Elevation of gangliosides in four brain regions from Parkinson’s disease patients with a GBA mutation

Shani Blumenreich1, Tamar Nehushtan1, Or B. Barav1, Jennifer T. Saville2, Tamir Dingjan3, John Hardy3, Maria Fuller2 and Anthony H. Futerman2,4✉

A number of genetic risk factors have been identified over the past decade for Parkinson’s Disease (PD), with variants in GBA prominent among them. GBA encodes the lysosomal enzyme that degrades the glycosphingolipid, glucosylceramide (GlcCer), with the activity of this enzyme defective in Gaucher disease. Based on the ill-defined relationship between glycosphingolipid metabolism and PD, we now analyze levels of various lipids by liquid chromatography/electrospray ionization-tandem mass spectrometry in four brain regions from age- and sex-matched patient samples, including idiopathic PD, PD patients with a GBA mutation and compare both to control brains (n = 21 for each group) obtained from individuals who died from a cause unrelated to PD. Of all the glycerolipids, sterols, and (glyco)sphingolipids (251 lipids in total), the only lipid class which showed significant differences were the gangliosides (sialic acid-containing complex glycosphingolipids), which were elevated in 3 of the 4 PD-GBA brain regions. There was no clear correlation between levels of individual gangliosides and the genetic variant in Gaucher disease (9 samples of severe (neuronopathic), 4 samples of mild (non-neuronopathic) GBA variants, and 8 samples with low pathogenicity variants which have a higher risk for development of PD). Most brain regions, i.e. occipital cortex, cingulate gyrus, and striatum, did not show a statistically significant elevation of GlcCer in PD-GBA. Only one region, the middle temporal gyrus, showed a small, but significant elevation in GlcCer concentration in PD-GBA. We conclude that changes in ganglioside, but not in GlcCer levels, may contribute to the association between PD and GBA mutations.

npj Parkinson’s Disease (2022) 8:99; https://doi.org/10.1038/s41531-022-00363-2

INTRODUCTION

The genetic association between genes related to lysosomal function and Parkinson’s disease (PD) is now well established, suggesting that the lysosome may play a crucial role in PD pathogenesis. In contrast, the mechanistic and biochemical association between lysosomal function and PD is unresolved and a matter of ongoing discussion. Of the lysosomal genes, GBA is the most prominent, with 5–25% of PD patients carrying a GBA mutation. GBA encodes the lysosomal hydrolase acid β-glucosidase (GCase), the enzyme defective in the inherited metabolic disease, Gaucher disease, Gaucher disease (Gaucher disease), and its deacetylated derivative, glucosylsphingosine (GlcSph). Gaucher disease is inherited in an autosomal recessive fashion, whereas most PD patients with GBA mutations (herein referred to as PD-GBA) are heterozygous GBA carriers.

A number of studies have investigated whether the mechanistic link between GBA and PD is related to altered levels of either GlcCer or GlcSph in the brain (reviewed in ref. 9). Many of these studies measured lipid levels in the substantia nigra, where loss of dopaminergic neurons occurs, and as a consequence interpretation of changes in GlcCer and GlcSph levels might be confounded by loss of cellular material due to loss of neurons rather than due to specific alterations in levels of a particular lipid class or species. To date, there is little evidence that GlcCer or GlcSph accumulate in brain samples from human PD or PD-GBA patients, although some Lewy body disease patients with a GBA mutation show small but non-significant changes in GlcCer levels. There is no evidence for GlcCer accumulation in the brains of heterozygous individuals with a GBA mutation, although there is a suggestion that GlcSph, but not GlcCer levels are elevated in the plasma of GBA carriers, while a different analysis reported normal plasma GlcSph levels in GBA carriers.

Despite the lack of evidence that the two direct substrates of GCase accumulate in GBA carriers or in PD-GBA patients, no systematic studies have been performed to determine whether levels of other GSLs in the same metabolic pathway might be altered, or whether levels of other brain lipids change in PD-GBA. This being the case, we have now systematically analyzed levels of the three major lipid classes found in the brain [i.e. glycerolipids, sphingolipids (SLs), and sterols] in a cohort of patient brain samples, which includes 21 idiopathic PD patients (IPD), 21 PD-GBA patients and 21 age and sex-matched controls. Levels of 251 individual lipid species were analyzed by liquid chromatography/electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). Rather than focusing on the substantia nigra, we used 4 other brain regions, including the striatum (STR), which is part of the nigrostriatal pathway and thus directly impacted by dopamine loss from the substantia nigra, while the three other regions, the occipital cortex (OCC), middle temporal gyrus (MTG) and cingulate gyrus (CG) mainly receive dopaminergic input from the ventral tegmental area and are thus not directly affected by dopamine loss in the nigrostriatal pathway.

Changes in levels of lipids between the various patient groups were relatively modest. Interestingly, concentrations of gangliosides (i.e. sialic-acid containing glycosphingolipids) were elevated...
by a small but statistically significant amount in most brain areas from PD-GBA samples, in such a way that suggests upregulation of the pathway of ganglioside metabolism. Our data are consistent with our recent suggestion that cellular pathways, other than changes in GlcCer or GlcSph levels, need to be unearthed in order to delineate the mechanistic association between GBA mutations and PD, including up-stream GSLs in the catabolic pathway such as gangliosides.

**RESULTS**

**Quality analysis and brain distribution of lipid classes**

Brain samples from four different brain regions (Fig. 1) were analyzed by LC-ESI-MS/MS. 251 lipid species were measured for most samples, including 6 sterols, 150 glycerophospholipids and lysoglycerophospholipids, and 95 SLs (Fig. 2). For the SL class, 4 simple SLs were measured (sphinganine, dihydroceramide, ceramide, and sphingosine), along with sphingomyelin (SM), sulfatide and a variety of gangliosides (sialic acid-containing GSLs), including GD1a/b, GD2, GD3, GM1, GM2, and GM3. The acyl chain distribution of all lipids was measured individually and also summed for each lipid class. The average levels of each of the SLs from 3 regions from control brain samples (Fig. 2) is consistent with published data on control human brains from elderly individuals, inasmuch as SM was the major SL, and gangliosides GD1a/b were the major gangliosides.

We first determined whether the age of the patients, their gender or the type of GBA variant influenced the lipid profile in each brain region by performing principal component analysis (PCA) on all lipid classes. No separation by group according to age, gender, or GBA mutation was detected, indicating that there is no correlation between lipid levels and age, gender, or GBA mutation (Supplementary Fig. 1).

To determine the difference in the distribution of the major lipid classes between the four brain regions, and more importantly for...
To determine changes in concentrations of individual lipids, data was interrogated by ANOVA followed by post-hoc pairwise multiple comparisons using the Tukey’s honestly significant difference test (Tukey HSD). Data was plotted as a heatmap to give an overview of changes in lipid concentrations between IPD samples compared to PD-GBA and controls, and between PD-GBA and IPD. With the exception of gangliosides, no consistent and overriding pattern of change was detected between 4 brain regions or between the sample groups (Fig. 3), indicating that any changes in lipid concentrations are brain region-dependent. One of the most noticeable changes was that concentrations of some lipids were reduced in both IPD and PD-GBA samples in the OCC, with a somewhat greater reduction in IPD. More specifically, concentrations of C32-C38-phosphatidylethanolamine (PE) (Supplementary Fig. 2a), C36:1- and C37:1-SM (Supplementary Fig. 2b), lysophosphatidylcholine (LPC) (Supplementary Fig. 2c) including ether LPC (Supplementary Table 1), C18:2- and C20:3-lysophosphatidylethanolamine (LPE) (Supplementary Fig. 2d), lysophosphatidylinositol (LPI) (Supplementary Fig. 2e), phosphatidylcholine (PC) (including ether PC (Supplementary Table 1), C23:0-, C24:0- and C25:0- ceramide (Supplementary Table 1) and C35:1-, C36:1- and C37:1-sphingomyelin (SM) (Supplementary Table 1) were reduced mostly in IPD but not in PD-GBA in the OCC.

### Analysis of glycosphingolipid levels

The most consistent change, as indicated in Table 1, was in ganglioside concentrations, which were increased in a number of brain areas in PD-GBA samples, and to a lesser extent in IPD, such that in almost all cases, the ratio of ganglioside concentration was higher in PD-GBA compared to IPD (Fig. 4). This was most noticeable in the MTG, where the mean concentration of all gangliosides was 2251 ± 611 pmol/mg protein in PD-GBA compared to 1984 ± 491 in IPD and 1808 ± 579 in controls (Fig. 4b) (p < 0.05 for PD-GBA versus controls). A similar result, and with a higher degree of statistical significance, was obtained when total ganglioside concentrations were analyzed, but excluding the most abundant gangliosides, namely GD1a/b (which were found at ~10-fold higher levels than the other gangliosides) (Fig. 4b). While statistical significance was not reached for total gangliosides in all regions, the effect of the ganglioside class did not differ between control, IPD, or PD-GBA samples, with the exception of gangliosides (p < 0.001) whereas SM levels are similar between regions (p = 0.534). Gangliosides were the only lipid class to change between sample groups (F(2,66) = 7.579, p = 0.001) irrespective of brain region (F(4,68,107,63) = 0.325, p = 0.887).

### Table 1. Effect of sample group and brain region on lipid levels.

| Lipid                        | Effect                           | F (DF)                     | p value |
|------------------------------|----------------------------------|----------------------------|---------|
| GlcCer                       | Brain region                     | 36.439 (2.47, 101.29)      | <0.001  |
|                              | Group                            | 2.174 (2, 41)              | 0.127   |
|                              | Group:brain region               | 0.358 (4.94, 101.29)       | 0.874   |
| Gangliosides                 | Brain region                     | 36.54 (2.54, 107.63)       | <0.001  |
|                              | Group                            | 7.579 (2, 46)              | 0.001   |
|                              | Group:brain region               | 0.325 (4.68, 107.63)       | 0.887   |
| SM                           | Brain region                     | 0.734 (3, 132)             | 0.534   |
|                              | Group                            | 1.0 (2, 44)                | 0.213   |
|                              | Group:brain region               | 1.275 (6, 132)             | 0.273   |
| Other SLs                    | Brain region                     | 4.953 (3, 138)             | 0.003   |
|                              | Group                            | 0.886 (3, 46)              | 0.447   |
|                              | Group:brain region               | 0.829 (6, 138)             | 0.684   |
| Glycerophospholipids         | Brain region                     | 8.398 (1, 92, 88.47)       | <0.001  |
|                              | Group                            | 0.719 (2, 46)              | 0.493   |
|                              | Group:brain region               | 1.528 (3.85, 88.47)        | 0.203   |
| Glycerolysophospholipids     | Brain region                     | 5.459 (1.12, 51.57)        | <0.001  |
|                              | Group                            | 1.292 (2, 46)              | 0.222   |
|                              | Group:brain region               | 0.524 (2.24, 51.57)        | 0.339   |
| Bis(monoacylglycerol)phosphate| Brain region                     | 16.9 (3, 138)              | <0.001  |
|                              | Group                            | 1.07 (2, 46)               | 0.352   |
|                              | Group:brain region               | 0.878 (6, 138)             | 0.513   |
| Cholesteryl ester            | Brain region                     | 166.205 (2.48, 109.02)     | <0.001  |
|                              | Group                            | 0.884 (2, 44)              | 0.42    |
|                              | Group:brain region               | 0.744 (4.96, 109.02)       | 0.591   |

The effect of the brain region (Brain region) and sample group (Group), i.e. control, IPD, or PD-GBA on lipid levels, and the interaction between them (Group:brain region) was analyzed by mixed ANOVA. F statistic, degrees of freedom (DF) and p value are reported. For instance, GlcCer levels differ significantly between brain regions (F(2,47,101.29) = 36.439, p < 0.001) whereas SM levels are similar between regions (F(3,132) = 0.734, p = 0.534). Gangliosides were the only lipid class to change between sample groups (F(2,66) = 7.579, p = 0.001) irrespective of brain region (F(4,68,107,63) = 0.325, p = 0.887).
DISCUSSION

The most notable finding of the current study is that the ganglioside concentrations were increased in most of the brain areas studied, in particular in PD-GBA, in a manner that is consistent with upregulation of the pathway of ganglioside metabolism rather than increases in levels of individual ganglioside species. In principle, changes in levels of gangliosides could come from either changes in their rate of biosynthesis, or from changes in their rate of degradation, although we cannot formally distinguish between these two possibilities. There were no significant differences in most of the other lipids measured in the four human brain regions between samples from control, PD, and PD-GBA patients, and moreover, concentrations of the lipid substrate of GCase were essentially unchanged, consistent with most studies.

One possible criticism of our study is that we did not analyze lipids in the main tissue affected in PD, namely the substantia nigra. The reason for this was two-fold. First, little tissue was available from the substantia nigra due to its obliterance during the progression of PD. Second, analysis of lipid concentrations in the substantia nigra would likely reflect tissue loss rather than loss...
of specific lipid species. Thus, we chose tissues that are less obviously affected by neuronal loss (with the exception of the STR, which is directly affected by axonal loss from the substantia nigra), but are nevertheless impacted by PD pathology, such as the OCC, MTG, and CG, which develop α-synuclein pathology at different stages of the disease and are involved in the manifestation of non-motor symptoms.

There are very few studies, if any, with which to compare our data, although some recent studies did measure gangliosides in the brain of PD patients, and demonstrated that ganglioside levels were lower in the substantia nigra of IPD patients compared to controls. These differences may be explained by the fact that both studies used a different brain region than used in our study. Since we have shown that lipid distribution differs between brain regions (Table 1), it is not unreasonable to suggest that the SN also has a different lipid composition and, therefore would show a different lipid pattern than detected in our study, which did not include the SN. In addition, since gangliosides are highly expressed in neurons, reduction of gangliosides in the SN could conceivably be explained by the neuronal death in this region. Furthermore, differences may also be attributed to the different methodologies used in the studies, whereby we used LC-ESI-MS/MS and not High-Performance Thin-Layer Chromatography or high-performance liquid chromatography.

In another study, GM1 and GD1a ganglioside levels were reduced in gray matter from the OCC of IPD patients, although this study used a relatively small sample size. Levels of C18-GM2 and GM3 were not affected in the putamen or cerebellum in IPD or PD-GBA, although a non-statistical trend of elevation was seen in the putamen of PD-GBA patients. GM3 levels were elevated in the motor cortex of patients with Lewy body disorders carrying a GBA mutation, but not in patients with Lewy body disorders which did not carry a GBA mutation. Finally, levels of several gangliosides were reduced in the cerebrospinal fluid of IPD samples with the exception of GA2 and GM1a, which were unchanged, and of ganglioside GM3 which was elevated. The latter is important, since GM3 is not normally present in the mature brain, and elevation of GM3 levels might be related to a survival mechanism based on the key role that GM3 plays in neurodevelopment. Elevated GM3 in the cerebrospinal fluid may also serve as a biomarker for disease severity. Elevation of brain gangliosides have also been reported in other neurodegenerative conditions.
Fig. 5  Individual ganglioside concentrations in IPD and PD-GBA brain. Boxplots of total ganglioside concentrations (pmol/mg protein) expressed according to the order of their lysosomal degradation. The box represents lower quartile, median, and upper quartile (black). The whiskers represent the minimum and maximum values, up to 1.5 times the interquartile range from the bottom or the top of the box to the furthest data point within that distance, thus excluding outliers. The mean is in red. *p ≤ 0.1; *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001.
Fig. 6  GlcCer concentrations in IPD and PD-GBA brain. Boxplots of concentrations of individual GlcCer species (pmol/mg protein) along with the sum of all species (Total). The box represents lower quartile, median and upper quartile (black). The whiskers represent the minimum and maximum values, up to 1.5 times the interquartile range from the bottom or the top of the box to the furthest data point within that distance, thus excluding outliers. No outliers were removed from the data, including sample PG6 which had concentrations of C18-GlcCer ~10-fold higher than most other samples in the striatum of PD-GBA; there is no experimental justification for removing this sample from the analysis although it is assumed to be due to an unidentified technical issue since concentrations of other lipids were not abnormal for PG6 in other brain regions. The mean is in red. *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001.
Fig. 7 Correlation between GlcCer, gangliosides, and ceramide. Matrices were generated using combined data from all four brain regions and all sample groups (i.e. control, IPD, and PD-GBA). Pearson correlation matrices of a GlcCer versus ceramide, b ceramide versus SM, and c GlcCer, and DHC versus total gangliosides. Correlation coefficient values are indicated. Axes represent log2 scale of lipid concentrations.

Fig. 8 Correlations between GlcCer and ganglioside concentrations and the GBA variant. a GlcCer (upper panel) and total ganglioside (lower panel) concentrations shown according to the different GBA mutations [severe mutations, i.e. G232E, R131C, L444P, R463C, RecA456P, RecNciI (red), mild mutations, i.e. N370S (orange) and risk factors E326K, T369M (blue)]. Each point indicates GlcCer or ganglioside concentrations in an individual patient. b Boxplots of total GlcCer (upper panel) and total ganglioside concentrations (lower panel) in the PD-GBA group according to mutation severity. The box represents lower quartile, median, and upper quartile (black). The whiskers represent the minimum and maximum values, up to 1.5 times the interquartile range from the bottom or the top of the box to the furthest data point within that distance, thus excluding outliers. The mean is in red. No statistical significance was reached using the t-test.
such as Alzheimer’s disease, and in several lysosomal storage diseases (reviewed in ref. 37).

It is difficult to compare studies from different tissues and different patient cohorts, but our systematic study suggests that, with one or two exceptions, ganglioside concentrations are elevated to a small extent in the IPD group, but elevated to a larger and statistically significant extent in the PD-GBA group, suggesting that gangliosides may play a specific role in the pathophysiology of PD-GBA. This is perhaps not surprising since gangliosides are enriched in the brain, and specifically in neurons. The precise role of gangliosides has proven difficult to define, but in general, they are involved in processes ranging from modulation of membrane properties to modulation of apoptosis, autophagy, and neurite outgrowth. In addition, ganglioside GM1 may have neurotrophic and neuroprotective properties, and modulation of GM1 levels was suggested as a treatment strategy for PD, although this approach has not been successful. Moreover, the addition of exosomes containing gangliosides may accelerate α-synuclein aggregation.

The latter may suggest that the long-term elevation of ganglioside levels in PD-GBA reported in our study may exacerbate α-synuclein pathology and/or cell death-related alterations. In gangliosides, rather than other lipids in lysosomal overload, is difficult to pin down. Interestingly, gangliosides, specifically GM1 and GM3, have been implied in amyloid pathology in Alzheimer’s disease.

Although a number of attempts have been made to measure whether GlcCer levels change in PD-GBA, most have reported little or no change (reviewed in ref. 38), consistent with our current data. In retrospect, this is perhaps not surprising since there is no evidence that GBA carriers (i.e. heterozygotes) display Gaucher disease symptoms which would reflect changes in GlcCer levels, and there is, therefore, no reason to assume that PD patients with a heterozygous GBA mutation would present with elevated GlcCer levels. We appreciate that our sample size was perhaps not large enough to detect a very small change in GlcCer levels in PD-GBA, and that in some cases a non-significant trend towards an increase was observed. However, even if increasing the sample size were to lead to a statistically-significant increase in GlcCer in PD-GBA patients, the question remains as to whether this change would be able to influence cellular pathology. Indeed, in Gaucher disease, GlcCer concentrations increase by at least ~10-fold, significantly higher than the small changes occasionally observed in our study. Moreover, basal GlcCer concentrations are in the region of ~20 pmol/mg protein whereas ganglioside concentrations are ~10-fold higher (2000–5000 pmol/mg protein), with the latter increasing by ~400 pmol/mg protein in PD-GBA versus control samples. One limitation of our study is that we did not measure GlcSph, which accumulates as a result of GBA mutations in Gaucher disease. However, since GlcCer accumulates to a larger extent than GlcSph in Gaucher disease, changes in GlcSph levels in PD-GBA are unlikely to be significant.

In summary, our data reveal a change in ganglioside levels in non-dopaminergic regions of the human brain in PD-GBA, and very few changes in other lipid classes, including GlcCer. This clearly has implications for therapeutic approaches that might target the GCase/GlcCer/GlcSph axis. It may, however, suggest that one or other aspect of ganglioside metabolism might be a suitable therapeutic target for PD-GBA patients.

**METHODS**

**Human brain samples**

Human brain samples were obtained as frozen tissue from the Queens Square Brain Bank of London, and include 21 idiopathic PD patients without a GBA mutation (IPD), 21 PD-GBA patients and 21 controls, from four different brain regions (OCC, MTG, CG, and STR). The CG region contained only 52 samples (15 controls, 18 IPD, and 19 PD-GBA), and the STR region contained 60 samples (19 controls, 21 IPD and 20 PD-GBA), for a total of 238 samples. The groups in this study were age- and sex-matched.

GBA was sequenced by the Queens Square Brain Bank of London and GBA mutations for each of the PD-GBA patients are documented (Table 2). The study was approved by the National Research Ethics Service (NRES) committee, London (127366). All participants gave written informed consent.

**Quantification of lipids by mass spectrometry**

Lipid extracts were prepared from frozen tissue samples of the CG, MTG, OCC and STR as described with addition of 10 pmol each of bis(monomonyristoylglycerophosphate) (BNP(14:0/14:0)), 1-myristoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine (PE(17:0/17:0)), and 1,2-dihexadecanoyl-sn-glycero-3-phospho-L-serine (PS(17:0/17:0)); 1 pmol each of D-erythro-sphinganine (C17 base) (Sphid17:0) and 1,2-dihexadecanoyl-sn-glycero-3-phospho-rac-1-glycerol (PG(17:0/17:0)); 5 pmol 1,2-ditetradecanoyl-sn-glycero-3-phosphocholine (PC(13:0/13:0)); 100 pmol of cholesteryl-2,2,3,4,4,6-d6 octadecanoate (CE 18:0 d6) and 20 pmol of 1,3-dipentadecanoyl-DG(15:0/15:0)) as internal standards. With the exception of CE 18:0 d6 (CDN Isotopes, Quebec, Canada) and DG(15:0/15:0) (Sigma Aldrich, St. Louis, MO), all lipid standards were purchased from Avanti Polar Lipids (Alabaster, AL). Lipid extracts were analysed by liquid chromatography electrospray ionisation tandem mass spectrometry (LC-ESI-MS/MS) with gangliosides quantified as in ref. 45, with the GD1a and 1b isoforms indistinguishable and therefore measured together, the remaining SLs were quantified as described in ref. 34. Total lipids for each class were determined by summing the concentrations of the individual species (see Supplementary Table 1 for the raw Lipidomics data).

For quantitation of GlcCer and its stereoisomer, galactosylsphingosine (GalCer), Agilent Bond Elut Lipid Extraction 96-well plates (Agilent Technologies, Santa Clara, CA) were utilised with lipid extraction. Brain homogenates (0.1 mg protein) were prepared in up to 0.1 mL of 0.9% saline. Samples were spiked with 10 pmol each of N-palmitoyl-D3-glucocerebroside (GlcCer(d18:1/16:0 d3)) and N-pentadecanoyl-psychosine (GalCer(d18:1/15:0)) (Matreya LLC, State College, PA) as internal standards. Protein was precipitated by the addition of 0.9 mL of ice-cold CH3CN:CH3OH (99:1). Samples were vortexed for 30 s and sonicated in a water bath for 14 min at room temperature, and then briefly vortexed again and transferred into a well of the extraction plate and eluted by gravity. This was followed by the addition of 2×1 mL CH3CN:CH3OH (9:1). Sorbent beds were dried under low vacuum pressure prior to elution of lipids with 2×1 mL CH3CN:CH3OH (1:1). The eluent was dried under N2 at 40 °C. Prior to LC-ESI-MS/MS, samples were reconstituted in 0.2 mL of the mobile phase, transferred to a v-bottom 96-well plate and heat-sealed with foil.

Separation of GlcCer and GalCer was achieved on a Phenomenex Omega SUGAR column (3 mm; 150 × 2.1 mm) maintained at 30 °C with an Agilent 1290 inline filter containing a 0.3 mm frit placed in front of the column. The sample (4 µL) was inject into an isotropic mobile phase of CH3CN:H2O (95:5) and 5 mM NH4OH, maintained at a flow rate of 0.7 mL/min. This was directed into the ESI source (ES 5500 V; ion source temperature 250 °C) of a SCIEX QTRAP 6500 tandem mass spectrometer in positive ion mode. Nitrogen (25 units) was used for the curtain gas; collision gas, medium; nebulizer gas 1, 20 units; auxillary gas 2, 40 units. The runtime for each sample was 5 min, and species were identified by MRM with GlcCer eluting approximately 0.1 min prior to GalCer. Authentic GlcCer(d18:1/16:0) and GalCer(d18:1/16:0) standards together with archived brain lipid extracts from a neuropaenic mouse model of Gaucher disease were used to aid in analyte identification. Individual lipids were quantified by relating the peak area to that of the corresponding internal standard using MultiQuant software (SCIEX, v. 3.0.1).

**Statistical analysis**

Seven datasets, comprising each lipid class, were analyzed using R version 3.6.1. Principal Component Analysis (PCA) was used to analyze the influence of various factors on the data (age, gender, and GBA mutation). For statistical analyses, data was transformed to a log2 scale (zero values were replaced with 0.001 and non-available (NA) data were removed). The mixed analysis of variance (mixed ANOVA), ‘rstatix’ package, was used to compare different lipid groups versus brain regions and samples.
### Table 2. Samples used in the study.

| Sample ID | Group | Gender | Age at death | GBA mutation |
|-----------|-------|--------|--------------|--------------|
| C1        | Control | M    | 81           |              |
| C2        | Control | M    | 95           |              |
| C3        | Control | M    | 87           |              |
| C4        | Control | M    | 63           |              |
| C5        | Control | F    | 64           |              |
| C6        | Control | F    | 53           |              |
| C7        | Control | F    | 85           |              |
| C8        | Control | F    | 56           |              |
| C9        | Control | F    | 86           |              |
| C10       | Control | F    | 68           |              |
| C11       | Control | M    | 43           |              |
| C12       | Control | M    | 71           |              |
| C13       | Control | F    | 87           |              |
| C14       | Control | F    | 83           |              |
| C15       | Control | F    | 80           |              |
| C16       | Control | M    | 89           |              |
| C17       | Control | F    | 92           |              |
| C18       | Control | M    | 87           |              |
| C19       | Control | F    | 78           |              |
| C20       | Control | M    | 88           |              |
| C21       | Control | M    | 84           |              |
| PD1       | IPD   | F    | 55           |              |
| PD2       | IPD   | M    | 67           |              |
| PD3       | IPD   | M    | 66           |              |
| PD4       | IPD   | F    | 61           |              |
| PD5       | IPD   | M    | 91           |              |
| PD6       | IPD   | M    | 85           |              |
| PD7       | IPD   | M    | 65           |              |
| PD8       | IPD   | M    | 68           |              |
| PD9       | IPD   | M    | 75           |              |
| PD10      | IPD   | M    | 63           |              |
| PD11      | IPD   | M    | 59           |              |
| PD12      | IPD   | M    | 81           |              |
| PD13      | IPD   | M    | 81           |              |
| PD14      | IPD   | M    | 81           |              |
| PD15      | IPD   | M    | 92           |              |
| PD16      | IPD   | M    | 82           |              |
| PD17      | IPD   | F    | 83           |              |
| PD18      | IPD   | M    | 80           |              |
| PD19      | IPD   | M    | 79           |              |
| PD20      | IPD   | F    | 73           |              |
| PD21      | IPD   | M    | 83           |              |

### Table 2 continued

| Sample ID | Group | Gender | Age at death | GBA mutation |
|-----------|-------|--------|--------------|--------------|
| PG1       | PD-GBA | M    | 62           | G232E         |
| PG2       | PD-GBA | M    | 55           | R131C         |
| PG3       | PD-GBA | F    | 67           | L444P         |
| PG4       | PD-GBA | M    | 91           | N370S         |
| PG5       | PD-GBA | M    | 85           | N370S         |
| PG6       | PD-GBA | M    | 65           | L444P         |
| PG7       | PD-GBA | M    | 68           | R463C         |
| PG8       | PD-GBA | M    | 57           | RecA456P      |
| PG9       | PD-GBA | F    | 64           | RecNci       |
| PG10      | PD-GBA | M    | 59           | L444P         |
| PG11      | PD-GBA | F    | 57           | L444P         |

Age, gender (M, male; F, female), and GBA mutations are shown. Tissues from 4 brain regions were available for most of the samples.

Heatmaps were created using the 'ComplexHeatmap' package. Statistical significance was evaluated by ANOVA, followed by post-hoc pairwise comparisons using the Tukey's honest significant difference test (Tukey HSD). Boxplots were created using the ‘ggplot2’ package. Pearson Correlation matrices were created using the ‘GGally’ package.

### DATA AVAILABILITY

The authors declare that all data supporting the findings of this study are available within the paper and the supplementary information files.

Received: 6 February 2022; Accepted: 11 July 2022; Published online: 06 August 2022

### REFERENCES

1. Robak, L. A. et al. Excessive burden of lysosomal storage disorder gene variants in Parkinson’s disease. Brain 140, 3191–3203 (2017).
2. Blumenreich, S., Barav, O. B., Jenkins, B. J. & Futerman, A. H. Lysosomal storage disorders shed light on lysosomal dysfunction in Parkinson’s disease. Int. J. Mol. Sci. 21, 1–12 (2020).
3. Balestrino, R. & Schapira, A. H. V. Parkinson disease. Eur. J. Neurol. 27, 27–42 (2020).
4. Futerman, A. H. & Hardy, J. Finding common ground. Nature 537, 6–7 (2016).
5. Balestrino, R. & Schapira, A. H. V. Glucocerebrosidase and Parkinson disease: Molecular, clinical, and therapeutic implications. Neuroscientist 24, 540–559 (2018).
6. Futerman, A. H. & Zimran, A. Gaucher Disease (CRC Press, 2006).
7. Hruska, K. S., LaMarca, M. E., Scott, C. R. & Sidransky, E. Gaucher disease. J. Neurochem. 718, 567–583 (2008).
8. Nilsson, O. & Svennerholm, L. Accumulation of glucosylceramide and glucosylsphingosine (Psychosine) in cerebrum and cerebellum in infantile and juvenile gaucher disease. J. Neurochem. 39, 709–718 (1982).
9. Milenkovic, I., Blumenreich, S. & Futerman, A. H. GBA mutations, glucosylceramide, and Parkinson’s disease. Curr. Opin. Neurobiol. 24, 148–154 (2022).
10. Wallom, J. S. & Sampson, E. L. Lipid composition of the normal human brain: Gray matter, white matter, and myelin. J. Lipid Res. 54, 537–544 (1965).
16. Tettamanti, G., Bonali, F., Marchesini, S. & Zambotti, V. A new procedure for the extraction, purification and fractionation of brain gangliosides. Biochim. Biophys. Acta 296, 160–170 (1973).

17. Chavko, M., Nemoto, E. M. & Melick, J. A. Regional lipid composition in the rat brain. Mol. Cell. Neurochem. 18, 123–131 (1993).

18. Fitzner, D. et al. Cell-type- and brain-region-resolved mouse brain lipidome. Cell Rep. 32, 108132 (2020).

19. Futterman, A. H. Biochemistry of Lipids, Lipoproteins, and Membranes (eds Ridgway, N. D. & McLeod, R. S.) 281–316 (Elsevier Science, 2021).

20. Beutler, E., Gelbart, T. & Scott, C. R. Hematologically important mutations: Gaucher disease. Blood Cells, Mol. Dis. 35, 355–364 (2005).

21. Lesage, S. et al. Large-scale screening of the Gaucher’s disease-related glucocerebrosidase gene in Europeans with Parkinson’s disease. Hum. Mol. Genet. 20, 202–210 (2011).

22. Ben Bidra, F. et al. Stabilization of glucocerebrosidase by active site occupancy. ACS Chem. Biol. 12, 1830–1841 (2017).

23. Sipione, S., Monyor, J., Galleguillos, D., Steinberg, N. & Kadam, V. Gangliosides in the brain: Physiology, pathophysiology, and therapeutic applications. Front. Neurosci. 14, 1–24 (2020).

24. Braak, H. et al. Staging of brain pathology related to sporadic Parkinson’s disease. Neurobiol. Aging 24, 197–211 (2003).

25. de Schipper, L. J. et al. Altered whole-brain and network-based functional connectivity in Parkinson’s disease. Front. Neuro. 9, 1–10 (2018).

26. Huebecker, M. et al. Reduced sphingolipid hydrolase activities, substrate accumulation and ganglioside decline in Parkinson’s disease. Mol. Neurodegener. 14, 1–21 (2019).

27. Seyfried, T. N. et al. Sex-related abnormalities in substantia nigra lipids in Parkinson’s disease. ASN Neuro 10, 1759091418781889 (2018).

28. Hadaczek, P. et al. GDNF signaling implemented by GM1 ganglioside; failure in Parkinson’s disease and GM1-deficient murine model. Exp. Neurol. 263, 177–189 (2015).

29. Gegg, M. E. et al. No evidence for substrate accumulation in Parkinson brains with GBA mutations. Mov. Disord. 30, 1085–1089 (2015).

30. Palmano, K., Rowan, A., Guillermo, R., Guan, J. & McJarrow, P. The role of gangliosides in neurodevelopment. Nutrients 7, 3891–3913 (2015).

31. Saville, J. T., Flanagan, K. M., Truxal, K. V., McBride, K. L. & Fuller, M. Evaluation of biomarkers for Sanfilippo syndrome. Mol. Genet. Metab. 128, 68–74 (2019).

32. Ariga, T. et al. Ganglioside metabolism in a transgenic mouse model of alzheimer’s disease: Expression of chol-1a antigens in the brain, ASN Neuro 2, 233–241 (2010).

33. Pember, Z., Blennow, K., Bogdanovic, N., Månsson, J. E. & Blomqvist, M. Altered distribution of the gangliosides GM1 and GM2 in Alzheimer’s disease. Dement. Geriatr. Cogn. Disord. 33, 174–188 (2012).

34. Saville, J. T. & Fuller, M. Sphingolipid dyshomeostasis in the brain of the mouse model of mucopolysaccharidosis type I. Mol. Genet. Metab. 129, 111–116 (2020).

35. Tobias, F., Pathmasiri, K. C. & Cologna, S. M. Mass spectrometry imaging reveals ganglioside and ceramide localization patterns during cerebellar degeneration in the Npc1−/− mouse model. Anal. Bioanal. Chem. 411, 5659–5668 (2019).

36. Nestrasis, I. et al. Distinct progression patterns of brain disease in infantile and juvenile gangliosidoses: Volumetric quantitative MRI study. Mol. Genet. Metabol. 123, 97–104 (2018).

37. Walkley, S. U. Secondary accumulation of gangliosides in lysosomal storage disorders. Semin. Cell Dev. Biol. 15, 433–444 (2004).

38. Chiricozzi, E. et al. GM1 ganglioside is a key factor in maintaining the mammalian neuronal functions avoiding neurodegeneration. Int. J. Mol. Sci. 21, 1–29 (2020).

39. Marie, G. et al. Acceleration of α-synuclein aggregation by exosomes. J. Biol. Chem. 290, 2969–2982 (2015).

40. Gaspar, R., Palbo, J., Weininger, U., Linse, S. & Sparr, E. Ganglioside lipids accelerate α-synuclein amyloid formation. Biochim. Biophys. Acta - Proteins Proteom. 1866, 1062–1072 (2018).

41. Tessitore, A. et al. GM1-ganglioside-mediated activation of the unfolded protein response causes neuronal death in a neurodegenerative gangliosidosis. Mol. Cell 15, 753–766 (2004).

42. Sano, R. et al. GM1-ganglioside accumulation at the mitochondria-associated ER membranes links ER stress to Ca(2+)-dependent mitochondrial apoptosis. Mol. Cell 36, 500–511 (2009).

43. De Maria, R. et al. Requirement for GD3 ganglioside in CD95- and ceramide-induced apoptosis. Science 277, 1652–1655 (1997).

44. Okawa, N. et al. Gangliosides determine the amyloid pathology of Alzheimer’s disease. Neuroreport 20, 1043–1046 (2009).

45. Saville, J. T., Thai, H. N., Lehmann, R. J., Derrick-Roberts, A. L. K. & Fuller, M. Subregional brain distribution of simple and complex glycosphingolipids in the mucopolysaccharidosis type I (Hurler syndrome) mouse: Impact of diet. J. Neurochem. 141, 287–295 (2017).

46. Apfel, A., Zhao, L. & Sartain, M. J. A novel solid phase extraction sample preparation method for lipidomic analysis of human plasma using liquid chromatography/mass spectrometry. Metabolites 11, 294 (2021).

47. Smith, N. J. C., Fuller, M., Saville, J. T. & Cox, T. M. Reduced cerebral vascularization in experimental neuropathic Gaucher disease. J. Pathol. 244, 120–128 (2018).

ACKNOWLEDGEMENTS

Funding for this work was obtained from the Michael J. Fox Foundation, the Rolf Willkund and Alice Willkund Parkinson’s Disease Research Fund and the Children’s Gaucher Research Fund. We would like to acknowledge Dr. Ron Rotkopf from the Department of Life Sciences Core Facilities in the Weizmann Institute of Science for help with statistical analysis, and Wan Ling Ng for technical assistance.

AUTHOR CONTRIBUTIONS

S.B. and A.H.F. wrote the manuscript. S.B. conducted the analysis of lipids together with T.N., O.B.B., and T.D. J.T.S. performed the lipidomics. J.H. obtained human brain samples. A.H.F. and M.F. designed experiments and obtained funding for the study.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41531-022-00363-2.

Correspondence and requests for materials should be addressed to Anthony H. Futterman.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2022