The Gut Microbiome: Markers of Human Health, Drug Efficacy and Xenobiotic Toxicity
Biomarkers of Toxicity and Disease

Identification of microbiome-based biomarkers and challenges associated with their application: Case studies from obesity, IBD, and cancer

25 June 2018 | Alexandria, VA

Emily Hollister
VP, Information Technology & Analytics
Background – Diversigen is a commercial microbiome service provider powered by CMMR

<table>
<thead>
<tr>
<th>MICROBIOME SERVICES</th>
<th>HEADQUARTERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metagenomic sequencing pipelines</td>
<td>Houston, Texas - located at the heart of the Texas Medical Center</td>
</tr>
<tr>
<td>Germ-free Project consulting Bioinformatics</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>YEAR FOUNDED</th>
<th>PEOPLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>12 highly talented and experienced individuals with a total of 8 PhD’s</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ORIGIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMC TEXAS MEDICAL CENTER</td>
</tr>
<tr>
<td>21 Renowned Hospitals</td>
</tr>
<tr>
<td>10 Academic Institutions</td>
</tr>
<tr>
<td>3 Dental Schools</td>
</tr>
</tbody>
</table>

Centre for Metagenomics and Microbiome Research (CMMR)

Microbiome Exploration | Microbial ecology modeling and dissection
Therapeutic development | Policy and outreach | Education | Translation
Metagenomics plays a key role in therapeutic and diagnostic development.

- Compare microbiota in healthy and ill subjects
- ID organisms / genes associated with health / disease
- Develop applications / solutions

**Community**
(100’s of strains, undefined composition)

**Consortium**
(defined composition of more than one strain, which together perform a function of interest)

**Single strain**
(one strain, pure isolate)

**Bioactive**
(molecule produced by strain that mediates effect of host)

- Diagnostic
- Probiotic / antibiotic
- Patient Stratification
  - Clinical trials
  - Personalized medicine
Microbiome-based biomarkers

• What are they? Do they exist?

• What are the challenges associated with their application?
  • Defining healthy has turned out to be difficult
  • Consensus/lack of across multiple studies – sources of bias, evolution of microbiome science/understanding

• They don’t have to be bacterial – although many purported biomarkers are

• The majority of the data are 16S-based
  • 16S lacks the resolution that we’d prefer to have (i.e., better than genus)

• The majority of data is stool-based
  • good for broad applicability and user acceptance; may not reflect biology at the disease interface. But…..at the end of the day, does it matter?
Obesity and the gut microbiome

- Summarizing the literature:
  - Data from animal studies is very compelling
  - Data from human studies is somewhat murky

- Consensus across studies is limited
  - Multiple potential pitfalls

Ley et al 2006; Turnbaugh et al 2006
Conferring obesity through FMT

Weight Gain After Fecal Microbiota Transplantation

Neha Alang¹ and Colleen R. Kelly²
¹Department of Internal Medicine, Newport Hospital, and ²Division of Gastroenterology, Center for Women’s Gastrointestinal Medicine at the Women’s Medicine Collaborative, The Miriam Hospital, Warren Alpert School of Brown University, Providence, Rhode Island

Fecal microbiota transplantation (FMT) is a promising treatment for recurrent Clostridium difficile infection. We report a case of a woman successfully treated with FMT who developed new-onset obesity after receiving stool from a healthy but overweight donor. This case may stimulate further studies on the mechanisms of the nutritional-neural-microbiota axis and reports of outcomes in patients who have used non-ideal donors for FMT.

Keywords. Clostridium difficile infection; fecal microbiota transplantation; gut microbiota; obesity.

Alang and Kelly 2015; Cox et al 2014
Microbiome alterations in are subtle and only partially reproducible

Table 2. Correlation of Operational Taxonomic Unit Abundances with Body Mass Index z-Scores Across Normal Weight (n = 22) and Obese (n = 42) Children

<table>
<thead>
<tr>
<th>OTU ID</th>
<th>Taxonomic identity</th>
<th>Spearman rho</th>
<th>q-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OTU_86</td>
<td>Unclassified proteobacteria</td>
<td>-0.59</td>
<td>0.0004</td>
</tr>
<tr>
<td>OTU_161</td>
<td>Paraprevotella clara</td>
<td>-0.55</td>
<td>0.0018</td>
</tr>
<tr>
<td>OTU_515</td>
<td>Unclassified bacterium</td>
<td>-0.52</td>
<td>0.0050</td>
</tr>
<tr>
<td>OTU_59</td>
<td>Bacteroides plebeius</td>
<td>-0.51</td>
<td>0.0054</td>
</tr>
<tr>
<td>OTU_216</td>
<td>Bacteroides thetaiotaomicron</td>
<td>-0.47</td>
<td>0.0170</td>
</tr>
<tr>
<td>OTU_476</td>
<td>Fusobacterium succinivorum</td>
<td>-0.47</td>
<td>0.0170</td>
</tr>
<tr>
<td>OTU_103</td>
<td>Prevotella stercorea</td>
<td>0.45</td>
<td>0.0282</td>
</tr>
<tr>
<td>OTU_107</td>
<td>Phascolarctobacterium succinutanis</td>
<td>0.45</td>
<td>0.0282</td>
</tr>
<tr>
<td>OTU_330</td>
<td>Unclassified bacterium</td>
<td>0.45</td>
<td>0.0282</td>
</tr>
<tr>
<td>OTU_98</td>
<td>Bacteroides salivarius</td>
<td>0.44</td>
<td>0.0312</td>
</tr>
<tr>
<td>OTU_409</td>
<td>Clostridium bolteae</td>
<td>-0.43</td>
<td>0.0407</td>
</tr>
<tr>
<td>OTU_355</td>
<td>Unclassified firmicutes</td>
<td>-0.43</td>
<td>0.0442</td>
</tr>
<tr>
<td>OTU_21</td>
<td>Unclassified bacteroidales</td>
<td>0.42</td>
<td>0.0546</td>
</tr>
<tr>
<td>OTU_13</td>
<td>Bacteroides massiliensis</td>
<td>0.40</td>
<td>0.0995</td>
</tr>
</tbody>
</table>

Spearman correlations were used to identify relationships between variables, and the Benjamini-Hochberg procedure was used to apply false discovery rate corrections.
Microbiome alterations in obesity are inconsistent

A

B

Sensitivity

Specificity

Accuracy

Baxter (AUC=0.52)
Escobar (AUC=0.57)
Goodrich (AUC=0.65)
HMP (AUC=0.60)
Ross (AUC=0.61)
Schubert (AUC=0.54)
Turnbaugh (AUC=0.57)
Wu (AUC=0.69)
Zeevi (AUC=0.55)
Zupancic (AUC=0.57)

Baxter (AUC=0.51)
Escobar (AUC=0.56)
Goodrich (AUC=0.62)
HMP (AUC=0.59)
Ross (AUC=0.51)
Schubert (AUC=0.52)
Turnbaugh (AUC=0.56)
Wu (AUC=0.59)
Zeevi (AUC=0.58)
Zupancic (AUC=0.65)

Sze and Schloss 2016
• IBD includes Crohn’s Disease and Ulcerative Colitis

• IBD affects 1.4M Americans, with increasing incidence in recent decades

• Greater incidence in Western nations and countries experiencing demographic/economic development

• Observed at greater rates in children immigrating to high(er) prevalence countries

• Concordance <50% in monozygotic twins

• Direct cost of treatment in the US represents an economic burden of ~$4 billion annually (UC)

• Greater risk for developing colorectal cancer

**IBD: Taxonomic patterns and trends**

**Taxonomic patterns:**
Imbalances/dysbiosis commonly described in the literature – largely 16S based:

- **CD:** ↓ Roseburia, ↓ Faecalibacterium prausntizii, ↑ Ruminococcus gnavus
- **UC:** not as clearly defined
  - ↑ sulfate-reducing Proteobacteria have been reported
  - ↓ Roseburia, ↓ Ruminococcaceae,

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Gevers et al 2014 Cell Host Microbe (CD)
Zhou et al 2018 mSystems
**Functional patterns:**
Fewer studies have used functional metagenomics.

- Gevers et al 2014: Species increased in CD contributed enrichment of glycerophospholipid and lipopolysaccharide metabolism

- Morgan et al 2012: Increased metabolism of sulfur-containing amino acids in IBD; shifted metabolism toward the degradation of mucin

- Alterations suggest an environment characterized by inflammation and oxidative stress

Morgan et al (2012) Genome Research
Gevers et al 2014 Cell Host Microbe
Does consensus exist with respect to IBD biomarkers?

Consensus?
To some degree, yes:

- Studies have repeatedly identified many of the same taxa (16S, WGS)
  - ↑ Enterobacteriaceae
  - ↑ Pasteurellaceae
  - ↑ Veillonellaceae
  - ↑ Fusobacteriaceae
  - ↓ Faecalibacterium
  - ↓ Roseburia
  - ↓ Clostridiales

- Unclear roles: Causal, contributory, coincident, consequential

Gut Microbiota Offers Universal Biomarkers across Ethnicity in Inflammatory Bowel Disease Diagnosis and Infliximab Response Prediction

Youlian Zhou, Zhenjiang Xu, Yan He, Yunsheng Yang, Le Liu, Qianyun Lin, Yuqiang Nie, Mingsong Li, Fachao Zhi, Side Liu, Amnon Amir, Antonio González, Anupriya Tripathi, Minhu Chen, Gary D. Wu, Rob Knight, Hongwei Zhou, Ye Chen

Zhou et al 2018 mSystems
IBD: How might microbiome-based biomarkers be used?

How might biomarker be used?
1. Early diagnosis
2. Monitoring of disease status/severity
3. Evaluating treatment success
4. Monitoring progression otherwise (e.g., toward relative health or other disease states)

Zhou et al 2018 mSystems
CRC & IBD: Risk association

- Increased risk of CRC in IBD recognized in late 80s

- Reported risk factors for CRC include
  - extensive disease
  - young age at diagnosis
  - family history of CRC
  - co-existing primary sclerosing cholangitis
  - **persistent inflammation of the colon**

- CRC in IBD carries a 2-fold increase in mortality compared with sporadic cancers.
  - No major differences between UC and CD except for a more advanced tumor stage at the time of diagnosis in CD patients.

- With IBD, the presence of CRC is often not known prior to surgery
Inflammation in CRC

Overall survivorship influenced by gene expression

A
- TCGA-COAD
- Survival measure - overall survival
- HDC gene expression
- Cohort divided at median of gene expression
- Overall survival
- Days
- HR: 0.69 (0.36-1.38), P=0.0901
- 3 Years
- HIGH EXPRESSION
- LOW EXPRESSION

B
- GSE17537
- Survival measure - overall survival
- HRH2 gene expression
- Cohort divided at median of gene expression
- Overall survival
- Days
- HR: 0.22 (0.05-0.91), P=0.0211746
- 3 Years
- 5 Years
- HIGH EXPRESSION
- LOW EXPRESSION

Histidine decarboxylase
Histamine H2 receptor

Gao et al 2017 American Journal of Pathology
Inflammation in CRC

Microbiome attenuation by addition of one organism

Model without chemical trigger

Model with chemical trigger

Model with chemical trigger and microbe

Model with chemical trigger and ko mutant microbe

Gao et al 2017 American Journal of Pathology
Colorectal cancer and the gut microbiome

- First CRC microbiome associations: adherent-invasive *Escherichia coli* is associated both with IBD and CRC

- Modern metagenomic techniques have yielded conflicting results
  - Most studies find associations
  - Different taxa are associated with CRC presence/absence, severity, disease progression, and survivorship

- Will microbiome be able to answer the question alone?
  - What about in high risk populations?

- CRC and the gut microbiome — Where are we with respect to describing it and identifying actionable biomarkers?

Rubin et al (2012) *Front Immunol*
Disparate CRC associated microbiota

Table 3. Bacterial species significantly over-represented in CRC stool samples.

<table>
<thead>
<tr>
<th>Bacterial Species</th>
<th>Avg. Healthy (%)</th>
<th>Avg. CRC (%)</th>
<th>Fold Change</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidaminobacter unclassified</td>
<td>0.05</td>
<td>0.39</td>
<td>7.7</td>
<td>0.0045</td>
</tr>
<tr>
<td>Phascolarctobacterium unclassified</td>
<td>3.31</td>
<td>11.0</td>
<td>3.2</td>
<td>0.0000</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>0.06</td>
<td>0.37</td>
<td>4.6</td>
<td>0.0050</td>
</tr>
<tr>
<td>Akkermansia muciniphila</td>
<td>3.54</td>
<td>12.8</td>
<td>3.6</td>
<td>0.0032</td>
</tr>
</tbody>
</table>

doi:10.1371/journal.pone.0070803.t003

When processed similarly concordance in associations

...mostly.

Shah et al 2017 Gut
Improvement in sensitivity by microbiome + risk factors


gFOBT, BMI, Wif1 methylation, fecal immunochemical test, viral clades…
Addition of metagenomic data

- 16S
- WGS
  - Bacterial
  - Viral
- Depth of sequencing

Hannigan (2017) bioRxiv
Multiple studies processed separately

Shah et al 2017 Gut
Multiple studies processed together

Shah et al. 2017 Gut
Microbiome-based biomarkers

Where do we go from here?

*Ideal biomarkers* should be able to:

- **Identify** disease at/prior to onset; **differentiate** between stages of disease (i.e., disease progression and/or response to treatment)
- **Be accessible and affordable** to facilitate broad acceptance for large scale screening
- **Work in high-risk populations** (e.g., T2DM, IBD are risk factors for CRC)
- **Distinguish** between disease of interest and other potentially microbiome-mediated conditions
- **Be robust** to lifestyle factors such as OTC or prescription drug consumption
Performing metagenomics at scale has many advantages

Studying multiple disease cohorts can **facilitate dissection of disease mechanisms and lead to better diagnostics and therapeutics**

Universal factors:
- Common exposures
- Common genetic risks

Unique regional factors:
- Diet
- Race/ethnicity
- Access to health care
- Environment

* Universal target discovery
* Personalized target discovery
Future outlook – efforts to accelerate and improve microbiome drug development

- **Access** to sequencing resources and comprehensive platforms
- **Utilize libraries** of microbiome samples and data
- **Improve study design** and share clinical knowledge & insights
- **Increase Black Box Methods** to identify/classify microbiomes
- **Incorporate machine learning** for predictive and association analysis
- **Adopt industry standards** e.g. CLIA, GLP
- **Grow functional data** (WGS, imputed analysis, IgASeq, RNAseq, etc)
- **Facilitate the shift** from association to causality
Thank You