Integrated systems biology to study non-alcoholic fatty liver disease in obese women


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Background
Non-alcoholic fatty liver disease (NAFLD) is a multi-factorial condition, the hepatic component of metabolic syndrome and one of the most common causes of chronic liver disease, with its prevalence increasing worldwide as a result of the obesity epidemic.2,3 Data are available from rodent models regarding the role of the gut microbiota and other microorganisms in liver disease and their contribution to NAFLD phenotype stratification (i.e., a comprehensive set of molecular phenotypes useful to identify subgroups of patients). Microbial factors associated with NAFLD include bacterial lipopolysaccharides (LPS) produced by Gram-negative bacteria and bile acid–FXR activators.1 The relevance of such factors in humans remains poorly understood. In addition, gut microbial populations of humans with NAFLD are inadequately characterized, and it is not known if changes in these populations and/or their functions contribute to initiation of NAFLD and/or its progression. We used an integrative multi-omics approach combining shotgun metagenomics (faecal microbiome) and molecular phenomics (liver transcriptome, plasma and urine metabolomes, clinical phenotyping) to decipher multi-omic interactions in NAFLD in obese women.

Objective
To integrate metagenomic, transcriptomic, metabonomic and clinical data to evaluate the contribution of the microbiome in liver disease and their contribution to NAFLD.

Methods
Fecal, liver biopsy, blood and urine samples and data for 28 clinical variables were collected for 56 obese (body mass index (BMI) >35 kg/m²) women from Italy (n = 31) and Spain (n = 25) who elected for bariatric surgery. Confounder analyses of clinical data were done using linear modeling. Histological examination of liver biopsies was used to grade NAFLD (NAFLD activity score: 0, 1, 2, 3). Confounder analyses of clinical data were done using linear modeling. Histological examination of liver biopsies was used to grade NAFLD (NAFLD activity score: 0, 1, 2, 3). Faecal metagenomes were generated and analysed using the SCaleable Automated Metagenomics Pipeline.6 Differentially expressed genes were identified in hepatic transcriptomes using limma, and analysed using Enrichr7, network analyses and Signalling Pathway Impact Analysis (SPIA).8 1H-NMR data were generated for plasma and urinary metabolomes.9 Clinical, metagenomic, transcriptomic and metabolic data were integrated using partial Spearman’s correlation, taking confounders (age, BMI and (SPIA) used to grade NAFLD (NAFLD activity score: 0, 1, 2, 3)

Results
1. Choline bioavailability was affected in obese NAFLD patients in a microbiota-independent manner.

2. Metabonomic profiles showed increased levels of branched-chain amino acids (BCAAs) in the plasma and urine of obese patients with NAFLD.

3. NAFLD activity score was significantly anti-correlated with microbial gene richness (MGR), and correlated with abundance of Proteobacteria. MGR was correlated with clinical markers of NAFLD.

4. KEGG analyses of metagenomic data showed increased endotoxin-related (LPS) processes related to Proteobacteria, as well as microbial processing of dietary lipids and amino acids.

5. NAFLD-associated hepatic transcriptomes were associated with BCAA metabolism, and endoplasmic reticulum/phagosome (not shown). Hepatic genes significantly correlated with NAFLD activity score and MGR were significantly associated with immune responses linked to non-specific microbial infections and insulin resistance, and the most connected gene was INSR (insulin receptor).

6. Molecular phenomic signatures were stable and predictive regardless of sample size, and consistent with the microbiome making a significant contribution to the NAFLD phenotype.

Conclusions
i) Low MGR is associated with NAFLD.
ii) There is disruption of the gut–liver axis in NAFLD, which can be seen in the faecal microbiome, hepatic transcriptome and urinary and plasma metabolomes.
iii) Consistency of phenome signatures strongly supports a relationship between microbial amino acid metabolism and MGR, hepatic gene expression and biofluid metabolomes, and ultimately NAFLD activity scores.
iv) There is a close association between the faecal microbiome, plasma/urinary metabolomes, hepatic steatosis, and clinical and molecular insulin resistance in morbid obesity.

v) Translational validation of rodent model data demonstrating an interplay between the microbiome and host gene expression in inflammation and host metabolism.

References

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