“Challenges in Developing Non-target Exposomics and Metabolomics Workflows for Cerebrospinal Fluid from Alzheimer’s disease and Mild Cognitive Impairment”

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[2] National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD 20894, USA

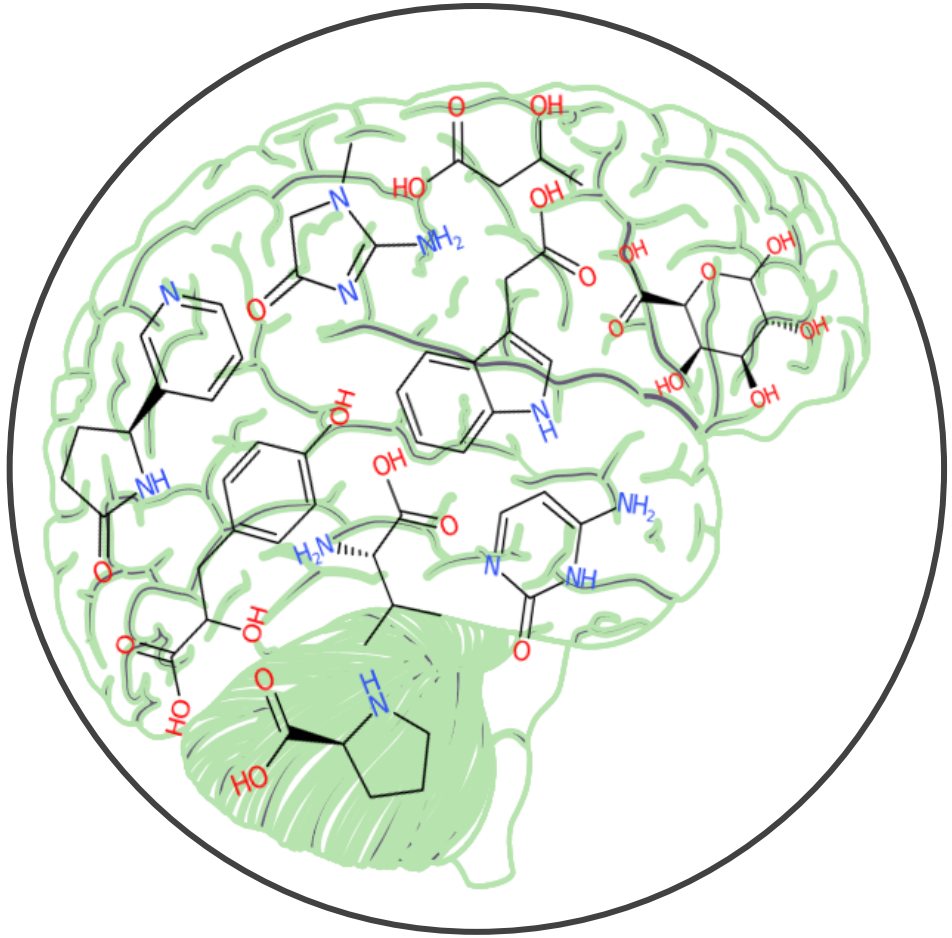
YOUNG NMC Symposium

MICROH

UNIVERSITÉ DU LUXEMBOURG

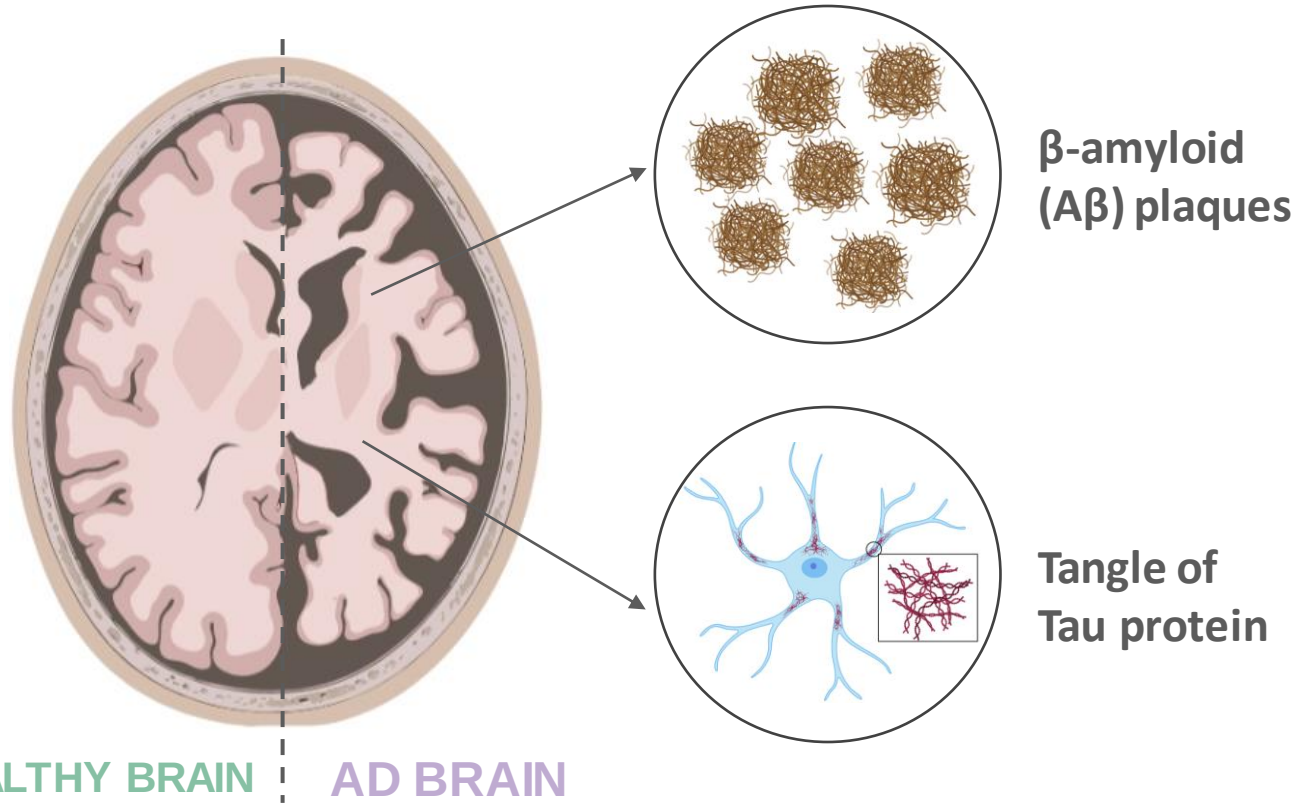
21st September 2023, Nijmegen

Outline



1. Introduction
2. Material and methods
3. Results and discussion
4. Conclusions and future perspectives

1.1. Alzheimer's Disease (AD) and Mild Cognitive Impairment (MCI)



AD is the most common cause of **dementia** accounting for **60-80 %** of dementia cases (Alzheimer's association, 2022)

AD pathology starts decades before the clinical symptoms & there is no curative treatment

Asymtomatic → Mild Cognitive Impairment (MCI) due to AD → Mild Dementia → Moderate Dementia → Severe Dementia

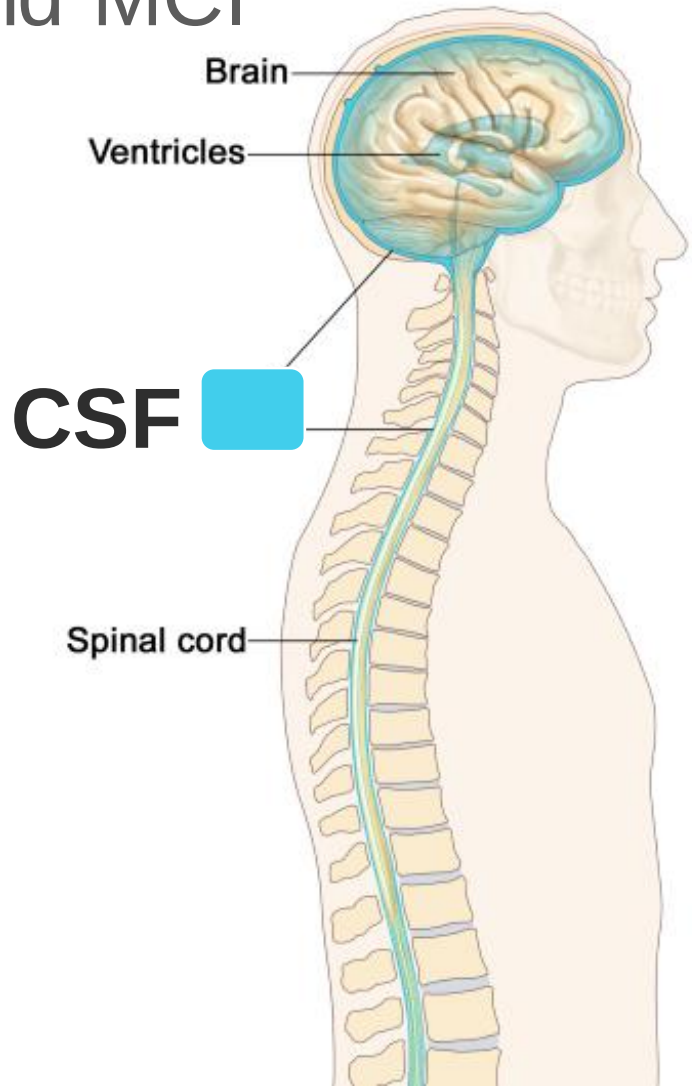
Knopman DS. *et al.* (2021). *Nat Rev Dis Primer* DOI: https://doi.org/10.1038/s41572-021-00269-y
Reveglia P. *et. al.* (2021). *Metabolomics* . DOI: https://doi.org/10.1007/s11306-021-01828-w
Alzheimer's association, 2022

1.2. Cerebrospinal Fluid (CSF) to study AD and MCI

CSF is the closest matrix to the brain

Abnormalities in this matrix are directly related to **pathological changes in the brain**

Metabolomics and exposomics studies of CSF might help us to better understand **disease progression**



https://www.cancer.gov/publications/dictionaries/cancer-terms/def/cerebrospinal-fluid

Athur David. *et al*. (2021). *Environment International*. DOI: https://doi.org/10.1016/j.envint.2021.106630
K.A. Lippa. *et. al*. (2022). *Metabolomics*. DOI: https://doi.org/10.1007/s11306-021-01848-6

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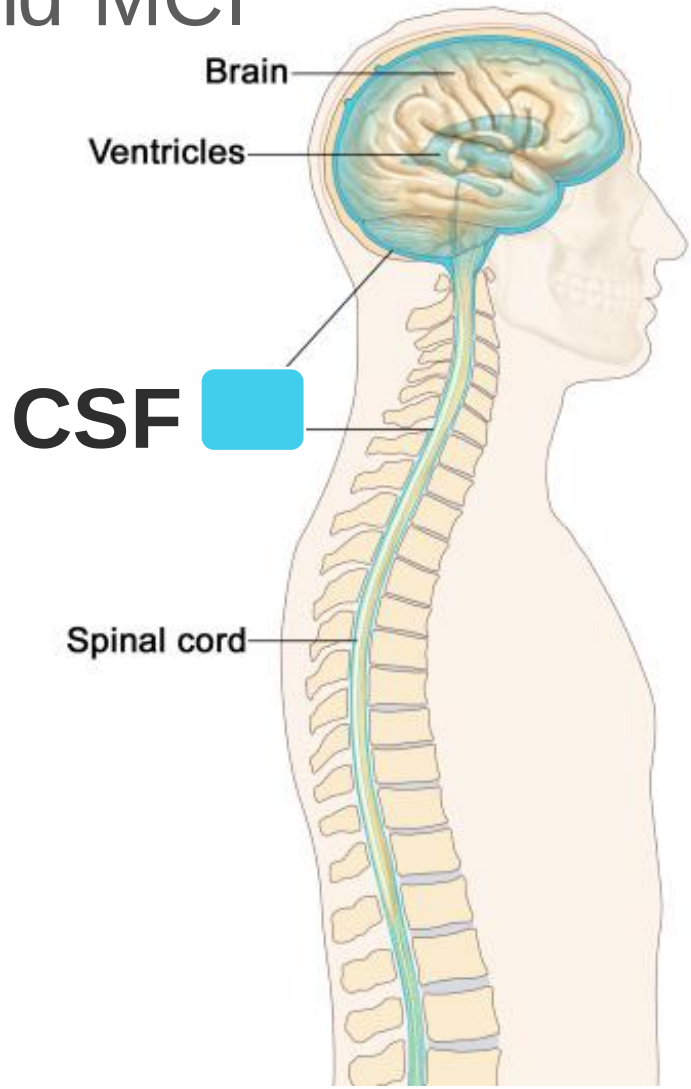
Lack of CSF standard **reference material**

Limited exposomics/ metabolomics **studies** in CSF

Most of the **exogenous** chemicals are known to be in **trace levels** compared to the endogenous (might be undetected with the current methods)

Obtaining **control samples** is **difficult** (invasive procedure)



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Athur David. *et al*. (2021). *Environment International*. DOI: https://doi.org/10.1016/j.envint.2021.106630
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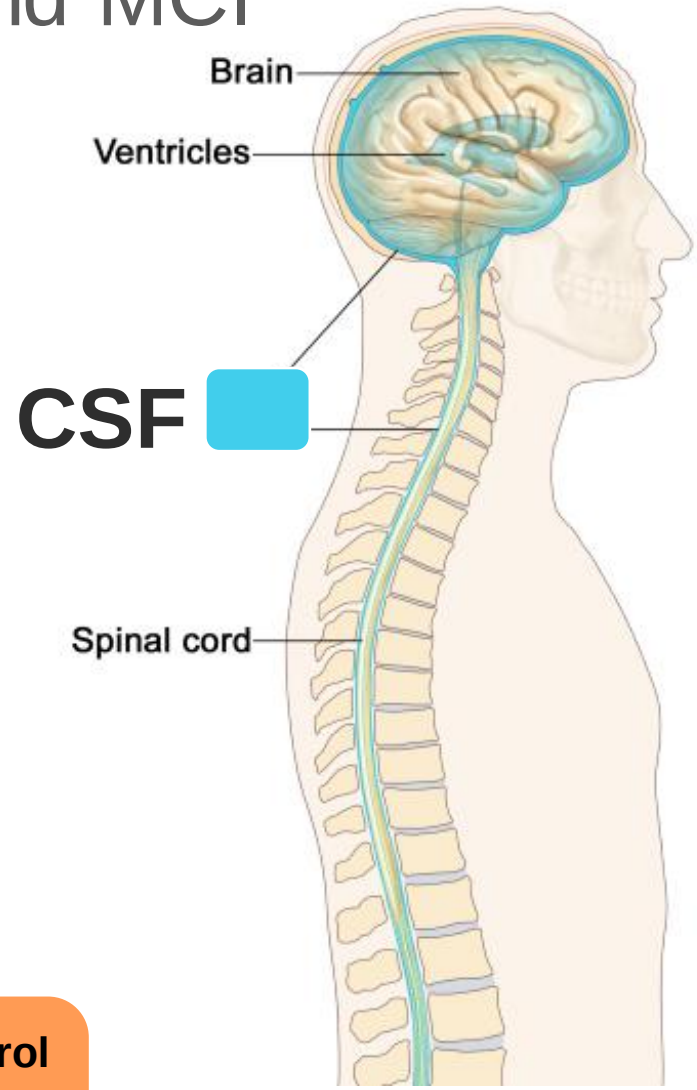

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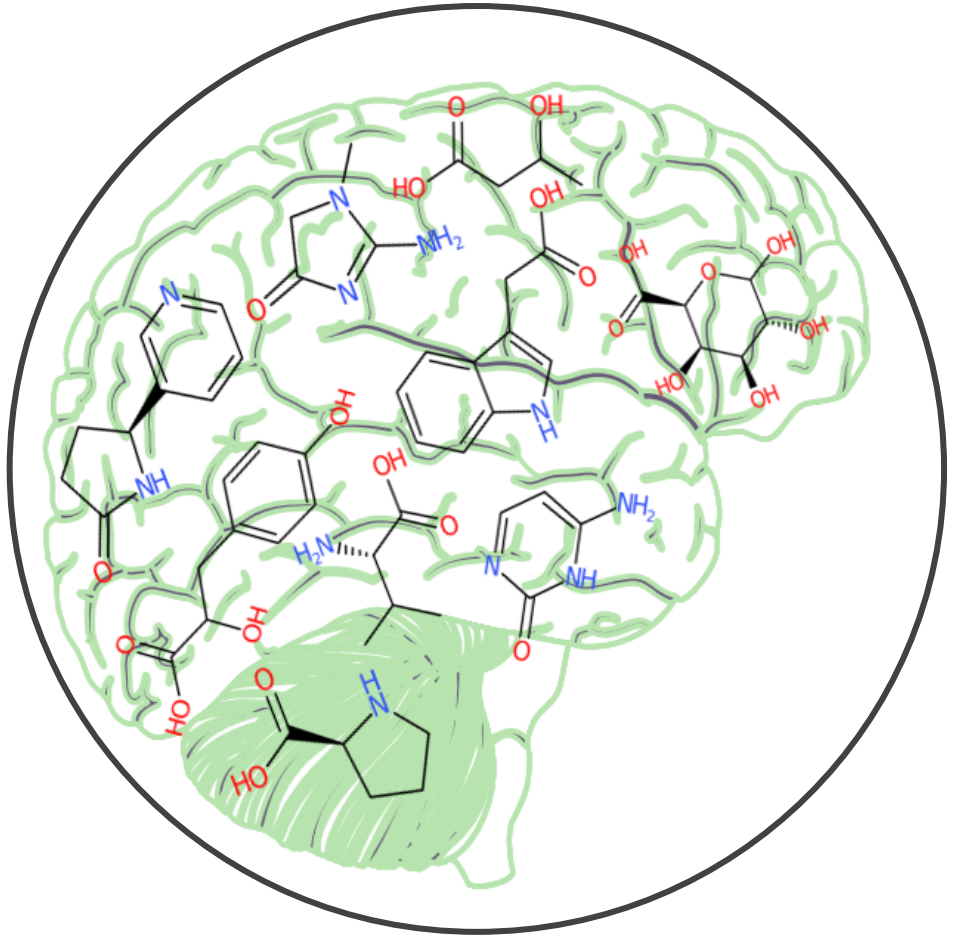
Outline



1. Introduction
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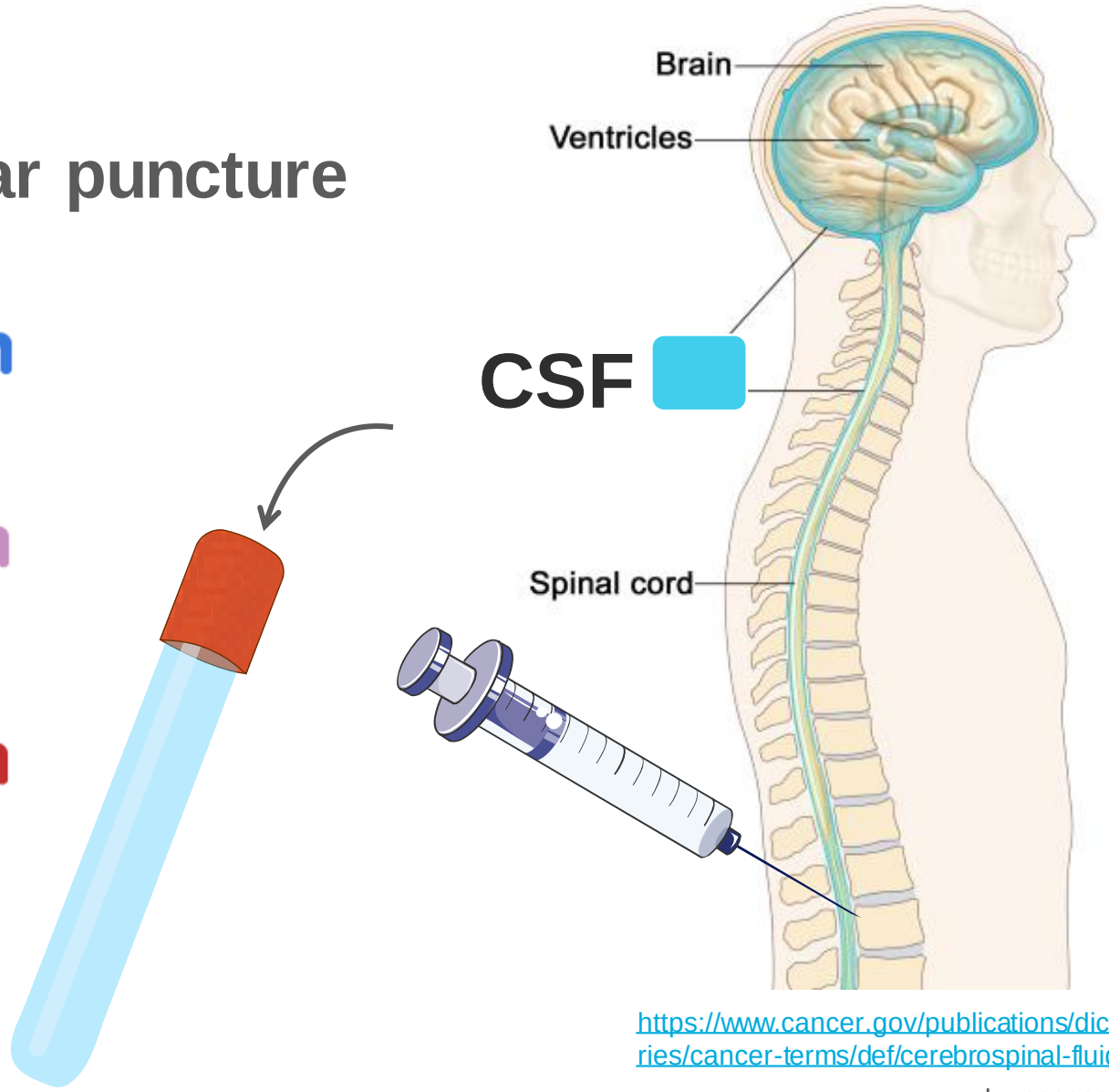
2.1. Sample collection

Samples were collected by **lumbar puncture**

ND

MCI

AD



https://www.cancer.gov/publications/dictionaries/cancer-terms/def/cerebrospinal-fluid

ND: non-demented (control group)
MCI: Mild cognitive impairment
AD: Alzheimer's disease

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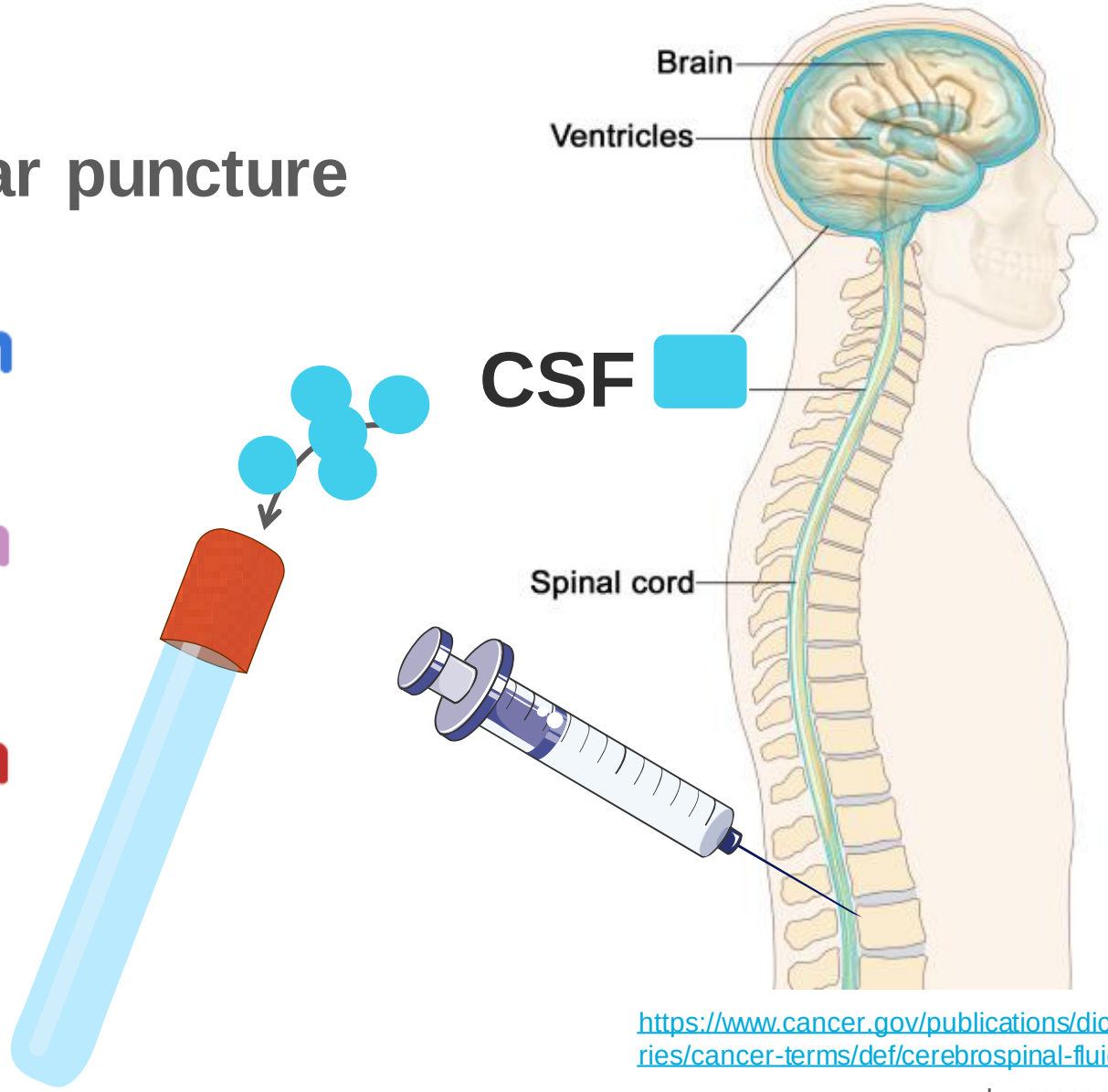

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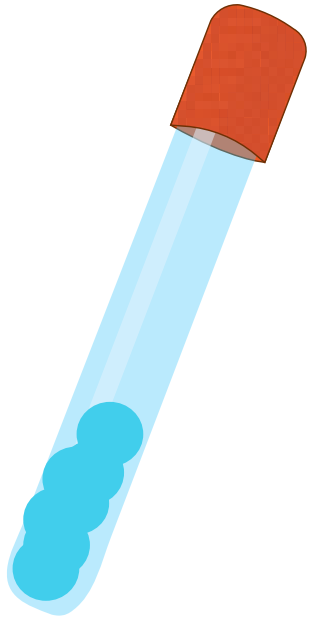
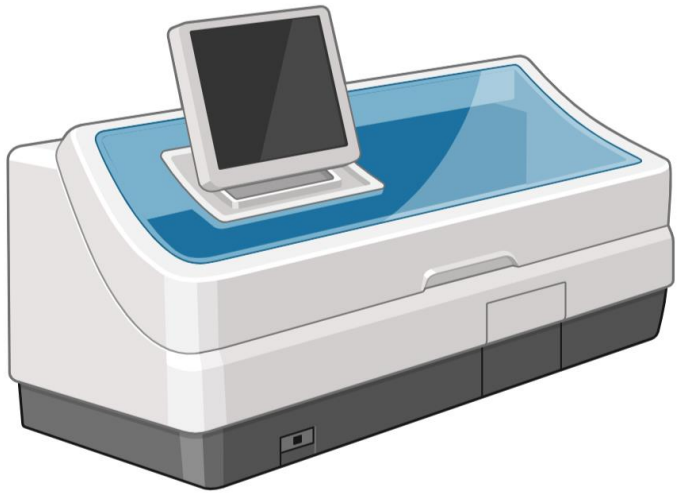
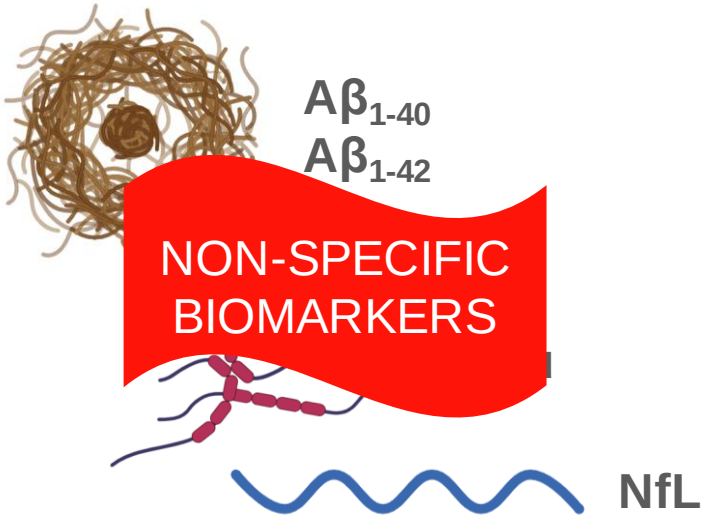
2.2. CSF analysis

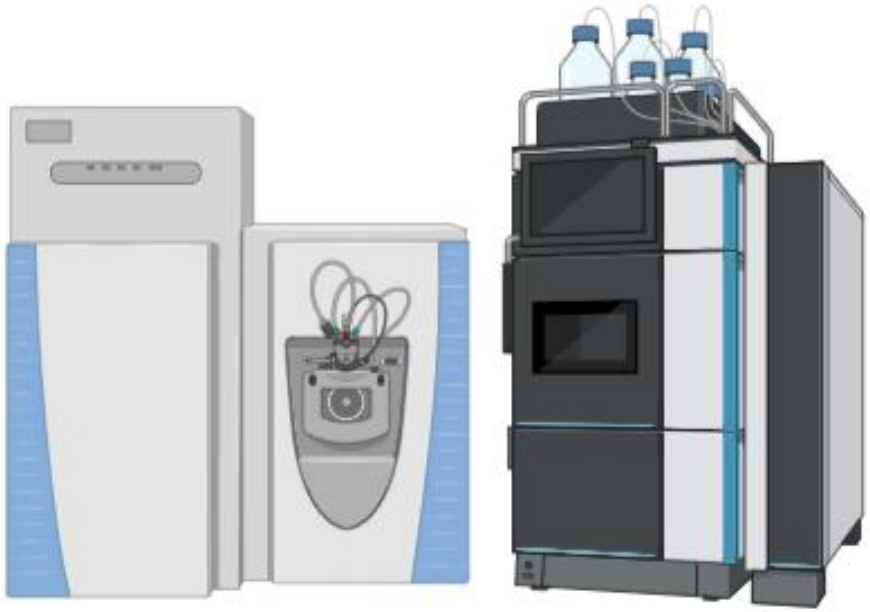


CSF
N = 30

Lumipulse G600II analyzer
Collaboration with Neuroinflammation group (LCSB)







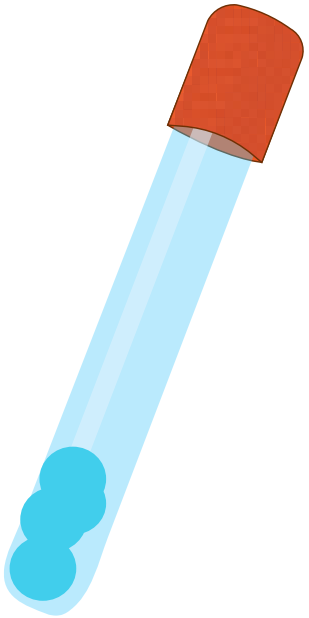
HRMS
Q Exactive™ HF

LC
RP and HILIC

NfL: Neurofilament Light

B. Talavera Andújar *et al.* (2022). *ABC.* DOI: 0.1007/s00216-022-04207-z

2.2. CSF analysis



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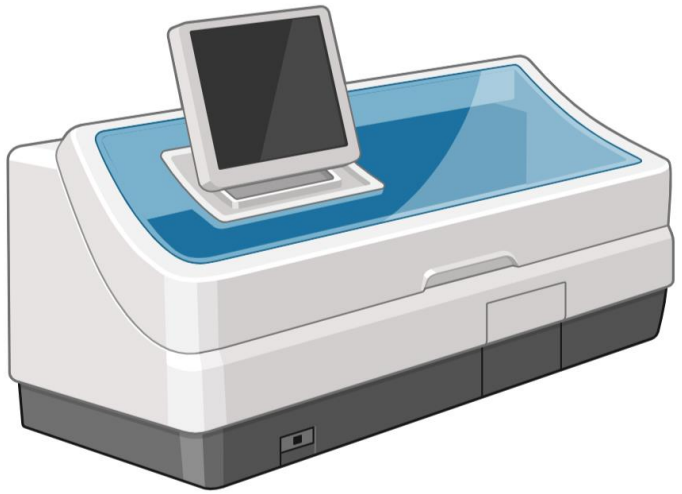
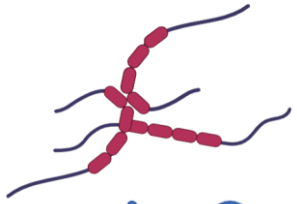
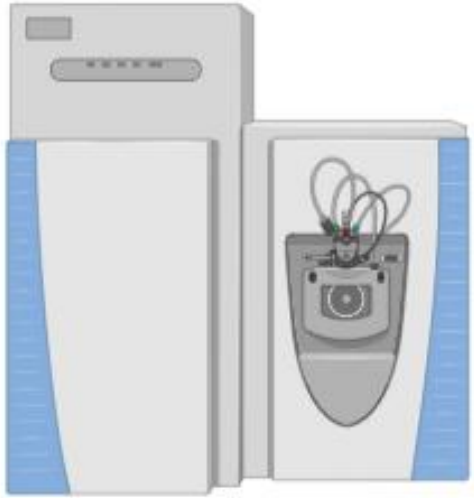


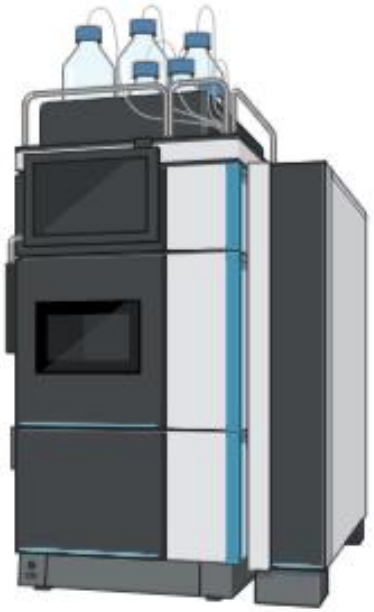
$A\beta_{1-40}$
$A\beta_{1-42}$



t-Tau
p-Tau

NfL





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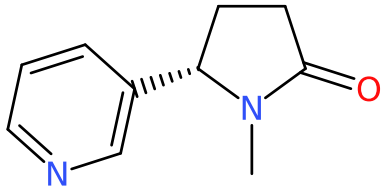
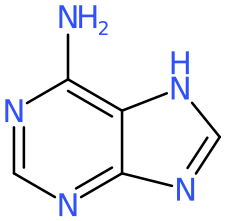
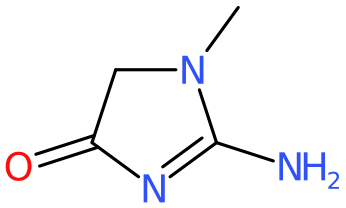
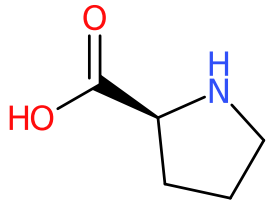
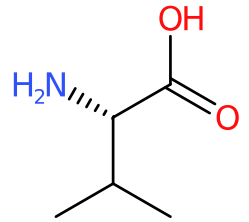








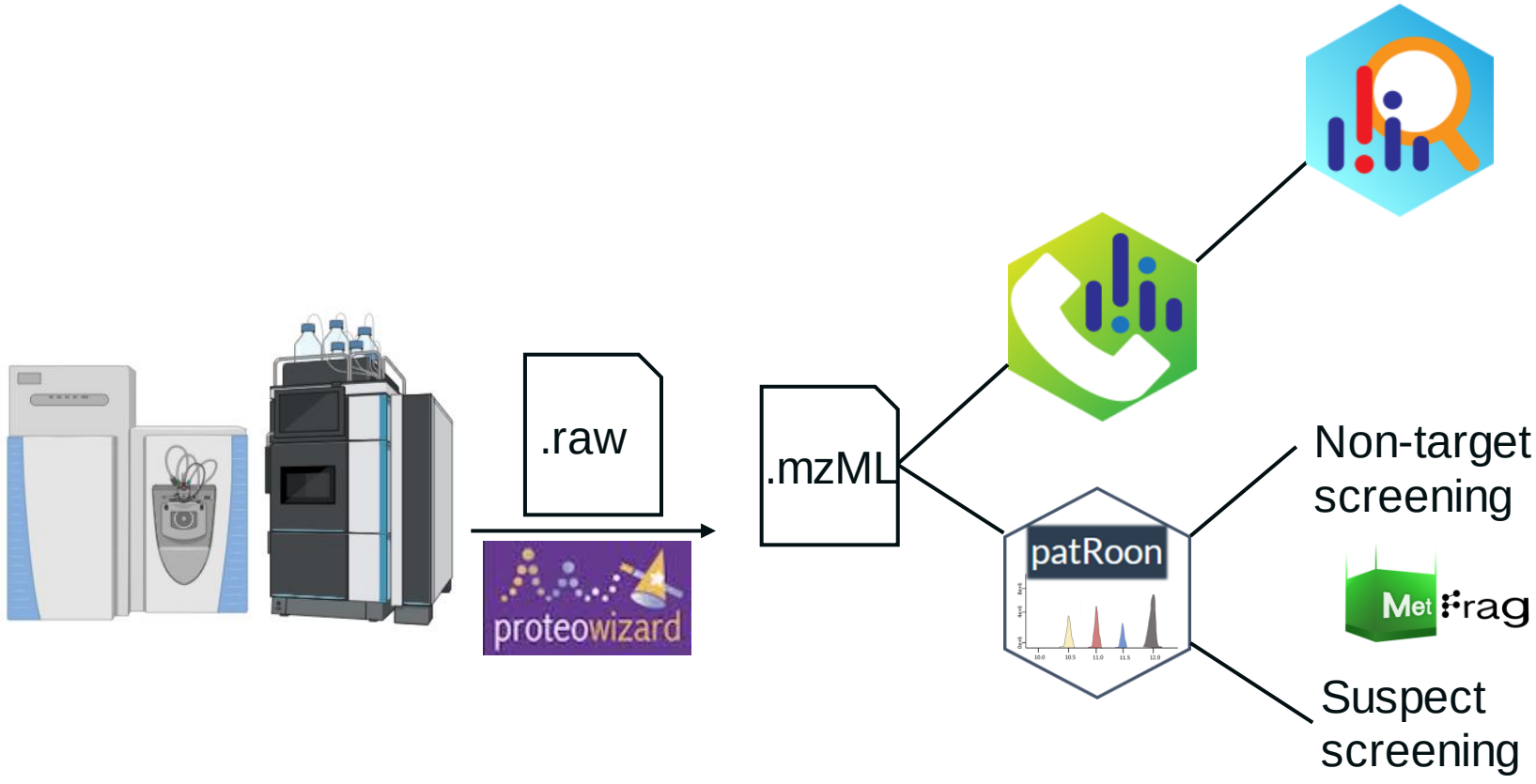


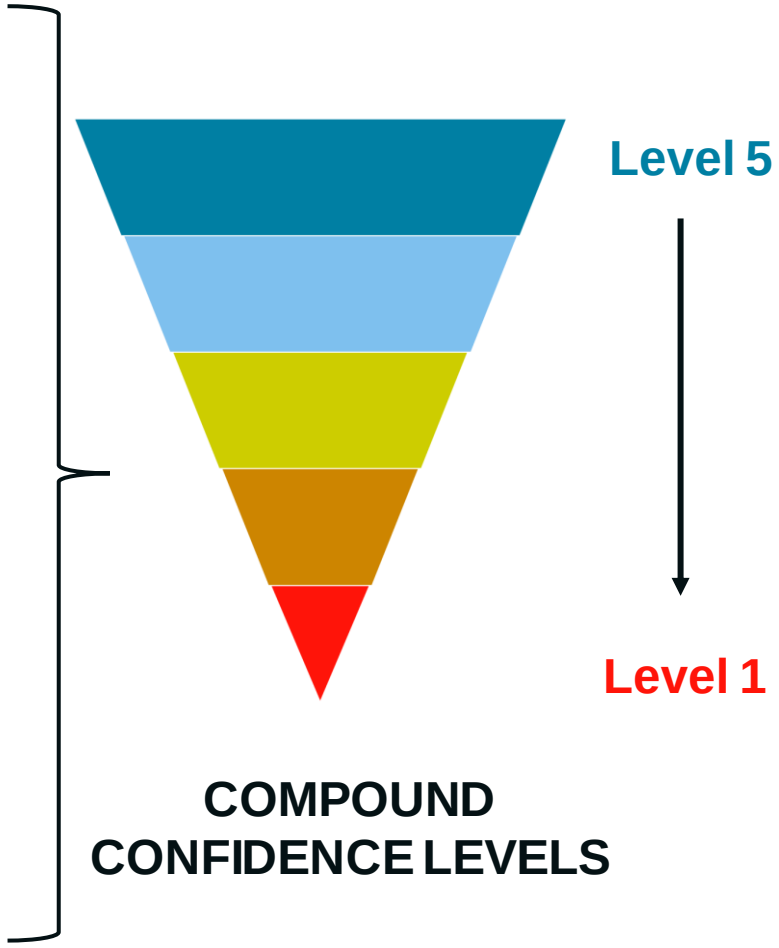
NON-TARGET ANALYSIS

NfL: Neurofilament Light

B. Talavera Andújar *et al.* (2022). *ABC.* DOI: 0.1007/s00216-022-04207-z

2.3. Data processing

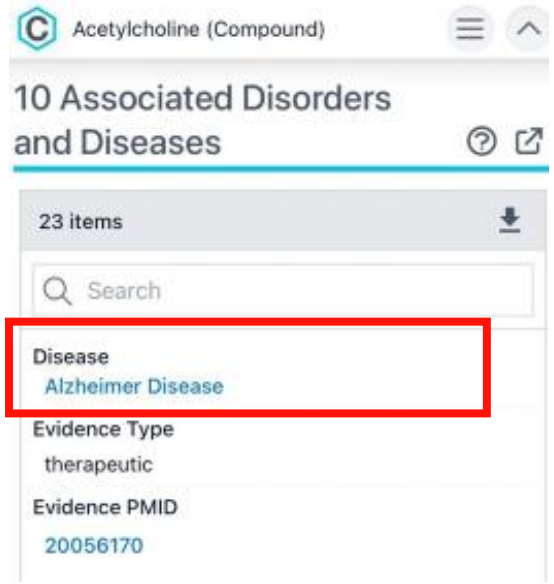


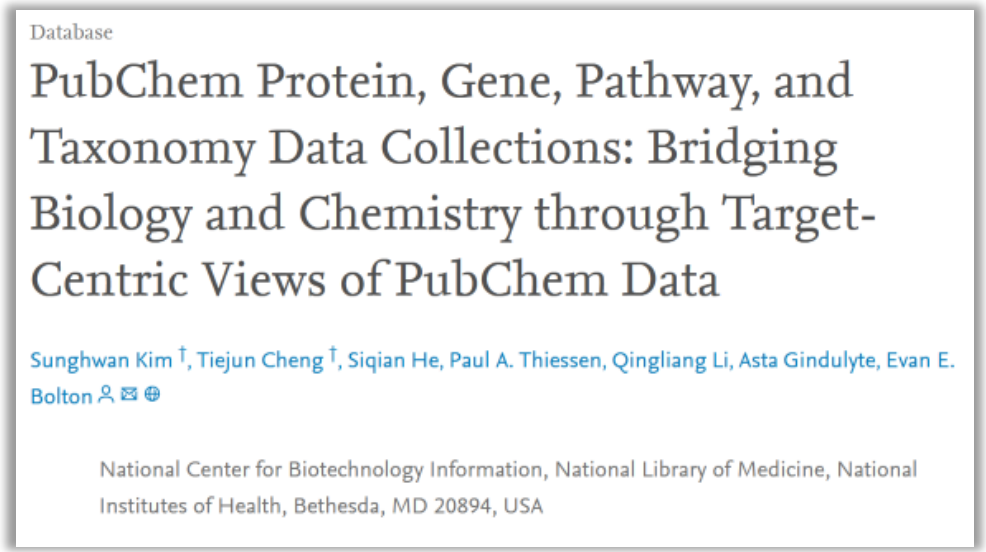


ANOVA with post-hoc Tukey's HSD
Linear multiple regression analysis

Schymanski et al, (2014) *ES&T*. DOI: 10.1021/es5002105
B. Talavera Andújar *et al*. (2022). *ABC*. DOI: 0.1007/s00216-022-04207-z
Tsugawa, H et at. (2015). *Nat Methods*. DOI: https://doi.org/10.1038/nmeth.3393
Helmus, R. et at. (2020). *J Cheminform*. DOI: https://doi.org/10.1186/s13321-020-00477-w

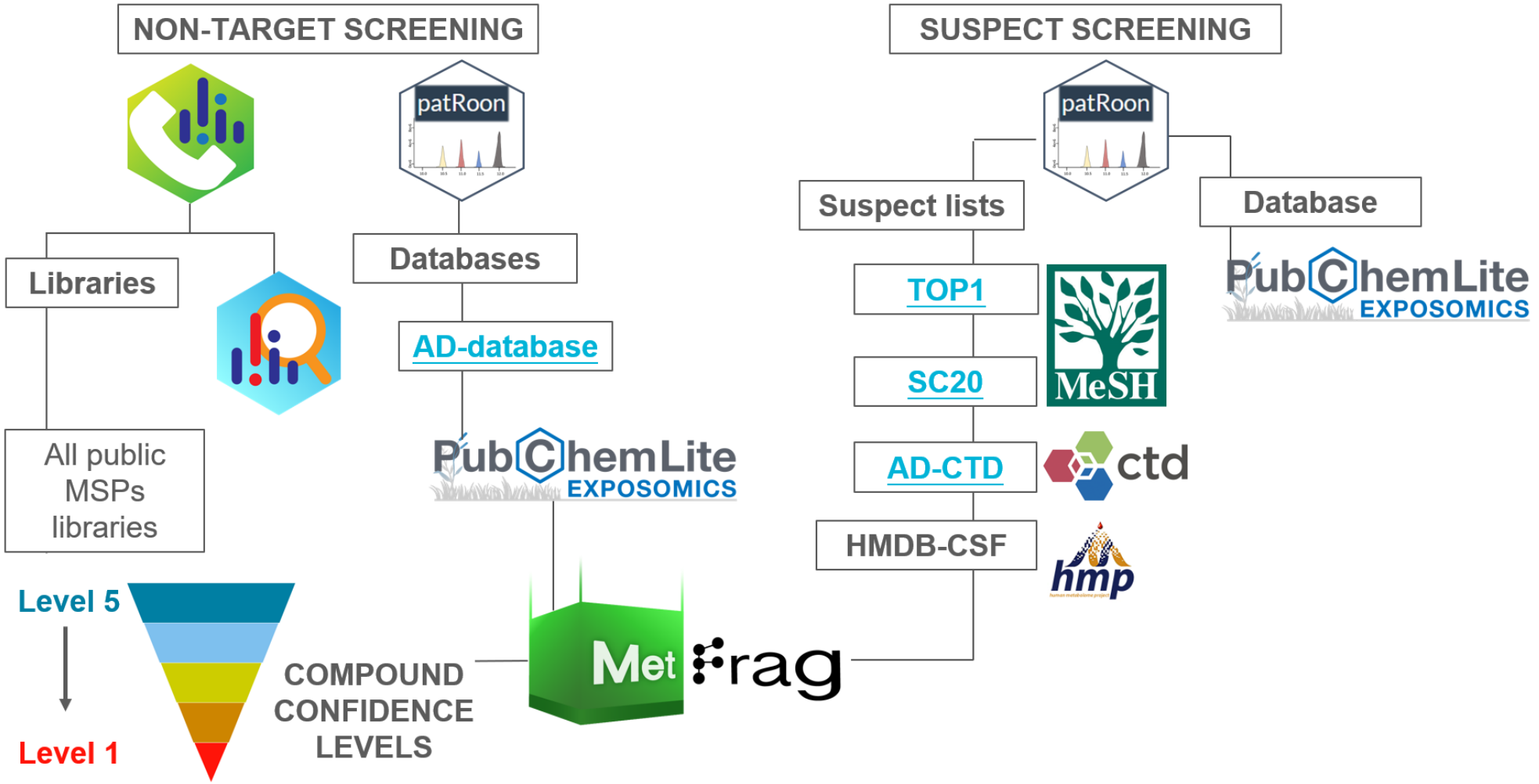
2.3. Data processing – Disease-specific information





Kim S. et al. (2022). DOI: 10.1016/j.jmb.2022.167514 & Zaslavsky *et al.* (2022) DOI: 10.3389/frma.2021.689059

2.3. Data processing



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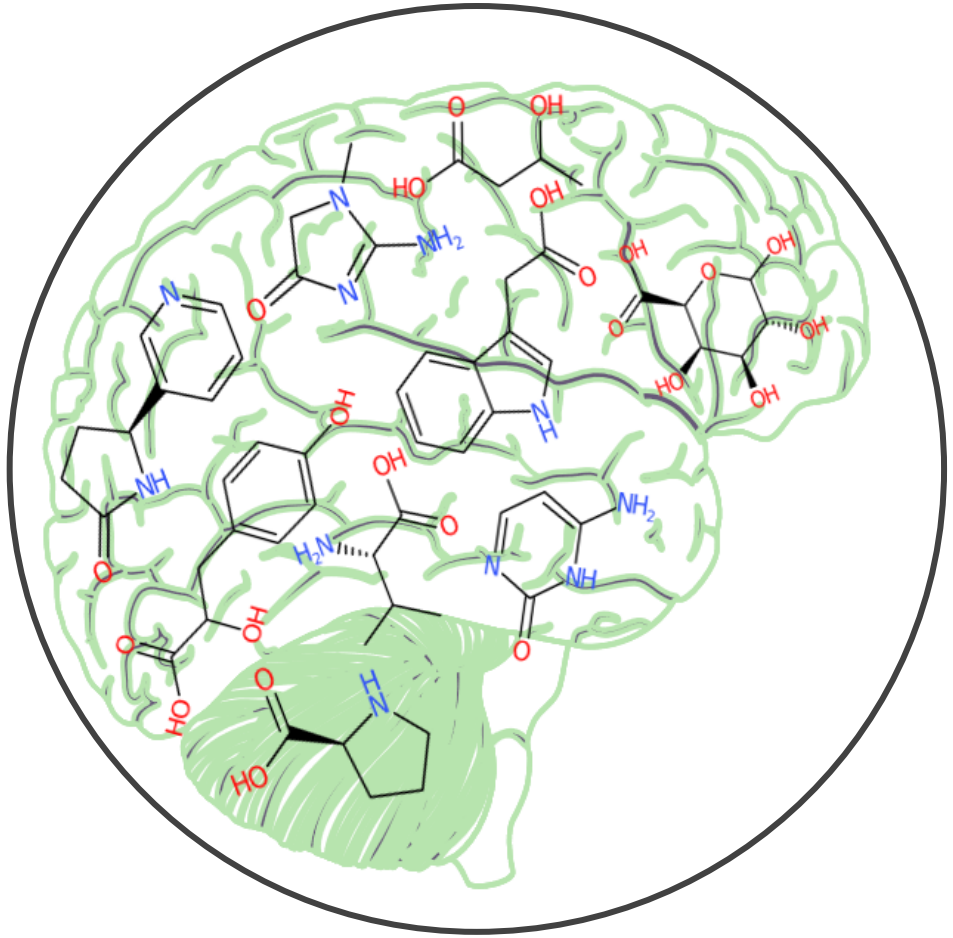
OPEN SCIENCE

CHECK THE CODES HERE! GitLab

Talavera Andújar B. et al. (2023). ChemRxiv. DOI: 10.26434/chemrxiv-2023-6j2gm

Outline



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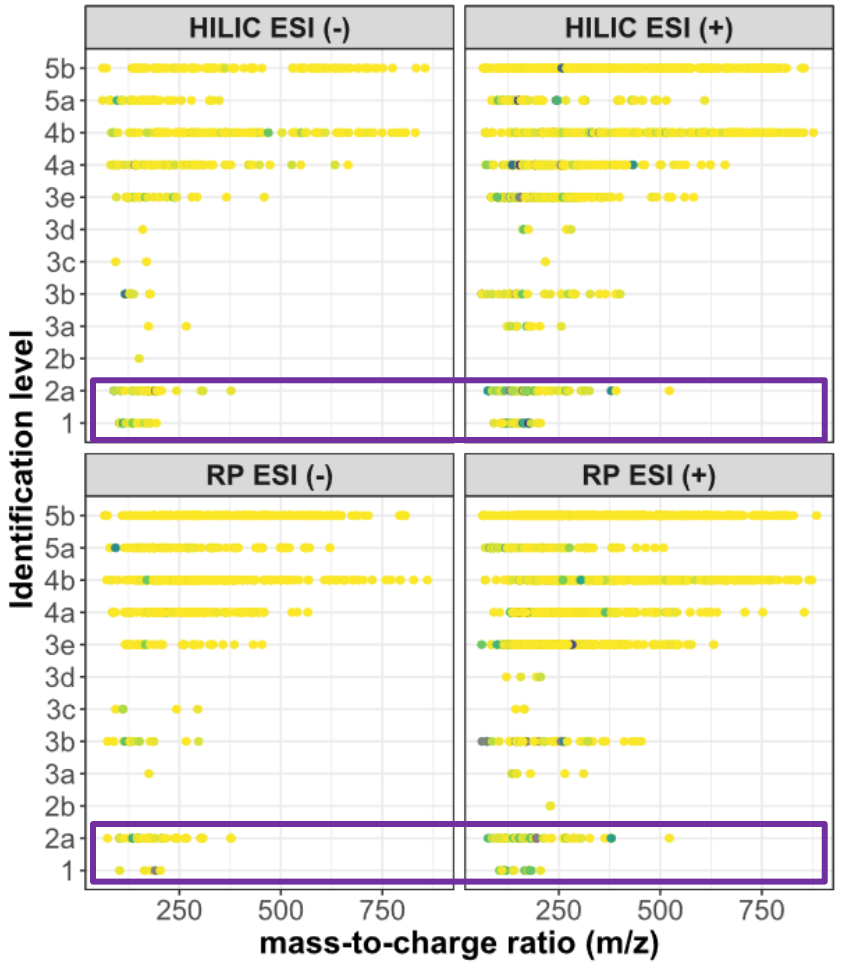
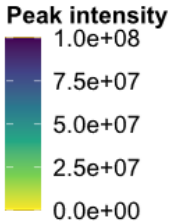
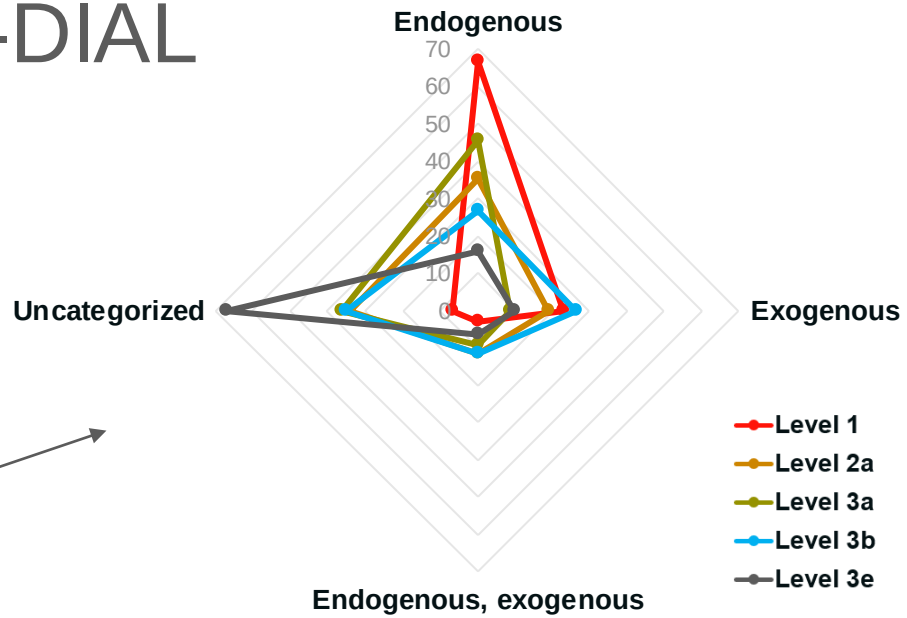
3.1. Compound coverage by MS-DIAL

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

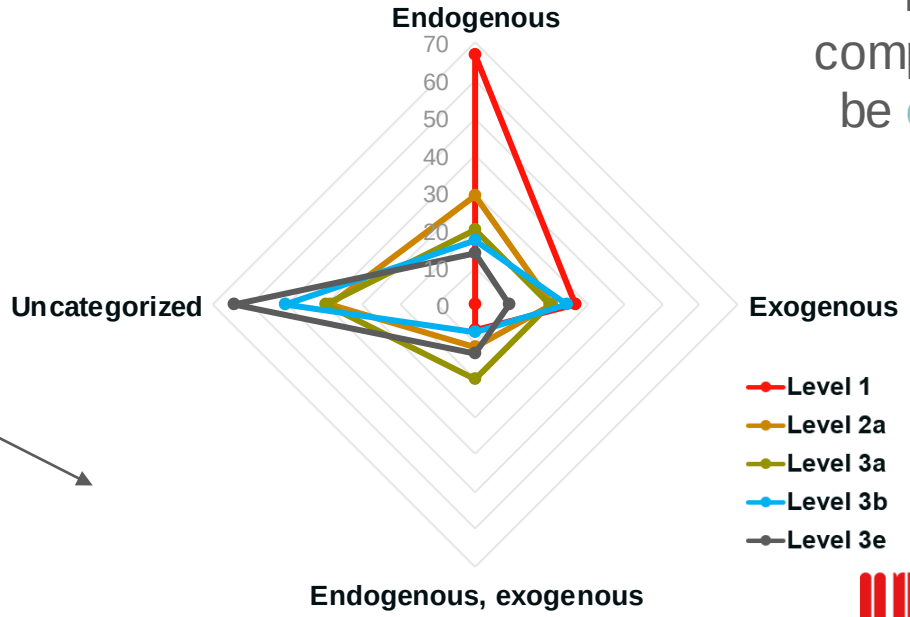




HILIC

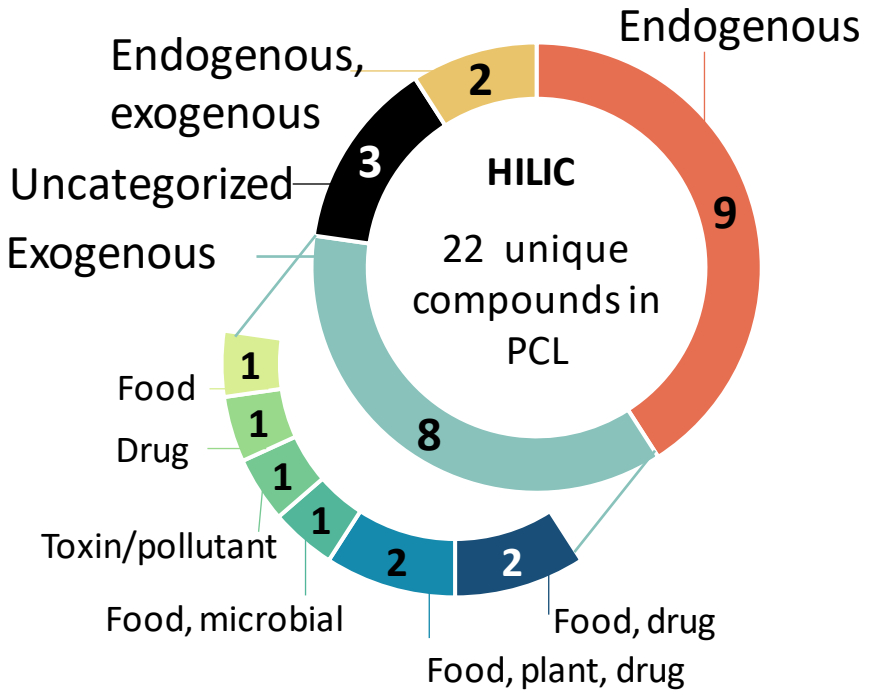


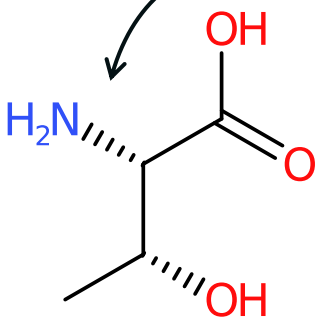
RP



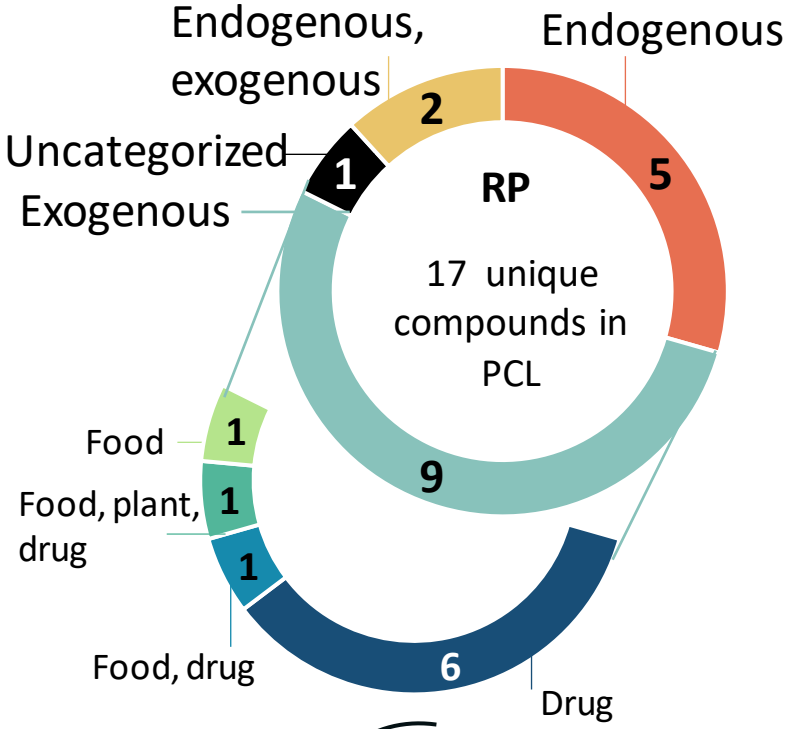
Level 1-2a compounds tend to be **endogenous**

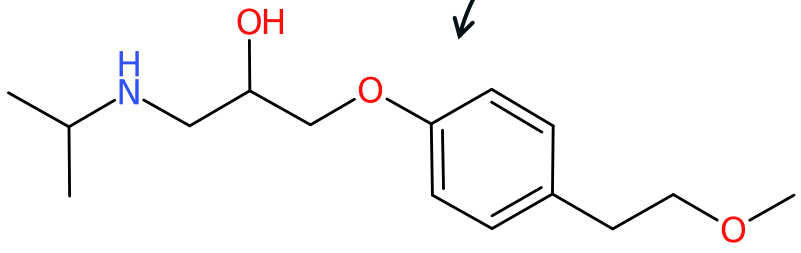
3.2. Compound coverage by patRoon





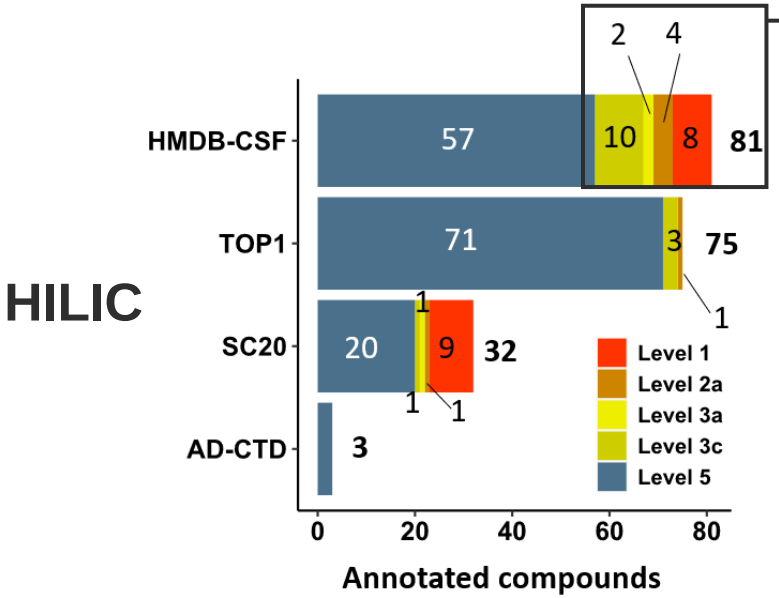
L-threonine

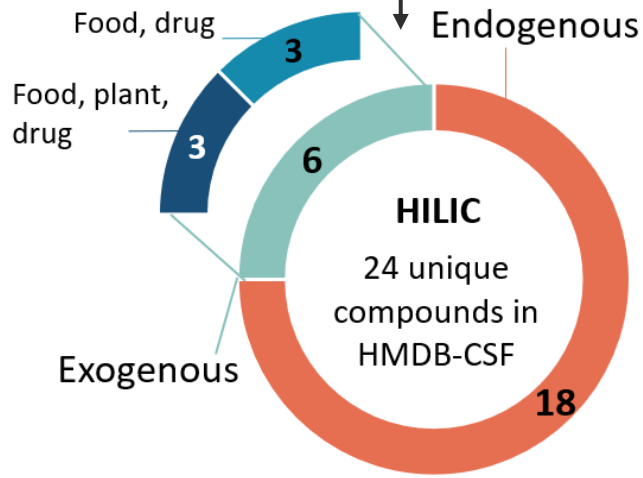


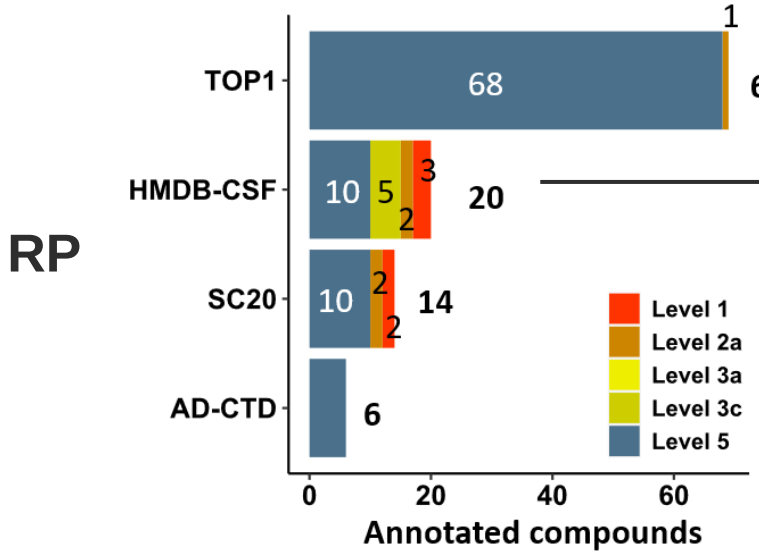


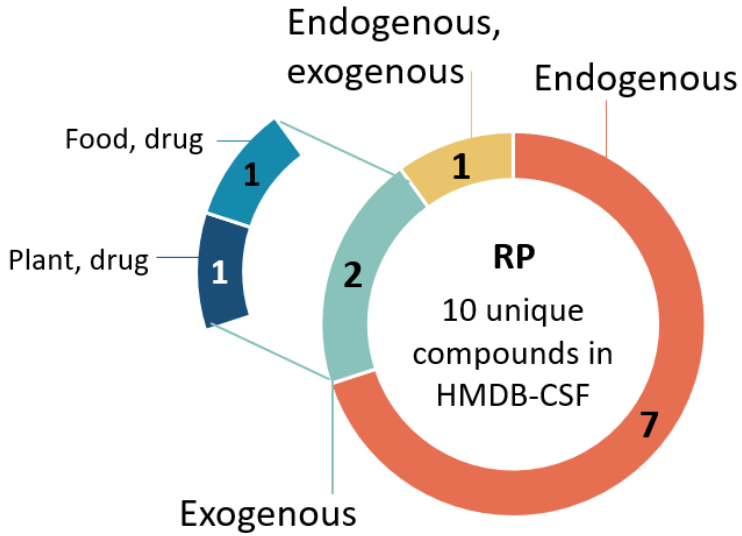
Metoprolol

3.2. Compound coverage by patRoon

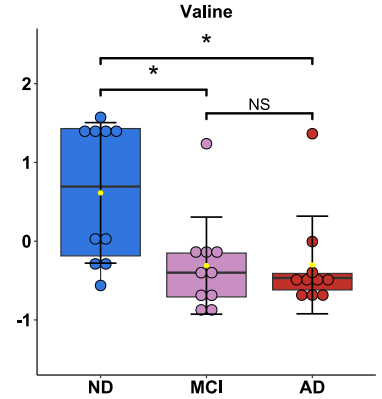


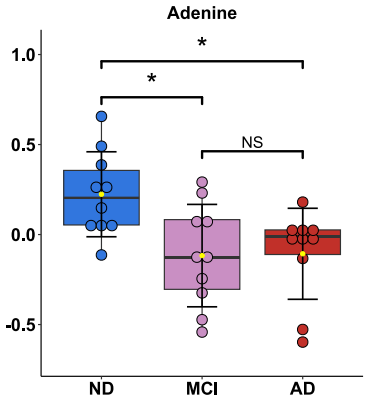


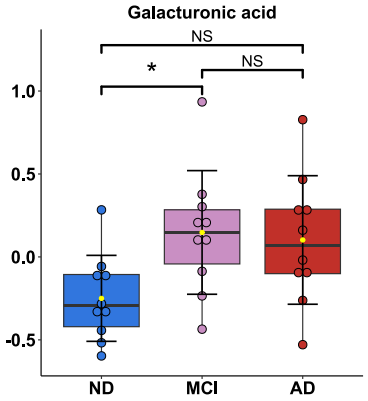




3.3. Statistically relevant compound found across groups







Amino acids and derivatives

01

Proline, valine, creatinine, N-acetylhistidine

Nucleobases

02

Adenine, cytosine

Sugars and sugar acids

03

Threonic acid, galacturonic acid, hexoses

Level 1-2a with post-hoc p-value < 0.05

Environmental, life style

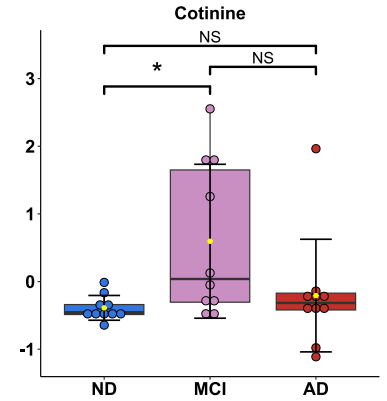
04

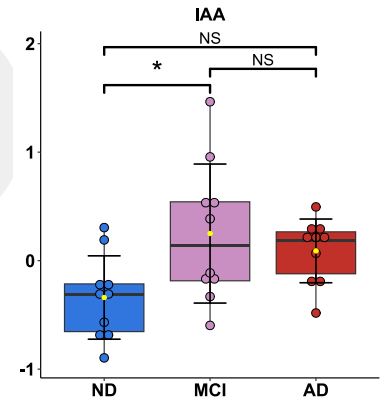
Cotinine, diazepam

Potential microbial metabolies

05

IAA, 4-HPLA and BHBA



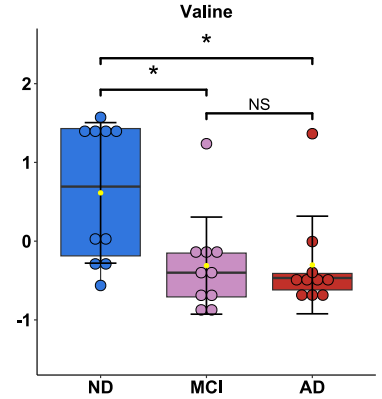


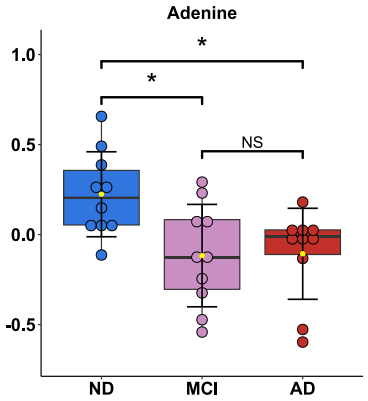
IAA: Indole-3-Acetic Acid
BHBA: β-hydroxybutyrate

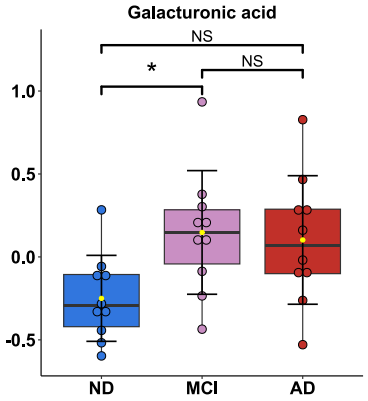
NS: Not-Significant differences

4-HPLA: 4-Hydroxyphenyllactic acid
*: post-hoc p-value <0.05

3.3. Statistically relevant compound found across groups







Amino acids and derivatives

01

Proline, valine, creatinine, N-acetylhistidine

Nucleobases

02

Adenine, cytosine

Sugars and sugar acids

03

Threonic acid, galacturonic acid, hexoses

Level 1-2a with post-hoc p-value < 0.05

Environmental, life style

04

Cotinine, diazepam

Potential microbial metabolies

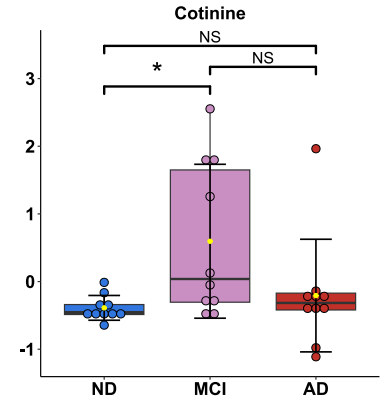
05

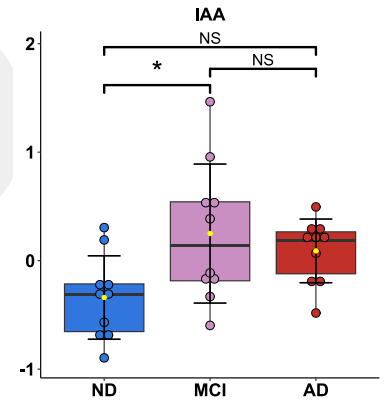
IAA, 4-HPLA and BHBA

Enrichment analysis

06

Most enriched pathways are related to the amino acid synthesis





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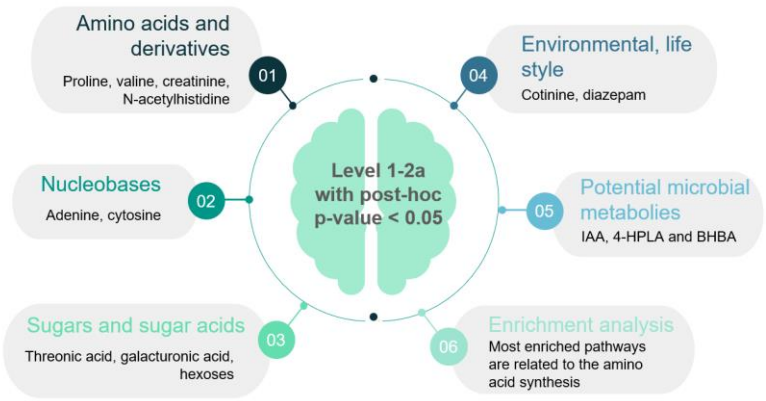
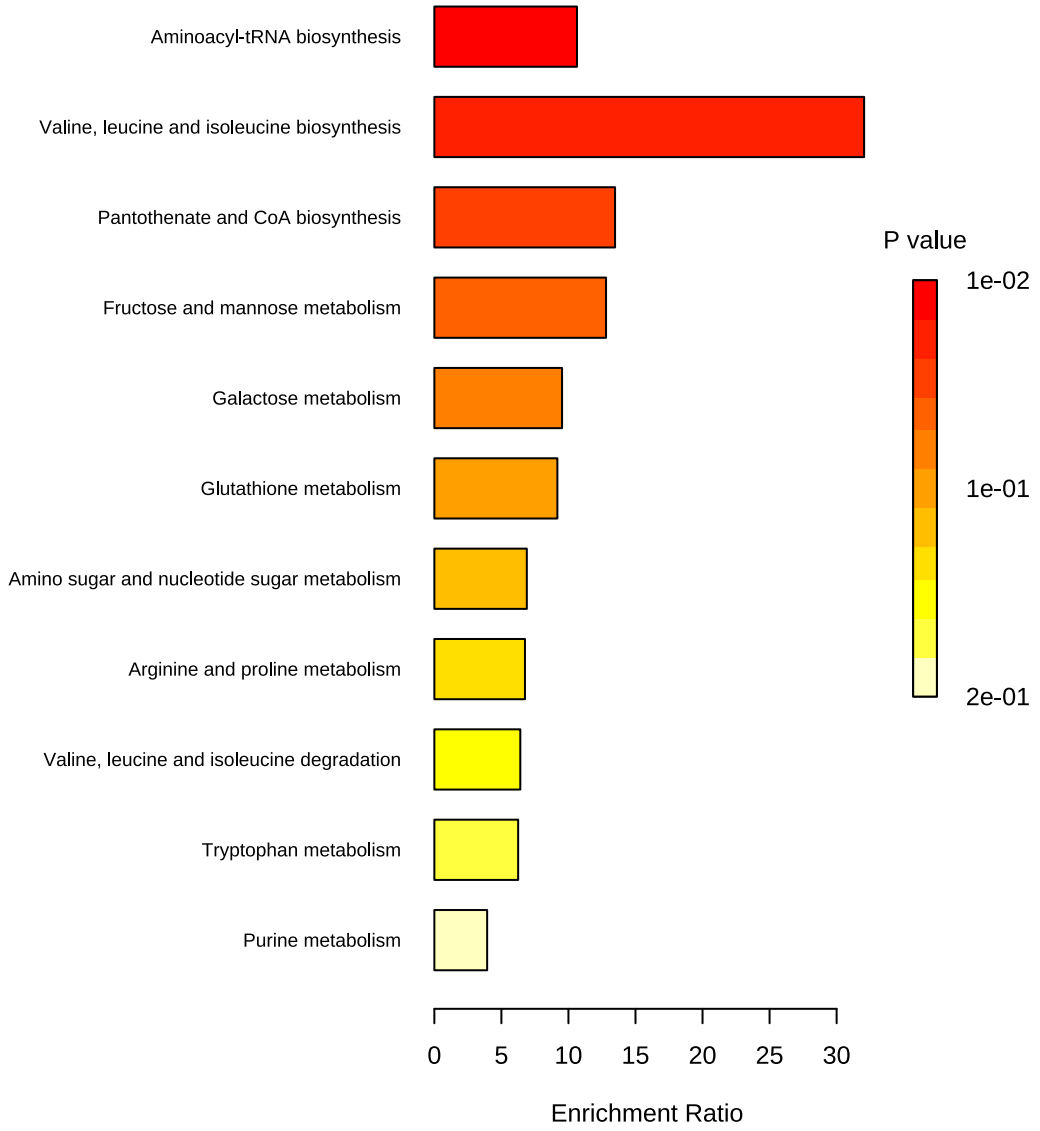
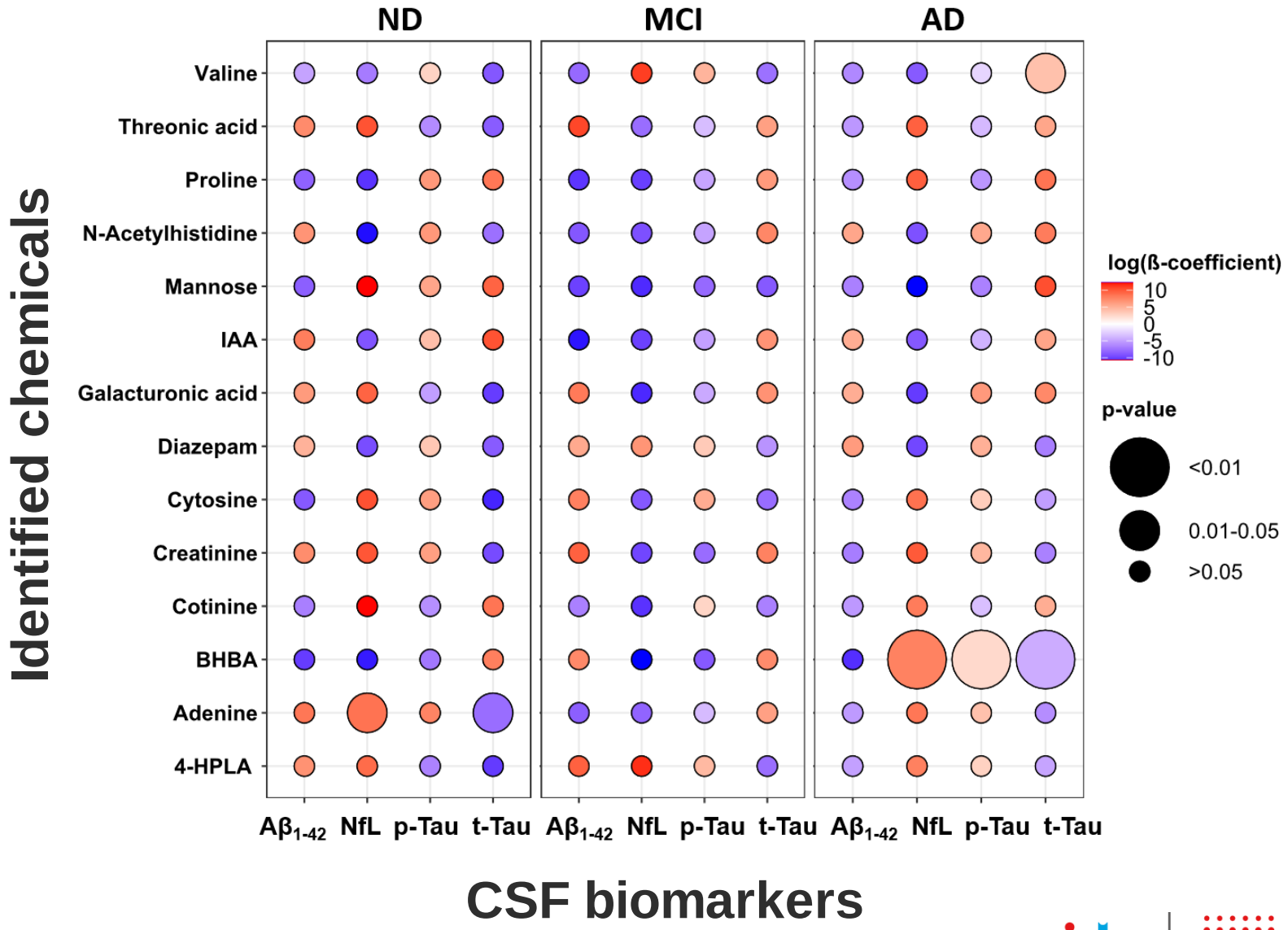




3.4. Studying potential association between chemicals and CSF disease biomarkers

The potential associations between the **CSF biomarkers** and the **identified chemicals** were studied



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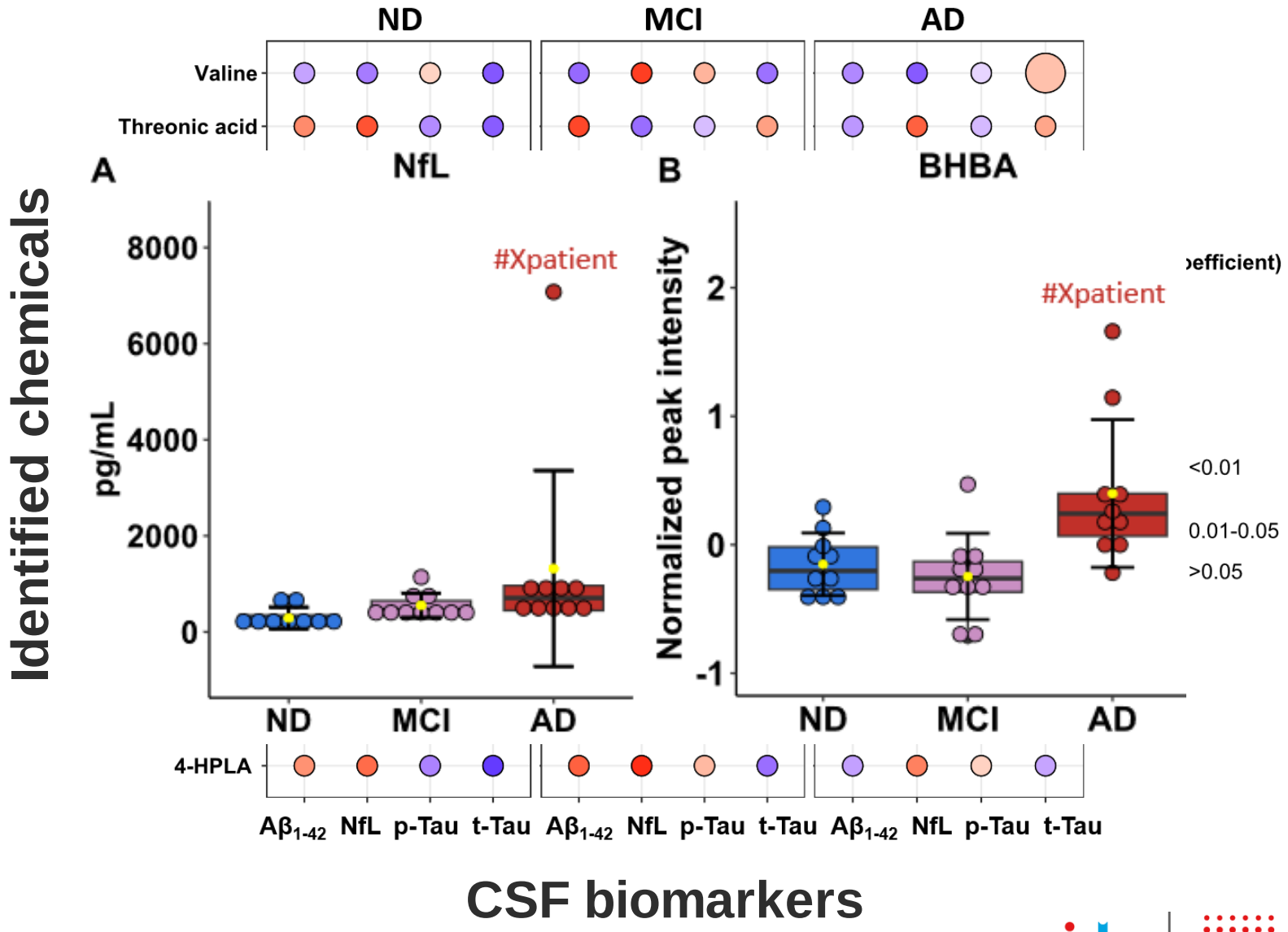

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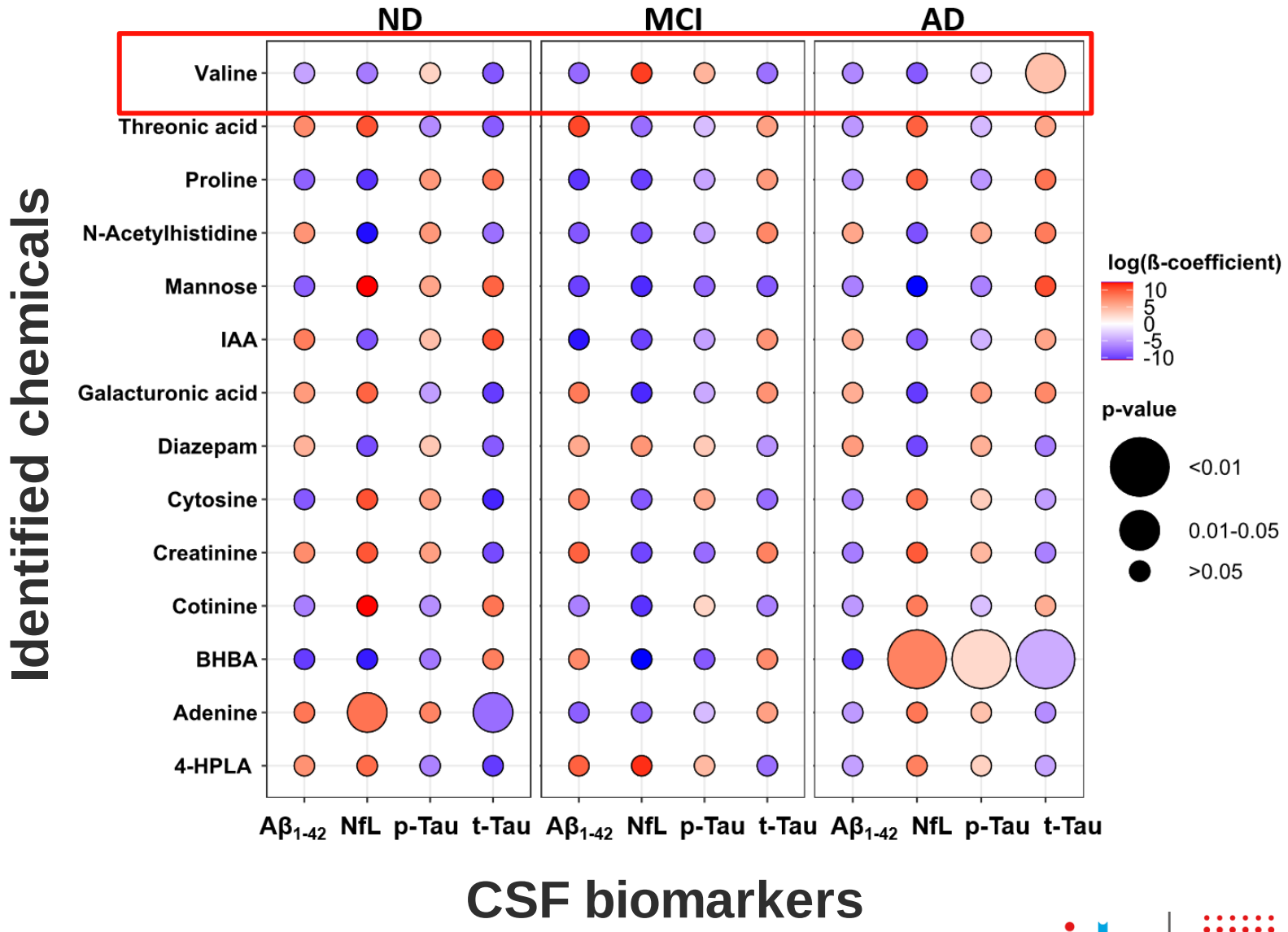

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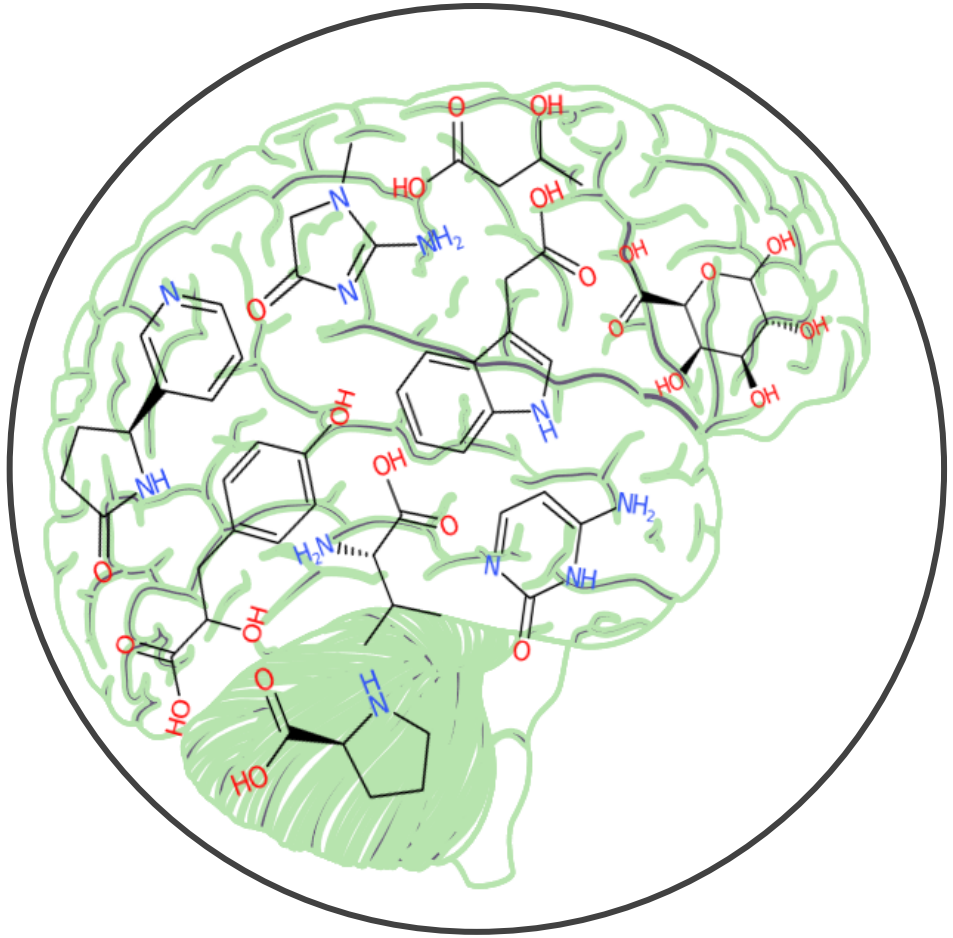
Outline



1. Introduction
2. Material and methods
3. Results and discussion
4. Conclusions and future perspectives

4. Conclusions and further perspectives

01 The combination of different analytical methods (RP+HILIC) and softwares allowed us to find a broader range of chemicals

02 Different compounds (amino acids, sugars, nucleobases) were found altered across groups, being promising targets for future experiments

03 Higher number of samples + different timepoints will be necessary to validate the findings presented here

04 Some compounds are not registered (yet) in the CSF-HMDB. **Share your data!**

THANK YOU FOR YOUR ATTENTION

Thank you to all ECI group!!

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Thank you all the YOUNG NMC members!

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