Comprehensive *in vivo* multiomics analysis of Alzheimer's disease pathogenesis in plasma

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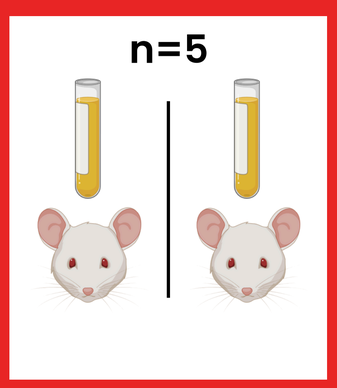
Introduction

Alzheimer's disease (AD), affecting over 24 million people worldwide, remains the leading cause of dementia, underscoring the critical need for new therapeutic targets. Conventional research has predominantly focused on the amyloid cascade hypothesis, which attributes AD pathogenesis to the aggregation of amyloid-β protein1. Yet, this framework inadequately captures the disease's multifaceted symptomology. This study advances our understanding by integrating high-throughput mass spectrometry-based proteomics, metabolomics and lipidomics, offering a comprehensive examination of AD's lipidome, proteome, and metabolome. We investigate the multiomic properties of the 5XFAD mouse model, which express human APP and PSEN1 transgenes with a total of five AD-linked mutations and recapitulate many AD-related phenotypes such as relatively early and aggressive presentation. Analysis of 5XFAD and wild-type control mouse plasma demonstrates notable differences in energy metabolism, heme metabolism, lipid transport and oxidative stress. These findings indicate the benefits of the inclusion of metabolomics and lipidomics in interpreting proteomics results.

Materials and methods

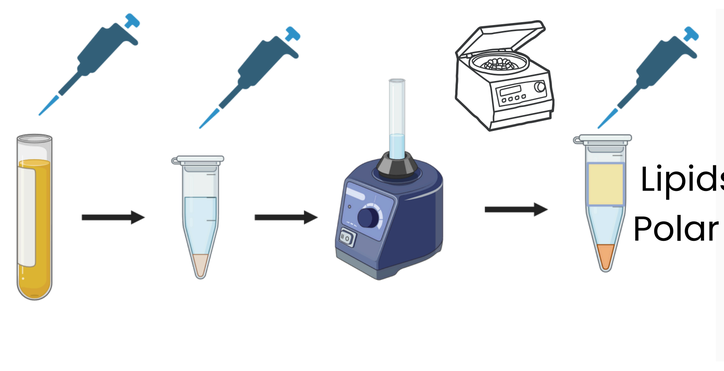
Metabolomics & Lipidomics Workflow



5XFAD vs Control

Proteomics Workflow

Sample Preparation

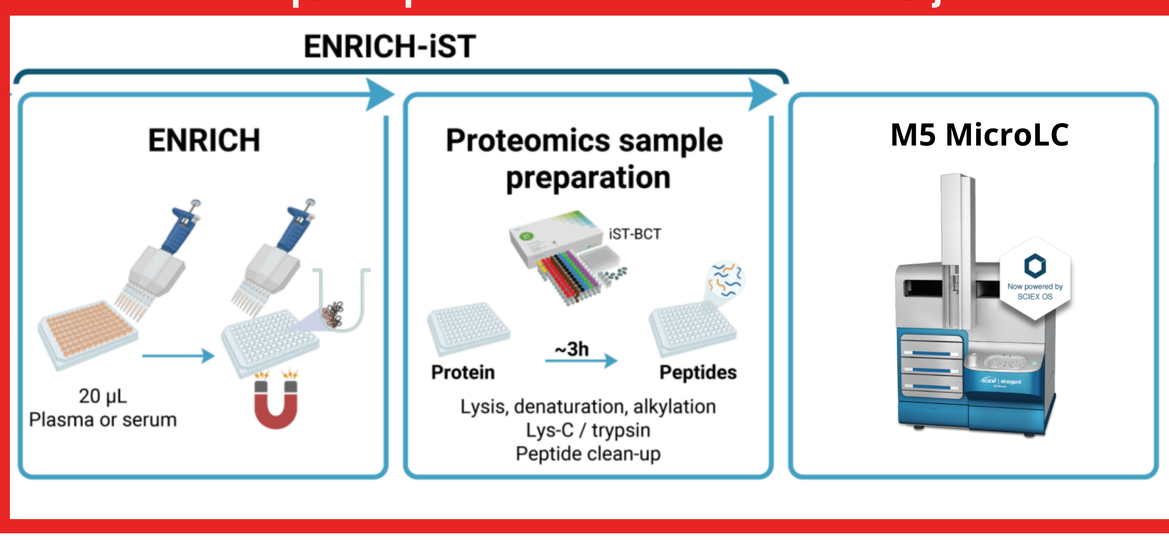


LC Injection

UHPLC 1290

Sample Preparation

LC Injection



MS Analysis

ZENOTOF 7600

- Increased Sensitivity and Depth (Zeno trap)
- SWATH-DIA MS/MS Acquisition on all precursors (DDA method)
- Improved Quantification and Identification

Data Analysis

oloMAP

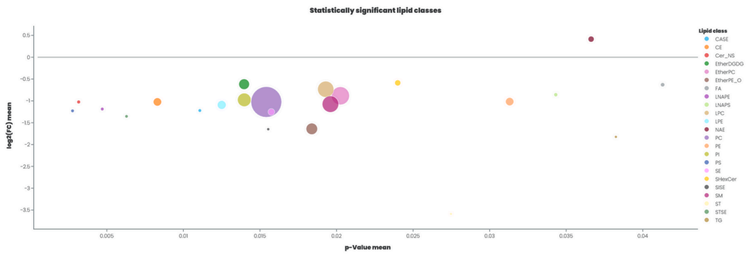
oloMAP v2.0 in-house sample managament system + data visualization and exploration platform for proteomics, metabolomics and lipidomics

DIA-NN

DIA-NN v1.8.1
Library-free search using UniProt UP000000589 proteome

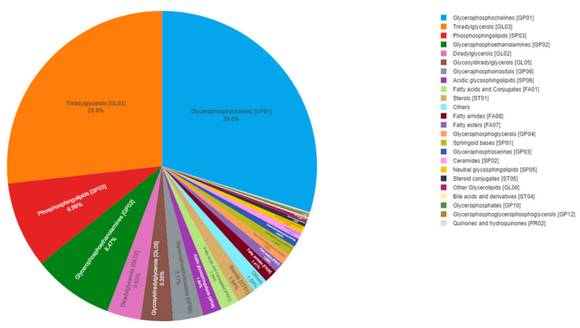
Results

Lipidomics (565 lipids)



47 LIPID SUB CLASS

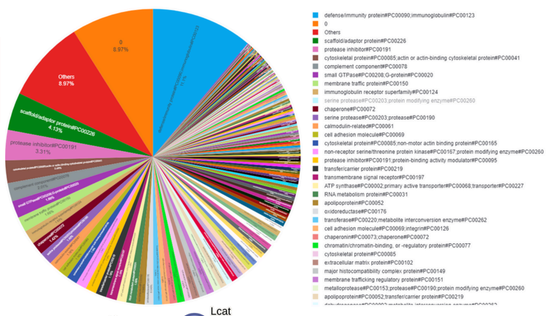
24 LIPID MAIN CLASS

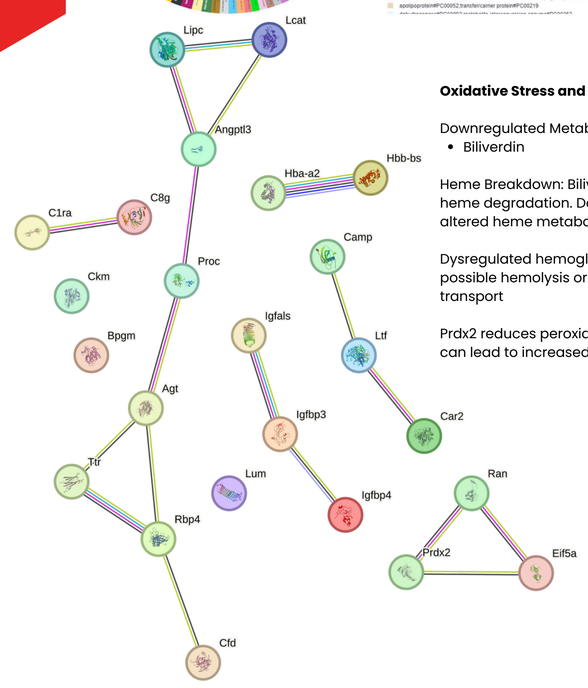


Proteomics (860 proteins)

122 protein class

801 Family





Oxidative Stress and Heme Metabolism

Downregulated Metabolites:
- Biliverdin

Heme Breakdown: Biliverdin is produced from heme degradation. Downregulation suggests altered heme metabolism

Dysregulated hemoglobin subunits indicate possible hemolysis or altered oxygen transport

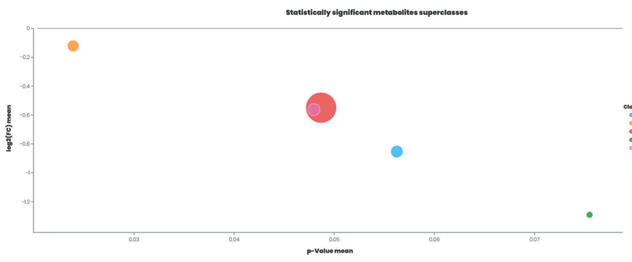
Prdx2 reduces peroxides; its dysregulation can lead to increased oxidative stress

Lipid Transport and Metabolism Proteins:
a. Angptl3 (Angiopoietin-like protein 3) regulates lipid metabolism by inhibiting lipoprotein lipase (LPL), affecting plasma triglyceride levels
- Downregulation of Angptl3 can result in increased LPL activity, enhancing the hydrolysis of triglycerides in lipoproteins
- This can lead to decreased levels of circulating triglycerides (TG) and associated lipid classes like phospholipids (PC, PE, PI, PS), and cholesteryl esters (CE)
- Implication: Altered lipid availability can affect neuronal membrane composition and function

b. Lipc (Hepatic triacylglycerol lipase) hydrolyzes triglycerides and phospholipids in lipoproteins
- Dysregulation affects the breakdown of triglycerides (TG) and phospholipids (PC, PE, LPC, LPE
- Reduced Lipc activity can contribute to decreased lipid turnover
- Implication: Accumulation or depletion of specific lipids can affect cellular signaling and membrane dynamics

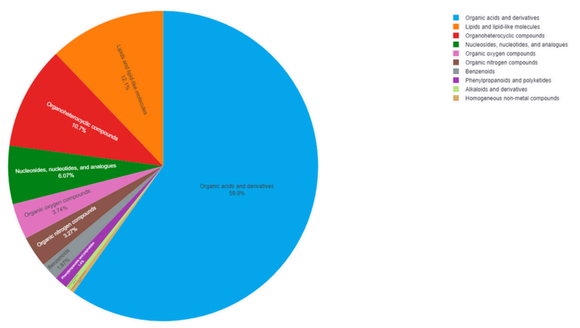
c. Lcat (Phosphatidylcholine-sterol acyltransferase) esterifies cholesterol to form cholesteryl esters (CE), which are important in lipid transport processes
- Dysregulation of Lcat can result in decreased CE formation and affect circulating PC levels
- Implication: Impaired cholesterol esterification affects cholesterol homeostasis and membrane composition

Metabolomics (214 polar metabolites)

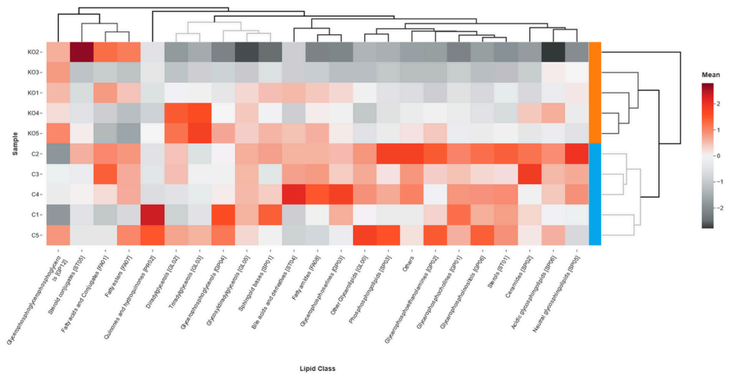


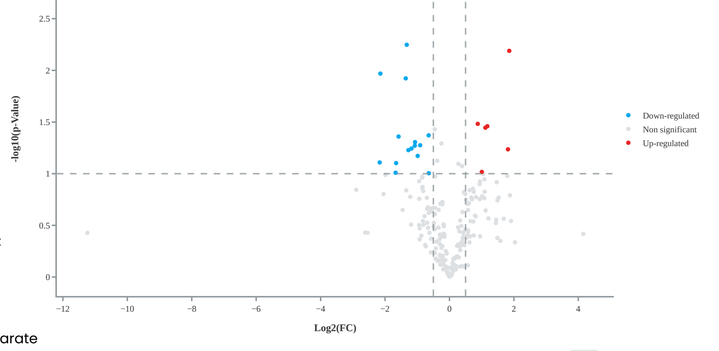
37 ClassyFire

10 Superclass



Correlation between the 3-omics





Energy Metabolism
Downregulated Metabolites:
- Glucose
- Citric acid
- Isocitric acid
- cis-Aconitic acid
- 3-Hydroxy-3-methylglutarate

Correlation and Interpretation:
- Glucose is the primary energy source for neurons, while Citric acid, isocitric acid, and cis-aconitic acid are key intermediates in the TCA cycle
  - Downregulation suggests altered energy production capabilities
  - Ckm is vital for ATP regeneration, and its dysregulation can exacerbate energy deficits
  - Bpgm regulates glycolysis through 2,3-BPG levels, affecting oxygen delivery and utilization

Conclusion

The identification of differentially expressed proteins, metabolites and lipids provides new insights into AD's molecular mechanisms, moving beyond the amyloid-centric paradigm. These findings emphasize the potential of uncovering novel AD therapeutic targets in preclinical drug development settings.

Ackowledgements

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References

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2. Tsugawa, H. et al. A lipidome atlas in MS-DIAL 4. Nat. Biotechnol. 38, 1159–1163 (2020).

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