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[1,2]. Data are available from rodent models regarding the role of the gut microbiota and microbiome in liver disease and their contribution to NAFLD phenome 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[2,3]. 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 metabonomes, clinical phenotyping) to decipher multi-scalar interactions in NAFLD in obese women.

Objective

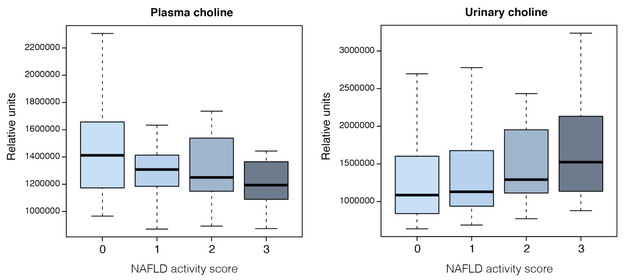
To integrate metagenomic, transcriptomic, metabonomic and clinical data to evaluate the contribution of the faecal microbiome to the molecular phenome (hepatic transcriptome, plasma and urine metabonomes) of NAFLD independent of clinical confounders in morbidly obese women recruited to the FLORINASH study.

Methods

Faecal, liver biopsy, blood and urine samples and data for 28 clinical variables were collected for 56 obese [body mass index (BMI) >35 kg/m^2] 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)[4]. Faecal metagenomes were generated and analysed using the SCaleable Automated Metagenomics Pipeline[5]. Differentially expressed genes were identified in hepatic transcriptomes using limma[6], and analysed using Enrichr[7], network analyses and Signaling Pathway Impact Analysis (SPIA)[8]. ^{1}H-NMR data were generated for plasma and urinary metabonomes[9]. Clinical, metagenomic, transcriptomic and metabonomic data were integrated using partial Spearman's correlation, taking confounders (age, BMI and cohort) into account. Interactions between the faecal microbiome and the clinical/molecular phenome were quantified, allowing generation of a Receiver Operating Characteristic (ROC) curve that confirmed the diagnostic power of molecular phenomic and metagenomic indices.

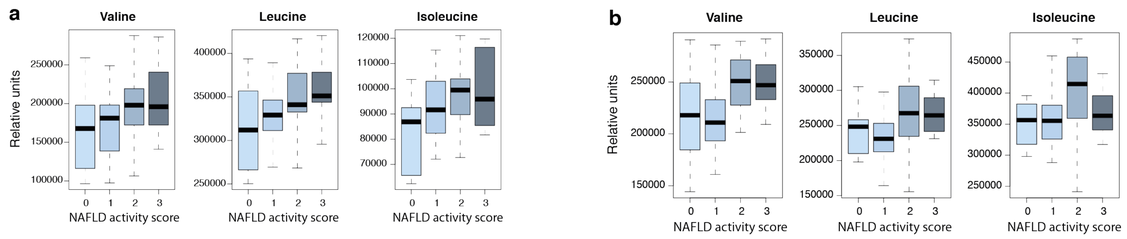
Results

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



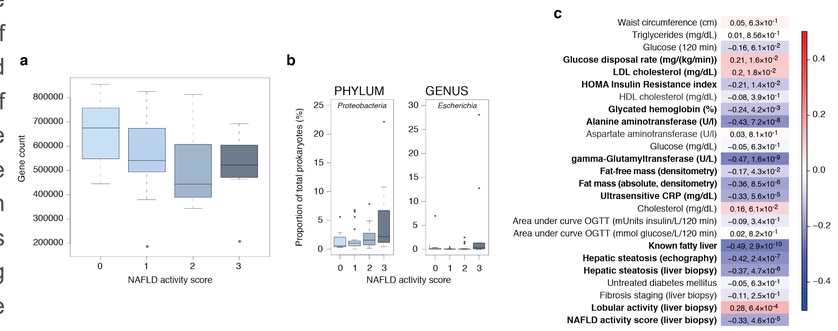
Serum plasma and urinary choline levels were significantly anti-correlated and correlated, respectively, with NAFLD activity score. No significant association was found between NAFLD activity score and trimethylamine and trimethylamine *N*-oxide levels, the gut-derived microbial breakdown products of choline, suggesting microbial use of choline is not associated with choline deficiency in obese women with NAFLD.

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



^{1}H-NMR data for BCAAs in (a) plasma and (b) urine samples. Levels of these metabolites were significantly correlated with NAFLD activity score.

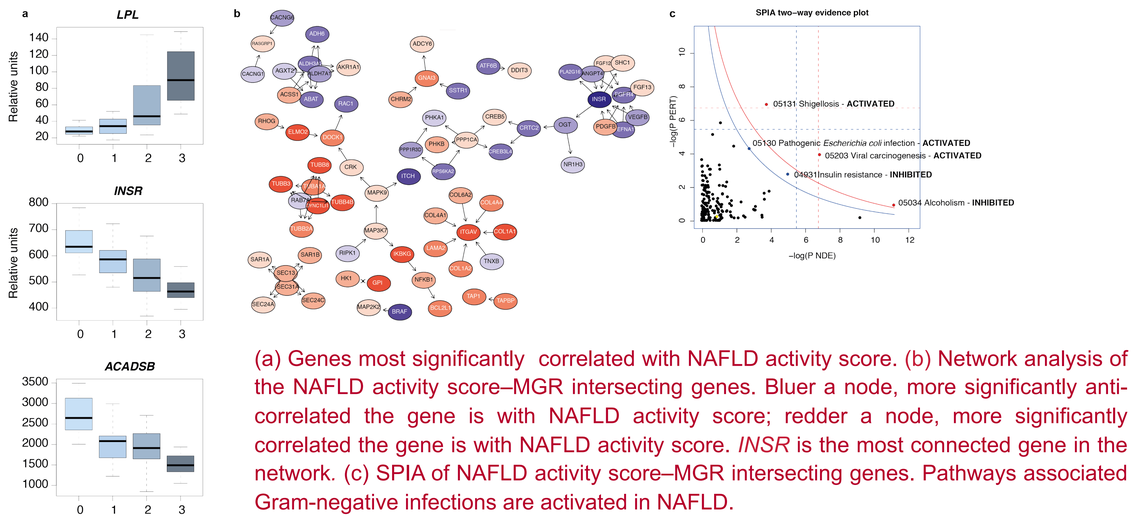
3. NAFLD activity score was significantly anti-correlated with microbial gene richness (MGR), and correlated with abundance of *Proteobacteria*. MGR was correlated with clinical markers of NAFLD.



Significant (a) association of MGR and (b) abundance of *Proteobacteria* in the faecal microbiota with NAFLD activity score. (c) Correlation of MGR with clinical data. *P* values shown adjusted for multiple correction testing (Benjamini–Hochberg).

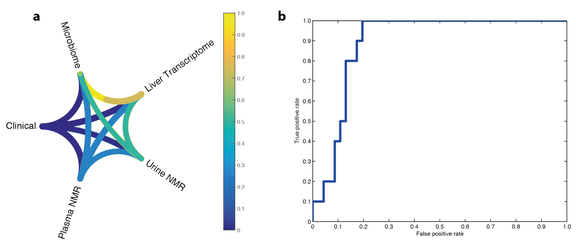
4. KEGG analyses of metagenomic data showed increased endotoxin-related (LPS) processes related to *Proteobacteria*, as well as microbial processing of dietary lipids and amino acids.

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).



(a) Genes most significantly correlated with NAFLD activity score. (b) Network analysis of the NAFLD activity score–MGR intersecting genes. Bluer a node, more significantly anti-correlated the gene is with NAFLD activity score; redder a node, more significantly correlated the gene is with NAFLD activity score. *INSR* is the most connected gene in the network. (c) SPIA of NAFLD activity score–MGR intersecting genes. Pathways associated Gram-negative infections are activated in NAFLD.

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



(a) Metagenome–phenome matrix correlation network computed for the patients using the modified Rv correlation matrix coefficient. (b) Performance of classification of NAFLD activity score 3 status (n = 9, vs others, n = 47) based on matching molecular phenome and faecal metagenome profiles. A ROC curve was obtained for the cross-validated model predictions derived from an O-PLS-DA model, reaching an AUC of 0.8913.

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 metabonomes.

iii) Consistency of phenome signatures strongly supports a relationship between microbial amino acid metabolism and MGR, hepatic gene expression and biofluid metabonomes, and ultimately NAFLD activity scores.

iv) There is a close association between the faecal microbiome, plasma/urinary metabonomes, 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.

*These authors made equal contributions to the work.

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