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MALDI mass spectrometry imaging in Alzheimer's disease mouse model Het CRND8 (+/-)

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Overview: First investigation of Alzheimer's disease mouse model Het CRND8 (+/-) using mass spectrometry imaging

1. Introduction

Mass spectrometry imaging (MSI) is an emerging technology for biomarker discovery and for assessing comprehensive molecular changes in complex biological matrices. It is a suitable technique for the identification of compounds such as proteins, peptides or lipids without an *a priori* knowledge of the composition of the sample. Alzheimer's disease is a pathology affecting more than 520,000 people in the UK. It primarily affects the hippocampus and cortex regions of the brain, probably by damaging and destroying the connections between brain cells and later by causing cell death. A better understanding of the molecular composition of diseased tissues compared to control samples are needed. Since the location of compounds on tissue sections is preserved, MSI allows identification of locally abundant species that could be lost if the whole tissue is homogenised.

In our study, we aimed to visualize the distribution of molecular entities in various brain regions during the progression of Alzheimer's disease using a well-known mouse model Het CRND8 (+/-) and the wild type (WT) as a control sample. We mainly focused on the hippocampus and the parietal cortex regions. The maintained spatial distribution of species allowed the detection of peaks from the in-situ digested tissue sections. Here, we present our initial results.

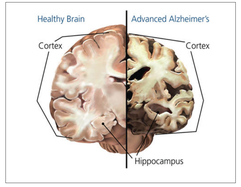


Figure 1 Main areas of the brain affected by Alzheimer disease

2. Material and methods

Sample collection

- 10 mice: 5 WT and 5 CRND8 (+/-)
- Snap frozen in liquid nitrogen after collection
- Serial sections @ 12 um: MALDI analysis and for Nissl staining
- Washing steps:

EtOH 70% 30 sec, EtOH 95% 30 sec, Carnoy's solution (= EtOH, chloroform, acetic acid (6:3:1)) 2 min, EtOH 95% 30 sec. H_2O MilliQ 30 sec then EtOH 95% 30 sec.

Sample preparation & Data acquisition

- Spraying the trypsin using **SunCollect** 100 ng/ul trypsin in 10/90 ACN/H2O, Octyl-Glucoside 0.5 %, 5 layers @ 5ul/min
- Rehydration in humidity chamber @37C overnight
- Spraying CHCA matrix (5 mg/ml) in 50/50 ACN/TFA 0.2%, 20 layers @ 20 ul/min
- Entire tissue sections acquired at 30 um spatial resolution using the **MALDI-7090** (Shimadzu-UK) in Reflectron positive ion mode m/z [700-3000]
- Data were visualized in **IonView™** software (Shimadzu)

Data analysis

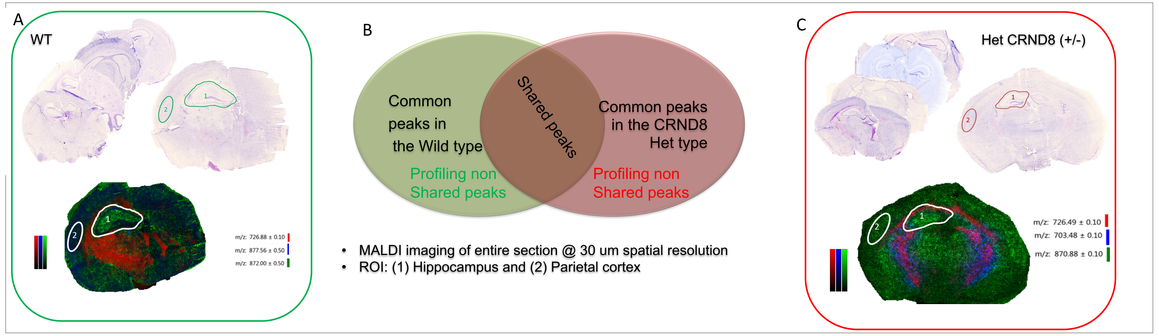
- Export of detected peaks/pixel point of ROI into MATLAB®
- Generate a list of common peaks of the WT and in CRND8 samples
- Identifying shared detected peaks in all samples within 0.2 Da tolerance
- Identifying NON shared peaks between WT common peaks **and** CRND8 common peaks

Figure 2 Workflow from sample collection to data analysis of mouse brain tissue sections (WT and HET CRND8)

3. Result

3-1. MALDI imaging of WT and CRND8 tissue sections

- MALDI imaging experiments were performed on full sections at 30 um spatial resolution (fig 3) in reflectron positive mode after optimising the conditions of trypsin and matrix spraying.
- Significant molecular changes on the full section and on regions of interest (ROI), hippocampus and cortex, have been investigated between the CRND8 and the WT mouse model (fig 3A and 3C).
- Differential peak detection plots of the regions of interest for both the wild type and the CRND8 type were generated: Common peaks found in the dataset of WT and common peaks found in the CRND8 with their corresponding average intensities were compared to identify the non shared peaks between the two mouse types (figure 3B) . Shared peaks and unique peaks detected in each dataset were plotted for the hippocampus (figure 4) and the parietal cortex region (figure 5). Potential species is shown as an illustration (Figure 6)



- MALDI imaging of entire section @ 30 um spatial resolution
- ROI: (1) Hippocampus and (2) Parietal cortex

Figure 3 MALDI imaging of the entire tissue sections of WT (Fig 3.A) and CRND8 (Fig 3.C), the region of interest are drawn on the Nissl stained images and the MALDI images. 30 peaks/pixel were exported per ROI. The approach used for profiling differential peaks is presented in fig 3B

3-2. Data analysis on ROI (hippocampus and parietal cortex)

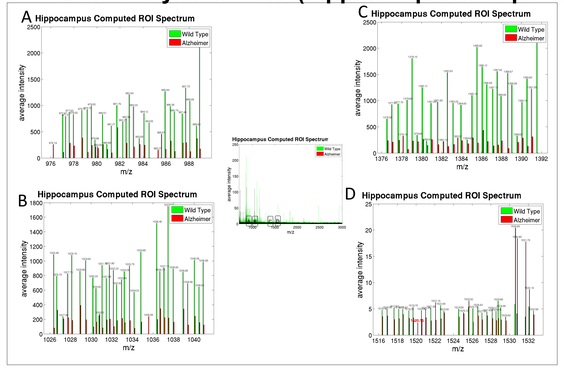


Figure 4 Shared peaks and unique peaks detected in the hippocampus region

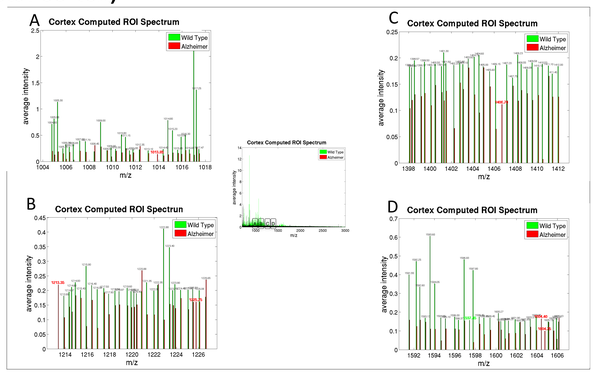


Figure 5 Shared peaks and unique peaks detected in the parietal cortex region

3-3. Example of MALDI images of potential candidate (m/z 1531.2)

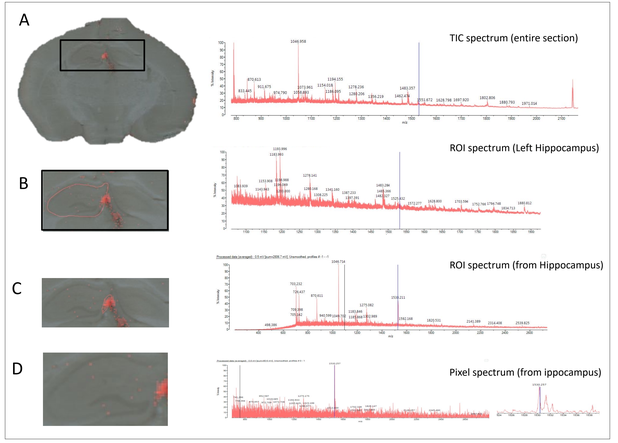


Figure 6 Overlay of optical scan of tissue section (CRND8) with the MALDI image of m/z 1531.3. (A) optical image of entire section with the co-localisation of species m/z 1531.3 and the corresponding TIC spectrum. (B) zoom on the hippocampus region and the corresponding ROI spectrum (left hippocampus). (D) a spectrum from a pixel point showing the species m/z 1531.3

4. Conclusion and perspectives

- MALDI imaging was performed on in-situ digested tissue sections, : Differences between the WT and the CRND8 model were investigated and potential m/z species are presented
- Spectra from specific ROI have been investigated and processed separately. The same approach could be performed on the entire section
- Selected candidates were ranked by intensity. The most intense ones will carry MS/MS on tissue (MS/MS imaging or MS/MS profiling). Further validation will be carried out in the next study. This will be done in conjunction with Mascot/Blast searches.
- Another level on investigation could be carried out on amyloid beta peptides antibodies to visualise co-loclalised species in the corresponding regions.
- More samples are required to have the possibility to run statistical analysis (T-Test)