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Association of GBA polymorphisms and mutations with dementia in incident Parkinson disease

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Abstract:	INTRODUCTION: Both polymorphisms and mutations in GBA may influence the development of dementia in patients with Parkinson's disease. METHODS: 442 patients and 419 controls were followed for seven years. Dementia was diagnosed using established criteria. Participants were analyzed for GBA genetic variants, including E326K, T369M and L444P. Associations between GBA carrier status and dementia were assessed with Cox survival analysis. RESULTS: A total of 12.0% of patients with Parkinson's disease carried a GBA variant, and nearly half (22/53) progressed to dementia during follow-up. Carriers of deleterious GBA mutations (adjusted HR 3.81, 95% CI 1.35 to 10.72; P = .011) or polymorphisms (adjusted HR 1.79; 95% CI 1.07 to 3.00; P = .028) progressed to dementia more rapidly than non-carriers. DISCUSSION: GBA variants are of great clinical relevance for the development of dementia in Parkinson's disease, especially due to the relatively higher frequency of these alleles compared to other risk alleles.

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Stavanger universitetssjukehus

January 18th 2018

Manuscript reference number: ADJ-D-17-00600
Title: Association of GBA polymorphisms and mutations with dementia in incident Parkinson disease
Decision: Revise

Dear Editor,

Thank you for allowing us the opportunity to re-submit our manuscript for consideration for publication in Alzheimer's and Dementia.

We have been able to address all the reviewer's comments, and a response to each of the points raised is included in this re-submission. We are pleased that they found the study to have many strengths and to be well written, and by addressing their comments we believe that we have significantly improved the manuscript.

I, the corresponding author, take full responsibility for the data, analysis and interpretation, and the conduct of the research. I have had full access to all the data, and I have permission to publish all data contained in this manuscript. Further, I hereby confirm that all authors have agreed with the contents of the manuscript, and none of the authors have any conflicts of interest related to the re-submission.

We have addressed all the reviewer's comments and given that the reviewers found our findings to be of interest for the field, we hope that you consider our improved manuscript for publication in Alzheimer's and Dementia.

Sincerely,

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Response to reviewers

Response to reviewer 1:

This is a well written paper and I do not have any major concerns. The authors examined the influence of variants in the GBA gene, including not only mutations previously widely studied, but also polymorphisms, on dementia in a multicenter PD patient cohort. The study has many strengths, including well characterized, population-based PD patient samples, clinically diagnosed dementia based on validated criteria, 7-years of follow-up starting early in PD diagnosis, eliminating issues of selective survival found in prevalent case cohorts, and GBA gene sequencing.

Specific comments:

1. I suggest naming the polymorphism in the abstract if there is space, or at least the most common ones (something like: Participants were analyzed for GBA genetic variants, including E326K, T36M, and L444P)

Response: We have now included information on the most common variants in this study in the abstract (page 3, paragraph 2). To achieve this, we slightly edited the text to reduce the word count by making the following changes:

page 3, paragraph 2 now reads "442 patients and 419 controls were followed for seven years. Dementia was diagnosed using established criteria. Participants were analyzed for *GBA* genetic variants, including E326K, T369M and L444P."

page 3, paragraph 3 now reads "A total of 12.0% of patients with Parkinson's disease carried a GBA variant, and nearly half (22/53) progressed to dementia during follow-up."

2. Section 2.1: Some information for participants enrolled in each study at baseline and the total N used in the analysis should be described in this section, and the reference table 1 should to link to the numbers. How did exclusions from the cohorts occur? Maybe material for genotyping was unavailable for the whole baseline sample of the different cohorts? From Caslake et al 2013, PINE enrolled 212 PD patients in its cohort, but only 118 are used in the current analysis (Table 1). From, Alves et al 2008, ParkWest 212 are included in the longitudinal study, and only 190 are used here. Linder et al 2010, reports 112 PD patients in

NYPUM, whereas 134 patients were used in the current study (Table 1); thus it seems more PD patients were recruited or did the authors also include Parkinsonism diagnoses? As the authors report, the included studies did a good job to minimize loss after baseline, and they seem to be able to use ~80% of the three cohorts here (442/(212+212+134)). A brief sentence on whether the loss was due to exclusion, loss to follow-up from refusal, illness/death, etc. or something else would be helpful for the discussion of selection bias (page 11).

Response: The reviewer is correct that in the manuscript and Table 1 we refer only to the numbers of individuals that were eligible for this study, and not to the numbers enrolled in each cohort study. We have followed the reviewer's recommendation and included the missing details in section 2.1 of the methods.

- The following section is added to page 5 paragraph 2:

"212 patients were enrolled in the ParkWest study, 211 in the PINE study and 182 in the NYPUM study. Of these 68 had a diagnosis other than PD during follow up, 57 declined genotyping, 31 had no available DNA sample or DNA was not extractable, and seven did not consent to follow up. The remaining 442 patients were eligible for this study and…"

- In addition, we include the missing reference for the NYPUM study (page 5, paragraph 2) that details the extended recruitment period (to the 30th of April 2009) and the 22 patients that joined the study during the additional 16 months (taking the total from 112 to 134):

Bäckström D et al. Polymorphisms in dopamine associated genes and cognitive decline in PD. Acta Neurol Scand 2018;137:91-98

- We have also changed the title of Table 1 to reflect that it contains the characteristics of the eligible participants:

"Table 1: Baseline characteristics and duration of follow up of the patients and controls included in the study"

- Regarding the losses to follow up, we included the details regarding the loss of the eligible patients in Figure 1, and we now refer to this figure in the discussion and have added the following text (page 12, paragraph 1):

"Each study is a representative incident cohort, designed to identify all new PD cases in a given population early in their disease, with high levels of consent and low levels of losses to follow up *for reasons other than death (Figure 1)*."

3. If GBA information is actually available for everyone at baseline, is GBA related to loss/refusal/exclusion? This could strengthen the argument against selection bias, or could suggest selection bias that may bias results towards the null, which would be expected if GBA positively influences both loss/exclusion and PDD.

Response: GBA information is only available for those participants who are included in this study. To make this clear we have included this statement in Methods, section 2.3 (page 6 paragraph 3): "Genomic DNA was extracted from peripheral blood samples *of eligible participants* using standard methods."

4. Section 2.1: On first read, I did not see any information about control selection/enrollment in the three papers referenced (15-17).

Response: The reviewer is correct, and we have rectified this mistake. In the manuscript, we had included the following sentence (page 5, paragraph 2):

"During the same time, normal control subjects were recruited in the same geographical areas from spouses or friends of PD patients, or unrelated persons."

Considering the reviewer's comments, and the fact that NYPUM have not described the controls in detail in a previous publication, we have expanded this to provide a more detailed outline of control recruitment and inclusion/exclusion criteria (page 5, paragraph 2):

"During the same time, normal control subjects were recruited in the same geographical areas where the cases were collected from spouses or friends of PD patients, or unrelated persons. They were clinically examined and had no signs of movement disorders or cognitive deficiencies. 201 controls were enrolled in the ParkWest study, 266 in the PINE study and 56 in the NYPUM study. Of these 68 had no available DNA sample or DNA was not extractable, 30 declined genotyping and 6 developed incident PD during follow up and were excluded. The remaining 419 consented to routine follow up with a standardized battery of clinical testing."

We also included the appropriate references from ParkWest, and PINE on page 5 paragraph 2:

Aarsland D, Bronnick K, Larsen JP, Tysnes OB, Alves G, Norwegian ParkWest Study G. Cognitive impairment in incident, untreated Parkinson disease: the Norwegian ParkWest study. Neurology. 2009;72:1121-6.

Fielding S, Macleod AD, Counsell CE. Medium-term prognosis of an incident cohort of parkinsonian patients compared to controls. Parkinsonism Relat Disord. 2016;32:36-41.

5. Section 3.3: page 9, line 6, do you mean Figure 2 not Figure 1?

Response: The reviewer is correct and we have corrected the text to read "Figure 2" (page 9, paragraph 2)

6. Section 3.3/Table 3: Each of the HRs was stronger after adjustment. This suggests some confounding towards the null. Is there one factor which made the difference? This comes to mind especially because of the relatively high GBA polymorphism variant frequency in the Swedish/Norwegian populations relative to other European populations, as discussed (pg 10, line 55/supplemental table 1). If so, this might be worth mentioning briefly, as something like "population stratification" may not be expected by readers when combining three populations from Northern Europe. However, small sample size for GBA carriers may limit these analyses.

Response: As noted by the reviewer, the effects of GBA on PDD risk are all strengthened when adjusting for other predictors. We found that most of the adjusting effect comes from including age at onset into the models. We take this to mean that the observed, unadjusted effect of GBA on PDD risk is attenuated by the additional effect that GBA carriers tend to be younger at onset of PD than non-carriers; the largest difference observed in our study was in carriers of GBA

mutations who were on average 9 years younger at diagnosis than non-carriers. It is well-known that dementia is strongly related to ageing, however, among those diagnosed with PDD before seven years of follow-up, the mean ages at PDD diagnosis were 78 (± 6), 76 (± 7) and 67 (± 6) for GBA non-carriers, polymorphism carriers and mutation carriers, respectively (p= .017, ANOVA with Welch correction). In sum, it seems, as pointed out in our discussion, that GBA carriers deviate from the typical idiopathic PD patient with a younger age of onset who is expected to have a more benign disease course with slower motor and cognitive decline as compared to patients with later onset PD.

To clarify the effect of covariables in the role of GBA in progression to PDD, we added a sentence to the end of section 3.3 (Page 9, paragraph 2):

"Adjusting with co-variables strengthened the effect of *GBA* on progression to PDD (Table 3). Most of the adjusting effect comes from including age at onset in the models, suggesting that the observed, unadjusted effect of GBA on PDD risk is attenuated by the additional effect that GBA carriers tend to be younger at onset of PD than non-carriers."

and have adjusted the discussion to reduce repetition (page 11, paragraph 2)

"*This is noteworthy* as idiopathic PD patients with a younger age of onset typically have a more benign disease course with slower motor and cognitive decline, when compared to patients with later onset PD [36, 37]."

7. Page 10, lines 8-11: "The largest multi-center study to date to analyze the role of polymorphisms did not find an effect on PDD risk [14]." I would suggest including the HR from Liu et al, as this study did estimate an increased risk associated with cognitive decline, even though the CI included the null but the weight of evidence from the CI/point estimate suggests a small increase in risk (nonpathogenic risk variants (6.6% of patients; HR, 1.36; 95% CI, 0.89-2.05), Liu et al, 2016)

Response: we agree that it is important to also include the data on cognitive decline and not only the endpoint of dementia, and have therefore modified the text to include this information (starting Page 10, paragraph 4):

"The largest multi-center study to date to analyze the role of polymorphisms did not find an effect on PDD risk*, however this study did estimate a small increased risk associated with cognitive decline (6.6% of patients; HR 1.36, 95% CI 0.89-2.05)*."

8. There is one more European study that examined GBA and PDD, but the patients are younger, prevalent, and recruited from tertiary care clinics (not population based), and they did conduct sequencing - this may be worth mentioning given your discussion of sequencing as a strength and your recommendation. Crosiers D, Verstraeten A, Wauters E, et al. Mutations in glucocerebrosidase are a major genetic risk factor for Parkinson's disease and increase susceptibility to dementia in a Flanders-Belgian cohort. Neurosci Lett. 2016;629:160-164.

Response: We have included this article in the discussion of the strategy to sequence GBA. In addition, we have included the other European based studies referenced in the manuscript that screened GBA, and feel that the comparison of these studies strengthens the discussion (page 11, paragraph 3):

"These results are similar to the overall *GBA* carrier frequency in other studies of European PD patients that have employed complete screening of *GBA* (range 9.3-12.2 %) [4-7], and…"

We have also referenced the Crosiers article in the discussion of previous works to study the role of deleterious mutations and the link to PDD (page 11, paragraph 2).

9. "Given that variants in GBA are the most common risk factor for PD, this places many individuals at risk of a more severe disease course." I suggest to moderate this statement since age and sex are more common idiopathic PD risk factors, and GBA variants are not the most common 'genetic risk factors' when we consider minor allele frequency. For example in the meta PD GWAS (Nalls et al, 2014), a number of polymorphisms are much more common than GBA variants, SNCA for example has more common variants. However, a GBA SNP did have the strongest association with PD in this meta-analysis, was this what the authors were referring too?

Nalls, M. A. et al. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. Nat. Genet. 46, 989-993 (2014).

Response: we agree that this statement should be modified, and have changed it to: "Given that variants in GBA are present in 12% of the PD population in this study, this places many individuals at risk of a more severe disease course." (page 13, paragraph 2).

Response to reviewer 2:

The role of GBA polymorphisms in Parkinson's disease dementia (PDD) is subject of debate in the field. The manuscript report a study targeting to understand the effects of GBA variants in PDD. The authors claim that this study is the largest population-based longitudinal multicenter study using a younger cohort and methods of assessment with lower sensitivity than the previously used in other studies. The major finding of this report is that PD patients carrying GBA variants, polymorphisms, or deleterious variants, have a faster progression to dementia, with carriers of deleterious mutations displaying a more rapid disease progression than carriers of polymorphisms. r the manuscript findings are of great interest in the field; it is well written and the methods used are sounds.

Specific comments

1. The authors highlighted and mention in the text that the effect of GBA variants are "on a continuum" that seems to imply a possible categorization of the variant effect in degrees going from none to worst for each of the mutations that is not presented and this reviewer is not sure whether it could.

Response: by this statement we intended to illustrate that the effect of each type of variant varies relative to the other types of variant based on the severity of the mutation, and that this is on a scale (or "continuum") "with carriers of deleterious mutations displaying a more rapid disease progression than carriers of *GBA* polymorphisms" (page 10, paragraph 2). Considering the reviewer's comment, we acknowledge that the manuscript has not made this point clearly, and that the use of the phrase "on a continuum" does not add significantly to the discussion, therefore, we have deleted this phrase (Discussion; page 10 paragraph 2).

Response to reviewer 4:

The authors report an interesting association between GBA variants and mutations with dementia, and earlier onset of dementia, in PD. The search for genetic associations with any feature of any neurodegenerative disease is an important line of research; the importance of this topic allows a more liberal review of the work than might be the case with more heavily studied topics.

That said, there are areas that need a bit of modification.

1. Sample: while the base sample from which these two cohorts were drawn may be population based, the cohorts are highly selected for the purposes of the study. You have basically done a 'screening study' to obtain proof of concept by enriching the total sample for PD, and this is justified because the GBA variants and mutations you identify are rare. But the process of cohort selection leads to the potential for inflating the association of your target genes with the outcome.

Response: This study was designed to obtain a population representative sample of PD patients and controls, as free from as much selection bias as possible. We have not enriched the sample for PD cases for the purpose of this study, and instead included all possible patients from those individuals enrolled by each of the three incident cohorts from their defined geographical regions during the recruitment period. The main reason that eligible individuals were not analysed for GBA is either because they were not available (e.g. lack of consent for genotyping or follow up, no sample available or DNA was not extractable), and is unlikely to lead to selection bias.

The reasons for exclusion from the study have now been included on page 5 paragraph 2 (please also see the response to Reviewer 1), and we hope that it is now transparent that we included all possible individuals from the original population based samples.

2. You state that no participant met criteria for Lewy body dementia. The boundaries between DLB and PDD are somewhat elastic. Please explicitly justify your statement and why it matters.

Response: The eligibility criteria for this study was to have a diagnosis of PD at baseline and patients with DLB were excluded. After considering the reviewer's comment, we agree that the original text was unclear and have modified it to be clear on the inclusion criteria (page 6, paragraph 2):

Original text: "No patients included in this study fulfilled criteria for dementia with Lewy bodies."

New text: "Patients with dementia with Lewy bodies (DLB), as defined by the development of dementia within one year of the onset of the motor features of PD, were not eligible for this study."

We have also added a sentence to the discussion. As the reviewer points out, the boundaries between PDD and DLB are not clear. We think that it is an important point to include for a readership with a broad interest in the dementias (page 12, paragraph 1)

"Furthermore, this study was designed to study the development of PDD, and patients with DLB were excluded. Given that DLB and PDD share many pathological and clinical features and probably represent two clinical entities on a spectrum of Lewy body disease, it will be of great interest to determine how different types of GBA variants affect disease progression in DLB. Finally, …"

3. Rate of dementia development: I don't see data on PD severity at endpoint (perhaps I missed it?). One assumes that development of dementia earlier in the course of PD reflects either more rapid dissemination of overall PD pathology or a different distribution of pathology (in brain regions most relevant to cognitive impairment) throughout the course of disease. Comment on whether your data also show more rapid progression of PD by conventional severity measures as well as a shift in the trajectory of only the cognitive components of PD.

Response: This is an important point and to address it we have performed mixed model analysis of Hoehn and Yahr progression. We found no significant differences between non-carriers and GBA carriers, or between non-carriers and either of the two GBA subgroups ($p>0.35$ for all comparisons), and conclude that our data shows that GBA variant carriers do not show a more rapid overall disease progression of PD by conventional severity measures.

We agree that this is of considerable interest to the field and therefore added a section to section 3.3 (page 10, paragraph 1):

"The effect of *GBA* on PDD was not reflected by an overall increased rate of disease progression, as we observed no significant differences in the progression of Hoehn and Yahr scores between *GBA* carriers and non-carriers during the first 7 years of disease, or between any of the *GBA* subgroups and non-carriers (all $p > 0.35$, data not shown)."

4. Please contextualize your work for a broader readership; most readers of the journal are not geneticists and will want to know whether the data you present should inform the way they practice or advise patients with PD. For researchers, you might suggest what kinds of strategies are needed to study important clinical effects of rare genetic variants - research tactics are crucial in this area.

Response: to address these important points, we have made three additions to the discussion:

Page 12, paragraph 1. We added the following text

"This will require further pooling of existing cohorts to give larger numbers or studying inception cohorts enriched for carriers of different GBA variants."

Page 13, paragraph 1. We added the following text:

"A range of different studies, including imaging and histopathological analyses of brains, and animal studies, will be required to improve understanding of precisely how each *GBA* variant increases risk of cognitive decline and affects the underlying spread of Lewy body pathology in PD.

Page 13, paragraph 2. We modified the conclusion:

"At present there are no preventative PD treatments that target GBA pathways and an individual's *GBA* status does not alter clinical management, which contend that screening for *GBA* variants should not yet be included in routine genetic testing in a clinical setting. However, future studies should focus on establishing the success of combining GBA mutations and polymorphisms with other predictors of dementia to gain a more precise indication of when PD patients get dementia. The ability to predict the development of dementia is already highly relevant for recruitment into and stratification of clinical trials, and the finding in this study that PD patients harboring either GBA mutations or polymorphisms are at significantly increased risk of early PDD, advocates that both groups are candidates for clinical PDD research studies. This will be especially important as interventions that target GBA to slow disease progression or to prevent dementia become available."

Further notes to the editor

We have identified two typing errors in the manuscript.

Table 3: row 2, column 4 should read 27.5 not 28.5

Figure 1: "dropouts" after the fifth-year assessment should read "withdrawals" to be in the same style as the rest of the figure.

Research In Context

Systematic review: The authors reviewed the literature using PubMed, and identified all studies in which the role of *GBA* variants on the development of dementia in Parkinson's disease was addressed. These mainly focused on the role of *GBA* mutations, and the question of whether *GBA* polymorphisms modify the development of dementia in Parkinson's disease remains unresolved.

Interpretation: Our findings show clearly, for the first time, that both carriers of *GBA* mutations and polymorphisms are at increased risk of developing dementia during the first seven years of Parkinson's disease.

Future directions: The manuscript proposes that carriers of both *GBA* mutations and polymorphisms should be considered for inclusion in future clinical trials targeting GCase dysfunction. This will require (1) establishment of the size of effect of each polymorphism on the rate of disease progression in larger cohorts, and (2) functional studies to determine the effect of each polymorphism of GCase activity.

Association of *GBA* polymorphisms and mutations with dementia in incident Parkinson's disease

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Abstract

INTRODUCTION: Both polymorphisms and mutations in *GBA* may influence the development of dementia in patients with Parkinson's disease.

METHODS: 442 patients and 419 controls were followed for seven years. Dementia was diagnosed using established criteria. Participants were analyzed for *GBA* genetic variants, including E326K, T369M and L444P. Associations between *GBA* carrier status and dementia were assessed with Cox survival analysis.

RESULTS: A total of 12.0% of patients with Parkinson's disease carried a *GBA* variant, and nearly half (22/53) progressed to dementia during follow-up. Carriers of deleterious *GBA* mutations (adjusted HR 3.81, 95% CI 1.35 to 10.72; $P = .011$) or polymorphisms (adjusted HR 1.79; 95% CI 1.07 to 3.00; $P = .028$) progressed to dementia more rapidly than non-carriers.

DISCUSSION: *GBA* variants are of great clinical relevance for the development of dementia in Parkinson's disease, especially due to the relatively higher frequency of these alleles compared to other risk alleles.

Key Words: Parkinson's disease; Parkinson's disease with dementia; GBA; Longitudinal; genetic association.

1 Background

Dementia is among the most common and severe non-motor symptoms of Parkinson disease (PD), affecting nearly 20% of all patients within the first 5 years of the disease, and the majority of patients if they survive for more than 10 years after diagnosis [1, 2]. Dementia in PD (PDD) has important adverse implications for quality of life, caregiver burden, and health-related costs [3]. The etiology of PDD remains poorly understood and no neuroprotective therapies are currently available.

Genetic factors undoubtedly play a role in modifying the rate of disease progression in PD, and identifying these is key to the early identification of patients at greatest risk of PDD. Genetic variants in glucocerebrosidase (*GBA*) have the strongest evidence for association with more rapid cognitive decline in PD. Homozygous mutations in *GBA* cause Gaucher disease (GD), and it is well established that some of the heterozygous mutations are associated with an increased risk of PD [4]. *GBA* variants associated with increased risk of PD chiefly fall into two categories: risk polymorphisms, the most common of which are E326K and T369M [5, 6]; and deleterious mutations, such as N370S and L444P, which in a homozygous state cause GD [7].

GBA variants have been shown to increase the risk of PDD in cross-sectional studies [8, 9], and longitudinal studies are starting to show how different *GBA* variants affect the rate of the development of dementia during the course of PD. Most longitudinal studies have found that carriers of deleterious *GBA* mutations are at increased risk of earlier PDD onset [10-13] or faster decline in global cognitive function [14]. To date, few studies have considered the effects of *GBA* risk polymorphisms on the development of PDD, and the only longitudinal studies to identify a significant association between *GBA* polymorphisms and progression to PDD did so only after controlling for the effect of *MAPT* genotype [10] or by including both mild cognitive impairment or PDD [6].

Therefore, we analyzed the *GBA* carrier frequencies of three deeply phenotyped, longitudinal PD cohorts of highly uniform design from Northern Europe, each of which uses established criteria for the diagnosis of PDD. Together, the Norwegian ParkWest Study [15], the Parkinsonism Incidence in Northeast Scotland (PINE) [16], and the New Parkinson Patient in Umeå (NYPUM) [17] studies represent the largest prospective population based longitudinal study of PD with age- and sex-matched

controls in which the effect of *GBA* variants on PD progression has been addressed. By determining the roles of *GBA* polymorphisms and deleterious mutations in the development of PDD, we provide important insights into the heterogeneity of disease progression in these subgroups.

2 Methods

2.1 Study Participants and Procedures

The ParkWest study, the NYPUM project, and the PINE study were initiated between 2002 and 2004. All are large, on-going, population-based multicenter studies of newly-diagnosed (incident) PD patients, designed to determine the incidence, neurobiology, and prognosis of PD, and are described in detail elsewhere [15-18]. Briefly, 212 patients were enrolled in the ParkWest study, 211 in the PINE study and 182 in the NYPUM study. Of these 68 had a diagnosis other than PD during follow up, 57 declined genotyping, 31 have no available DNA sample or DNA was not extractable, and seven did not consent to follow up. The remaining 442 patients were eligible for this study and underwent comprehensive and standardized clinical examinations before drug treatment was initiated if possible (98% drug-naïve). During the same time, normal control subjects were recruited in the same geographical areas from spouses or friends of PD patients, or unrelated persons [19, 20]. They were clinically examined and had no signs of movement disorders or cognitive deficiencies. 201 controls were enrolled in the ParkWest study, 266 in the PINE study and 56 in the NYPUM study. Of these 68 had no available DNA sample or DNA was not extractable, 30 declined genotyping and 6 developed incident PD during follow up and were excluded. The remaining 419 consented to routine follow up with a standardized battery of clinical testing. PD patients are currently under continued follow-up, and only those with a confirmed clinical or pathological (if performed post-mortem) diagnosis of PD according to the UK brain bank criteria at their latest or final clinical visit were included. All participants signed written-informed consent. The Western Norway Regional Committee for Medical and Health Research Ethics, the Regional Ethics Review Board in Umeå, and the Multi Centre Research Ethics Committee for Scotland, approved the respective studies.

2.2 Clinical assessments in PD

The data was analyzed with the focus on PD risk, age at symptom onset or diagnosis, and the development of dementia. PD patients were examined at time of diagnosis by experienced study neurologists and research nurses. Clinical evaluations made up to the 7-year visit are included in this study. Motor severity was rated using the motor section (part III) of the Unified Parkinson Disease Rating Scale (UPDRS), and disease stage using the Hoehn and Yahr staging. Global cognitive decline was measured by the Mini-Mental State Examination (MMSE) [21]. Dementia diagnosis was set according to Movement Disorder Society criteria [22] (ParkWest and NYPUM) or DSM-IV [23] (PINE), using a combination of clinical history from the patient and carer, and cognitive testing. Patients with dementia with Lewy bodies (DLB), as defined by the development of dementia within one year of the onset of the motor features of PD, were not eligible for this study.

2.3 Genetic analysis

Genomic DNA was extracted from peripheral blood samples of eligible participants using standard methods. Large-scale allelic discrimination analysis was performed for all patients and controls using a pre-designed TaqMan SNP genotyping assay for rs75548401/T369M, and custom assays for rs369068553/V460L, rs2230288/E326K, rs76763715/N370S, and rs781152868/Y135C (ThermoFisher Scientific) as described [24, 25]. The call rate was 99.8%.

A total of 188 patients of the ParkWest cohort were also characterized by whole exome sequencing (unpublished material). Variants falling within the *GBA* region were identified and analysed using Ingenuity Variant Analysis (Qiagen, CA). Five nonsynonymous variants were detected: N370S, T369M, E326K and two additional mutations, rs369068553/V460L and rs781152868/Y135C, which were confirmed by sequencing as described [26, 27]. V460L and Y135C were genotyped in the remaining samples, and the carrier of V460L confirmed by sequencing.

For rs421016/L444P genotyping, a fragment of 960 base pairs was amplified as described (primer sequences and reaction conditions available on request) [26, 28]. PCR products were analyzed by restriction fragment length polymorphism in all three cohorts using *Nci*I [29]. Ten samples failed genotyping, giving a success rate of 98.8%. All mutations were confirmed by direct sequencing of the PCR product [29].

All amino acid substitutions are numbered excluding the 39-residue signal peptide.

2.4 Statistical methods

"*GBA* carriers" included all patients carrying any of the detected nonsynonymous *GBA* variants. *GBA* carriers were further split into "polymorphism carriers" (E326K, T369M or V460L), and "deleterious carriers" (Y135C, N370S or L444P), based on published reports and predicted pathogenicity as described [13].

Between-group differences were compared using t-tests, Mann-Whitney tests and X^2-tests as appropriate. For age of PD onset, we performed multiple linear regression analysis adjusted for study cohort and sex. We used logistic regression to calculate odds ratios (ORs) with 95% confidence intervals (CIs) for incident PD by different *GBA* carrier groups, without and with adjustment for study cohort, age at baseline and sex. For incident PDD cases, we assigned time of dementia onset to the midpoint of the interval between assessments at which dementia was diagnosed, as described previously [1]. We performed Cox regression analysis to assess the role of *GBA* carrier status on the evolution of PDD in the patient group, adjusting for study cohort, age at baseline, sex and years of education. Censoring occurred due to deaths and losses to follow up, and at end of study i.e. at the 7 year visit. Assumption of proportionality was assessed and deemed to hold using log–log plots. There was no statistical evidence of differential effect of *GBA* on PDD free survival between cohorts. We considered two-tailed values of $p < .05$ significant and conducted all statistical analyses using IBM SPSS Statistics (Armonk, NY), version 21.0.

3 Results

Of 861 study participants, 419 were controls and 442 were patients with PD. Their baseline characteristics are listed in Table 1. No statistical differences were detected between patients and controls in any demographic or clinical variables at baseline. For PD patients, mean age at baseline was 69.81 (±9.62) years, with 60.4% (267) males. During follow-up, 115 (26.0%) patients deceased, while 24 (5.4%) patients dropped out of the study for reasons other than death (Figure 1).

3.1 Genetic analysis

We identified a total of 82 carriers of *GBA* variants in this cohort (Supplementary Table 1). In addition to analysis of the four most frequently studied *GBA* variants in PD, E326K, T369M, N370S and L444P, whole exome sequencing of patients from ParkWest identified two further mutations: V460L, previously identified in PD and healthy controls [30, 31] and; Y135C, which has been identified in GD (Clinvar accession 280972) but not PD.

One patient was homozygous for E326K, and one patient carried both the E326K and the T369M variant. No other homozygous subjects or carriers of complex alleles were identified. For further analysis patients and controls were classified as non-carriers or carriers of any *GBA* variant, or further subdivided into carriers of polymorphisms or carriers of deleterious mutations (Table 2 and Supplementary Table 1).

3.2 GBA and PD risk

Of the 82 *GBA* carriers in the cohort, 53 were patients (12.0% of patients) and 29 controls (6.9% of controls). Logistic regression analysis showed that both carriers of any *GBA* variant and carriers of polymorphisms had an increased risk of PD (all *GBA* carriers OR 1.83; 95% CI 1.14 to 2.94; $P = .012$, and polymorphism carriers OR 1.73; 95% CI 1.05 to 2.86; $P = .033$). After adjusting for study cohort, age and sex, the associations remained significant for carriers of any *GBA* variant (all *GBA* carriers:

OR 1.70; 95% CI 1.05 to 2.77; P = .032, and polymorphism carriers: OR 1.63; 95% CI 0.97 to 2.73; P = .065). Furthermore, linear regression analysis revealed a significant effect of *GBA* variants on age of PD diagnosis, reducing age at onset by 3.4 years on average in patients with any *GBA* variant (β = -3.38; 95% CI - 6.13 to -0.63; P = .016) and 9.1 years in patients with a deleterious mutation (β = -9.10; 95% CI -15.80 to -2.40; P = .008), compared with non-carriers (70.2 ± 9.50 years). These associations remained significant when adjusting for study cohort and sex (all *GBA* carriers: β = -3.55; 95% CI -6.28 to -0.81; P = .011, and mutation carriers: β = -9.56; 95% CI -16.23 to -2.88; P = .005). No associations were identified between *GBA* carrier status and demographic or clinical baseline variables other than age (all P > .050) (Table 2).

3.3 GBA and PDD risk

Progression to PDD was more frequent in the *GBA* carrier group compared to non-carriers. By 7 years of follow-up, 22 (41.5%) of the 53 *GBA* carriers had progressed to dementia vs. 107 (27.5%) of the 389 non-carriers. To assess the longitudinal effects of *GBA* carrier status on progression to dementia, we performed a Cox regression analysis (Table 3, Figure 2). We observed a significant association between *GBA* carrier status and progression rate to dementia when adjusting for age at baseline, years of education, study cohort and sex (HR 1.98, 95% CI 1.23 to 3.18, P = .005). When assessed separately, the polymorphism carrier group showed a significant but more moderate association with progression rate to dementia (adjusted HR 1.79, 95% CI 1.07 to 3.00, P = .028), while the deleterious carriers had a higher risk of a progression to dementia (adjusted HR = 3.81, 95% CI 1.35 to 10.72, P = .011) compared to non-carriers. Adjusting with co-variables strengthened the effect of *GBA* on progression to PDD (Table 3). Most of the adjusting effect comes from including age at onset in the models, suggesting that the observed, unadjusted effect of *GBA* on PDD risk is attenuated by the additional effect that *GBA* carriers tend to be younger at onset of PD than non-carriers.

The effect of *GBA* on PDD was not reflected by an overall increased rate of disease progression, as we observed no significant differences in the progression of Hoehn and Yahr scores between *GBA* carriers

and non-carriers during the first 7 years of disease, or between any of the *GBA* subgroups and non-carriers (all $p > 0.35$, data not shown).

4 Discussion

In this study, we examined the association of *GBA* variants with the development of dementia in the largest population-based longitudinal multicenter study of incident PD to date. We show that PD patients carrying *GBA* variants have a faster progression to dementia, with carriers of deleterious mutations displaying a more rapid disease progression than carriers of *GBA* polymorphisms.

Of the 129 patients who progressed to PDD within the 7-year visit, 17.1% were carriers of a *GBA* variant, compared to 9.9% of the PD patients without dementia. We observed a differential effect of the type of *GBA* variant on the risk for dementia; multivariable survival analysis showed that the risk of progressing to PDD within the 7-year visit for *GBA* polymorphism carriers was nearly doubled compared to non-carriers, whereas deleterious mutation carriers were at nearly 4-times greater risk.

The role of *GBA* polymorphisms in disease heterogeneity as PD progresses is a disputed topic: The first longitudinal study to analyze *GBA* found that mutations predicted increased risk of dementia, but when comparing 7 polymorphism carriers to 117 non-carriers, an association with dementia was only found after adjusting for *MAPT* haplotype [10]. Subsequently, two studies found that E326K is associated with a higher prevalence of dementia [8], or with conversion to mild cognitive impairment or dementia [6], but unexpectedly in these studies carriers of *GBA* mutations were at similar or lower risk than E326K carriers. Conversely, a Spanish retrospective study observed increased risk of PDD in *GBA* mutation carriers, but no effect in patients carrying potentially benign variants [13]. However, the benign group included 13 synonymous and non-coding sequence variants in addition to E326K and T396M, which could mask the effect of missense variants. The largest multi-center study to date to analyze the role of polymorphisms did not find an effect on PDD risk, however this study did estimate a small increased risk associated with cognitive decline (6.6% of patients; HR 1.36, 95% CI 0.89-2.05) [14]. Of notable difference to our study was the considerably younger PD sample, as well

as the use of MMSE for the detection of PDD, which has lower sensitivity compared to established diagnostic criteria for PDD [32]. Considering these inconsistent findings, this study, which is composed of cohorts specifically designed to study the incidence and prognosis of PD, provides new and important insights into the role of *GBA* polymorphisms in PDD.

Significant attention has been paid to the phenotypic characterization of PD patients with the most frequent pathogenic *GBA* mutations [10-12, 14, 33-35], and our findings reaffirm the role of damaging mutations in the development of dementia in PD. The clinical relevance of these results is augmented by the additional effect of *GBA* carrier status on age at PD onset: We show that in addition to more rapid dementia development, patients with PD who carried a *GBA* variant had a younger age of onset. The largest difference was observed in carriers of *GBA* mutations who were on average 9 years younger at diagnosis than non-carriers. This is noteworthy as idiopathic PD patients with a younger age of onset typically have a more benign disease course with slower motor and cognitive decline, when compared to patients with later onset PD [36, 37].

Information on the frequency of *GBA* variants in Northern Europe is scarce. Previous studies reported a low carrier frequency of genetic variants in PD patients, with the exception of L444P in Northern Sweden, which is more prevalent in this region consistent with a higher incidence of GD in this part of the country [25, 29, 38]. Here we performed complete screening of *GBA* in a population based cohort of PD patients from Norway, and find a comparatively high frequency of *GBA* variants (12.0%). These results are similar to the overall *GBA* carrier frequency in other studies of European PD patients that have employed complete screening of *GBA* (range 9.3-12.2 %) [9, 10, 33, 35], and highlight the importance of complete sequencing and cataloguing of all variants in population based cohorts to avoid underestimating variant frequency. Although not the main focus of this study, we further show that both carriers of any variant and polymorphism carriers were at increased risk of PD. These data reinforce the role of T369M and E326K as risk factors in PD [5, 38, 39], and extend the finding that *GBA* is a major risk factor in PD to Northern European populations [25].

This work has considerable strengths: Each study is a representative incident cohort, designed to identify all new PD cases in a given population early in their disease, with high levels of consent and

low levels of losses to follow up for reasons other than death (Figure 1). Furthermore, each study made substantial efforts to follow all participants until death, including home visits for those no longer willing or able to attend clinic visits, and telephone follow up for dropouts, greatly reducing the problem of selection bias. Of equal importance are the uniformities in data-ascertainment methods, with each study center using validated criteria to diagnose dementia, and a median prospective follow up period of 7 years from PD diagnosis. These methods minimize the risk of erroneous inclusion of non-PD cases, and provide information about individual conversion to PDD from the time of PD diagnosis. Potential limitations of our study include the modest number of carriers of individual variants, which prevented us from analyzing the effect of each variant separately. In the future, it will be important to determine if individual *GBA* polymorphisms have differential effects on disease severity, as no comparisons between E326K and T369M have been performed in unselected PD populations. This will require further pooling of existing cohorts to give larger numbers, or studying inception cohorts enriched for carriers of different *GBA* variants. Furthermore, this study was designed to study the development of PDD, and patients with DLB were excluded. Given that DLB and PDD share many pathological and clinical features, and probably represent two clinical entities on a spectrum of Lewy body disease, it will be of great interest to determine how different types of *GBA* variants affect disease progression in DLB. Finally, complete information on the development of dementia in the control population during follow up was not available, preventing the analysis of the role of *GBA* in the non-PD population.

Dementia typically occurs in advanced stages of idiopathic PD, and is often related to the advanced spread of Lewy bodies to cortical areas. Reduced glucocerebrosidase enzyme activity leads to accumulation and oligomerization of α-synuclein. Several studies have shown that the effect of *GBA* variants on enzyme activity varies according to the nature of the variant: individuals with *GBA* mutations display the lowest glucocerebrosidase activity, whilst polymorphism carriers display a level of activity intermediate between non-carriers and mutants [40, 41]. This suggests a disease mechanism by which *GBA* mutations ultimately drive the development of dementia by increasing the accumulation and spread of α-synuclein. In support of this, autopsies of *GBA* mutation-carriers

revealed a more widespread Lewy body-type pathology in the neocortex than that of matched PD controls [27]. Our work suggests that polymorphic variants also play a role in the hastened development of PDD, although the effect on glucocerebrosidase activity might be milder, and the subsequent rate of α-synuclein accumulation and spread lower, leading to the observed milder disease phenotype. A range of different studies, including imaging and histopathological analyses of brains, and animal studies, will be required to improve understanding of precisely how each *GBA* variant increases risk of cognitive decline and affects the underlying spread of Lewy body pathology in PD.

4.1 Conclusion

In this population-based study, we present the first comprehensive overview of *GBA* variants in Northern Europe, and show that in our unselected multicenter cohort of patients with incident PD, *GBA* carrier status has important implications for the development of dementia. Given that variants in *GBA* are present in 12% of the PD population in this study, this places many individuals at risk of a more severe disease course. At present there are no preventative PD treatments that target *GBA* pathways and an individual's *GBA* status does not alter clinical management, which contend that screening for *GBA* variants should not yet be included in routine genetic testing in a clinical setting. However, future studies should focus on establishing the success of combining *GBA* mutations and polymorphisms with other predictors of dementia to gain a more precise indication of when PD patients develop dementia. The ability to predict the development of dementia is highly relevant for recruitment into and stratification of clinical trials, and the finding in this study that PD patients harboring either *GBA* mutations or polymorphisms are at significantly increased risk of early PDD, advocates that both groups of patients are candidates for clinical PDD research studies. This will be especially important as interventions that target *GBA* to slow disease progression and to prevent dementia become available.

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Figures captions

Figure 1. Flowchart of patient inclusion over time in this study.

Overview of patient inclusion from baseline until the 7-year visit. The number of patients attending each visit is shown and the cumulative number of patients diagnosed with PDD is shown in brackets. Withdrawals and deaths between visits are shown in dashed boxes. *Withdrew from study visits but dementia and death status known. **7 patients had not yet attended the 7-year visit, but were not lost to the study. The flowchart is simplified for readability.

Figure 2: Survival analysis for dementia onset. Cox regression models show the effects of *GBA* carrier status on the outcome of dementia over time in patients with PD, with adjustment for covariates (study cohort, age, sex and years of education). (A) Analysis of time to PDD for non-carriers and patients with *GBA* variants, or (B) non-carriers and patients with deleterious *GBA* mutations or polymorphic variants.

Table 1: Baseline characteristics and duration of follow up of the patients and controls included in the study

	ParkWest		PINE		NYPUM		All	
	NC	PD	NC	PD	NC	PD	NC	PD
N total*	192	190	171	118	56	134	419	442
Male, N (%)	97 (50.5)	115 (60.5)	109 (63.7)	72 (61.0)	38 (67.9)	80 (59.7)	244 (58.2)	267 (60.4)
Age at baseline, years, mean (±SD)	66.26 (9.53)	67.96 (9.11)	74.79 (9.32)	72.16 (9.89)	64.84 (7.72)	70.37 (9.61)	69.55 (10.19)	69.81 (9.62)
Age at first symptoms, mean (±SD)		65.68 (9.21)		69.98 (9.87)		68.43 (9.67)		67.67 (9.68)
≤ 65 years at baseline, N (%)	82 (42.7)	69 (36.3)	24 (14.0)	27 (22.9)	26 (46.4)	43 (32.1)	132 (31.5)	139 (31.4)
Positive family history, N (%)†	31 (16.1)	42 (22.1)	9 (5.3)	24 (20.3)	0*	38 (28.4)	40 (9.5)	104 (23.5)
Education, years mean (±SD)	12.22 (3.70)	11.13 (3.30)	12.84 (1.64)	13.36 (1.90)	11.94 (3.85)	9.92 (3.83)	12.46 (3.02)	11.42 (3.40)
UPDRS III, mean (±SD) ‡	-	23.54 (11.24)	-	24.12 (11.60)	-	26.83 (11.29)	-	24.69 (11.41)
Hoehn & Yahr, mean (±SD) ‡	-	1.92 (0.63)	-	2.22 (0.76)	-	2.26 (0.69)	-	2.10 (0.70)
MMSE score, mean (±SD)	28.56 (1.49)	27.73 (2.40)	28.84 (1.18)	28.43 (1.64)	29.24 (0.83)	28.60 (1.44)	28.20 (2.18)	28.17 (2.00)
Duration of follow up, years, mean (±SD)	5.96 (2.18)	6.34 (1.58)	5.97 (1.63)	5.92 (1.74)	3.1 (3.6)	6.38 (1.36)	5.97 (1.95)	6.24 (1.57)

* N total gives the number for which *GBA* genotyping was performed.

† Self-reported family history. *Available for 30/56 controls where none had a positive family history for PD

‡ UPDRS III and Hoehn and Yahr were not measured in control subjects.

Abbreviations: MMSE, Mini-Mental State Examination; NA, not available; NC, Normal controls; NYPUM, the New Parkinson Patient in Umeå project; ParkWest, the Norwegian ParkWest study; PD Patients with Parkinson Disease; PINE, the Parkinsonism Incidence in Northeast Scotland study; UPDRS III, Unified Parkinson Disease Rating Scale Part III.

Table 2. Demographic and clinical baseline features of patients with PD

Clinical variables,	**Non-carriers**	***GBA* carriers**	***P****	**Polymorphism carriers**	***P****	**Deleterious mutation carriers**	***P****
Male, N (%)	233 (59.9)	34 (64.2)	0.654	28 (62.2)	0.873	6 (75.0)	0.486
Age at baseline, mean (±SD)	70.22 (9.49)	66.84 (10.07)	**0.016**	67.84 (10.27)	0.115	61.21 (6.97)	**0.008**
Age at first symptoms, mean (±SD)	68.03 (9.63)	64.98 (9.79)	**0.031**	65.95 (10.06)	0.172	59.54 (5.90)	**0.013**
Positive family history, N (%)	93 (23.9)	11 (20.8)	0.861	10 (22.2)	1.000	1 (12.5)	0.686
Education, years, mean (±SD)	11.39 (3.44)	11.63 (3.17)	0.637	11.61 (3.22)	0.693	11.75 (3.06)	0.768
UPDRS III, mean (±SD)	24.99 (11.63)	22.49 (9.52)	0.135	23.00 (9.72)	0.271	19.63 (8.26)	0.195
Hoehn & Yahr, mean (±SD)	2.11 (0.72)	2.08 (0.61)	0.761	2.09 (0.62)	0.871	2.0 (0.60)	0.676
MMSE score, mean (±SD)	28.15 (2.05)	28.34 (1.62)	0.532	28.36 (1.69)	0.530	28.25 (1.28)	0.892

* Unadjusted *p* values were calculated using t-tests, Mann-Whitney U-tests and X^2-tests as appropriate.

Abbreviations: MMSE, Mini-Mental State Examination; UPDRS III, Unified Parkinson Disease Rating Scale Part III.

Significant *P* values (< 0.05) are highlighted in bold

Table 3: Survival analysis for dementia on the disease duration timescale

	Total PD, n	PDD, n	% PDD	Unadjusted HR (95% CI)	*P*	Adjusted HR (95% CI)*	*P*
Non-carriers	389	107	27.5	Ref†		Ref†	
GBA carriers	53	22	41.5	1.53 (0.97 – 2.43)	0.068	1.98 (1.23 – 3.18)	**0.005**
Polymorphism carriers	45	18	40.0	1.48 (0.90 – 2.44)	0.124	1.79 (1.07 – 3.00)	**0.028**
Deleterious mutation carriers	8	4	50.0	1.82 (0.67 – 4.94)	0.242	3.81 (1.35 – 10.72)	**0.011**

*adjusted for study cohort, sex, age at baseline and years education

† Non-carrier group used as reference group for statistical analysis

Abbreviations: CI, confidence interval; HR, hazard ratio: PDD, Parkinson disease with dementia

Significant *p* values (< 0.05) are highlighted in bold

Figure(s)

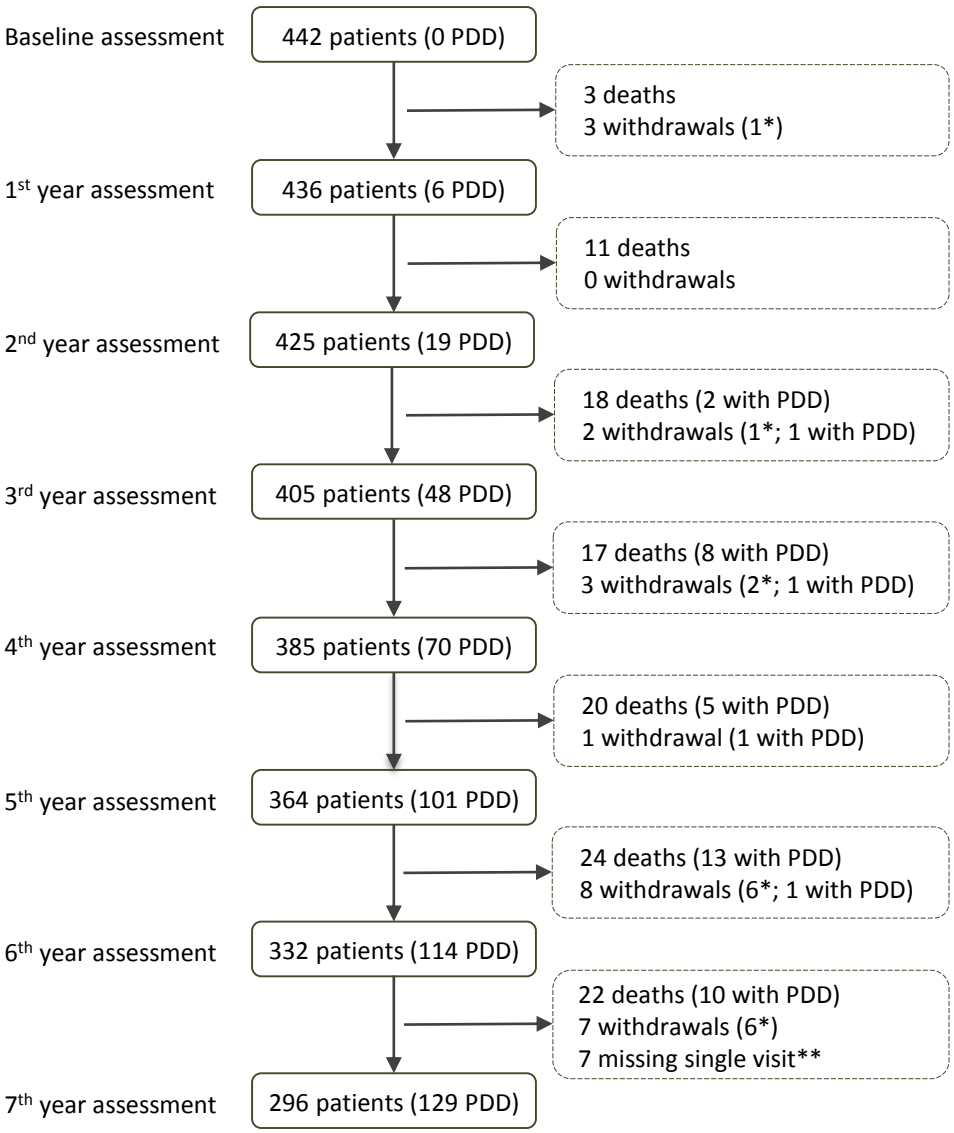

Figure 1

Figure 2

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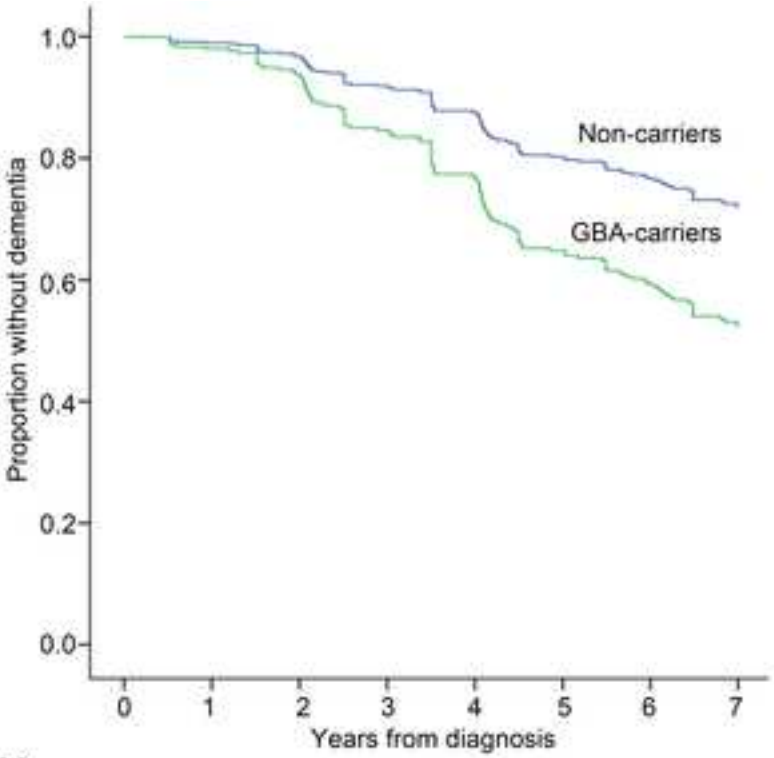
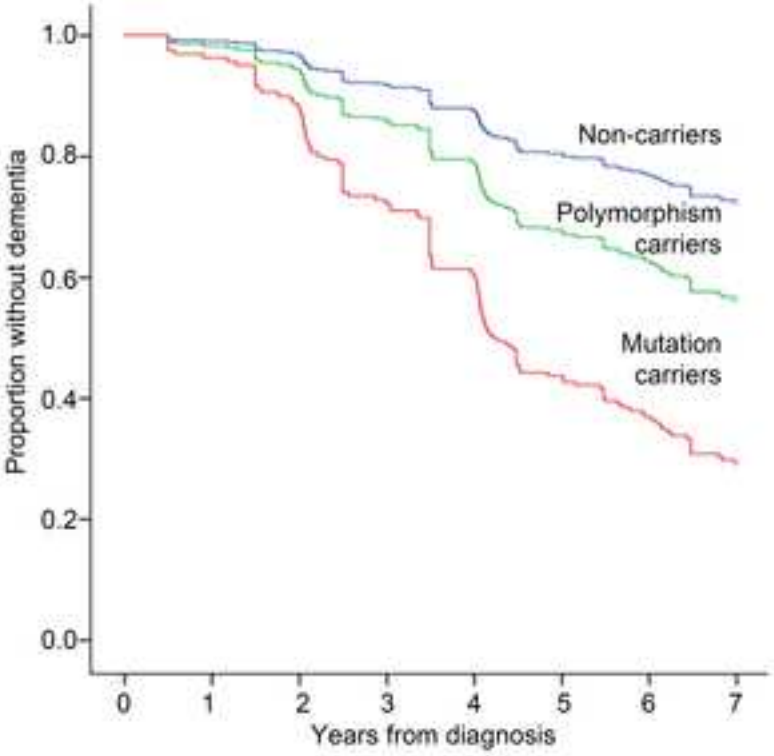


No. at risk								
Non-carriers:	389	379	359	319	295	251	229	143
GBA carriers:	53	53	51	45	38	33	30	18



No. at risk								
Non-carriers:	389	379	359	319	295	251	229	143
Polymorphism carriers:	45	45	43	39	32	28	25	15
Mutation carriers:	8	8	8	6	6	5	5	3

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Marked Revision

Association of *GBA* polymorphisms and mutations with dementia in incident Parkinson's disease

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Abstract

INTRODUCTION: Both polymorphisms and mutations in *GBA* may influence the development of dementia in patients with Parkinson's disease.

METHODS: 442 patients and 419 controls were followed for seven years. Dementia was diagnosed using established criteria. Participants were analyzed for *GBA* genetic variants, including E326K, T369M and L444P. Associations between *GBA* carrier status and dementia were assessed with Cox survival analysis.

RESULTS: A total of 12.0% of patients with Parkinson's disease carried a *GBA* variant, and nearly half (22/53) progressed to dementia during follow-up. Carriers of deleterious *GBA* mutations (adjusted HR 3.81, 95% CI 1.35 to 10.72; $P = .011$) or polymorphisms (adjusted HR 1.79; 95% CI 1.07 to 3.00; $P = .028$) progressed to dementia more rapidly than non-carriers.

DISCUSSION: *GBA* variants are of great clinical relevance for the development of dementia in Parkinson's disease, especially due to the relatively higher frequency of these alleles compared to other risk alleles.

Key Words: Parkinson's disease; Parkinson's disease with dementia; GBA; Longitudinal; genetic association.

1 Background

Dementia is among the most common and severe non-motor symptoms of Parkinson disease (PD), affecting nearly 20% of all patients within the first 5 years of the disease, and the majority of patients if they survive for more than 10 years after diagnosis [1, 2]. Dementia in PD (PDD) has important adverse implications for quality of life, caregiver burden, and health-related costs [3]. The etiology of PDD remains poorly understood and no neuroprotective therapies are currently available.

Genetic factors undoubtedly play a role in modifying the rate of disease progression in PD, and identifying these is key to the early identification of patients at greatest risk of PDD. Genetic variants in glucocerebrosidase (*GBA*) have the strongest evidence for association with more rapid cognitive decline in PD. Homozygous mutations in *GBA* cause Gaucher disease (GD), and it is well established that some of the heterozygous mutations are associated with an increased risk of PD [4]. *GBA* variants associated with increased risk of PD chiefly fall into two categories: risk polymorphisms, the most common of which are E326K and T369M [5, 6]; and deleterious mutations, such as N370S and L444P, which in a homozygous state cause GD [7].

GBA variants have been shown to increase the risk of PDD in cross-sectional studies [8, 9], and longitudinal studies are starting to show how different *GBA* variants affect the rate of the development of dementia during the course of PD. Most longitudinal studies have found that carriers of deleterious *GBA* mutations are at increased risk of earlier PDD onset [10-13] or faster decline in global cognitive function [14]. To date, few studies have considered the effects of *GBA* risk polymorphisms on the development of PDD, and the only longitudinal studies to identify a significant association between *GBA* polymorphisms and progression to PDD did so only after controlling for the effect of *MAPT* genotype [10] or by including both mild cognitive impairment or PDD [6].

Therefore, we analyzed the *GBA* carrier frequencies of three deeply phenotyped, longitudinal PD cohorts of highly uniform design from Northern Europe, each of which uses established criteria for the diagnosis of PDD. Together, the Norwegian ParkWest Study [15], the Parkinsonism Incidence in Northeast Scotland (PINE) [16], and the New Parkinson Patient in Umeå (NYPUM) [17] studies represent the largest prospective population based longitudinal study of PD with age- and sex-matched

controls in which the effect of *GBA* variants on PD progression has been addressed. By determining the roles of *GBA* polymorphisms and deleterious mutations in the development of PDD, we provide important insights into the heterogeneity of disease progression in these subgroups.

2 Methods

2.1 Study Participants and Procedures

The ParkWest study, the NYPUM project, and the PINE study were initiated between 2002 and 2004. All are large, on-going, population-based multicenter studies of newly-diagnosed (incident) PD patients, designed to determine the incidence, neurobiology, and prognosis of PD, and are described in detail elsewhere [15-18]. Briefly, 212 patients were enrolled in the ParkWest study, 211 in the PINE study and 182 in the NYPUM study. Of these 68 had a diagnosis other than PD during follow up, 57 declined genotyping, 31 have no available DNA sample or DNA was not extractable, and seven did not consent to follow up. The remaining 442 patients were eligible for this study and underwent comprehensive and standardized clinical examinations before drug treatment was initiated if possible (98% drug-naïve). During the same time, normal control subjects were recruited in the same geographical areas from spouses or friends of PD patients, or unrelated persons [19, 20]. They were clinically examined and had no signs of movement disorders or cognitive deficiencies. 201 controls were enrolled in the ParkWest study, 266 in the PINE study and 56 in the NYPUM study. Of these 68 had no available DNA sample or DNA was not extractable, 30 declined genotyping and 6 developed incident PD during follow up and were excluded. The remaining 419 consented to routine follow up with a standardized battery of clinical testing. PD patients are currently under continued follow-up, and only those with a confirmed clinical or pathological (if performed post-mortem) diagnosis of PD according to the UK brain bank criteria at their latest or final clinical visit were included. All participants signed written-informed consent. The Western Norway Regional Committee for Medical and Health Research Ethics, the Regional Ethics Review Board in Umeå, and the Multi Centre Research Ethics Committee for Scotland, approved the respective studies.

2.2 Clinical assessments in PD

The data was analyzed with the focus on PD risk, age at symptom onset or diagnosis, and the development of dementia. PD patients were examined at time of diagnosis by experienced study neurologists and research nurses. Clinical evaluations made up to the 7-year visit are included in this study. Motor severity was rated using the motor section (part III) of the Unified Parkinson Disease Rating Scale (UPDRS), and disease stage using the Hoehn and Yahr staging. Global cognitive decline was measured by the Mini-Mental State Examination (MMSE) [21]. Dementia diagnosis was set according to Movement Disorder Society criteria [22] (ParkWest and NYPUM) or DSM-IV [23] (PINE), using a combination of clinical history from the patient and carer, and cognitive testing. Patients with dementia with Lewy bodies (DLB), as defined by the development of dementia within one year of the onset of the motor features of PD, were not eligible for this study.

2.3 Genetic analysis

Genomic DNA was extracted from peripheral blood samples of eligible participants using standard methods. Large-scale allelic discrimination analysis was performed for all patients and controls using a pre-designed TaqMan SNP genotyping assay for rs75548401/T369M, and custom assays for rs369068553/V460L, rs2230288/E326K, rs76763715/N370S, and rs781152868/Y135C (ThermoFisher Scientific) as described [24, 25]. The call rate was 99.8%.

A total of 188 patients of the ParkWest cohort were also characterized by whole exome sequencing (unpublished material). Variants falling within the *GBA* region were identified and analysed using Ingenuity Variant Analysis (Qiagen, CA). Five nonsynonymous variants were detected: N370S, T369M, E326K and two additional mutations, rs369068553/V460L and rs781152868/Y135C, which were confirmed by sequencing as described [26, 27]. V460L and Y135C were genotyped in the remaining samples, and the carrier of V460L confirmed by sequencing.

For rs421016/L444P genotyping, a fragment of 960 base pairs was amplified as described (primer sequences and reaction conditions available on request) [26, 28]. PCR products were analyzed by restriction fragment length polymorphism in all three cohorts using *Nci*I [29]. Ten samples failed genotyping, giving a success rate of 98.8%. All mutations were confirmed by direct sequencing of the PCR product [29].

All amino acid substitutions are numbered excluding the 39-residue signal peptide.

2.4 Statistical methods

"*GBA* carriers" included all patients carrying any of the detected nonsynonymous *GBA* variants. *GBA* carriers were further split into "polymorphism carriers" (E326K, T369M or V460L), and "deleterious carriers" (Y135C, N370S or L444P), based on published reports and predicted pathogenicity as described [13].

Between-group differences were compared using t-tests, Mann-Whitney tests and X^2-tests as appropriate. For age of PD onset, we performed multiple linear regression analysis adjusted for study cohort and sex. We used logistic regression to calculate odds ratios (ORs) with 95% confidence intervals (CIs) for incident PD by different *GBA* carrier groups, without and with adjustment for study cohort, age at baseline and sex. For incident PDD cases, we assigned time of dementia onset to the midpoint of the interval between assessments at which dementia was diagnosed, as described previously [1]. We performed Cox regression analysis to assess the role of *GBA* carrier status on the evolution of PDD in the patient group, adjusting for study cohort, age at baseline, sex and years of education. Censoring occurred due to deaths and losses to follow up, and at end of study i.e. at the 7 year visit. Assumption of proportionality was assessed and deemed to hold using log–log plots. There was no statistical evidence of differential effect of *GBA* on PDD free survival between cohorts. We considered two-tailed values of $p < .05$ significant and conducted all statistical analyses using IBM SPSS Statistics (Armonk, NY), version 21.0.

3 Results

Of 861 study participants, 419 were controls and 442 were patients with PD. Their baseline characteristics are listed in Table 1. No statistical differences were detected between patients and controls in any demographic or clinical variables at baseline. For PD patients, mean age at baseline was 69.81 (±9.62) years, with 60.4% (267) males. During follow-up, 115 (26.0%) patients deceased, while 24 (5.4%) patients dropped out of the study for reasons other than death (Figure 1).

3.1 Genetic analysis

We identified a total of 82 carriers of *GBA* variants in this cohort (Supplementary Table 1). In addition to analysis of the four most frequently studied *GBA* variants in PD, E326K, T369M, N370S and L444P, whole exome sequencing of patients from ParkWest identified two further mutations: V460L, previously identified in PD and healthy controls [30, 31] and; Y135C, which has been identified in GD (Clinvar accession 280972) but not PD.

One patient was homozygous for E326K, and one patient carried both the E326K and the T369M variant. No other homozygous subjects or carriers of complex alleles were identified. For further analysis patients and controls were classified as non-carriers or carriers of any *GBA* variant, or further subdivided into carriers of polymorphisms or carriers of deleterious mutations (Table 2 and Supplementary Table 1).

3.2 GBA and PD risk

Of the 82 *GBA* carriers in the cohort, 53 were patients (12.0% of patients) and 29 controls (6.9% of controls). Logistic regression analysis showed that both carriers of any *GBA* variant and carriers of polymorphisms had an increased risk of PD (all *GBA* carriers OR 1.83; 95% CI 1.14 to 2.94; $P = .012$, and polymorphism carriers OR 1.73; 95% CI 1.05 to 2.86; $P = .033$). After adjusting for study cohort, age and sex, the associations remained significant for carriers of any *GBA* variant (all *GBA* carriers:

OR 1.70; 95% CI 1.05 to 2.77; $P = .032$, and polymorphism carriers: OR 1.63; 95% CI 0.97 to 2.73; $P = .065$). Furthermore, linear regression analysis revealed a significant effect of *GBA* variants on age of PD diagnosis, reducing age at onset by 3.4 years on average in patients with any *GBA* variant ($\beta = -3.38$; 95% CI - 6.13 to -0.63; $P = .016$) and 9.1 years in patients with a deleterious mutation ($\beta = -9.10$; 95% CI -15.80 to -2.40; $P = .008$), compared with non-carriers (70.2 ± 9.50 years). These associations remained significant when adjusting for study cohort and sex (all *GBA* carriers: $\beta = -3.55$; 95% CI -6.28 to -0.81; $P = .011$, and mutation carriers: $\beta = -9.56$; 95% CI -16.23 to -2.88; $P = .005$). No associations were identified between *GBA* carrier status and demographic or clinical baseline variables other than age (all $P > .050$) (Table 2).

3.3 GBA and PDD risk

Progression to PDD was more frequent in the *GBA* carrier group compared to non-carriers. By 7 years of follow-up, 22 (41.5%) of the 53 *GBA* carriers had progressed to dementia vs. 107 (27.5%) of the 389 non-carriers. To assess the longitudinal effects of *GBA* carrier status on progression to dementia, we performed a Cox regression analysis (Table 3, Figure 2). We observed a significant association between *GBA* carrier status and progression rate to dementia when adjusting for age at baseline, years of education, study cohort and sex (HR 1.98, 95% CI 1.23 to 3.18, $P = .005$). When assessed separately, the polymorphism carrier group showed a significant but more moderate association with progression rate to dementia (adjusted HR 1.79, 95% CI 1.07 to 3.00, $P = .028$), while the deleterious carriers had a higher risk of a progression to dementia (adjusted HR = 3.81, 95% CI 1.35 to 10.72, $P = .011$) compared to non-carriers. Adjusting with co-variables strengthened the effect of *GBA* on progression to PDD (Table 3). Most of the adjusting effect comes from including age at onset in the models, suggesting that the observed, unadjusted effect of *GBA* on PDD risk is attenuated by the additional effect that *GBA* carriers tend to be younger at onset of PD than non-carriers.

The effect of *GBA* on PDD was not reflected by an overall increased rate of disease progression, as we observed no significant differences in the progression of Hoehn and Yahr scores between *GBA* carriers

and non-carriers during the first 7 years of disease, or between any of the *GBA* subgroups and non-carriers (all $p > 0.35$, data not shown).

4 Discussion

In this study, we examined the association of *GBA* variants with the development of dementia in the largest population-based longitudinal multicenter study of incident PD to date. We show that PD patients carrying *GBA* variants have a faster progression to dementia, with carriers of deleterious mutations displaying a more rapid disease progression than carriers of *GBA* polymorphisms.

Of the 129 patients who progressed to PDD within the 7-year visit, 17.1% were carriers of a *GBA* variant, compared to 9.9% of the PD patients without dementia. We observed a differential effect of the type of *GBA* variant on the risk for dementia; multivariable survival analysis showed that the risk of progressing to PDD within the 7-year visit for *GBA* polymorphism carriers was nearly doubled compared to non-carriers, whereas deleterious mutation carriers were at nearly 4-times greater risk.

The role of *GBA* polymorphisms in disease heterogeneity as PD progresses is a disputed topic: The first longitudinal study to analyze *GBA* found that mutations predicted increased risk of dementia, but when comparing 7 polymorphism carriers to 117 non-carriers, an association with dementia was only found after adjusting for *MAPT* haplotype [10]. Subsequently, two studies found that E326K is associated with a higher prevalence of dementia [8], or with conversion to mild cognitive impairment or dementia [6], but unexpectedly in these studies carriers of *GBA* mutations were at similar or lower risk than E326K carriers. Conversely, a Spanish retrospective study observed increased risk of PDD in *GBA* mutation carriers, but no effect in patients carrying potentially benign variants [13]. However, the benign group included 13 synonymous and non-coding sequence variants in addition to E326K and T396M, which could mask the effect of missense variants. The largest multi-center study to date to analyze the role of polymorphisms did not find an effect on PDD risk, however this study did estimate a small increased risk associated with cognitive decline (6.6% of patients; HR 1.36, 95% CI 0.89-2.05) [14]. Of notable difference to our study was the considerably younger PD sample, as well

as the use of MMSE for the detection of PDD, which has lower sensitivity compared to established diagnostic criteria for PDD [32]. Considering these inconsistent findings, this study, which is composed of cohorts specifically designed to study the incidence and prognosis of PD, provides new and important insights into the role of *GBA* polymorphisms in PDD.

Significant attention has been paid to the phenotypic characterization of PD patients with the most frequent pathogenic *GBA* mutations [10-12, 14, 33-35], and our findings reaffirm the role of damaging mutations in the development of dementia in PD. The clinical relevance of these results is augmented by the additional effect of *GBA* carrier status on age at PD onset: We show that in addition to more rapid dementia development, patients with PD who carried a *GBA* variant had a younger age of onset. The largest difference was observed in carriers of *GBA* mutations who were on average 9 years younger at diagnosis than non-carriers. This is noteworthy as idiopathic PD patients with a younger age of onset typically have a more benign disease course with slower motor and cognitive decline, when compared to patients with later onset PD [36, 37].

Information on the frequency of *GBA* variants in Northern Europe is scarce. Previous studies reported a low carrier frequency of genetic variants in PD patients, with the exception of L444P in Northern Sweden, which is more prevalent in this region consistent with a higher incidence of GD in this part of the country [25, 29, 38]. Here we performed complete screening of *GBA* in a population based cohort of PD patients from Norway, and find a comparatively high frequency of *GBA* variants (12.0%). These results are similar to the overall *GBA* carrier frequency in other studies of European PD patients that have employed complete screening of *GBA* (range 9.3-12.2 %) [9, 10, 33, 35], and highlight the importance of complete sequencing and cataloguing of all variants in population based cohorts to avoid underestimating variant frequency. Although not the main focus of this study, we further show that both carriers of any variant and polymorphism carriers were at increased risk of PD. These data reinforce the role of T369M and E326K as risk factors in PD [5, 38, 39], and extend the finding that *GBA* is a major risk factor in PD to Northern European populations [25].

This work has considerable strengths: Each study is a representative incident cohort, designed to identify all new PD cases in a given population early in their disease, with high levels of consent and

low levels of losses to follow up for reasons other than death (Figure 1). Furthermore, each study made substantial efforts to follow all participants until death, including home visits for those no longer willing or able to attend clinic visits, and telephone follow up for dropouts, greatly reducing the problem of selection bias. Of equal importance are the uniformities in data-ascertainment methods, with each study center using validated criteria to diagnose dementia, and a median prospective follow up period of 7 years from PD diagnosis. These methods minimize the risk of erroneous inclusion of non-PD cases, and provide information about individual conversion to PDD from the time of PD diagnosis. Potential limitations of our study include the modest number of carriers of individual variants, which prevented us from analyzing the effect of each variant separately. In the future, it will be important to determine if individual *GBA* polymorphisms have differential effects on disease severity, as no comparisons between E326K and T369M have been performed in unselected PD populations. This will require further pooling of existing cohorts to give larger numbers, or studying inception cohorts enriched for carriers of different *GBA* variants. Furthermore, this study was designed to study the development of PDD, and patients with DLB were excluded. Given that DLB and PDD share many pathological and clinical features, and probably represent two clinical entities on a spectrum of Lewy body disease, it will be of great interest to determine how different types of *GBA* variants affect disease progression in DLB. Finally, complete information on the development of dementia in the control population during follow up was not available, preventing the analysis of the role of *GBA* in the non-PD population.

Dementia typically occurs in advanced stages of idiopathic PD, and is often related to the advanced spread of Lewy bodies to cortical areas. Reduced glucocerebrosidase enzyme activity leads to accumulation and oligomerization of α-synuclein. Several studies have shown that the effect of *GBA* variants on enzyme activity varies according to the nature of the variant: individuals with *GBA* mutations display the lowest glucocerebrosidase activity, whilst polymorphism carriers display a level of activity intermediate between non-carriers and mutants [40, 41]. This suggests a disease mechanism by which *GBA* mutations ultimately drive the development of dementia by increasing the accumulation and spread of α-synuclein. In support of this, autopsies of *GBA* mutation-carriers

revealed a more widespread Lewy body-type pathology in the neocortex than that of matched PD controls [27]. Our work suggests that polymorphic variants also play a role in the hastened development of PDD, although the effect on glucocerebrosidase activity might be milder, and the subsequent rate of α-synuclein accumulation and spread lower, leading to the observed milder disease phenotype. A range of different studies, including imaging and histopathological analyses of brains, and animal studies, will be required to improve understanding of precisely how each *GBA* variant increases risk of cognitive decline and affects the underlying spread of Lewy body pathology in PD.

4.1 Conclusion

In this population-based study, we present the first comprehensive overview of *GBA* variants in Northern Europe, and show that in our unselected multicenter cohort of patients with incident PD, *GBA* carrier status has important implications for the development of dementia. Given that variants in *GBA* are present in 12% of the PD population in this study, this places many individuals at risk of a more severe disease course. At present there are no preventative PD treatments that target *GBA* pathways and an individual's *GBA* status does not alter clinical management, which contend that screening for *GBA* variants should not yet be included in routine genetic testing in a clinical setting. However, future studies should focus on establishing the success of combining *GBA* mutations and polymorphisms with other predictors of dementia to gain a more precise indication of when PD patients develop dementia. The ability to predict the development of dementia is highly relevant for recruitment into and stratification of clinical trials, and the finding in this study that PD patients harboring either *GBA* mutations or polymorphisms are at significantly increased risk of early PDD, advocates that both groups of patients are candidates for clinical PDD research studies. This will be especially important as interventions that target *GBA* to slow disease progression and to prevent dementia become available.

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Figures captions

Figure 1. Flowchart of patient inclusion over time in this study.

Overview of patient inclusion from baseline until the 7-year visit. The number of patients attending each visit is shown and the cumulative number of patients diagnosed with PDD is shown in brackets. Withdrawals and deaths between visits are shown in dashed boxes. *Withdrew from study visits but dementia and death status known. **7 patients had not yet attended the 7-year visit, but were not lost to the study. The flowchart is simplified for readability.

Figure 2: Survival analysis for dementia onset. Cox regression models show the effects of *GBA* carrier status on the outcome of dementia over time in patients with PD, with adjustment for covariates (study cohort, age, sex and years of education). (A) Analysis of time to PDD for non-carriers and patients with *GBA* variants, or (B) non-carriers and patients with deleterious *GBA* mutations or polymorphic variants.

Table 1: Baseline characteristics and duration of follow up of the patients and controls included in the study

	ParkWest		PINE		NYPUM		All	
	NC	PD	NC	PD	NC	PD	NC	PD
N total*	192	190	171	118	56	134	419	442
Male, N (%)	97 (50.5)	115 (60.5)	109 (63.7)	72 (61.0)	38 (67.9)	80 (59.7)	244 (58.2)	267 (60.4)
Age at baseline, years, mean (±SD)	66.26 (9.53)	67.96 (9.11)	74.79 (9.32)	72.16 (9.89)	64.84 (7.72)	70.37 (9.61)	69.55 (10.19)	69.81 (9.62)
Age at first symptoms, mean (±SD)		65.68 (9.21)		69.98 (9.87)		68.43 (9.67)		67.67 (9.68)
≤ 65 years at baseline, N (%)	82 (42.7)	69 (36.3)	24 (14.0)	27 (22.9)	26 (46.4)	43 (32.1)	132 (31.5)	139 (31.4)
Positive family history, N (%)†	31 (16.1)	42 (22.1)	9 (5.3)	24 (20.3)	0*	38 (28.4)	40 (9.5)	104 (23.5)
Education, years mean (±SD)	12.22 (3.70)	11.13 (3.30)	12.84 (1.64)	13.36 (1.90)	11.94 (3.85)	9.92 (3.83)	12.46 (3.02)	11.42 (3.40)
UPDRS III, mean (±SD) ‡	-	23.54 (11.24)	-	24.12 (11.60)	-	26.83 (11.29)	-	24.69 (11.41)
Hoehn & Yahr, mean (±SD) ‡	-	1.92 (0.63)	-	2.22 (0.76)	-	2.26 (0.69)	-	2.10 (0.70)
MMSE score, mean (±SD)	28.56 (1.49)	27.73 (2.40)	28.84 (1.18)	28.43 (1.64)	29.24 (0.83)	28.60 (1.44)	28.20 (2.18)	28.17 (2.00)
Duration of follow up, years, mean (±SD)	5.96 (2.18)	6.34 (1.58)	5.97 (1.63)	5.92 (1.74)	3.1 (3.6)	6.38 (1.36)	5.97 (1.95)	6.24 (1.57)

* N total gives the number for which *GBA* genotyping was performed.

† Self-reported family history. *Available for 30/56 controls where none had a positive family history for PD

‡ UPDRS III and Hoehn and Yahr were not measured in control subjects.

Abbreviations: MMSE, Mini-Mental State Examination; NA, not available; NC, Normal controls; NYPUM, the New Parkinson Patient in Umeå project; ParkWest, the Norwegian ParkWest study; PD Patients with Parkinson Disease; PINE, the Parkinsonism Incidence in Northeast Scotland study; UPDRS III, Unified Parkinson Disease Rating Scale Part III.

Table 2. Demographic and clinical baseline features of patients with PD

Clinical variables,	**Non-carriers**	***GBA* carriers**	***P****	**Polymorphism carriers**	***P****	**Deleterious mutation carriers**	***P****
Male, N (%)	233 (59.9)	34 (64.2)	0.654	28 (62.2)	0.873	6 (75.0)	0.486
Age at baseline, mean (±SD)	70.22 (9.49)	66.84 (10.07)	**0.016**	67.84 (10.27)	0.115	61.21 (6.97)	**0.008**
Age at first symptoms, mean (±SD)	68.03 (9.63)	64.98 (9.79)	**0.031**	65.95 (10.06)	0.172	59.54 (5.90)	**0.013**
Positive family history, N (%)	93 (23.9)	11 (20.8)	0.861	10 (22.2)	1.000	1 (12.5)	0.686
Education, years, mean (±SD)	11.39 (3.44)	11.63 (3.17)	0.637	11.61 (3.22)	0.693	11.75 (3.06)	0.768
UPDRS III, mean (±SD)	24.99 (11.63)	22.49 (9.52)	0.135	23.00 (9.72)	0.271	19.63 (8.26)	0.195
Hoehn & Yahr, mean (±SD)	2.11 (0.72)	2.08 (0.61)	0.761	2.09 (0.62)	0.871	2.0 (0.60)	0.676
MMSE score, mean (±SD)	28.15 (2.05)	28.34 (1.62)	0.532	28.36 (1.69)	0.530	28.25 (1.28)	0.892

* Unadjusted *p* values were calculated using t-tests, Mann-Whitney U-tests and X^2-tests as appropriate.

Abbreviations: MMSE, Mini-Mental State Examination; UPDRS III, Unified Parkinson Disease Rating Scale Part III.

Significant *P* values (< 0.05) are highlighted in bold

Table 3: Survival analysis for dementia on the disease duration timescale

	Total PD, n	PDD, n	% PDD	Unadjusted HR (95% CI)	*P*	Adjusted HR (95% CI)*	*P*
Non-carriers	389	107	27.5	Ref†		Ref†	
GBA carriers	53	22	41.5	1.53 (0.97 – 2.43)	0.068	1.98 (1.23 – 3.18)	**0.005**
Polymorphism carriers	45	18	40.0	1.48 (0.90 – 2.44)	0.124	1.79 (1.07 – 3.00)	**0.028**
Deleterious mutation carriers	8	4	50.0	1.82 (0.67 – 4.94)	0.242	3.81 (1.35 – 10.72)	**0.011**

*adjusted for study cohort, sex, age at baseline and years education

† Non-carrier group used as reference group for statistical analysis

Abbreviations: CI, confidence interval; HR, hazard ratio: PDD, Parkinson disease with dementia

Significant *p* values (< 0.05) are highlighted in bold