Inter- and intra-day variability in β-glucocerebrosidase activity and pathway biomarkers in healthy volunteers and patients with Parkinson's disease with a *GBA1* mutation

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Background

Heterozygous mutations in the *GBA1* gene, which result in reduced β-glucocerebrosidase (GCase) activity, are a major risk factor for Parkinson's disease (PD). Decreased GCase activity is associated with impaired lysosomal function and alpha synuclein aggregation. A challenge for therapies targeting GCase is the measurement of target and pathway engagement. In this study we used a novel approach to measure lysosomal GCase assay to determine the suitability of this approach for target engagement with a GCase targeted therapeutic and assessed plasma glucosyl β-sphingosine (GluSphing) as a potential pathway engagement marker.

Objectives

1. Evaluate a novel approach to assess lysosomal GCase activity in whole blood samples from healthy volunteers (HVs) and PD patients with GBA mutation (GBA-PD), including inter- and intra-day variability
2. Determine plasma GluSphing levels in HVs and GBA-PD patients, including inter- and intra-day variability

Methods

- Whole blood was obtained from 8 HVs and 12 GBA-PD patients at 3 time points (Day 1, 0 and 4h, and Day 8, time matched to 0h).
- Lysosomal GCase activity was assessed using the GCase substrate PFB-FDGlu in whole blood, followed by flow cytometry analysis to assess activity in monocytes (Figure 1).
- A mass spectrometry assay was developed and qualified to enable quantification of plasma Glucosyl- and Galactosyl-Sphingosine.

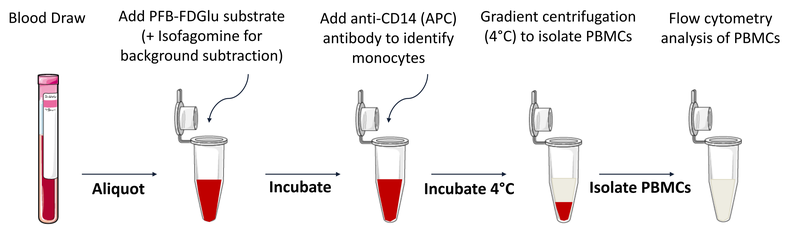

Figure 1. Overview of the approach to directly assess lysosomal GCase activity in monocytes from whole blood samples

Results

Participant Information

	HVs	GBA-PD patients
Number	8	12
Age (y)	71.1 (2.6)	67.3 (6.6)
BMI (kg/m²)	25.9 (2.1)	24.9 (3.5)
Gender, male (%)	88%	42%
Hoehn and Yahr	-	2.2 (0.9)

Table 1. Demographics

Mutation	Count (%)
E326K	3 (25%)
E326K/D140H	3 (25%)
T369M	3 (25%)
L444P	1 (8.3%)
R120W	1 (8.3%)
H490R	1 (8.3%)

Table 2. *GBA1* mutations

Lysosomal GCase activity is reduced in GBA-PD relative to HVs

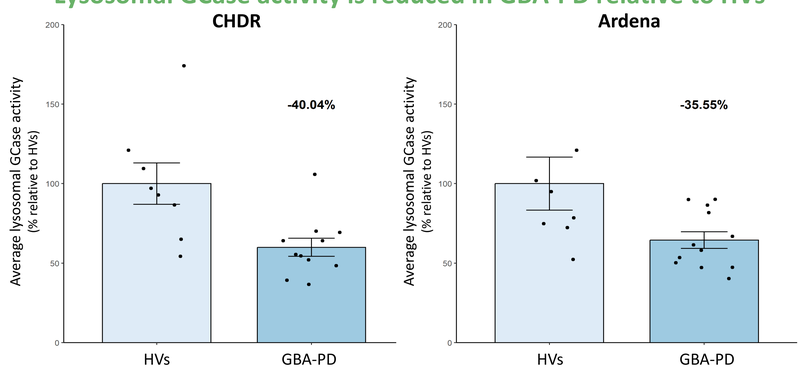

Figure 2. Lysosomal GCase activity in GBA-PD patients is significantly reduced in matched blood samples analysed at two independent labs. Data is presented as the percent GCase activity relative to HV average, ± SEM, p<0.05.

GCase activity is consistently reduced in GBA-PD over time

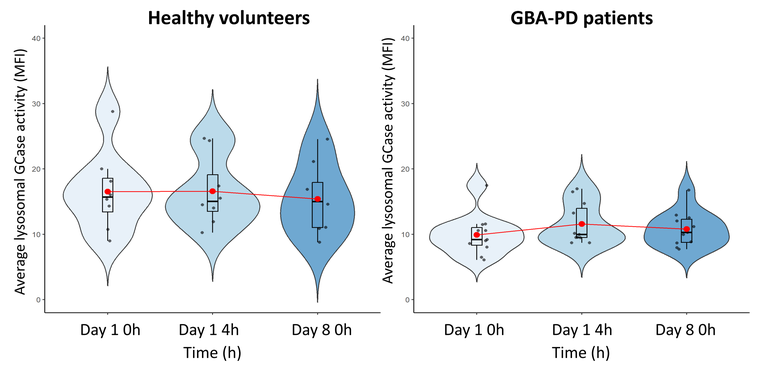

Figure 3. Average lysosomal GCase activity measured in blood samples on Day 1 (0 and 4h) and Day 8 (0 h) was 33% lower across all time points in GBA-PD compared to HVs with minimal inter- and intra-day variability (p=0.004).

Plasma Glucosylsphingosine is elevated in GBA-PD patients

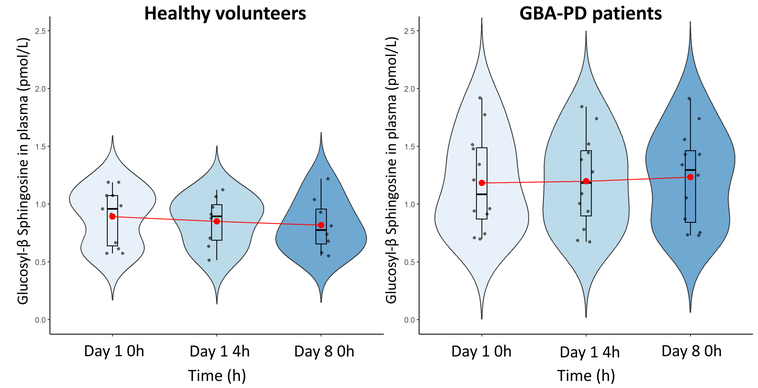

Figure 4. Plasma GluSphing concentrations in HVs and GBA-PD patients on Day 1, (0 and 4h), and Day 8, (time matched to 0h). GluSphing is 42% higher in GBA-PD but not statistically significant relative to HVs (0.31 pmol/mL (95% CI: -0.014, 0.64), p=0.06).

	GCase activity		Glucosyl-β Sphingosine	
Group	**HV**	**GBA-PD**	**HV**	**GBA-PD**
Inter-day (%CV)	24.0	37.1	2.4	1.7
Intra-day (%CV)	16.8	25.9	0.0	0.0

Table 3. Variability (%CV) in GCase activity and Glucosyl-β sphingosine in HVs and GBA-PD

Conclusion

- Using a novel lysosomal GCase activity assay, we observe biochemically relevant reductions in GCase activity in whole blood samples from GBA-PD patients relative to HVs with minimal inter- and intra-day variability.
- Comparable GCase activity data was generated by two independent labs demonstrating that this approach is transferrable and reproducible.
- Plasma glucosylsphingosine was increased in GBA-PD but was not statistically significant in this small cohort.
- These results support the use of this assay to assess target engagement of GCase therapeutics in early clinical development.
- A further optimized and validated version of this assay is currently being used to assess target engagement of an allosteric GCase activator in Phase 1 clinical studies.

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