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Alpha galactosidase A activity in Parkinson's disease

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Abstract

Glucocerebrosidase (GCase, deficient in Gaucher disease) enzymatic activity measured in dried blood spots of Parkinson's Disease (PD) cases is within healthy range but reduced compared to controls. It is not known whether activities of additional lysosomal enzymes are reduced in dried blood spots in PD. To test whether reduction in lysosomal enzymatic activity in PD is specific to GCase, we measured GCase, acid sphingomyelinase (deficient in Niemann–Pick disease types A and B), alpha galactosidase A (deficient in Fabry), acid alpha-glucosidase (deficient in Pompe) and galactosylceramidase (deficient in Krabbe) enzymatic activities in dried blood spots of PD patients (n=648) and controls (n=317) recruited from Columbia University. Full sequencing of glucocerebrosidase (*GBA*) and the *LRRK2* G2019S mutation was performed. Enzymatic activities were compared between PD cases and controls using *t* test and regression models adjusted for age, gender, and *GBA* and *LRRK2* G2019S mutation status. Alpha galactosidase A activity was lower in PD cases compared to controls both when only non-carriers were included (excluding all *GBA*

and *LRRK2* G2019S carriers and PD cases with age-at-onset below 40) [2.85 $\mu\text{mol/l/h}$ versus 3.12 $\mu\text{mol/l/h}$, $p=0.018$; after controlling for batch effect, $p=0.006$ (468 PD cases and 296 controls)], and when including the entire cohort (2.89 $\mu\text{mol/l/h}$ versus 3.10 $\mu\text{mol/l/h}$, $p=0.040$; after controlling for batch effect, $p=0.011$). Because the alpha galactosidase A gene is X-linked, we stratified the analyses by sex. Among women who were non-carriers of *GBA* and *LRRK2* G2019S mutations (PD, $n=155$; control, $n=194$), alpha galactosidase A activity was lower in PD compared to controls (2.77 $\mu\text{mol/l/h}$ versus 3.10 $\mu\text{mol/l/h}$, $p=0.044$; after controlling for a batch effect, $p=0.001$). The enzymatic activity of acid sphingomyelinase, acid alpha-glucosidase and galactosylceramidase was not significantly different between PD and controls. In non-carriers, most lysosomal enzyme activities were correlated, with the strongest association in GCase, acid alpha-glucosidase, and alpha galactosidase A (Pearson correlation coefficient between 0.382–0.532). In a regression model with all five enzymes among non-carriers (adjusted for sex and age), higher alpha galactosidase A activity was associated with lower odds of PD status (OR= 0.54; 95% CI:0.31–0.95; $p=0.032$). When *LRRK2* G2019S PD carriers ($n=37$) were compared to non-carriers with PD, carriers had higher GCase, acid sphingomyelinase and alpha galactosidase A activity. We conclude that alpha galactosidase A may have a potential independent role in PD, in addition to GCase.

Keywords

Parkinson's disease; lysosomal storage disease; movement disorders; neurodegeneration; biomarkers

INTRODUCTION

The association between mutations in glucocerebrosidase (*GBA*)¹, which encodes the lysosomal enzyme glucocerebrosidase (GCase), and Parkinson's disease (PD) has highlighted the importance of lysosomal function in PD pathogenesis. Studies have shown that mutations in *SMPD1*, which encodes the lysosomal enzyme acid sphingomyelinase (Dagan et al., 2015; Foo et al., 2013; Gan-Or et al., 2013), and variants in *SCARB2* (Alcalay et al., 2016), which encodes the glucocerebrosidase chaperone LIMP-2, may also be associated with PD. Since the lysosome is involved in the degradation of alpha-synuclein (Webb et al., 2003), it is hypothesized that lysosomal dysfunction may lead to alpha-synuclein accumulation, and subsequently to PD (Moors et al., 2016).

We have previously shown that measuring GCase activity in dried blood spots demonstrated significant differences in mean enzymatic activity between *GBA* heterozygotes and non-carriers of *GBA* mutations or variants, and a modest reduction (roughly 5%) in PD cases relative to controls (Alcalay et al., 2015). Here, we aimed to use the same cohort and methodology of mass-spectrometry based measurement in dried blood spots, to test for an association between PD and the activities of four additional lysosomal enzymes (Figure), acid sphingomyelinase (ASM, EC 3.1.4.12, deficient in Niemann–Pick disease types A and

¹PD = Parkinson's disease; *GBA* = glucocerebrosidase gene; GCase = glucocerebrosidase enzyme; ASM = acid sphingomyelinase; GLA = acid alpha galactosidase; GAA = acid alpha- glucosidase; GALC = galactocerebrosidase; UPDRS = United Parkinson's Disease Rating Scale; MoCA = Montreal Cognitive Assessment

B), acid alpha galactosidase (GLA, EC 3.2.1.22 deficient in Fabry disease), acid alpha-glucosidase (GAA, EC 3.2.1.20, deficient in Pompe disease) and galactocerebrosidase (GALC, EC 3.2.1.46, deficient in Krabbe disease).

METHODS AND PARTICIPANTS

PARTICIPANTS AND CLINICAL EVALUATION

Participants in the Spot study included PD patients and non-blood related controls (primarily spouses) from the Center for Parkinson's Disease at Columbia University Medical Center in New York, NY (Alcalay et al., 2015; Sakanaka et al., 2014). In brief, a blood sample and demographics, medical history, medications, PD family history (Marder et al., 2003), the Unified Parkinson's Disease Rating Scale (UPDRS) in the "on" state, and the Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005) were collected from consecutive PD cases, as defined by the United Kingdom PD brain bank criteria (however we did not exclude cases with a family history of PD) (Hughes et al., 1992). A convenience sample of non-blood related individuals served as controls. Participants were fully sequenced for *GBA* and genotyped for the *LRRK2* G2019S mutation as previously described (Alcalay et al., 2015). For the purpose of the analyses in this report, we considered all carriers of Gaucher causing mutations and the E326K and T369M variants, which have been associated with PD (although T369M inconsistently) (Mallett et al., 2016), *GBA* mutation/variant carriers. All study procedures were approved by the Columbia University IRB, and all participants signed informed consent.

ENZYMATIC ACTIVITY ASSAY

Dried blood spots were obtained as previously described (Olivova et al., 2008; Reuser et al., 2011). Enzymatic activities of the five enzymes – GCase, ASM, GLA, GAA and GALC – were measured using a previously published protocol as part of a multiplex assay by Genzyme/Sanofi (Zhang et al., 2008). In summary, the GCase, ASM, GLA and GAA enzymes were extracted from a 3.2 mm-diameter punch in 70 µl of 20 mM sodium phosphate buffer (pH 7.1) on a 96-well plate. Ten µl of dried blood spot extract was mixed with 15 µl of substrate/internal standard mixtures (The Center for Disease Control and Prevention, Georgia, Atlanta). For GALC, a full 3.2 mm dried blood spots punch was combined with 30 µl of its S/IS mixture.

The GCase final S/IS mixture contained 0.67 mM of C12-glucocerebroside and 13.33 µM C14-ceramide in 0.31/0.62 M citrate-phosphate buffer with 16 g/L sodium taurocholate, pH 5.1. The ASM final S/IS mixture contained 0.33 mM of C-6 sphingomyelin and 6.67 µM C-4 ceramide in 0.92 M sodium acetate with 1.0 g/L sodium taurocholate and 0.6 mM of zinc chloride, pH 5.7. The GLA final S/IS mixture contained 3.33 mM of (6-Benzoylamino-hexyl)-{2-[4-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-phenylcarbamoyl]-ethyl}-carbamic acid *tert*-butyl ester and 6.67 µM of (6-d5-benzoylamino-hexyl)-[2-(4-hydroxy-phenylcarbamoyl)-ethyl]-carbamic acid *tert*-butyl ester in 0.142 M sodium acetate with 3 g/L sodium taurocholate and 160 mM GalNAc, pH 4.6. The GAA final S/IS mixture contained 0.67 mM of (7-Benzoylamino-heptyl)-{2-[4-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydro-pyran-2-yloxy)-phenylcarbamoyl]-ethyl}-carbamic

acid *tert*-butyl ester and 6.67 μ M of (7-d5-benzoylamino-heptyl)-[2-(4-hydroxy-phenylcarbamoyl)-ethyl]-carbamic acid *tert*-butyl ester in 0.30/0.15 M citrate-phosphate buffer with 10 g/L CHAPS hydrate and 13.3 μ M acarbose, pH 4.0. The GALC final S/IS mixture contained 1 mM of C8-galactosylceramide and 6.67 μ M of C10-ceramide in 0.09/0.18M citrate-phosphate with 9.6 g/L sodium taurocholate and 1.2 mM of oleic acid, pH 4.4.

Sealed plates were incubated on an orbital shaker at 37°C for 20 hours. Reactions were quenched with 100 μ l of organic solution (ethyl acetate:methanol, 1:1), combined in a deep well plate and extracted by 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 μ l of a solvent mixture (80:20 acetonitrile:water containing 0.2% formic acid). All solvents and oleic acid were purchased from Honeywell, Morris Plains, NJ, acarbose from Toronto Research Chemicals, Toronto, Canada, and the remaining chemicals were purchased from Sigma, St. Luis, MI.

All analytes were monitored on an API 4000 triple quadrupole mass spectrometer (ABSciex, Framingham, Massachusetts, USA) 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 liter of whole blood per hour (μ mol/l/h).

STATISTICAL ANALYSIS

Demographics, activity of each enzyme, and frequency of *GBA* and *LRRK2* G2019S mutations were compared between PD cases and controls using the Student *t* test for continuous variables, and the chi-square and Fisher exact tests for categorical variables. Enzymatic activity was expressed in two ways, the first in μ mol/l/h units, and the second adjusted for a batch effect. To do so, enzymatic activity was normalized to the batch in which the samples were analyzed. Each batch included at least 3 replicates of two QC controls with previously established activity ranges. The activity of each sample was divided by the mean activity of the QC controls from the same run.

We compared PD patients to controls twice. First, we included all participants. Second, we excluded *GBA* and *LRRK2* G2019S mutation carriers, as well as PD patients with disease age at onset below 40 to exclude potential PD cases with *Parkin*, *PINK-1* or *DJ-1* mutations, which were not tested for. Given that Fabry's disease is X-linked, we stratified the analyses by sex.

In non-carriers, to test the correlation between the different enzymatic activities in dried blood spots, we used Pearson correlation coefficient, correlating normalized to batch values of each enzyme. Since most enzymes were correlated to one another, we used a logistic regression model in which PD status was the outcome and the lysosomal enzymatic activities were the predictors, adjusted for age and gender.

Given the previously reported association between *LRRK2* G2019S mutations and high GCase activity (Alcalay et al., 2015), we compared lysosomal enzymatic activity between G2019S carriers and non-carriers (excluding *GBA* mutation and variant carriers) using Student *t*-test. We ran the analyses twice: first, including both cases and controls who were not *GBA* mutation carriers (n=835) and then including only PD cases (excluding controls) who were not *GBA* mutation carriers (n=537). Lastly, we compared carriers of two *GBA* mutations or variants (homozygotes and compound heterozygotes) to non-carriers of *GBA* or *LRRK2* mutations.

Analyses were performed using SPSS Statistics version 23.0 software (Chicago, IL).

RESULTS

Cohort characteristics and GLA activity

The study included 648 PD cases and 317 controls. The demographics and GCase activity of most participants (PD, n=517; controls, n=252) were previously reported (Alcalay et al., 2015). PD cases and controls were similar in age and education. Controls were more likely to be women because we used spouse controls and PD is more prevalent in males (Table 1). PD participants were more likely than controls to carry the *GBA* mutations and variants and the *LRRK2* G2019S mutation. The mean age-at-PD onset (AAO) was 59.1 (SD: 11.6), and the mean levodopa equivalent daily dose among PD cases was 526 mg (SD: 467 mg). The levels of lysosomal enzymes in all participants was within healthy range and did not meet criteria for published corresponding disease (e.g., Fabry or Krabbe; except for 10 cases of reduced GCase activity who met diagnostic criteria for Gaucher's). In addition to the previously reported GCase activity, GLA levels were reduced in PD compared to controls (2.89 $\mu\text{mol/l/h}$ versus 3.10 $\mu\text{mol/l/h}$, $p=0.040$; after controlling for a batch effect, $p=0.011$).

The difference in GLA activity between PD cases and controls was even more pronounced when mutation carriers (all *GBA* and *LRRK2* G2019S carriers and PD cases with age-at-onset below 40) were excluded (2.85 $\mu\text{mol/l/h}$ versus 3.12 $\mu\text{mol/l/h}$, $p=0.018$; after controlling for a batch effect, $p=0.006$; including 468 PD cases and 296 controls), hereinafter non-carriers. We stratified the analyses by sex because GLA is encoded by the alpha galactosidase A gene, which is X-linked. Among non-carrier men (PD = 313, controls = 102), GLA activity was not significantly reduced in PD (2.90 $\mu\text{mol/l/h}$ versus 3.17 $\mu\text{mol/l/h}$, $p=0.123$; after controlling for a batch effect, $p=0.282$). Among non-carrier women (PD = 155, controls = 194), GLA activity was significantly lower in PD compared to controls (2.77 $\mu\text{mol/l/h}$ versus 3.10 $\mu\text{mol/l/h}$, $p=0.044$; after controlling for a batch effect, $p=0.001$).

Given the association between GLA and PD status, we examined the association between GLA and age and sex. Among non-carrier controls (n=296), GLA activity was similar in men and women [men: n=102, 3.12 $\mu\text{mol/l/h}$ (1.75 $\mu\text{mol/l/h}$); women: n=194, 3.10 $\mu\text{mol/l/h}$ (1.71 $\mu\text{mol/l/h}$); $p=0.738$, when normalized to batch: $p=0.836$]. GLA activity was not associated with age among controls (n=296; Pearson Correlation, $r=0.022$, $p=0.704$).

Correlation among lysosomal enzymes

All five lysosomal enzymes were correlated with one another, except for ASM and GALC. The strongest association was between GCase, GAA and GLA, with Pearson correlation coefficient ranging between 0.154–0.532.

Because of the correlations between activity of the lysosomal enzymes, we used a logistic model to test the association between enzymatic activity and PD status in non-carriers, adjusted for age and sex. Higher GLA activity was associated with lower odds of PD status (OR= 0.54; 95% CI: 0.31–0.95; p=0.032).

Enzymatic activity among *LRRK2* G2019S carriers

Enzymatic activity was available for 39 *LRRK2* G2019S carriers who were not also *GBA* carriers, including 2 without PD and 37 *LRRK2*/PD. In addition to GCase activity, which was elevated in *LRRK2* carriers compared to non-carriers (Alcalay et al., 2015), ASM and GLA activity were also elevated in *LRRK2* G2019S carriers. In fact, the differences were greater in ASM (121%, p=0.001) than in GCase (116%, p=0.002). Results were similar when all (non-*GBA* carriers) participants were included and when analyses were restricted to PD cases only.

Enzymatic activity among *GBA* homozygotes/compound heterozygotes

To test the specificity of the enzymatic activity measurements, we compared carriers of two *GBA* mutations, including homozygotes and compound heterozygotes (n=10; including 7 patients with Gaucher disease and 3 who carried both a Gaucher causing mutation and either the E326K or the T369M variants in trans), to non-carriers of *LRRK2* G2019S and *GBA* mutations or variants. Mean GCase activity was significantly different between the groups (12.02 $\mu\text{mol/l/h}$ versus 1.28 $\mu\text{mol/l/h}$; p<0.001), but there were no significant differences in enzymatic activities of all other enzymes. ASM and GAA activities were insignificantly higher in carriers of two *GBA* mutations, and GLA and GALC were insignificantly higher in the non-carrier group.

DISCUSSION

In the current study we found an association between lower GLA activity and PD status, which is stronger than the association between lower GCase activity and PD. The link between PD and *GBA* mutations highlights the role of lysosomal dysfunction in the pathogenesis of PD (Sidransky and Lopez, 2012). An unresolved question is whether the association between *GBA* mutations and PD is gene or enzyme-specific, or if other abnormalities of lysosomal dysfunction may also contribute to PD. *GBA* mutations are by far more common than mutations in other lysosomal genes. Specifically, the E326K variant is present in up to 3% of all healthy Caucasians (Duran et al., 2013) and pathogenic (i.e. Gaucher-causing) mutations are present in about 6–7% of all healthy Ashkenazi Jews (Gan-Or et al., 2008). Therefore, genetic studies are better powered to identify a *GBA*-PD link than a link between any other lysosomal gene and PD. A strength of this study is that rather than test genetic association, we tested enzymatic activities directly. Reduced GLA activity was associated with PD status when *LRRK2* and *GBA* mutation carriers were included, and

this association was even stronger in non-carriers. The association was present in both sexes, but reached significance among women only. This finding is noteworthy especially because the gene encoding GLA, galactosidase alpha (*GLA*) is on the X chromosome. Mutations in *GLA* cause Fabry's disease, an X-linked disease, which may present with rash, heart disease, renal failure, neuropathy or stroke, due to accumulation of the GLA substrate, globotriaosylceramide (GB3) (Desnick et al., 2003) and globotriosylsphingosine (Aerts et al., 2008). To date, over 900 *GLA* mutations have been reported in Fabry disease patients, and are present in all ethnicities (Stenson et al., 2017). The phenotype in men is more severe, and the range of Fabry phenotype in women can vary from asymptomatic to severely affected. The estimated median cumulative survival for Fabry disease patients, based on questionnaire data prior to enzyme replacement therapy (Eng et al., 2001), is 50 years for affected men and 70 years for affected women (MacDermot et al., 2001a; MacDermot et al., 2001b). Currently, Fabry can be treated with enzyme replacement therapy, which does not penetrate the blood brain barrier, but substrate reduction therapy by small molecules which may penetrate the blood brain barrier are in development (Ashe et al., 2015).

Fabry, GLA and PD

This is the largest study to date linking GLA activity and PD. Others have also noted an association between reduced GLA activity (or Fabry's disease) and PD. Wu et al. (Wu et al., 2008) measured GLA activity in leukocytes of PD cases (n=38) and controls (n=258), and found that PD patients had lower GLA activity compared to controls (18.56 ± 1.49 units versus 22.24 ± 0.60 units, $p < 0.05$). Pchelina et al. (Pchelina et al., 2017) measured GLA activity in Gaucher patients (n=42), *GBA*/PD (n=21), non-carriers with PD (n=84) and controls (n=62). Compared to controls, they noted reduced GLA activity in *GBA*/PD ($p = 0.001$) and in Gaucher patients ($p = 0.019$), and a non-significant trend among PD non-carriers ($p = 0.178$). Two case reports of progressive parkinsonism in Fabry patients were published (Borsini et al., 2002; Buechner et al., 2006; Orimo et al., 1994), although neuropathological examination was not available. Furthermore, Lohle et al. (Lohle et al., 2015) examined 110 Fabry patients and 57 controls with a motor and non-motor examination. Patients with Fabry demonstrated slower hand and gait speed, but did not manifest non-motor symptoms such as hyposmia or REM sleep behavior disorder, which are often present in idiopathic PD or in *GBA*-PD. We also demonstrated, using a survey study, an estimated higher prevalence of PD in Fabry patients and families, but PD diagnosis was not clinically confirmed (Wise et al., 2018). Lastly, in a mouse model of Fabry, Nelson et al. (Nelson et al., 2014) found aggregates of phosphorylated alpha-synuclein in the pons.

Alpha galactosidase A, other lysosomal enzymes and PD

Lysosomal enzymatic activities have been compared between PD and controls in brain tissue, CSF and blood. In brain tissue, GCase activity was consistently reduced in PD and in *GBA*/PD compared to controls (Chu et al., 2009; Clark et al., 2015; Gegg et al., 2012; Murphy et al., 2014), and more recently Nelson and colleagues demonstrated reduced GLA activity in the temporal cortex of PD brains compared to controls (Nelson et al., 2018). However, GLA was never reported in studies on lysosomal enzymes in CSF (Balducci et al., 2007; Parnetti et al., 2014; Parnetti et al., 2017; van Dijk et al., 2013). Hexosaminidase activity (not measured here) measurements demonstrated mixed and conflicting results

between PD and controls (Balducci et al., 2007; Parnetti et al., 2014; Parnetti et al., 2017; van Dijk et al., 2013).

In this study, GLA activity was associated with all other enzymes measured (Pearson correlation coefficient ranging between 0.154–0.532). While only GLA and GCase were significantly associated with PD status, there was a non-significant trend for reduced enzymatic activity in all lysosomal enzymes in PD compared to controls. It is possible that a larger study would have detected these other enzymatic activity differences, as genetic studies have associated both *SMPD1* (which encodes ASM) and *GALC* (which encodes GALC) with PD (Chang et al., 2017; Gan-Or et al., 2015).

While the lysosomal enzymatic activities are correlated with one another, there is clear specificity for the different enzymes. For example, while mean GCase activity is almost 10 fold lower in carriers of two *GBA* mutations than in non-carriers, there was no significant difference in the activity of the other enzymes between carriers of two *GBA* mutations and non-carriers. Furthermore, Pchelina et al. (Pchelina et al., 2017) reported elevated GALC and reduced GLA in Gaucher's patients compared to controls. These findings suggest that global lysosomal dysfunction may be an upstream event that affects many of the enzymatic activities, but when the activity of one of the enzymes is severely reduced due to mutation of a specific enzyme's gene, it does not necessarily influence other enzymes.

Lysosomal enzymatic activity in *LRRK2* G2019S carriers

Like our previous finding in this cohort that GCase activity was higher in *LRRK2* G2019S carriers than in non-carriers (Alcalay et al., 2015), we found that ASM and GLA activity are also higher in carriers than in non-carriers, with the highest effect size in ASM (Table 4). It should be noted that only two G2019S carriers without PD were included in this analysis, and comparison between carriers with and without PD should be conducted in future studies. The mechanism of elevated GCase, ASM and GLA in *LRRK2* G2019S carriers compared to non-carriers is unknown. It is possible that *LRRK2* mutations upregulate lysosome function and cause PD in a different way than the lysosomal dysfunction in idiopathic PD. The effect may be compensatory to accommodate *LRRK2* activity upregulation in the endolysosomal system. However, it is unclear why this effect would be stronger in ASM, GLA and GCase than in other enzymes such as GAA and GALC.

The strengths of our study include the large number of participants and the uniform clinical and laboratory evaluation of a peripheral biomarker. Our finding of reduced GLA activity in PD is supported by prior literature (Buechner et al., 2006; Lohle et al., 2015; Nelson et al., 2014; Orimo et al., 1994; Pchelina et al., 2017; Wu et al., 2008), but should be replicated, as our analyses were not corrected for multiple comparisons. Although as an association study our study design limits our ability to infer about mechanism, it is interesting that GLA is closely related to GCase: both have a significant role in the conversion of GB3 to ceramide (Figure 1). Therefore, dysregulation in either enzyme may change lysosomal membrane lipid concentration, which in turn may modify alpha-synuclein degradation (Grey et al., 2015). Alternatively, it is possible that reduced GLA activity reflects lysosomal dysfunction, rather than being its proximate cause. Therefore, future studies are required. Specifically, one needs to test for the presence of genetic variants in *GLA*, which may explain reduced

activity even in the absence of Fabry's disease. Replication of our findings is required, most importantly in CSF and in brain tissue, where to the best of our knowledge GLA activity has never been compared between PD and controls.

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References

- Aerts JM, et al. Elevated globotriaosylsphingosine is a hallmark of Fabry disease. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; 105:2812–2817. [PubMed: 18287059]
- Alcalay RN, et al. Glucocerebrosidase activity in Parkinson's disease with and without GBA mutations. *Brain*. 2015
- Alcalay RN, et al. SCARB2 variants and glucocerebrosidase activity in Parkinson's disease. *NPJ Parkinsons Dis*. 2016; 2
- Ashe KM, et al. Efficacy of Enzyme and Substrate Reduction Therapy with a Novel Antagonist of Glucosylceramide Synthase for Fabry Disease. *Mol Med*. 2015; 21:389–99. [PubMed: 25938659]
- Balducci C, et al. Lysosomal hydrolases in cerebrospinal fluid from subjects with Parkinson's disease. *Mov Disord*. 2007; 22:1481–4. [PubMed: 17546678]
- Borsini W, et al. Anderson-Fabry disease with cerebrovascular complications in two Italian families. *Neurol Sci*. 2002; 23:49–53. [PubMed: 12235491]
- Buechner S, et al. Parkinsonism and Anderson Fabry's disease: a case report. *Mov Disord*. 2006; 21:103–7. [PubMed: 16149089]
- Chang D, et al. A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci. *Nat Genet*. 2017; 49:1511–1516. [PubMed: 28892059]
- Chu Y, et al. Alterations in lysosomal and proteasomal markers in Parkinson's disease: relationship to alpha-synuclein inclusions. *Neurobiol Dis*. 2009; 35:385–98. [PubMed: 19505575]
- Clark LN, et al. Gene-wise association of variants in four lysosomal storage disorder genes in neuropathologically confirmed Lewy body disease. *PLoS One*. 2015; 10:e0125204. [PubMed: 25933391]

- Dagan E, et al. The contribution of Niemann-Pick SMPD1 mutations to Parkinson disease in Ashkenazi Jews. *Parkinsonism Relat Disord*. 2015; 21:1067–71. [PubMed: 26169695]
- Desnick RJ, et al. Fabry disease, an under-recognized multisystemic disorder: expert recommendations for diagnosis, management, and enzyme replacement therapy. *Ann Intern Med*. 2003; 138:338–46. [PubMed: 12585833]
- Duran R, et al. The glucocerebrosidase E326K variant predisposes to Parkinson's disease, but does not cause Gaucher's disease. *Movement Disorders*. 2013; 28:232–236. [PubMed: 23225227]
- Eng CM, et al. Safety and efficacy of recombinant human alpha-galactosidase A replacement therapy in Fabry's disease. *N Engl J Med*. 2001; 345:9–16. [PubMed: 11439963]
- Foo JN, et al. Rare lysosomal enzyme gene SMPD1 variant (p.R591C) associates with Parkinson's disease. *Neurobiol Aging*. 2013; 34:2890 e13–5.
- Gan-Or Z, et al. Genotype-phenotype correlations between GBA mutations and Parkinson disease risk and onset. *Neurology*. 2008; 70:2277–83. [PubMed: 18434642]
- Gan-Or Z, et al. The p.L302P mutation in the lysosomal enzyme gene SMPD1 is a risk factor for Parkinson disease. *Neurology*. 2013; 80:1606–1610. [PubMed: 23535491]
- Gan-Or Z, et al. The emerging role of SMPD1 mutations in Parkinson's disease: Implications for future studies. *Parkinsonism Relat Disord*. 2015; 21:1294–5. [PubMed: 26320887]
- Gegg ME, et al. Glucocerebrosidase deficiency in substantia nigra of parkinson disease brains. *Ann Neurol*. 2012; 72:455–63. [PubMed: 23034917]
- Grey M, et al. Acceleration of alpha-synuclein aggregation by exosomes. *J Biol Chem*. 2015; 290:2969–82. [PubMed: 25425650]
- Hughes AJ, et al. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry*. 1992; 55:181–4. [PubMed: 1564476]
- Lohle M, et al. Clinical prodromes of neurodegeneration in Anderson-Fabry disease. *Neurology*. 2015; 84:1454–64. [PubMed: 25762709]
- MacDermot KD, Holmes A, Miners AH. Anderson-Fabry disease: clinical manifestations and impact of disease in a cohort of 98 hemizygous males. *J Med Genet*. 2001a; 38:750–60. [PubMed: 11694547]
- MacDermot KD, Holmes A, Miners AH. Anderson-Fabry disease: clinical manifestations and impact of disease in a cohort of 60 obligate carrier females. *J Med Genet*. 2001b; 38:769–75. [PubMed: 11732485]
- Mallett V, et al. GBA p.T369M substitution in Parkinson disease: Polymorphism or association? A meta-analysis. *Neurol Genet*. 2016; 2:e104. [PubMed: 27648471]
- Marder K, et al. Accuracy of family history data on Parkinson's disease. *Neurology*. 2003; 61:18–23. [PubMed: 12847150]
- Moors T, et al. Lysosomal Dysfunction and alpha-Synuclein Aggregation in Parkinson's Disease: Diagnostic Links. *Mov Disord*. 2016; 31:791–801. [PubMed: 26923732]
- Murphy KE, et al. Reduced glucocerebrosidase is associated with increased alpha-synuclein in sporadic Parkinson's disease. *Brain*. 2014; 137:834–48. [PubMed: 24477431]
- Nasreddine ZS, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc*. 2005; 53:695–9. [PubMed: 15817019]
- Nelson MP, et al. Autophagy-lysosome pathway associated neuropathology and axonal degeneration in the brains of alpha-galactosidase A-deficient mice. *Acta Neuropathol Commun*. 2014; 2:20. [PubMed: 24529306]
- Nelson MP, et al. The lysosomal enzyme alpha-Galactosidase A is deficient in Parkinson's disease brain in association with the pathologic accumulation of alpha-synuclein. *Neurobiol Dis*. 2018; 110:68–81. [PubMed: 29196214]
- Olivova P, et al. An improved high-throughput dried blood spot screening method for Gaucher disease. *Clin Chim Acta*. 2008; 398:163–4. [PubMed: 18812173]
- Orimo S, et al. An autopsied case of Fabry's disease presenting with parkinsonism and cardiomegaly as a cardinal clinical manifestation. *Rinsho Shinkeigaku*. 1994; 34:1003–7. [PubMed: 7834942]

- Parnetti L, et al. Cerebrospinal fluid lysosomal enzymes and alpha-synuclein in Parkinson's disease. *Mov Disord.* 2014; 29:1019–27. [PubMed: 24436092]
- Parnetti L, et al. Cerebrospinal fluid beta-glucocerebrosidase activity is reduced in parkinson's disease patients. *Mov Disord.* 2017
- Pchelina S, et al. Oligomeric alpha-synuclein and glucocerebrosidase activity levels in GBA-associated Parkinson's disease. *Neurosci Lett.* 2017; 636:70–76. [PubMed: 27780739]
- Reuser AJ, et al. The use of dried blood spot samples in the diagnosis of lysosomal storage disorders—current status and perspectives. *Mol Genet Metab.* 2011; 104:144–8. [PubMed: 21831684]
- Sakanaka K, et al. Knowledge of and interest in genetic results among Parkinson disease patients and caregivers. *J Genet Couns.* 2014; 23:114–20. [PubMed: 23748874]
- Sidransky E, Lopez G. The link between the GBA gene and parkinsonism. *Lancet Neurology.* 2012; 11:986–998. [PubMed: 23079555]
- Stenson PD, et al. The Human Gene Mutation Database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. *Hum Genet.* 2017; 136:665–677. [PubMed: 28349240]
- van Dijk KD, et al. Changes in endolysosomal enzyme activities in cerebrospinal fluid of patients with Parkinson's disease. *Mov Disord.* 2013; 28:747–54. [PubMed: 23712522]
- Webb JL, et al. alpha-synuclein is degraded by both autophagy and the proteasome. *Journal of Biological Chemistry.* 2003; 278:25009–25013. [PubMed: 12719433]
- Wise AH, et al. Parkinson's disease prevalence in Fabry disease: A survey study. *Mol Genet Metab Rep.* 2018; 14:27–30. [PubMed: 29159076]
- Wu G, et al. Decreased activities of lysosomal acid alpha-D-galactosidase A in the leukocytes of sporadic Parkinson's disease. *J Neurol Sci.* 2008; 271:168–73. [PubMed: 18495164]
- Zhang XK, et al. Multiplex enzyme assay screening of dried blood spots for lysosomal storage disorders by using tandem mass spectrometry. *Clinical Chemistry.* 2008; 54:1725–1728. [PubMed: 18719200]

Figure 1.

Hydrolytic enzymes tested in this study participate in sphingolipid metabolism in the lysosome as indicated by their respective gene names (blue). Homozygous or compound heterozygous mutations in these genes are known to cause lysosomal storage disorders (LSD, red) due to a deficiency in enzymatic activity. A validated multiplex assay for screening the activity of these enzymes was developed in Genzyme and included the four enzymes involved in the sphingolipid pathway as outlined: acid sphingomyelinase (ASM), glucocerebrosidase (GCase), acid alpha galactosidase (GLA) and galactocerebrosidase (GALC). The figure also depicts beta-galactosidase (GLB1). An additional lysosomal hydrolase acid alpha-glucosidase (GAA), deficient in Pompe disease, was included as part of the validated multiplex assay.

Table 1

Demographics and lysosomal enzymatic activity in Parkinson's disease cases and controls

	Parkinson's cases (n=648)	Controls (n=317)	p-value
Mean age in years (SD)	65.8 (10.6)	65.0 (9.6)	0.287
Percent male (n)	65.6% (425)	34.1% (108)	<0.001
<i>LRRK2</i> G2019S carriers (n) ¹	6.5% (42)	0.6% (2)	<0.001
<i>GBA</i> mutation/variant carriers (n) ^{1,2}	17.0% (110)	6.0% (19)	<0.001
Education in years (SD)	16.8 (3.1)	16.8 (2.8)	0.958
UPDRS- part III (SD)	18.2 (10.5)	0.9 (1.7)	<0.001
Montreal Cognitive Assessment (SD)	25.2 (3.6)	27.0 (2.3)	<0.001
Mean acid sphingomyelinase (ASM) enzymatic activity in $\mu\text{mol/l/h}$ (SD)	4.67 (1.74)	4.64 (1.56)	0.806
Mean ASM normalized activity	1.18 (0.44)	1.19 (0.41)	0.796
Mean glucocerebrosidase (GCase) enzymatic activity in $\mu\text{mol/l/h}$ (SD)	11.34 (3.81)	11.90 (3.43)	0.026
Mean GCase normalized activity	1.05 (0.37)	1.11 (0.32)	0.007
Mean acid alpha-glucosidase (GAA) enzymatic activity in $\mu\text{mol/l/h}$ (SD)	8.59 (2.46)	8.65 (2.59)	0.719
Mean GAA normalized activity	1.25 (0.35)	1.26 (0.36)	0.477
Mean alpha galactosidase A (GLA) enzymatic activity in $\mu\text{mol/l/h}$ (SD)	2.89 (1.41)	3.10 (1.68)	0.040
Mean GLA normalized activity	1.04 (0.32)	1.10 (0.35)	0.011
Mean galactosylceramidase (GALC) enzymatic activity in $\mu\text{mol/l/h}$ (SD)	3.00 (1.45)	3.08 (1.30)	0.385
Mean GALC normalized activity	1.26 (0.55)	1.30 (0.54)	0.333

GBA: glucocerebrosidase gene. SD: standard deviation. UPDRS: Unified Parkinson's Disease Rating Scale.

¹The genotype of one PD participant was not available.

²Includes carriers of *GBA* variants and mutations previously linked to Parkinson's and 10 PD cases who were homozygotes/compound heterozygous

Table 2Correlation between enzymatic activity of lysosomal enzymes in dried blood spots¹

	Acid sphingomyelinase (ASM)	Glucocerebrosidase (GCase)	Alpha-glucosidase (GAA)	Alpha galactosidase A (GLA)	Galactosylceramidase (GALC)
Acid sphingomyelinase (ASM)	X	0.208 <0.001	0.234 <0.001	0.154 <0.001	0.036 0.318
Glucocerebrosidase (GCase)	0.208 <0.001	X	0.526 <0.001	0.532 <0.001	0.283 <0.001
Alpha glucosidase (GAA)	0.234 <0.001	0.526 <0.001	X	0.382 <0.001	0.203 <0.001
Alpha galactosidase A (GLA)	0.154 <0.001	0.532 <0.001	0.382 <0.001	X	0.212 <0.001
Galactosylceramidase (GALC)	0.036 0.275	0.283 <0.001	0.203 <0.001	0.212 <0.001	X

¹Correlation was measured using the normalized to batch enzymatic activity in non-carriers (n=764).

Table 3

The association between enzymatic activity of lysosomal enzymes in dried blood spots with PD status in non-carriers of *GBA* or *LRRK2* G2019S mutations^I

	Odds Ratio	95% CI	p-value
Sex	0.257	0.188–0.352	<0.001
Age	1.015	0.998–1.032	0.081
Acid sphingomyelinase (ASM)	0.801	0.551–1.232	NS
Glucocerebrosidase (GCase)	1.015	0.529–1.949	NS
Alpha glucosidase (GAA)	0.946	0.558–1.604	NS
Alpha galactosidase A (GLA)	0.543	0.311–0.949	0.032
Galactosylceramidase (GALC)	0.989	1.739–13.23	NS

^I Including 764 participants without *GBA* or *LRRK2* mutations and age at PD onset 40 or above; NS = not significant

Table 4Lysosomal enzymatic activity in dried blood spots in *LRKK2* G2019S carriers and non-carriers with PD

	<i>LRKK2</i> G2019S non carriers (n=500)	<i>LRKK2</i> G2019S carriers (n=37)	p-value
Mean age in years (SD)	65.7 (10.9)	69.4 (10.5)	0.049
Percent male (n)	67.0% (335)	45.9% (17)	0.012
Mean acid sphingomyelinase enzymatic (ASM) activity in $\mu\text{mol/l/h}$ (SD)	4.63 (1.63)	5.60 (2.78)	0.001
Mean acid sphingomyelinase normalized activity	1.17 (0.40)	1.42 (0.76)	0.001
Mean glucocerebrosidase enzymatic (GCase) activity in $\mu\text{mol/l/h}$ (SD)	11.97 (3.22)	13.78 (4.88)	0.002
Mean glucocerebrosidase normalized activity	1.11 (0.31)	1.29 (0.48)	0.001
Mean acid alpha-glucosidase (GAA) enzymatic activity in $\mu\text{mol/l/h}$ (SD)	8.62 (2.48)	9.08 (2.56)	0.271
Mean acid alpha-glucosidase normalized activity	1.25 (0.35)	1.30 (0.36)	0.459
Mean alpha galactosidase A (GLA) enzymatic activity in $\mu\text{mol/l/h}$ (SD)	2.89 (1.48)	3.23 (1.62)	0.180
Mean alpha galactosidase A normalized activity	1.03 (0.32)	1.15 (0.33)	0.032
Mean galactosylceramidase (GALC) enzymatic activity in $\mu\text{mol/l/h}$ (SD)	3.00 (1.49)	3.43 (1.50)	0.092
Mean galactosylceramidase normalized activity	1.25 (0.56)	1.39 (0.54)	0.143