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Abstract

Introduction: Variants in the *GBA1* gene have been identified as a common risk factor for Parkinson's disease (PD). In addition to pathogenic mutations (those associated with Gaucher disease), a number of "non-pathogenic" variants also occur at increased frequency in PD. Previous studies have reported that pathogenic variants adversely affect the clinical course of PD. The role of "non-pathogenic" *GBA1* variants on PD course is less clear. In this study we report the effect of *GBA1* variants in incident PD patients with long-term follow up.

Methods: The study population consisted of patients in the CamPaIGN and PICNICS cohorts. Patients were grouped into non-carriers, carriers of "non-pathogenic" *GBA1* variants, and carriers of pathogenic *GBA1* mutations. Survival analyses for time to development of dementia, postural instability and death were carried out. Cox-regression analysis controlling for potential confounders were used to determine the impact of *GBA1* variants on these outcome measures.

Results: *GBA1* variants were identified in 14.4% of patients. Pathogenic and "non-pathogenic" *GBA1* variants were associated with the accelerated development of dementia and a more aggressive motor course. Pathogenic *GBA1* variants were associated with earlier mortality in comparison to non-carriers, independent of the development of dementia.

Discussion: *GBA1* variants, including those not associated with Gaucher disease, are common in PD, and result in a more aggressive disease course.

Introduction

Heterozygous mutations in the *GBA1* gene have been identified as a common risk factor for Parkinson's disease (PD), being found in approximately 5 % of patients¹⁻⁴. Biallelic pathogenic mutations result in the lysosomal storage disorder, Gaucher disease (GD)⁵. In addition to the known pathogenic mutations associated with GD, there are a number of "non-pathogenic" *GBA1* variants that are not associated with

GD, but that are found at increased frequency in PD patients, including the common E326K and T369M variants⁶⁻⁹. Carrying a pathogenic *GBAI* mutation conveys an earlier age of onset^{10 11} and adversely affects the clinical course of PD, manifested by an increased risk of cognitive decline and dementia^{1 10 12 13 14-18} and increased rate of motor progression^{1 10 12 19 20}. However, some studies have found no effect on motor progression in *GBAI* mutation-associated PD^{14 15 17}. Few studies have commented on the effect of *GBAI* mutations on mortality in PD, though some have suggested that survival time is reduced in *GBAI* mutation carriers^{10 16}. The clinical significance of carrying a “non-pathogenic” *GBAI* variant is disputed^{14 21} though the E326K variant in particular has been associated with an adverse clinical course in PD in some studies^{20 22}.

We previously reported on the epidemiology of *GBAI* mutation-associated PD in incident PD¹. Here we present long-term follow-up data of incident PD cases carrying pathogenic and “non-pathogenic” variants in the *GBAI* gene, from two community-based cohorts: the “Cambridgeshire Incidence of Parkinson’s disease from General Practice to Neurologist” (CamPaIGN) and “Parkinsonism: Incidence, Cognition and Non-motor heterogeneity in Cambridgeshire” (PICNICS) cohorts. Advantages of this study over some previously published studies include the long duration of follow-up, along with the fact that both cohorts consisted of community-based patients with newly diagnosed PD.

Methods

Patients

The study population consisted of patients in the CamPaIGN and PICNICS cohorts – both community-based incident PD cohorts (table 1)²³. PD diagnosis was determined by the UK Parkinson’s Disease Society Brain Bank Criteria. The CamPaIGN cohort was recruited between 2000 and 2002 and subjects were followed up at 2 yearly intervals for up to 18 years. The PICNICS cohort was recruited between 2006 and 2013 and subjects have been followed up at 18 monthly intervals, so far for up to 11.4 years. Inclusion and exclusion criteria for these cohorts are summarised in supplementary table 1. The

CamPaIGN and PICNICS studies were both approved by the Cambridgeshire Local Research Ethics Committee, and written informed consent was obtained from all subjects.

Motor, cognitive and neuropsychiatric assessments were completed at each time point including the Unified Parkinson's Disease Rating Scale (UPDRS) in the CamPaIGN cohort, Movement Disorders Society UPDRS (MDS-UPDRS) in the PICNICS cohort, Mini-Mental State Examination (MMSE), Hoehn and Yahr (HY) stage, levodopa equivalent dose, Beck Depression Inventory (BDI), National Adult Reading Test (NART) and Parkinson's Disease Questionnaire-39 (PDQ-39). For the CamPaIGN cohort, estimated MDS-UPDRS scores were calculated as described previously²⁴. Postural instability and gait disability (PIGD) and tremor scores were calculated for the CamPaIGN cohort as described here²⁵. We adapted this method to allow calculation of similar motor phenotypes in the PICNICS cohort, using the mean value of the UPDRS-MDS scores for questions 2.10 (tremor history), 3.15 (postural tremor), 3.16 (kinetic tremor) and 3.17 (rest tremor) for tremor score, and questions 2.12 (walking and balance history), 2.13 (freezing history), 3.10 (gait examination) and 3.13 (posture examination) for PiGD score. *GBA1* variants were identified through sequencing of the *GBA1* gene targeted at exons 1 to 11 in 250 patients, as described previously¹. A further 127 patients underwent targeted screening using a Illumina Multi-Ethnic Genotyping Array (MEGA) ChIP for specific *GBA1* variants (supplementary figure 1)²⁶.

Survival and regression analyses

Subjects were grouped into non-carriers, and carriers of any *GBA1* variant (*GBA1*-PD). The *GBA1*-PD group was further stratified into carriers of pathogenic mutations, and carriers of "non-pathogenic" variants, as indicated below. Hereafter, variants referred to as "pathogenic" are those associated with the development of GD in the homozygous or compound heterozygous state, and variants referred to as "non-pathogenic" are those that are not associated with the development of GD. All patients in whom sequencing had been undertaken were included in survival analyses, as well as additional patients in

whom any *GBAI* variant was detected on the MEGA CHIP array. Patients in which only MEGA CHIP screening was performed that were not found to carry a *GBAI* variant were excluded, given the possibility that they carried an undetected *GBAI* variant that had not been screened for. For subgroup analysis, , subjects that only had genotyping with the Mega CHIP array in which no *GBAI* variant was identified, or in which a “non-pathogenic” variant was identified (n=6) were excluded, to ensure that there no patients with missed pathogenic mutations were included in the non-carrier or “non-pathogenic” variant-carrier groups.

Time to mortality, postural instability and dementia were selected as outcome measures, as these represent important clinical milestones in the progression of PD. Kaplan-Meier curves, and Cox Regression analyses controlling for potential confounders as detailed below, were used for comparison between the groups. Development of postural instability was defined as time to reach HY stage three (HY3) or PIGD score of two or more, and dementia was defined as a MMSE score of 24 or less, with fulfilment of Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria (functional impairment (as defined by Schwab and England score of 70% or less) with cognitive deficits in at least two domains and exclusion of other causes of cognitive dysfunction). The CamPaIGN and PICNICS cohort studies predated establishment of the Movement Disorder Society PD dementia criteria. Patients were only considered to have reached these outcomes if these criteria were fulfilled at all subsequent visits.

Statistical analysis

All statistical tests were performed on SPSS 25 (IBM) software. Independent samples T-tests were used to compare between non-carriers and carriers of all *GBAI* variants. Levene’s test was first performed to ensure that equality of variance could be assumed. One-Way ANOVA tests were used for comparison between non-carriers and carriers of “non-pathogenic” or pathogenic *GBAI* variants. Chi-squared tests

were used to compare sex distribution. For survival analysis, log-rank values are reported, with comparison to the non-carrier group.

	Non-carriers	All <i>GBAI</i> variants	P-value	<i>GBAI</i> non-pathogenic variant carriers	Pathogenic <i>GBAI</i> mutation carriers	P-value
Number	214	48	-	31	17	-
Sex M:F	127:87	32:16	0.348	22:9	10:7	0.459
Age at diagnosis	69.2 (9.8)	68.3 (8.9)	0.569	68.7 (10.1)	67.5 (6.3)	0.785
Years of follow-up	6.9 (4.2)	6.1 (3.4)	0.255	6.8 (3.6)	6.2 (2.8)	0.791
Years in education	11.9 (3.1)	11.3 (2.5)	0.253	11.3 (2.5)	11.5 (2.7)	0.510
Total UPDRS at baseline	51.8 (19.6)	54.2 (16.8)	0.431	56.2 (18.3)	50.0 (15.2)	0.452
Motor UPDRS at baseline	31.6 (12.5)	35.1(12.0)	0.072	35.7 (12.1)	35.1 (13.2)	0.168
Hoehn & Yahr at baseline	1.7 (0.7)	2.0 (0.8)	0.02	2.0 (0.74)	2.0 (0.8)	0.067
PIGD score at baseline	0.75 (0.55)	0.85 (0.47)	0.225	0.86 (0.52)	0.79 (0.34)	0.563
Tremor score at baseline	0.59 (0.41)	0.61 (0.41)	0.744	0.75 (0.42)	0.51 (0.35)	0.119
Baseline levodopa equivalent dose	185.9 mg (238.7)	204.4 mg (241.1)	0.594	166.1 mg (214.1)	274.1 mg (277.0)	0.290
MMSE at baseline	28.5 (1.4)	28.2 (1.9)	0.274	27.9 (2.1)	28.6 (1.4)	0.150
BDI at baseline	7.3 (5.3)	6.8 (5.0)	0.629	7.8 (5.6)	4.9 (2.9)	0.180
NART at baseline	116.1 (61.9)	111.3 (11.3)	0.609	109.7 (10.7)	113.2 (12.0)	0.873
PDQ-39 at baseline	24.7 (19.8)	26.4 (19.8)	0.602	32.5 (22.3)	17.5 (9.2)	0.057

Table 1 – Patient demographics. P-values are shown for comparison between non-carriers and all variant-carriers as determined by independent samples T-tests and Chi-squared tests, and for comparison between non-carriers, “non-pathogenic” variant-carriers, and pathogenic variant carriers, as determined by One-Way ANOVA and Chi squared tests. Standard deviations shown in brackets. Abbreviations: BDI = Beck Depression Inventory; F = female; M = male; MMSE = mini mental state

examination; NART = National Adult Reading Test; PDQ-39 = Parkinson's disease questionnaire 39; PIGD = postural instability and gait disability; UPDRS = Unified Parkinson's Disease Rating Scale.

Results

Incidence and spectrum of GBA1 abnormalities

GBA1 variants were present in 36 out of the 250 patients in which sequencing was performed (14.4%). Pathogenic mutations were present in 11 of these patients (4.4 %), which included N370S (n=3), L444P (n=3), R463C (n=1), G10S (n=1), N426K (n=1), R48W (n=1) and R257Q (n=1). Isolated “non-pathogenic” variants were present in 25 of these patients (10.0 %). These included c.762 18T>A (n=8), E326K (n=7), T369M (n=7), E388K (n=1), L119L (n=1) and c.589 86A>G (n=1). The carrier of the pathogenic R48W variant carried a concomitant E326K variant, and the carrier of the pathogenic N426K variant carried a concomitant c.762 18T>A variant. These were both included in the pathogenic variant group for subgroup analyses. Variants were identified in 12 of the 127 patients (9.4%) in which MEGA ChIP analysis was performed. These included the pathogenic N370S (n=4), R463C (n=1) and G10S (n=1) variants, and the “non-pathogenic” T369M (n=3), E326K (n=2) and E388K (n=1) variants.

After excluding those patients in which only MEGA ChIP genotyping was performed that were not identified to carry a *GBA1* variant, these combined cohorts contained 214 non-carriers and 48 carriers of *GBA1* variants. Mean follow-up times were 8.0 years (maximum 18.0 years) for the CamPaIGN cohort (n=115) and 5.8 years (maximum 11.4 years) for the PICNICS cohort (n=147).

Cognitive decline and risk of dementia

Baseline cognitive function did not differ between variant carriers and non-carriers, with no significant differences in MMSE scores at initial assessment (table 1). Carriers of any *GBA1* variant had an increased risk of progression to dementia compared to non-carriers (table 2 and figure 1). Cox regression analysis controlling for age at diagnosis, sex and baseline MMSE score revealed that development of dementia was significantly more likely in carriers of any *GBA1* variant, compared to non-carriers, with a hazard ratio (HR) of 2.1 (95 % confidence interval (CI) 1.2-3.9, p=0.013). At the 10 year time point, fewer than half of the variant carriers remained dementia-free, in comparison to 68.4 % of non-carriers. At 15 years, 64.2 % of surviving non-carriers remained dementia-free. All patients in the *GBA1* carrier group had developed dementia or died prior to the 15 year time point. The estimated mean time to dementia was approximately five years earlier in variant carriers, compared to non-carriers (table 2).

Sub-classification of the variant-carrier group into those with pathogenic mutations and those with “non-pathogenic” variants revealed a significantly increased risk of progression to dementia in carriers of pathogenic variants, with a HR of 3.6 (95 % CI 1.5-8.3, p=0.003). In carriers of “non-pathogenic” variants there was a non-significant trend towards increased risk of dementia (HR 1.8 (95% CI 0.9-3.8, p=0.11).

	Non-carriers	All <i>GBA1</i> variants	Non-pathogenic variants	Pathogenic mutations
Number	214	48	25	17
Estimated mean time to dementia (years)	13.7 (12.6-14.7)	8.6 (7.2-10.0)	8.3 (6.4-10.2)	8.3 (6.2-10.3)
Estimated median time to dementia (years)	*	8.5 (6.9-10.1)	8.3 (5.4-11.3)	8.5 (4.1-12.9)
5 year survival without dementia (%)	85.3 (SE 2.7)	80.2 (SE 6.3)	82.3 (SE 8.0)	74.0 (SE 11.2)
10 year survival without dementia (%)	68.4 (SE 4.3)	45.7 (SE 9.8)	43.2 (SE 12.5)	43.2 (SE 16.0)
15 year survival without dementia (%)	64.2 (SE 5.8)	0	0	0

Log-rank p-value	-	0.013	0.014	0.109
Hazard ratio	-	2.1 (1.2-3.9; p=0.013)	1.8 (0.9-3.8; p=0.110)	3.6 (1.6-8.3; p=0.003)

Table 2 – Time to dementia. Long-term follow-up data for progression to dementia in PD patients without *GBAI* variants compared to those carrying any *GBAI* variant, a pathogenic *GBAI* mutation, or a *GBAI* “non-pathogenic” variant. HR determined by Cox regression analysis controlling for age at diagnosis, sex and baseline MMSE score. 95 % CI for HR and estimated mean and median shown in brackets. Log-rank p-values shown for comparison between the *GBAI* groups and the non-carrier group. Abbreviations: SE = standard error of the mean. *fewer than half reached outcome.

Motor progression

The baseline HY stage was lower in non-carriers compared to carriers (table 1), though there were no significant differences between the subgroups. There were no differences in baseline UPDRS motor score, PIGD score or levodopa equivalent dose between any of the groups. The risk of progression to postural instability was increased in the *GBAI*-PD group in comparison to non-carrier group (table 3 and figure 2). At five years from diagnosis, 67.5 % of the *GBAI*-PD group had reached HY3, compared to 43 % of non-carriers. After controlling for potential confounders (age at diagnosis, sex, baseline HY score, baseline UPDRS motor score, and baseline PIGD score) the risk of progression to HY3 was significantly greater in both carriers of “non-pathogenic” variants and pathogenic mutations, with a HR of 1.7 (95 % CI 1.0-2.9, p=0.035) and 1.9 (95 % CI 1.0-3.4, p=0.036) respectively (figure 2 and table 3).

	Non-carriers	All <i>GBAI</i> variants	Non-pathogenic variants	Pathogenic mutations
Number	213	48	25	17
Estimated mean time to HY3 (years)	6.8 (5.9-7.6)	4.7 (3.4-5.9)	4.6 (3.0-6.3)	4.4 (3.0-5.9)
Estimated median time to HY3 (years)	6.0 (4.6-7.4)	3.0 (2.5-3.5)	2.8 (2.5-3.1)	4.4 (2.6-6.2)
5 year survival without postural instability (%)	57.0 (SE 3.6)	32.5 (SE 7.3)	32.0 (SE 10.0)	32.4(SE 11.8)

10 year survival without postural instability (%)	28.7 (SE 4.1)	9.6 (SE 5.9)	10.7 (SE 7.0)	0
15 year survival without postural instability (%)	7.7 (SE 3.9)	9.6 (SE 5.9)	10.7 (SE 7.0)	0
Log-rank p-value	-	0.013	0.074	0.095
Hazard ratio	-	1.7 (1.2-2.6; p=0.006)	1.7 (1.0-2.9; p=0.035)	1.9 (1.0-3.4; p=0.036)

Table 3 – Time to HY3. Long-term follow-up data for progression to postural instability in PD patients without *GBAI* variants compared to those carrying any *GBAI* variant, a pathogenic *GBAI* mutation, or a *GBAI* “non-pathogenic” variant. HR determined by Cox regression analysis controlling for age at diagnosis, sex, baseline HY score, baseline UPDRS motor score, and baseline PIGD score. 95 % CI for HR and estimated mean and median shown in brackets. Log-rank p-values shown for comparison between the *GBAI* groups and the non-carrier group. Abbreviations: HY3 = Hoehn and Yahr stage three; SE = standard error of the mean.

Time to PIGD score of two or more was also determined as a measure of motor progression. Pathogenic variants were associated with an increased risk of progression to PIGD score of two or more, with a HR of 2.8 (95% CI 1.4-5.7, p=0.005). There was no significant increased risk of progression to PIGD score of two or more in carriers of “non-pathogenic” variants (table 4).

	Non-carriers	All <i>GBAI</i> variants	Non-pathogenic variants	Pathogenic mutations
Number	214	48	25	17
Estimated mean time to PIGD\geq2 (years)	7.7 (7.2-8.3)	6.8 (5.6-7.9)	6.9 (5.3-8.5)	5.7 (4.4-7.0)
Estimated median time to PIGD\geq2 (years)	8.6 (7.4-9.9)	6.0 (5.1-7.0)	6.0 (5.1-7.0)	4.9 (3.1-6.6)
5 year survival to PIGD\geq2 (%)	76.1 (SE 3.2)	62.6 (SE 8.1)	71.5 (SE 9.9)	48.8 (SE 13.9)
10 year survival to PIGD\geq2 (%)	40.9 (SE 5.6)	37.9 (SE 8.8)	39.0 (SE 12.0)	30.5 (SE 13.5)
Log-rank p-value	-	0.094	0.335	0.066
Hazard ratio	-	1.9 (1.1-3.2; p=0.015)	1.6 (0.8-3.1; p=0.178)	2.8 (1.4-5.7; p=0.005)

Table 4 – Time to PIGD score of two or more. Long-term follow-up data for progression to postural instability in PD patients without *GBAI* variants compared to those carrying any *GBAI* variant, a

pathogenic *GBAI* mutation, or a *GBAI* “non-pathogenic” variant. HR determined by Cox regression analysis controlling for age at diagnosis, sex, baseline HY score, baseline UPDRS motor score, and baseline PIGD score. 95 % CI for HR and estimated mean and median shown in brackets. Log-rank p-values shown for comparison between the *GBAI* groups and the non-carrier group. Abbreviations: HY3 = Hoehn and Yahr stage three; PIGD = postural instability and gait difficulty score; SE = standard error of the mean.

Mortality

Mean time to mortality was approximately one year earlier in carriers of any *GBAI* variant compared to non-carriers (table 5 and figure 3). At 15 years from diagnosis, only 5.3% of *GBAI* variant carriers were surviving, compared to 20.1% of non-carriers. When controlling for age and sex, the risk of mortality was increased in carriers of pathogenic mutations in comparison to non-carriers with a HR of 2.1 (95 % CI 1.0 to 4.1, p=0.043), but not in those carrying “non-pathogenic” variants (table 5).

To explore whether the increase in mortality associated with pathogenic *GBAI* variants was attributable to the development of dementia, the dementia outcome was added into the Cox regression model, along with age and sex. The increased risk of mortality associated with carrying any *GBAI* variant persisted after controlling for dementia status (HR 2.0 (95 % CI 1.0-4.1, p=0.046)), suggesting that the relationship was independent of the development of dementia. Causes of death in each group are shown in supplementary table 2.

	Non-carriers	All <i>GBAI</i> variants	Non-pathogenic variants	Pathogenic mutations
Number	214	48	25	17
Estimated mean time to death (years)	10.6 (10.0-11.3)	9.4 (8.4-10.5)	10.0 (8.7-11.3)	9.5 (7.8-11.3)
Estimated median time to death (years)	10.0 (9.3-10.7)	9.4 (8.3-10.6)	10.0 (8.9-11.1)	8.5 (7.7-9.3)

5 year survival (%)	89.2 (SE 2.2)	93.4 (SE 3.7)	96.0 (SE 3.9)	100.0
10 year survival (%)	49.6 (SE 3.9)	39.8 (SE 8.8)	49.7 (SE 11.6)	29.8 (SE 14.1)
15 year survival (%)	20.1 (SE 3.9)	5.3 (SE 5.0)	7.8 (SE 7.3)	0
Log-rank p-value	-	0.169	0.639	0.275
Hazard ratio	-	1.5 (1.0-2.3; p=0.061)	1.2 (0.7-2.0; p=0.567)	2.1 (1.0-4.1; p=0.043)

Table 5 – Time to mortality. Long-term follow-up data for progression to time to mortality in PD patients without *GBAI* variants compared to those carrying any *GBAI* variant, a pathogenic *GBAI* mutation, or a *GBAI* “non-pathogenic” variant. HR determined by Cox regression analysis controlling for age at diagnosis and sex. 95 % CI for HR and estimated mean and median shown in brackets. Log-rank p-values shown for comparison between the *GBAI* groups and the non-carrier group. Abbreviations: SE = standard error of the mean.

Discussion

In this study, we have described the clinical course of incident *GBAI*-PD for up to 18 years from the time of diagnosis, which is a longer follow-up period than any previous study. We have demonstrated that *GBAI* variants confer an increased risk of dementia, faster motor progression, and mortality in PD, in keeping with previous reports. Importantly, in addition to pathogenic *GBAI* mutations, variants generally considered to be “non-pathogenic”, were found to adversely affect disease course in *GBAI*-PD.

By studying patients with incident PD for up to 18 years, this study has uniquely described the natural history of *GBAI*-PD. Most prior longitudinal studies of *GBAI*-PD have employed prevalent cohorts in which variation in disease duration may confound the results, and many other studies have used clinic-based cohorts which may be disproportionately represented by atypical or severe cases. The two cohorts analysed in this study were community-based and thus more representative of the general PD

population. Another strength of this study was the inclusion of patients with *GBAI* variants that do not cause GD, which occur at increased incidence in PD. These variants were termed “non-pathogenic” to differentiate them from variants that cause GD, though taking into account results from this and previous studies, they should not be considered to be non-deleterious.

Of the patients that had undergone genetic sequencing, 14% carried a *GBAI* variant, with 4.4% carrying a pathogenic mutation^{2-4,27} previously associated with GD. The frequency of pathogenic mutations was similar to that seen in previous studies. The prevalence of any *GBAI* variant was higher than that seen in other studies, probably due to the fact that we included non-coding variants such as c.762 18T>A, which has been reported as a potential risk factor for PD, though its importance is not clear²⁸. The most common variants were c.762 18T>A, E326K, and T369M which accounted for almost two thirds of all variants. Carriers of these variants are a particularly interesting group to study, as their clinical significance is much less clear than that of so called “pathogenic” *GBAI* mutations.

We found that *GBAI* variants were generally associated with a more aggressive disease course. Diagnosis of dementia and development of postural instability were chosen as outcome measures, since these represent important milestones in the clinical progression of PD²⁹. Consistent with previous studies, carriers of pathogenic mutations had an increased risk of reaching both of these milestones. Carriers of any *GBAI* variant had a projected mean time to dementia that was approximately five years earlier than in non-carriers, whilst the estimated mean time to development of postural instability was approximately two years earlier than that seen in non-carriers. Carriers of pathogenic mutations had a 3.6-fold greater risk of development of dementia than in non-carriers, and a 1.9-fold greater risk of progression to HY3. Importantly, carriers of variants not associated with GD conveyed a similar risk of development of postural instability in comparison to non-carriers, with a trend towards greater risk of dementia. These variants have previously been reported to have little effect on clinical course in PD¹⁴, though it is possible that such effects would be missed due to short follow-up times, and heterogeneous

populations. Carriers of the common E326K variant have been shown to have an increased risk of dementia and motor progression in comparison to non-carriers, in one US-based study²⁰. However, in a large meta-analysis of *GBAI* variants in PD, there was no association between development of PD and the E326K variant⁴. This study population was enriched for individuals from Jewish, Portuguese, and Asian populations, in which the E326K accounts for a lower proportion of *GBAI* variants than in other populations, which may account for the lack of association in this analysis^{3 30}.

We also found a significantly increased risk of mortality over the study period in carriers of pathogenic *GBAI* variants, in which the risk of death was approximately two-fold greater than that in non-carriers, with a mean time to mortality approximately one year earlier. This observation was not attributable to the increased incidence of dementia in *GBAI* variant carriers, since the effect persisted after controlling for dementia status. Other factors (such as immobility, adverse drug effects, and non-motor features) may contribute to the observed increase in mortality in *GBAI*-PD. “Non-pathogenic” variants were not associated with an increased risk of mortality, though the time to mortality was non-significantly reduced in this group. The impact of *GBAI* variants on PD progression may lie on a spectrum, with pathogenic mutations conveying a greater biological effect than “non-pathogenic” variants. As such our study may have been underpowered to detect a small reduction in time to mortality associated with “non-pathogenic” variants, and it would be interesting to explore this in a larger cohort.

Age of disease onset has previously been reported to be reduced in PD associated with *GBAI* mutations^{10 13 27 31}. In this study, age of onset did not differ based on *GBAI* status. The mean age of onset in both carriers of pathogenic mutations and carriers of “non-pathogenic” variants in *GBAI* was approximately two years earlier than in non-carriers, and it may be that this study was underpowered to detect such a difference in age at onset.

The observation that “non-pathogenic” *GBAI* variants adversely affect the prognosis of PD patients is important. This significantly increases the proportion of PD patients in which *GBAI* abnormalities play a potentially pathogenic role, in comparison to considering GD-causative mutations as the only variants that alter PD course. Given that *GBAI* mutations are associated with the specific pathology of lysosome-autophagy system dysfunction³², it is possible that the optimal treatment approach in this group of patients will involve drugs that are able to restore activity of this system. As such treatments emerge, it may become important to screen PD patients for variants in the *GBAI* gene, so that precision medicine can be applied, and our data suggests that it will be important to screen for a much broader range of variants than those previously identified as “pathogenic”.

The “pathogenic” mutations associated with GD result in significant reduction in glucocerebrosidase enzyme activity, which underlies its clinical manifestations^{5 33}. However, polymorphisms such as E326K result in a relatively minimal reduction in enzyme activity²², and the fact that these predispose to PD without being associated with GD, supports the concept that the pathogenic mechanisms of *GBAI*-PD are distinct from those of GD. This means that putative treatments for *GBAI*-PD may need to correct glucocerebrosidase folding and structure, or restore activity of dysfunctional pathways (such as the lysosome-autophagy system), rather than simply restoring glucocerebrosidase enzyme activity.

We acknowledge that there are a number of limitations with our study. While we have controlled for relevant potential confounders in our survival analyses, data regarding prodromal features of PD that may influence prognosis (e.g. REM sleep-behaviour disorder) were not available. We have reported on the time from diagnosis, rather than time from symptom onset, to the development of clinical milestones, which may not truly reflect disease duration. However, the time of symptom onset is difficult to determine accurately, since many prodromal features may not be attributed to PD, which would potentially introduce bias.

These cohorts predated the development of the MDS criteria for PD dementia. Cognitive status was measured in these cohorts with MMSE, though other cognitive examinations are now favoured. In order to maximise the accuracy of dementia diagnosis in these cohorts we employed strict criteria that reflect the MDS criteria for PD dementia, including a requirement for irreversible deficits in multiple cognitive domains accompanied by functional impairment. Though we included baseline MMSE as a covariate in our Cox regression model for time to dementia, it is not known how many patients had mild cognitive impairment at baseline, which may be expected to influence prognosis. Because assessments were performed every two years for the CamPaIGN cohort, and every 18 months for the PICNICS cohort, it was not possible to determine the exact time of the onset of dementia or progression to HY3 or PIGD score of two or more, which was determined as the mid-point between the assessment at which the patient had reached the outcome measure, and the prior assessment.

Another limitation of our study is the relatively limited sample size. We have attempted to address this by using two homogeneous incident cohorts from the same population base but separated in time²³. However, 115 patients were excluded from survival analyses as they had only undergone limited genetic screening, meaning that it was possible that they harboured unidentified, potentially clinically significant *GBA1* variants. The sequencing of *GBA1* conferred major benefits in this study, as a more comprehensive method for identifying both presence of absence of *GBA1* variants. Whilst we acknowledge that complete sequencing reads could not be achieved in all patients (as previously described in Winder-Rhodes et al 2013), we feel that the use of sequencing data allowed us to assign *GBA1* variant carrier status with maximum possible accuracy. Further strengths of this study are the long duration of follow-up of up to 18 years, and the fact that our cohorts consisted of incident PD patients recruited from the community, allowing follow up from diagnosis to the development of key clinical milestones in a representative population. As such we have presented the first long-term data on the effect of *GBA1* variants on mortality in an incident PD population, and detected significant differences in the natural history of PD associated with “non-pathogenic” *GBA1* variants, which may have been missed in previous studies, probably due to their limited follow-up durations.

In conclusion, long-term follow-up of the CamPaIGN and PICNICS cohorts confirms that *GBAI* mutations adversely affect clinical course. Importantly, carriers of *GBAI* variants that are often considered to be “non-pathogenic” had a significantly worse clinical course than non-carriers. *GBAI* variants therefore contribute to the pathogenesis and clinical features of more than 10 % of PD patients, making them a particularly important genetic sub-group. Since *GBAI* abnormalities appear to be associated with specific pathogenic mechanisms, it may be that the optimal disease-modifying approach differs in this group than in the wider PD population, with important implications for future clinical trials.

Competing interests

The authors declare no competing interests.

Contributorship Statement

TBS wrote the manuscript. TBS, MC, SWR, GL, CS, TF, JE, and DB performed the research. RAB and CHW-G conceived the ideas for this study and provided critical review of the manuscript.

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Figure Legends

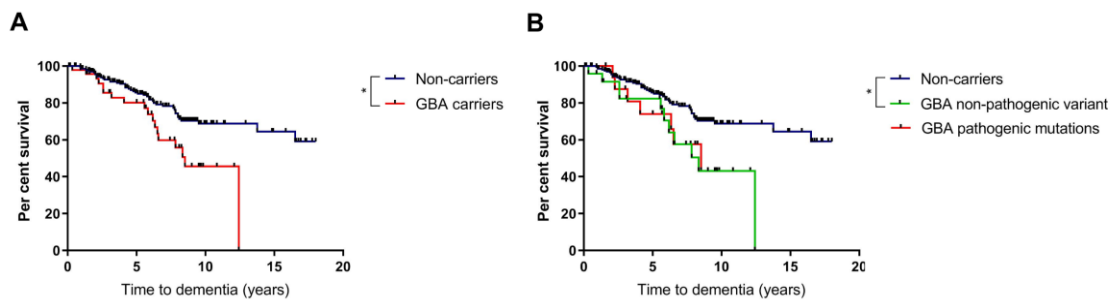


Figure 1 - Effect of *GBA1* variants on development of dementia in PD. A) Survival analysis for time to dementia in carriers of any *GBA1* variant compared to non-carriers. B) Subgroup survival analysis for time to dementia in carriers of pathogenic *GBA1* mutations, “non-pathogenic” variants, and non-carriers. Statistical significance as determined by log-rank tests indicated by asterixes.

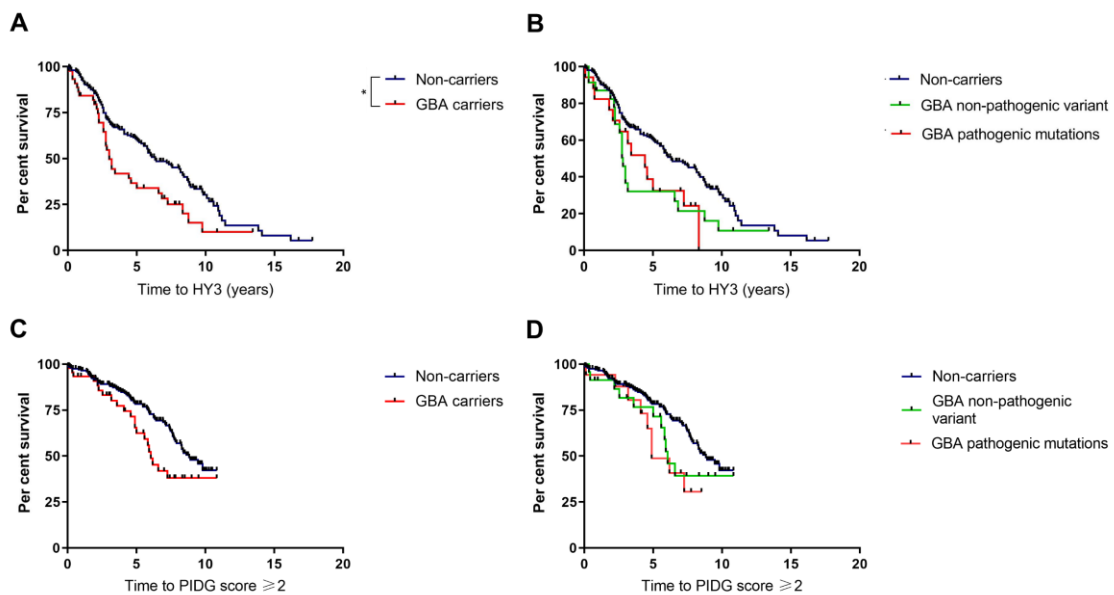


Figure 2 - Effect of *GBA1* variants on development of postural instability in PD. A) and C) Survival analysis for time to HY3 and PIGD score of two or more respectively, in carriers of any *GBA1* variant compared to non-carriers. B) and D) Subgroup survival analysis for time to HY3 and PIGD score of two or more respectively, in carriers of pathogenic *GBA1* mutations, “non-pathogenic” variants, and non-carriers. Statistical significance as determined by log-rank tests indicated by asterixes.

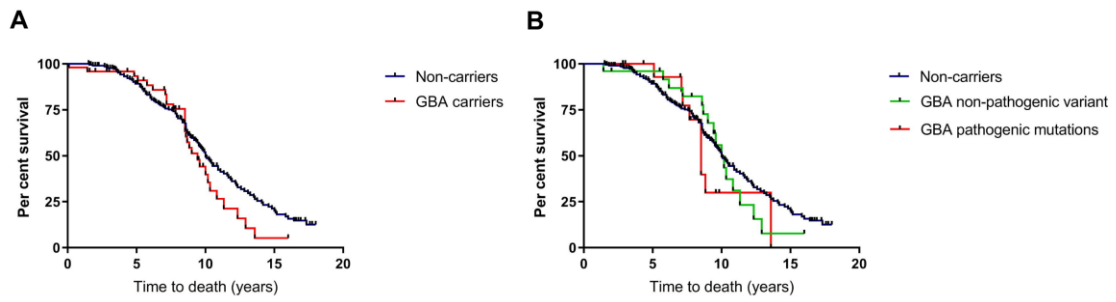


Figure 3 - Effect of *GBA1* variants on time to death in PD. A) Survival analysis for time to death in carriers of any *GBA1* variant compared to non-carriers. B) Subgroup survival analysis for time to death in carriers of pathogenic *GBA1* mutations, "non-pathogenic" variants, and non-carriers.

Supplementary Material

	CamPaIGN	PICNICS
Inclusion criteria	<ul style="list-style-type: none"> • New cases with Parkinsonism diagnosed December 1 2000 to December 31 2002 • Recruited from community (Cambridgeshire) • Diagnosis validated by a movement disorder specialist using UKPD Brain Bank criteria at baseline assessment (with re-validation at 3.5 years) 	<ul style="list-style-type: none"> • New cases of Parkinson's disease diagnosed between November 1 2006 and May 8 2013 • Recruitment from community, Cambridgeshire • UKPD BB criteria at baseline
Exclusion criteria	<ul style="list-style-type: none"> • MMSE of < 24 at baseline • Failure to meet UKPD Brain Bank criteria at subsequent follow-up • Diagnosis other than idiopathic PD 	<ul style="list-style-type: none"> • Dementia at baseline • Failure to meet UKPD Brain Bank criteria at subsequent follow-up • Diagnosis other than idiopathic PD

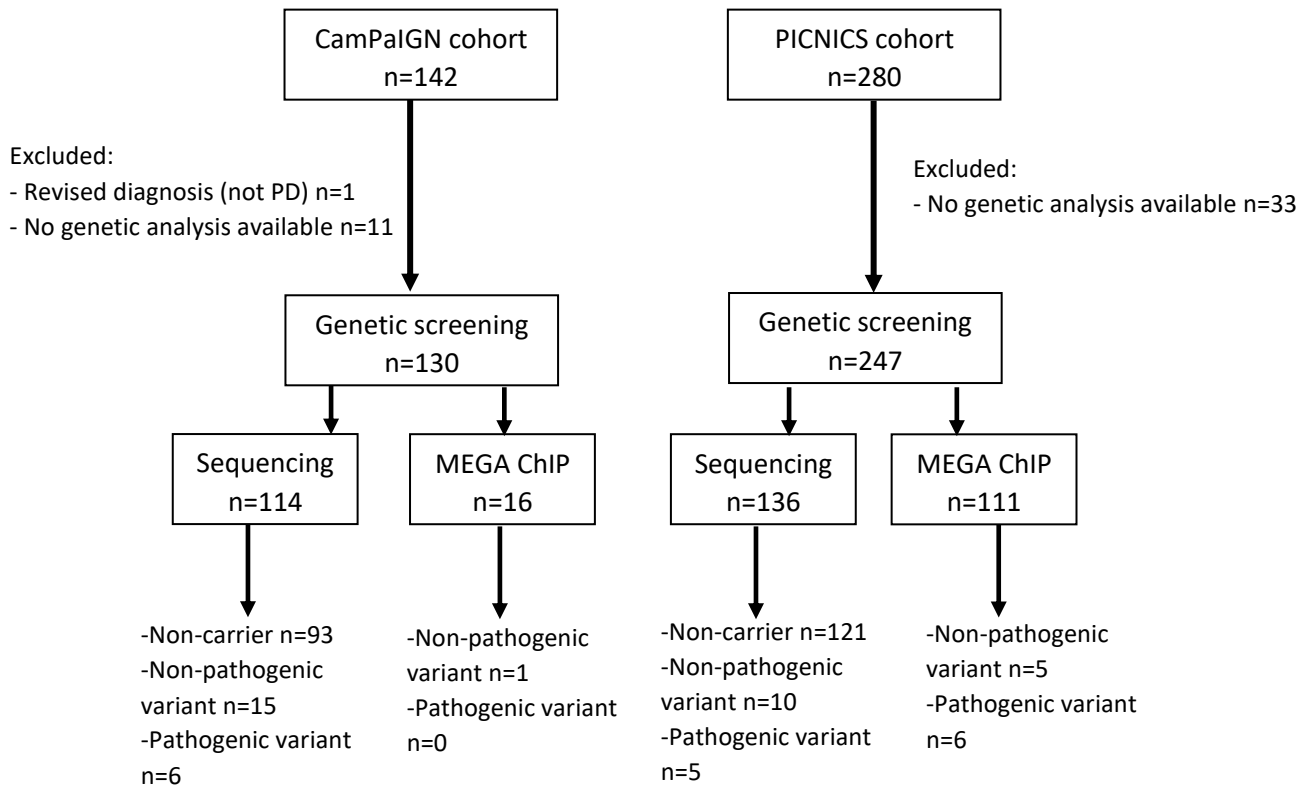
Supplementary table 1. Inclusion and exclusion criteria for CamPaIGN and PICNICS cohorts.

	Non-carriers	<i>GBA1</i> non-pathogenic variant carriers	Pathogenic <i>GBA1</i> mutation carriers
Infection	41 (32.5%) - Respiratory n=36 - UTI n=4 - UTI + pneumonia n=1	5 (26.3%) - Respiratory n=5	2 (22.2%) - Respiratory n=1 - Infected fracture n=1
Parkinson's disease	14 (11.1%)	-	1 (11.1%)
Cardiovascular	8 (6.3%) - Congestive cardiac failure n=3 - Myocardial / pericardial fibrosis n=2 - HOCM n=1 - Cardiac arrest n=1 - Ruptured AAA n=1	2 (10.5%) - Myocardial infarction n=2	-
Malignancy	5 (4.0%) - Prostate n=1 - Bowel n=1 - Renal sarcoma n=1 - Lung n=1 - Not-specified n=1	-	-
Old age / frailty	4 (3.2%)	-	-
Stroke	2 (1.6%)	1 (5.3%)	-
Gastrointestinal	2 (1.6%) - Stercoral bowel perforation n=1 - Gastrointestinal haemorrhage n=1	-	1 (11.1%) - Sigmoid volvulus n=1
Other	5 (4.0%) - COPD n=2 - Liver failure n=1 - Subdural haematoma n=1 - Suicide n=1	-	-
Unknown	45 (35.7%)	11 (57.9%)	5 (55.6%)

Supplementary table 2. Causes of death for non-carriers and carriers of “non-pathogenic” and pathogenic *GBA1* variants with Parkinson’s disease. Causes of death were determined through analysis of medical certificates of the cause of death where accessible. Abbreviations: AAA = Abdominal aortic aneurysm; COPD = Chronic obstructive pulmonary disease; HOCM = Hypertrophic obstructive cardiomyopathy; UTI = Urinary tract infection.

	Non-carriers	All <i>GBA1</i> variants	P-value	<i>GBA1</i> non-pathogenic variant carriers	Pathogenic <i>GBA1</i> mutation carriers	P-value
Years from diagnosis to baseline visit (SD)	0.23 (0.33)	0.28 (0.33)	0.344	0.31 (0.35)	0.24 (0.28)	0.534

Supplementary table 3. Time from diagnosis to first assessment for patients in CamPaIGN and PICNICS cohorts.



Supplementary figure 1. Genetic analysis in CamPaIGN and PICNICS cohorts.