Quantitative Mass Spectrometry Imaging to Study Lipid Metabolism in Parkinson's Disease Model

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Introduction

Mass spectrometry imaging (MSI) provides insight into the molecular distribution of a broad range of compounds. This work investigates the suitability of MSI for quantitatively visualizing disease-related alterations in brain lipid concentration and distribution related to Parkinson's disease (PD). For this purpose, a new MALDI internal standard (IS) mix was developed for the simultaneous quantitative MSI of multiple lipid classes. As proof-of-principle, the IS mix was applied for quantitative detection of glycosphingolipids (GSL) in a transgenic mouse models carrying D409V mutations in the glucocerebrosidase gene (GBA1) that encodes the enzyme glucocerebrosidase (GCase). Alterations in GSL concentration and distribution were compared across brain sections from wild-type and heterozygous or homozygous D409V GBA mutant mice to determine spatial lipid phenotypes across conditions of increasing GBA mutation severity.

Method

12µm longitudinal cryosections at -20° C

1. Spraying of IS mixture 60 µl/min at 50° C
2. Spraying of 15mg/mL DHB 50° C at 120 µl/min

MALDI-TOF acquisitions 30µm spatial resolution

TimsTOF MALDI-2

Results

Compound	Exact Mass
15:0-18:1 (d7) PC	752.606
18:1-18:1 SM (d9)	737.64
15:0-18:1 (d7) PA (Na salt)	689.499
15:0-18:1 (d7) PE	710.559
15:0-18:1 (d7) PG (Na salt)	763.536
15:0-18:1 (d7) PI (NH4 salt)	846.596
17:0-16:1 (d5) PS (Na salt)	774.52
17:0 (d5) Lyso PE	472.33
C12 Mono-Sulfo Galactosyl(β) Ceramide (d18:1/12:0)	740.486
C15 Lactosyl(β) Ceramide (d18:1-d7/15:0)	854.65
C18 Ceramide-d7 (d18:1/18:0)	572.587
C17 Glucosyl(β) Ceramide (d18:1/17:0)	713.581

Figure 1. Final composition of splashmix that covers 12 lipid classes with relative concentrations optimized for quantitative MALDI/MALDI-2-MSI analyses in brain tissue sections

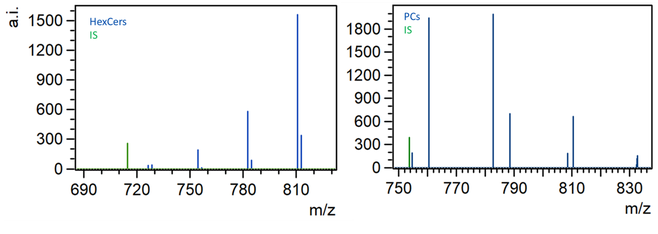

Figure 2. Average on tissue spectra of HexCers (top), PCs (bottom) in blue and their respective internal standards in green.

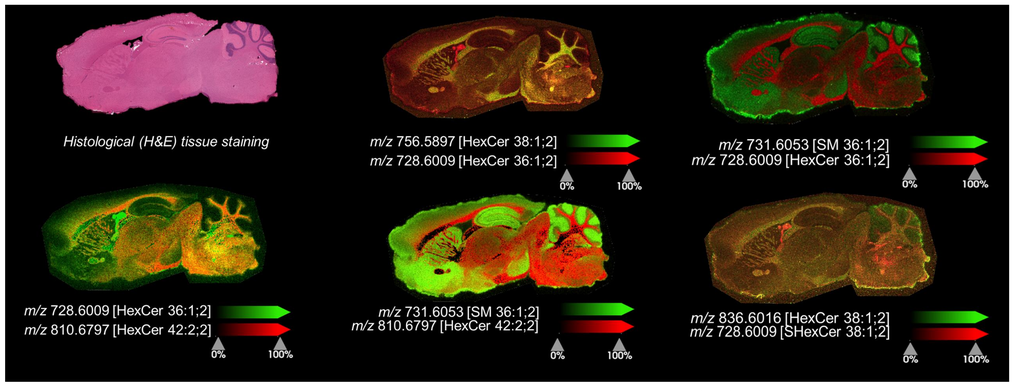

Figure 3. Overlay of ion images for HexCer, SM and SHexCer based on ion intensities. Evidently, these examples show the highly selective distribution of different sphingolipids, even inside classes where only the ceramide backbone composition differ.

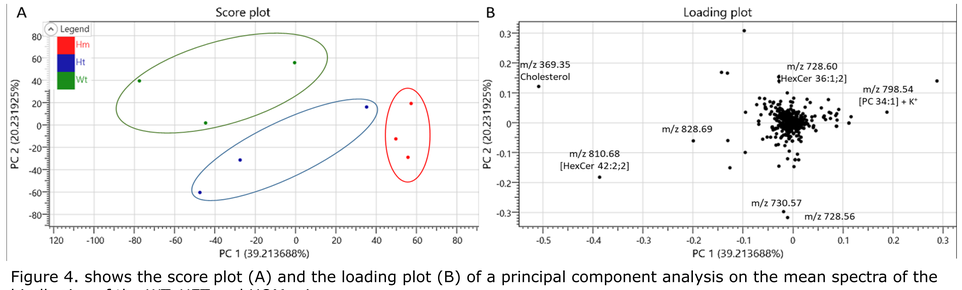

Figure 4. shows the score plot (A) and the loading plot (B) of a principal component analysis on the mean spectra of the hindbrains of the WT, HET and HOM mice.

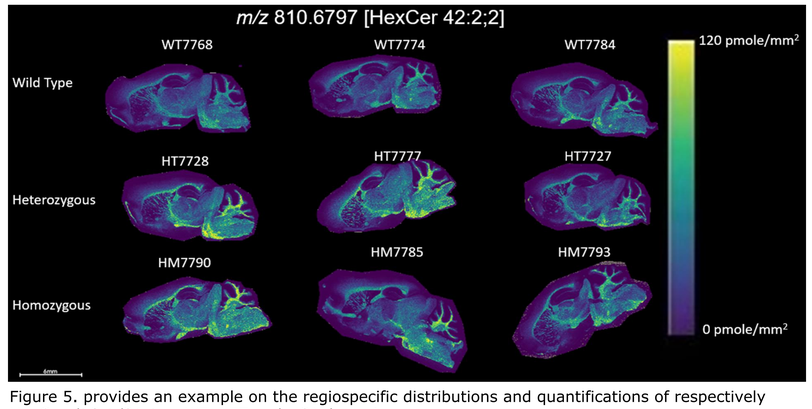

Figure 5. provides an example on the regiospecific distributions and quantifications of respectively HexCer d18:1/24:1 in WT, HET and HOM brain tissues.

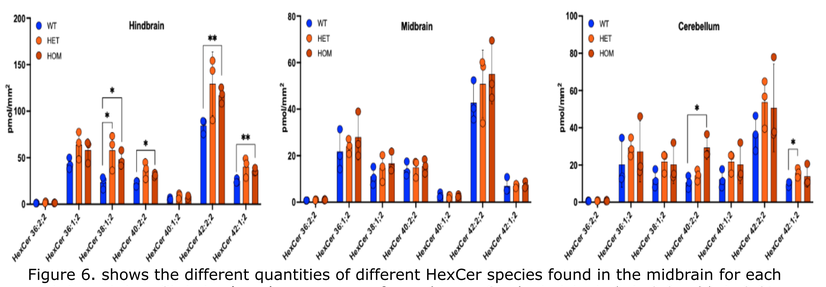

Figure 6. shows the different quantities of different HexCer species found in the midbrain for each mutation status. Statistical analyses were performed using Students t-test. * P<0.05, ** P<0.01.

Conclusions

Initial investigations have revealed fatty acid chain length-dependent distribution of hexosylceramide (HexCer) species such as HexCer (d18:1/20:0) and HexCer (d18:1/24:1) across the brain that represent the combined signal of glucose- and galactose containing. Both HexCer (d18:1/20:0) and HexCer (d18:1/24:1) appear concentrated within white matter fiber tracts in the midbrain, cerebellum and possibly brain stem but with different relative peak intensities. In addition, the data showed clear elevation of the HexCer brain levels correlating with GBA mutation status. Furthermore, other lipids such as ceramides, Hex2Cer, sphingomyelins (SM), phospatidylcholines (PC) and phosphatidylethanolamines (PE) could be quantified simultaneously using this approach. In conclusion, our presented IS-based Q-MSI approach provides a selective and high-throughput analytical platform to study quantitative alterations in lipid composition throughout biological tissues.

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