Investigating systemic gut microbiome derived metabolites from IL18-/- mice as potential mechanisms in health and disease

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Overview

- In this work wild type as well as Interleukin-18 knock-out (IL18 -/-) specific pathogen free (spf) and germ-free (gf) mice have been studied to highlight an end-to-end untargeted metabolomics workflow using high resolution HILIC-LC-DIA-MS/MS.
- Plasma profiles separated predominantly based on the presence (spf) or absence (gf) of a gut microbiome, although it was also possible to identify metabolites significantly different in wt and IL-18 -/- mice, some of which were specific to spf animals suggesting a potential interaction between the gut microbiome and IL-18.

1. Introduction

The gut microbiome has a significant role in health and disease. Inflammation and altered gut microbiota have been implicated in several disorders including obesity, diabetes, inflammatory bowel disease and arthritis. One mechanism by which gut microbes could alter systemic disease is through generating metabolites from dietary substances which go into the systemic circulation to have pleotropic effects. This proof-of-concept study considered the impact of the pro-inflammatory cytokine Interleukin-18 (IL-18) on the gut microbiome by assessing the changes in the systemic circulating metabolites by high resolution LC-MS/MS analysis.

2. Materials and Methods

The plasma profiles of four groups of mice (wild type specific pathogen free (spf; n=5), IL-18-/- spf (n=6), wild type germ free (gf; n=11), and IL-18-/- gf (n=11)) were analysed using an untargeted HILIC-DIA-MS/MS method (LCMS-9030 Q-TOF system, Shimadzu Corporation, Japan).

- **HILIC LC Separation**.
  - Shim-pack Velox HILIC (2.1x100mm 2.7µm); 40ºC, flow rate 0.3 mL/min
  - Binary gradient; water + 10mM ammonium formate 0.1% formic acid, and acetonitrile:water + 10mM ammonium formate 0.1% formic acid [92:8]
  - Cycle time 18 minutes.
- **Mass Spectrometry Detection**. QTOF LCMS-9030 using external mass calibration
  - MS mass scan m/z 60-1000; 100 msecs; positive and negative ion modes
  - DIA-MS/MS mass scans m/z 60-1000; precursor isolation window 35 Da collision energy spread 5-55V
  - DDA-MS/MS; 20 MS/MS mass scans
  - Cycle time less than 1 second for MS and MS/MS mass scans.
- **Data processing**.
  - **MS-DIAL |** raw data file processing, feature detection, alignment. Subsequent filters applied to retain components detected in at least 80% of one of the four groups: wt spf, IL-18 -/- spf, wt gf and IL-18 -/- gf.
  - **MetaboAnalyst 5.0 |** used to find features that were found to be statistically significantly different by univariate and multivariate methods. Further filters were applied to retain components with a QC RSD<20%.
  - **LabSolutions Insight |** used for compound Verification/Identification. Statistically significant features were identified to MSI level 1 or 2 using MS/MS library matching (in house and external data repositories including MS-DIAL metabolomics MSP spectral kit (http://prime.psc.riken.jp/compms/msdial/main.html).

2.2 Data processing workflow

One approach for processing untargeted metabolomics data;

- **MS-DIAL |** raw data file processing, feature detection, alignment, filters applied.
- **MetaboAnalyst 5.0 |** PCA and T-test.
- **Compound Identification |** LabSolutions Insight.

2.3 Identifying features of statistical significance

For feature identification, a pooled QC sample was used to identify metabolites with MS/MS library matching in LabSolutions Insight.

- Transfer the significant feature list into LabSolutions Insight.
- Use the default metabolomics library search methods, search multiple libraries in a single method;
  - In house metabolomics library with HILIC retention times.
  - MS-DIAL metabolomics MSP spectral kit which includes repositories from MassBank, GNPS, RIKEN and CASMI (converted .msp files http://prime.psc.riken.jp/compms/msdial/main.html). Elution order was considered for metabolites or lipids within the same chemical class to increase the reporting confidence of MSI level 2 annotations.
- Reporting confidence based on meeting specific criteria for mass error, isotope agreement, retention time and library similarity.

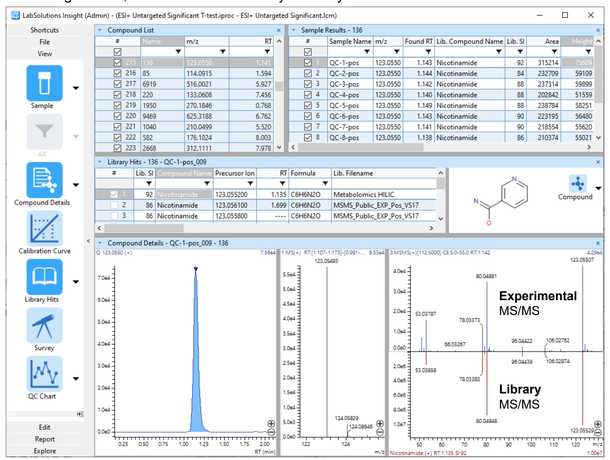

Figure 1. In this example, the ion at m/z 123.0550 (determined as an ion of statistical significance) corresponded to nicotinamide based upon a high reporting confidence (accurate mass error, isotopic pattern matching score, HILIC retention time and product ion spectral library verification using in house developed MS/MS libraries or external MS/MS libraries).

3. Results

Following raw data file processing using MS-DIAL (to detect features, align features across data sets), the dataset was exported to MetaboAnalyst for statistical analysis.

- **MetaboAnalyst 5.0 | Principal components analysis** was applied to give an overview of group distributions .

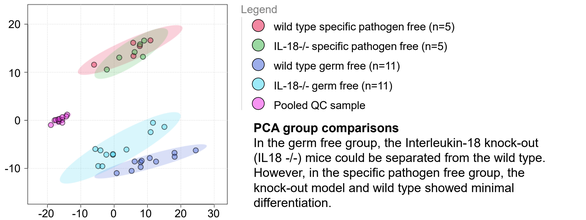

Figure 2. Principal components analysis of all samples (Log_{10} transformed) highlighting the differentiation between pathogen free and germ free groups.

3.1 Gut microbiome interaction with IL-18

Group comparison considered differences between wild type spf controls and IL-18-/- spf, as well as between wild type gf controls and IL-18-/- gf.

- **Presence of a gut microbiome** (spf groups). The IL-18-/- spf phenotype showed increases in a number metabolites including arginine, citrulline, methionine, phenylalanine, proline and valine in the plasma when compared to wild type spf mice (t-test p<0.05).
- **Absence of a gut microbiome** (germ free groups). These metabolites were not significant in the germ-free mice suggesting a potential interaction between IL-18 and the gut microbiome.

Identified metabolites that differed significantly in the spf group including a number of amino acids.
T-test - wt spf vs IL18 -/- spf

Metabolites common to both spf and gf including nicotinamide and LPC 18:1 sn-1 were also significant in wt vs IL18 -/- in the gf mice.

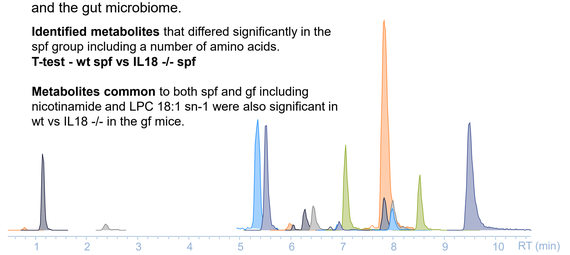

Figure 3. Mass chromatograms for 20 annotated metabolites in a QC sample. Metabolites identified as being significantly different in the IL18 -/- spf group compared to the wild type spf group.

3.2 Confidence in metabolite identification

Metabolite identification was performed in LabSolutions Insight using automated searching against an in-house MS/MS library of authentic standards with defined retention times for this analytical method (MSI-level 1), or by MS/MS spectral comparison to the MS-DIAL metabolomics MSP spectral kit (MSI-level 2). Figure 4 shows three examples of statistically significant metabolites with MSI level 1 identifications, representing the highest confidence.

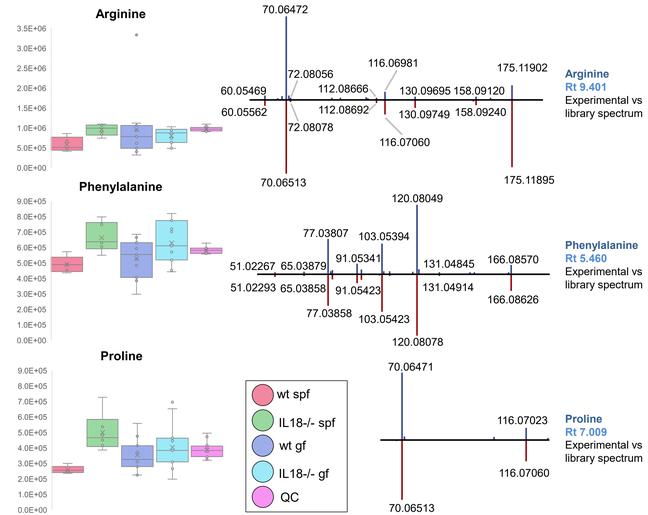

Figure 4. Box and whisker plots for the relative abundances of arginine, phenylalanine and proline in each group. Identifications were made by library search against MS/MS from authentic reference material acquired using the HILIC LC-MS/MS method.

4. Conclusions

- An untargeted metabolomics workflow has been applied to investigate the plasma profiles of mice with and without a gut microbiome and interleukin-18 (IL-18).
- Statistical tests revealed several metabolites which could be identified in the plasma profiles that were statistically significantly different between groups (specific pathogen free and germ free mice; wild type or IL-18 -/-).
- The workflow will now be applied to a larger cohort to validate these early findings which may be indicative of IL-18 and gut microbiome interaction.