Diet-health-microbiota interactions in older persons
- the ELDERMET study

Paul W. O’Toole
Dept. Microbiology, Univ. College Cork, Ireland
Alimentary Pharmabiotic Centre, Univ. College Cork, Ireland
http://apc.ucc.ie
http://eldermet.ucc.ie

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The human gut microbiota

- 10-100 trillion microbes in GI tract
- ~10 times more bacterial cells in GIT than human cells in body
- ~ 100 times more bacterial genes than human genes
- Variations in the gut microbiota in disease
Diseases with microbiota linkages

**Irritable Bowel Syndrome**

**Obesity**

**Atherosclerosis**

- normal human artery
- artery narrowed by atherosclerotic plaque
- endothelium
- smooth muscle
- damaged endothelium
- smooth muscle cells
- macrophages transformed into foam cells
- lipids, calcium, cellular debris
- fibrous cap

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Microbiota 16S gene profiling – who is there?

- "Sample"
- DNA
- PCR
  - Universal 16S gene primers
- 16S amplicon
- Pyrosequence
  - ca. 40,000 reads/subject
- Assign reads to genera or OTUs
Microbiota shotgun sequencing – what are they doing?

1. **Sample**
2. **DNA**
3. **Fragment**

   - **PCR Universal 16S gene primers**
   - **16S amplicon**
   - **Pyrosequence ca. 40,000 reads/subject**
   - **Assign reads to genera or OTUs**

   - **Shot-gun sequence**
   - **Assemble**
   - **Annotate**

   - **Cluster of COGs in the core**
     - Information storage and processing
     - Cellular processes
     - Replication, recombination and repair
     - Post-translational modification, protein turnover, chaperones
     - Gene expression
     - Cell motility and chemotaxis
     - Energy production and conversion
     - Amino acid transport and metabolism
     - Nucleotide transport and metabolism
     - Coenzyme transport and metabolism
     - Basal metabolism
     - Inorganic ion transport and metabolism
     - Translation, ribosomal structure and biogenesis
     - Transcription
     - Extracellular structures
     - Signal transduction mechanisms
     - Cell cycle control, cell division, chromosome partitioning
     - Intracellular transport and secretion
     - Cell wall/membrane/envelope biogenesis
     - General function unknown
     - Not assigned
ELDERMET

Amended from Nature, May 2008
ELDERMET

What did we do?

• Composition of faecal microbiota 500 subjects >65 years, $T_0$, $T_3$, $T_6$

• Measure specific clinical/health parameters

• Microbial metagenome & metabolome - test for correlations with health indices

• Stratification

<table>
<thead>
<tr>
<th>STRATUM</th>
<th>SUBJECTS</th>
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<tbody>
<tr>
<td>Long stay</td>
<td>100</td>
</tr>
<tr>
<td>Rehab (&lt;6 wks)</td>
<td>50</td>
</tr>
<tr>
<td>Day Hospital</td>
<td>50</td>
</tr>
<tr>
<td>Community</td>
<td>50</td>
</tr>
<tr>
<td>Community –antibiotic</td>
<td>100</td>
</tr>
<tr>
<td><em>Clostridium difficile</em> positive</td>
<td>100</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>50</td>
</tr>
<tr>
<td>TOTAL</td>
<td>500</td>
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Elderly subjects – sampling

- Faeces
- Blood
- Urine
- Saliva
- Anthropometrics
- Food Frequency Questionnaire (FFQ)
- MMT, FIT, Geriatric Depression
Aggregate microbiota composition is different in younger versus older subjects

Claesson et al., 2011. PNAS USA.
The *Bacteroidetes* : *Firmicutes* ratio varies considerably in elderly subjects. (14-91)% *Bacteroidetes* : (81-10%) *Firmicutes*
Diet-related gut organisms vary considerably in Elderly Irish subjects

\[ n = 160 \]
Is variation in microbiota composition related to community location, diet or metadata?

• 83 Community-dwelling
• 20 Day hospital (out-patient)
• 15 Rehabilitation (≤6 weeks)
• 60 Long-stay (>6 weeks)
• (13 Young healthy controls)

191

Mean age 78+/- 8; 65-102 yrs.
Gut bacteria depend on where you live

Microbiota composition separates subjects by community location

Hierarchical Ward-linkage clustering based on Spearman correlation coefficients of the proportion of OTUs for each subject

Unweighted UniFrac OTU PCoA
Diet co-segregates with microbiota and residence location

**DG1:** “low fat / high fibre”
**DG2:** “moderate fat / high fibre”
**DG3:** “moderate fat / low fibre”
**DG4:** “high fat / moderate fibre”
Compliance with dietary guidelines

Foods High in Fat and/or Sugar (use sparingly) 19%

Meat, Fish, Poultry & Alternatives (2 servings/day) 34%

Milk, Cheese, Yoghurt products (3 servings/day) 10%

Fruit & Vegetables (5+ servings/day) 64%

Cereals, Breads, Potatoes, Rice & Pasta (6+ servings/day) 18%

Foods High in Fat and/or Sugar (use sparingly) 13%

Meat, Fish, Poultry & Alternatives (2 servings/day) 33%

Milk, Cheese, Yoghurt products (3 servings/day) 10%

Fruit & Vegetables (5+ servings/day) 28%

Cereals, Breads, Potatoes, Rice & Pasta (6+ servings/day) 8%

Community

Long-stay care
Patients being fed junk food diet in our hospitals

Frozen pizza, battered sausages and chips are all on HSE menu

The HSE will buy almost 90 tonnes of frozen chips, 62,000 sausage rolls and other highly processed foods for patients in the public health system over the next four years.

Tender documents seen by the Irish Independent show the frozen food requirements for more than 100 hospitals and nursing homes over the next four years.
Microbiota diversity correlates with diet diversity

**D**

**Number of Unique OTUs**
- Kruskal-Wallis: p-value = 5.18e-12

**Shannon Index**
- Kruskal-Wallis: p-value = 6.68e-07

**Phylogenetic Diversity**
- Kruskal-Wallis: p-value = 2.026e-11

**Healthy Food Diversity**
- Kruskal-Wallis: p-value = 8.82e-07

---

**Unique OTUs vs. Healthy Food Diversity**
- p-value = 1.21e-05 R = 0.331

**Shannon Index vs. Healthy Food Diversity**
- p-value = 3.12e-06 R = 0.351

**Phylogenetic Diversity vs. Healthy Food Diversity**
- p-value = 3.46e-07 R = 0.381
Procrustes: Microbiota & diet correlate, & by community location

Unweighted UniFrac PCoA vs. FFQ PCA

Weighted UniFrac PCoA vs. FFQ PCA

FFQ
Community
Day Hospital
Rehab
Long-stay
Microbiota diet correlation by duration in long-stay care

FFQ

N/A (C+DH)  Week0to6 (Rehab)  Week6toYear1  Year1+  Microbiota
Separation of residence location by faecal water NMR metabolome

Long-stay Community Rehab Community

Dr. Martina Wallace and Dr. Lorraine Brennan, Univ. College Dublin
Integrating metabolome, metabolites & genus-level microbiota

Co-inertia of microbiota & metabolome → by location

NMR spectrum metabolite PCA
Shotgun metagenome: differentially abundant SCFA genes

- BCoAt: Butyryl-CoA transferase/Acetyl-CoA hydrolase
- ACS: Acetate-formyltetrahydrofolate synthetase/Formate-tetrahydrofolate ligase
- PCoAt: Propionyl-CoA:succinate-CoA transferase/Propionate CoA-transferase

Who cares?

Inflammatory markers vary by community location
Microbiota-health correlations

Health/clinical markers
- BMI: Body Mass Index
- CC: Calf Circumference
- MAC: Mid-Arm Circumference
- SBP: Systolic Blood Pressure
- DBP: Diastolic Blood Pressure
- CCI: Charlson Comorbidity Index
- Barthel Index of Activities of Daily Living
- FIM: Functional Independence Measure
- MMSE: Mini-Mental State Exam
- MNA: Mini-Nutritional Assessment

Possible confounders
- Antibiotics:
  - exclude <1mo
  - >1mo had no sign. effect on µ-biota
- Adjust quantile regression model for:
  - Age and gender
  - Location
  - Medication
Microbiota separation correlates with health measures


Table 1 | Regression tests of associations between clinical measurements and microbiota composition.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>RC range</td>
<td>RC s.d.</td>
<td>P</td>
</tr>
<tr>
<td>----------------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>GDT</td>
<td>-0.42</td>
<td>-0.11</td>
<td>0.6</td>
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<tr>
<td>Diastolic blood pressure</td>
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<td>0.25</td>
<td>0.81</td>
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<tr>
<td>Weight</td>
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<td>0.033</td>
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<tr>
<td>CC</td>
<td>-3.9</td>
<td>-1.01</td>
<td>0.022</td>
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<tr>
<td>IL-6</td>
<td>6.71</td>
<td>1.7</td>
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<td>IL-8</td>
<td>4.23</td>
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<td>TNF-α</td>
<td>1.1</td>
<td>0.28</td>
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<td>----------------------------</td>
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<tr>
<td>MNA</td>
<td>-1.1</td>
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<td>Diastolic blood pressure</td>
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<td>GDT</td>
<td>-0.13</td>
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Table 1a Unweighted UniFrac PCoA for all four residence locations

Table 1b Unweighted UniFrac PCoA for community-only subjects

Table 1c Unweighted UniFrac PCoA for long-stay-only subjects

Quantile (median) regression tests of associations between clinical measurements and microbiota composition as measured by unweighted UniFrac PCoA across all four residence locations (that is, all subjects (a), community-only subjects (b), and long-stay-only subjects (c)). Column headings are: RC range, regression coefficients scaled to the full variation along each PCoA axis, thus indicating relative magnitude and direction of the health association; RC s.d., regression coefficients scaled to one standard deviation; P, quantile regression P values generated by boot-strap analysis. Significant associations are in bold. An additive model was used to adjust for the effects of age, sex, residence location, relevant medication and the two other principal coordinates. CC, calf circumference; IL, interleukin; MMSE, mini-mental state examination.
Microbiota changes across location are mirrored by changes in health

Summary

• Microbiota composition correlates with habitual diet
• Movement from community dwelling to residential care associated with altered diet
• Diet changes; microbiota follows
• Microbiota alterations correlate with health changes especially in long-stay
• Metagenomics and metabolomics support a diet-microbiota-health axis
n = 1,250

UK, NL, FR, IT, PL

T₀  12 mo.’s

5 x 25 subjects
Future perspectives in microbiota health research

• Prospective / longitudinal studies
• Interventions
• Microbiota modulation / restoration
• Genome / epigenome interactions
• Integrated metabolomics, nutrition and microbial ecology
• Dietary guidelines informed by knowledge of effects on microbiota
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