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Integrated analysis of LC/MS and GC/MS data in NASH and NAFLD model mice

Yutaka Umakoshi[1], Tsutomu Matsubara[2], Chiho Kadono[2], Yuki Sakamoto[1]
(1) Shimadzu Corporation, Analytical & Measuring Instruments Division, Japan; (2) Graduate School of Medicine, Osaka Metropolitan University, Japan.

1. Introduction

- Metabolomics is a technology for comprehensively analyzing metabolites in living organisms. In metabolomics, liquid chromatography/ mass spectrometry (LC/MS) and gas chromatography/ mass spectrometry (GC/MS) are commonly used. The metabolites they can measure are slightly different, so the simultaneous use of LC/MS and GC/MS can complement each other's data.
- This study shows integrated analysis of LC/MS and GC/MS data in non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver disease (NAFLD) model mice (**Fig. 1**).

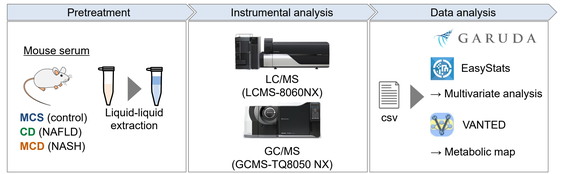

Fig. 1 Workflow of this research

2. Methods

NASH, NAFLD and control mice serum were prepared (n= 5-6/group). NASH was induced by **methionine- and choline-deficient diet (MCD)**, and NAFLD was induced by **choline-deficient diet (CD)**. A control group was prepared with **methionine- and choline-supplemented diet (MCS)**. These models were prepared according to previous research[1)].

Each serum was deproteinized and delipidated by liquid-liquid extraction using a slightly modified Bligh and Dyer's method. For LC/MS analysis, the residue was resuspended in water after centrifugal concentration of the aqueous layer and “LC/MS/MS Method Package for Primary Metabolites Ver.3” was used as the analytical method. For GC/MS analysis, samples were derivatized by methoxyamine and MSTFA after centrifugal concentration and “Smart Metabolites Database™ Ver.2” was used as the analytical method. MRM mode was used for both methods. “Multi-omics Analysis Package” was used for data analysis.

3. Results

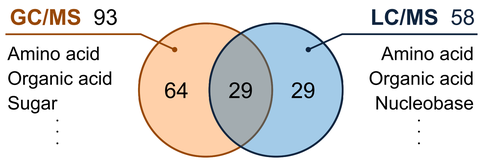

Fig. 2 Number of compounds detected by LC/MS and GC/MS

- A total of 122 components were detected using LC/MS and GC/MS (**Fig. 2**). LC/MS can detect amino acids, organic acids, and nucleobases with high sensitivity, while GC/MS can detect amino acids, small organic acids, and sugars, comprehensively. Amino acids and some organic acids were detected by both methods. For metabolites detected in both, sensitive LC/MS data were used in subsequent data analysis.

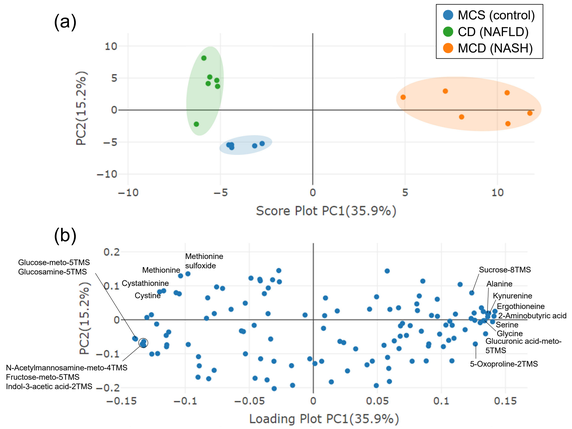

Fig. 3 Principal component analysis (a) score plot (b) loading plot

- Principal component analysis was performed by integrating the LC/MS and GC/MS data (**Fig. 3**). Three groups were plotted separately on the score plot. The first principal component separated the MCD group, and the second principal component separated CD group from the control group.
- The loading plot shows that low molecular weight amino acids, such as glycine, alanine, serine, 2-aminobutyric acid were increased in the MCD group compared to the control group. In contrast, sugar and sugar-related metabolites were decreased in the MCD group.
- Sulfur-related metabolites, such as methionine, methionine sulfoxide, cystathionine and cystine were increased in the CD group, compared with the control group.

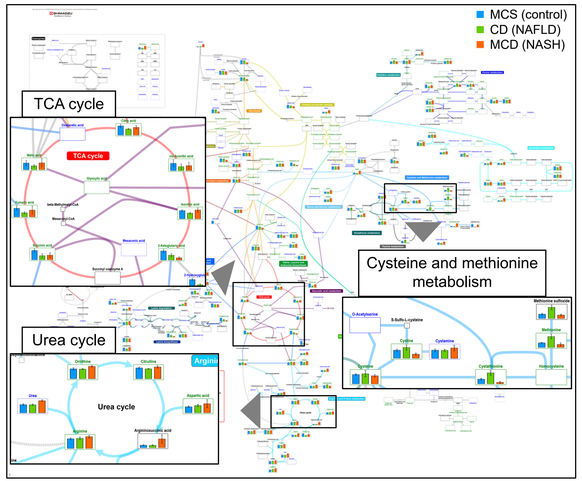

Fig. 4 Metabolic map displaying LC/MS and GC/MS data

- In order to visualize the obtained data, a metabolic map was made on VANTED software (**Fig. 4**). Metabolite names targeted by LC/MS, GC/MS, and both analytical methods are shown in black, blue, and green, respectively. This metabolic map contains major metabolic pathways, including glycolysis, amino acid metabolism, the TCA cycle, and the urea cycle.
- Some of the altered metabolites are excerpted (**Fig. 5**). Sugars, such as glucose, had decreased in the MCD group, as in previous studies[2)]. In contrast, amino acids, such as serine, glycine and threonine, had significantly increased in the MCD group. Sulfur-related metabolites had characteristically increased in the CD group.

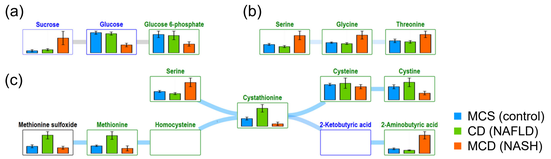

Fig. 5 Metabolic map (a) glucose-related metabolites (b) low molecular weight amino acids (c) Sulfur-related metabolites

4. Conclusion

- Using LC/MS and GC/MS, 122 components were detected in mouse serum. Both data were visualized using a newly developed metabolic map. The simultaneous use of two methods can complement each other's data.
- Metabolic map displays showed that some compounds, such as sugars, amino acids and sulfur-related metabolites, were altered in the CD and MCD groups.

Reference
1) HEPATOLOGY, (2012) Vol. 56, No. 1, Tanaka *et al.*
2) Biochimica et Biophysica Acta (2014) 1841, 1596–1607, Tanaka *et al.*

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