The Human Microbiome and [Faecal] Microbial Transplantation

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Why?

- Increasing interest in the role of the microbiome in shaping health and disease
- Rapid technological advances
- Stand-out example of a therapeutic effect
- Tsunami of chronic disease fuelled by obesity
What?

- Definitions and technology
- Microbial functions
- Age-related change
- Factors that influence the microbiome
- Microbial imbalance – “dysbiosis”
- Therapeutic strategies
Definitions

- Microbiome – microbial encoding genes
- Microbiota – micro-organisms
- Hologenome – Host and microbial genes
- Holobiont – an individual host and its microbial community
- Metagenome – all the genetic material in a given sample (e.g. faecal sample)
- Pathobiont – a benign commensal microbe that can elicit pathogenesis under certain environmental conditions
Technology

<table>
<thead>
<tr>
<th>Approach</th>
<th>Data</th>
<th>Platform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomarker sequencing (e.g., 16S rRNA gene or</td>
<td>Community composition</td>
<td>Next-generation sequencing</td>
</tr>
<tr>
<td>internal transcribed spacer region)*</td>
<td></td>
<td>Semi-quantitative, genus level resolution</td>
</tr>
<tr>
<td>Metagenomics</td>
<td>Generation of draft genomes, functional</td>
<td>Next-generation sequencing</td>
</tr>
<tr>
<td>Metatranscriptomics (RNA sequencing)</td>
<td>capacity, growth dynamics</td>
<td>Strain-level, quantitative, functional data</td>
</tr>
<tr>
<td>Metaproteomics</td>
<td>Gene expression</td>
<td></td>
</tr>
<tr>
<td>Metabolomics</td>
<td>Protein expression</td>
<td>Liquid or gas chromatography–mass spectrometry</td>
</tr>
<tr>
<td>Metabolomics</td>
<td>Metabolic productivity</td>
<td>Liquid or gas chromatography–mass spectrometry or magnetic resonance spectroscopy</td>
</tr>
</tbody>
</table>

Detects metabolites from microbes, diet, host; depends on data dictionary
Microbial functions

Influences
- Immune maturation and homeostasis
- Host cell proliferation
- Vascularization
- Neurologic signalling
- Pathogen burden
- Intestinal endocrine functions
- Bone density
- Energy biogenesis

T regulatory cells
- Butyrate

Biosynthesis
- Vitamins
- Steroid hormones
- Neurotransmitters

Metabolism
- Branched-chain and aromatic amino acids
- Dietary components
- Bile salts
- Drugs
- Xenobiotics

5-10% of daily requirement
Age-related development of the intestinal microbiome

- Mode of delivery
- Major adult microbiota are Bacteroidetes and Firmicutes
- Enormous variation between individuals
- Functionally similar
Microbiome changes after birth
Cesarean delivery and childhood disorders

<table>
<thead>
<tr>
<th>Cesarean Delivery Associated Childhood Diseases</th>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Allergic Rhinitis</strong></td>
<td></td>
</tr>
<tr>
<td>All Cesareans</td>
<td>1.37 (1.14-1.63)</td>
</tr>
<tr>
<td>Repeat Cesareans Only</td>
<td>1.78 (1.34-2.37)</td>
</tr>
<tr>
<td><strong>Asthma</strong></td>
<td></td>
</tr>
<tr>
<td>All Cesareans</td>
<td>1.24 (1.01-1.53)</td>
</tr>
<tr>
<td>Female</td>
<td>1.53 (1.10-2.10)</td>
</tr>
<tr>
<td>Female &amp; Repeat Cesarean</td>
<td>1.83 (1.13-2.97)</td>
</tr>
<tr>
<td><strong>Celiac Disease</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.80 (1.13-2.88)</td>
</tr>
<tr>
<td><strong>Diabetes Mellitus (Type 1)</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.19 (1.04-1.36)</td>
</tr>
<tr>
<td><strong>Gastroenteritis</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.31 (1.24-1.38)</td>
</tr>
<tr>
<td>Gastroenteritis AND Asthma</td>
<td>1.74 (1.36-2.23)</td>
</tr>
</tbody>
</table>

From Neu J, Rushing J. Clinical Perinatology 2011

Cesarean delivery in US increased 48% between 1996 and 2007 – to 31.8%
Influences on the microbiome

- Host genetic factors
- Diet (including infant feeding, supplements)
- Host immune response
- Smoking
- Antibiotics and other drugs
- Infections
- Diurnal rhythm
- Environmental microbial exposures
- Nod2 is an intracellular sensor of muramyl dipeptide, a component of bacterial cell walls
- It is a major susceptibility gene for Crohn’s disease – homozygotes for a disease-associated mutation carry a 20-40 fold increased disease risk
- Both mouse models and human studies show a significantly increased load of commensal bacteria in those carrying Nod2 mutations
Relative abundance of microbiota in human subjects with or without Nod2 mutations

Results for the mucosa-associated microbiota using tissue biopsies taken from the terminal ileum.
Smoking Cessation Induces Profound Changes in the Composition of the Intestinal Microbiota in Humans

Luc Biedermann¹, Jonas Zeitz², Jessica Mwinyi¹,³, Eveline Sutter-Minder⁴, Ateequr Rehman⁵α

- Stool samples
- 9 week period
- Small numbers (10 + 10)
- Increased microbial diversity with smoking cessation
- Similar shifts to obese→lean
PPIs alter the intestinal microbiome

- PPI data within the TwinsUK cohort
- Self-reported
- 1827 individuals (90% female)
- Average age – 62 years
- PPI users significantly:
  - Older ($p<10^{-6}$)
  - Frailer ($p<10^{-15}$)
  - Higher BMI ($p=0.0003$)

Major findings in PPI users as compared to not:

- Lower abundance of commensals
- Reduced microbial diversity
- Increased oral and pharyngeal commensals
- ↑ Streptococcaceae
- Replicated in independent study of MZ twins discordant for PPI use
Dysbiosis – microbial imbalance ± maladaptation

- Change in microbial composition relative to healthy individuals
- What is a healthy human microbiome?

<table>
<thead>
<tr>
<th>Terms</th>
<th>Publications</th>
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<tbody>
<tr>
<td>All</td>
<td>2011–2016</td>
</tr>
<tr>
<td>Gut</td>
<td>colon</td>
</tr>
<tr>
<td>Oral</td>
<td>mouth</td>
</tr>
<tr>
<td>Urogenital</td>
<td>vaginal</td>
</tr>
<tr>
<td>Skin</td>
<td>cutaneous</td>
</tr>
<tr>
<td>Airway</td>
<td>lung</td>
</tr>
<tr>
<td>Placenta</td>
<td>breast milk</td>
</tr>
<tr>
<td>Ocular</td>
<td>eye</td>
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</tbody>
</table>
The “Common Ground” Hypothesis of disease attributed to dysbiotic intestinal microbiota
(from: Lynch S 2016)
Disease associations

• Atherosclerosis
• Metabolic disorders (obesity, Type II diabetes)
• Asthma
• Autism spectrum disorder
• Inflammatory Bowel Diseases
Human studies

• Majority used 16S ribosomal RNA sequencing

• Useful insights but multiple issues:
  variability within and across individuals
  lifestyle factors – diet, smoking
  reproducibility
  sampling
  statistical power
  heterogeneity within and between cases/controls
  adjustment for drug effects
  need for longitudinal and interventional studies
Coprophagia

I HAVE THE RESULTS FROM YOUR STOOL SAMPLE...
DELICIOUS
Fecal transplant: Sounds gross but saves lives

ERIK ORTIZ
Thursday, September 27, 2012

An Atlanta-area woman found relief from a bacterial infection in her colon when her mom provided a life-saving transplant — involving fecal matter.

While a fecal-matter transplant sounds stomach turning, it ensured 20-year-old Kaitlin Hunter a normal life again after a devastating car accident that nearly killed her.

“I’ve been so happy,” Hunter, of Marietta, told CNN on Wednesday after the July surgery. “I’m cured.”

Hunter’s ordeal began after a June 2011 car accident in California that left her with a fractured spine and damaged liver and colon, CNN said.
Longitudinal studies

Transfer of Intestinal Microbiota From Lean Donors Increases Insulin Sensitivity in Individuals With Metabolic Syndrome

ANNE VRIEZE,* ELS VAN NOOD,* FRITS HOLLEMAN,* JARKKO SALOJÄRVI,† RUUD S. KOOTTE,§
Interventional studies - FMT

Established role in Clostridium difficile dysbiosis

Success rate close to 90% [CI, 84-93%]

Three randomized, controlled trials (to 2016)

127 CDI cases – NNT = 2!
FMT in IBD – meta-analysis

- 18 studies to Q4 2014
- 79 UC and 39 CD described
- 45% of cases achieved clinical remission (CR)
- 36.2% CR in 9 cohort studies
- 24% CR in UC
- 60.5% CR in CD

- Limited data on mucosal healing

RJ, Rubin DT. JCC 2014
Forest plot of 9 cohort studies
Update on Fecal Microbiota Transplantation

67 A New Genetic Cause of Small Intestinal Carcinoid

102/110 Two Clinical Trials of Fecal Transplantation in Ulcerative Colitis

130 A Randomized Trial of Plastic Versus Metal Stents in Biliary Obstruction

177 VEGFR2 Signaling Inhibits Senescence and Promotes Colorectal Cancer

ALSO:
• RESEARCH PRIORITIES FOR ALCOHOLIC HEPATITIS
• REVIEW: AUTOIMMUNE PANCREATITIS
Fecal Microbiota Transplantation Induces Remission in Patients With Active Ulcerative Colitis in a Randomized Controlled Trial

Paul Moayyedi, Michael G. Surette, Peter T. Kim, Josie Libertucci, Melanie Wolfe

Findings From a Randomized Controlled Trial of Fecal Transplantation for Patients With Ulcerative Colitis

Noortje G. Rossen, Susana Fuentes, Mirjam J. van der Spek, Jan G. Tijssen

Multi-Donor Intense Faecal Microbiota Transplantation for Resistant Ulcerative Colitis: A Randomised Controlled Trial (The FOCUS Study)

Sudarshan Paramsothy

on behalf of the FOCUS Study Group
<table>
<thead>
<tr>
<th>Factor</th>
<th>Canada</th>
<th>Netherlands</th>
<th>Australia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease severity</td>
<td>All-comers</td>
<td>Mild-moderate</td>
<td>Mild-moderate</td>
</tr>
<tr>
<td>Concomitant meds</td>
<td>Any – stable doses</td>
<td>Not biologic</td>
<td>Not biologic</td>
</tr>
<tr>
<td>Sites</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Number of cases</td>
<td>75</td>
<td>48</td>
<td>81</td>
</tr>
<tr>
<td>Donors</td>
<td>Single HV</td>
<td>Known or HV</td>
<td>Mixed HV</td>
</tr>
<tr>
<td>Preparation</td>
<td>None</td>
<td>Bowel lavage</td>
<td>None</td>
</tr>
<tr>
<td>Route</td>
<td>Enema</td>
<td>NG</td>
<td>Scope, then enemas</td>
</tr>
<tr>
<td>Volume</td>
<td>50 ml</td>
<td>120 ml</td>
<td>150 ml</td>
</tr>
<tr>
<td>Frequency/duration</td>
<td>OW – 6 weeks</td>
<td>Weeks 0 and 3</td>
<td>5/week – 8 weeks</td>
</tr>
<tr>
<td>Primary outcome</td>
<td>Total Mayo≤2, Endo 0</td>
<td>SCCAI≤2, Endo improved by ≥1</td>
<td>Total Mayo≤2, Endo improved by ≥1</td>
</tr>
<tr>
<td>Time point</td>
<td>Week 7</td>
<td>Week 12</td>
<td>Week 8</td>
</tr>
</tbody>
</table>
Meta-analysis of 3 RCTs

- Based on clinical remission
- RR = 0.81 [95% CI = 0.71-0.92]
- NNT = 6 [95% CI = 4-14]
- All 3 studies had similar RR – Dutch study lacked sufficient power
- Safe approach – AEs related to deterioration in UC, one C.difficile
Other strategies

- OpenBiome – capsule technology
DIY – “the power of poop”

• Stool by stool instructions.....
Future work

- Further RCT including CD
- Capsules for CD (ileum)
- Enemas for UC – twice a week
- For 6-8 weeks
- HV
- Single donor – 7 of 9 cases in remission received FMT from one donor

Predictors:
- Disease duration
- Immunosuppression
- Corticosteroids at baseline
- Disease severity

![Graph showing Bray-Curtis dissimilarity with P = 0.02]
Summary

• Understanding of the human microbiome rapidly advancing
• Increasing attention being given to study design
• Key roles for diet and other lifestyle interventions that can occur in primary care