Functional Role of Gut Microbiota in Chronic Liver Disease

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## Agenda

<table>
<thead>
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<th>The Intestinal Microbiome</th>
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<td>The Microbiome and Cholestatic Liver Disease: Plausible Interactions</td>
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<td>Effect of Microbiome on Bile Acids and Potential Therapeutic Interventions</td>
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</table>
The Human Microbiome

- Comprised of bacteria, viruses, and other micro-organisms\(^1\)
- Distinctive microbiomes at each body site (e.g., gut, lung, skin, mucosa)\(^2\)
- The gut microbiota
  - Human gut is home to ~100 trillion bacterial cells\(^3\)
  - Density of \(10^{11}\) to \(10^{12}\) per gram in the colon\(^4\)
  - Large numbers of species present, many uncultured

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Host-Microbial Mutualism in the Gut

Host benefits to bacteria
- Provides unique niche
- Intestinal mucus provides source of nutrition

Bacteria benefits to host
- Fermentation of indigestible carbohydrates and production of SCFAs
- Biotransformation of conjugated bile acids
- Urease activity participates in nitrogen balance
- Synthesis of certain vitamins
- Metabolize drugs
- Education of the mucosal immune system

Abbreviations: AMP, antimicrobial peptides; Ig, immunoglobulin; PSA, polysaccharide A; SCFA, short-chain fatty acids; SFB, segmented filamentous bacteria; Treg, regulatory T-cells.

Agenda

The Intestinal Microbiome

The Microbiome and NAFLD/NASH: Plausible Interactions

Effect of Microbiome on Bile Acids and Potential Therapeutic Interventions
The Gut Microbiome and Liver Disease

- Liver is first portal that emerges from intestinal mucosal surface
  - Receives approximately 75% of blood supply from splanchnic circulation

- Potential liver diseases affected by gut microbiome
  - NASH and NAFLD
  - Cholestatic liver disease (PBC and PSC)
  - Cirrhosis
  - Hyperammonemia and hepatic encephalopathy
  - Hepatic drug metabolism

Abbreviations: NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis.
NAFLD Spectrum

<table>
<thead>
<tr>
<th>Type 1</th>
<th>NAFL, steatosis alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 2</td>
<td>NAFL, steatosis plus inflammation</td>
</tr>
<tr>
<td>Type 3</td>
<td>NASH, steatosis plus hepatocyte injury (ballooning)</td>
</tr>
<tr>
<td>Type 4</td>
<td>NASH, steatosis plus fibrosis</td>
</tr>
</tbody>
</table>

NAFL – nonalcoholic fatty liver
NASH – nonalcoholic steatohepatitis

Obesity / Overnutrition Sedentary Lifestyle
Genet. Predisposition

Normal Liver → NAFLD 25% western pop. appr. 300 millions → NASH 5-10% western pop. 50-100 millions → Cirrhosis 1-3 millions → Liver Cancer → Acute-on-Chronic Liver Failure
HIV and Liver Disease

Joshi et al. Lancet 2011;377:1198
The Gut Lumen Contents and its Role in Energy Balance and Metabolic Function

- Central mechanisms:
  - Appetite, food reward
  - Energy expenditure
  - Metabolic function

Central mechanisms influence:
- Liver
- Pancreas

GI Lumen contains:
- Nutrients
- Microbiota
- Bile acids
- Mucus

GI tissue:
- Immune cells
- Gut hormones
- Efferent neurons

Energy balance influences:
- Metabolic function

Metabolic syndrome (Syndrome X):
- Central obesity
- High blood pressure
- High triglycerides
- Low HDL-cholesterol
- Insulin resistance
# Murine Models Demonstrating Cause-and-Effect Relationships Between the Gut Microbiome and the Development of Obesity

## Table 1. Metabolic Consequences of Specified Host and Dietary Interactions with the Microbiome

<table>
<thead>
<tr>
<th>Host State</th>
<th>Diet (SCFAs, Immune Function, Anatomic)</th>
<th>Phenotype with Microbiota</th>
<th>Phenotype in GF Mice or with Reduced Microbiota&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Major Microbial Changes</th>
<th>Transfer&lt;sup&gt;b&lt;/sup&gt;</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>ob&lt;sup&gt;/-&lt;/sup&gt;</em></td>
<td>Diet</td>
<td>↑ Weight and adiposity</td>
<td>Resistance to GIO&lt;sup&gt;a&lt;/sup&gt;</td>
<td>↑ Firmicutes ↓ Bacteroidetes</td>
<td>Yes</td>
<td>Ley et al., 2005; Turnbaugh et al., 2006; Cani et al., 2008</td>
</tr>
<tr>
<td>High-calorie diet</td>
<td>Diet</td>
<td>↑ Weight and adiposity</td>
<td>Resistance to DIO</td>
<td>↑ Firmicutes ↓ Bacteroidetes</td>
<td>Yes</td>
<td>Turnbaugh et al., 2008</td>
</tr>
<tr>
<td><em>Gpr41&lt;sup&gt;−/−&lt;/sup&gt;</em></td>
<td>Diet</td>
<td>Lean</td>
<td>No difference from controls</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Samuel et al., 2008</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Diet</td>
<td>↑ Weight and adiposity</td>
<td>Unknown</td>
<td>↑ Lactic acid bacteria ↓ Butyrate producers</td>
<td>Yes</td>
<td>Koren et al., 2012</td>
</tr>
<tr>
<td><em>Tlr5&lt;sup&gt;−/−&lt;/sup&gt;</em></td>
<td>Diet</td>
<td>Obese, insulin resistant, hyperphagic</td>
<td>No difference from controls&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Changes at the OTU level, no changes at the phylum level</td>
<td>Yes</td>
<td>Vijay-Kumar et al., 2010</td>
</tr>
<tr>
<td>Inflammomasomes <em>Asc&lt;sup&gt;−/−&lt;/sup&gt;, Casp1&lt;sup&gt;−/−&lt;/sup&gt;, Nlp3&lt;sup&gt;−/−&lt;/sup&gt;, IL18&lt;sup&gt;−/−&lt;/sup&gt;</em></td>
<td>Diet</td>
<td>↑ NASH</td>
<td>No difference from controls&lt;sup&gt;a&lt;/sup&gt;</td>
<td>↑ Prevotellaceae and Porphyromonadaceae</td>
<td>Yes</td>
<td>Henao-Mejia et al., 2012</td>
</tr>
<tr>
<td>Lymphotoxin-deficient&lt;sup&gt;c&lt;/sup&gt; <em>Ltbr&lt;sup&gt;−/−&lt;/sup&gt;, Lta&lt;sup&gt;−/−&lt;/sup&gt;, Ltb&lt;sup&gt;−/−&lt;/sup&gt;</em></td>
<td>Diet</td>
<td>Resistant to DIO</td>
<td>Unknown</td>
<td>↑ SFB and Cytophaga ↓ Erysipelotrichi</td>
<td>Yes, but transient, obese WT → <em>Ltbr&lt;sup&gt;−/−&lt;/sup&gt;</em></td>
<td>Upadhyay et al., 2012</td>
</tr>
<tr>
<td>Roux-en-Y gastric bypass</td>
<td>Diet</td>
<td>Lean</td>
<td>Unknown</td>
<td>↑ Gammaproteobacteria ↑ <em>Akermansia</em></td>
<td>Yes</td>
<td>Liou et al., 2013</td>
</tr>
</tbody>
</table>

<sup>a</sup>Reduction in microbiota by high-dose broad-spectrum antibiotics.

<sup>b</sup>Transfer of the phenotype to conventional mice has been accomplished.

<sup>c</sup>Resistance to DIO also achieved by deleting IL23a and RORγt, elements downstream of the lymphotoxin pathway.

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Dietary Fiber, the Production of Short Chain Fatty Acids, and Their Effects on the Host

Colon
- Dietary fibre
  - Microbial degradation
  - Oligo-, monosaccharides
    - Short-chain fatty acids
      - GPCR signalling
      - HDAC inhibition
      - Energy source

Liver
- Lipogenesis and gluconeogenesis
  - Trevisan et al.
  - Nature 2012;489:242
Mechanisms by Which Gut Microbes May Influence the Development of Metabolic Syndrome

Effect of Microbial Metabolites (i.e. SCFAs)

Direct Effect of Microbes on Immune Activation

Dietary Effects on Human Gut Microbiome and its Association with Disease

• Individuals with marked obesity, insulin resistance, dyslipidemia, and inflammatory phenotype have low bacterial richness

• Increased consumption of an agrarian diet, rich in fruits and vegetables with higher fiber, is associated with increased bacterial gene richness

• Energy-restricted diets increase bacterial gene richness

Decrease gut microbiome “richness” (decreased number of various bacteria and their genes) is associated with both disease states and the consumption of a Westernized diet

Wu et al. Science 2011;334:105-8
Cell Metabolism

Dietary Fiber-Induced Improvement in Glucose Metabolism Is Associated with Increased Abundance of Prevotella

Graphical Abstract

Authors
Petia Kovatcheva-Datchary, Anne Nilsson, Rozita Akrami, ..., Eric Martens, Inger Björck, Fredrik Bäckhed

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In Brief
Diet affects the gut microbiota composition, though large inter-individual variations exist. Kovatcheva-Datchary et al. reveal that subjects with improved glucose metabolism after barley kernel supplementation have increased Prevotella in their gut microbiota. Prevotella plays a direct role in the beneficial response, supporting the importance of personalized approaches to improve metabolism.

Highlights
- Prevotella/Bacteroides is associated with a beneficial response to barley kernels
- Prevotella-enriched microbial interactions are higher in barley kernel responders
- Prevotella protects against Bacteroides-induced glucose intolerance
- Prevotella promotes increased hepatic glycogen storage in mice
Prescreening of donors to prevent transmission of currently known pathogens

Homogenization, filtration, and administration usually through a colonoscope.

Success rate of around 90% when fecal microbiota transplantation (FMT) is used to treat CDI

FMT and the Treatment of Type 2 Diabetes

BRIEF REPORT

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

FMT: Clinical trials

- *C difficile* infection (38)
- Crohn’s (5)
- Ulcerative Colitis (15)
- Pouchitis (3)
- IBD (9)
- IBS (6)
- Constipation (4)
- **NAFLD/NASH (3)**
  - PSC
  - Intestinal pseudo-obstruction
  - Autologous FMT (preventative)
- Obesity/metabolic syndrome (5)
- HIV
- DM-II (2)
- Pancreatitis (2)
- Hepatitis B
- MRSA enterocolitis
- Drug-resistant organisms (4)
- Hepatic encephalopathy (2)
- Post-stem cell transplant (2)

Clinicaltrials.gov
06/02/2016
Alterations or “Dysbiosis” of the Gut Microbiota in NAFLD and NASH in Humans

Schnabl and Brenner. Gastro 2014

The Gut Microbiome as a Biomarker of Cirrhosis

Table 1. Changes in the Intestinal Microbiota Associated With NAFLD and NASH in Human Beings

<table>
<thead>
<tr>
<th>Disease</th>
<th>Comparison</th>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus</th>
<th>Implicated microbiota</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy (n = 30)</td>
<td>Healthy vs NAFLD</td>
<td>Firmicutes</td>
<td>Bacilli</td>
<td>Lactobacillales</td>
<td>Lactobacillaceae</td>
<td>Lactobacillus</td>
<td>16S rRNA gene pyrosequencing</td>
</tr>
<tr>
<td>NAFLD (n = 30)</td>
<td></td>
<td>Clostridia</td>
<td>Lachnospiraceae</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>Clostridia</td>
<td>Oscillibacter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children:</td>
<td>Healthy vs obese</td>
<td>Bacteroidetes</td>
<td>Prevotellaceae</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy (n = 16)</td>
<td></td>
<td>Firmicutes</td>
<td>Rikenellaceae</td>
<td></td>
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<tr>
<td>Obese (n = 25)</td>
<td></td>
<td>Bacteroidetes</td>
<td>Ruminococcaceae</td>
<td></td>
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<tr>
<td>NASH (n = 22)</td>
<td></td>
<td>Firmicutes</td>
<td>Lachnospiraceae</td>
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<td>Healthy (n = 17)</td>
<td>Healthy vs NASH</td>
<td>Bacteroidetes</td>
<td>Enterobacteriaceae</td>
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<td></td>
</tr>
<tr>
<td>Steatosis (n = 11)</td>
<td></td>
<td>Proteobacteria</td>
<td>Enterobacteriaceae</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>NASH (n = 22)</td>
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<td>Enterobacteriaceae</td>
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A comparison of condition A vs condition B: †, increase in condition B relative to condition A; ‡, decrease in condition B relative to condition A; ns, no significant difference.

Taxonomy was updated using the National Center for Biotechnology Information (NCBI) Taxonomy Browser.

References focus on microbiota changes associated with liver disease rather than obesity or the metabolic syndrome.

ARTICLE

Alterations of the human gut microbiome in liver cirrhosis

Nan Qin1,2,*, Fengling Yang1,*, Ang Li1,*, Edi Prifti1,*, Yanfei Chen1,*, Li Shao1,2,*, Jing Guo1, Emmanuelle Le Chatelier2, Jian Yao5,2, Lingliao Wu1, Jiawei Zhou2, Shujun Ni1, Lin Liu1, Nicolas Pons1, Jean Michel Bato1, Sean P. Kennedy1, Pierre Leonard1, Chunhui Yuan1, Wenchao Ding1, Yuanting Chen1, Xinjun Hu1, Beiwen Zheng1,*, Guirong Qian1, Wei Xu1, S. Dusko Ehrlich1,4, Shusen Zheng1,5 & Lanjuan Li1,2

doi:10.1038/nature13568
Agenda

The Intestinal Microbiome

The Microbiome and NAFLD/NASH: Plausible Interactions

Effect of Microbiome on Bile Acids and Potential Therapeutic Interventions
Role of Bile Acids and Microbiota Effect

• Produced from cholesterol as conjugated bile acids in liver to be secreted into small intestine\(^1\)
  – Absorbed in terminal ileum and returned to liver

• Important for\(^1\)
  – Absorption of dietary fat and vitamins
  – Direct bacteriostatic effect
  – Ligands for FXR and TGR5

• Intestinal microbiota may affect liver by modifying intestinal bile acids and altering FXR signaling\(^1\)
  – The gut microbiota converts primary into secondary bile acids\(^1\)
  – Bile acid production in germ-free vs conventionally housed mice is altered by differential bile acid activation of FXR\(^2\)
  – Activation of FXR enhances small intestinal barrier function\(^3\)

Abbreviation: FXR, farnesoid X receptor.
Bile Salt Transformations by the Gut Microbiome and FXR

Ridlon et al. J. Lipid Res. 2006

Obetacholic Acid (OCA)
Activation of FXR is beneficial in the treatment of liver disease in both rodents and humans.

- OCA produced marked reduction in high rate of gut bacterial translocation in Cirrhotic rats via:
  - Reduction of fecal bacterial load
  - Partial recovery of intestinal dysbiosis
  - Improvements in intestinal barrier function and gut inflammation
  - Reduced liver fibrogenesis

![Obeticholic acid reduces bacterial translocation and inhibits intestinal inflammation in cirrhotic rats](image)

![Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial](image)

Central mechanisms:
- Appetite, food reward
- Energy expenditure
- Metabolic function

Weight Control:
- Liver Pancreas

Energy balance Metabolic function

Diet and Weight Control

GI Lumen
- Nutrients
- Bile acids
- Microbiota
- Mucus

Reverse Dysbiosis

Enhance Barrier Function

FXR