Role of Microbiome and DNA testing in Pain Management (Interstitial Cystitis / Pelvic Pain)

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<table>
<thead>
<tr>
<th>Commercial Interest</th>
<th>Nature of Relevant Financial Relationship (Include all those that apply)</th>
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<tr>
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<td>What was received</td>
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<tr>
<td>• Petrichor Analytics, LLC</td>
<td>• Consulting Fees</td>
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<td>• Thorne/WellnessFX</td>
<td>• Consulting Fees, Service Agreement</td>
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It’s a Microbial World

<table>
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<tr>
<th>People = $7.43 \times 10^9$</th>
<th>7,432,663,275</th>
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<tbody>
<tr>
<td>Microbes in Human Gl Tracts $1.5 \times 10^{22}$</td>
<td>15,000,000,000,000,000,000,000,000,000</td>
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<tr>
<td>Stars = $10^{24}$</td>
<td>1,000,000,000,000,000,000,000,000,000,000</td>
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<tr>
<td>Microbes = $5 \times 10^{30}$</td>
<td>500,000,000,000,000,000,000,000,000,000,000</td>
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Microbes make up the majority of biomass, diversity, species, and organisms


The human microbiome or, the “other human genome”

- $1 \times 10^{13}$ human cells
- $2.5 \times 10^4$ human genes
- $1 \times 10^{14}$ microbial cells (microbiome)
- $3 \times 10^6$ microbial genes (metagenome)

Image courtesy of the NIH HMP website http://nihroadmap.nih.gov/hmp/
Microbiome Questions – A Census

• **Composition**: Whom are present in a microbiome?

• **Abundance**: How many of each microbe is present in a microbiome?

• **Function**: What is the microbiome (capable of ) doing?

• **Goals**: Identify the role of the microbiome (including metabolites) in disease and develop specific predictive, diagnostic, theranostic and prognostic tests for the clinical practice
Consequences of a Perturbed Microbiome?

- Peptic ulcers
- Kidney Stones
- Osteoporosis
- Obesity
- Diabetes
- Bowel Disorders
- Cancer
- Pre-term birth
How are we doing this?

1. **Taxonomy**
   We extract bacterial DNA, sequencing DNA coding regions V3-V5 of the 16S rRNA molecule.

2. **Metagenomics**
   We then re-sequence the data to detect genes that conduct microbial gene functions.
“Healthy Cohort” Body Sites

- Saliva
- Tongue dorsum
- Hard palate
- Buccal mucosa
- Keratinized (attached) gingiva
- Palatine tonsils
- Throat
- Supragingival plaque
- Subgingival plaque

- Retroauricular crease, both ears (2)
- Antecubital fossa (inner elbow), both arms (2)

- Anterior right and left nares (pooled)

- Stool

- Posterior fornix, vagina
- Midpoint, vagina
- Vaginal introitus

Slide courtesy of NHGRI
In adults, each part of the body supports a distinct microbial community.

With no apparent relationship with gender, age, weight, ethnicity or race.

Microbiome is acquired anew each generation.

1) Infants obtain microbes from mother or environment.

2) Microbial succession over ~1-2 yrs.

3) Microbiome becomes “adult-like” in ~1-2 yrs.
The Gut Brain Axis
Diet and Evolution
Gut Microbes as a Components of Dietary Adaptations esp. maternally-derived
What is the link?

Even though resistant fibers are good for us, our bodies delegate dealing with them to the gastro-intestinal microbiome. Our overall question: how important is the GI microbiome in brain function?

Microbiomes bring good things to life

- 6-10% of our daily energy supply
- Produce short-chain fatty acids (SCFAs), nutrients directed to intestinal lining, limit inflammation
- Neurohormones, vitamins, (folate, B6, B12)
- Affect obesity and appetite control
Why do we think knowing about microbes in brain function is important?

Nutrients from microbiomes add up, impact many tissues

Microbial products promote brain growth, and impact longevity (e.g. SCFA cancer inhibitors)

*Did they have roles in brain development and function?*

Some of our ancestors ate high fiber diets

*Did we have to negotiate new arrangements with microbes? Were we occupied by novel microbes?*

Microbes swap genes easily (horizontal gene transfer)

*Could this support changes in brain development and function?*
Germ-free in early life – effects on the social brain?

**Microbiome-gut-brain axis**

- Hypothalamus
- CRH
- Pituitary
- ACTH
- Adrenal
- Cortisol
- Vagus Nerve
- Enteric muscle
- Epithelium
- Short-chain fatty acids
- Enteric microbiota
- Neurotransmitters
- S-Hydroxy Dopamine
- 5-Hydroxytryptamine
- Serotonin

**Germ-free mouse**

- Altered gut-brain communication
  - ↓ memory
  - ↑ brain serotonin
  - Altered social behaviours??

- Mood
- Cognition
- Emotion
- Immune cells
- Cytokines
Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease


null, Volume 167, Issue 6, 2016, 1469-1480.e12; http://dx.doi.org/10.1016/j.cell.2016.11.018
Microbiomes of Interstitial Cystitis and Altered Microbiome of Chronic Pelvic Pain

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UTI vs. Asymptomatic Bacteriuria

Pain Perception

UPEC

ASB E. coli

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Referred Visceral Pain: Mechanical Allodynia

Von Frey filaments
- graded fiber stiffness
- assess tactile sensitivity
- e.g., neuropathy

Stimulate 10x each fiber
- Jump / retract / groom
- Response frequency

Pelvic vs footpad

Stimulus areas

DJ Klumpp. All rights reserved.
UPEC Induces Acute Pelvic Allodynia

- Mice mimic human responses
- Pain independent of mast cells

UPEC Differs from ASB E. coli

- Similar overall genetic structure
- Virulence factors not expressed in ASB
- “Mellowing out” to commensalism (Klemm 2007)
Lipopolysaccharide (LPS)

- O-antigen (?)
- OAg monomer (~ 40x)  
  180+ serotypes
- Core polysaccharide  
  R1-R4, K12
- Disaccharide diphosphate
- Lipid A (inflammation)
- Fatty acids
Purified NU14 LPS Induces Pain

ASB LPS Blocks UPEC Pain


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Intravesical ASB Reduces Cystitis Pain

Time (days)

Allodynia (% baseline)

Cipro

Saline

2% lidocaine

ASB

Rudick et al (in revision)
LPS in UTI Pain

FimH

LPS

Pathology

Inflamm

Pain

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O-antigen Modulates Pain Phenotype

Spinal Hyper-Excitability Underlies Pain Behavior

- Spontaneous firing in pain states

Peripheral and Central Mechanisms in Post-UTI Pain

Pain + Voiding dysfunction + Depression = IC-like model

Sensory Neuron

CCR2

TRPV1

TLR4

LPS

MCP-1

GABA

Glutamate

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• *E. coli* induce and modulate pelvic pain in mice

• Is IC associated with microbiome changes that promote pain?
Microbiomes of Interstitial Cystitis and Altered Microbiome of Chronic Pelvic Pain

Approach

- IC patients (n=8), healthy subjects (n=7)
- Fecal and vaginal samples
- 16S rRNA amplicon sequencing, metagenomics, metabolomics
- Anaerobic cultures
- Gnotobiotic mice
Global Differences: 16S Phyla

- Firmicutes
- Unclassified
- Fusobacteria
- D. from Bacteria
- Proteobacteria
- Actinobacteria
- Tenericutes
- Chloroflexi
- Verrucomicrobia
- Spirochaetes
- Cyanobacteria
- Aquificae
- Chlorobi
- Deferribacteres
- Chrysiogenetes
- Synergistetes
- Thermotogae
- Elusimicrobia
- Acidobacteria
- Fibrobacteria
- Planctomycetes

Legend:
- Control
- IC
ERF of 16S: Significant Features in IC

Likelihood of relevance

100% Likelihood

70% Likelihood

Variable Importance Metric
Odoribacter in Clinical Samples

Data for OTU 112

<table>
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<th>ICF-1</th>
<th>ICF-2</th>
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Mouse vs human taxa

OTU12 abundance (16S reads)

P=0.0002
IC Microbiota: DIPP Species

Species of differential abundance within OTU
• Deficient in IC Pelvic Pain (DIPP)
• Key features associate (community)

Braundmeier-Fleming et al. 2016. Stool-based biomarkers of interstitial cystitis/bladder pain syndrome. Scientific Reports. 6:26083 | DOI: 10.1038/srep26083
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**DIPP Species**

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<th>Species</th>
<th>AUC (ave)</th>
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<tbody>
<tr>
<td><em>Colinsella aerofaciens</em></td>
<td>0.86</td>
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<tr>
<td><em>Eggerthella sinensis</em></td>
<td>0.84</td>
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<tr>
<td><em>Faecalibacterium prasunitzii</em></td>
<td>0.79</td>
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<tr>
<td><em>Odoribacter splanchnicus</em></td>
<td>0.72</td>
</tr>
<tr>
<td><em>Lactonifactor longoviformis</em></td>
<td>0.55</td>
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- ROC shows biomarker potential
Stool Metabolites as Clinical Biomarkers

Glyceraldehyde

Braundmeier-Fleming et al. 2016. Stool-based biomarkers of interstitial cystitis/bladder pain syndrome. Scientific Reports. 6:26083 | DOI: 10.1038/srep26083
Microbiomes of Interstitial Cystitis

- 16S/ERF identifies key features (OTUs = genera)
- qPCR identifies DIPP species
- DIPP species and metabolites as novel IC biomarkers
- Human flora modulate pelvic pain in mice
Microbial Modulation of Pelvic Pain

- Pain response determined *a priori*, central & peripheral mechs
- ASB *E. coli* attenuate pain (periph?)
- Microbiota attenuate pain (crosstalk? fatty acids?)
- Therapy through mechanistic probiotics

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Words of Wisdom

“I have finally come to the conclusion that a good reliable set of bowels is worth more to a man than any quantity of brains”

Josh Billings 1818-1885
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