QUANTITATIVE SPATIAL PROFILING OF GANGLIOSIDES ACROSS HUMAN AND MURINE BRAIN TISSUES

Maria José Q Mantas[1]; Shadrack M Mutuku[2]; Nicolas Tomasiello[2]; Greg Sutherland[3]; Caine Smith[3]; Nathan Heath Patterson[1]; Marc Claesen[1]; Alice Ly[1]; Nathan G. Hatcher[4]; Nico Verbeeck[1]; Kim Ekroos[5]; Shane R. Ellis[2]

[1] Aspect Analytics, Genk, Belgium;
[2] Molecular Horizons and School of Chemistry and Molecular Bioscience, University of Wollongong, Wollongong, Australia;
[3] New South Wales Brain Tissue Research Centre, Charles Perkins Centre and School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Sydney, Australia;
[4] Merck & Co., Inc., West Point, PA;
[5] Lipidomics Consulting Ltd, Esbo, Finland

Introduction

Lipids play a critical role in influencing structural integrity, signalling pathways, and metabolic processes. Attaining spatially resolved quantitative information on lipid content is essential for understanding brain organization and function. We previously developed a novel workflow using MALDI mass spectrometry imaging (MSI) combined with the MSI SPLASH™ (Avanti) lipid standard mixture, where lipid classes were normalized by representative standards sprayed onto tissue, enabling the generation of spatially resolved quantitative lipidomics profiles in mouse brain[1]. In this study, we expand on this methodology by utilizing ganglioside-specific standards to provide quantitative lipid profiles for gangliosides using quantitative mass spectrometry imaging (Q-MSI). This workflow is applied to determine and compare ganglioside compositions in midbrain and cortical areas of murine and human tissues, screening and quantizing for 246 ganglioside species.

Methods:

Human brain tissues were provided by the UCL Queen Square Brain Bank for Neurological Disorders. Mouse and human tissue sections were coated with a three component ganglioside internal standard mixture, consisting of GM3, GM1 and GD1 standards, using a TM-sprayer (HTX Technologies, LLC), followed by application of 2,5-DHA matrix doped with ammonium sulphate. Tissues were analysed using an AP-MALDI source (UHR, MassTech) coupled to an Orbitrap Fusion in negative mode. After MALDI-MSI analysis, tissues were stained with Luxol Fast Blue (LFB) and the slides digitised. MSI and accompanying histology data were imported into the Weave platform, and tagged with relevant metadata to enable downstream data analysis. Ganglioside ion images were extracted and normalized to their corresponding lipid standards.

Results

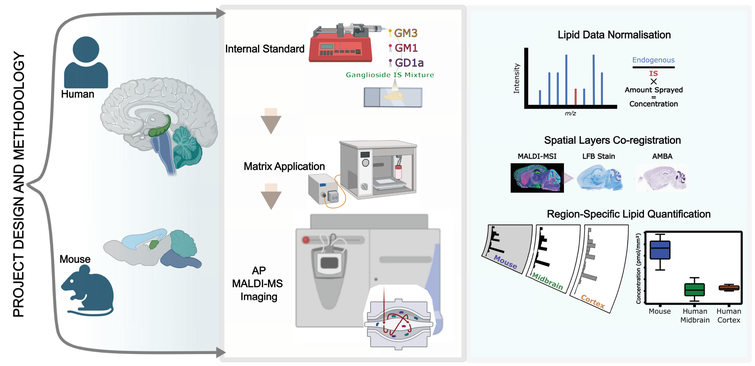

Figure 1: Q-MSI Workflow
Murine and human sections were sectioned and coated with a three component ganglioside internal standard mixture, consisting of GM3, GM1 and GD1 standards. Ganglioside ion images were extracted and normalized to their corresponding lipid standards, enabling direct quantification of gangliosides in tissue.

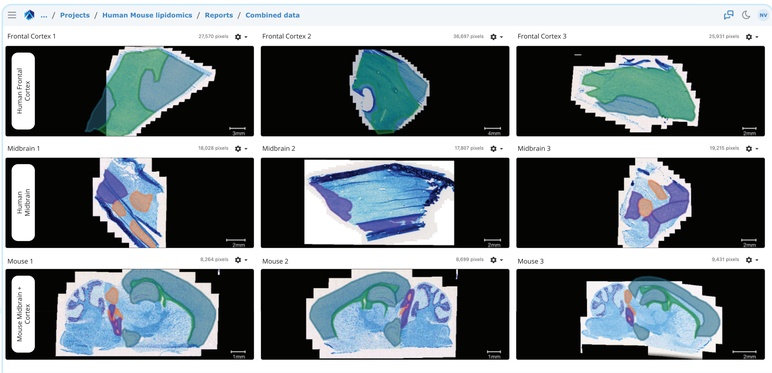

Figure 2: Pathologist tissue annotations performed in Weave platform.
Histological annotations of brain regions in the human and mouse samples were performed in Weave on the post-measurement LFB-stained, full resolution microscopy images. Samples were annotated for 'Cortex white matter' (green), 'Cortex grey matter' (blue), 'Midbrain white matter' (purple) and 'Midbrain grey matter' (orange). MSI and microscopy measurements were spatially co-registered using non-rigid registration algorithms, after which annotations were transferred to the MSI data.

References
[1] Mutuku et al., Toward Omics-Scale Quantitative Mass Spectrometry Imaging of Lipids in Brain Tissue Using a Multiclass Internal Standard Mixture. Anal. Chem. **2023**, 95 (50). https://doi.org/10.1021/acs.analchem.3c02724

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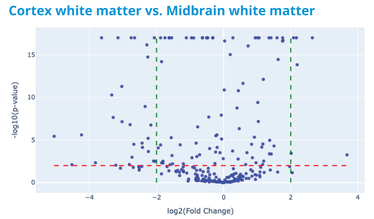
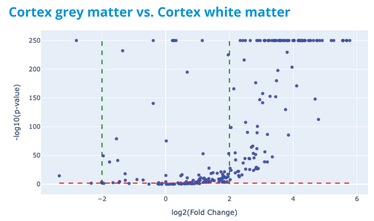
Figure 3: Comparison of ganglioside expression in human tissue based on extracted gangliosides.

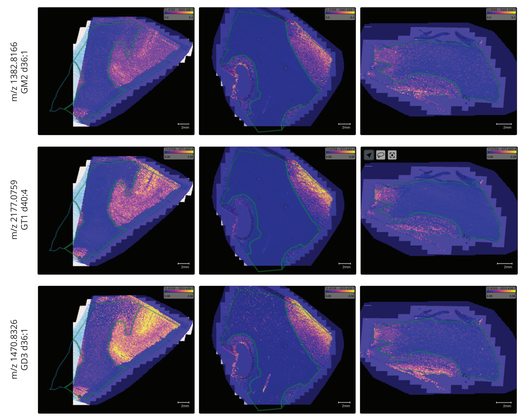
Left: We conducted a t-test-based ranking of all extracted and normalized gangliosides across brain regions to identify differentially expressed species. In this analysis, each pixel in the MSI dataset was treated as an independent observation, which resulted in the displayed pseudo-volcano plots.

These pseudo-volcano plots can assist in interactive data exploration in Weave. Here we show differential ganglioside expression between: Cortex white matter vs. midbrain white matter (left), and Cortex grey matter vs. cortex white matter (right). From this analysis, we see differentially expressed gangliosides across anatomical regions, notably a substantial upregulation of a wide number of ganglioside species in the cortex grey matter compared to the cortex white matter.

Right: Ion images showing selected gangliosides with differential expression between cortex white matter (green) and grey matter (blue).







Mean tissue concentration of selected differentially expressed gangliosides in frontal cortex grey matter

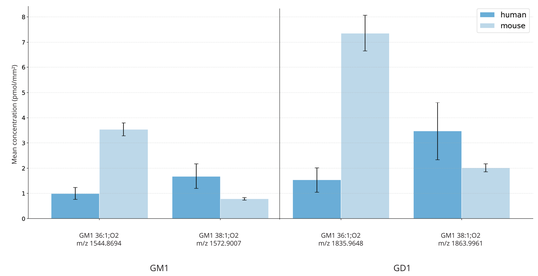
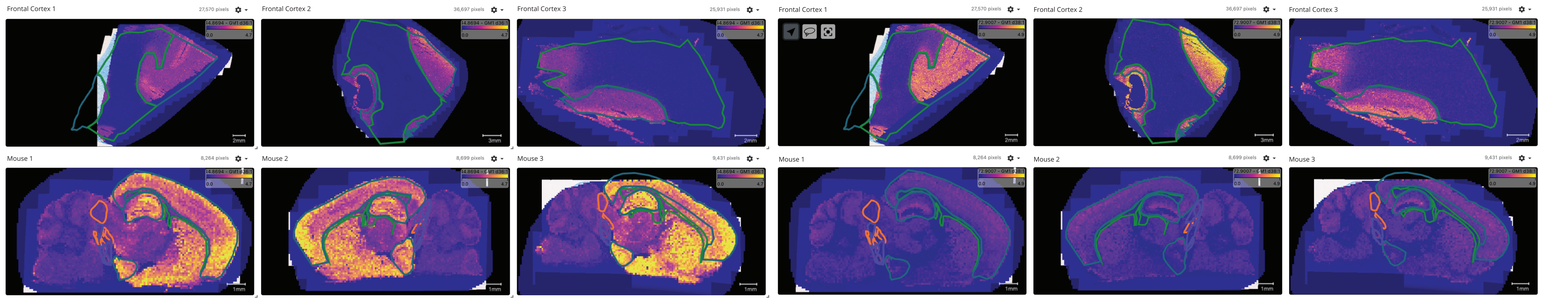


Figure 4: Differential analysis mouse-human samples.
The Q-MSI workflow allows direct quantitative comparison of ganglioside expressions across species. Region-specific differential expression analysis between mouse and human brain tissue shows a preference for longer chain gangliosides in human brain tissue compared to mouse.

Left: Mean concentration in tissue of **GM1 36:1;O2 vs GM1 38:1;O2** and **GD1 36:1;O2 vs GD1 38:1;O2** across regions of interest. The ratio of **GM1 36:1;O2/GM1 38:1;O2** and **GM1 36:1;O2/GM1 38:1;O2** respectively is inverted between mouse and human brain tissue.

Bottom: Standard-normalized expression of GM1 36:1;O2 vs GM1 38:1;O2 in tissue. Pronounced differences in expression in the Cortex grey matter (blue annotation) between human and mouse tissue, highlighting structural and metabolic variations between human and murine brains.



m/z 1544.8694 - GM1 36:1;O2
(intensity relative to IS)

m/z 1572.9007 - GM1 38:1;O2
(intensity relative to IS)

Conclusion & Outlook

- We present a robust framework for spatially resolving the ganglioside organization and function of the brain in both human and murine models.
- First application of ganglioside-specific normalization and quantitation in mouse and human brain tissue reveals novel insights into interspecies differences in brain lipid content and distributions.
- These results provide the most defined and quantitative ganglioside atlases to date of selective brain regions of human and mouse.