Glucocerebrosidase activity in Parkinson’s disease with and without GBA mutations

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Glucocerebrosidase (GBA) mutations have been associated with Parkinson’s disease in numerous studies. However, it is unknown whether the increased risk of Parkinson’s disease in GBA carriers is due to a loss of glucocerebrosidase enzymatic activity. We measured glucocerebrosidase enzymatic activity in dried blood spots in patients with Parkinson’s disease (n = 517) and controls (n = 252) with and without GBA mutations. Participants were recruited from Columbia University, New York, and fully sequenced for GBA mutations and genotyped for the LRRK2 G2019S mutation, the most common autosomal dominant mutation in the Ashkenazi Jewish population. Glucocerebrosidase enzymatic activity in dried blood spots was measured by a mass spectrometry-based assay and compared among participants categorized by GBA mutation status and Parkinson’s disease diagnosis. Parkinson’s disease patients were more likely than controls to carry the LRRK2 G2019S mutation (n = 39, 7.5% versus n = 2, 0.8%, P < 0.001) and GBA mutations or variants (seven homozygotes and compound heterozygotes and 81 heterozygotes, 17.0% versus 17 heterozygotes, 6.7%, P < 0.001). GBA homozygotes/compound heterozygotes had lower enzymatic activity than GBA heterozygotes (0.85 μmol/l/h versus 7.88 μmol/l/h, P < 0.001), and GBA heterozygotes had lower enzymatic activity than GBA and LRRK2 non-carriers (7.88 μmol/l/h versus 11.93 μmol/l/h, P < 0.001). Glucocerebrosidase activity was reduced in heterozygotes compared to non-carriers when each mutation was compared independently (N370S, P < 0.001; L444P, P < 0.001; 84GG, P = 0.003; R496H, P = 0.018) and also reduced in GBA variants associated with Parkinson’s risk but not with Gaucher disease (E326K, P = 0.009; T369M, P < 0.001). When all patients with Parkinson’s disease were considered, they had lower mean glucocerebrosidase enzymatic activity than controls (11.14 μmol/l/h versus 11.85 μmol/l/h, P = 0.011). Difference compared to controls persisted in patients with idiopathic Parkinson’s disease (after exclusion of all GBA and LRRK2 carriers; 11.53 μmol/l/h, versus 12.11 μmol/l/h, P = 0.036) and after adjustment for age and gender (P = 0.012). Interestingly, LRRK2 G2019S carriers (n = 36), most of whom had Parkinson’s disease, had higher enzymatic activity than non-carriers (13.69 μmol/l/h versus 11.93 μmol/l/h, P = 0.002). In patients with idiopathic Parkinson’s, higher glucocerebrosidase enzymatic activity was associated with longer disease duration (P = 0.002) in adjusted models, suggesting a milder disease course. We conclude that lower glucocerebrosidase enzymatic activity is strongly associated with GBA mutations, and modestly with idiopathic Parkinson’s disease. The association of lower glucocerebrosidase activity in both GBA mutation carriers and Parkinson’s patients without GBA mutations suggests that loss of glucocerebrosidase function contributes to the pathogenesis of Parkinson’s disease. High glucocerebrosidase enzymatic activity in LRRK2 G2019S carriers may reflect a distinct pathogenic mechanism. Taken together, these data suggest that glucocerebrosidase enzymatic activity could be a modifiable therapeutic target.
Introduction

Mutations in the glucocerebrosidase (GBA) gene are associated with Parkinson’s disease. This association has been established in patients with mutations in two GBA alleles (i.e. patients with Gaucher disease; Neudorfer et al., 1996; Mitsui et al., 2009), and in carriers of a single GBA mutation (i.e. GBA heterozygotes; Aharon-Peretz et al., 2004; Lwin et al., 2004; Halperin et al., 2006; Goker-Alpan et al., 2008; Bras et al., 2009; Kalinderi et al., 2009; Mitsui et al., 2009; Sidransky et al., 2009; Giraldo et al., 2011). GBA encodes the lysosomal enzyme glucocerebrosidase, a glucosylceramide hydrolase that plays an important role in sphingolipid degradation, especially in the macrophage/monocyte cell lineage. In Gaucher disease, diminished glucocerebrosidase enzymatic activity accounts for glucocerebroside accumulation in the spleen, liver and bone marrow. Gaucher disease is the most common lysosomal storage disease in the general population and the most common genetic disorder in Ashkenazi Jews, up to 6% of whom are carriers of GBA mutations (Horowitz et al., 1996). Among Ashkenazi Jews, the vast majority of patients with Gaucher disease carry at least one N370S mutation, a missense mutation that retains 30% of glucocerebrosidase enzymatic activity and is linked to Type 1 Gaucher disease, the milder non-neuronopathic form. Here and thereafter, we use the term GBA mutation to describe pathogenic changes in the GBA gene (previously associated with Gaucher disease), a polymorphism if it is clearly not associated with Gaucher disease, or a variant if it is unclear or controversial (e.g. E326K).

The mechanism by which GBA mutations are linked to Parkinson’s disease remains unknown. As mutations lead to a reduction in glucocerebrosidase enzymatic activity, and more ‘severe’ mutations (e.g. 84GG) are associated with higher risk for Parkinson’s disease compared to ‘milder’ mutations (e.g. N370S; Gan-Or et al., 2008), loss of glucocerebrosidase enzymatic activity may be a pathogenic mechanism. However, the fact that most GBA homozygotes and compound heterozygotes (i.e. patients with Gaucher disease) will never develop Parkinson’s disease (Rosenbloom et al., 2011; Alcalay et al., 2014) in spite of diminished glucocerebrosidase enzymatic activity, while GBA heterozygotes are at a higher risk for Parkinson’s disease compared to controls, suggests an additional mechanism (Mazzulli et al., 2011; Sidransky and Lopez, 2012). Little is known about glucocerebrosidase enzymatic activity in carriers of specific heterozygous GBA mutations, and there is currently no evidence of sphingolipid accumulation in heterozygotes (as opposed to patients with Gaucher disease); however, increased production of cytosolic reactive oxygen species has been demonstrated in fibroblasts from GBA heterozygotes with and without Parkinson’s disease (McNeill et al., 2014). Glucocerebrosidase enzymatic activity measurement in dried blood spots is a validated methodology (Zhang et al. 2008), which is clinically approved to screen for Gaucher disease. Here, our aims were to measure glucocerebrosidase enzymatic activity in dried blood spots from Parkinson’s disease patients with and without GBA mutations and from controls to (i) assess whether glucocerebrosidase enzymatic activity is reduced in heterozygote GBA carriers with Parkinson’s disease compared to non-carriers; and (ii) explore the association between glucocerebrosidase enzymatic activity, Parkinson’s disease risk, and Parkinson’s disease severity.

Materials and methods

Participants and clinical evaluation

Participants in the study (‘SPOT’) included Parkinson’s disease patients and non-blood related controls (mostly spouses) from the Center for Parkinson’s Disease at Columbia University Medical Center in New York, NY. The cohort’s demographics (but not genotype) were previously described in a report of participants’ attitude towards genetic testing (Sakanaka et al., 2014). In brief, a blood sample and demographics including Ashkenazi Jewish ancestry, medical history, medication, Parkinson’s disease family history (Marder et al., 2003), the Unified Parkinson’s Disease Rating Scale (UPDRS; Fahn, 1987) in the ‘ON’ state and the Montreal Cognitive Assessment (Nasreddine et al., 2005) were collected from consecutive Parkinson’s disease cases, as defined by the United Kingdom Parkinson’s Disease Brain Bank criteria (except that we did not exclude cases with a family history of Parkinson’s disease) (Hughes et al., 1992), and a convenience sample of consecutive non-blood related control individuals, mostly spouses.
Evaluation of Parkinson’s cases and controls was identical. All study procedures were approved by the Columbia University institutional review board, and all participants signed informed consent.

Sequencing and genotyping of GBA mutations

DNA was extracted using a standard salting-out method. All participants were genotyped for GBA mutations in two ways. First, participants were screened as previously described for 10 GBA mutations and variants (Alcalay et al., 2013, 2014), and, given the high prevalence of LRRK2 G2019S in the Ashkenazi Jewish population (Alcalay et al., 2013), all study participants were genotyped for LRRK2 G2019S mutation (Alcalay et al., 2013).

We subsequently fully sequenced the GBA gene in all patients with Parkinson’s disease and controls, regardless of whether any of the 10 GBA mutations/variants were found. Supplementary Table 1 details the primers and conditions used for the amplification and sequencing of the GBA gene. PCRs were performed using the AmpliTaq Gold® DNA Polymerase (Applied Biosystems) according to the manufacturer’s instructions. PCR products were sequenced using a 3730XL DNA Analyzer (Applied Biosystems) and the chromatograms were analysed using the Genalys 3.3b software (Takahashi et al., 2003). The success rate of the sequencing was 96%, and all mutations detected by screening were replicated by sequencing. For Parkinson’s disease cases with compound heterozygote GBA mutations/variants, family members were analysed to confirm whether the mutations were in cis (same allele) or trans (opposite allele).

Glucocerebrosidase enzymatic activity assay

Dried blood spots were obtained as previously described (Olivova et al., 2008; Reuser et al., 2011). In brief, blood samples were collected in a 10 cm$^3$ EDTA tube. Seventy-five microlitres of blood were 'spotted' on each of five circles on a filter paper (Whatman® 903 protein saver card) and dried at room temperature for at least 4 h. Absorbent filter paper was then stored in a sealed plastic bag with desiccants and a humidity indicator in a −20°C freezer and later shipped to the laboratories at room temperature. Upon receipt, the samples were stored at −80°C before analysis.

Glucocerebrosidase enzymatic activity was measured using a previously published protocol as part of a multiplex assay together with four additional lysosomal enzymes (Zhang et al., 2013). In summary, glucocerebrosidase was extracted from a 3.2-mm diameter punch from a dried blood spot sample in 70 μl of 20 mM sodium phosphate buffer (pH 7.1) on a 96-well plate. Ten microlitres of dried blood spot extract was added to 15 μl of glucocerebrosidase substrate/internal standard mixtures (The Center for Disease Control and Prevention, Georgia, Atlanta), 0.67 mM of C12-glucocerebroside and 13.33 μM C14-ceramide in the citrate-phosphate (0.31/0.620 M) buffer (Sigma) with sodium taurocholate (16 g/l, Sigma). The substrate has previously been selected because it does not exist in human blood, and due to the fact it has a similar structure to the smallest natural glucocerebrosidase substrate. Sealed plates were incubated on an orbital shaker at 37°C for 20 h. Reactions were quenched with 100 μl of organic solution (ethyl acetate:methanol, 1:1) following liquid-liquid and solid phase extractions. The samples were dried under nitrogen, sealed and stored at −20°C. Prior to tandem mass spectrometry (MS/MS) analysis, plates were thawed and reconstituted with 200 μl of a solvent mixture (80:20 acetonitrile:water containing 0.2% formic acid).

All analyses were monitored on an API 4000 triple quadrupole mass spectrometer (ABScicex) by selected ion monitoring mode (Multiple Reaction Monitoring, MRM). The enzyme activity of each sample was calculated from the ion abundance ratio of product to internal standard as measured by the mass spectrometer. Background activity of a blank filter paper was subtracted from the dried blood spot activity. Activity was expressed as micromoles of product per litre of whole blood per hour (μmol/l/h). Two quality control samples with previously established activity levels for each enzyme and disease positive samples were included in each plate for quality control. All Genzyme scientists were blinded to Parkinson’s disease and genetic status.

Statistical analysis

Demographics, level of glucocerebrosidase enzymatic activity and frequency of GBA mutations were compared between Parkinson’s disease cases and controls using the Student t-test for continuous variables, and the chi-square and Fisher’s exact tests for categorical variables.

Including Parkinson’s disease cases and controls, glucocerebrosidase enzymatic activity was compared among individuals with two GBA mutations (Gaucher disease patients and compound heterozygotes of a mutation and a variant), GBA heterozygotes and non-carriers by the Student t-test and ANOVA. In addition, we compared heterozygote carriers of each GBA mutation and variant, as well as of the LRRK2 G2019S mutation, separately to non-GBA non-LRRK2 carriers.

To test whether glucocerebrosidase enzymatic activity was associated with demographic characteristics, we analysed glucocerebrosidase enzymatic activity by age, sex and Ashkenazi Jewish ancestry using the Pearson correlation coefficient and t-tests as appropriate. Analyses were performed on controls only and repeated including the entire cohort (including Parkinson’s disease cases and GBA and LRRK2 mutation carriers).

We used the Student t-test to compare glucocerebrosidase enzymatic activity between Parkinson’s disease cases and controls, first including all participants, and second after excluding: (i) GBA and LRRK2 G2019S mutation carriers; (ii) those with a family history of Parkinson’s disease in a first degree relative; and (iii) patients with Parkinson’s disease with disease age at onset ≤40 years to exclude potential Parkinson’s disease cases with parkin (PARK2), PINK1 (also known as DJ-1) mutations, which were not tested. To examine the association between glucocerebrosidase enzymatic activity (predictor) and Parkinson’s disease status (outcome), we constructed logistic regression models. To do so, glucocerebrosidase enzymatic activity was normalized to the batch in which the samples were analysed. Each batch included at least three replicates of two quality control controls with previously established activity
ranges. The activity of each sample was divided by the mean activity of the quality control samples from the same run. Logistic models included normalized glucocerebrosidase enzymatic activity, age, and gender as predictors.

To test the association between glucocerebrosidase enzymatic activity and Parkinson’s disease characteristics in non-GBA non-LRRK2 G2019S carriers we divided the non-GBA non-LRRK2 G2019S Parkinson’s disease cohort into tertiles based on glucocerebrosidase enzymatic activity. The Student t-test and chi square test were used to compare demographics and disease characteristics among the tertiles. A multivariate logistic regression model was constructed to test the association between membership in the higher tertile of glucocerebrosidase activity (outcome) and demographics and disease characteristics (predictor).

Analyses were performed using SPSS Statistics version 19.0 software.

Results

Cohort characteristics

The study included 517 Parkinson’s disease patients and 252 controls. Parkinson’s disease patients and controls were similar in age, education and Ashkenazi Jewish ancestry, but by design (using spouse controls) patients with Parkinson’s disease were more likely to be male than controls (Table 1). Patients with Parkinson’s disease had mean disease duration of 6.8 ± 6.3 years and were more likely than controls to carry the LRRK2 G2019S mutation (7.5% versus 0.8%, P < 0.001) and GBA mutations or variants (17.0% versus 6.7%, P < 0.001). Among GBA variant/mutation carriers, 53.4% (47 of 88) of Parkinson’s disease participants and 35.2% (6 of 17) of control participants (carriers without Parkinson’s) reported at least one Ashkenazi Jewish grandparent.

GBA mutations and glucocerebrosidase enzymatic activity

Seven patients with Parkinson’s disease were found to have two GBA mutations or variants. Four of these seven were N370S homozygotes and one was a compound heterozygote for the N370S and A456P/L444P mutations, consistent with Gaucher disease. Two carried a T369M variant in addition to either N370S or 84GG. In both cases, analysis of family members confirmed that the T369M variant was in trans to the other mutation. Two N370S homozygotes and the 84GG/T369M compound heterozygote Parkinson’s disease patients were first identified through this study and were referred for clinical testing and evaluation. The frequency of GBA and LRRK2 G2019S mutations by Parkinson’s disease status is presented in Table 2.

As expected, glucocerebrosidase enzymatic activity in GBA homozygotes/compound heterozygotes was significantly lower and did not overlap with activity in GBA heterozygotes or non-carriers (Fig. 1). Mean glucocerebrosidase enzymatic activity in heterozygotes [including patients with Parkinson’s (n = 81) and heterozygotes without Parkinson’s disease (n = 17), n = 98] was lower than in non-GBA non-LRRK2 carriers (7.88 μmol/l/h versus 11.93 μmol/l/h, P < 0.001) and higher than in GBA homozygotes/compound heterozygotes (7.88 μmol/l/h versus 0.85 μmol/l/h, P < 0.001). When each GBA mutation or variant group was compared independently to non-GBA non-LRRK2 carriers, glucocerebrosidase enzymatic activity was lower in each of the mutations or variants which were present in at least four participants (N370S, L444P, 84GG, R496H, E326K, T369M; Table 3 and Supplementary Table 3), and was higher when compared independently with glucocerebrosidase enzymatic activity in GBA homozygotes/compound heterozygotes. Glucocerebrosidase enzymatic activity was higher in the 36 LRRK2 G2019S carriers who did not carry GBA mutations than in non-GBA non-LRRK2 carriers.

Glucocerebrosidase enzymatic activity by age, gender, ancestry and Parkinson's disease status

We examined whether age and gender are associated with glucocerebrosidase enzymatic activity among controls with no GBA and no LRRK2 G2019S mutations. There was no correlation between glucocerebrosidase enzymatic activity and age (Pearson correlation < 0.001, P = 0.998) and there was no difference in enzymatic activity by gender (males 12.03 μmol/l/h, females 12.08 μmol/l/h, P = 0.914). There was no association between age, gender and glucocerebrosidase enzymatic activity when analyses were repeated including all participants (i.e. including Parkinson’s cases and mutation carriers). We further compared glucocerebrosidase enzymatic activity between those with four Ashkenazi Jewish grandparents (n = 222) and those without Ashkenazi Jewish grandparents (n = 381), excluding those with mixed ancestry, GBA or LRRK2 mutations. There was no difference in glucocerebrosidase activity (11.99 μmol/l/h versus 11.73 μmol/l/h, P = 0.343) between Ashkenazi Jews and non-Jews.

Mean glucocerebrosidase enzymatic activity was modestly lower in Parkinson’s disease patients (n = 517) than in controls (n = 252) (11.14 μmol/l/h versus 11.85 μmol/l/h, P = 0.011), but ranges overlap (Parkinson’s disease glucocerebrosidase enzymatic range: 0.005–30.30 μmol/l/h; control range: 4.45–25.95 μmol/l/h). When analyses were repeated excluding GBA and LRRK2 G2019S mutation carriers, all those with a family history of Parkinson’s disease, and Parkinson’s disease patients with disease age at onset ≤ 40, glucocerebrosidase enzymatic activity was lower in Parkinson’s participants (n = 301) than in controls (n = 219) (11.53 μmol/l/h, range: 5.20–24.27 μmol/l/h versus 12.11 μmol/l/h, range: 5.07–25.95 μmol/l/h).
Glucocerebrosidase enzymatic activity and Parkinson’s phenotype in idiopathic Parkinson’s disease

To test the association between glucocerebrosidase enzymatic activity and Parkinson’s disease phenotype, we divided the Parkinson’s disease cases who were non-GBA non-LRRK2 G2019S carriers into tertiles based on glucocerebrosidase enzymatic activity. Comparison of the demographics and disease characteristics among the tertiles is presented in Table 4. Higher glucocerebrosidase enzymatic activity was associated with male gender, younger age at onset, longer disease duration, higher daily levodopa

Table 4

<table>
<thead>
<tr>
<th>Table 1 Demographics, GBA mutation status and glucocerebrosidase enzymatic activity in Parkinson’s disease cases and controls</th>
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<tbody>
<tr>
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<tr>
<td>Mean age in years, (SD)</td>
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<tr>
<td>Males, % (n)</td>
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<tr>
<td>Subjects with at least one Ashkenazi Jewish grandparent, % (n)</td>
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</tbody>
</table>
| Subjects with family history of Parkinson’s disease in first-degree relative, % (n)
LRRK2 G2019S carriers, % (n)
GBA mutation/variant status, % (n)
Mean glucocerebrosidase enzymatic activity in μmol/l/h, (SD) | 18.2 (92) | 4.8 (12) | < 0.001 |
| 7.5 (39) | 0.8 (2) | < 0.001 |
| 1.4 (7)* | 0.0 (0) | < 0.001 |
| 15.7 (81) | 6.7 (17) | 0.011 |
| 83.0 (429) | 93.3 (235) | 0.001 |
| 11.14 (3.77) | 11.85 (3.40) | 0.011 |
| Education in years, (SD) | 16.6 (2.9) | 16.7 (2.7) | 0.746 |
| UPDRS part III, (SD) | 18.0 (10.6) | 1.0 (1.9) | < 0.001 |
| Montreal Cognitive Assessment, (SD) | 25.2 (3.7) | 27.0 (2.2) | < 0.001 |
| Mean Parkinson’s age-at-onset, (SD) | 59.2 (11.6) | | |
| Levodopa equivalent daily dose in mg, (SD) | 539 (461) | | |

*Family history information was not available on 12 Parkinson’s disease cases and four controls.

Table 2 Frequency of GBA and LRRK2 G2019S mutations and variants by Parkinson’s disease status

<table>
<thead>
<tr>
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<tr>
<td>GBA homozygotes and compound heterozygotes</td>
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<tr>
<td>All GBA heterozygotes</td>
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<tr>
<td>N370S</td>
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<tr>
<td>L444P</td>
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<tr>
<td>84GG</td>
</tr>
<tr>
<td>R496H</td>
</tr>
<tr>
<td>IVS2 +1</td>
</tr>
<tr>
<td>K-27R</td>
</tr>
<tr>
<td>E326K variant</td>
</tr>
<tr>
<td>T369M variant</td>
</tr>
<tr>
<td>Other GBA variants or mutations</td>
</tr>
<tr>
<td>LRRK2 G2019S</td>
</tr>
<tr>
<td>Non-GBA non-LRRK2 carriers</td>
</tr>
</tbody>
</table>

*Four LRRK2 G2019S carriers also carried the GBA N370S mutation and one carried the GBA R44C variant.

**Family history information was not available on 12 Parkinson’s disease cases and four controls.**
dose and worse performance on the UPDRS and Montreal Cognitive Assessment.

Given the different characteristics among the tertiles we constructed a multivariate logistic regression model in which membership in the highest glucocerebrosidase enzymatic activity tertile (versus the lower and middle tertiles) was the outcome, and sex, age, disease duration, family history of Parkinson’s disease, education, levodopa equivalent daily dose, UPDRS-III and Montreal Cognitive Assessment were the predictors. In the model, younger age at exam (OR = 0.98, 95% CI = 0.95–1.00, P = 0.048), longer Parkinson’s disease duration (OR = 1.07, 95% CI = 1.03–1.11, P = 0.002), and lower Montreal Cognitive Assessment scores (OR = 0.92, 95% CI = 0.86–0.99, P = 0.026) were significantly associated with the highest glucocerebrosidase enzymatic activity tertile.

Discussion

Better understanding of the link between GBA mutations and Parkinson’s disease may shed light on the pathophysiology of Parkinson’s disease and lead to novel therapeutic approaches for treating the disease. This is the first study to measure glucocerebrosidase enzymatic activity in dried blood spots in Parkinson’s disease cases with and without GBA mutations and in controls. Our findings show that GBA heterozygotes with Parkinson’s disease have lower glucocerebrosidase enzymatic activity than controls, and that low enzymatic activity is modestly associated with Parkinson’s disease even among those without GBA or LRRK2 mutations. These findings suggest that low glucocerebrosidase enzymatic activity may be a risk factor for Parkinson’s disease.

Glucocerebrosidase enzymatic activity by mutation status

Glucocerebrosidase enzymatic activity is routinely tested during the workup for Gaucher disease, but little is known about enzymatic activity in healthy individuals or in heterozygotes; it is unknown how glucocerebrosidase enzymatic activity is regulated, or how gender and age affect enzymatic activity. The reports on glucocerebrosidase enzymatic activity in GBA heterozygotes predate genetic testing, and describe reduced activity in family members who are obligate carriers (Raghavan et al., 1980; Svennerholm et al., 1980; Grabowski et al., 1982). Consistent with these reports, we observed lower mean glucocerebrosidase enzymatic activity in heterozygotes when compared to non-carriers, with overlapping ranges of activity. Here, we report that glucocerebrosidase enzymatic activity in GBA heterozygotes predate genetic testing, and describe reduced activity in family members who are obligate carriers (Raghavan et al., 1980; Svennerholm et al., 1980; Grabowski et al., 1982). Consistent with these reports, we observed lower mean glucocerebrosidase enzymatic activity in heterozygotes when compared to non-carriers, with overlapping ranges of activity. Here, we report that glucocerebrosidase enzymatic activity in GBA heterozygotes predate genetic testing, and describe reduced activity in family members who are obligate carriers (Raghavan et al., 1980; Svennerholm et al., 1980; Grabowski et al., 1982).
activity than heterozygote carriers of ‘severe’ mutations (including L444P, 84GG and IVS-2 + 1). In addition, we show that two GBA variants, E326K and T369M, whose contribution to Gaucher disease in the homozygote state is controversial (Horowitz et al., 2011), are associated with lower enzymatic activity than non-carriers. The GBA E326K and T369M variants have been associated with Parkinson’s disease in some but not all studies (Clark et al., 2007, 2009; Nichols et al., 2009; Duran et al., 2013). The observation in this study may help explain the reported association between these mutations and Parkinson’s disease (Clark et al., 2007; Duran et al., 2013), if this link is mediated through reduced glucocerebrosidase enzymatic activity. It is important to note that most heterozygotes in this study (82.7%, Table 1) are Parkinson’s disease cases, and glucocerebrosidase enzymatic activity in heterozygotes without Parkinson’s disease was available only in 17 cases, most of whom carried the E326K and T369M variants or variants of unknown significance (Supplementary Table 2).

### Glucocerebrosidase enzymatic activity and Parkinson’s disease

When the entire cohort was included, mildly lower glucocerebrosidase enzymatic activity was observed in Parkinson’s disease after adjustment for age and gender. The association persisted after excluding GBA mutation carriers, suggesting that reduced glucocerebrosidase enzymatic activity may be independently associated with Parkinson’s disease. However, the mechanism of the association was not studied here. We did not test whether glucocerebrosidase expression was reduced in Parkinson’s cases compared to controls. Other factors (e.g. genes that modify glucocerebrosidase enzymatic activity such as

<table>
<thead>
<tr>
<th>Mutation status</th>
<th>n</th>
<th>Mean glucocerebrosidase enzymatic activity in μmol/l/h (SD)</th>
<th>Range</th>
<th>P-value (compared to non-carriers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBA homozygotes and compound heterozygotes</td>
<td>7</td>
<td>0.85 (1.11)</td>
<td>0.005–3.00</td>
<td>&lt;0.001</td>
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<td>N370S</td>
<td>40</td>
<td>6.42 (1.72)</td>
<td>3.22–11.72</td>
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<td>L444P</td>
<td>8</td>
<td>7.66 (2.28)</td>
<td>4.88–11.26</td>
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<tr>
<td>84GG</td>
<td>4</td>
<td>7.13 (1.29)</td>
<td>5.46–8.47</td>
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<td>R496H</td>
<td>4</td>
<td>8.10 (2.52)</td>
<td>4.83–10.92</td>
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<tr>
<td>IVS2 + 1</td>
<td>2</td>
<td>7.97 (1.00)</td>
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<tr>
<td>E326K</td>
<td>16</td>
<td>9.81 (2.87)</td>
<td>5.2–16.37</td>
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<tr>
<td>T369M</td>
<td>9</td>
<td>7.64 (1.71)</td>
<td>6.12–11.10</td>
<td>&lt;0.001</td>
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<tr>
<td>LRRK2 G2019Sb</td>
<td>36</td>
<td>13.69 (4.84)</td>
<td>8.70–30.30</td>
<td>0.002</td>
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<tr>
<td>Non-GBA and non-LRRK2 carriers</td>
<td>628</td>
<td>11.93 (3.21)</td>
<td>4.17–25.95</td>
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</tbody>
</table>

*Including both Parkinson’s disease and control participants.

*Four LRRK2 G2019S carriers that carried the N370S mutation and one that carried the R44C variant were removed from the analysis. Their glucocerebrosidase enzymatic activity was similar to other GBA mutation carriers (mean 7.3 ± 2.7 μmol/l/h).
SCARB2; Velayati et al., 2011), may also increase the risk for Parkinson’s disease. In addition, the role of the glucocerebrosidase activators—e.g. Saposin C (Salvioli et al., 2000)—in Parkinson’s risk is unknown. Measuring Saposin C levels in lysosomes of patients with Parkinson’s disease and controls may further clarify the role of Saposin C in Parkinson’s risk. Of special interest are those without GBA mutations who have glucocerebrosidase enzymatic activity lower than heterozygotes. Exploring the cause of lower enzymatic activity in this group (Supplementary material) may shed light on glucocerebrosidase modifiers. However, reduced glucocerebrosidase enzymatic activity alone is not a sufficient cause of Parkinson’s disease, given that most patients with Gaucher disease, who by definition have diminished enzymatic activity, will never develop Parkinson’s disease (Rosenbloom et al., 2011; Alcalay et al., 2014). Moreover, reduced glucocerebrosidase enzymatic activity is not a necessary cause of Parkinson’s disease, as there is a significant overlap in glucocerebrosidase enzymatic activity between Parkinson’s disease cases and controls. Therefore, glucocerebrosidase enzymatic activity alone cannot be used as a biomarker or a screening tool to identify Parkinson’s disease.

The pathophysiological mechanism of the link between GBA mutations, reduced glucocerebrosidase activity and Parkinson’s disease remains unclear. Several studies have found an increase in alpha synuclein levels and/or aggregation, both in vitro and in vivo, in the setting of mutant GBA expression or glucocerebrosidase loss of function (Cullen et al., 2011; Mazzulli et al., 2011; Sardi et al., 2011; Woodard et al., 2014). However, the relative contribution of loss of glucocerebrosidase activity versus toxic gain of function remains controversial. Interestingly, one study found that the glucocerebrosidase substrate glucosylceramide can bind to alpha-synuclein oligomers (Mazzulli et al., 2011). Here, we did not measure glucocerebrosidase substrates in our samples, and future studies are needed to evaluate if the reduced glucocerebrosidase activity is also associated with higher levels of substrate accumulation.

Low glucocerebrosidase enzymatic activity has been previously reported in Parkinson’s disease and Lewy body dementia in CSF (Balducci et al., 2007; Parnetti et al., 2009, 2014) and in two brain autopsy studies (Gegg et al., 2012; Murphy et al., 2014), findings which are consistent with our observation. Gegg et al. measured glucocerebrosidase enzymatic activity in brains of GBA heterozygotes (n = 14), sporadic Parkinson’s disease (n = 14) and controls (n = 10). Compared to controls, they found that GBA heterozygotes had decreased glucocerebrosidase enzymatic activity in all brain regions except for the frontal cortex, and sporadic Parkinson’s disease brains also showed lower glucocerebrosidase enzymatic activity in the substantia nigra and cerebellum. Murphy et al. (2014) examined neuropathological tissue of sporadic Parkinson’s brains (n = 19) and age matched controls (n = 10) all without GBA mutations. Both glucocerebrosidase protein and enzymatic activity were reduced in the anterior cingulate gyrus of Parkinson’s cases when compared to controls. The importance of our finding that glucocerebrosidase enzymatic activity is lower in dried blood spots of Parkinson’s disease cases versus controls is that blood samples can be obtained prospectively and longitudinally (as opposed to the retrospective nature of autopsy samples), and in a simpler and less invasive procedure than lumbar puncture (Olivova et al., 2008; Reusser et al., 2011). The marked differences in mean glucocerebrosidase enzymatic activity among patients with Gaucher disease, heterozygotes and non-GBA carriers further validates the reliability of glucocerebrosidase enzymatic activity measurement in dried blood spots. The correlation between glucocerebrosidase enzymatic activity in dried blood spots and glucocerebrosidase enzymatic activity in spinal fluid, and more importantly in brain parenchyma, remains unknown and should be explored in follow-up studies.

We also tested the association between glucocerebrosidase enzymatic activity and Parkinson’s disease characteristics and severity markers. Based on the more severe Parkinson’s disease phenotype of GBA carriers compared to non-carriers (Neumann et al., 2009; Alcalay et al., 2012), we hypothesized that among non-carriers, high glucocerebrosidase enzymatic activity would be associated with milder disease severity. Higher glucocerebrosidase enzymatic activity was associated with male gender, younger age at onset, longer disease duration, higher levodopa dose and worse performance on the UPDRS and Montreal Cognitive Assessment, which are markers of more advanced Parkinson’s disease. In regression models, only age, disease duration and Montreal Cognitive Assessment performance were associated with high glucocerebrosidase enzymatic activity. Worse Montreal Cognitive Assessment score may represent worse cognitive functioning. However, longer Parkinson’s disease duration in a cross-sectional study may be viewed as a marker of a more benign form of Parkinson’s disease, where those with the potentially protective effect of high glucocerebrosidase enzymatic activity were more likely to survive and participate in the study. A similar phenomenon was observed in our report on parkin-Parkinson’s disease (Alcalay et al., 2012). Therefore, our finding of longer disease duration among Parkinson’s disease cases in the higher tertile of glucocerebrosidase enzymatic activity may be interpreted as a marker of a milder disease course. However, we cannot conclude whether higher glucocerebrosidase enzymatic activity is associated with a milder course (earlier age-at-onset, but longer disease duration and slower progression). Longitudinal follow up including comprehensive evaluation is required to conclude whether glucocerebrosidase enzymatic activity may be a marker of disease severity in idiopathic Parkinson’s disease.

Glucocerebrosidase enzymatic activity in LRRK2 G2019S carriers

Interestingly, we found that LRRK2 G2019S carriers have higher glucocerebrosidase enzymatic activity than
non-carriers (Table 3), even higher than controls without Parkinson’s disease. This finding will require replication. The mechanism by which glucocerebrosidase enzymatic activity is increased in dried blood spots of LRRK2 carriers is unknown. One possible explanation is that mutant LRRK2 may cause lysosomal compartment expansion (Orenstein et al., 2013), which in turn is associated with increased glucocerebrosidase enzymatic activity. Alternatively, it is possible that mutated LRRK2 affects retromer function and thus alters sorting and turnover of glucocerebrosidase (MacLeod et al., 2013).

The strengths of our study include the large number of participants, all of whom were carefully evaluated by a single movement disorders specialist for research purposes, and the simplicity of obtaining the biological product, the dried blood spot. However, we acknowledge that the Parkinson’s disease and control groups were not matched for gender (which is not associated with glucocerebrosidase activity) and we also have a relatively smaller number of male controls (n = 84). The differential glucocerebrosidase enzymatic activity in heterozygotes and non-carriers (as performed in a laboratory that was blinded to genotype) further validates the glucocerebrosidase enzymatic activity measurement.

The major limitation of our study is its cross-sectional nature. We cannot conclude if high enzymatic activity is associated with a milder disease (Alcalay et al., 2012). The lack of longitudinal data limits our ability to assess potential changes in glucocerebrosidase enzymatic activity because of Parkinson’s disease treatment (levodopa) or as Parkinson’s disease progresses. We cannot conclude if low glucocerebrosidase enzymatic activity is an independent risk factor for Parkinson’s disease unless controls or GBA carriers with low activity are followed. Comparison of glucocerebrosidase enzymatic activity between older GBA heterozygotes with and without Parkinson’s disease would help determine if lower glucocerebrosidase enzymatic activity is associated with Parkinson’s disease in this population and may be used as a biomarker for increased Parkinson’s disease risk.

Longitudinal follow-up would allow us to conclude whether higher glucocerebrosidase enzymatic activity is associated with a slower rate of Parkinson’s disease progression and whether progression differs by type of GBA mutation.

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Conflict of interest

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Supplementary material

Supplementary material is available at Brain online.

References


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