GUT MICROBIOME
CONFERENCE 2015

SEPTEMBER 26-27, 2015
HYATT REGENCY HUNTINGTON BEACH

A CME conference presented by the GastroIntestinal Health Foundation.

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New Developments in Irritable Bowel Syndrome

Mark Pimentel, MD, FRCP(C)
Director, GI Motility Program
Cedars-Sinai Medical Center
IBS – Forks in the Road

- Not all decisions in how to handle IBS made things better.
- What seems like the easiest road to diagnose, characterize and treat IBS may have led us into problems which now have to be fixed.
Stress and IBS: Cause and Effect?

- **Level 1 Evidence:** Apply severe psychological stress to people and watch them develop IBS over time.
- **Level 2 Evidence:** Compare psychological scores in IBS and compare to a disease comparable to IBS.
- **Level 3 Evidence:** Compare psychological scores between IBS and healthy people.
Stress and IBS

• Military study:
  – Shooting gun in combat
  – Shooting another human
  – Active combat
  – Injured in combat

  – Only Gastroenteritis was associated with IBS

Stress Due to Chronic Disease Not IBS?

IBS – Timeline

- 1950’s - Spastic Colitis
- 1960’s - Spastic Colon
- 1970’s - Irritable Bowel
- 1980’s - Irritable bowel syndrome
- 1990’s - Functional Bowel Disease – IBS type
- 2000’s - IBS – IBS-D, IBS-C, IBS-A
Acute Gastroenteritis

CdtB Toxin

Autoimmunity to vinculin

Gut ICC/neuronal Changes

Bacterial Overgrowth

IBS Microbial Hypothesis
Non-Constipation-IBS

D-IBS  M-IBS  C-IBS
Breath Testing is Abnormal in IBS

Forest plot of all age-sex matched studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Breath Test</th>
<th>OR (95% CI)</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grover</td>
<td>sucrose</td>
<td>2.29 (0.89, 5.87)</td>
<td>18.65</td>
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<tr>
<td>Lupascu</td>
<td>glucose</td>
<td>10.89 (3.52, 33.71)</td>
<td>16.82</td>
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<tr>
<td>Pimentel</td>
<td>lactulose</td>
<td>20.67 (5.29, 80.69)</td>
<td>14.68</td>
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<tr>
<td>Parodi</td>
<td>glucose</td>
<td>4.30 (1.24, 14.98)</td>
<td>15.71</td>
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<tr>
<td>Scarpellini</td>
<td>lactulose</td>
<td>24.27 (7.35, 80.15)</td>
<td>16.20</td>
</tr>
<tr>
<td>Collin</td>
<td>lactulose</td>
<td>18.04 (6.55, 49.71)</td>
<td>17.94</td>
</tr>
<tr>
<td>Overall</td>
<td>(I-squared = 67.9%, p = 0.008)</td>
<td>9.64 (4.26, 21.82)</td>
<td>100.00</td>
</tr>
</tbody>
</table>

NOTE: Weights are from random effects analysis
Small Bowel Culture in IBS

SIBO and IBS

27.3% N=77 non-D-IBS, N=35 D-IBS

60%

P=0.004

N=77 non-D-IBS, N=35 D-IBS

Pyleris. et al. DDS. 2012.
Single Organism PCR in IBS

**DUODENAL ASPIRATES**

- **E. coli**
  - Healthy
  - Not-IBS
  - IBS
  - $P < 0.05$

- **Klebsiella**
  - Healthy
  - Not-IBS
  - IBS
  - $P < 0.05$

$N=77$ non-D-IBS, $N=35$ D-IBS

Agilent Plots of Normal and IBS

Normal Subjects
### Primary Outcome (4 Weeks After Tx)

#### Efficacy Outcome

<table>
<thead>
<tr>
<th>Study</th>
<th>Odds Ratio</th>
<th>(95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGA-IBS Weekly</td>
<td>1.53</td>
<td>(1.10, 2.12)</td>
<td>0.0125</td>
</tr>
<tr>
<td></td>
<td>1.45</td>
<td>(1.05, 2.01)</td>
<td>0.0263</td>
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<td></td>
<td>1.49</td>
<td>(1.18, 1.88)</td>
<td>0.0008</td>
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<tr>
<td><strong>Key Secondary</strong></td>
<td></td>
<td></td>
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<tr>
<td>IBS Bloating Weekly</td>
<td>1.62</td>
<td>(1.16, 2.27)</td>
<td>0.0045</td>
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<td></td>
<td>1.49</td>
<td>(1.08, 2.06)</td>
<td>0.0167</td>
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<td></td>
<td>1.56</td>
<td>(1.23, 1.96)</td>
<td>0.0002</td>
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<tr>
<td><strong>Other Secondary</strong></td>
<td></td>
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<tr>
<td>SGA-IBS Daily</td>
<td>1.76</td>
<td>(1.26, 2.47)</td>
<td>0.0009</td>
</tr>
<tr>
<td></td>
<td>1.59</td>
<td>(1.13, 2.24)</td>
<td>0.0072</td>
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<tr>
<td></td>
<td>1.61</td>
<td>(1.28, 2.04)</td>
<td>&lt;0.0001</td>
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<tr>
<td><strong>FDA Proposed</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ab Pain &amp; Stool Daily (FDA)</td>
<td>1.40</td>
<td>(1.02, 1.92)</td>
<td>0.0401</td>
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<td></td>
<td>1.46</td>
<td>(1.02, 1.37)</td>
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<td>1.47</td>
<td>(1.17, 1.84)</td>
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<td>Ab Pain Daily (FDA)</td>
<td>1.46</td>
<td>(1.02, 2.03)</td>
<td>0.0157</td>
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<td>1.46</td>
<td>(1.06, 2.00)</td>
<td>0.0194</td>
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<td>1.46</td>
<td>(1.17, 1.83)</td>
<td>0.0009</td>
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<tr>
<td>Stool Consist. Daily (FDA)</td>
<td>1.80</td>
<td>(1.25, 2.59)</td>
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<td>1.57</td>
<td>(1.12, 2.21)</td>
<td>0.0086</td>
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<td>1.67</td>
<td>(1.31, 2.14)</td>
<td>&lt;0.0001</td>
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</tbody>
</table>

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*Pimentel. et al. NEJM. 2011.*
### Durability of Response (3 Months)

**Key**
- **Primary**
- **Secondary**
- **Other Secondary**
- **FDA Proposed**

<table>
<thead>
<tr>
<th>Efficacy Outcome</th>
<th>Study</th>
<th>Odds Ratio (95% CI)</th>
<th>p-value</th>
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<tbody>
<tr>
<td><strong>Primary</strong></td>
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<td></td>
<td></td>
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<tr>
<td>SGA-IBS Weekly</td>
<td>TARGET 1</td>
<td>1.35 (1.00, 1.82)</td>
<td>0.0477</td>
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<tr>
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<td>TARGET 2</td>
<td>1.52 (1.13, 2.03)</td>
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<td></td>
<td>Combined</td>
<td>1.44 (1.17, 1.77)</td>
<td>0.0007</td>
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<tr>
<td><strong>Secondary</strong></td>
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<tr>
<td>IBS Bloating</td>
<td>TARGET 1</td>
<td>1.28 (0.95, 1.73)</td>
<td>0.1042</td>
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<td>Weekly</td>
<td>TARGET 2</td>
<td>1.56 (1.16, 2.09)</td>
<td>0.0031</td>
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<tr>
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<td>Combined</td>
<td>1.42 (1.15, 1.75)</td>
<td>0.0011</td>
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<tr>
<td>Other Secondary</td>
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<tr>
<td>SGA-IBS Daily</td>
<td>TARGET 1</td>
<td>1.60 (1.18, 2.18)</td>
<td>0.0025</td>
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<td>TARGET 2</td>
<td>1.47 (1.09, 1.99)</td>
<td>0.0127</td>
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<tr>
<td></td>
<td>Combined</td>
<td>1.48 (1.20, 1.83)</td>
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<tr>
<td>IBS Bloating</td>
<td>TARGET 1</td>
<td>1.50 (1.10, 2.04)</td>
<td>0.0103</td>
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<tr>
<td>Daily</td>
<td>TARGET 2</td>
<td>1.67 (1.24, 2.25)</td>
<td>0.0008</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>1.53 (1.24, 1.89)</td>
<td>-0.0001</td>
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<tr>
<td>IBS Ab Pain</td>
<td>TARGET 1</td>
<td>1.35 (1.00, 1.83)</td>
<td>0.0495</td>
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<tr>
<td>Daily</td>
<td>TARGET 2</td>
<td>1.35 (1.01, 1.81)</td>
<td>0.0435</td>
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<tr>
<td></td>
<td>Combined</td>
<td>1.31 (1.06, 1.61)</td>
<td>0.0118</td>
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<tr>
<td><strong>FDA Proposed</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ab Pain &amp; Stool</td>
<td>TARGET 1</td>
<td>1.36 (1.01, 1.83)</td>
<td>0.0396</td>
</tr>
<tr>
<td>Daily (FDA)</td>
<td>TARGET 2</td>
<td>1.44 (1.08, 1.92)</td>
<td>0.0141</td>
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<td></td>
<td>Combined</td>
<td>1.40 (1.14, 1.72)</td>
<td>0.0014</td>
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<tr>
<td>Ab Pain Daily</td>
<td>TARGET 1</td>
<td>1.31 (0.96, 1.75)</td>
<td>0.0725</td>
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<tr>
<td>(FDA)</td>
<td>TARGET 2</td>
<td>1.37 (1.03, 1.83)</td>
<td>0.0098</td>
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<tr>
<td></td>
<td>Combined</td>
<td>1.33 (1.09, 1.64)</td>
<td>0.0058</td>
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<tr>
<td>Stool Consist.</td>
<td>TARGET 1</td>
<td>1.70 (1.24, 2.33)</td>
<td>0.0009</td>
</tr>
<tr>
<td>Daily (FDA)</td>
<td>TARGET 2</td>
<td>1.48 (1.09, 2.00)</td>
<td>0.0114</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>1.58 (1.27, 1.97)</td>
<td>-0.0001</td>
</tr>
</tbody>
</table>

*Pimentel. et al. NEJM. 2011.*
Alosetron

Patients With Adequate Relief, %

* $P<.05$; †Diarrhea-predominant intention to treat (ITT) population.

Screening

Open-Label Treatment Phase

**Study Day 1**

- **7-13 d PBO**
- **2w RFX**
- **4w f/u**

Maintenance Phase

- **Variable up to 18w**
- **Only patients with recurrent symptoms randomized**

Follow-up

- **Target 3 Trial**

Open-Label Rifaximin

550 TID x 2 weeks

Maintenance Phase

**Primary Evaluation Period**

First Repeat Treatment

- **2w RFX**
- **4w f/u**
- **6w**
- **2w RFX**
- **4w f/u**

Double-blind Treatment Phases

Maintenance Phase

- **6w**

Second Repeat Treatment

- **2w PBO**
- **4w f/u**

Follow-up

- **4 W**

Only patients with recurrent symptoms randomized

Non-Responders Withdrawn and proceed to EOS Phase

**Stool sample collection**
Comparability of Symptom Severity

TARGET 3

TARGET 3: All DBR Subjects (n=636)
Worst abdominal pain over the last 24hr

Subjects Do Not Return to Baseline Symptom Severity Following Initial Therapy with Rifaximin
### Meta-analysis of studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>OR (95% CI)</th>
<th>Weight</th>
</tr>
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<tbody>
<tr>
<td>Peled</td>
<td>1987</td>
<td>0.83 (0.20, 3.56)</td>
<td>8.43</td>
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<tr>
<td>Fiedorek</td>
<td>1990</td>
<td>4.32 (1.60, 11.68)</td>
<td>12.03</td>
</tr>
<tr>
<td>Pimentel</td>
<td>2003</td>
<td>5.58 (2.22, 14.03)</td>
<td>12.68</td>
</tr>
<tr>
<td>Pimentel</td>
<td>2003</td>
<td>44.23 (2.48, 788.51)</td>
<td>3.18</td>
</tr>
<tr>
<td>Majewski</td>
<td>2007</td>
<td>1.81 (0.70, 4.67)</td>
<td>12.46</td>
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<tr>
<td>Bratten</td>
<td>2008</td>
<td>2.22 (1.14, 4.33)</td>
<td>15.14</td>
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<tr>
<td>Parodi</td>
<td>2009</td>
<td>1.89 (0.79, 4.51)</td>
<td>13.17</td>
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<tr>
<td>Hwang</td>
<td>2009</td>
<td>47.67 (8.73, 260.41)</td>
<td>6.99</td>
</tr>
<tr>
<td>Attaluri</td>
<td>2009</td>
<td>3.70 (2.06, 6.66)</td>
<td>15.92</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>3.51 (2.00, 6.16)</td>
<td>100.00</td>
</tr>
</tbody>
</table>

NOTE: Weights are from random effects analysis
Methane Slows Intestinal Transit

69% mean slowing of transit with CH$_4$

n=5, p<0.0001

Methanobrevibacter Smithii - the Cause of Constipation

M. Smithii Is the Source of Methane in C-IBS

- **Stool M. smithii level by qPCR**
  - 10^6
  - 10^4
  - 10^2
  - 0

- **Breath Methane Positive (>3ppm)**
  - Constipated

- **Breath Methane Negative (<3ppm)**
  - Normal
**Methanobrevibacter Smithii** or Methane as a Test Dictates Treatment

CH4 Eradication

P=0.001

Clinical Response

P=0.01

---

Methane Positive C-IBS
Double Blind Placebo Controlled Trial

P=0.01

After Treatment Bloating Level by Group

Final Visit Constipation Severity Based on Methane >3ppm

Analysis of the Neomycin + Rifaximin Group

P=0.04

SIBO Treatment Strategy

IBS SYMPTOMS

LACTULOSE BREATH TEST

- Hydrogen Positive
  - Rifaximin
- Methane Positive
  - Rifaximin+Neomycin

Symptom Re-evaluation/Retest

- Prokinetic
- No Response

Investigate

Acute Gastroenteritis

CdtB Toxin

Autoimmunity to vinculin

Gut ICC/neuronal Changes

Bacterial Overgrowth

IBS Microbial Hypothesis
Risk of PI-IBS Increases 7-Fold After Infectious Gastroenteritis*

Characteristics of Acute Illness
Identify Patients at Risk for PI-IBS


*OR for IBS After Acute Gastroenteritis*
Post-Infectious IBS = IBS

Mechanism: Rat Model (SIBO = IBS)

n=33
Stool = Campy-
No Acute Gastroenteritis

3 Months After Recovery
Stool Consistency Evaluation

Bacterial Quantitation by RT-PCR of Duodenum, Jejunum, Ileum

n=33
Stool = Campy+
Acute Gastroenteritis

C. jejuni

Stool = Campy-
Recovery

Mechanism: Rat Model (SIBO = IBS)

3 months after *Campylobacter jejuni* 81-176 infection

Persistent altered stool consistency

Increased rectal lymphocytes

Mechanism: Rat Model (SIBO = IBS)

CD117 immunostain of duodenal, jejunal, ileal cross-sections (Dako Cytomation, Inc; Carpinteria, CA)

Randomized and coded

2 independent, blinded readers reported as DMP ICC per villus
ICC Level and SIBO

Jee et al. World J Gastroenterol. 2010.
Ileal ICC in Controls

Ileal ICC in C+/SIBO-

Ileal ICC in C+/SIBO+

Cytolethal Distending Toxin (CDT)

- *C. jejuni*
- *E. Coli*
- *Salmonella*
- *Shigella*

All cause IBS
3 months later stool evaluation

Bacterial quantitative PCR of small bowel

Cytolethal Distending Toxin (CDT)

Wild type Campy

CdtB (-) Campy

Campy + Rifaximin

Daily stool for Campy (length of colonization)
### CDT and Rifaximin in IBS: Rat Model

<table>
<thead>
<tr>
<th></th>
<th>Campy</th>
<th>CdtB-</th>
<th>P-value</th>
<th>Rifaximin</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stool % wet weight</td>
<td>60.1±6.8</td>
<td>60.8±3.6</td>
<td>0.47</td>
<td>61.1±3.8</td>
<td>0.33</td>
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<tr>
<td>Consistency</td>
<td>1.51±0.37</td>
<td>1.23±0.27</td>
<td>&lt;0.00001</td>
<td>1.15±0.30</td>
<td>&lt;0.0000001</td>
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<tr>
<td>Standard Deviation</td>
<td>8.4±6.4</td>
<td>4.2±2.4</td>
<td>&lt;0.0001</td>
<td>4.1±2.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Proportion with normal bowel form all 3 days</td>
<td>17.8%</td>
<td>50.0%</td>
<td>&lt;0.01 OR=4.63</td>
<td>59.3%</td>
<td>&lt;0.00001 OR=6.7</td>
</tr>
</tbody>
</table>

Note: No significant differences were seen between CDT- and Rifaximin treated arms of the study.

Neurogastroenterol Motil 2013.
Molecular Mimicry

Pre-immune Serum

Anti-CDT Antibodies
IBS Mechanism/Nerve Damage

GANGLIA OF HUMAN ILEAL SECTIONS

Anti-CdtB is also Anti-glial in humans

Vinculin- The Link to IBS

ZONULA ADHERENS

plasma membrane

cadherin

p120ctn
α-actinin

α-actinin

actin

vinculin

catenins
Vinculin

- Focal adhesion plaques
- Actin filaments
Molecular Mimicry/Autoimmunity

Cytolethal Distending Toxin B

Human Vinculin
Molecular Mimicry/Autoimmunity

Cytolethal Distending Toxin B → Human Vinculin
Vinculin Expression and SIBO

Rats with single or double infection, SIBO or no SIBO
Vinculin Expression and Small Bowel Bacterial Levels

% of rats with Elevated Small Bowel Bacteria

CdtB Inoculated

Control

P = 0.03

Above Threshold
Below Threshold

Triantafyllou. DDW, 2014.
Blood Test For IBS

- D-IBS subjects (N=2375)
- Subjects with IBD (N=142) which included Crohn’s disease (N=73) and ulcerative colitis (N=69)
- Subjects with celiac disease (N=121)
- Healthy subjects (N=43)
Blood Test For IBS

AUC=0.62 (95% CI, 0.58-0.67)
AUC=0.81 (95% CI, 0.77-0.84)

Sensitivity

0 1

1-specificity

Anti-vinculin Ab
Anti-CdtB Ab
marker

AUC=0.81 (95% CI, 0.77-0.84)

AUC=0.62 (95% CI, 0.58-0.67)

0 1

1-specificity

Anti-CdtB
Anti-vinculin
IBS Pathophysiologic Sequence

Food Poisoning

Bacterial Toxin

Autoimmunity

Gut Nerve Damage

Bacterial Overgrowth

IBS

E. Coli
C. jejuni
Shigella
Salmonella

Cytolethal Distending Toxin (CDT B)

Anti-vinculin

Reduced ICC
Reduced MMC

Breath testing
Culture
qPCR
deep sequencing

Antibiotics
Conclusions

- Small intestinal bacterial changes are seen in at least 60% of IBS.
- *M. smithii* (methane production) slows transit and is associated with constipation
- Breath testing strategizes the treatment for IBS
- Acute gastroenteritis causes IBS
- *C. jejuni* gastroenteritis causes SIBO in rats through CdtB neurotoxicity
- CdtB can cause autoimmunity to vinculin via molecular mimicry
- Serum anti-CdtB and anti-vinculin can distinguish IBS from IBD
- IBS is an organic disease
# Acknowledgements

## Motility Research Team/Endocrine Team

<table>
<thead>
<tr>
<th>Gene Kim</th>
<th>Ruchi Mathur, MD, FRCP(C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stacy Weitsman</td>
<td>Mark Goodarzi, MD, PhD</td>
</tr>
<tr>
<td>Walter Morales</td>
<td>Michelle Jones, PhD</td>
</tr>
<tr>
<td>Shari Chua</td>
<td>Zachary Marsh</td>
</tr>
<tr>
<td>Gillian Barlow, PhD</td>
<td>Emily Marsh</td>
</tr>
<tr>
<td>Chris Chang, MD, PhD</td>
<td>Venkata Pokkunuri</td>
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<td>Ali Rezaie, MD, MPH</td>
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## Sequencing Team

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<tr>
<th>Vincent Funari, PhD</th>
<th>Pat Guerry, PhD</th>
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<tbody>
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<td>Jie Tang, PhD</td>
<td>Mark Riddle, MD</td>
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<td>Jordan Brown</td>
<td>Michael Prouty, MD</td>
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<td>Brooks Cash, MD</td>
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<td>Katerina Pistikis, MD</td>
<td>Kostas Triantafyllou, MD</td>
</tr>
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