Non-target LC-HRMS to Study the Exposome of Mild Cognitive Impairment and Alzheimer's Disease on Cerebrospinal Fluid

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INTRODUCTION

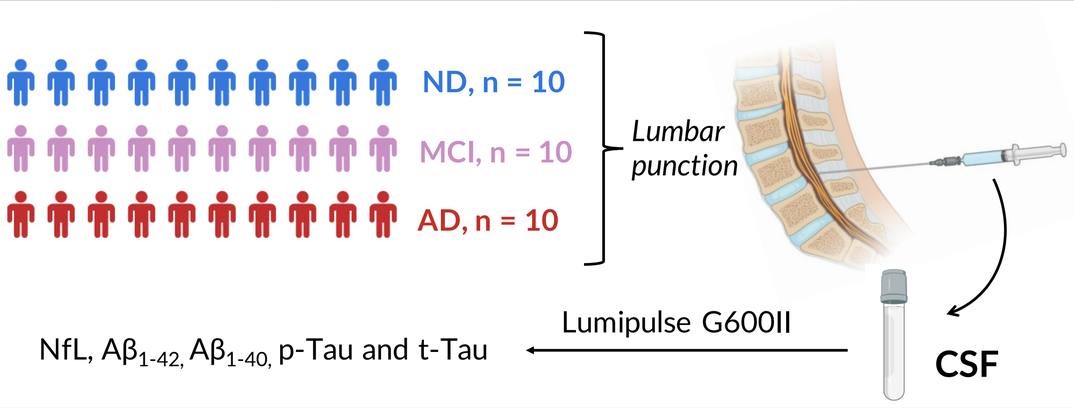
- Alzheimer's disease (AD) is the **most common form of dementia,** and its prevalence is expected to increase from 50 million people (2010) to 113 million by 2050.
- The current diagnosis is based on clinical symptoms and pathological alterations: ↓$A\beta_{1\text{-}42,}$ ↑ p-Tau, ↑ t-Tau, and ↑ Neurofilament Light (NfL) in Cerebrospinal Fluid (CSF).
- However, these are **nonspecific biomarkers**, resulting in a high rate of **misdiagnosis in the early stages**.
- Research is needed to find **disease-specific biomarkers.**
- **Studying the exposome** could be one way to accomplish this challenge.

AIMS

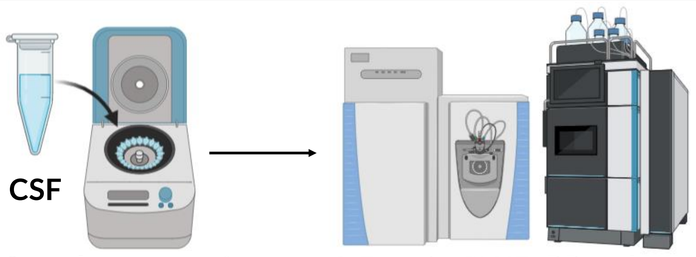
1. Study the CSF of three groups of patients: **AD**, Mild Cognitive Impairment (**MCI**), an intermediate predementia stage, and a non-demented (**ND**) control group by liquid chromatography coupled to high resolution mass spectrometry (**LC-HRMS**)
2. Understand the CSF chemical composition of the CSF samples and investigate the differences across groups
3. Study the associations between the altered chemicals and the known CSF biomarkers

METHODS

(1) Sample collection and biomarker assessment



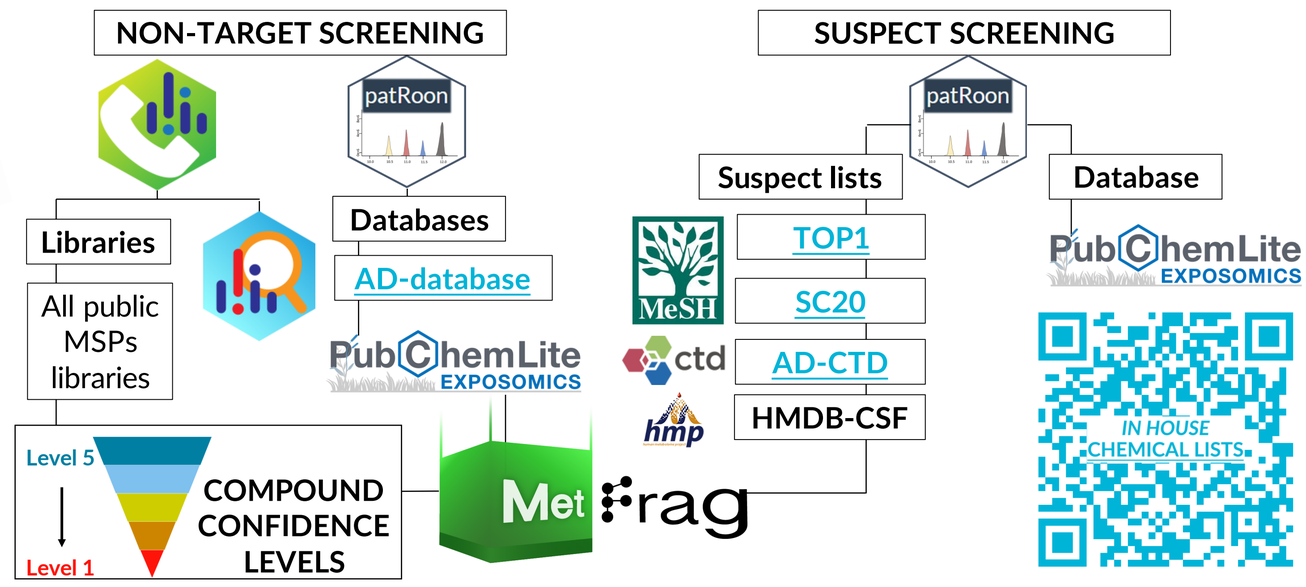
(2) LC-HRMS analysis



***RP column:** BEH C18*
(A) 0.1 % FA in MilliQ H_2O
(B) MeOH
***HILIC column:** SeQuant® ZIC-pHILIC*
(A) 20mM NH_4Ac in ACN
(B) 20mM NH_4Ac in MilliQ H_2O

*RP and HILIC columns**

(3) Data processing and compound identification



(4) Statistical analysis

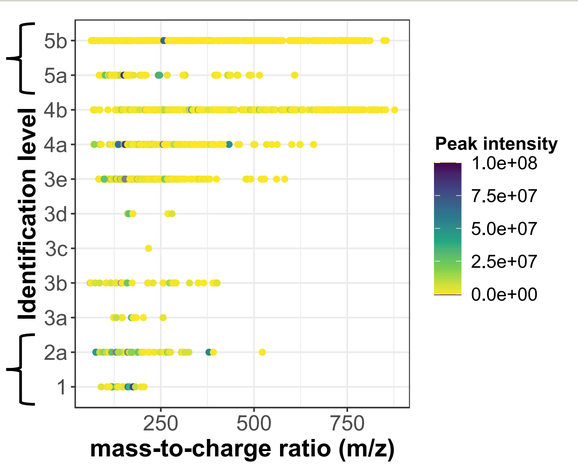
- Data filtration, normalization by sum, log transformation and pareto scaling
- **ANOVA** with post-hoc Tukey's HSD test and **linear multiple regression analysis**

RESULTS AND DISCUSSION

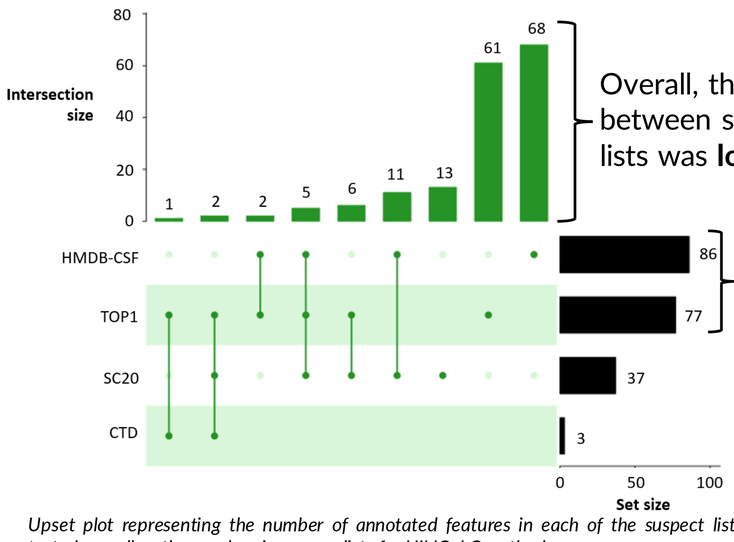
MS-DIAL and patRoon identifications

Level 5 features are more **diverse** (from low to high mases), and the **intensities** are mainly **low.**

High confidence features (level 1-2a) consisting primarily of **small** molecules, generally exhibit **higher peak intensities.**



Dot plot showing the m/z distribution of the tentative annotated features, shaded according to peak intensity. Only MS-DIAL and MS-FINDER annotations separated by HILIC (+) are considered.

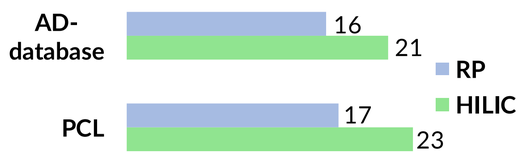


Overall, the **overlap** between suspect lists was **low**

Most of the unique features were found with these two lists

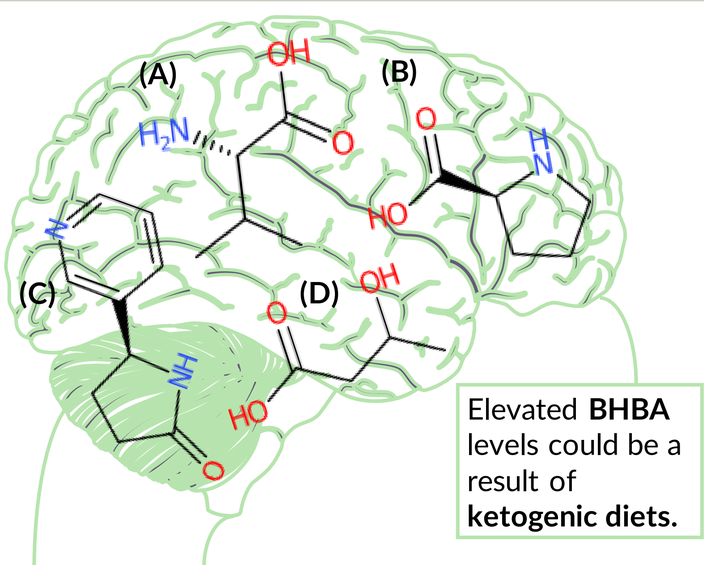
Upset plot representing the number of annotated features in each of the suspect lists tested as well as the overlapping across lists for HILIC LC method.

AD-database is smaller compared to the PCL but provides similar results, suggesting an **adequate chemical coverage**



Bar plots showing the unique features annotated by the two databases employed for the non-target screening approaches in patRoon. PCL: PubChemLite for Exposomics

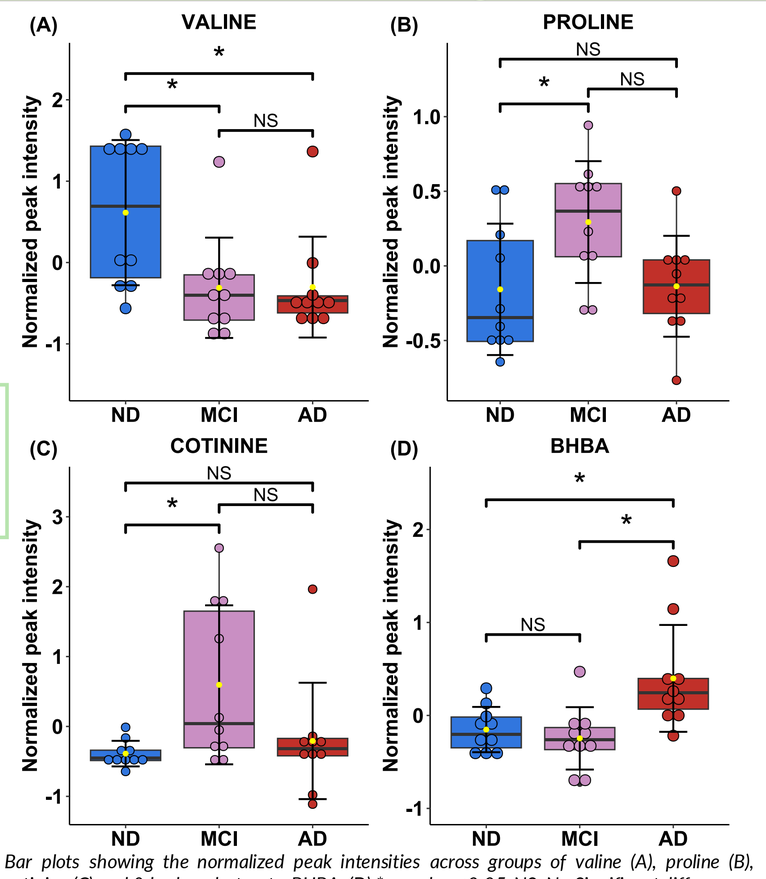
Different classes of chemicals were found to be altered across groups



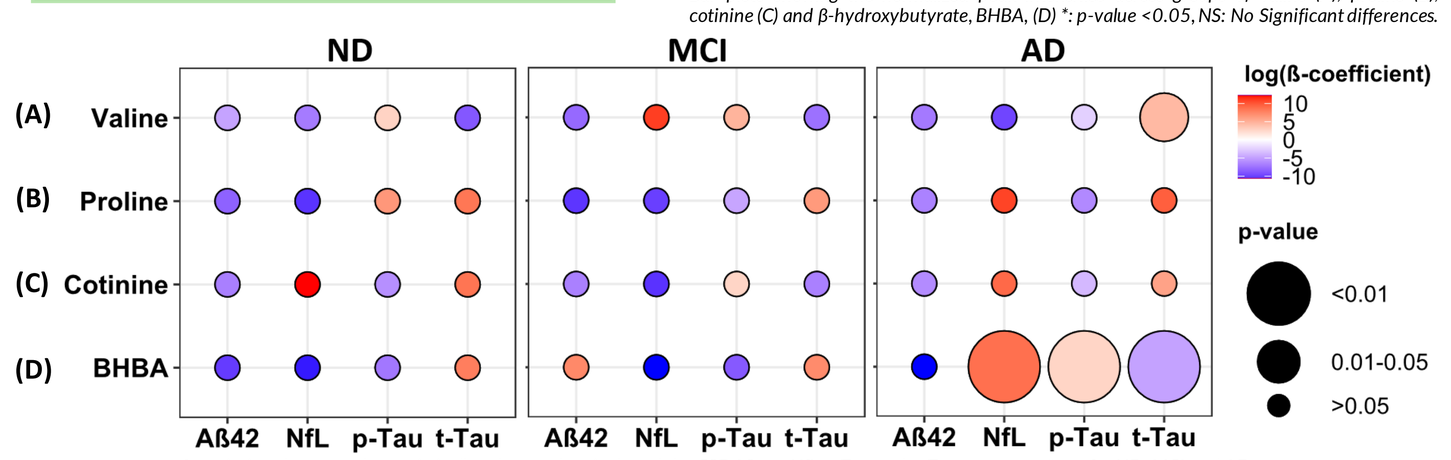
Elevated **BHBA** levels could be a result of **ketogenic diets.**

Alterations in **amino acids** levels (A and B) might be explained by the brain **glucose hypometabolism in AD**.

Statistically significant associations were found between the **clinical biomarkers in AD** and some chemicals (**valine and BHBA**) found in the CSF of the AD group.



*Bar plots showing the normalized peak intensities across groups of valine (A), proline (B), cotinine (C) and β-hydroxybutyrate, BHBA, (D) *: p-value <0.05, NS: No Significant differences.*



Associations between the statistically relevant chemicals and the CSF $A\beta_{1\text{-}42}$, NfL, t-Tau and p-Tau concentrations, for ND, MCI and AD group.

CONCLUSIONS

- This study showed different chemical alterations in the CSF of AD, MCI and ND groups. The incorporation of the MCI group allowed us to explore biomarkers of disease progression, as some chemicals were found with higher levels in this group compared to the others (e.g., proline).
- Environmental and lifestyle factors might explain some chemical differences found across the different groups (e.g., BHBA, cotinine).
- This pilot study aims to establish methodologies and hypotheses that can be examined and confirmed in future studies involving a larger patient cohort.

References

Talavera Andújar B. *et al.* Non-target LC-HRMS to Study the Exposome of Mild Cognitive Impairment and Alzheimer's Disease on Cerebrospinal Fluid. *In prep.*

Funding

BTA is part of the "Microbiomes in One Health" (MICROH) PhD training program, which is supported by the PRIDE doctoral research funding scheme of the Luxembourg National Research Fund (FNR).