The Influence of Polyphenol-Rich Diets in Mice –
A Multicompartmental LC-QTOF-Based Metabolomics Approach

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References


original publication
Introduction

• study the physiological response to a given nutritional intervention

• usually been limited to changes in either plasma or urine

• LC-Q-TOF-based metabolome analyses (foodstuff, plasma, urine, and caecal content metabolomes) in mice

• offer higher order information

• intra- and intercompartment relationships

• a 3 week intervention study with three different phenolic-rich extracts in mice
Study Design

- three control experiments (almond, hazelnut and carob)
- n=7 randomly assigned mice per group.
- supplemented with 3% almond, hazelnut or carob over a period of three weeks.
- overnight fasting
- urine, plasma and caecal samples were collected
- storage @-20°C until measurement
GeneSpring: A Bioinformatics Suite of Integrated Modules

GX
- mRNA
- Alternative Splicing
- microRNA
- Genome-wide association
- Copy Number Variation

NGS
- SureSelect Target Enrichment
- Whole Genome Sequencing
- DNA Variation
- Chromosomal Rearrangements
- RNA-Seq
- Gene Fusion Detection
- Alternative Splicing

MPP
- MS-Proteomics
- MS-Metabolomics

Integrated Biology
- Joint Pathway Analysis
- Computational Network Discovery

Intuitive Data Management

Easy to use
Wizard-Driven Workflows

Powerful data visualization

Integrated Pathway Analysis
Merge And Analyze Multi-omic Data

LC/MS GC/MS

MassHunter Qual/Quant

GeneSpring Platform

Pathway Architect

Microarrays

Feature Extraction

Alignment to Reference Genome

NGS

Alignment to Reference Genome

generic import

NMR

The Measure of Confidence

Agilent Technologies
MPP Workflow Overview

GCMS
- GC/MSD
- GC-QQQ
- AMDIS
- Mass Profiler Pro
- ID Browser
- Pathway Analysis

LCMS
- LC-TOF
- LC-Q-TOF
- MFE
- ID Browser
- Pathway Analysis
From Ions to Pathways
A Discovery Workflow Overview

- When starting from raw data, we perform feature extraction to extract and express ion signals as chemical abundances
- The statistics and pathway analysis can only happen if features are first extracted
- If we perform feature extraction first in a discovery workflow with no a priori chemical knowledge, we refer to this as “untargeted feature extraction”
If you have a database of known target metabolites you are extracting, feature extraction can use this information in a "targeted feature extraction".

There is no need of an identification step – simply map to pathways.
An accurate mass LC-MS/MS library (QTOF based)
- MS/MS spectra from mono-isotopic ion
- MS/MS spectra are collected in ESI positive and negative ion mode
- Fragmentation data is collected at three collision energies: 10, 20 and 40
- MS/MS spectra are curated for quality
  - Fragment ions are confirmed
  - Fragment ions are mass corrected
  - Noise ions removed
  - Manually reviewed
- MS/MS searches use MassHunter Qual
METLIN Compounds With Known HMDB Super-classes

METLIN content is human focused

- Covers primary metabolism
  - Amino acids, carbohydrates, lipids, nucleosides…
  - Plus it includes human secondary metabolism
- Plant primary metabolism is similar to mammalian primary metabolism
- Plant secondary metabolism is not covered
Interrogating the "unknown unknowns"

What it can do:
- Interpret MS/MS data based on fragmentation rules
- Link fragment patterns to potential parent structures
- Search custom libraries and/or public databases (ChemSpider)

What it cannot do:
- De novo structural determination
Example of Structure Correlation
Pathway Architect

Canonical pathway visualization
Single or multi-omic analysis
Biochemical, metabolomic, & signaling pathways from publicly reviewed databases
- Wikipathways
- Biopax (OWL)
- GPML
- Custom - personalized

Easy pathway browsing, filtering, navigating and searching
Convenient export of compound list from pathways
BridgeDb: Mapping Entities Onto Pathways
Resolves the mapping problem between databases

Metabolites Identifiers
- KEGG
- HMDB
- ChEBI
- CAS

Proteins Identifiers:
- Swiss-Prot
- UniProt
- UniProt/TrEMBL

Genes Identifiers:
- Entrez Gene, GenBank, Ensembl
- EC Number, RefSeq, UniGene, HUGO
- HGNC, EMBL
Multi-Omics Analysis in GeneSpring / MPP
Integrated Biology: Enabling pathway-based multi-omic discovery-to-validation

MULTI-OMIC DISCOVERY

1. HYPOTHESIS-FREE
   - Design Experiment
   - Data Acquisition
   - Data Analysis
   - Build Model

2. PATHWAY-CENTRIC HYPOTHESIS-DRIVEN

VALIDATION

EXPERIMENTATION
What is the Next Experiment?

- Missense or nonsense mutation?
- Transcriptional regulation?
- Splice variants?
- Translational regulation?
- Differential PTM?
- Known agonists or antagonists?

From a metabolic pathway I’ve identified…
Metabolomics Workflow Details – This Study

1. Separate and detect
   - LC-TOF/QTOF

2. Find peaks and quantitate
   - MassHunter Qual

3. Multivariate analysis
   - Mass Profiler Pro

4. Identify peaks
   - ID Browser

5. Analyze pathways
   - Pathway Architect
## Chromatographic Method Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Columns used:</td>
<td>Phenomenex Luna C18 3µ pfp(2), 2×100 mm, 100A, operated at 40°C Guard column: AJO-8326 pfp(2) 4×2 mm</td>
</tr>
</tbody>
</table>
| Mobile phase     | A: Water with 0.1% formic acid (positive ion detection), 0.1% acetic acid (negative ion detection)  
B: Acetonitrile/water 95/5 v/v                                                                 |
| Gradient condition | Time (min) % mobile phase B  
0    5  
20   100  
25   100  
6 min post time |
| Flow             | 0.2 mL/min                                                                                                                                 |

- Generic linear gradient  
  total run time 31 min  
- Separation relatively equally spaced across entire run time (see following slide)
Compounds extracted from all compartments (almond diet and control samples) after alignment and binning: More than 41000 compounds
Entities by Compartment and Diet

Hierarchical Clustering:
Distribution of compounds as a function of compartment and diet.
Mouse Metabolite Database from Pathways

• The PMDC tool creates accurate mass databases from all pathway-related compounds.
• Pathway selection by species or common compound.
Unsupervised Data Analysis – PCA

- PCA analysis of all three diet data vs. control after outlier filtering and 2-way ANOVA significance analysis (p=0.01)
- Best diet/control separation for urine and caecal samples
- Plasma results less significant
Pathway Analysis Example

Most prominent pathways:
- amino acid metabolism
- biogenic amine synthesis
- folic acid network
- glutathione metabolism
- TCA cycle
- glycogen metabolism

urine
Diet-induced commonalities/differences

hazelnut (A), almond (B), carob (C), plasma (D), urine(E), caecal content (F)
Summary and Conclusion

• For pattern recognition, unsupervised (PCA) and supervised (PLSDA) multivariate analyses were used. Both approaches revealed noticeable effects of diet in plasma, urine, and caecal contents.

• Dietary intake of phenolic-rich extract affects pathways such as amino acid, bile acid, glutathione, taurine metabolism and TCA cycle.

• The pathways influenced by the diet have been retrieved by a novel “pathway architect” software.

• Metabolomics based on HPLC-QTOF/MS demonstrated that the number of correlations is higher in caecal contents and urine than in plasma.

• Moreover, intercompartment correlations showed that caecal contents – plasma correlations are the most frequent in mice, followed by plasma – urine ones.

• We found that diet significantly affects the number of inter- and intracompartment correlations.

• These analyses reveal the complexity of interorgan metabolic relationships and their sensitivity to dietary changes.
Thank You