Urinary metabolic profiling in inflammatory bowel disease

Dr Horace Williams
Clinical Research Fellow
Imperial College London
Background: Metabolic profiling

- Metabolic profiling or “metabonomics” describes the generation of metabolic information from biofluids or tissues.

- NMR spectroscopy (NMRS)
  - simultaneous acquisition of multiple biochemical parameters

- Urinary metabolic profiling
  - study specific diseases based on underlying metabolic processes
  - no such application to IBD
Rationale for metabolic profiling in IBD

**Gut microbiota** differ between CD, UC and healthy controls

**Gut microbiota** have important influences on specific urinary metabolites:
- Hippurate
- Formate
- 4-cresol sulphate
- Dimethylamine
- TMAO
**Experimental design: subject groups**

<table>
<thead>
<tr>
<th></th>
<th>Crohn’s disease</th>
<th>Ulcerative colitis</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number (Male/Female)</strong></td>
<td>86 (47/39)</td>
<td>60 (30/30)</td>
<td>60 (30/30)</td>
</tr>
<tr>
<td><strong>Disease location</strong></td>
<td>L1: 18</td>
<td>E1: 14</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>L2: 25</td>
<td>E2: 18</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>L3: 43</td>
<td>E3: 28</td>
<td>-</td>
</tr>
<tr>
<td><strong>Longitudinal Samples</strong></td>
<td>35</td>
<td>14</td>
<td>26</td>
</tr>
</tbody>
</table>

- Urinary NMR Spectra acquired using JEOL 500MHz Eclipse+ NMR spectrometer
- Largest urinary metabonomic study in any disease to date
Hypothesis-driven analysis

- **HIPPURATE relative index**
  - Controls: p<0.0001
  - UC patients: p=0.001
  - CD patients: p=0.0001

- **FORMATE relative index**
  - Controls: p=0.82
  - UC patients: p=0.001
  - CD patients: p=0.98

- **4-CRESOL SULPHATE relative index**
  - Controls: p=0.0007
  - UC patients: p=0.0003
  - CD patients: p<0.0001

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Multivariate factor analysis

Principal Components Analysis (PCA)

– Assumes no *a priori* knowledge
– Overview of the samples, analysing the whole spectrum
– Principal components are linear combinations of variables (metabolites) accounting for the greatest variation within the dataset
– Scores plot: each sample represented in the new co-ordinate space
– Loadings plot: combination of metabolites responsible for the scores plot
Multivariate factor analysis

A: PCA scores plot: 86 CD patients and 60 controls.
B: PCA scores plot: 68 CD patients and 60 controls (excluding outliers).
Multivariate factor analysis

Partial least squares discriminant analysis (PLS-DA)

- NMR spectroscopic variables (metabolites) are related to class membership
- Scores plots generated
- Loadings plots and regression vector: identification of spectral regions (metabolites) responsible for separation between groups
- Orthogonal Signal Correction (OSC)
- Rigorous validation techniques
Multivariate factor analysis

<table>
<thead>
<tr>
<th></th>
<th>CD</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>61</td>
<td>7</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>50</td>
</tr>
</tbody>
</table>

- Sensitivity: 90%
- Specificity: 83%
**PLS-DA models: CD vs. UC**

<table>
<thead>
<tr>
<th></th>
<th>All individuals</th>
<th>Individuals on no medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD: UC</td>
<td>L2 CD: UC</td>
<td>CD: UC</td>
</tr>
<tr>
<td>Sensitivity: specificity</td>
<td>86: 82</td>
<td>79: 83</td>
</tr>
</tbody>
</table>

Results compare favourably with predictive abilities of ASCA / pANCA

**Metabolites primarily responsible for distinguishing CD and UC:**
- HIPPURATE, citrate, methylhistidine, guanidoacetate, 4-cresol sulphate
Hippurate Metabolism

Investigating Hippurate Metabolism in IBD

- 16 CD patients, 16 healthy controls
- Low benzoate diet
- Administered 5mg/kg sodium benzoate.
Results

A. HIPPURATE relative index (to total spectral integral)

B. HIPPURATE excretion (1 hour - baseline)

C. % CHANGE in hippurate excretion (baseline to 1 hour)

Controls

CD patients

p=0.53

p=0.0007
Discussion

- Significant differences in IBD for specific urinary metabolites whose concentration is modulated according to make-up of the intestinal microbiota:
  - Hippurate, formate, 4-cresol sulphate
- Multivariate analysis (PLS-DA modelling):
  - Able to distinguish between cohorts, even colonic CD vs UC
  - Hippurate of major importance
- Elucidation of hippurate metabolism in IBD
  - Gut microbiota implicated in the lower levels found in CD
Acknowledgements

- Broad Medical Research Program

- Collaborators/Researchers
  - Dr. Bernard North
  - Dr. David Walker
  - Prof. Ken Welsh
  - Dr. Hiroe Sato
  - Ms. Vicky Loh
  - Miss. Venisha Patel
  - Dr. Simon Jakobovits
Urine NMRS from male healthy control

KEY:
1: lactate
2: alanine
3: 4-cresol
4: citrate
5: DMA
6: creatine
7: creatinine
8: TMAO
9: glycine
10: hippurate
11: formate

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Addressing potential confounders

Subject selection

Caucasian

Exclusion criteria

Comorbidity

Intercurrent illness

Antibiotic usage

Subject questionnaire

Diet

Drugs
Subject groups

• No significant differences between groups in terms of dietary constituents, EtOH intake, exercise, smoking

• Female subjects matched for reproductive status and use of hormonal therapies
Addressing potential confounders

- Recent studies:
- Inter-individual variation > intra-individual variation
- Good reproducibility between individuals
- First void urine samples exhibit greatest variability
- Random urine samples, 13.00 ± 3 hours