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COMPREHENSIVE PROFILES OF THE MICROBIOME AND METABOLOME WITHIN THE GUT-LIVER AXIS IN WESTERN DIET-FED MC4R KNOCKOUT MICE

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AIM

The aim of this study was to clarify comprehensive profiles of the metabolome and microbiome in Metabolic dysfunction-associated steatohepatitis (MASH) model mice. The key objectives were:

- To analyze the metabolome and microbiome profiles in MASH model mice with obesity and insulin resistance like human MASH patients.
- To explore the relationships between their profiles within the gut-liver axis

These results will contribute to future exploratory studies of clinical markers or therapeutic targets.

INTRODUCTION

MASH is highly associated with obesity and metabolic diseases. Although the gut-liver axis is critical in the pathogenesis, the molecular perturbations within the axis and their relationships still need to be fully understood. Using plasma, liver and intestinal contents from MASH model mice that mimic the human MASH pathology with obesity and insulin resistance, we conducted microbiome and metabolome analysis. Here, we show the results of multi-omics analyses of the gut-liver axis in MASH.

METHOD

Melanocortin 4 receptor knockout (MC4R KO) mice and wild-type (WT) mice were fed a Western diet (WD, D12079B, Research diets) or a normal diet (ND, 98121701, Research diets) for 12 weeks[1]. Plasma, liver, and small and large intestinal contents were obtained from each group.

For Metabolomics and Microbiome analysis, LC/MS and GC/MS were applied to each tissue. 16S rDNA amplicon sequencing was applied to small and large intestinal contents.

REFERENCES

1) Matsumoto M et al. *Plos One* 2020; 28; e0228212, 2) Segata N et al. *Genome Biol. 2011; 12; R60*, 3) Langfelder P and Horvath S. *BMC Bioinformatics 2008; 9; 559*

ACKNOWLEDGMENTS

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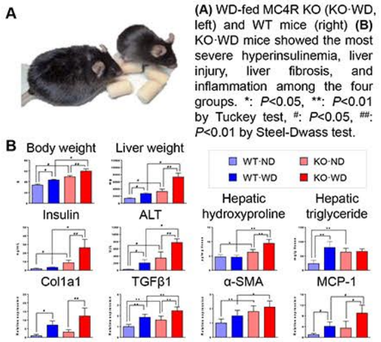
RESULTS

Fig. 1 Plasma and liver phenotypic profiles



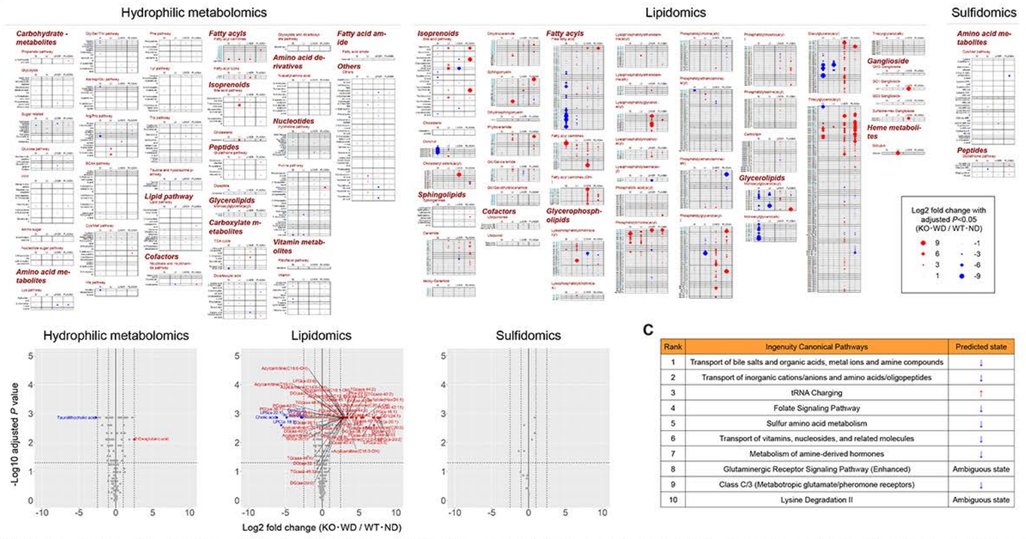
(A) WD-fed MC4R KO (KO·WD, left) and WT mice (right) **(B)** KO·WD mice showed the most severe hyperinsulinemia, liver injury, liver fibrosis, and inflammation among the four groups. *: $P<0.05$, **: $P<0.01$ by Tuckey test, #: $P<0.05$, ##: $P<0.01$ by Steel-Dwass test.

Table. 1. Characteristics of MASH model mice used in this study

Model	Increased ALT	Steatosis	Fibrosis	Inflammation	Insulin resistance	Obesity
WD-fed MC4R KO mice	○	○	◎	○	○	○
ND-fed MC4R KO	○	○	○	Δ	ND	○
CDAHFD-fed WT	○	○	○	○	×	×
CCl4-treated mice	○	×	○	○	ND	×

ND: not determined

Fig. 2 Metabolome profiles in MASH model mice

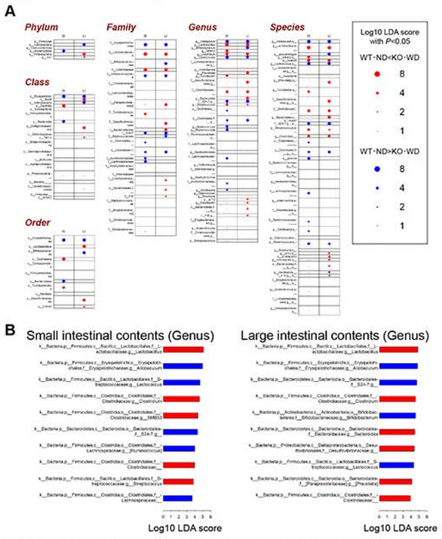


C

Rank	Ingenuity Canonical Pathways	Predicted state
1	Transport of bile salts and organic acids, metal ions and amine compounds	↓
2	Transport of inorganic cations/anions and amino acids/oligopeptides	↓
3	tRNA Charging	↑
4	Folate Signaling Pathway	↓
5	Sulfur amino acid metabolism	↓
6	Transport of vitamins, nucleosides, and related molecules	↓
7	Metabolism of amine-derived hormones	↓
8	Glutaminergic Receptor Signaling Pathway (Enhanced)	Ambiguous state
9	Class C/3 (Metabotropic glutamate/pheromone receptors)	↓
10	Lysine Degradation II	Ambiguous state

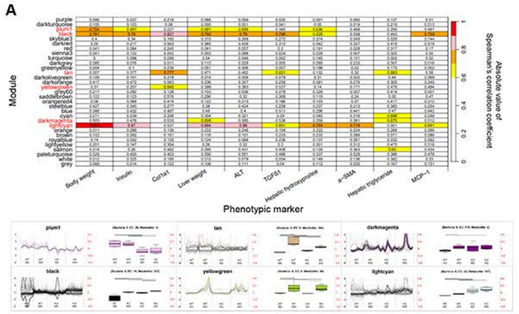
(A) Metabolome profiles of small and large intestinal contents (shown as SI and LI, respectively), liver, and plasma. Each dot represents a molecule with a statistically significant difference in signal intensity between KO·WD and WT mice fed ND (WT·ND). A hyphen (-) indicates molecules undetected in the respective tissue. Molecules highlighted in blue-green represent saturated lipids. **(B)** Volcano plot showing differential metabolites between KO·WD and WT·ND in the liver. Statistical analysis was performed using the Wilcoxon rank-sum test, with *P* value adjustment conducted using the Benjamini-Hochberg method. **(C)** Top 10 canonical pathways perturbed in the liver, identified using Ingenuity Pathway Analysis.

Fig. 3 Microbiome profiles in MASH model mice



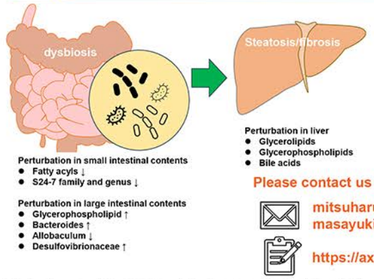
(A) Microbiome profiles of small and large intestinal contents (shown as SI and LI, respectively) analyzed using LEfSe[2]. Each dot represents a bacterium with a statistically significant difference in the LDA score between WT·ND and KO·WD mice. **(B)** Top 10 bacteria with the highest LDA scores ($P<0.05$).

Fig. 4 Gut-liver axis associated with highly perturbed hepatic metabolites



(A) Correlation between each module classified by WGCNA[3] and phenotypic markers (top panel) and each perturbation profiles of modules correlated with phenotypic markers (bottom panel). **(B)** Bacteria and metabolites in modules correlated with phenotypic markers and associated with highly perturbed hepatic metabolites (|Spearman's correlation coefficient|>0.6).

CONCLUSIONS



We identified correlations between these bacteria, metabolites, and phenotypic markers, suggesting their potential relevance as biomarkers or therapeutic targets in future studies.

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