# Comparative Analysis of Lipids in a Metabolic Dysfunction-Associated Steatohepatitis (MASH) Mouse Model using Imaging MS and LCMS

**Doug Carlton[1]**, Jeffrey Dahl[1], Toshiya Matsubara[1], Kyounghee Min[2], Ei-ichi Matsuo[3], Philipp E. Scherer[2], Ruth Gordillo[2]

1. Shimadzu Scientific Instruments, Columbia, MD. 2. University of Texas Southwestern Medical Center, Dallas, TX. 3. Shimadzu Corporation, Kyoto, Japan

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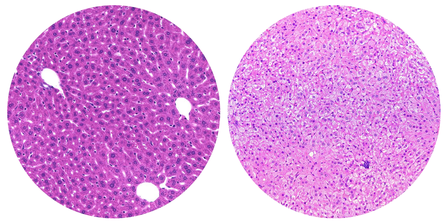
## INTRODUCTION

Preclinical mouse models of MASH are needed to characterize the disease and develop treatments in humans. Abnormal ratios of lipids such as phosphatidylcholine (PC) to phospatidylethanolamine (PE) are associated with steatogenesis and inflammation [1]. To characterize the levels of several hundred potential lipid species of interest, we utilized a combined genetic and diet–induced MASH mouse model.



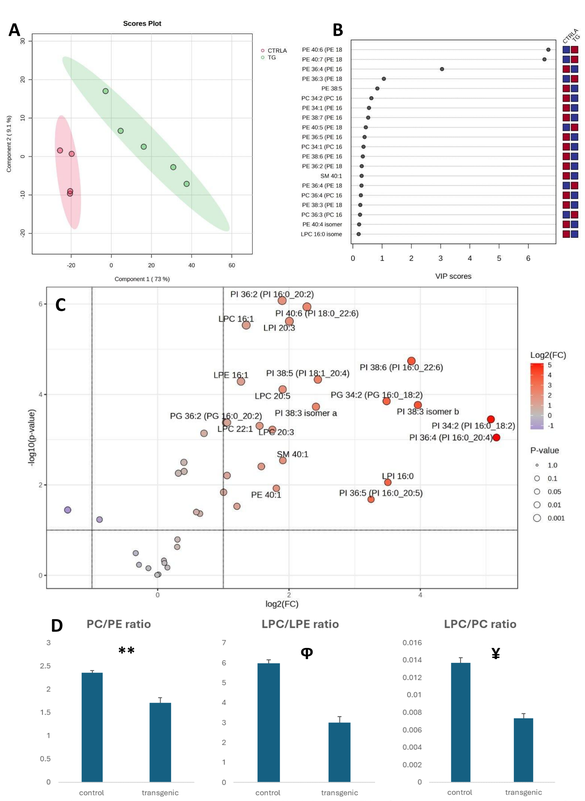
Control Transgenic (MASH)

## METHODS

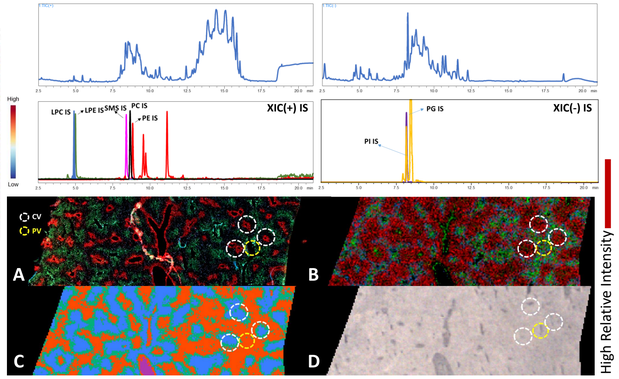
- LCMS analysis was performed on the Shimadzu LCMS-9030 QTOF mass spectrometer (Shimadzu Corporation, Kyoto, Japan) on internal standard (SPLASH LipidoMIX™; Avanti Research, Alabaster, AL) fortified liver homogenate extracts. Samples were analyzed in both positive and negative mode. Binary gradient elution using a Phenomenex Kinetex (Phenomenex, Torrance, CA) column was performed. Targeted lipidomics data analysis was conducted with the Shimadzu Library for phospholipids profiling.
- For lipidomics imaging frozen liver sections (10-µm) were mounted on conductive glass slides. Matrix was deposited using an automated matrix vapor deposition system iMLayer™ (Shimadzu Corporation). 1,5-diaminonaphthalene (DAN) was used for negative mode, and α-cyano-4-hydroxycinnamic acid (CHCA) for positive mode. Atmospheric Pressure IMS analysis was carried out using the Shimadzu iMScope™ QT.


Imaging Mass Microscope iMScope™ QT | UHPLC-QTOF LCMS™-9030 | Matrix Vapor Deposition System iMLayer™

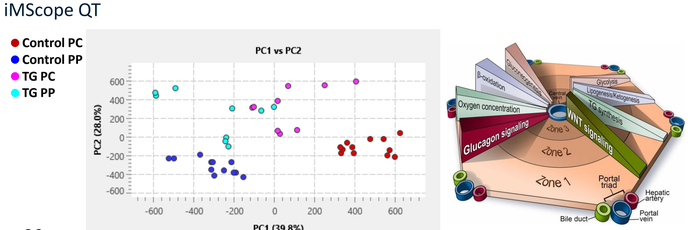
## RESULTS



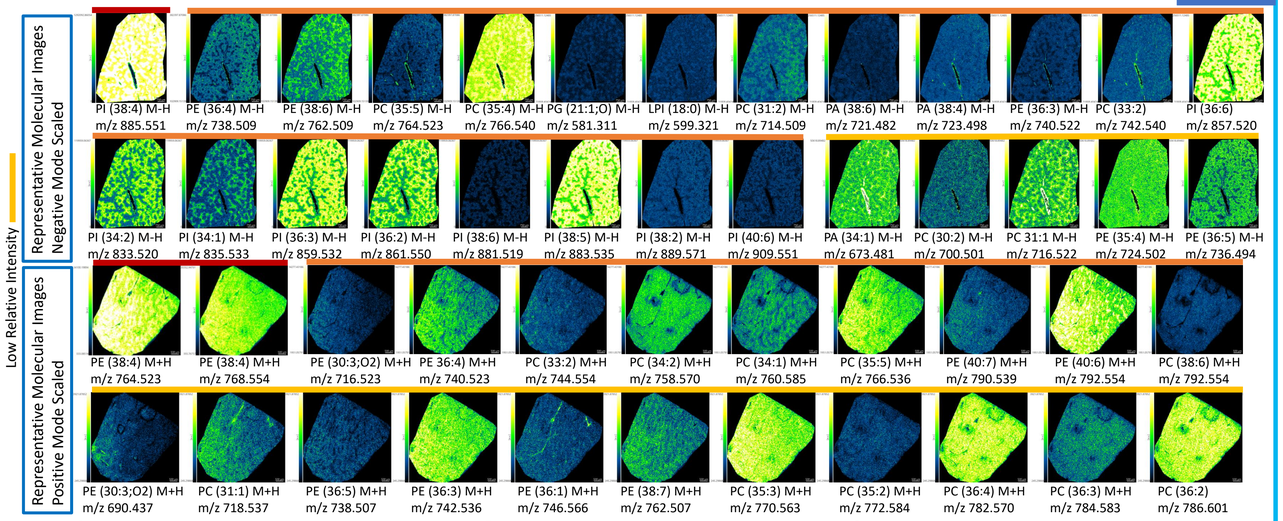

**A)** Unsupervised PCA components of lipids profiled in positive mode. **B)** Top 20 lipids identified in positive mode with relatively higher VIP score. **C)** Volcano plot TG over control (lipids profiled in negative mode) **D)** Phospholipids ratios associated with the diagnosis and persistence of MASH (Φ ≤ 0.001, ¥≤ 0.0001)[1-4]

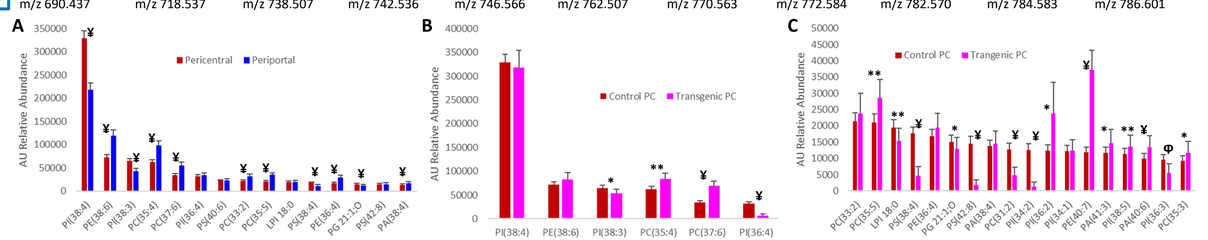



Determination of liver lobule functional zones regions of interest (ROI) [5] was performed using IF-staining, overlay of reported lipid molecules localized in the functional zones [6], and computational determination of image clusters **A)** IF- staining: Glutamine synthetase (pericentral), E-cadherin (periportal) and DAPI **B)** Overlay of false color molecular images PI (38:3) (pericentral), PI (36:3) (midzone), and PE (38:6) (periportal) **C)** Unsupervised image clustering (4 clusters) pericentral and periportal zones **D)** Optical image obtained with iMScope QT




Unsupervised PCA components of lipids (negative mode) in the different ROIs. Controls PC and PP zones are well differentiated. Control PC and TG PC are also well differentiated




Relative concentration differences **A)** Pericentral vs periportal zones in control livers **B)** Control pericentral vs TG pericentral (high abundance lipids) **C)** control pericentral vs TG pericentral (intermediate abundance lipids)

**References.** [1] 10.1016/j.cmet.2006.03.007 [2] https://doi.org/10.1016/j.bbamem.2017.04.006 [3] https://doi.org/10.3897/folmed.64.e59297 [4] https://doi.org/10.1016/j.dld.2024.05.015 [5] https://doi.org/10.1073/pnas.1804203115 [6] https://doi.org/10.1016/j.jhepr.2023.100725

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