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 μmol/l/h versus 3.12 μmol/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 μmol/l/h versus 3.10 μmol/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 μmol/l/h versus 3.10 μmol/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 and 0.532). In a regression model with all 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.
1. 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 (Fig. 1), acid sphingomyelinase (ASM, EC 3.1.4.12, deficient in Niemann–Pick disease types A and 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).

2. Methods and participants

2.1. Participants and clinical evaluation

Participants in the Spot study included PD patients and 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.

2.2. 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)-carboxylic acid tert-butyil ester and 6.67 μM of (6-d5-benzoylamino-hexyl)-(2-[4-hydroxy-phenylcarbamoyl]-ethyl)-carboxylic acid tert-butyil 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)-carboxylic acid tert-butyil ester and 6.67 μM of (7-d5-benzoylamino-heptyl)-(2-[4-hydroxy-phenylcarbamoyl]-ethyl)-carboxylic acid tert-butyil ester in 0.30/0.15 M citrate-phosphate buffer with 10 g/l CHAPS hydrate and 13.3 μM acarbose, pH 4.0. The GALC final S/IS mixture contained 1 mM of C8-galactosylceramide and 6.67 μM of C10-ceramide in 0.09/0.18 M citrate-phosphate with 9.6 g/l sodium taurocholate and 1.2 mM of oleic 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 was included as part of the validated multiplex assay. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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1 PD = 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.
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