High-Throughput µLC-MS/MS Lipidomics of 3D *In Vitro* Disease Models to Investigate Lipid Dysregulation

MUNI

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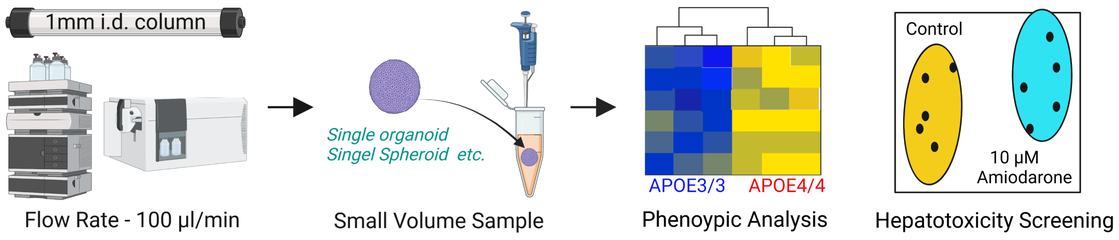
Background and Aim

- µLC-MS/MS is increasingly applied for the high-throughput profiling of biological samples and has proven an acceptable tradeoff between sensitivity and reproducibility.
- However, application in lipid profiling is limited.

Aims

- Optimization of sensitive and robust microflow lipidomics (µLC-MS/MS) workflow
- Demonstration of application in iPSC derived cerebral organoid and 3D-hepatospheroids

Optimization of µLC-MS/MS **Single Step Lipid Extraction** **Applications in 3D-*In Vitro* Models**



Flow Rate - 100 µl/min | Small Volume Sample | Phenoypic Analysis | Hepatotoxicity Screening

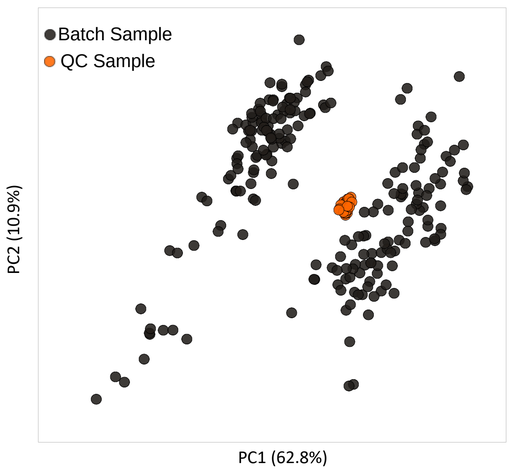
Optimization & Performance Evaluation

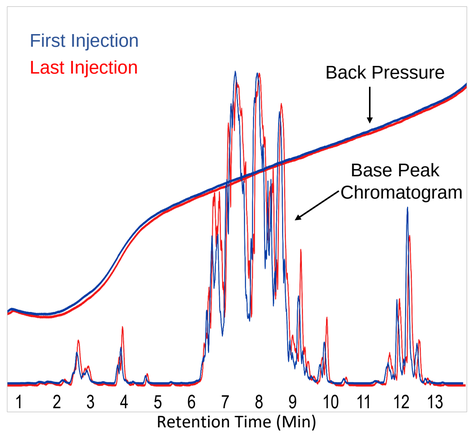
MicroLC-MS/MS Conditions

- *In vitro* samples extracted using isopropanol injected on 1290 Infinity II UHPLC coupled to 6469 QQQ LC-MS (Agilent). Reverse phase separation was performed using microbore column (CSH, 1 mm *100 mm, 1.7µm, Waters).

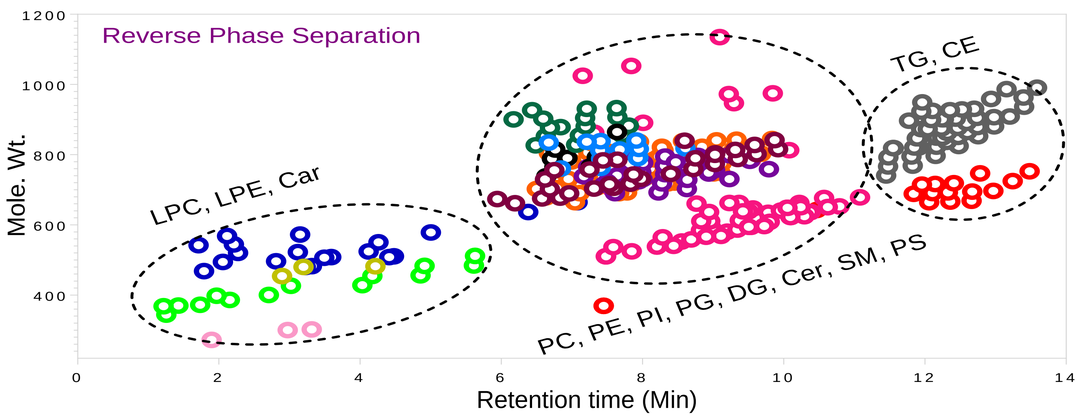
~300 sample injected for performance evaluation

- Pool QC samples injected throughout batch analysis, tight clustering in PCA plot demonstrate excellent reproducibility.
- Overlay of BPC and back pressure of first and last injection of batch analysis shows stable instrument performance.



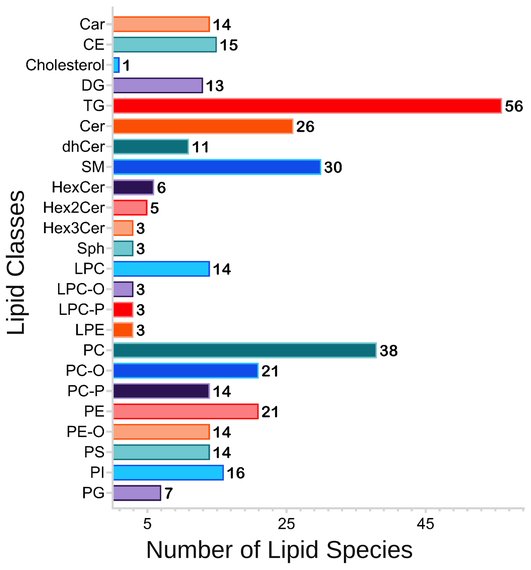


Chromatographic elution pattern of different lipid classes

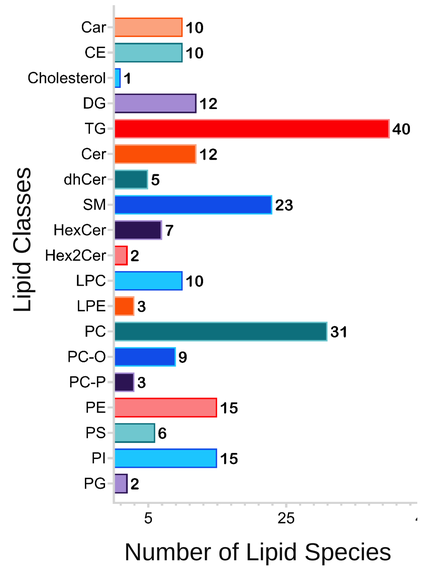


Sensitive and High-throughput Lipid Quantification from *In Vitro* sample

~350 Lipid species *measured from single cerebral organoid*



~216 Lipid species measured from single hepatospheroid

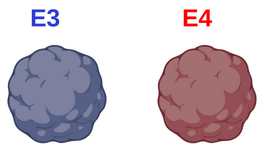


Application of Workflow

Exp. I – Understanding the lipid metabolism of APOE phenotypes (E3 vs E4) of iPSC derived cerebral organoid

Collaborator – Prof. Jiri Damborsky

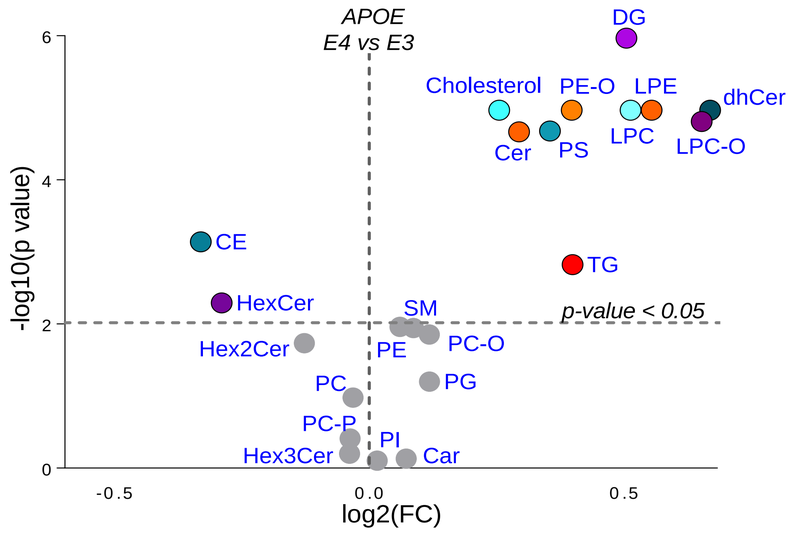
Cerebral Organoids
APOE Phenotypes (n=10)



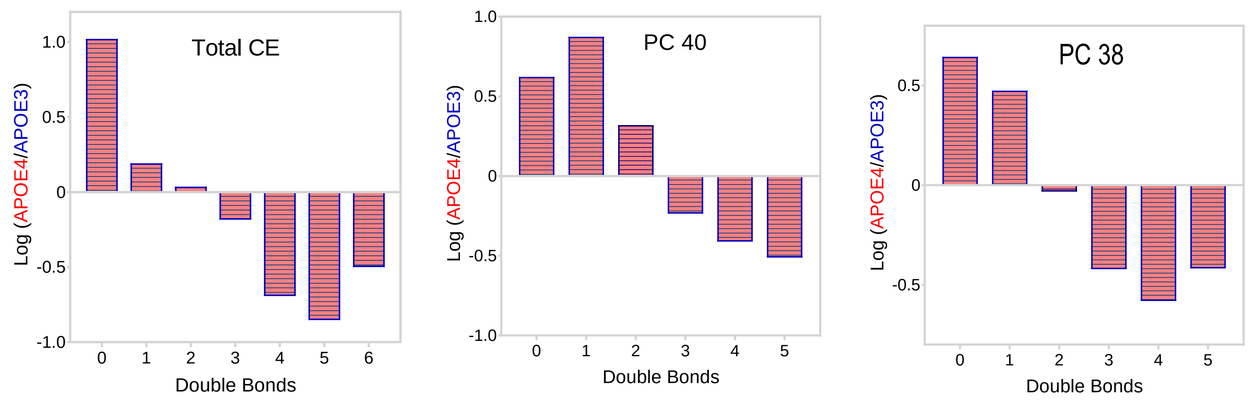
µLC-MS/MS

Volcano plot shows dysregulation of

- Cholesterol Transport
- Sphingolipid metabolism
- Phospholipid Metabolism

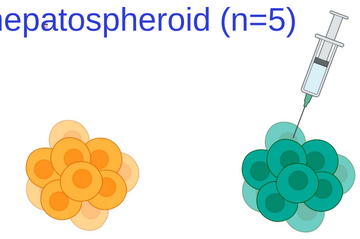


Intersestingly, lipid species with the 4-7 double bonds were downregulated in the E4 phenotype, while lipid species with 0 to 3 double bonds were sowing no change or upregulated (Shown below)



Exp. II – In depth characterization of amiodarone induce lipid accumulation in 3D-hepatospheroid (n=5)

Collaborator – Marina Grossi, Dr. Pavel Babica group

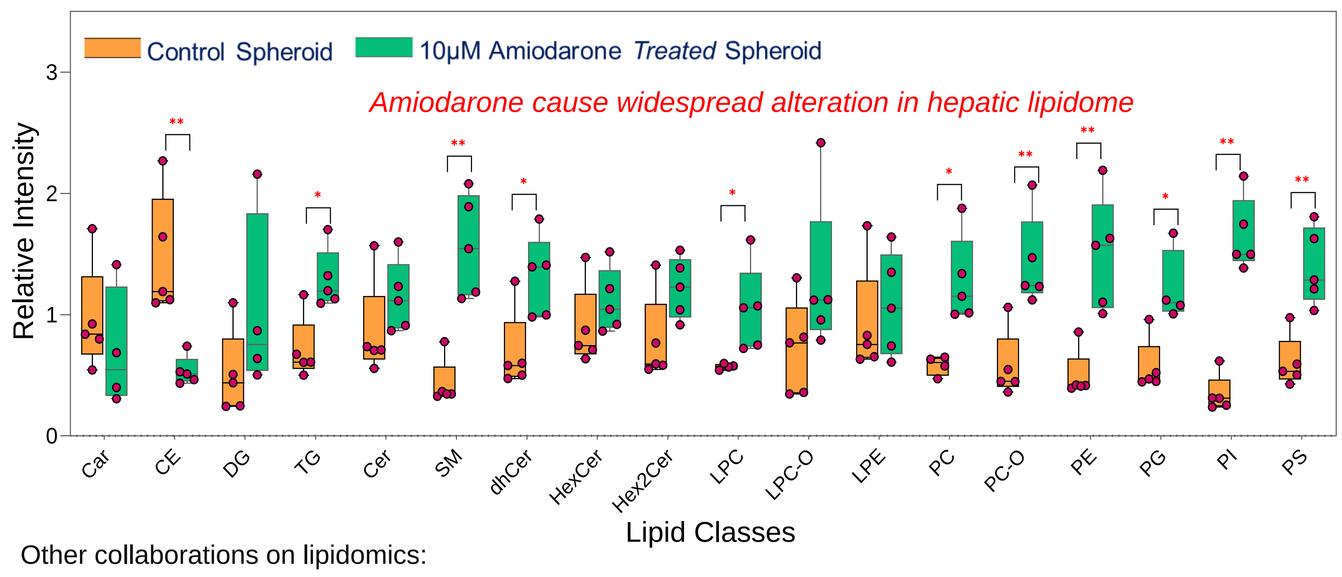


Control Spheroid | Spheroid + 10µM Amiodarone

µLC-MS/MS

Dysregulated lipid classes upon Amiodarone treatment

Glycerolipids (↑**TG,**↑**DG**)
Phospholipids (↑**PC,**↑**PC-O,**↑**PE,**↑**PG,**↑**PI and**↑**PS**)
Sphingolipids (↑**dhCer**,↑**SM**)
Sterol (↓CE)



Other collaborations on lipidomics:

Hepatic lipid dysregulation of flame retardants – Chander Negi, Prof. Ludek Blaha's group
Liidomics of TM3 and TM4 cell line to investigate chemical mixture – Eiska Sychrova, Dr. Iva Sovadinova group

Conclusion and Future Directions

- Optimized µLC-MS/MS lipidomics workflow allows sensitive, high-throughput and robust measurement of lipidome from small volume *in vitro* samples such as single hepatospheroid or single cerebral organoid.
- Our study revealed widespread lipid dysregulation in APOE4 cerebral organoid, and another study demonstrated alteration in lipid composition of hepatospheroid after amiodarone treatment
- This work paves the way for a more routine application of µLC-MS/MS lipidomics in high-throughput in vitro toxicity screening.

Acknowledgements

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