



# Boosting the profiling of clinical metabolomics projects using a novel trapped ion mobility spectrometry qTOF-MS

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## Introduction

- **Untargeted metabolomics** of biofluids via LC-MS is more and more applied in **clinical research**, e.g. breast cancer[1], Alzheimer's disease[2] and ischemic stroke[3]
- Often, **more than 80%** of all features in a sample are **not annotated** [4], which raises the **need for better annotation** algorithms, higher accuracy and sensitivity.
- Trapped Ion Mobility Spectrometry (e.g. TIMS) brings in a new and 4th dimension to annotation, however lacks sensitivity for especially very small metabolites

## Data Acquisition

- LC-timsTOF measurement of **three typical clinical biofluid samples**: urine, blood serum and cerebrospinal fluid (CSF)
- Sample preparation: protein precipitation (serum, CSF) or dilution (urine) with ACN, centrifugation,
- LC-MS mode: HILIC (Mobile phase A: 10 mM Ammonium formate + 0.1% FA, mobile phase B: 90% ACN in 10 mM Ammonium formate + 0.1 % FA), positive acquisition mode, *m/z* range 50-1,300
- Measurement on **timsTOF Pro 2** (Tübingen, GER) and new **timsMetabo** (Bremen, GER) for increased coverage
  - Combination of TIMS (Ion Mobility -> CCS) and tandem mass spectrometry (-> *m/z*, MS/MS)
- Annotation via in-house libraries (Annot. Level 1, Pro 2) + commercial spectral libraries (Annot. Level 2, Pro 2 + Metabo)



## Results

### Cerebrospinal fluid (CSF)

- Alzheimer's disease (AD) patients (n= 15) + Parkinson's disease (PD) patients (n = 15)
- **334 annotations** from both instruments combined
- **Increased sensitivity** of timsMetabo, especially for amino acids, lyso-PCs (Fig. 1)

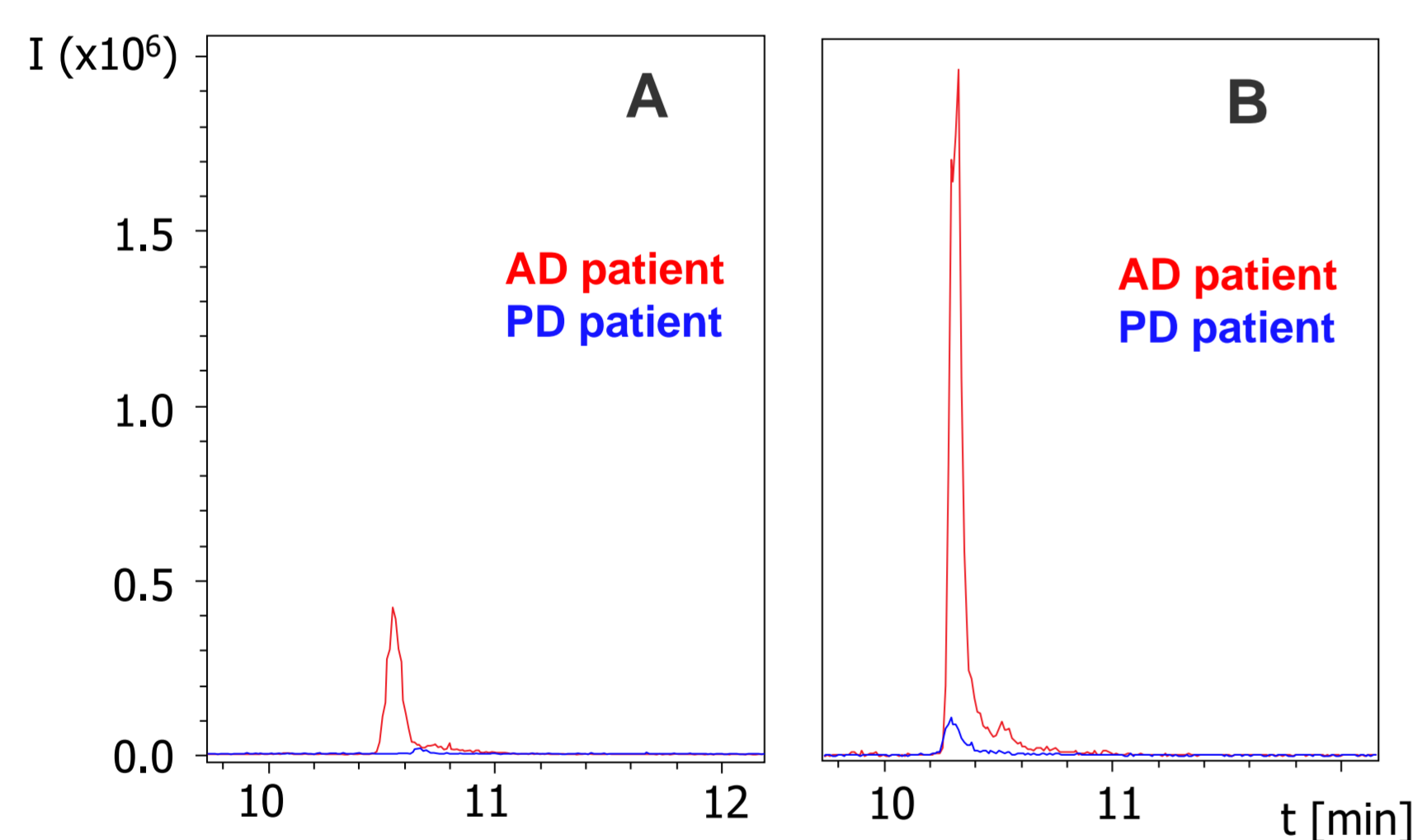


Fig. 1: EICs of L-Arginine ([M+H]<sup>+</sup>, *m/z* 175.11952) from measurements on A) timsTOF Pro 2 and B) timsMetabo

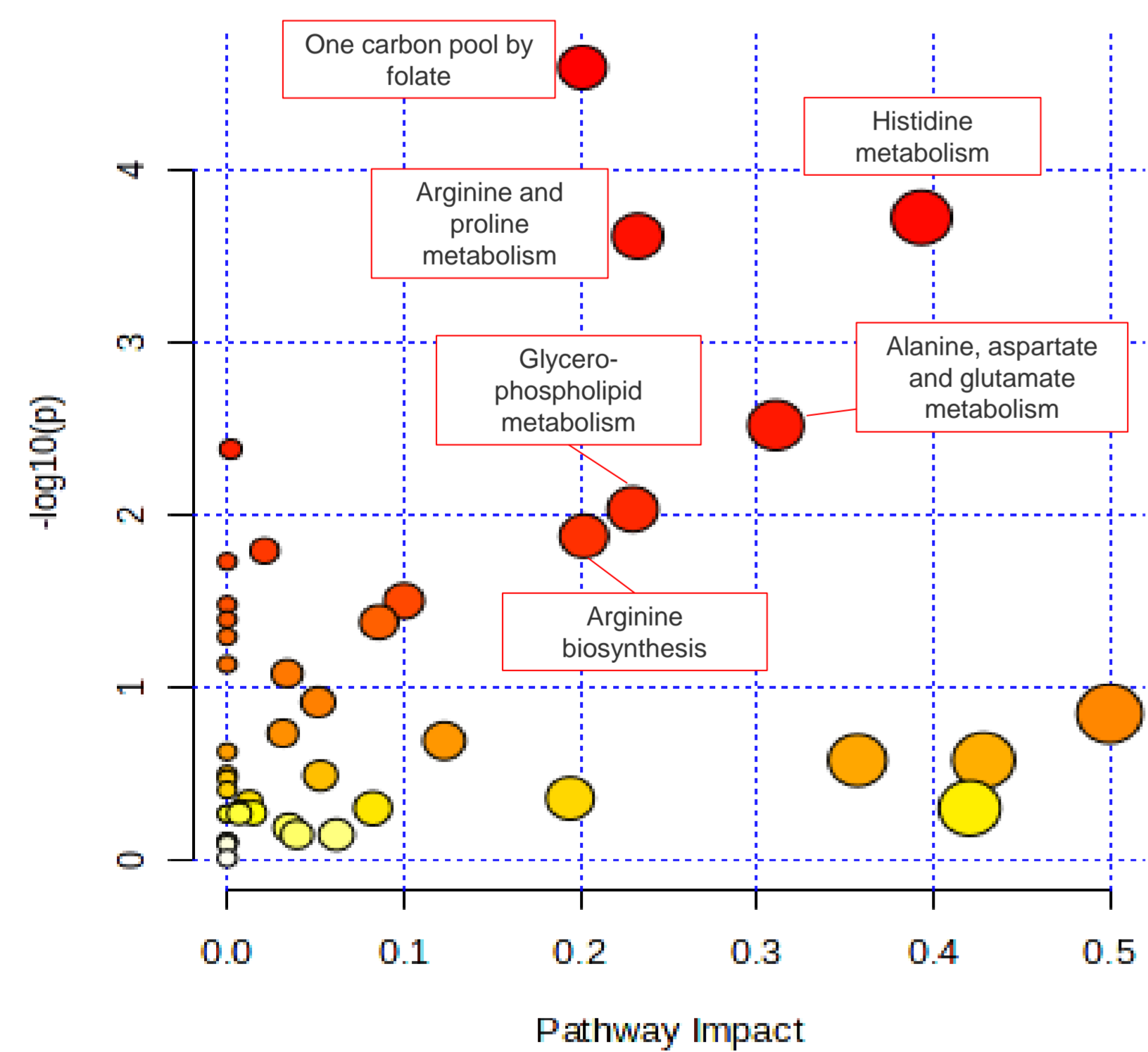


Fig. 2: Metabolic pathway analysis of identified compounds in CSF from AD and PD patients (combined data from timsTOF Pro 2 and timsMetabo)

### Urine

- Metachromatic Leukodystrophy (MLD)
  - Progressive nervous system degradation
- Samples from n = 25 patients
- In-house libraries (MS, MS<sup>2</sup>, IM, RT) account for **57% of annotations**
  - Internal library still gold standard
- Metabolic differences consistent with **build-up of waste products and motor dysfunction** (Fig. 3)

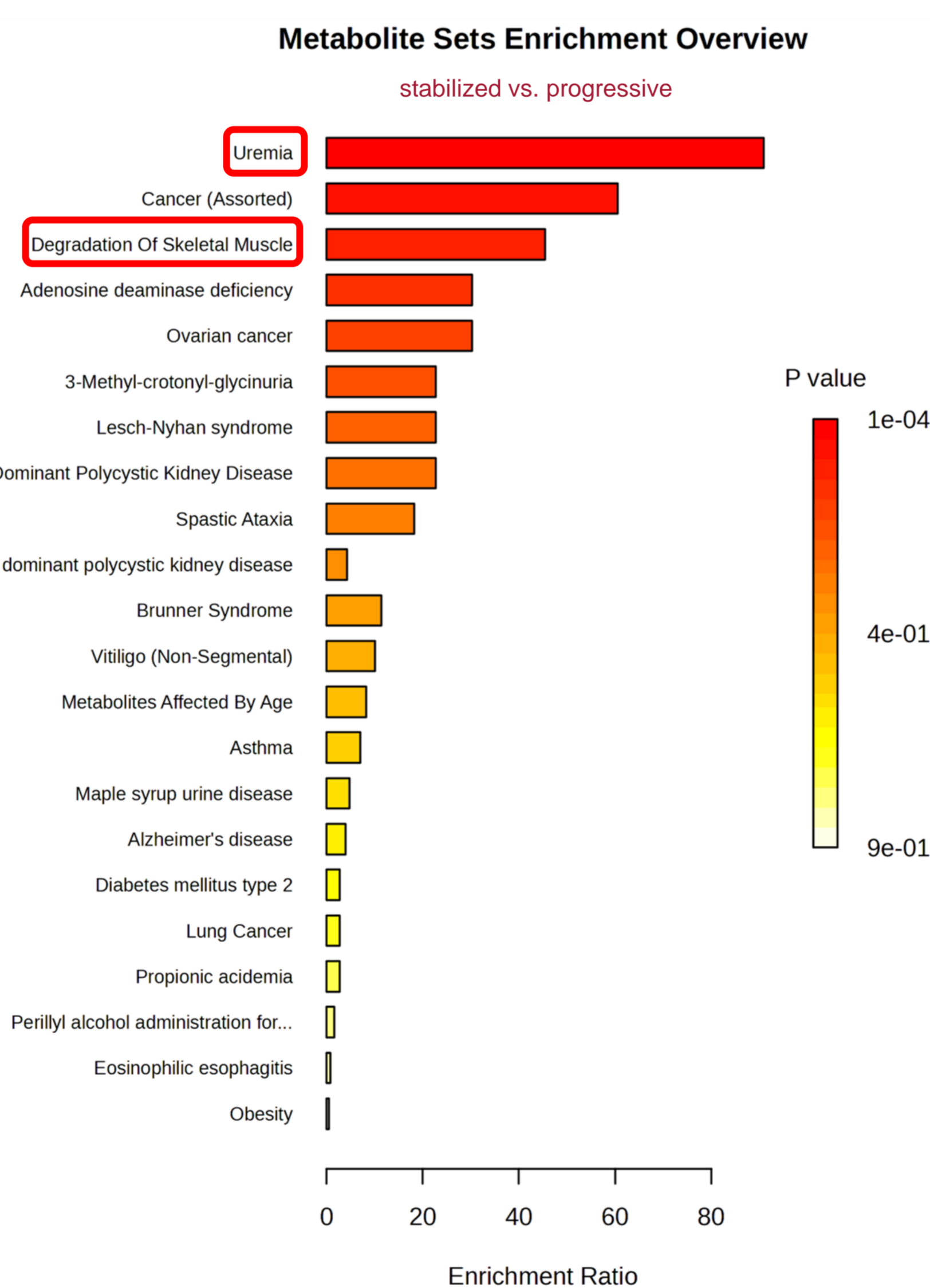


Fig. 3: Phenotype-based enrichment analysis of stabilized vs progressive patients

### Blood serum

- Endometriosis patients (n=4) and healthy control (n = 9); measured on timsMetabo
- Differences between both phenotypes (Fig. 4)

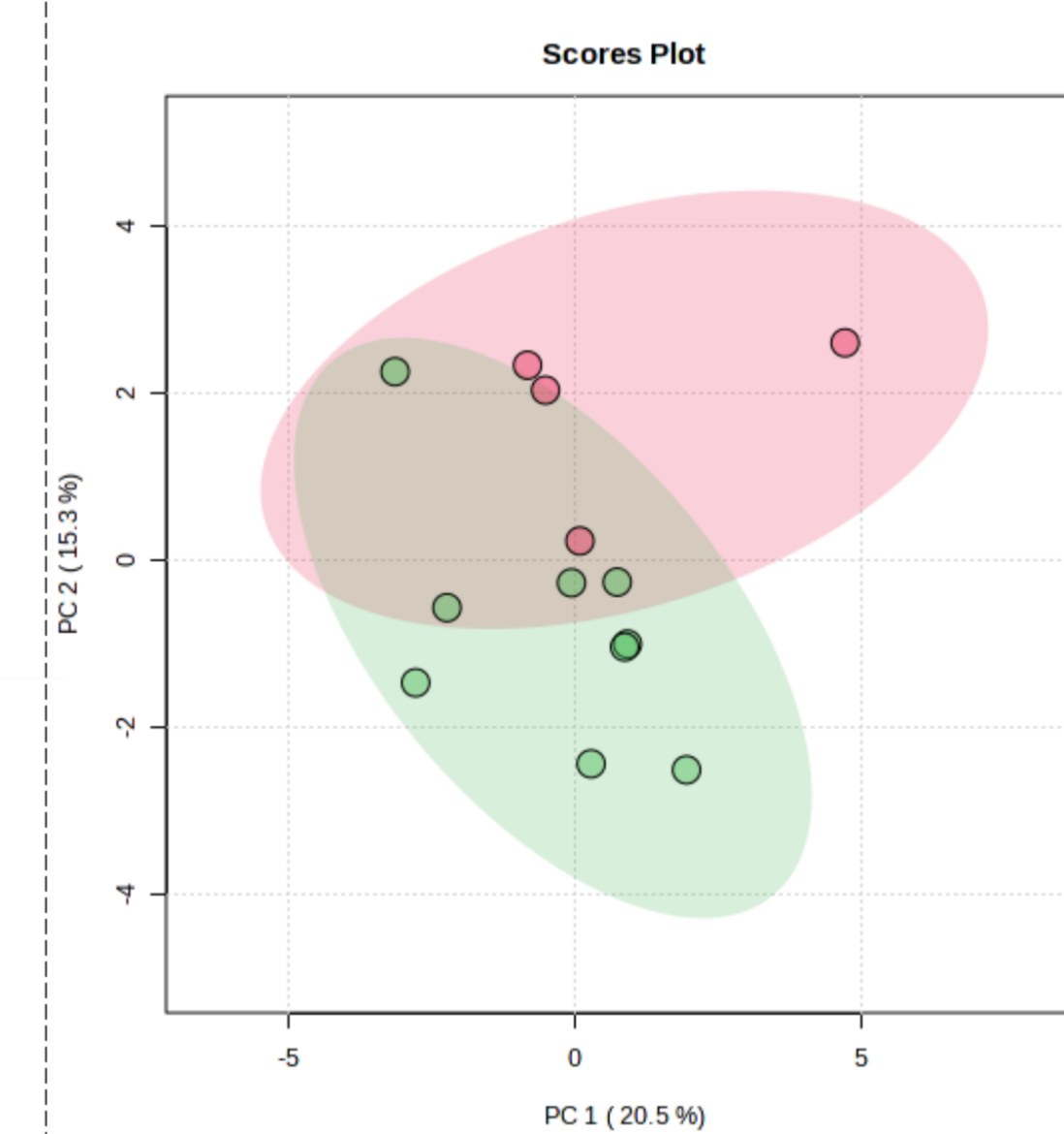


Fig. 4: Phenotype-based PCA 2D scores plot

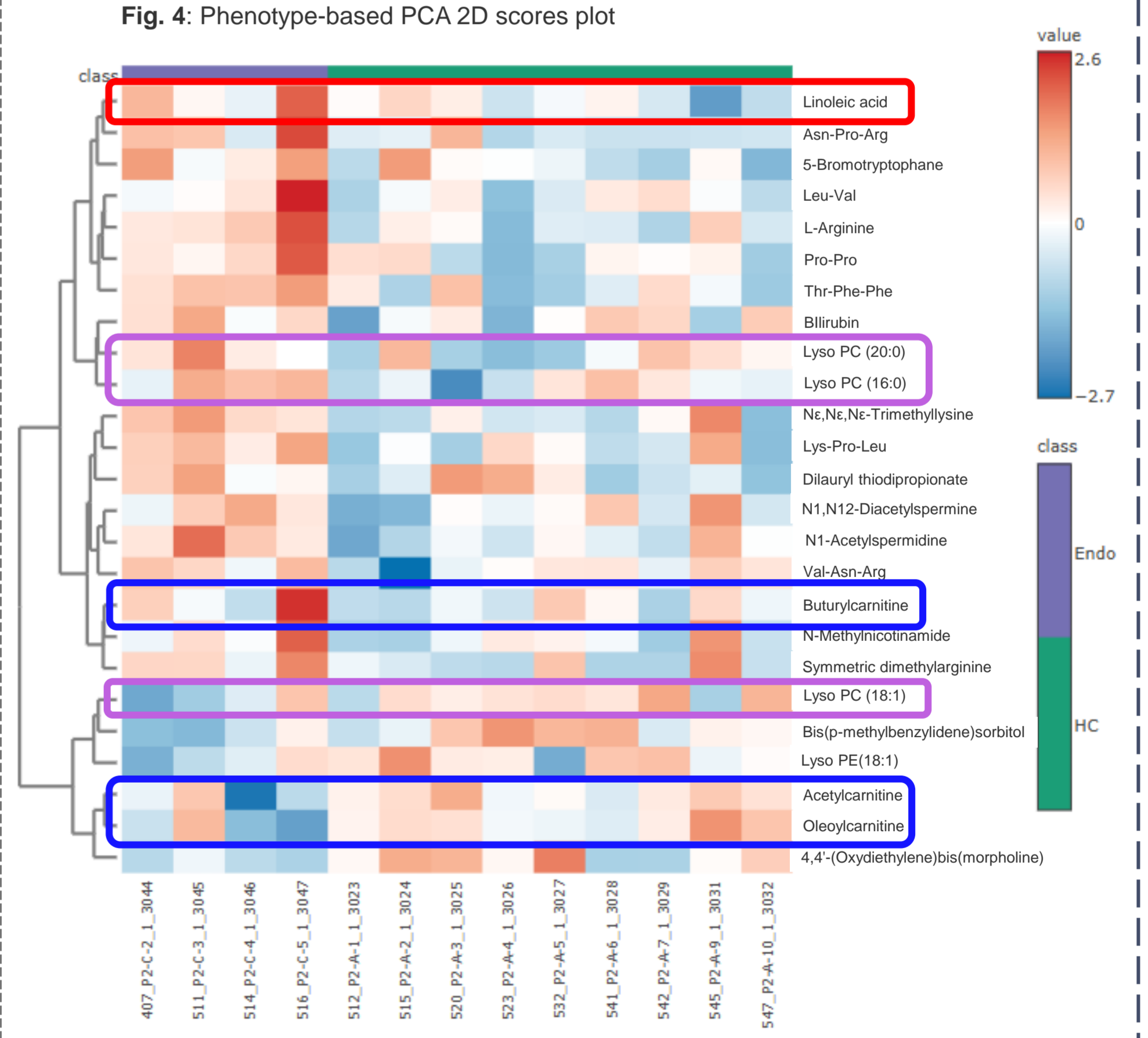
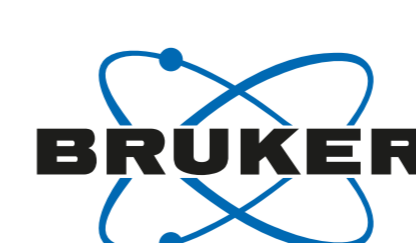


Fig. 5: Top 25 significant metabolites of the serum samples from endometriosis and HC samples

## Summary

- LC-timsTOF based analysis of typical biofluids indicated **improved phenotype stratification**, covering both polar metabolites and lipid compounds.
- New hardware developments can further **increase coverage** of metabolite annotations, especially for low molecular weight compounds.
  - Optimized workflow and inclusion of **ion mobility technology** increases both number and confidence in annotations



### References

- [1] N. Amiri-Dashatan *et al.*, Int. J. Biol. Markers. 2022, 37(4):349-359, doi:10.1177/03936155221123343
- [2] T Milos *et al.*, Prog Neuropsychopharmacol. Biol. Psychiatry 2023, 127:110830, doi:10.1016/j.pnpbp.2023.110830
- [3] X Wang X *et al.*, Front. Neurosci. 2021 14:580929. doi: 10.3389/fnins.2020.580929
- [4] Y. El Abiead *et al.*, Nat Metab. 2025, 7(3):435-437, doi: 10.1038/s42255-025-01239-4