



# Human Microbiome Science

## VISION FOR THE FUTURE

JULY 24 - 26, 2013

Program Guide

# **Human Microbiome Science: Vision for the Future**

Bethesda North Marriott Hotel & Conference Center  
5701 Marinelli Road  
North Bethesda, Maryland 20852

July 24 – 26, 2013

This meeting was partially sponsored by an NIH grant 3U01HG004866-05S1 to the University of Maryland School of Medicine and with additional support from private sponsors (see page 11).

\*Please refer to the meeting website for the most up-to-date information.\*

## **HUMAN MICROBIOME SCIENCE: Vision for the Future**

Bethesda, Maryland | July 24 – 26, 2013

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## HUMAN MICROBIOME SCIENCE: Vision for the Future

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### Venue

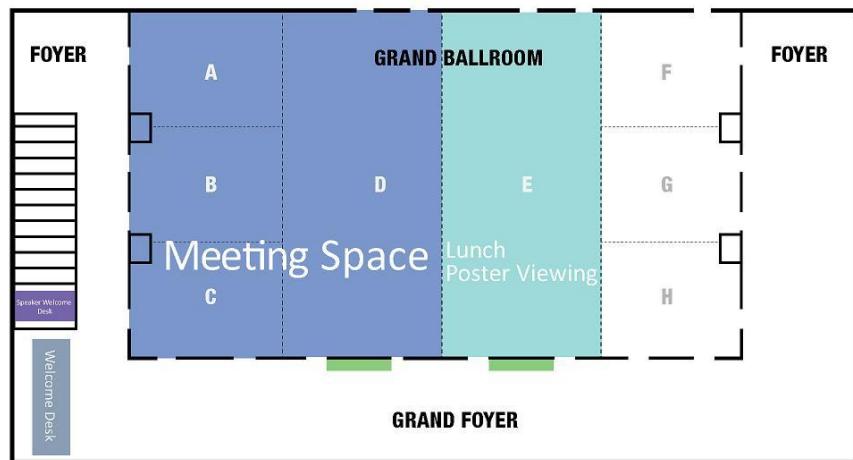
**Bethesda North Marriott Hotel & Conference Center**  
5701 Marinelli Road, North Bethesda, Maryland 20852 USA  
(301) 822-9200

All scientific sessions of the meeting will be held in Salons A-D. All posters will be set up in Salon E, which will also be set up with tables for all breaks and lunch sessions. Lunch will be served, buffet-style, in the Foyers.

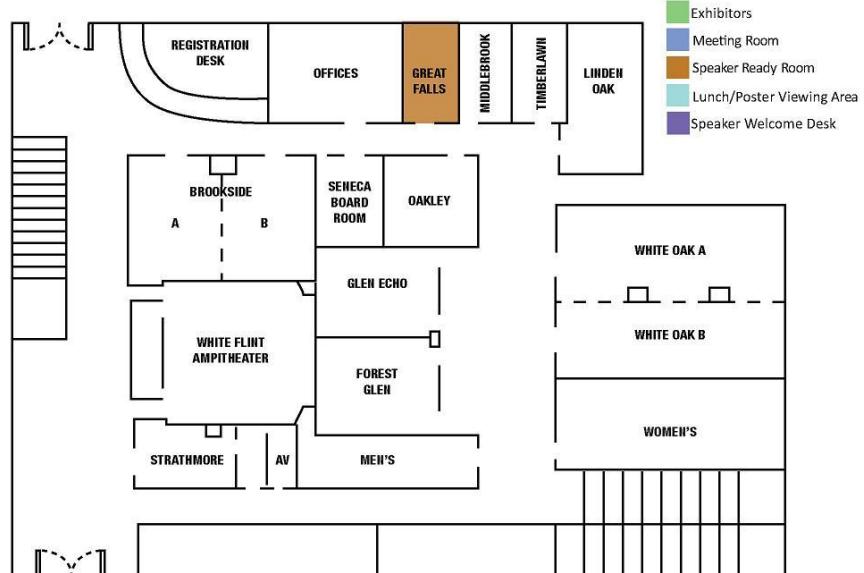
Media personnel are invited to use the Great Falls room on the Lower Level of the Conference Center for interviews.

Parking is available at the Conference Center and is free for all meeting participants.

**MAIN LEVEL**



**LOWER LEVEL**



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### **Meeting Information**

#### **Food and Beverage Service**

The full meeting package includes lunch for all three days of the meeting. The food and beverage service will be located in the **Foyers**, outside of the main meeting room. There will be four food/beverage stations: two outside the doors to Salon D, one outside the doors to Salon B, and one outside the doors to Salon A. The station outside of Salon A will only be in use during lunch, break refreshments will only be at the other three stations. Lunch will be served buffet-style. Please line up on both sides of the tables to facilitate everyone getting their food in a timely manor. Tables to sit at while eating will be available in **Salon E, along with the posters**, each day for your convenience. If you have any specific dietary restrictions, please let the planners know in advance by emailing [janine@strategicresults.com](mailto:janine@strategicresults.com).

For your convenience, the conference center's Starbucks will be open from 6:00 AM through 10:00 PM each day of the meeting. The Starbucks is located on the main level of the conference center.

#### **Internet Access & Charging Stations**

There will not be internet access for participants in the meeting space. However, complimentary wireless internet access is available in the hotel lobby and in all public areas of the Bethesda North Marriott Hotel & Conference Center.

Charging stations will be set up in the meeting space for your convenience. These are meant to assist in charging any laptops or tablets that are being used for note-taking.

#### **Poster Sessions**

Posters will be presented on all three days of the meeting in **Salon E**. At a minimum, poster presenters are asked to be present at their poster during the lunch-time poster session on their assigned day starting 40 minutes into the lunch session. Presenters with even-numbered posters should staff their posters on Day 1 and those with odd-numbered posters should staff their posters on Day 2. We encourage all poster presenters to staff their posters on Day 3. These lunchtime Poster Viewings will provide a forum for investigators from diverse disciplines to exchange new knowledge, findings, and ideas, and to lay the groundwork for future collaborations. All poster abstracts are available and can be found at the end of the Program Guide.

#### **Poster Award**

We will be awarding a certificate to the most outstanding poster of the conference. The winning poster will be determined based on votes from meeting attendees. Ballots will be available at poster board space #58. The winner will be announced Friday afternoon.

#### **Registration**

Registration/Check-In for the Human Microbiome Vision meeting is located in the hallway on the Main Level of the Conference Center, near the escalators. Please check in at the Registration Desk in order to pick up your event name badge.

#### **Return Travel**

For all non-locals attending the meeting, please let the staff at the registration desk know your departure time and airport in order to assist in scheduling a taxi service for you.

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### **Meeting Information, continued**

#### **Social Media**

Attendees and those viewing the webcast can submit questions for speakers and for consideration at the daily open floor discussions to **#Microbiome** or by emailing [HMVision@mail.nih.gov](mailto:HMVision@mail.nih.gov).

#### **Webcast**

Please feel free to share with your colleagues that the event is being broadcasted live by the NIH Communications department. Please [click here](#) to view the broadcasting.

Also, the event is being recorded and will be available after the conclusion of the meeting for your reference.

## **Welcome and Meeting Charge**

Research into the human microbiome and its relationship to human health and disease is expanding at a phenomenal rate. The time is right to assess the state of the science across this diverse field of many disciplines. This meeting has been organized to provide an overview of cutting-edge work in NIH-supported microbiome research and to identify both the obstacles to, and opportunities for, progress in this emerging area of biomedical research.

### **Meeting Charge:**

**We enthusiastically challenge** all meeting attendees to participate in a collegial discussion that will:

- Recognize that the study of the human microbiome, in disease and in health, is of relevance to the missions of all NIH Institutes and Centers;
- Increase awareness across all NIH Institutes and Centers of gaps, needs and challenges faced by the broad microbiome research community;
- Facilitate coordination between the NIH Institutes and Centers to promote coherent oversight for policies and approaches which will maximally benefit microbiome-related biomedical research;
- Identify areas where common resources or partnerships would benefit microbiome-related biomedical research;
- Explore how NIH and other government funding agencies can collaborate to build capacity for integrating the microbiome into studies of human health and more broadly into studies of human interactions with their environment and the Earth's microbial communities;
- Foster understanding of the current state of microbiome research, and shape an overall vision for future directions of the field over the next 10 years.

## HUMAN MICROBIOME SCIENCE: Vision for the Future

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### Agenda, Day 1 - Wednesday, July 24, 2013

Presentation	Time	Session chair(s)/Co-moderator
<b>Theme of the Day: Overview and Approaches</b>		
<b>Call to order, charge for the meeting</b> Owen R. White, PhD   University of Maryland School of Medicine	8:00 AM - 8:05 AM	
<b>"Supercharging Science for the Superorganism"</b> Francis S. Collins, MD, PhD   NIH, DHHS	8:05 AM - 8:20 AM	Owen R. White, PhD   University of Maryland School of Medicine
<b>"The NIH Human Microbiome Project: An Update"</b> Eric D. Green, MD, PhD   NHGRI, NIH, DHHS	8:20 AM - 8:30 AM	Owen R. White, PhD   University of Maryland School of Medicine
<b>Keynote 1, "Diversity, Stability and Resilience of the Microbiome"</b> David A. Relman, MD   Stanford School of Medicine	8:30 AM - 9:15 AM	Melody Mills, PhD   National Institute of Allergy and Infectious Diseases
<b>"ELSI Issues and Microbiome Studies" (Tentative Title)</b> Rosalmond Rhodes   Mt Sinai School of Medicine (Substitute for Richard Sharp)	9:15 AM - 9:45 AM	Jean McEwen, JD, PhD   National Human Genome Research Institute
<b>Title To Be Confirmed</b> Robbie Barbero, PhD   Office of Science and Technology Policy	9:45 AM - 10:00 AM	Owen R. White, PhD   University of Maryland School of Medicine
<b>Break</b>	10:00 AM - 10:30 AM	
<b>Basic Biology of the Microbiome</b>		
<b>"Microbiome Colonization and Assembly" (Tentative Title)</b> Ruth E. Ley, PhD   Cornell University	10:30 AM - 11:00 AM	Mike Reddy, PhD   National Institute of General Medical Sciences & TBD
<b>"Microbiome Dynamics in Adults" (Tentative Title)</b> Jacques Ravel, PhD   University of Maryland School of Medicine	11:00 AM - 11:30 AM	
<b>"Composition and Dynamics of the Human Virome"</b> Frederic D. Bushman, PhD   University of Pennsylvania	11:30 AM - 12:00 AM	
<b>Lunch/Poster session</b>	12:00 PM - 1:30 PM	
<b>State of the Art Microbiome Tools, Technologies, Approaches - part 1</b>		
<b>"Multi-omics of the Human Microbiome – Filling in the Missing Links"</b> Janet Jansson, PhD   Lawrence Berkeley National Lab	1:30 PM - 2:00 PM	Gabriela Riscuta, MD, CNS   National Cancer Institute
<b>"Approaches for Host Immune/Microbiome Studies" (Tentative Title)</b> Dan Rudolf Littman, MD, PhD   New York University	2:00 PM - 2:30 PM	
<b>"Functional Analysis of Human Microbiome Metagenomes, Metatranscriptomes, and Multi'omics"</b> Curtis Huttenhower, PhD   Harvard School of Public Health	2:30 PM - 3:00 PM	
<b>Break</b>	3:00 PM - 3:30 PM	
<b>State of the Art Microbiome Tools, Technologies, Approaches - part 2</b>		
<b>"From Correlation to Causation in Human Microbiome Studies"</b> Rob Knight, PhD   University of Colorado, Boulder	3:30 PM - 4:00 PM	Maria Giovanni, PhD   National Institute of Allergy and Infectious Diseases & Michelle Giglio, PhD   University of Maryland School of Medicine
<b>"Large Data Management, Data Standards, Data Sharing"</b> Owen R. White, PhD   University of Maryland School of Medicine	4:00 PM - 4:30 PM	
<b>Open floor discussion</b> Ed Yong   Nature/National Geographic, moderator	4:30 PM - 5:15 PM	Lita Proctor, PhD   National Human Genome Research Institute

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### Agenda, Day 2 – Thursday, July 25, 2013

Presentation	Time	Session chair(s)/Co-Moderator
<b>Theme of the Day: Interactions of the Host/Microbiome System</b>		
<b>Keynote 2, "The Modern vs Ancestral Microbiome"</b> Maria Dominguez-Bello, PhD   New York University	8:00 AM - 8:45 AM	Rob Knight, PhD   University of Colorado, Boulder
<b>Host Immune System/Microbiota Interactions</b>		
"Bacterial Colonization Factors Control Specificity and Stability of the Gut Microbiota" Sarkis K. Mazmanian, PhD   California Institute of Technology	8:45 AM - 9:15 AM	Michael Grey, PhD   National Institute of Diabetes and Digestive and Kidney Diseases, & Annette Rothermel, PhD   National Institute of Allergy and Infectious Diseases
"Ground Zero – the Impact of the Gut Microbiome on Host Epithelial Functions and Responses" Eugene B. Chang, MD   University of Chicago	9:15 AM - 9:45 AM	
"Wound Healing to Longevity: Harnessing Microbe-Induced Hormonal and Immune Proficiency for Human Health" Susan E. Erdman, DVM   Massachusetts Institute of Technology	9:45 AM - 10:15 AM	
<b>Break</b>	10:15 AM - 10:45 AM	
<b>Microbiome and Disease Associations</b>		
"Eczema, Immunity and the Skin Microbiome" Heidi H. Kong, MD   NCI, NIH, DHHS	10:45 AM - 11:15 AM	Lisa Chadwick, PhD   National Institute of Environmental Health
"The Lung Microbiome: Challenging Old Paradigms about Microbes and the Host Respiratory Tract" Gary B. Huffnagle, PhD   University of Michigan	11:15 AM - 11:45 AM	Science & Young Kim, MD, PhD   National Cancer Institute
"The Microbiome in Infectious and Noninfectious Gut Inflammation" Vince Young, MD, PhD   University of Michigan	11:45 AM - 12:15 PM	
<b>Lunch/Poster session</b>	12:15 PM - 1:45 PM	
<b>Functional Interactions between Host and Microbiome</b>		
"Control of Epithelial Proliferation by the Microbiome" Andrew S. Neish, MD   Emory University School of Medicine	1:45 PM - 2:15 PM	Phil Daschner, PhD   National Cancer Institute & Beena Akolkar, PhD   National Institute of Diabetes and Digestive and Kidney Diseases
"Moving towards a Metagenomic Basis of Therapeutics" Peter J. Turnbaugh, PhD   Harvard University	2:15 PM - 2:45 PM	
"Microbial Metabolites and their Regulation of Colonic Regulatory T Cell Homeostasis" Wendy S. Garrett, MD, PhD   Harvard School of Public Health	2:45 PM - 3:15 PM	
<b>Break</b>	3:15 PM - 3:45 PM	
<b>Diet and the Microbiome</b>		
"Diet-Microbiota Interactions and The Elderly" Ian B. Jeffery, PhD   University College Cork, Ireland	3:45 PM - 4:15 PM	Cindy Davis, PhD   Office of Dietary Supplements & Kathryn Camp, MS, RD, CSP   Office of Dietary Supplements
"Diet, Childhood Nutrition and the Microbiome" Kathryn G. Dewey, PhD   University of California, Davis	4:15 PM - 4:45 PM	
"Gut Microbial Metabolism of Food Constituents: Modulating Human Dietary Exposures" Johanna W. Lampe, PhD   Fred Hutchinson Cancer Research Center	4:45 PM - 5:15 PM	
<b>Open floor discussion</b> Ed Yong   Nature/National Geographic, moderator	5:15 PM - 6:00 PM	Padma Maruvada, PhD   National Institute of Diabetes and Digestive and Kidney Diseases

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### Agenda, Day 3 – Friday, July 26, 2013

Presentation	Time	Session chair(s)/Co-Moderator
<b>Theme of the Day: Translational Research and the Microbiome</b>		
<b>Keynote 3, "Translational Science and the Microbiome"</b> Jonathan Braun, MD, PhD   University of California, Los Angeles	8:00 AM - 8:45 AM	Bob Karp, PhD   National Institute of Diabetes and Digestive and Kidney Diseases
<b>"The Microbiome: Getting to Products that Benefit Patients"</b> Jesse L. Goodman, MD   FDA, DHHS	8:45 AM - 9:15 AM	Jacques Ravel, PhD   University of Maryland School of Medicine
<b>Translation - Body/Microbiome Axis</b>		
<b>"Microbiome, Brain and Behavior"</b> Ted Dinan, MD, PhD   University College Cork, Ireland	9:15 AM - 9:45 AM	Carl Baker, MD, PhD   National Institute of Arthritis and
<b>"Microbiome and Obesity" (Tentative Title)</b> Martin J. Blaser, MD   New York University	9:45 AM - 10:15 AM	Musculoskeletal and Skin Diseases & Nancy Desmond, PhD   National
<b>"Microbiome and Cardiovascular Disease Biomarkers" (Tentative Title)</b> Stanley Hazen, MD, PhD   Cleveland Clinic	10:15 AM - 10:45 AM	Institute of Mental Health
<b>"From Associative Microbial Dysbiosis Observation to Functional Cancer Studies: What have we Learned from Colorectal Cancer?"</b> Christian Jobin, PhD   University of Florida, School of Medicine	10:45 AM - 11:15 AM	
<b>"Harvesting the Molecular Wealth of Microbiomes"</b> Julian Davies, PhD   The University of British Columbia, Canada	11:15 AM - 11:45 AM	
<b>Lunch/Poster session</b>		
<b>Translation - Probiotics, Microbiome Vaccines, Fecal Transplants</b>		
<b>"State of the Science and Obstacles: Fecal Microbiota Transplantation" (Tentative Title)</b> Alexander Khoruts, MD   University of Minnesota	12:45 PM - 1:15 PM	Linda Duffy, PhD   National Center for Complementary and Alternative Medicine & Mukesh Verma, PhD
<b>"Use of Microbial Ecosystems to Treat Recurrent Clostridium difficile Infection"</b> Elaine Petrof, MD   Queen's University, Canada	1:15 PM - 1:45 PM	National Cancer Institute
<b>"A Milk-Oriented-Microbiota (MOM) in Infants—How Babies Find their MOMs: Insights for Next Generation Prebiotics and Probiotics"</b> David A. Mills, PhD   University of California, Davis	1:45 PM - 2:15 PM	
<b>"Microbiota and Vaccines"</b> Eric Brown   The University of British Columbia, Canada (Substitute for Brett Finlay)	2:15 PM - 2:45 PM	
<b>Final open floor discussion</b> Ed Yong   Nature/National Geographic, moderator	2:45 PM - 3:30 PM	TBD

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### **Human Microbiome Science Planning Committee**

**Michelle Giglio, PhD** | Institute for Genome Sciences, University of Maryland School of Medicine

**Rob Knight, PhD** | University of Colorado, Boulder

**Jacques Ravel, PhD** | Institute for Genome Sciences, University of Maryland School of Medicine

**Owen White, PhD** | Institute for Genome Sciences, University of Maryland School of Medicine

**Lita Proctor, PhD** | National Human Genome Research Institute, NIH, DHHS

**Beena Akolkar, PhD** | National Institute of Diabetes and Digestive and Kidney Diseases, NIH, DHHS

**Carl Baker, MD, PhD** | National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, DHHS

**Lisa Chadwick, PhD** | National Institute of Environmental Health Science, NIH, DHHS

**Shaila Chhibba** | National Human Genome Research Institute, NIH, DHHS

**Phil Daschner, PhD** | National Cancer Institute, NIH, DHHS

**Cindy Davis, PhD** | Office of Dietary Supplements, NIH, DHHS

**Nancy Desmond, PhD** | National Institute of Mental Health, NIH, DHHS

**Nick DiGiacomo** | National Human Genome Research Institute, NIH, DHHS

**Linda Duffy, PhD** | National Center for Complementary and Alternative Medicine, NIH, DHHS

**Irene Eckstrand, PhD** | National Institute of General Medical Sciences, NIH, DHHS

**Maria Giovanni, PhD** | National Institute of Allergy and Infectious Diseases, NIH, DHHS

**Gilman Graves, MD** | National Institute of Child Health and Human Development, NIH, DHHS

**Michael Grey, PhD** | National Institute of Diabetes and Digestive and Kidney Diseases, NIH, DHHS

**Max Guo, PhD** | National Institute on Aging, NIH, DHHS

**Bob Karp, PhD** | National Institute of Diabetes and Digestive and Kidney Diseases, NIH, DHHS

**Ron Kohanski, PhD** | National Institute on Aging, NIH, DHHS

**Dwayne Lunsford, PhD** | National Institute of Dental and Craniofacial Research, NIH, DHHS

**Padma Maruvada, PhD** | National Institute of Diabetes and Digestive and Kidney Diseases, NIH, DHHS

**Jean McEwen, JD, PhD** | National Human Genome Research Institute, NIH, DHHS

**Pamela McInnes, DDS, MSc** | National Institute of Dental and Craniofacial Research, NIH, DHHS

**Julia Puzak** | National Institute of Allergy and Infectious Diseases, NIH, DHHS

**Mike Reddy, PhD** | National Institute of General Medical Sciences, NIH, DHHS

**Pat Reichelderfer, PhD** | National Institute of Child Health and Human Development, NIH, DHHS

**Gabriela Riscuta, MD** | National Cancer Institute, NIH, DHHS

**Shiva Singh, PhD** | National Institute of General Medical Sciences, NIH, DHHS

**Pothur Srinivas, PhD** | National Heart, Lung, and Blood Institute, NIH, DHHS

**Chris Wellington** | National Human Genome Research Institute, NIH, DHHS

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### Sponsors

The Organizers of the Human Microbiome Science meeting would like to thank all of our sponsors! Their generosity is greatly appreciated. Sponsors will be exhibiting throughout the duration of the event. These exhibits will be located in the lobby outside of the main meeting room near the doors to Salon A. Please take some time to visit them. Additionally, several sponsors are also presenting posters on scientific research from their respective organizations.

#### Silver level sponsors:



#### Bronze level sponsors:



#### Other supporters:



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### Keynote and Distinguished Speakers



**Robbie Barbero**

AAAS Fellow

Technology and Innovation Division

Office of Science and Technology Policy

Executive Office of the President



**Jonathan Braun, MD, PhD\***

Professor

Chair, Department of Pathology & Laboratory Medicine

David Geffen School of Medicine

University of California, Los Angeles



**Francis S. Collins, MD, PhD**

Director

National Institutes of Health, DHHS

\*Abstract follows

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### Keynote and Distinguished Speakers, continued



#### Maria Dominguez-Bello, PhD\*

Associate Professor

Department of Medicine (Clin Pharm Div)  
New York University



#### Jesse L. Goodman, MD

Chief Scientist

Food and Drug Administration, DHHS



#### Eric D. Green, MD, PhD

Director

National Human Genome Research Institute, NIH, DHHS

\*Abstract follows

## HUMAN MICROBIOME SCIENCE: Vision for the Future

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### Keynote and Distinguished Speakers, continued



**David A. Relman, MD\***

Professor, Medicine - Infectious Diseases

Professor, Microbiology & Immunology

Stanford School of Medicine

\*Abstract follows

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### Session Speakers

#### Martin J. Blaser, MD \*

Principal Investigator

Frederick H. King Professor of Internal Medicine and Chairman of the Department of Medicine;  
Professor of Microbiology  
Departments of Medicine (Administration) and Microbiology  
New York University

#### Frederic D. Bushman, PhD \*

Professor, Department of Microbiology  
Perelman School of Medicine  
University of Pennsylvania

#### Eugene B. Chang, MD \*

Martin Boyer Professor of Medicine  
Associate Section Chief for Research  
University of Chicago

#### Julian Davies, PhD

Principal Investigator  
Professor Emeritus  
Department of Microbiology and Immunology  
The University of British Columbia

#### Kathryn G. Dewey, PhD \*

Distinguished Professor, Department of Nutrition  
Director of Program in International and Community Nutrition  
University of California, Davis

#### Ted Dinan, MD, PhD \*

Professor of Psychiatry  
Department of Psychiatry and Alimentary Pharmabiotic Centre  
University College Cork

#### Susan E. Erdman, DVM \*

Principal Research Scientist  
Assistant Director, Division of Comparative Medicine  
Massachusetts Institute of Technology

#### Brett Finlay, PhD \*

Professor  
Michael Smith Laboratories  
Departments of Biochemistry and Molecular Biology, and Microbiology and Immunology  
The University of British Columbia

\*Abstract follows

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### Session Speakers, continued

#### Wendy S. Garrett, MD, PhD \*

Assistant Professor of Medicine  
Harvard School of Public Health

#### Stanley Hazen, MD, PhD

Director, Center for Cardiovascular Diagnostics and Prevention  
Director, Cleveland Clinic Mass Spectrometry Core Facilities  
Department Chair, Department of Cell Biology  
Cleveland Clinic

#### Gary B. Huffnagle, PhD \*

Professor  
Department of Microbiology and Immunology  
University of Michigan Medical School

#### Curtis Huttenhower, PhD \*

Associate Professor of Computational Biology and Bioinformatics  
Department of Biostatistics  
Harvard School of Public Health

#### Janet Jansson, PhD \*

Professor and Senior Staff Scientist  
Ecology Department  
Lawrence Berkeley National Laboratory

#### Ian B. Jeffery, PhD \*

Department of Microbiology  
Alimentary Pharmabiotic Centre  
University College Cork, Ireland

#### Christian Jobin, PhD \*

Associate Professor  
Department of Medicine  
University of Florida

#### Alexander Khoruts, MD

Associate Professor of Medicine  
Department of Gastroenterology, Hepatology and Nutrition  
University of Minnesota

\*Abstract follows

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### Session Speakers, continued

#### Rob Knight, PhD \*

Associate Professor

Howard Hughes Medical Institute

Department of Chemistry and Biochemistry

University of Colorado, Boulder

#### Heidi H. Kong, MD \*

Investigator, Dermatology Branch

National Cancer Institute, NIH, DHHS

#### Johanna W. Lampe, PhD \*

Research Professor

Department of Epidemiology

Fred Hutchinson Cancer Research Center

#### Ruth E. Ley, PhD

Assistant Professor

Department of Microbiology

Cornell University

#### Dan Rudolf Littman, MD, PhD

Helen L. and Martin S. Kimmel Professor of Molecular Immunology

Professor, Departments of Pathology (Skirball), Microbiology (Microbiology ) and Molecular

Pathogenesis

New York University

#### Sarkis K. Mazmanian, PhD \*

Professor

Division of Biology

California Institute of Technology

#### David A. Mills, PhD \*

Professor, Department of Viticulture and Enology

College of Agricultural and Environmental Sciences

University of California, Davis

#### Andrew S. Neish, MD \*

Professor

Pathology & Laboratory Medicine

Emory University School of Medicine

\*Abstract follows

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### Session Speakers, continued

#### Elaine Petrof, MD \*

Associate Professor

Gastrointestinal Disease Research Unit

Queen's University

#### Jacques Ravel, PhD

Professor, Microbiology and Immunology

Associate Professor, Institute for Genome Sciences

University of Maryland School of Medicine

#### Richard R. Sharp, PhD

Director of Bioethics Research

Mayo Clinic

#### Peter J. Turnbaugh, PhD \*

Bauer Fellow

FAS Center for Systems Biology

Harvard University

#### Owen R. White, PhD

Professor, Epidemiology & Public Health

Associate Director, Institute for Genome Sciences

University of Maryland School of Medicine

#### Ed Yong

Science Writer

Nature/National Geographic

#### Vince Young, MD, PhD \*

Associate Professor

Department of Microbiology and Immunology

University of Michigan Medical School

\*Abstracts follow

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### Speaker Abstracts

#### Martin J. Blaser, MD

*NYU Medical Center, New York, NY*

#### Microbiome and Obesity

Obesity has been epidemic in the United States and other developed countries of the world for the past several decades, and appears to be worsening. Obesity also is increasing in developing countries. Such a worldwide phenomenon occurring in a relatively short time-frame indicates important environmental causation, but the calorie hypothesis has been insufficient to fully explain it. Because the roots of obesity often begin in early life, and since farmers routinely feed antibiotics to their livestock early in life to promote their growth, we have been interested in the effects of the changing early life microbiome on obesity risk.

Our hypothesis is that perturbation of the microbiome in early life by antibiotics and other insults, is creating conditions that drive the propensity to obesity. Support for this hypothesis comes from review of data on antibiotic usage patterns in the United States, which indicates widespread and intensive early life, and continuing, antibiotic usage. We directly tested the hypothesis in a series of mouse models in which mice were given antibiotic regimens in their drinking water or not, combined with dietary interventions or not. Results of the models show that low-dose (sub-therapeutic levels of) antibiotics, as given on the farm, are growth-promoting in mice, with changes in fat, muscle and bone development. Intermediate carbohydrate and lipid metabolism in the liver are changed. Changes in the composition of the microbiome accompany and precede the changes in host phenotype. As on the farm, early exposures have maximal effects.

These data provide evidence that supports the hypothesis that early life antibiotic exposures are causing adipose phenotypes, via their effects on the microbiome during a critical developmental stage in childhood.

Gaps, needs, and challenges in this field include:

- Epidemiologic studies in humans to explore the consequences of early life factors that affect microbiome development.
- Model systems that identify critical early life factors and that characterize the interactions with the microbiota.

- Mechanistic studies that establish the full causal pathways, and identify potential loci for intervention.
  - Development of clinical trials in children for the prevention of obesity through microbiome interventions.
  - Trials to assess whether and when interventions based on microbiome manipulation can affect existing obesity.
- 

#### Jonathan Braun, MD, PhD

*University of California, Los Angeles, Los Angeles, CA*

#### Translational Science and the Microbiome

Among the hopes for microbiome discovery are new insights on translational science- research on the basis of human diseases and their treatment. In particular, the question is whether microbial species or their products may play a causal role in certain diseases, and if so, how they may be monitored and modified to restore health.

The early news is that this may be so. Examples include inflammatory diseases of the mucosal surface (*C. difficile* colitis and inflammatory bowel disease), remote immune disease (type 1 diabetes), lymphoma, atherosclerosis, and elements of behavior and cognition.

In the context of highly networked systems of human physiology and microbial ecosystems, there are two fundamental gaps. First, how to design experimental and bioinformatic strategies that can uncover causation versus correlation. And second, how to achieve a description of the network that is robust and predictive for therapeutic intervention. These will be illuminated by the examples of California pacific fisheries, and how biologists fix radios.

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### Composition and dynamics of the human virome

Viruses are the most abundant and arguably the most successful biological entities on earth. The virome of humans consists of viruses persistently and transiently infecting animal cells, viruses integrated in the human genome, and bacteriophage preying on human-associated bacteria and archaea. The human gut is one of the most densely inhabited bacterial communities known, and this community is similarly densely inhabited by viruses that prey on these bacteria. We and others have purified gut virome samples and begun to investigate their composition and dynamics using deep DNA sequencing. Assembly and analysis shows that most of the resulting virome contigs do not resemble previously described strains, and virome communities differ dramatically between individuals. Results from recent analysis of these communities will be presented in the lecture. In one recent study, virome populations were studied during a dietary intervention, revealing that the viral communities changed dectably associated with diet, and communities from individuals on the same diets converged. A second study investigated the sources of variation in deeply sequenced virome samples (~40 billion bases of sequence from 12 different individuals), revealing notable short hypervariable regions associated with reverse transcriptase genes in DNA phage. A third study characterized longitudinal variation within one individual (50 billion bases of sequence), illustrating how virome communities change over time. Community membership was mostly stable over the 2.5 years sampled, but some viral lineages changed extensively. These findings help understand why viral communites are so different between individuals. This must be in part because different humans harbor quite different bacterial communities, so these will naturally support different viral predators. However, once in an individual, some viruses evolve rapidly, also helping to account for inter-individual variation. Despite the progress in the field, many central issues remain to be addressed:

- Methods for virome analysis, both on the wet and dry sides, need considerable further validation and development.
  - The ecology of bacteriophage predation in the human microbiome is just beginning to be studied.
  - Methods for distinguishing animal cell viruses and associating newly detected viruses with disease states need considerable development.
  - Viral nucleic acid covalent modifications are diverse and extensive, and only starting to be studied in metagenomic samples.
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### Eugene B. Chang, MD

*University of Chicago, Chicago, IL*

### Ground Zero – the impact of the gut microbiome on host epithelial functions and responses.

**Background:** The gut epithelium sits at the interface with the outside (or inside) microbial world providing numerous functions vital for maintenance of health and disease prevention. In this regard, there is no doubt that many of these functions are dependent on cues from the gut microbiota which are essential for normal gut epithelial development, self renewal, selective ion/nutrient/water transport, barrier function (including junctional complexes and mucus), and anti-microbial defense. These cues include PAMPs, small bioactive molecules, metabolites, and other poorly and yet-to-be-defined factors. At the same time, the gut epithelium possesses a plasticity enabling it to respond to changes in the microbial community in a measured, yet appropriate way and serves as the mediator to regulate both adaptive and innate immune states. Given the complexity of the host-microbe interactions required to develop and maintain these functions, it is not surprising that the inherent or acquired abnormalities of the gut epithelium are believed to be key elements in the etiopathogenesis of many disorders, including complex immune diseases, cancer, infectious diseases, and metabolic disorders.

#### Gaps:

- Rudimentary knowledge and inventories of bioactive microbe-derived factors involved in the development, maintenance, and perturbation of gut epithelial functions.
- Incomplete understanding of the complexity and heterogeneity of gut epithelial functions, particularly as they relate to microbial

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selection, assemblage and region-specific interactions.

- Incomplete vetting and understanding of the above in the context of human biology and pathobiology

### Challenges:

- Co-study of microbial/host dynamic and system heterogeneity (spatial and temporal)
- Inadequate access to, funds, and resources for rapid analysis and integration of large microbial and host 'omic' datasets.
- Limited technologies for assessment of microbial function and for establishing causal relationships – experimentally and for human based studies
  - Small Biomass requiring biased amplification
  - Inconsistent and unvetted sampling methodologies for both host and microbe analyses.
- Dialogue and interactions between basic, translational, and clinical investigators

### Needs

- Advances, access to, and more affordable "omics" technologies – with resources and available personnel to assist or with whom to network to rapidly analyze and integrate large datasets.
  - Scalable technologies for small sample biomass
- Improved animal and experimental models of human disease (e.g. human-derived tissues, organoids, etc) that faithfully recapitulate an individual's biology, genetics and physiological responses, yet scalable to examine complex microbial and intercellular interactions.
- More opportunities for large scale multi-disciplinary team approaches for discovery
- Translation of knowledge and technology for development of interventional and therapeutic strategies for human disease – tools preparing us for the era of targeted and precision Medicine.

**Kathryn G. Dewey, PhD**

*UC Davis*

### Diet, child nutrition and the microbiome

#### Key questions relevant to this topic include:

- 1) How does diet in early life influence the microbiome?
- 2) How does the microbiome influence child nutritional status?
- 3) How do differences in microbiome structure and function affect functional outcomes during the first 2-3 years of life, as well as long-term health and developmental outcomes?

#### Gaps identified:

- a) Apart from studies comparing breastfed and formula-fed infants, there is very little information on how dietary composition or nutrient intake affects the microbiome of children.
  - b) The etiology of the emerging link between malnutrition and the microbiome, and the prevention or treatment of abnormal status via dietary or pre/probiotic interventions, require further investigation.
  - c) Prospective studies, particularly long-term follow-up of intervention trials, are needed to identify the short- and long-term consequences of differences in microbiome structure and function in early life.
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**Ted Dinan, MD, PhD**

*University College Cork, Cork, Ireland*

### Microbiome, Brain and Behaviour

The fields of microbiology and neuroscience in modern medicine have largely developed in distinct trajectories, with the exception of studies focused on the direct impact of infectious agents on brain function, including early investigations of syphilis and, more recently, studies of the neurological complications of AIDS. However, it has recently become evident that the microbiota, especially microbiota within the gut, can greatly influence all aspects of physiology, including gut–brain communication, brain function and even behaviour. A variety of strategies have been used to investigate the impact of the microbiome on the brain and behaviour. These include germ free studies, probiotic and antibiotic

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treatments, infective models and faecal microbiota transplantation.

Germ free (GF) studies clearly indicate that in the absence of a microbiota brain development is altered, as is the neuroendocrine response to stress. Specifically, decreased levels of brain derived neurotrophic factor (BDNF) in key brain regions such as the hippocampus are reported, together with alterations in the activity of the serotonergic system. In response to stress GF animals have an exaggerated release of corticosterone. Probiotic studies indicate that certain strains are capable of altering central neurotransmitters. *Lactobacillus rhamnosus* alters the expression of GABA-A and GABA-B receptors in diverse brain regions and the effect is correlated with reduced anxiety (Bravo et al, 2011). Vagotomised animals do not demonstrate such a probiotic action. Faecal transplantations studies indicate that an anxious phenotype can be transferred from an anxious to a non-anxious animal following a microbiome transplant (Neufeld et al, 2011). Antibiotic studies suggest efficacy of minocycline (which impacts on gram positive and negative bacteria) in depression and perhaps also in schizophrenia (Soczynska et al, 2012).

The major gap in knowledge at presents lies in the paucity of human studies especially in patient populations. Do patients with major depression or anxiety disorders have a distinct microbiota fingerprint? We do have data on animal models of depression, which indicate alterations (O'Mahony et al, 2009). Do probiotics produce the anxiolytic or antidepressant effects in humans that have been reported in rodents? There are many potential bidirectional routes of communication between the brain and the gut. Which is the most important route of communication between gut microbes and the brain?

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**Maria G Dominguez-Bello, PhD**

*NYU School of Medicine, New York, NY*

### **The Modern vs Ancestral Microbiome**

Our complex human microbiota has coevolved with us. Its structure maintains phylogenetic signal, providing evidence of exclusive intra-species genetic transfer, in which vertical transmission should play an important role. For all mammals, the mother must be an important source of microbiome constituents, since she

exclusively provides the first inoculum at birth, and continuing during early development of her child. Modern practices disrupt the microbiome, including C-section, which precludes the newborn from obtaining the original inoculum. Westernized peoples have less diverse microbiota than those in less modernized societies. Unless corrected, future generations will receive impacted microbiotas from impacted mothers. Restoration involves understanding the lost microbiome diversity. In the short term, we can decrease anti-microbiome practices, restore to newborns delivered by C-section the natural maternal inoculum and promote maternal breastfeeding. However, we may need a deeper restoration of the lost diversity, for which the source would be microbiomes of unimpacted peoples, presumably more similar to our ancestral state. Research should identify the real human probiotics, and children of the future may have timely exposure to them, reversing the increased risks of diseases related to Westernization.

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**Susan Erdman, DVM**

*Massachusetts Institute of Technology, Cambridge, MA*

### **Wound healing to longevity: Harnessing microbe-induced hormonal and immune proficiency for human health**

**Abstract:** The number of individuals diagnosed with inflammatory health disorders is increasing at an alarming rate with devastating consequences. Chronic inflammatory conditions underlie many of the deadliest human diseases, according to epidemiological and scientific studies. A possible causal link between microbial ecology and inflammatory disorders such as diabetes, some types of cancer, and various autoimmune disorders has emerged. While no definitive connections exist, recent research correlates intestinal microbes with potent anti-inflammatory T regulatory (TREG) immune cells that control deleterious chronic inflammation, thus imparting good health.

Key questions involve whether microbe-based restructuring of host immune networks described here may ultimately help to remedy our public health inflammatory disease crisis. Do compulsive societal hygiene practices eliminate beneficial microbial ecology and consequently subvert immune balance needed for good health, as displayed in animal studies? Do microbial pathogens actually have important roles in

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stimulating TREG and beneficial immune tolerance in maintaining our good health? Do recent provocative studies using purified microbes in animal models translate into tangible health benefits for human subjects? Does human maternal and infant microbial ecology, in particular, offer niches with opportunity to impart sustained good health for future generations?

The state of our science in animal models links specific microbe exposures with enhanced immune cell functions, and more recently with regulation of hormones including the neuropeptide oxytocin. Accumulated data support a model whereby microbial symbionts recruit beneficial host immune cells in a hormone-dependent fashion, at least in part involving TREG cells that inhibit chronic inflammatory pathology, consequently bestowing good health. These microbe-hormone-immune interplays have profound impacts on mammalian reproductive fitness, social engagements, mental health, and basic physiology such as with wound repair capacity and ultimately healthful longevity. Microbe-induced benefits may be immediate, and may also extend from oxytocin-potent mother animals with improved infant health outcomes in subsequent generations. Knowing that microbes induce host hormonal and immune changes that convey well-being to their progeny implies far-reaching societal potential.

A fundamental challenge is to address remarkably difficult and urgent public health problems through innovative science and translational medicine. How do we identify and harness the power of microbes for public health? Possibilities include:

- embarking on low-risk high-reward clinical trials using well-characterized organisms with global benefits, ie., do lactic acid bacteria immediately impart benefits to whole body health?
- integrating high-throughput microbe-hormone-immune profiling into prospective epidemiology to identify hygienic signatures for risk assessment and microbial rescue strategies, ie., after Cesarian births, antimicrobials, or illness.
- utilizing invertebrate and rodent models with rapid generation times to probe genetic impact of microbes upon hosts and their offspring.

An emerging microbe-mind-body connection first identified in animal models highlights the vast

translational potential for applying microbiota to impart powerful public health benefits to our grandchildren and beyond.

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**B. Brett Finlay, PhD**

*University of British Columbia, Vancouver, BC, Canada*

### Microbiota and Vaccines

It is well established that vaccine responses to infectious agents poorer in less developed countries when compared to developed countries. It is also well known that microbiota impact on the development of many key immune pathways. Hence it is probable that the microbiota impact on vaccine responses, which suggests that the microbiota could be used to alter vaccine responses. Some members of the microbiota (probiotics) have been engineered to express pathogen antigens as a potential vaccination strategy. The need to strategically target specific members of the microbiota is becoming increasingly important as key members are identified. There have been some attempts at targeting pathogens (microbiota members that can cause disease) indicating that microbiota species can be successfully targeted with antibiotics. Specific oral microbiota species have been successfully targeted to decrease periodontal disease, and work is underway to target a microbiota species associated with autism. Two main themes will be discussed: a) the impact of the microbiota on vaccine responses, and how this might be exploited; and b) the potential to use vaccines to specifically target particular members of the microbiome for therapeutic purposes.

Major questions relating to these themes are:

- 1) can the microbiota be altered to improve vaccine responses?
  - 2) can the microbiota be used to deliver antigens?
  - 3) can specific vaccines be designed to target particular microbiota strains?
  - 4) would there be downstream community consequences to this targeting.?
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**Wendy Garrett, MD, PhD**

*Harvard School of Public Health, Boston, MA*

### **Microbial metabolites and their regulation of colonic regulatory T cell homeostasis**

**Gaps/Needs/Challenges:** Microbiome studies have yielded clades and species associated with health and disease states. Critical knowledge gaps and challenges include identification of microbe-associated metabolites important in immune-microbiota co-adaption and their mechanisms of action.

**Abstract:** Regulatory T cells ( $T_{regs}$ ) that express the transcription factor Foxp3 are critical for intestinal homeostasis and dampening intestinal inflammation. Candidate microbe approaches have identified bacterial species and strain-specific molecules that can affect intestinal immune responses, including species that modulate  $T_{reg}$  responses. Because neither all humans nor mice harbor the same bacterial strains, we posited that more prevalent factors exist that modulate colonic  $T_{regs}$ . We determined that short chain fatty acids, gut microbiota-derived bacterial fermentation products, regulate the size and function of the colonic  $T_{reg}$  pool and protect against colitis in a *Ffar2* (*GPR43*) - dependent manner in mice. Short-chain fatty acids, abundant microbial metabolites in the colon, underlie adaptive immune microbiota co-adaptation and promote colonic homeostasis and health.

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**Gary Huffnagle, PhD**

*University of Michigan, Ann Arbor, MI*

### **The Lung Microbiome: Challenging old paradigms about microbes and the host respiratory tract**

Our understanding of our interaction with the microbial world has changed markedly since the dawn of modern microbiology and the identification of "germs" in the latter half of the 19th century. The focus for the first 100 years of human microbiology was on transmissible microbial agents of disease and on pathogens. Unresolved questions involved the mechanisms of carriage of disease-causing microbes. The next thirty years witnessed the application of molecular biology to understand pathogenic mechanisms and the rise of "opportunistic infections" as AIDS, therapies for immune modulation, improved acute care medicine and a sharp rise in chronic inflammatory diseases resulted in

an explosive growth in the population of susceptible individuals. Now, over the past decade, has come an appreciation that our interaction with the microbial world is even greater than previously appreciated as culture-independent techniques and high-throughput sequencing have allowed us to follow the unseen world of our own microbiology and redefine the bacterial genome not as a static blueprint for virulence but as a dynamic field plan for host-microbe symbiosis that adapts to a changing environment through horizontal gene transfer in polymicrobial biofilms. The indigenous microbial communities of the body are now recognized to be both beneficial to health and a potential source of pathogens.

The term "microbiome" has been used to describe the resident microbial communities of a surface, such as the mucosa, as if membership and interactions are more static than dynamic. But what about a surface such as the lung, that is continuously exposed to live microbes but the exposures to the wide array of microbes range markedly from brief to quickly transient to slowly transient to colonization AND include both replicating and viable-but-not-replicating cells AND changes (transient or permanent) in the physiologic state of that surface markedly alter the host-microbe relationship and microbial persistence? What is the collection of microbes on that surface called? There can be no argument that the host-microbe interactions in the gut are different from those in airways, but microbe-host interactions can certainly drive respiratory disease and, as an example, have been strongly associated with acute exacerbation of COPD. The lower airways are also coated with surfactant, not mucus, making them significantly different than the rest of the mucosal surfaces. In this talk, I am going to extend the definition of the "human microbiome" to include the microbial communities of the healthy and diseased lung as I discuss various aspects of bacterial community dynamics and host-microbe interactions in the lung during health and disease. This includes the role of inflammation in changing microbial community structure, the low and likely heterogeneous density of pulmonary microbial communities, as well as discussing the implications of readily culturable bacterial species becoming non-culturable during growth in the lungs.

#### **Gaps**

- understanding the implications of culturable and non-culturable states of bacteria in the lungs
- visualization of microbes in the lungs

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- documenting the degree of bacterial transience vs. persistence vs. colonization in the airways

### Needs

- new cultivation strategies
- identification of the strengths and shortcomings of various animal models for bridging the concepts of human respiratory microbiology
- microbial metabolomic analysis of the airways during health and disease

### Challenges

- more consistent and supportive peer review of lung microbiome proposals
- accepting that sampling of the lower respiratory tract in humans will be imperfect while at the same time continuing to perform experiments
- understanding the relationship between the microbiology of a bronchoalveolar lavage specimen and regional heterogeneity of microbial communities in the lungs

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**Curtis Huttenhower, PhD**

*Harvard University, Boston, MA*

### Functional analysis of human microbiome metagenomes, metatranscriptomes, and multi'omics

Decades of molecular biology and microbiology have established the biomolecular systems driving individual microbes in exquisite detail, as well as many routes by which pathogens interact with the human immune system. While new molecular tools have now made the broader human microbiome accessible to investigation in increasing mechanistic detail, corresponding new computational tools are needed to manipulate and interpret the resulting high-throughput data. This is particularly true for shotgun metagenomic and metatranscriptomic sequencing, which together provide one of the currently most cost-effective routes by which host and microbial function can be interrogated in the microbiome. Study designs must also be developed that further integrate these with broader, highly scalable approaches such as amplicon profiling, and with deeper mechanistic information such as metabolomics and single cell sequencing. Integrated functional analysis methods are needed to model the biomolecular networks driving emergent phenotypes in the microbiome and their influences on human health.

Current work in the field includes many bioinformatic steps between raw high-throughput data, integrative models of biomolecular function in the microbiome, and human health. I will briefly discuss the state-of-the-art in metagenome and metatranscriptome analysis, particularly the degree to which specific microbial genes and gene products can be accurately identified in such data and combined into meaningful pathways. It is also important to emphasize that these techniques yield information not only on bacteria in the human microbiome, but archaea, viruses, and eukaryotes as well. I will summarize the amazing surge in microbial genomics and functional genomics that has made this possible, particularly as it has allowed increasingly detailed models of microbe-microbe and host-microbe interactions. I'll conclude with examples integrating sequence-based and multi'omic assays to characterize microbiome function in health, such as the Human Microbiome Project, and in dysbiotic conditions such as inflammatory bowel disease.

Gaps remaining to be filled by computational methods for functional analysis of the microbiome include:

- Tools for making metagenomes and metatranscriptomes as readily accessible as 16S amplicon surveys or microarrays are today - on the web for data organization and acquisition, on the desktop for visualization and manipulation, and on the cloud for scalability.
- Resources for systematic, cross-species microbial protein function prediction, cataloging, and validation.
- Computationally tractable databases of microbes, microbial genes, microbial pathways, and host immune pathways of particular relevance in the human microbiome.
- High-quality quantitative models of microbial community metabolic and regulatory networks, comparable to the state-of-the-art in individual microbes and human cell types.
- Comprehensive catalogs of microbe-microbe and host-microbe interaction mechanisms, including small molecule signals, bioactive metabolites, and secreted and cell surface peptides.
- Detailed "microbiogeography" to provide an understanding of who's where and when, enabling the practical fitting and application of quantitative models.
- In vitro models of human-associated microbial communities amenable to highly controlled per-microbe and per-gene knock-in and knock-out experiments.

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- High standards for reproducibility of all aspects of human microbiome bioinformatics in order to ensure translation-quality results.
  - Broad functional multi'omic studies in large, prospective human cohorts with defined phenotypes so that computational models can be accurately applied to specific diseases' diagnosis and therapy.
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**Janet K. Jansson, PhD**

*Lawrence Berkeley National Laboratory, Berkeley, CA*

### Multi-omics of the human microbiome – Filling in the missing links

- **Gaps.** Great advances have recently been achieved in understanding the composition of the human gut microbiome in healthy humans across a range of body sites through the NIH Human Microbiome Project. The data based on high throughput sequencing of 16S rRNA genes has revealed distinct microbiome communities for different body sites and has also revealed that there is considerable variability within individuals for a given body site. By contrast, shotgun metagenome sequencing data has revealed that there is far less variability in predicted categories of gene functions across human populations for a given body site. A current knowledge gap is how the function of the human microbiome is correlated to health, environment, diet and disease. Although functional information based on gene abundances in metagenome data is useful as a starting point, the DNA sequence data does not reveal whether the genes are expressed.
- **State of the science:** It is known from studies of microorganisms in pure culture and in simple microbial communities that not all functional genes are expressed at a given time; instead a specific gene repertoire is expressed in response to the environmental conditions encountered at a specific time. To date there have been few studies that have tried to determine which microbial functional genes are expressed and translated into proteins in humans and to link different omics datasets to understand what functions are correlated to health of the host. One recent example is the use of multi-omics to gain an understanding of the correlation of the gut microbiome to Crohn's Disease. A combination of microbiomics, metagenomics, metaproteomics and metabolomics revealed that

individuals with inflammation in the ileum clustered separately from those with inflammation in the colon and with healthy individuals or those with different IBD phenotypes, such as ulcerative colitis. Several bioindicators of ileal Crohn's disease (ICD) were found at the microbial, proteome and metabolite levels.

- **Needs and Challenges.** A current need and challenge is to link these different omics datasets to gain a better picture of the human microbiome at the systems biology level. This will require more multi-omics datasets combined with new bioinformatics and biostatistical approaches to analyze the enormous datasets that are obtained.
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**Ian B. Jeffery, PhD**

*University College Cork, Cork, Ireland*

### Diet-Microbiota Interactions and The Elderly

Life expectancy is increasing due to modern medicine, improved nutrition and increasing awareness of healthy lifestyle practices. Therefore the number of elderly individuals as a proportion of society is increasing. This ageing cohort is faced with a unique set of challenges related to maintaining their health and therefore their independence. In particular, clinical issues such as inflamm-aging, sarcopenia and depression are of particular importance.

The study of the microbiota and the microbiome is an area that is gaining recognition due to the potential of the microbiome to influence healthy ageing. There is a growing acceptance of the influence of diet upon the microbiome and the importance of this on clinical measures.

A number of studies, the ELDERMET study being the largest, have investigated the microbiota composition at the elderly extreme of life. These studies have shown that the microbiota composition is associated with long-term diet and residence location, as well as the health status of these individuals within these residence locations. Indeed the large differences in the microbiota composition changes between subjects of different ages, gender, and nationality are almost certainly partially due to factors including diet.

Therefore defining what constitutes a microbial system that can be termed as in "dysbiosis" as opposed to being part of the core microbiota is a challenging task.

However many studies report that one property of a system that is prone to dysbiosis is a low diversity microbiota. A number of studies have found this to be an exacerbating feature of a number of disorders including obesity, inflammatory bowel disease and accelerated aging-related health loss, as shown by the ELDERMET study. Although these changes in older subjects are associated with diet, it has not yet been shown if dietary modulation can restore microbial diversity. There may also be physiological or financial barriers to overcome in any diet-based microbiota modification.

Therefore in the future, large multinational longitudinal microbiota studies carried out in well phenotyped individuals combined with high quality dietary information, as well as dietary interventions, will be needed to fully access the diet-microbiota-health relationships. This is the goal of the European Commission funded NuAge project ([www.nu-age.eu](http://www.nu-age.eu)) which will conduct a dietary intervention in 1,250 subjects across 5 European cities. This study will record extensive physical and clinical measurements and will seek to correlate observed changes with alterations in the microbiome, inflammasome and peripheral blood lymphocyte epigenome in the subjects.

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**Christian Jobin, PhD***University of Florida, Gainesville, FL***From associative microbial dysbiosis observation to functional cancer studies: What have we learned from colorectal cancer?**

Microbiota and host form a complex “supra-organism” in which symbiotic relationships confer beneficial effects to the host in many key aspects of life, including metabolism and immunity. This relationship can be disturbed by defects in the host’s regulatory circuits controlling bacterial sensing and homeostasis, or alterations of the microbiome caused by changes in environment, diet or lifestyle. Notably, host genetics, dietary habits and life style are known risk factors for carcinogenesis. An estimated bacterial community ranging from  $10^{13}$ - $10^{14}$  in the colon and a collective genome evaluated at  $3 \times 10^6$  genes lives in relative close proximity to the intestinal epithelium. Our understanding of the functional impact of this microbial community on cancer prevention/development or treatment responses is currently limited. Recent

reports has identified microbial dysbiosis between CRC patients and controls, suggesting a potential link between microbial composition and carcinogenesis. Preclinical models combined with gnotobiotic technology have showed the key role of bacteria in promoting development of CRC, including the commensal strain *Escherichia coli*. In addition, microbial genetic manipulation has successfully identified genotoxins such as colibactin as key factor in cancer development. Higher prevalence of some of these genotoxins, including the *E.coli pks* island were observed in CRC patients, suggesting a potential role for microbial genes in human carcinogenesis. Despite these advances, a number of questions remained unanswered.

- Impact of the microbiota on development of various forms of cancer (lung, skin, oral cavity, pancreas)?
- Impact of the microbiome on anti-tumor drug treatment efficacy?
- Relationship between diet, microbial community and cancer development?
- Functional contribution of the microbiota to cancer (genes, toxins, metabolites)?
- Long-distance effect of the intestinal microbiota on extra-intestinal cancer?
- Cancer-promoting and cancer-protecting bacterial strains co-exist in the microbiome? Which factors (genetic, diet, lifestyle) influence this delicate balance?
- Manipulation of microbial composition for therapeutic purposes?
- Generation of microbial biomarkers for disease prevention and treatment?

Establishing the functional contribution of the microbiota to human cancer development would require a multi-faceted and large-scale approach where microbial-derived carcinogenic pathways are established from bacterial meta-transcriptomic, metagenomic and metabolomic analysis using microbiomes from patients at different stages and control populations. Furthermore, this systems biology approach would need to be married to in vitro microbiological techniques and gnotobiotic models to establish functional links. The inclusion of novel cultivation media, in particular for anaerobic conditions and innovative chemostats culture techniques will be necessary to overcome the limited spectrum of bacteria

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that can currently be cultured and functionally characterized in vitro or in gnotobiotic animal models.

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### Rob Knight, PhD

*HHMI and University of Colorado at Boulder, Boulder, CO*

#### From correlation to causation in human microbiome studies

Human microbiome studies have made tremendous progress in the past few years, yet many of these studies are still correlative in nature. Moving from correlation to causation requires that we go beyond the paradigm of finding differences between cases and controls: instead, we must engage in prospective longitudinal studies, and test putative causal associations in vitro and in animal models. Two crucial aspects of successful studies, especially those that seek to understand causation, are the effect size of technical versus biological parameters and good clinical metadata. Here I describe these principles with specific application to malnutrition, obesity, IBD, and Type I diabetes, and discuss the possibilities of crowdfunded science such as the American Gut project as a resource for hypothesis generation.

#### Gaps:

- We don't know the effect sizes we expect for different pathological or physiological states, or for different analytical techniques
- Techniques for statistical analysis of dynamic microbiome data are just emerging
- Whether prospective studies or mechanistic studies will provide more useful information, or whether both will typically be needed, remains unknown
- Most "metadata" (i.e. per-sample or per-individual data) are not available in the public databases in a standardized form

#### Needs:

- Standardized scale for effect sizes in microbiome studies, and improved methods of performing power calculations
- Better ways to relate human data to animal model data, especially in terms of relevant spatial and temporal scales
- Prospective longitudinal studies

- Library of microbial strains of known provenance and environmental/clinical distribution for testing hypotheses about microbial function
- Better annotations at all scales from genes to organism

#### Challenges:

- How can we balance subject privacy issues with the scientific utility of rich, publicly available clinical data?
  - How can we integrate data from multiple omics levels in longitudinal studies? How much data do we need of each type?
  - How can we integrate the knowledge of gene functions produced by multiple researchers using multiple techniques in an efficient way?
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### Heidi H. Kong, MD, MHSc

*National Cancer Institute, Bethesda, MD*

#### Eczema, immunity and the skin microbiome

The skin serves not only as a barrier against invading pathogens and moisture loss, but also as a host to microbial communities. Skin is distinct from other human epithelia, e.g. gut, oral, or vaginal mucosa, with regards to resident microbes and immune system. Investigating the skin microbiome is important for understanding our outermost epithelial surface, especially in relationship to skin immune system development and homeostasis in health and in disease.

Microbiome surveys of adult healthy skin have demonstrated that skin microbes are more diverse than previously determined via traditional culture-based studies and that the skin microbiome is highly site-specific with distinct environments having different microbial communities. Microbiome surveys have also characterized the differences in the skin bacterial microbiome in neonates, infants, and older children. A skin fungal microbiome survey in healthy adults showed that while *Malessezia* predominates in many sites, there is marked site-specificity in the skin fungal communities. Surveys of the skin microbiome provide a necessary foundation for investigations on the microbiota in dermatologic disorders.

Atopic dermatitis (AD, a common form of eczema) is a skin disease affecting 15% of the U.S. population. The innate and adaptive immune systems are dysregulated in these patients. AD patients are frequently colonized by *Staphylococcus aureus* (76-100%) and have relatively

high rates of *S. aureus* skin infections as well as susceptibility to dissemination of cutaneous herpes simplex virus and smallpox infections. Using genomics, we have shown that there is a marked upward shift in the proportion of both *S. aureus* and *S. epidermidis* on the skin during AD disease flares. By understanding how the skin microbiome shifts in AD, we may be able to improve our understanding of AD pathophysiology and develop novel management strategies.

Several primary immunodeficiency syndromes also present with skin disease, including AD-like dermatitis. These patients provide an opportunity to investigate monogenic disorders with defined immune defects and associated skin disorders. Patients with *STAT1* mutations (chronic mucocutaneous candidiasis) and *STAT3* mutations (autosomal dominant Hyper IgE Syndrome) harbor different skin microbiomes from controls, including increased *Acinetobacter* spp. Prestimulation of healthy volunteer PBMCs with *Acinetobacter* augmented production of IL-22, IFN- $\gamma$  and TNF- $\alpha$  in response to *Candida albicans* and *S. aureus*, suggesting that certain bacteria can influence cytokine production by healthy circulating blood cells. Exploring how skin microbes influence human skin immunity is important for understanding skin health and disease.

**Challenges and gaps:**

- moving from correlation to causality;
  - understanding the specific immune factors and relationships with microbiota that are important in skin;
  - determining how skin barrier influences the interaction between host immunity and microbes; and
  - quantitation of microbes on the skin
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**Johanna W. Lampe, PhD** and **Meredith AJ Hullar**  
*Fred Hutchinson Cancer Research Center, Seattle, WA*

**Gut Microbial Metabolism of Food Constituents:  
Modulating Human Dietary Exposures**

Epidemiologic evidence suggests that the interaction between humans, their food choices, and metabolism by their commensal gut microorganisms has the capacity to influence health and disease risk. Host diet can influence the amount and types of microbes present in the gut, and gut microbial metabolism of dietary constituents produces compounds that may

protect or harm the host. Bacteria metabolize both organic and inorganic constituents of diet that are indigestible by human enzymes or that escape digestion in the upper gastrointestinal tract. They have unique metabolic functions and their end-products can: 1) supply energy to host cells; 2) act as signaling molecules in host pathways; 3) be genotoxic or beneficial to host cells. Carbohydrates (i.e., dietary fiber, resistant starch, and oligosaccharides), are fermented to short chain fatty acids (SCFA; primarily acetate, propionate, and butyrate). Butyrate is a key fuel source for gut epithelial cells and also regulates their proliferation and apoptosis. It and the other SCFA can also have farther reaching signaling effects on hepatic lipogenesis and other pathways. Nitrogenous compounds (i.e., proteins and amino acids) similarly reach the colon and are fermented and further metabolized to a range of metabolites, depending on the amino-acid precursors. Many amino acids are deaminated and fermented to hydrogen, carbon dioxide, SCFA, and other organic acids. Microbial metabolism of aromatic amino acids produces phenols (phenol and p-cresol) and indoles—groups of compounds that can be genotoxic and co-carcinogenic. Sulfur-containing amino acids, as well as sulfates from other dietary sources, are metabolized by sulfate-reducing bacteria to hydrogen sulfide, which has been associated with gut epithelial inflammation and genotoxicity. Another class of mutagenic compounds, N-nitroso compounds (NOC) are formed when nitrate is reduced to nitrite by gut bacterial nitrate reductase and nitrite subsequently interacts with organic compounds to form NOC. Microbial transformation of other compounds associated with high-protein foods can also influence human health. For example, choline, released from phosphatidylcholine during lipolysis, is metabolized by gut bacteria to trimethylamine and is converted in the liver to trimethylamine *N*-oxide (TMAO). Increased plasma TMAO concentrations are associated with increased risk of incident major adverse cardiovascular events. Many bioactive compounds in plant foods that have been shown to be disease preventive in experimental animal models and associated with lower disease risk in humans are also extensively metabolized by gut microbes to a variety of metabolites. In humans, wide ranges in circulating levels of these metabolites among individuals in response to a standard phytochemical dose suggest large inter-individual variation in gut microbial capacity to metabolize the parent phytochemicals. Overall, elucidating the complex interplay of host diet and the gut microbiome on human dietary exposures, as well as

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better understanding the impact of the bacterial metabolites on regulatory pathways, may help to guide future disease prevention strategies.

### Gaps, Needs and Challenges

- Need to facilitate transdisciplinary research to allow for integrated breadth of knowledge.
- Need for prospective cohorts with measures of exposure (i.e., diet, etc) and samples for gut microbiome characterization.
- Need for well-controlled dietary interventions to understand the inter-individual variation in bacterial metabolic phenotypes in the context of diet.
- Need for accurate model systems of human dietary metabolism and associated microbiota.
- Need for methods of assessing composite functionality of the gut microbiome and integration of the structure and function of microbial systems.
- Need for computational methods to integrate high-dimensional microbiome and metabolome data.
- Challenge of testing causality of the gut microbiome's contribution to health and disease in healthy humans.

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**Sarkis K. Mazmanian, PhD**

*California Institute of Technology, Pasadena, CA*

### Bacterial colonization factors control specificity and stability of the gut microbiota

Mammals harbor a complex gut microbiome, comprised of bacteria that confer immunologic, metabolic and neurologic benefits. Despite advances in sequence-based microbial profiling and myriad studies defining microbiome composition during health and disease, little is known about the molecular processes employed by symbiotic bacteria to stably colonize the gastrointestinal (GI) tract. We sought to define how mammals assemble and maintain the *Bacteroides*, one of the most numerically prominent genera of the human microbiome. While the gut normally contains hundreds of bacterial species, we surprisingly find that germ-free mice mono-associated with a single *Bacteroides* are resistant to colonization by the same, but not different, species. To identify bacterial mechanisms for species-specific saturable colonization, we devised an *in vivo* genetic screen and discovered a unique class of Polysaccharide Utilization Loci (PUL) that are conserved among intestinal *Bacteroides*. We named this genetic locus the commensal colonization factors

(ccf). Deletion of the ccf genes in the model symbiont, *Bacteroides fragilis*, results in colonization defects in mice and reduced horizontal transmission. The ccf genes of *B. fragilis* are up-regulated during gut colonization, preferentially at the colonic surface. When we visualize microbial biogeography within the colon, *B. fragilis* penetrates the colonic mucus and resides deep within crypt channels, while ccf mutants are defective in crypt association. Remarkably, the CCF system is required for *B. fragilis* colonization following microbiome disruption with *Citrobacter rodentium* infection or antibiotic treatment, suggesting the niche within colonic crypts represents a reservoir for bacteria to maintain long-term colonization. These findings reveal that intestinal *Bacteroides* have evolved species-specific physical interactions with the host that mediate stable and resilient gut colonization, and the CCF system represents a novel molecular mechanism for symbiosis.

### Gaps, needs, challenges:

- What are the molecular mechanisms that control long-term colonization by the microbiota?
- How can we enhance resilience / repopulation of the human microbiota following a disturbance (antibiotics, gastroenteritis, etc.)

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**David Mills, PhD**

*University of California – Davis, Davis, CA*

### A milk-oriented-microbiota (MOM) in infants—How babies find their MOMs: Insights for next generation prebiotics and probiotics

One goal of the application of probiotics and prebiotics is to direct the gut microbiota into a more healthful state. While a variety of clinical trials suggest both prebiotics and probiotics can have a positive impact on various conditions, an understanding of the mechanisms driving such benefits is lacking. We have examined the enrichment of specific beneficial populations of bifidobacteria within breast fed infants as a model for both prebiotic (milk glycans) and probiotic (bifidobacteria) application in humans.

Human milk contains numerous components that shape the microbial content of the developing infant gastrointestinal tract. A prominent feature of milk is an array of complex glycans and glycoconjugates that serve a passive immune function by sequestering and

deflecting pathogens while simultaneously enriching a protective microbiota often dominated by bifidobacteria. Infant-borne bifidobacteria are able to utilize human milk oligosaccharides (HMOs) as a sole carbon source however the specific mechanisms to deconstruct complex HMOs vary by species. These different infant-borne bifidobacteria contain specific glycosidases and transport systems required to utilize free HMOs or glycoconjugates. Additional research has shown that growth on milk glycans enhances bifidobacterial interaction with the infant host through both direct and indirect routes. Growth on HMO results in increased bifidobacterial binding to epithelial cells and beneficially modulates intestinal barrier function. In aggregate, these studies suggest a co-evolutionary relationship between mammalian milk glycans, infant-borne bifidobacteria and the infant host that has enabled a programmed enrichment of a protective bifidobacterial-dominant microbiota during a critical stage of infant development. Importantly, analysis of this natural system serves as a key model for design of pro/prebiotic-based manipulation of the gut microbiota in a range of health settings.

Some questions/gaps/challenges in probiotic and prebiotic research

- What are the factors driving host specific responses to pre/probiotic applications
- How do pre/probiotics influence the host microbiota at the systems level
- Do pre/probiotics drive metabolic changes in the gut microbiota and systemically
- What are the mechanisms by which probiotics persist within the gut (or do we want them to persist?) and are they important for probiotic activity?
- Can we tailor pre/probiotic type/dosage using personalized information (host genotype, enterotype, glycotype etc.)?

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**Andrew S. Neish, MD***Emory University, Atlanta, GA*

#### **Control of Epithelial Homeostasis by the Microbiota**

The resident prokaryotic microbiota of the mammalian intestine is a numerically vast and taxonomically complex symbiotic community that influences diverse homeostatic functions including maintenance of the epithelial barrier, modulation of immune responses, and

control over cellular growth/differentiation and restitutive pathways. Specifically, recent advances have implicated the commensal microbiota in regulating epithelial cell cycle and stem cell dynamics in wide variety of organisms, thus indicating a role in normal gut growth and development and suggesting that “dysbiosis” of the bacterial community may influence initiation and progression of GI cancers. However, there is a *gap in the knowledge* concerning a mechanistic understanding of how the commensal microbiota influences regulation of cellular growth signaling networks in health and disease. Insight has come from studies of lower organisms, which have revealed a common paradigm wherein contact of prokaryotic organisms stimulate the enzymatic generation of reactive oxygen species (ROS) in the host, as an anti-microbial effector, or more significantly, as a cellular signaling intermediate. In mammals, while the induced generation of ROS via stimulation of formyl peptide receptors (FPRs) is a cardinal feature of the cellular response of phagocytes to all bacteria, evidence is accumulating that ROS are also similarly elicited in other cell types, including intestinal epithelia, also in response to microbial signals and FPRs.

Physiological ROS generation occurs via the action of highly conserved NADPH oxidase (Nox) enzymes, including Nox2 in phagocytes and Nox1 in intestinal epithelia. Interestingly, a subset of highly conserved bacteria, predominately lactobacilli and bifidobacteria, potently stimulates Nox dependent ROS generation in the guts of animals as disparate as mammals and invertebrates. This physiologically-generated ROS is known to function in cellular signaling via the rapid and transient oxidative inactivation of an expanding class of sensor proteins bearing oxidant-sensitive thiol groups. These proteins include tyrosine phosphatases that serve as regulators of MAP kinase pathways, cytoskeletal dynamics, as well as components involved in control of ubiquitination-mediated NF- $\kappa$ B activation.

Germ free flies and mice are known to exhibit suppressed epithelial proliferation and sensitivity to injury. We have demonstrated aberrant crypt dynamics in epithelial-specific Nox1 and Fpr1 null mice, and impaired gut stem cell proliferation in *Drosophila* when the orthologous dNox is genetically suppressed in discrete stem cell microenvironments. We hypothesize that ROS generated by NADPH oxidases function as signaling molecules during normal development and proliferation of intestinal enterocytes. In addition,

specific commensal bacteria (and fMLP) accelerate epithelial cell movement in a redox dependent fashion. Similarly, mucosal wound closure *in vivo* is enhanced by certain members of the microbiota, and these effects are abolished in Nox1 and Fpr1 (but not MyD88) null animals. These results demonstrate how enteric microbiota influence highly conserved regulatory networks of the intestinal epithelia.

Future challenges include the characterization of novel redox sensitive pathways that are influenced by microbially induced ROS. Additionally, delineation of members of the microbiota that potently stimulate ROS, and the microbiological determinants in these organisms responsible for ROS induction, will enable a mechanistic description of an important host-microbial relationship. Other important gaps include characterizing the temporal acquisition of pro-proliferative members of the microbiota, their role in the development of the neonatal intestine and their activities in modulating proliferation/homeostasis in normal conditions, restitution post injury or during oncogenesis.

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**Elaine Petrof, MD**

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### **Use of Microbial Ecosystems to treat recurrent *Clostridium difficile* infection**

Microbial ecosystem therapy (MET), is a means of replacing a dysfunctional, damaged ecosystem with a fully developed and healthy ecosystem composed of dozens of strains of “native” intestinal bacteria [1]. Proof of principle for microbial ecosystem therapy has already been established in the form of fecal microbiota transplantation (infusing donor stool into the intestine of the recipient to re-establish normal bacterial composition), or FMT, for the treatment of recurrent *Clostridium difficile* infection (CDI) [2]. This infection, which can be life-threatening, is most often acquired after a patient receives antibiotics for another unrelated infection, usually while in hospital. A continuing problem due to emergence of virulent strains over the past decade [3,4], US data from the Centers for Disease Control indicate that CDI affects 336,000 people and results in 14,000 deaths annually. Recurrent CDI (resolution of disease while on appropriate therapy, followed by recurrence of CDI after treatment has been stopped) [5] is also on the rise. Recurrent CDI is thought

to result from a “dysfunctional”, low-diversity microbiota, i.e. there appears to be an inability of certain individuals to “re-establish” their normal protective bacterial microbiota [5,6]. Hence, FMT is being increasingly used when standard medical therapies fail. However, while FMT has been shown to be very effective for recurrent CDI [7,8], little is known about its mechanisms of action, it is not a very palatable procedure, and concerns exist about donor transmission of infection and reproducibility of the fecal “product” [2, 9, 10].

Our group has developed a “synthetic stool” (“RePOOPulate”) to repopulate the colon with a healthy ecosystem of native intestinal bacteria [11]. This strategy was developed to help address some of the challenges of using FMT for recurrent CDI. Since our group has experience with both “conventional” FMT as well as “synthetic stool” approaches, the challenges of microbial ecosystem therapy in general, as well as future directions in the field, will be highlighted.

Some of the specific gaps, needs and challenges of microbial ecosystem therapy that will be covered in this session include:

- How can efficacy and stability be optimized?
  - ◆ What is the ideal formulation - how many strains needed to create the most robust ecosystem?
  - ◆ How do we gain alignment of the QC approach?
- How can both long and short-term safety be optimized?
  - ◆ What are the best ways to track long term safety?
  - ◆ How can we optimize the formulation of bacteria transplanted as we learn more about the potential pathogenic characteristics of different strains?

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**David A. Relman, MD**  
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### Diversity, Stability, and Resilience of the Microbiome

Recent advances in the study of the human microbiota and their collective genomes have highlighted the diversity of these communities, features of individuality, conserved as well as personalized predicted functional attributes, and the intimate relationship of these communities to host physiology. Yet, questions remain about the ecological processes that establish and maintain the human microbiota throughout life, as well

as the features of this ecosystem that are associated with stability, recovery after disturbance, and with ‘colonization resistance’ to pathogens. As with other ecosystems, early stages of assembly may have disproportionate impact on later aspects of function. The human microbiome in adults at baseline is dynamic but also displays features of stability. Short, pulse disturbances may cause at least transient alterations in structure and function; compounded or sustained disturbances may lead to persistent, alternate states. We need to be able to describe the current ecological fitness landscape in an individual and the kinds of forces necessary to induce shifts towards ecological states associated with health. Our long-term goal is a predictive understanding of the microbiome and the mechanisms that underlie resilience, as well as well-informed strategies for its manipulation, so as to maintain or restore health, and avoid or mitigate disease.

### Gaps, needs and challenges

- How to assess the functions and activities of the microbiota?
  - Understanding variation in microbiome structure and function across multiple spatial and temporal scales (most relevant scales?)
  - Descriptions of the habitat-specific ecological fitness landscape in individuals (so as to be able to predict response to disturbance)
  - Long-term health consequences for an individual of their early childhood microbiome?
  - Biological roles played by low abundance members of the human microbiota?
  - Comprehensive descriptions and analyses of the interactions between human and microbiota
  - Effective methods for integrating multiple types of data from the human-microbial ecosystem
  - Features associated with, and mechanistic basis for ecosystem stability and resilience?
  - Effective strategies for targeted, precise manipulation of the microbiome
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**Peter J. Turnbaugh, PhD**

*FAS Center for Systems Biology, Harvard University,  
Cambridge, MA*

### Moving towards a metagenomic basis of therapeutics

Many widely used pharmacology textbooks include a short paragraph stating that gut microbes influence the efficacy and toxicity of xenobiotics, including host-targeted drugs, antibiotics, and diet-derived bioactive compounds. However, the molecular mechanisms responsible often remain unknown, making it challenging to translate these findings to new therapies and diagnostics, or to appreciate the broader biological, ecological, and evolutionary implications. A major need is to develop translational research pipelines that integrate metagenomic sequencing of clinical cohorts, single cell analyses such as flow cytometry and microfluidics, metabolomics, culture collections, and gnotobiotic animal models. These methods promise to aid in addressing critical gaps in our current knowledge: (1) elucidating the bacterial taxa and metabolic pathways responsible for xenobiotic metabolism; (2) determining how microbial communities adapt during exposure to xenobiotics; and (3) testing the relative importance of host, microbial, and environmental factors for pharmacokinetics and dynamics. A long-term challenge is to obtain a more comprehensive, “metagenomic”, basis for the use of therapeutics, yielding fundamental insights into host-microbial interactions, and providing new approaches to predict and/or manipulate the metabolic activities of our resident gut microbes. If successful, the human microbiome will likely warrant more than a footnote in the pharmacology manuals of the future.

**Keywords:** human microbiome, metagenomics, xenobiotic metabolism, nutrition, microbial ecology.

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**Vincent B. Young, MD, PhD**

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### The Microbiome in Infectious and Non-infectious Gut Inflammation

The intestinal microbiota is large collection of microbes that is in contact with a tremendous mucosal surface. As such, there is a multitude of interactions that have to be maintained in a delicate equilibrium in order to maintain homeostasis of this environment. Disturbances

in this equilibrium, which can arise from alteration of the indigenous microbiota or the host can lead to loss of homeostasis. One common response to this altered homeostasis is the development of mucosal inflammation. The inflammatory bowel diseases ulcerative colitis and Crohn’s disease have traditionally been thought of as “idiopathic” as typical pathogens (i.e. fulfilling Koch’s postulates) have generally not been identified in these conditions. More recently, it has become apparent that the microbiome plays a significant role in the etiopathogenesis of inflammatory bowel disease and in inflammatory intestinal disorders resulting from infection from a “classical” infectious agent. In this talk, I will review the evidence that is beginning to determine the role the indigenous microbiota has in the pathogenesis of both infectious and non-infectious intestinal inflammatory diseases. Evidence is accumulating that understanding the normal interactions between the intestinal microbiota and mucosa may lead to novel means to prevent and treat diseases characterized by the development of acute and chronic gut inflammation.

### Gaps, needs and challenges to be discussed:

- There needs to be a move from associations between disease states and specific microbiota community structures (for example as measured by 16S rRNA-encoding gene sequence analysis) towards an understanding of the *functional consequences* of these community alterations.
  - Results from experimentation with model microbial communities (for example in continuous flow culture systems, animal and invertebrate systems) need to be validated and correlated with carefully designed studies in human subjects.
  - There is a need for the development and validation of analytic methods to process data derived from “multi-omic” datasets, which include 16S rRNA-encoding gene sequences, metagenomic sequences, host response data, proteomic and metabolomics data.
  - Results from microbiome studies need to be developed into novel therapeutics, which will require the ability to cultivate specific members of the microbiota deeper understanding of how to administer cultivars as potential therapies.
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### Poster Presenters

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Asquith	Mark	P1	<b>HLA B27 Expression Predisposes to Spondyloarthritis and Influences the Gut Microbiome</b>
Auchtung	Jennifer	P2	<b>Studying interactions between <i>C. difficile</i> and complex microbial communities in human fecal bioreactors</b>
Borozan	Ivan	P3	<b>CaPSID: A bioinformatics platform for computational pathogen sequence identification in human genomes and transcriptomes</b>
Chaston	John	P4	<b>The genetic basis for microbiota-dependent variation in host nutrition and health</b>
Cooper	Alan	P5	<b>The evolution of the oral microbiome, and human pathogen genomes</b>
Cox	Laura	P6	<b>Antibiotic Disruption of the Microbiome During a Critical Developmental Window has Lasting Metabolic Consequences</b>
Cullen	Thomas	P7	<b>Mechanisms Used by Human Commensal Organisms to Survive Host Immune Defenses</b>
Dale	Jennifer	P8	<b>A toolbox for analysis of enterococcal genetic determinants for colonization and adaptation to host-associated and environmental microbiomes</b>
de la Cruz	Diomel	P9	<b>Meconium Microbiome and Implications to Prematurity</b>
Demoruelle	M. Kristen	P10	<b>Lung Microbiome Differs in Asymptomatic Subjects at Elevated Risk for Future Rheumatoid Arthritis Compared to Healthy Controls</b>
Dichosa	Armand	P11	<b>Dissecting the Human Microbiome Using Gel Microdroplets and Single Cell Genomics</b>
Fettweis	Jennifer	P50	<b>Species diversity of the human vaginal microbiome</b>
Frank	Lily	P12	<b>Human Microbiome Research and De Minimis Risk</b>
Gajer	Pawel	P13	<b>A systems biology approach to understand the interaction between the vaginal microbiome and its metabolome</b>
Haiser	Henry	P14	<b>Predicting and manipulating drug inactivation by the human gut microbiome</b>

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Hamilton	Mary	P15	Progressive Increase in Large Intestine Transcellular but not Paracellular Permeability Correlates with Plasma Endotoxemia in Diet-Induced Obese Rats.
Hemarajata	Peera	P16	Lactobacillus reuteri-mediated Immunomodulation is Controlled by Regulation of Histidine Decarboxylation
Holscher	Hannah	P17	Novel Fibers Shift Human Fecal Microbiota in Healthy Adult Males
Hsiao	Elaine	P18	A Commensal Bacterium of the Gut Microbiome Ameliorates Behavioral Abnormalities in A Mouse Model of an Autism Risk Factor
Jaroszewski	Lukasz	P19	Structural characterization of proteins families from human gut microbiome
Jones	Rheinallt	P20	Role of the Microflora in the Etiology of Gastro-Intestinal Cancer
Kaiser	Brooke	P21	A Multi-Omic View of Host-Pathogen-Commensal Interplay in Salmonella-Induced Intestinal Inflammation
Kempainen	Kaisa	P22	An Analysis of Gut Microbial Diversity of Non-Autoimmune Subjects Genetically at High-Risk for Type 1 Diabetes
Leone	Vanessa	P23	Antibiotic-induced Gut Microbiota Alters Period-2 Circadian Gene Expression in the Liver
Lewis	Zachery	P24	Maternal FUT2 Polymorphisms Influence the Gut Microbial Communities of Breastfed Infants
Ma	Liang	P25	Using Microfluidics for Targeted Cultivation of Human Gut Microbes
Ma	Yingfei	P26	Novel Insights of Human Papillomavirus Infection Revealed by Metagenomic Analysis
Mishima	Yoshiyuki	P27	Impact of a simplified human microbiota consortium on a gnotobiotic murine model of colitis and intestinal mucosal homeostasis
Moore	Aimee	P28	The Pediatric Fecal Resistome Is Established in Early Infancy
Nakatsuji	Teruaki	P29	The Microbiome Extends to Subepidermal Compartments of Human Skin: Correlation with Skin Barrier Function

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Nasko	Daniel	P30	Assessing CRISPR Spacer Composition in the Vaginal Microbiome
Oh	Julia	P31	The Altered Landscape of the Human Skin Microbiome in Patients with Primary Immunodeficiencies
Ross	Matthew	P32	Viral Metagenomics for Etiologic Agent Discovery
Shankar	Jyoti	P33	Differential Effect of Antibiotics on the Gastrointestinal Bacterial and Fungal Microbiomes and their Influence on Colonization with <i>Candida</i> .
Shi	Baochen	P34	Dynamic Changes in the Periodontitis Metagenome
Song	Yang	P35	Microbiota Changes and Colonization During Treatment of Clostridium difficile Infection With Fecal Microbiota Transplantation
Stucker	Karla	P36	Sequencing the Enteric Microbiome to Understand Pediatric Diarrheal Disease in South Africa
Theriot	Casey	P37	Antibiotic-mediated Shifts in the Gut Microbiome and Metabolome Leads to Susceptibility to Clostridium difficile Infection
Toborek	Michal	P38	Exercise Attenuates Changes in the Gut Microbiome Induced by Polychlorinated Biphenyls
Ursell	Luke	P39	Microbial Communities of the Human Gastrointestinal Tract Demonstrate Individualized Responses to Low Molecular Weight Substrate Perturbations
Venkataraman	Arvind	P40	Niche vs. Neutrality in microbial communities
Vital	Marius	P41	Investigating the role of butyrate-producing bacterial communities in the development of Ulcerative Colitis
Vlasova	Anastasia	P42	Lactobacilli and Bifidobacteria promote immune homeostasis and modulate innate immune responses to human rotavirus vaccine and challenge in neonatal gnotobiotic pigs
Wang	Ana	P43	Chemical-based Metaproteomics of the Healthy Human Distal Gut Microbiome
Wang	Sida	P44	Microfluidic droplet enabled co-cultivation and characterization of microbial communities

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### Poster Presenters, continued

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Warinner	Christina	P51	<b>Extending Oral Microbiome Research into the Human Evolutionary Past</b>
Weingarden	Alexa	P45	<b>Bile Acid Metabolism Changes Following Fecal Microbiota Transplantation for Clostridium difficile Infection</b>
Weyrich	Laura	P46	<b>From Neanderthals to Chimpanzees: Origins of the Oral Microbiome</b>
Whiteson	Katrine	P47	<b>Breath gasses as biomarkers in Cystic Fibrosis</b>
Wylie	Kristine	P48	<b>The Human Virome in Immunocompromised and Immunocompetent Children</b>
Zoh	Roger	P49	<b>Probabilistic Correlation Analysis of the Metagenome and Host Transcriptome: A Tale of Two Non-Normal Data Sets</b>
Qiagen		P52	<b>Identification of antibiotic resistance genes in Klebsiella pneumoniae isolates and metagenomic samples using real-time PCR arrays</b>
Roche		P53	<b>800+ Base 16S and 18S rRNA Gene Sequencing using the GS FLX+ and GS Junior Systems</b>
Metabolon		P54	<b>Metabolomic Profiling of Gut Microflora Activity</b>

## Poster Presentation Abstracts

**P1. Mark Asquith<sup>1</sup>, Sean Davin<sup>1</sup>, Patrick Stauffer<sup>1</sup>, Phoebe Lin<sup>1</sup>, Eric Cambronne<sup>1</sup>, Mary Bach<sup>2</sup>, Russell Van Gelder<sup>3</sup>, Joel Taurog<sup>4</sup>, Rob Knight<sup>5</sup>, Stephen R. Planck<sup>1,6</sup>, James T. Rosenbaum<sup>\*1,6</sup>.**

### HLA B27 Expression Predisposes to Spondyloarthritis and Influences the Gut Microbiome

<sup>1</sup>Oregon Health and Science University, Portland, OR; <sup>2</sup>University of Washington, Seattle, WA; <sup>3</sup>UW Medicine Eye Institute, Seattle, WA; <sup>4</sup>University of Texas Southwestern, Dallas, TX; <sup>5</sup>University of Colorado at Boulder, Boulder, CO; <sup>6</sup>Devers Eye Institute, Portland, OR

**Background/Hypothesis:** The correlation between the human leukocyte antigen (HLA) B27 allele and ankylosing spondylitis (AS) is the strongest association of an HLA class molecule and disease. The mechanism by which B27 influences disease susceptibility is unknown. The bacteria of the digestive tract influence the development and maintenance of a healthy immune system. Dysbiosis of the microbiome is implicated with conditions such as ulcerative colitis and Crohn's disease, which share some clinical symptoms with AS. Fischer 33-3 rats express HLA B27 and spontaneously develop diarrhea and arthritis unless they are bred in a germ-free environment. We hypothesized that HLA B27 expression influences the gut microbiota in these animals.

**Methods/Microbiome analysis:** We analyzed the microbiota in ileum, cecum, transverse colon, and stool samples from 4.3 to 32 week old transgenic rats (n=19) and age-matched, wild type controls (n=7) by 16s ribosomal DNA amplification, mass DNA sequencing, and UNIFRAC analysis. Results were confirmed by RT-PCR using species-specific primer/probe sets.

**Results:** Principal component analysis of preliminary data shows that the B27+ rats have a gastrointestinal microbiome distinct from littermate controls ( $p<0.0001$ ). Compared to these controls, transgenic rats had higher frequencies of Tenericutes (5.0% to

0.6%) and Verrucomicrobia (15.4% to 4.9%) and a reduced frequency of Firmicutes (39.8% to 61.8%). Highly represented spp. Akkermansia muciniphilia and Bacteroides fragilis were significantly more abundant in the intestine of B27+ animals relative to WT controls.

**Conclusions/Significance:** HLA B27 expression profoundly shapes the gut microbiota as it predisposes for microbiota-dependent intestinal and systemic disease. These findings signify expression of HLA risk alleles in humans may also modulate their gut microflora and susceptibility to spondyloarthropathy. The data encourage further research to determine whether particular gut bacteria promote or suppress colitis and arthritis in B27 transgenic rats in addition to characterizing B27-dependent alterations to the microbiota in humans.

**Major past and future challenges to progress:** The relationship between human HLA expression, microbiota composition/function and disease pathogenesis remains unknown. While novel and improved molecular biology techniques have revealed the complexity of the gut microbiota with increased resolution, longitudinal studies of the microbiota before and during disease development are urgently needed. This is particularly challenging with respect to spondyloarthropathies - chronic diseases which may take years to develop. Moreover, as/when candidate organisms with therapeutic potential become identified, successful translation to the clinic faces formidable (and possibly unforeseen) technical, regulatory and practical obstacles ahead.

#### Keywords:

Immune system  
Host/microbiome interaction  
Disease association

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**P2. Jennifer Auchtung**, Catherine Robinson, Robert Britton\*.

**Studying interactions between *C. difficile* and complex microbial communities in human fecal bioreactors**

*Michigan State University, East Lansing, MI*

*Clostridium difficile* is a gastrointestinal pathogen estimated to cause ~25% of antibiotic-associated diarrhea. Although *C. difficile* disease is typically self-limiting, severe recalcitrant disease can occur in some patients. The primary risk factor for *C. difficile* infection is antibiotic treatment, which disrupts the microbiome and allows *C. difficile* to invade the unstable gut ecosystem and cause disease. Two important questions regarding *C. difficile* infection are how the microbiome normally resists *C. difficile* invasion and how the microbiome can be restored to inhibit *C. difficile*. The primary objective of our research is to develop a moderate-throughput, *in vitro* system for studying the interactions between *C. difficile* and fecal communities in order to identify and cultivate members of the microbiome important for resisting *C. difficile* invasion.

Therefore, we developed mini-bioreactors for continuous-flow cultivation of up to 48 microbial communities in parallel. We collected fecal samples from twelve healthy donors and used these samples as inocula for mini-bioreactors. After establishing fecal microbial communities in the mini-bioreactors, we introduced high levels of *C. difficile* and monitored its abundance through *C. difficile*-specific plating and qPCR. We found that communities treated with antibiotic prior to *C. difficile* challenge were invaded by *C. difficile*, whereas unperturbed communities resisted invasion. We also examined the composition of the microbial communities in the mini-bioreactors by pyrosequencing the V3-V5 regions of the 16S rRNA genes of these communities. Using mothur to analyze the sequence data, we compared shared community structure between samples at the level of operational taxonomic units (OTUs) with ≤3% sequence identity between OTUs. We found that most mini-bioreactor

communities were initially highly similar and were a subset of the fecal inoculum. Over time, the communities diverged to form unique microbial communities that maintained significant shared community composition. Antibiotic treatment consistently eliminated a specific subset of the microbial community. We identified several microbes closely related to this subset lost during antibiotic treatment in our fecal microbiome isolate collection. We plan to test the ability of these isolates to restore the ability of mini-bioreactor microbial communities to inhibit *C. difficile* proliferation following perturbation. From our studies, we can conclude that mini-bioreactors are an effective tool for testing the role of specific members of the microbiome in resisting infection by *C. difficile*. However, we expect that our mini-bioreactor setup could have greater significance to microbiome research community as a useful tool for studying other aspects of microbiome dynamics. A primary challenge that we encountered in our work was optimizing our mini-bioreactor operating conditions to maintain sterility and prevent equipment malfunction due to hydrogen sulfide production by the microbial communities. A second challenge was to identify appropriate continuous-culture conditions that allowed cultivation of complex communities. Although the medium composition, inoculum, and flow rate conditions we selected have worked well for our initial studies on *C. difficile*, identifying other culture conditions that allow cultivation of different subsets of microbial communities represents a future challenge to be pursued.

**Keywords:**

Ecology  
Probiotics  
*Clostridium difficile*

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**P3. Ivan Borozan<sup>1</sup>, Shane Wilson<sup>1</sup>, Stuart N. Watt<sup>1</sup>, Paola Blanchette<sup>2</sup>, Philip E. Branton<sup>2</sup>, Vincent Ferretti<sup>1</sup>.**

### **CaPSID: A bioinformatics platform for computational pathogen sequence identification in human genomes and transcriptomes**

<sup>1</sup>*Ontario Institute for Cancer Research, Toronto, ON, Canada;* <sup>2</sup>*McGill University, Montreal, QC, Canada*

Progress to identify viruses as causative agents of human cancers has been slow and made difficult by the lack of good methods to rigorously detect these organisms. Next generation sequencing technologies offer a desirable new solution with major advantages being the unbiased detection of known pathogens, even when present in minute amounts in sequenced samples, and the ability to discover completely novel organisms. The increasing number of cancer sequence databases such as the one currently build by the International Cancer Genome Consortium [1] will allow for the first time an in-depth analysis of the viral sequence content of thousands of complete human tumor genomes and transcriptomes. This represents a unique opportunity for the discovery of new tumor-associated human pathogens. Recently our group has designed CaPSID [2] (Computational Pathogen Sequence Identification) a comprehensive bioinformatics platform for identifying, querying and visualizing both exogenous and endogenous pathogen nucleotide sequences in human tumour transcriptomes and genomes. We demonstrate the high accuracy with which CaPSID's bioinformatics pipeline is capable of detecting viral transcripts, even when those transcripts are present in low abundance in human cancer transcriptome samples, and show that CaPSID's predictions can be successfully validated in vitro. Furthermore CaPSID offers new and useful features that are not available in any current software used in metagenomic analyses of high-throughput sequencing data. CaPSID is also suitable for collaborative types of projects between teams of scientists, for example between bioinformaticians and molecular virologists, through its web interface allowing researchers without expert knowledge in

computational techniques to analyze sequencing results stored in the CaPSID's database. The major challenge we faced when developing CaPSID was to provide a high performance platform for metagenomic analysis that could efficiently process, analyze and store relevant genomic data produced by large scale next generation sequencing projects. Finally we identify two major future challenges. One is to improve our analysis method for more systematic identification of those pathogen sequences that have more obvious relation to cancer. Second is to improve our current methodology for discovering entirely novel pathogen sequences in sequenced samples. The CaPSID platform is currently used to analyze the viral content of various cancer data-sets generated by the Ontario Institute for Cancer Research genomics platform. CaPSID is free and open source code, documentation and tutorial can be found at <https://github.com/capsid/capsid>.

#### References:

- 1.Nature, 464:993-998 (15 April 2010)
- 2.BMC Bioinformatics, 13:206 (2012 Aug 17)

#### Keywords:

Bioinformatics/computational tools  
Methods  
Disease association

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**P4. John M. Chaston, Adam J. Dobson, Peter D. Newell, Adam (Chun-Nin) Wong, David R. Sannino, Sara L. Hermann, Angela E. Douglas\*.**

### **The genetic basis for microbiota-dependent variation in host nutrition and health**

*Cornell University, Ithaca, NY*

The impact of the resident microbiota on animal nutrition can be substantial. Gastrointestinal microbes can modify ingested food and exchange metabolites with their animal hosts. Variation in the nutritional status among individual animals may result from differences in host genotype, bacterial complement and function, or both. To test the hypothesis that host

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genotype and microbiota composition each contribute to host nutrition, we studied *Drosophila melanogaster* and its gastrointestinal tract bacterial community, which is dominated by just five bacterial taxa (>99% numerical representation). We measured host nutritional indices in 150 genetically diverse *D. melanogaster* fly lines to assess the impact of host genotype on host nutritional indices (lipid, glucose, glycogen, and protein content) and related traits (weight, feeding rate, growth rate, resistance to starvation). We determined the bacterial contributions to fly nutrition by quantifying the same nutritional indices in experimentally-generated germ-free flies. Our results demonstrate that the nutritional status of an animal can be shaped by the interaction between host genotype and microbial colonization. Specifically, the magnitude of each nutritional index varies widely across different host genotypes, and most of the indices vary significantly with microbial treatment (conventional versus germ-free). We performed genome-wide association of fly-line allelic variation with each nutritional index. We identified genes that are known to affect host nutrition (e.g. TOR, insulin signaling, growth factors) or dictate host-microbe interactions (e.g. innate immune signaling pathway). Genes with no previously documented relationship to nutrition and microbiota-responsiveness were also associated with bacterial-dependent nutritional status. These experiments, in combination with an analysis of the *Drosophila* microbiota-dependent transcriptional network, reveal how similar biological processes can be mediated by different host-microbe interactions in genetically-distinct individuals. We are currently verifying the interactions of these genes with the microbiota to alter host nutritional status. One extension of this study is to determine the reliability of host genotype as a predictor of microbial impacts on host nutritional health, as a test-bed for incorporating microbe-dependent effects on host health into genome-based personalized medicine.

### Keywords:

Host/microbiome interaction  
Diet  
Biomarkers

P5. Alan Cooper<sup>1</sup>, Laura Weyrich<sup>1</sup>, Keith Dobney<sup>2</sup>.

### The evolution of the oral microbiome, and human pathogen genomes

<sup>1</sup>University of Adelaide, Adelaide, Australia; <sup>2</sup>University of Aberdeen, Aberdeen, United Kingdom

We examined a range of ancient skeletons to determine whether calcified plaque (dental calculus) might act as a source of preserved human oral microbial DNA, and to examine the diversity of pathogenic and commensal organisms present. Dental calculus is potentially the only routinely fossilised source of human microbiome information, providing a unique means to examine evolutionary history, response to environmental changes (eg antibiotics), paleoepidemiology through time, and specific genomic loci associated with phenotype.

We extracted and analysed DNA from 50 ancient humans ranging from pre-farming, through the Neolithic and Industrial revolutions, to the modern day, and examined changes in microbial composition and diversity through time. We also examined specimens from different parts of the world, and close outgroups such as Neandertals and chimps, to examine how the human oral microbiome has evolved and differentiated.

We observed major changes in microbial composition and diversity around 7,500 yrBP at the Mesolithic/Neolithic transition (associated with the introduction of farming and a major increase in dietary carbohydrates), and at 1850 AD during the Industrial Revolution (when processed sugar and flour became widely available). Both transitions were characterised by major changes in diversity, associated with increases in disease-associated bacteria, and consistent with increased signs of skeletal pathologies (periodontal disease, caries, dietary stress).

These data indicate that shifts in diet and culture have had major impacts on the human microbiome and may have interrupted co-evolutionary relationships and/or otherwise contributed to modern chronic oral disease. Similarly, it is likely these changes are also contributory to other systemic disorders, and may have altered the

ability of pathogens to infect humans.

Until recently, it has not been possible to use ancient DNA methods to accurately characterise ancient dental calculus, but hybridization-enrichment and HTS now provide the unique opportunity to track the evolution and paleoepidemiology of the human oral microbiome, and examine genomic changes in key pathogens such as *Mycobacterium*, *Staphylococcus*, *Streptococcus*, and *Bordetella* species through time.

**Keywords:**

Evolution

Disease association

Diet

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**P6. Laura M. Cox**<sup>1</sup>, Ilseung Cho<sup>2</sup>, Jiho Sohn<sup>3</sup>, Kartik Raju<sup>2</sup>, Isabel Teitler<sup>2</sup>, Elisa Venturini<sup>4</sup>, Sarah Owens<sup>5</sup>, Alexander V. Alekseyenko<sup>6</sup>, Martin J. Blaser<sup>7</sup>.

**Antibiotic Disruption of the Microbiome During a Critical Developmental Window has Lasting Metabolic Consequences**

<sup>1</sup>NYU Sackler Institute of Graduate Biomedical Sciences, New York, NY; <sup>2</sup>NYU Langone Medical Center, New York, NY; <sup>3</sup>NYU College of Arts and Science, New York, NY; <sup>4</sup>NYU Joint Genome Institute, New York, NY; <sup>5</sup>Argonne National Laboratory, Lemont, IL; <sup>6</sup>NYU Center for Health Informatics and Bioinformatics, New York, NY; <sup>7</sup>NYU Langone Medical Center, New York, NY

Acquisition of the intestinal microbiota begins at birth and a stable microbial community develops from a succession of key organisms. Antibiotic disruption of the co-evolved microbiota during maturation can increase weight and adiposity. To investigate the role of timing, diet, and causation, C57B/L6J mice received sub-therapeutic antibiotic treatment (STAT) and control mice did not receive antibiotics in several murine models of growth promotion. We found that STAT increased weight gain and adiposity compared to controls on both normal chow and high-fat diets, and the greatest weight and adiposity resulted from the

combination of STAT and high-fat diet. STAT administered during the first 4 weeks of murine development was both optimal and sufficient for increasing adult total and fat mass. To characterize changes in the intestinal microbiome over time, the V4 region of the 16S rRNA gene was sequenced at an average depth of > 5,000 sequences/sample in longitudinally collected fecal samples and cecal and ileal samples collected at sacrifice. Principal coordinate analysis of unweighted UniFrac distances revealed that the microbiome differed between control and STAT mice that received continuous antibiotics. For mice receiving STAT limited to the first 4 or 8 weeks of life, the intestinal microbiota recovered after cessation of antibiotics, despite lasting changes in metabolic phenotypes. The growth promotion phenotype was transferrable to germ-free hosts by microbiota alone. When transferred to new hosts, STAT microbiota had a greater loss in  $\alpha$ -diversity and was less resilient. Microbiome compositional differences were maintained in control and STAT recipients over time. This work demonstrates that the STAT-selected microbiota, not the antibiotics per se, alter host metabolism that early life exposure to antibiotics has long-term metabolic consequences which are additive to diet-induced changes. This work begins to identify candidate biomarker organisms that influence host metabolism and identifies a critical window of host microbiome interaction. By sampling at multiple time points, this work overcomes the challenge of studying a dynamic system with great variability. The future challenges will be to biologically validate the candidate organisms associated with promoting or protecting against obesity, to further characterize the mechanism by which specific microorganisms effect host metabolism, and to model these findings to predict health outcomes.

**Keywords:**

Host/microbiome interaction

Disease association

Ecology

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**P7. Thomas W. Cullen**, Whitman B. Schofield, Andrew L. Goodman\*.

### Mechanisms Used by Human Commensal Organisms to Survive Host Immune Defenses

*Yale University, West Haven, CT*

Trillions of microorganisms colonize the human gastrointestinal tract and live in a mutually beneficial relationship with their host. To maintain this homeostasis, mammals have evolved elaborate physical and immune barriers that allow the growth of commensal organisms (primarily belonging to two phyla, Bacteroidetes and Firmicutes) while eliminating pathogens. However, homostasis is often disrupted by diverse events (e.g. antibiotics) resulting in overgrowth of opportunistic pathogens such as *Clostridium difficile*, exploiting dysbiosis. The mechanisms used by human gut microbes to survive host defenses during homeostasis and/or repeated inflammatory events are not well understood. We tested several prominent *Bacteroides* spp. for resistance to antimicrobial peptides (AMPs) secreted by the host and found extraordinarily high resistance. To determine the mechanisms used by human gut microbes to resist defensins and other AMPs secreted by the host, we mutagenized several prominent human gut species with a broad-host-range transposon and used insertion sequencing (INSeq) to identify mutants with altered sensitivity to Polymyxin B (PMB), a polypeptide whose mechanism of action is identical to that of human defensins. In each species tested, insertions in a gene with distant homology to a *Helicobacter pylori* lipopolysaccharide modifying enzyme result in high levels of sensitivity. This protein, which we named LpxF, provides a ~1000-fold increase in resistance to PMB and several other AMPs produced by the intestinal epithelium, which is consistent with its function in *H. pylori*. Fourier transform mass spectrometry of lipid A purified from an LpxF mutant strain reveals that this enzyme is required for removal of the 4'-phosphate group from this molecule. LpxF orthologs are found in most commensal Bacteroidetes, which universally lack a 4'-phosphate on their lipid A

and exhibit high PMB resistance, suggesting that the mechanism identified in these studies is broadly conserved. Animal studies are currently underway to understand the role of AMP resistance in mediating the interaction between human gut microbes and their host.

#### Keywords:

Host/microbiome interaction

Immune system

Homeostasis

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**P8. Jennifer L. Dale**<sup>1</sup>, Kristi L. Frank<sup>1</sup>, Dawn A. Manias<sup>1</sup>, Nicholas A. Dillon<sup>1</sup>, Patrick M. Schlievert<sup>2,1</sup>, Jo Handelsman<sup>3,4</sup>, Jonathon F. Holt<sup>3,4</sup>, Qinghong Ran<sup>5</sup>, Michael J. Sadowsky<sup>5</sup>, Gary M. Dunny\*<sup>1</sup>.

### A toolbox for analysis of enterococcal genetic determinants for colonization and adaptation to host-associated and environmental microbiomes

<sup>1</sup>*University of Minnesota, Minneapolis, MN*; <sup>2</sup>*University of Iowa, Iowa City, IA*; <sup>3</sup>*Yale University, New Haven, CT*; <sup>4</sup>*University of Wisconsin - Madison, Madison, WI*; <sup>5</sup>*University of Minnesota, St. Paul, MN*

*Enterococcus faecalis* and *Enterococcus faecium* persist (frequently at low population density) in a remarkable diversity of host-associated and non-associated microbial communities, and they display an extraordinary propensity to become predominant when their ecological niche is disrupted. Enterococcal proliferation in disturbed host-associated communities often has dire pathogenic consequences for the host, whether it be a mammal, an insect or a nematode. The high level of innate and acquired resistance of enterococci to antibiotics seriously impairs our ability to control these infections in humans and animals. On the other hand, non-pathogenic enterococci may have potential use as probiotics. In this presentation, we describe a comprehensive approach for the elucidation and analysis of enterococcal genetic determinants required for survival and adaptation to multiple

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ecological niches. Our approach includes both gene expression profiling, and random insertional mutagenesis followed by phenotypic screening. We have employed multiple techniques to identify core genome determinants involved in *in vitro* and *in vivo* adaptation under a multitude of growth conditions. We summarize the limitations of current methods and suggest enhancements that promise to overcome current limitations. Using powerful new genetic methods combined with high-throughput parallel sequencing, which are readily adaptable to multiple strains, we can enhance current conventional techniques. In addition, we are optimizing approaches that should facilitate real-time analysis of Enterococci in their natural habitats at the single cell level. Applying this combinatorial approach of techniques will help elucidate genes facilitating the transition of Enterococci from a non-pathogen to pathogen.

### **Keywords:**

Enterococcus

Methods

Host/microbiome interaction

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**P9.** Alexandria Ardissoni<sup>\*1</sup>, Kevin Rechcigl<sup>1</sup>, **Diomel de la Cruz**<sup>2</sup>, Roberto Murgas-Torrazza<sup>2</sup>, Josef Neu<sup>2</sup>, Eric W. Triplett<sup>1</sup>.

### **Meconium Microbiome and Implications to Prematurity**

<sup>1</sup>University of Florida, Gainesville, FL; <sup>2</sup>University of Florida, Gainesville, FL

**Background/Significance:** Preterm birth is the second leading cause of death in children under the age of 5 years old worldwide. However, the underlying mechanisms leading to preterm labor are not well understood. It is known that a fetus swallows amniotic fluid in utero, and studies have shown that amniotic fluid is not sterile as previously believed. This not only suggests that the human intestinal microbiome may begin in utero, but also that the in utero microbial

environment may affect immune response and potentially induce preterm labor. Meconium samples represent the initial microbe colonizers and potentially represent the *in utero* environment.

**Purpose/Hypothesis:** By sampling meconium from infants of various gestational ages, microbial differences corresponding with gestational age would be indicative of organisms that may lead to premature labor or provide a more tolerogenic response. Thus, the aim of this study is two-fold: determine if there are microbes indicative of preterm labor and get a better understanding of initial human intestinal microbial colonization.

**Methods:** Meconium from 47 infants ranging in gestational age from 23–41 weeks was collected and 16S rRNA analyses of the V4 region were performed. Amplicons were sequenced on the IonTorrent PGM platform.

**Results:** Of 16 near full/full term subjects (greater than 33 weeks gestational age), 16S rRNA was detected in only 9 (56.25%); whereas, 16S rRNA was detected in 26/31 (83.87%) of preterm infants (less than 33 weeks gestational age). When compared to other microbiome studies of amniotic fluid, meconium, maternal vaginal and oral locales, and colostrum, a larger proportion of sequences from this meconium study were attributed to genera that were also found in amniotic fluid. Taken together, this suggests that preterm subjects are more likely to encounter microbes in utero, which may trigger a cascade of events implicating preterm birth. Firmicutes and Proteobacteria comprised the majority of sequences, median 32.8% and 26.0%, respectively. Principle component analysis revealed that samples from near full/full term infants clustered together while samples from preterm infants were less similar. Several genera were negatively correlated with gestational age, including *Lactobacillus*.

**Conclusions:** These findings suggest that meconium microbial communities are representative of an *in utero* environment. Microbial colonization is more frequent in preterm meconium samples than near full/full term suggesting that colonization occurs *in utero* and possibly initiates events leading to preterm delivery. In particular, *Lactobacillus* species have been reported to induce IL-6 production (augmented in the

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amniotic fluid of mothers who deliver prematurely) and is frequently detected in the vaginal canal, providing a likely source of colonization in the womb. Quantifying microbial communities without sacrificing breadth of community information remains a major challenge to human microbial ecology. Therefore, the quantitative vs. qualitative degree to which microbial exposure in utero contributes to preterm delivery remains unclear. This information is useful in advancing the understanding of the potential role of the human microbiome in prematurity as well as the establishment of the human microbiome in utero.

### Keywords:

Disease association

Host/microbiome interaction

Ecology

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**P10. M. Kristen Demourelle<sup>1</sup>, Jill M. Norris<sup>2</sup>, V. Michael Holers<sup>1</sup>, J. Kirk Harris<sup>1</sup>, Kevin D. Deane<sup>1</sup>.**

### Lung Microbiome Differs in Asymptomatic Subjects at Elevated Risk for Future Rheumatoid Arthritis Compared to Healthy Controls

<sup>1</sup>University of Colorado School of Medicine, Aurora, CO;

<sup>2</sup>Colorado School of Public Health, Aurora, CO

**Purpose/Hypothesis:** A dysregulated immune response to the lung microbiome initiates mucosal inflammation and autoimmunity in the development of rheumatoid arthritis (RA). **Background/Significance:** RA is an autoimmune disease for which the etiology is unknown; however, there is a “preclinical” period of RA development during which autoantibodies highly specific for RA, including anti-cyclic citrullinated peptide (anti-CCP), are elevated in the circulation years before the onset of clinical signs/symptoms of arthritis. These findings suggest that the RA disease process begins at an extra-articular site. Additionally, emerging data suggest that microorganisms and mucosal inflammation are involved in early RA development (Scher 2012, Mikuls 2012). Our recently published data

demonstrating inflammatory airways disease on imaging in arthritis-free subjects at high risk for future RA, including several that later developed joint inflammation classifiable as RA, suggest that the lung may be a mucosal site where RA begins. Therefore, investigations of the lung microbiome in subjects at-risk for future RA may provide insight into RA pathogenesis.

#### **Microbiomes: Lung.**

**Methods:** The Studies of the Etiology of RA (SERA) is a unique prospectively followed cohort created to study the natural history of RA, from which we selected 13 arthritis-free cases positive for anti-CCP (IgA/IgG ELISA) and 9 healthy controls. Using established protocols, we collected an induced sputum sample (<10 squamous epithelial cells per high-powered field) and performed microbiome analysis with 16S rRNA gene amplification and 454 pyrosequencing. We used barcoded PCR primers to construct multiplexed amplicon pools and assign sequences to the appropriate subject sample. Nonparametric testing was used to compare microbial prevalence and median relative abundance between groups.

**Results:** Cases were older, more frequently male, and more frequently ever smokers, although all cases quit smoking a median 9 years prior to sputa collection. We identified >1,000 sequences per sample (Good's coverage >98.9%) and 80 genera across all samples (mean 30 per sample). In cases v. controls, relative abundance was elevated for *Haemophilus* (2.0% v. 0.6%, p=0.01) and *Streptococcus* (30.4% v. 18.0%, p=0.07), but lower for *Prevotella* (12.4% v. 25%, p=0.07). Other studies have associated *Porphyromonas* with increased RA risk, but we did not find a significant difference in abundance between groups (1.1% v. 1.5%, p=0.43).

**Conclusions:** Herein, we identify differences in lung microbiota between anti-CCP positive cases that are at high-risk for future RA and controls. In our prior work, 9 of these autoantibody positive cases had inflammatory airways disease on lung imaging raising the possibility of a mechanistic link between the lung microbiome, mucosal inflammation and generation of RA-related autoimmunity. Currently, we are finalizing methodologies to detect lung generation of RA-related

autoantibodies, and in future studies we will evaluate the relationship between the microbiome and generation of these autoantibodies within the lung.

**Significance:** If microorganisms causing lung mucosal inflammation is an initiating step in the etiology of RA, future lung-targeted interventions could be applied to treat and prevent RA.

**Major past and future challenges to progress:** The unknown influence of age, sex and smoking on the lung microbiome, and potential contamination of samples by oro-pharyngeal organisms.

**Keywords:**

Disease association

Immune system

Rheumatoid Arthritis

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**P11. Armand E. Dichosa<sup>1</sup>, Michael S. Fitzsimons<sup>2</sup>, Cliff S. Han\*<sup>1</sup>.**

**Dissecting the Human Microbiome Using Gel Microdroplets and Single Cell Genomics**

<sup>1</sup>*Los Alamos National Laboratory, Los Alamos, NM;*

<sup>2</sup>*NuGen Technologies, San Carlos, CA*

The collective bacteria, archaea, and viruses that comprise the many facets of the human microbiome significantly impact the overall health of the host. Equally important to understanding the host-microbe interactions is the microbe-microbe dynamics. Specifically, genetic exchange via natural transformation, transduction, and conjugation allows for inter/intra-species transfer and integration of genetic material, thereby altering the microbe's overall functional activities and fitness through variations of individual genomes. The challenge, therefore, is to obtain multiple, [near] complete genomes from representative cells for in-depth comparative genome analyses. As single cell genomics (SCG) offers rapid, culture-independent means to determine both the taxonomic identity and potential physiology from the genomic perspective, obtaining the complete genome

from an isolated single cell remains elusive. Our prior work has demonstrated that multiple, clonal genomic templates (via whole cells) greatly improve genomic assemblies. However, as a vast majority of cells cannot grow in culture, possibly due to necessary growth signals and/or specific co-microbial influences, obtaining substantial genomic template is impossible. To simultaneously overcome the amplification bias inherent in multiple displacement amplification (MDA) and to broadly enrich for cells under more favorable growth conditions, we hypothesized that capturing single cells in gel microdroplets (GMD; one cell in a GMD), for growth among its native community will yield clonal microcolonies sufficient to achieve complete genomes.

We tested our hypothesis on human oral and gut microbiomes by singly capturing cells in agarose-based GMD spheres (~40 µm) via an emulsion matrix process, for in vitro co-cultivation, respectively, in brain heart infusion media. Because of the porous properties of each GMD, cell-to-cell communication and nutrient exchange occurs during co-cultivation. Thus, single cells form clonal microcolonies conveniently packaged in each GMD, which was amenable for subsequent MDA. Our results yielded near complete genomes from several oral and gut microbes, which provided remarkable insight into intragenomic species variation from the Streptococcus oral inhabitants, which was likely due to homologous recombination, and little genomic variation from the Enterococcus gut inhabitants. Significant regions in the oral Streptococcus genomes were highly conserved and are likely essential to growth under our experimental conditions, while the regions containing more differences were involved with pathogenicity and energy metabolism.

Our findings show how significantly active (or inactive) the members of microbiomes are in recombining specific segments of DNA with each other. Our work also raises questions as to how we identify a "species", since completed genomes provides more taxonomic resolution than the standard 16S rRNA phlotyping. These and other insights about the biology and functional roles bacteria play in their respective microbiomes are revealed from the perspective of

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complete genomes, which could not have been achieved without the use of GMD.

As our demonstration GMD with SCG holds much promise for more intensive studies of the human microbiome, our biggest challenge is to adapt this technology *in situ* (e.g., gut, and other wet environments) as, clearly, we have only just begun to dissect the complex human microbiome and determine their vital contributions to the human host.

### **Keywords:**

Gel microdroplets  
Single cell genomics  
Genomic variations

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**P50. Jennifer M. Fettweis<sup>1</sup>, Gregory A. Buck<sup>2</sup>, .. Vaginal Microbiome Consortium<sup>1</sup>.**

### **Species diversity of the human vaginal Microbiome**

<sup>1</sup>*Virginia Commonwealth University, Richmond, VA;*

<sup>2</sup>*Virginia Commonwealth University, Richmond, VA*

**Purpose/Hypothesis:** The overall goal of the study is to determine how the vaginal microbiome is associated with common physiological conditions, environmental exposures, and relevant infectious diseases. We also hypothesize that the genes of the host contribute to the composition of the vaginal microbiome.

**Background/Significance:** The human vagina is colonized by an array of bacterial taxa that have clear implications for the health of the host. Vaginal dysbiosis can lead to increased risk for sexually transmitted infections, increased risk of acquisition of HIV and other viral diseases, pregnancy complications and other pathological conditions. However, the relationships of the human vaginal microbiome with infectious and physiological states and host genetic factors are still poorly understood.

**Microbiomes:** Our study focuses on the vaginal human microbiome. The majority of samples that we analyze are obtained from the mid-vaginal wall. For a subset of subjects, we also examine the microbiome of the

vaginal introitus, the cervix, the perianal region and the buccal mucosa.

**Methods:** To date, we have collected more than 40,000 samples from more than 6,000 women. We have recruited hundreds of twin pairs into the study, and we are now in the final stage of recruitment for the twin cohort. Moreover, we have analyzed metagenomic rDNA sequences from more than 3,000 samples from the mid-vaginal wall, cervix, introitus, perianal region or mouth. We have sequenced ~80 of these samples by whole metagenomic shotgun sequencing, and we have isolated and sequenced hundreds of bacterial isolates of interest from these samples.

**Results:** A species-level analysis of 16S rRNA data suggests that vaginal microbiome profiles can be classified into major, minor and rare ‘vagotypes’, which are associated with various host correlates. We have characterized the genomes of several species of interest including *Sneathia amnii*, an emerging pathogen implicated in a variety of clinical manifestations including bacterial vaginosis and spontaneous abortion. We have also identified a new uncultivated species that is strongly associated with a sexually transmitted infection. This organism encodes several putative virulence factors of interest. We have used whole metagenome sequences to assemble the complete genome of this uncultivated species and to perform strain-level comparisons. We are also examining other bacterial genomes for pathogenicity determinants, and we are assessing the metabolic potential of entire bacterial communities from select samples.

**Conclusions and significance:** Vaginal microbiome profiles can be classified into ‘vagotypes’, which are correlated with phenotypic data. Moreover, several bacterial species that have yet to be cultivated or characterized have important implications for women’s health.

**Major past and future challenges to progress:** The development and dissemination of new bioinformatics tools arising from biologically driven inquiries into microbiome datasets will help to accelerate the field.

### **Keywords:**

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Reproductive health

Disease association

Vaginal microbiome

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**P12. Lily Frank\***, Rosamond Rhodes.

### **Human Microbiome Research and De Minimis Risk**

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**Purpose:** This Project focuses on developing insight into the ethical, legal, and social implications (ELSI) of human microbiome research. The work presented in this poster specifically focuses on reassessing current research ethics regulations and conceptual foundations from the perspective of human microbiome research.

**Background/Significance:** Human microbiome research often involves the collection of a large number of samples, requires participation from a broad spectrum of participants, and generates tremendous amounts of information. Each kind of research with human participants involves a different degree of risks and burdens, therefore raises different kinds of ethical considerations and calls for different levels of oversight. Considering the existing research ethics regulations from the perspective of microbiome research reveals some limitations of the existing regulations and Institutional Review Board processes.

**Microbiomes:** No samples were taken. This was not a clinical or animal model study.

**Methods:** Our study involved a dialogue among a multi-disciplinary group of 27 academics with the aim of achieving consensus positions on ethical, legal, and social issues related to human microbiome research. This poster presentation represents a position on research risk endorsed by 26 of the study participants.

**Results:** Scientists need to collect data and samples from a broad swath of participants to advance the HMP. Yet, U.S. research regulations have narrowly focused on protecting participants from very small risks and may inhibit some groups' participation in research. We explain these issues and why federal

regulators should transform the Common Rule to accommodate microbiome and genomic research. We propose that research regulations should add a new sub-category of minimal risk, de minimis risk. De minimis risks are the hard to imagine or extremely unlikely risks, including negligible physical risks, in which nothing dangerous is done to the participant's body and there are no foreseen likely or significant social or psychological harms to participants. Most microbiome studies involve only de minimis risk, such as swabbing a participant's cheek to sample their oral microbiome or using discarded blood samples that have been collected in clinical care.

**Conclusions:** Adding de minimis risk as a new category of risk refocuses regulations on the assessment of the risks and benefits of different kinds of studies. This proposal also has the potential to reduce the likelihood of bias in population studies by increasing broad participation. It can reduce obstacles that have inhibited research and that it would strike a sensible balance between advancing science and respecting research participants.

**Significance:** We argue that informed consent should not be the default requirement for studies that involve only de minimis risk. Nor would these studies require a waiver of informed consent from the IRB. It may still be appropriate in some cases to obtain oral agreement or blanket consent from participants when it is feasible and the burdens are reasonable.

**Major past and future challenges to progress:** A future area of inquiry is to consider the nature of the institutional gatekeeper boards that will determine which studies present de minimis risk.

### **Keywords:**

ELSI

Minimal Risk

Research Regulations

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**P13. Paweł Gajer<sup>1</sup>, Bing Ma<sup>1</sup>, Rebecca M. Brotman<sup>1</sup>, Douglas Fadrosh<sup>1</sup>, Ryan D. Michalek<sup>2</sup>, Larry J. Forney<sup>3</sup>, Jacques Ravel<sup>\*1</sup>.**

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### Unraveling interactions between the microbiome and metabolome in the human vagina

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Characterization of the species composition and structure of vaginal microbial communities by classification of 16S rRNA gene sequences has provided insight to changes in the vaginal microbiota that occur in health and disease. However, characterizing the function of the bacteria in the vaginal ecosystem is critical for understanding ecological interactions among community members and the effects these organisms have on the host. Analysis of the metabolome provides chemical fingerprints that reflect specific physiological processes in the microbial populations and the human host. In this study, we explored correlations between the vaginal microbiota and their metabolomes in 36 samples collected from five women representing samples with high ( $>5.5$ ) or low ( $<4.5$ ) pH, which is of interest because an elevated vaginal pH is associated with bacterial vaginosis. Metabolome analysis was performed by coupling both liquid chromatography (LC) and gas chromatography (GC) to mass spectrometry (MS), while the composition and structure of the vaginal microbiota was established by pyrosequencing barcoded 16S rRNA gene fragments (V1-V3). We used the Sparse Correlations for Compositional data (SparCC) algorithm to construct correlations network between metabolites and bacterial phylotypes abundances. Metabolome analysis documented 414 known compounds in all samples, of which 256 differed significantly between experimental groups ( $p \leq 0.05$ ). The concentrations of 68 compounds were higher, while those of 188 were lower. The metabolome profiles reflected differences in the metabolism of glucose and glycogen, the TCA cycle, polyamine production, amino acid catabolism, lipid oxidation, purine degradation, redox homeostasis and inflammation status of the host. The bacterial

composition and structure of these communities was also very distinctive: vaginal samples with low pH were dominated by species of *Lactobacillus*, while those with high pH consisting of diverse strict anaerobes. Correlation profiles between phylotypes and metabolites identified strong relationships between specific bacteria and metabolites. These associations between biochemical signatures, specific phylotypes and clinical symptoms that accompany elevated vaginal pH will be discussed, and these may provide a better understanding of the interaction between the host and the vaginal microbiota.

#### Keywords:

Vaginal microbiota

Metabolomics

Bioinformatics/computational tools

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**P14. Henry J. Haiser**, David B. Gootenberg, Kelly Chatman, Gopal Sirasani, Emily P. Balskus, Peter J. Turnbaugh.

### Predicting and manipulating drug inactivation by the human gut microbiome

*Harvard University, Cambridge, MA*

The trillions of microorganisms in the human gastrointestinal tract are an underexplored aspect of pharmacology. Despite numerous examples of microbial effects on drug efficacy and toxicity, there is often an incomplete understanding of the underlying mechanisms. Previous work has demonstrated that *Escherichia coli*, a common member of the human gastrointestinal tract, is capable of inactivating the widely used cardiac drug, digoxin, through an unknown mechanism. Using a transcriptomic approach, we identified a two-gene cytochrome-encoding operon in *E. coli* that is transcriptionally upregulated in the presence of digoxin. Comparative genomics revealed that the upregulated operon is absent in two non digoxin-inactivating *E. coli* strains. We demonstrate that the abundance of the cytochrome operon in

metagenomic DNA isolated from human fecal samples serves as a predictive biomarker for ex vivo digoxin inactivation. Using culture-based studies we note that digoxin inactivation is enhanced by microbial interactions, while it is inhibited by the amino acid arginine. Pharmacokinetic studies using gnotobiotic mice revealed that increasing dietary protein reduces the in vivo metabolism of digoxin by *E. lenta*, with significant changes to drug concentration in the urine and serum. This work demonstrates the power of combining metagenomics and gnotobiotics to understand the metabolism of clinically relevant drugs by the human gut microbiome. We have established an experimental framework for the mechanistic dissection of microbial drug metabolism that might be widely applied to the >40 known drugs subject to biotransformation, resulting in altered efficacy and toxicity across a wide range of diseases—including cardiac disease, inflammatory bowel disease, and cancer. Our results emphasize that a comprehensive view of pharmacology should include the structure and activity of our resident microbial communities, and a deeper understanding of their interactions with each other; with their host habitat; and with the nutritional milieu of the gastrointestinal tract.

**Keywords:**

Clinical applications  
Biomarkers  
Ecology

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**P15. Mary K. Hamilton<sup>\*</sup><sup>1</sup>, Gaelle Boudry<sup>2</sup>, Helen Raybould<sup>1</sup>.**

**Progressive Increase in Large Intestine Transcellular but not Paracellular Permeability Correlates with Plasma Endotoxemia in Diet-Induced Obese Rats.**

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<sup>2</sup>INRA, St Gilles, France

**Purpose:** The relationship between the gut microbiome and passage of microbial components, such as lipopolysaccharide (LPS), across the gut epithelium with metabolic endotoxemia and the obese phenotype is unclear. We hypothesize that high fat feeding changes the microbiota, increases transcellular permeability and passage of LPS. The aim of the study was to determine the temporal relationship between impaired small or large intestinal permeability with metabolic endotoxemia and the obese phenotype in rodent diet-induced obesity.

**Background:** Obesity is characterized by altered gut microbiota, increased intestinal permeability, increased plasma LPS, low-grade inflammation and metabolic alterations in both humans and rodents. Whether the microbiota shift or increased intestinal permeability leads to the increase in plasma LPS is unclear. Moreover, the contribution of altered paracellular versus transcellular transport in different intestinal regions to the obese phenotype is unknown.

**Microbiomes:** We have shown that high fat diet-induced obese rodents have an increase in the Enterobacteriales order within the cecal contents, consisting primarily of Gram-negative bacteria containing LPS in the outer membrane.

**Methods:** Diet-induced obesity was induced by feeding rats a high fat diet (HF, 45% fat) or normal chow (NC, 10% fat) for 1, 3 or 6 weeks. Intestinal tissues (ileum, cecum, and colon) was mounted in Ussing chambers; flux of horseradish peroxidase (HRP, marker of transcellular permeability) and FITC-dextran 4000 (FD-4, marker of paracellular permeability) was measured to evaluate permeability. LPS-binding protein (LBP) in plasma was measured by ELISA.

**Results:** In the small intestine, FD-4 flux was higher in HF than NC rats at wk1 (ileum p=0.004) then returned to normal values; HRP flux was unaffected by HF diet. In the large intestine, HRP flux was increased in the cecum and colon of HF compared to NC rats at wk3 (p=0.03 and 0.04) and wk6 (p=0.009 and 0.02) but not wk1. Plasma LBP was increased in HF rats at wk3 and

wk6 and correlated significantly with cecal and colonic HRP flux ( $p=0.004$  and  $0.0005$ ), which correlated significantly with adiposity ( $p<0.001$ ).

**Conclusion:** High fat feeding induces a late onset (wk3) increase in transcellular permeability in the large intestine that correlates with plasma LBP and adiposity. These data support the hypothesis that alteration of transcellular but not paracellular transport in the large intestine is involved in metabolic endotoxemia and the obese phenotype in rats.

**Significance:** An understanding of how changes in the gut microbiome and passage of LPS across the gut epithelium lead to changes in homeostatic pathways controlling food intake and body weight will provide new preventative and treatment options for maintenance of a healthy body weight. Moreover, maintenance of gut barrier function may be important in other conditions associated with changes in gut microbiota and increased passage of microbial products.

**Major Challenges:** Studies of changes in the gut microbiota and alteration of homeostatic pathways are largely correlative due to the interconnected ecology of the gut microbiome. Mechanistic studies are now required to provide a better understanding of the interactions between the microbiome and host in complex metabolic diseases.

**Keywords:**

Diet

Host/microbiome interaction

Obesity

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**P16. Peera Hemarajata, Chunxu Gao, Kathryn Pflughoef, Carissa Thomas, Jennifer Spinler, Delphine Saulnier, James Versalovic\*.**

***Lactobacillus reuteri*-mediated Immunomodulation is Controlled by Regulation of Histidine Decarboxylation**

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**Background:** Perturbations of the intestinal microbiota may alter the function of the microbial community and result in increased predisposition to different diseases. Dietary nutrients may be converted into active metabolites by intestinal microbes and may affect regulatory functions in the host. Probiotics may restore the composition of the gut microbiome and introduce beneficial functions to gut microbiota, resulting in amelioration of gut inflammation and other intestinal or systemic diseases. **Microbiome:** Human microbiome-derived *Lactobacillus reuteri* strains potently suppress pro-inflammatory cytokines such as tumor necrosis factor (TNF) by converting the amino acid, L-histidine, to histamine. Histamine suppresses mitogen-activated protein (MAP) kinase activation and cytokine production via histamine receptor 2 (H2) on myeloid cells.

**Hypothesis:** *L. reuteri* ATCC PTA 6475 may contain genetic elements involved in regulation of histamine production.

**Methods and Results:** Two independent strategies were used to identify genes required for *L. reuteri*-mediated immunomodulation. For the gene-targeted approach, we performed comparative transcriptomics analysis of *L. reuteri* 6475 during exponential phase and stationary phase (when TNF-inhibitory factors are produced). Among the markedly upregulated genes, one gene, renamed the putative histidine decarboxylase gene cluster regulator (*phdR*), was a primary regulator of genes involved in histamine biosynthesis. The *phdR* gene is essential for TNF suppression by *L. reuteri* and expression of the histidine decarboxylase (*(hdc)* gene cluster. Inactivation of *phdR* resulted in diminished TNF suppression *in vitro* and reduced anti-inflammatory effects *in vivo* in a trinitrobenzene sulfonic acid (TNBS) mouse model of acute colitis. The *phdR* mutant strain did not suppress colitis and resulted in greater concentrations of serum amyloid A (SAA) in affected animals. Promoter region affected by *phdR* was determined by reporter gene experiments. These studies support the presence of a master regulatory gene, *phdR*, which modulates expression of a gene cluster known to mediate immunoregulation by probiotics at the transcriptional

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level. For the global approach, we identified a proton-chloride antiporter gene (*eriC*) as a genetic element necessary for *L. reuteri*-mediated immunomodulation and histamine production. We developed a single-plasmid *Himar1* transposon mutagenesis system for *L. reuteri* 6475 using the *Himar1* transposon in conjunction with a nisin-inducible *Himar1* transposase. The *eriC* gene was identified through a combination of high-throughput selective screening and whole genome shotgun sequencing transposon mutants. Mutation in *eriC* resulted in *L. reuteri* 6475 no longer being able to suppress TNF production by myeloid cells, a diminished production of histamine, and downregulation of *hdc* genes.

**Conclusion and significance:** We identified novel regulatory mechanisms of histamine production by *L. reuteri*, which is an indigenous member of the normal intestinal microbiota. This research provides a better understanding of the immunomodulatory gene network important for amino acid metabolism in intestinal microbes using *L. reuteri* as a model organism.

**Future challenges:** Further characterization of these regulatory mechanisms could lead to development of probiotics with increased capacity for producing immunomodulatory factors, which may be used to treat chronic inflammatory gastrointestinal diseases. These findings may point the way towards new strategies for controlling gene expression in probiotics by dietary interventions or microbiome manipulation.

### Keywords:

Probiotics  
Host/microbiome interaction  
Amino acid decarboxylation

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**P17. Hannah D. Holscher<sup>1</sup>, J. Gregory Caporaso<sup>2,3</sup>, Jennifer M. Brulc<sup>4</sup>, Kelly S. Swanson<sup>\*5</sup>.**

### Novel Fibers Shift Human Fecal Microbiota in Healthy Adult Males

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*Laboratory, Lemont, IL;* <sup>4</sup>*General Mills Inc., Minneapolis, MN;* <sup>5</sup>*University of Illinois, Urbana, IL*

**Background:** The gastrointestinal microbiome plays an integral role in human health. Microbiome diversity and composition is influenced by a number of factors, most notably, diet. Incorporation of dietary fiber is one way the microbiota can be manipulated for therapeutic potential. The impact of novel fibers on gut microbiota remains under investigated.

**Purpose:** This study aimed to determine the impact of polydextrose (PDX) and soluble corn fiber (SCF) on the human fecal microbiota using shotgun 454 pyrosequencing. We hypothesized that consumption of PDX and SCF would beneficially shift gastrointestinal microbiota compared to no fiber controls (NFC).

**Methods:** Healthy adult males (n=21) were enrolled in a prospective, randomized, double-blind, placebo-controlled crossover trial consisting of three 21-day periods. Participants were randomly assigned to 1 of 3 treatments during each period: 1) NFC; 2) PDX (21 g/d); and 3) SCF (21 g/d). Fresh fecal samples were collected during days 16 to 21 of each period. Following extraction, DNA was subjected to shotgun 454 pyrosequencing. Data were analyzed with QIIME 1.6.0, using the QIIME-IMG 25Oct2012 reference sequence collection for read mapping. Predominant taxa were analyzed by ANOVA, with post-hoc Tukey adjustment to determine treatment effects.

**Results:** Firmicutes was the most abundant phyla (~61% of sequences) followed by Bacteroidetes (~32%), Proteobacteria (~2%), Actinobacteria (~2%), and Verrucomicrobia (~1%). Abundance of Firmicutes was lower (P<0.001) when men consumed PDX (56%) and SCF (55%) than when they consumed NFC (68%). PDX and SCF supplementation also resulted in a greater (P<0.001) proportion of fecal Bacteroidetes (36% and 38%, respectively) than NFC (25%). Among Firmicutes, the Clostridia class constituted ~90% of sequences, being dominated by Ruminococcaceae, Lachnospiraceae, Eubacteriaceae, and Clostridiaceae. Ruminococcaceae, Lachnospiraceae, and Eubacteriaceae decreased (p<0.05) with fiber supplementation. Within Bacteroidetes, the Bacteroidia class constituted ~98% of sequences, being

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dominated by Bacteroidaceae, Porphyromonadaceae, Prevotellaceae, and Rikenellaceae.

Porphyromonadaceae increased ( $p<0.001$ ) with fiber supplementation. Among Actinobacteria, Bifidobacteriaceae and Coriobacteriaceae families dominated. Fecal Coriobacteriaceae was lower ( $p<0.05$ ) with fiber supplementation. Among genera, fiber supplementation resulted in decreased ( $p<0.05$ ) *Eubacterium* and increased ( $p<0.05$ ) *Parabacteroides*.

**Conclusions:** Shotgun 454 pyrosequencing allowed us to discern the impact of fiber supplementation on the Firmicutes:Bacteroidetes ratio in healthy adults.

Elevated Firmicutes:Bacteroidetes has been associated with obesity, with diet-induced weight loss decreasing this ratio. Herein, we demonstrated a beneficial microbial shift within healthy males by adding novel fibers to the diet without caloric restriction.

**Significance:** Understanding the impact of novel fibers on the gut microbiota may allow us to optimize strategies to improve human health. This study has used next generation sequencing to fill gaps in knowledge on the impact of novel fibers on gastrointestinal microbiota.

**Challenges:** A past challenge to testing the impact of fiber on gastrointestinal microbiota was known primer bias and underestimation of certain taxa using 16S rRNA gene-based approaches. The current study utilized shotgun sequencing to obtain a less biased characterization of bacterial populations; however, shotgun sequencing greatly increases computational time and resource allocation over amplicon sequencing, so future studies interested in taxonomy need to use less-biased 16S primers.

### Keywords:

Diet

Clinical applications

Host/microbiome interaction

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**P18. Elaine Y. Hsiao<sup>1</sup>, Sara McBride<sup>1</sup>, Sophia Hsien<sup>1</sup>, Julian Codelli<sup>1</sup>, Janet Chow<sup>1</sup>, Gil Sharon<sup>1</sup>, Sara E. Reisman<sup>1</sup>, Joseph Petrosino<sup>2</sup>, Paul H. Patterson<sup>1</sup>, Sarkis K. Mazmanian<sup>1</sup>.**

### A Commensal Bacterium of the Gut Microbiome Ameliorates Behavioral Abnormalities in A Mouse Model of an Autism Risk Factor

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<sup>2</sup>Baylor College of Medicine, Houston, TX

**Purpose/Hypothesis:** The goal of the study is to explore the potential contribution of gastrointestinal abnormalities to the manifestation of core symptoms of autism spectrum disorder.

**Background/Significance:** While autism is a neurodevelopmental disorder characterized by language and social deficits, recent studies have highlighted striking gastrointestinal abnormalities in subsets of autistic individuals. We use a mouse model of an ASD risk factor, maternal immune activation (MIA), to assess whether offspring, which display core behavioral and neuropathological features of autism, also display ASD-associated GI symptoms. To explore the potential connections between GI problems and the brain and behavior, we test whether postnatal administration of a probiotic influences GI and ASD-related behaviors.

**Microbiomes:** Using a mouse model of an autism risk factor, we assess fecal samples for microbiome analysis.

**Methods:** Pregnant mice are injected with poly(I:C) (to evoke a maternal inflammatory response) or saline on E12.5. Adult poly(I:C) offspring are confirmed to exhibit autism-related behavioral abnormalities and neuropathology. Offspring are fed three doses of probiotic bacteria at weaning. Adult offspring are assessed for a) intestinal barrier integrity by measuring leakage of FITC-dextran through the intestinal epithelium and tight junction expression, b) enteric immune abnormalities by assessing profiles and function of leukocytes derived from the mesenteric lymph nodes, c) GI inflammation by cytokine Luminex array and histology, d) composition of the intestinal microbiota by metagenomic sequencing, e) serum metabolome profiles by GC/LC-MS.

**Results:** MIA offspring display decreased intestinal barrier integrity and corresponding changes in levels of

tight junction proteins. These symptoms are associated with altered expression of colon cytokines and changes in serum metabolite levels, as well as global changes in the composition of the microbiome. Postnatal probiotic treatment ameliorates these GI abnormalities, restores changes in the intestinal microbiome, normalizes certain serum metabolites, and corrects several ASD-related behaviors. Elevating serum levels of a specific microbially-modulated metabolite sufficiently induces some autism-related behavioral abnormalities.

**Conclusions:** In a mouse model of a primary autism risk factor, mice that exhibit neuropathological and behavioral features of autism also display ASD-related gastrointestinal abnormalities, including alterations in the composition of the microbiome. Treatment with a commensal bacterium shifts the microbiome toward that seen in controls, and improves gastrointestinal symptoms and autism-related behaviors. We provide evidence that microbiome-mediated changes in the serum metabolome may underlie the effect of probiotic treatment on behavior.

**Significance:** These studies highlight the importance of the gut-brain axis, where primary manipulations of the intestinal microbiome can influence GI physiology and behavioral performance. The results raise the possibility of testing a probiotic therapy in individuals with autism and co-morbid GI problems. Also, findings of altered serum metabolite profiles in the MIA mouse model raise the possibility of testing particular metabolites as candidate biomarkers for subsets of human ASD.

**Major past and future challenges to progress:** Future challenges include the identification of specific species or communities of the commensal microbiome that elicit a targeted function and the ability to recapitulate community structures in animal models.

**Keywords:**

Host/microbiome interaction  
Probiotics  
Disease association

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**P19. Lukasz Jaroszewski<sup>1,2,3</sup>, Adam Godzik<sup>1,2,3</sup>.**

**Structural characterization of proteins families from human gut microbiome**

<sup>1</sup>*Sanford-Burnham Medical Research Institute, La Jolla, CA;*

<sup>2</sup>*Joint Center for Structural Genomics, La Jolla, CA;*

<sup>3</sup>*UCSD, La Jolla, CA*

Human gut microbiome contains very large number of novel, uncharacterized proteins. Disproportional percentage of them are predicted to be extracellular or secreted, and thus, involved in interactions between bacteria and its environment, including its host. A first step in studying uncharacterized proteins is to group them into families that contain proteins with common evolutionary ancestry and therefore similar functions and structures. Thanks to this, structural characterization of at least one member of a family provides us with a framework to interpret available functional information for all members of the family. In the case no such information is available, analysis of the 3D structure may identify relations between this and other families and directly or indirectly provide hypothesis about function of the proteins from this family.

With a goal of extending knowledge about uncharacterized proteins present in the Human Gut Microbiome (HGM) Joint Center for Structural Genomics focused on first identifying novel protein families and then their structural characterization by means of high throughput crystallography. As of today, JCSG identified over 200 of novel protein families present only (or mostly) in HGM and determined first structural representatives for over 50 of them. Here we present examples of functional insights gained from the analysis of these structures. As hoped, for a large number of these families their three-dimensional structures suggest their functions. One of the largest groups are previously unrecognized metabolic proteins that play a role in binding and/or metabolism of carbohydrates. Another large group are proteins from the lipocalin superfamily involved in binding and transport of small hydrophobic molecules such as steroids, bilins, retinoids, and lipids. However, by far the most interesting group contains protein families

whose structures suggest interaction with the host's immunological system and/or other microorganisms. This group contains predicted adhesins, toxins, proteins related to antibiotic resistance and proteins predicted to interact with human immunity receptors. We present several examples of more detailed functional hypotheses based on structural similarities to known families, predicted active and binding sites together with available information about genomic context and expression data.

**Keywords:**

Protein structure  
Bioinformatics/computational tools  
Host/microbiome interaction

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**P20. Rheinallt M. Jones\***, Andrew S. Neish.

**Role of the Microflora in the Etiology of Gastro-Intestinal Cancer**

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**Purpose/Hypothesis:** We hypothesize that microbially stimulated ROS acts on multiple signaling molecules to transduce the influence of the microbiota on cell cycle and proliferative signaling pathways.

**Background/Significance:** Recent advances have implicated a role for the intestinal luminal microbiota in epithelial cell cycle regulation and stem cell dynamics, as well as suggested a "dysbiosis" of this relationship in the initiation and progression of GI cancers. However, there is a gap in the knowledge concerning a mechanistic understanding of how the commensal microbiota influences these processes. We previously showed that a subset of highly conserved symbiotic bacteria potently stimulates intestinal cells to induce the deliberate enzymatic generation of physiological levels of reactive oxygen species (ROS). It also is well established that physiological generation of low levels of ROS in distinct subcellular domains act as critical second messengers in multiple signaling networks. This is achieved by their ability to reversibly

oxidize low pKa cysteines ("sulfur switches") of specific sensor target proteins. One pathway shown to be modulated in this manner by ROS is WNT/β-catenin signaling, which is critical for ISC proliferation and differentiation. However, the extent to which ROS participate as signaling molecules controlling proliferation and differentiation in the intestine is still an open question.

**Microbiomes:** Our investigations focus on the intestinal microflora of mice and *Drosophila*.

**Methods:** As an innovative approach to testing our hypothesis, we employ knockout murine models, and the genetically tractable *Drosophila* model whose biology can be manipulated to a far greater extent than mammalian models. Low pKa cysteines within proteins within the intestinal tissues of mice and *Drosophila* colonized with lactobacilli were identified using a novel proteomic technique known as Redox Isotope-Coded Affinity Tag (ICAT).

**Results:** Our results show that lactobacilli are potent inducers of endogenous ROS generation, and of ROS-dependent cellular proliferation within intestines of *Drosophila* and mice. Closer examination of gut tissues revealed discrete localization of cellular ROS generation within the intestinal stem cell microenvironment, with low ROS levels detected in stem cells and elevated levels of ROS detected in differentiated cells. Redox I-CAT analysis identified lactobacilli-induced oxidation of cysteine residues in the domain of β-catenin that controls nuclear import/export activity. Furthermore, alterations in the distribution of β-Catenin in the midgut of germ-free *Drosophila*, and in colonic crypts of antibiotic-treated mice we detected.

**Conclusions:** Collectively, these data demonstrate compelling evidence for an essential and dynamic role of commensal gut microbiota in controlling the activity of proteins that regulate intestinal growth and development.

**Significance:** These investigations are significant because identifying the molecular mechanisms of the beneficial influence of the microbiota on stem cell dynamics, which is the next logical step in the research for the development of probiotics for the prevention or treatment of neoplastic disease.

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**Major past and future challenges to progress:** The current investigation is the springboard to attaining the next challenge in the field which is to identify regulatory cysteine residues within proteins which control signaling events that lead to carcinogenic outcomes following altered microbiota or due to host genetic susceptibility.

**Keywords:**

Host/microbiome interaction  
Probiotics  
Reactive Oxygen Species (ROS)  
Evolution

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### A Multi-Omic View of Host-Pathogen-Commensal Interplay in Salmonella-Induced Intestinal Inflammation

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<sup>3</sup>J. Craig Venter Institute, Rockville, MD; <sup>4</sup>J. Craig Venter Institute, Rockville, MD

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The potential for the commensal microbiota to alter infection outcome by influencing host-pathogen interplay is largely unknown. We used a multi-omics “systems” approach, incorporating proteomics, metabolomics, glycomics, and metagenomics, to explore the molecular interplay between the murine host, the pathogen *Salmonella enterica* serovar *Typhimurium* (*S. Typhimurium*), and commensal gut microorganisms during gastroenteritis. The gastrointestinal phase of *S. Typhimurium* infection is often overlooked due to the challenges of establishing infection in the gut of most murine hosts, a process which requires antibiotic pre-treatment to deplete

commensal organisms. However, by utilizing the 129/SvJ mouse model, in which *Salmonella* persists in the gastrointestinal tract without antibiotic pre-treatment, we were able to overcome this obstacle and investigate the complexities of *Salmonella* pathogenesis, microbe-microbe interactions, and microbe-host interactions. We found proteomic evidence that *S. Typhimurium* thrives within the infected mouse gut, inducing an inflammatory response and disrupting the intestinal microbiome. Alteration of the host microbiome population structure was highly correlated with gut environmental changes, including the accumulation of metabolites normally consumed by commensal microbiota. Finally, both proteomic and glycomic evidence suggest *S. Typhimurium* may metabolize increased fucose moieties within the gut during infection. The application of multiple omics measurements to *Salmonella*-induced gastroenteritis provides insights into complex molecular strategies employed during pathogenesis between host, pathogen, and the microbiome.

**Keywords:**

Host/microbiome interaction  
Infection  
Omics Technologies

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**P22. Kaisa M. Kemppainen<sup>1</sup>, Alexandria Ardisson<sup>1</sup>, Austin Davis-Richardson<sup>1</sup>, Jannie Fagen<sup>1</sup>, Kelsey Gano<sup>1</sup>, Luis Leon-Novelo<sup>2</sup>, George Casella<sup>3</sup>, Olli Simell<sup>4</sup>, Anette G. Ziegler<sup>5</sup>, Marian J. Rewers<sup>6</sup>, Åke Lernmark<sup>7</sup>, William Hagopian<sup>8</sup>, JinXiong She<sup>9</sup>, Jeffrey P. Krischer<sup>10</sup>, Beena Akolkar<sup>11</sup>, Desmond Schatz<sup>12</sup>, Mark Atkinson<sup>13</sup>, Eric W. Triplett\*<sup>1</sup>.**

### An Analysis of Gut Microbial Diversity of Non-Autoimmune Subjects Genetically at High-Risk for Type 1 Diabetes

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**Purpose:** The objective of this study was to characterize the gut microbiome of the control population of a large international type 1 diabetes study consortium - the Environmental Determinants of Diabetes in the Young (TEDDY) study and to identify characteristics that define a healthy microbiome.

**Background and Significance:** The human gut microbiome is associated with the development of several autoimmune diseases, including type 1 diabetes (T1D). The incidence of this disease is increasing in many developed countries at a rate that cannot be explained by genetics alone. TEDDY, composed of six study centers Europe and the United States, was formed to identify potential environmental triggers of T1D. Before a thorough analysis of case and control gut microbiomes can be performed, the control gut microbiome must be characterized.

**Methods:** Stool samples were collected monthly from 15 non-autoimmune infants at each study site. High-throughput barcoded sequencing of fecal bacterial 16S

rRNA gene amplicons was performed on the Illumina IIx platform on a total of 1141 samples from 90 children. A custom PANGEA program was used to assign taxonomic groups to sequencing reads. Data analysis was performed using XLSTAT-pro and R statistical software. Shannon diversity index calculations were used to analyze bacterial diversity and heatmaps and correlation analyses were used to determine temporal changes in the gut microbiome. **Results:** Significant site-specific differences exist in the composition and development of gut microbiomes of TEDDY controls. The microbiomes from Sweden and Washington develop as expected based on previous studies, while those from Colorado and Finland are dominated by the genus *Bacteroides*. Despite these differences, the microbiome profiles converge over time and resemble each other by 19 months. Bacterial community diversity differed significantly between all sites across time. A common trend among all sites was the increase in the proportion of butyrate-producing bacteria and a decrease in the proportion of potentially harmful Enterobacteria.

**Conclusions:** This study highlights the great variability in the gut microbial composition and diversity of European and American children but also suggests that TEDDY controls develop an increasingly healthy microbiota over time.

**Significance:** While numerous studies have examined the impact of geographical location on the gut microbiome, our study is the first to consider the differences between European and North American children. Our study also gives new insight into the structure of the gut microbiome of children genetically at high risk for T1D. This study is an important step in utilizing the vast amount of data that will be available soon through the TEDDY study. The underlying differences among control subjects identified in this study will benefit case-control studies in the future. Determining the role of the gut microbiome in T1D development has been hampered by insufficient sample size and lack of methods to determine causality. The complexity of the gut microbiome makes it difficult to determine the role of specific microbes in disease, but large study cohorts and clinical trials will help to characterize these interactions.

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### Keywords:

Ecology  
Bioinformatics/computational tools  
Host/microbiome interaction

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**P23. Vanessa Leone<sup>1</sup>, Edmond Huang<sup>1</sup>, Yunwei Wang<sup>1</sup>, Suzanne Devkota<sup>2</sup>, Elizabeth Zale<sup>1</sup>, Sushila Dalal<sup>1</sup>, Eugene B. Chang<sup>1</sup>.**

### **Antibiotic-induced Gut Microbiota Alters Period-2 Circadian Gene Expression in the Liver**

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The circadian gene network in peripheral tissues has been shown to play a crucial role in driving and maintaining host metabolism. Studies suggest that gut microbiota also play a crucial role in host metabolism. A clear link between circadian gene network, host metabolism, and gut microbiota has yet to be elucidated. Our lab has shown circadian gene expression is altered in the absence of gut microbiota (germ-free; GF). Liver samples from GF mice exhibited significant upregulation of circadian genes Cryptochrome (Cry) 2, Period (Per) 1-3, and D site of albumin promoter binding protein (DBP), while Clock and Bmal-1 expression were downregulated when compared to specific pathogen-free mice (SPF). We then tested whether reducing gut microbes via antibiotic treatment alters circadian clock gene expression, similar to that seen in GF mice. SPF mice were exposed to antibiotic drinking water for 10 days and activity was monitored pre and post-treatment. Mice were then harvested every 4 hours over a 24-hour period and liver was analyzed for circadian gene expression. Cecal contents were collected at the time of sacrifice. DNA was extracted and primers specific to the V3-4 regions of the 16S rRNA gene were used. Data was analyzed using QIIME software. No differences in activity were exhibited between control and antibiotic-treated animals. Analysis of 16s rRNA of cecal contents showed that 10-day antibiotic treatment significantly

reduced overall community diversity relative to that seen in untreated animals. Only Per2 gene expression was significantly downregulated at t16:00 as compared to controls, resulting in a phase shift and reduced amplitude. While the gene expression results in antibiotic-treated mice differ from those seen in GF mice, this data suggests that alterations in gut microbiota may play a role in maintaining circadian gene expression in peripheral tissues, which could have implications in downstream metabolism. Further studies are needed to determine if antibiotic treatment elicits a direct effect on host circadian gene expression through xenobiotic metabolism or alternatively, if the gut microbiota selected via antibiotic treatment could lead to alterations in host circadian gene expression in the liver.

### Keywords:

Host/microbiome interaction  
Antibiotics  
Metabolism

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**P24. Zachery T. Lewis<sup>1</sup>, Sarah G. Totten<sup>2</sup>, Jennifer Smilowitz<sup>3</sup>, Danielle G. Lemay<sup>4</sup>, Karen M. Kalanetra<sup>1</sup>, Mariya Ryazantseva<sup>1</sup>, J. Bruce Greman<sup>3</sup>, Carlito B. Lebrilla<sup>2</sup>, David A. Mills<sup>\*5</sup>.**

### **Maternal FUT2 Polymorphisms Influence the Gut Microbial Communities of Breastfed Infants**

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Select bifidobacteria are known to possess the ability to consume 2' fucosylated glycans, such as the oligosaccharides found in the breast milk of a mother with an active fucosyltransferase 2 gene (FUT2; termed "secretor"). These and other human milk oligosaccharides are hypothesized to selectively enrich bifidobacteria in infants. We sought to determine if infants fed by non-secretor mothers are under-colonized in bifidobacteria due to their lack of 2' fucosylated glycans. We examined the fecal

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communities of a cohort of exclusively breast-fed infants using next-generation sequencing, *Bifidobacterium*-specific qPCR and a bifidobacteria-focused terminal restriction fragment length polymorphism analysis. Metadata collected included the mothers' secretor genotype determined via FUT2 gene analysis and secretor phenotype determined by mass spectrometry on breast-milk samples. The microbiota data indicated that, on average, bifidobacteria are established in infants fed by secretor mothers earlier, more often, and at higher levels than infants fed by non-secretor mothers. Moreover, infants lacking high levels of bifidobacteria in their feces were colonized by *Escherichia* and *Streptococcus*, bacterial genera considered less desirable than bifidobacteria. These results suggest non-secretor status signals a disadvantage for the establishment of a healthy, bifidobacterial-laden, microbiota early in life. In addition, this work provides mechanistic insight into how milk enriches beneficial bacterial populations in infants and reveals translational clues for glycan-based enrichment of bifidobacterial populations in at risk populations--such as premature infants.

### Keywords:

Host/microbiome interaction  
Ecology  
Probiotics

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**P25. Liang Ma<sup>1</sup>, Mikhail A. Karymov<sup>1</sup>, Qichao Pan<sup>2</sup>, Stefano Begolo<sup>1</sup>, James Q. Boedicker<sup>2</sup>, Rustem F. Ismagilov\*<sup>1</sup>.**

### Using Microfluidics for Targeted Cultivation of Human Gut Microbes

<sup>1</sup>California Institute of Technology, Pasadena, CA; <sup>2</sup>The University of Chicago, Chicago, IL

We developed a microfluidic platform for genetically targeted isolation and cultivation of previously “unculturable” bacteria from the human colon. The gut microbiome plays a critical role in maintaining human

health. While metagenomic studies have advanced our knowledge of this microbial community, obtaining pure cultures of these microbes is still crucial for understanding their genomics and physiology.

Microbial targets containing genes of interest are often identified in metagenomic data sets, but are not yet culturable using traditional methods. Thus, we sought to create a technology that enhances the success rate of cultivating microbes and rapidly identifies and isolates targets of interest.

We hypothesized that cultivation and growth of microbes depends on the microenvironment around the cells, and that this environment can be controlled by microfluidic tools. For instance, some microbes grow only in high densities. Confinement of single cells in small volumes allows crucial signals to accumulate and activate high-density-dependent behavior. Other strains flourish only within a particular microbial community. By controlling the strength of interaction among members in a synthetic community, we learned that spatial structure can achieve stable coexistence of interacting species. We used these findings to guide cultivation on microfluidic devices and design a targeted cultivation approach that enables rapid cultivation and genetically targeted isolation of microbes from clinical samples. We implemented this strategy on SlipChip, a handheld microfluidic platform that manipulates thousands of miniaturized droplets in parallel.

Microbial suspensions were obtained from a mucosal biopsy from the colon of a healthy human volunteer, and single cells were stochastically confined on SlipChip and incubated in an anaerobic chamber to allow growth of colonies. The SlipChip was then split to make two copies of the same colony. On one half of the chip, target colonies were identified using PCR assay with primers that target genes of interest based on metagenomic data. Then, the droplet containing the target colony on the other half of the chip was retrieved for a scale-up culture. We validated this method by isolating *Bacteroides vulgatus*, a core gut microbe, from the clinical sample.

We applied these approaches to cultivate microbes from the Human Microbiome Project’s “Most Wanted” list, which contains targets that are identified from

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16S-based metagenomic studies but not yet cultured or sequenced. We focused on cultivating targets from the human gut microbiome. Four healthy human volunteers participated, and we obtained samples from four sites of the colon: the cecum, hepatic flexure, transverse colon, and sigmoid rectum. To maximize performance, we developed high-throughput and multiplexed methods for identifying targets from the list that can be cultured by our approach. We obtained one member from the high priority group on the “Most Wanted” list, and several members from the medium priority group.

This work provides a successful framework for cultivating and isolating previously “unculturable” bacteria from clinical samples. While additional questions remain, such as the precise effects these isolates have on colon health, this method may become useful for isolation of specific beneficial microbes from donor or patient samples for therapeutic purposes.

### Keywords:

Microfluidics  
Cultivation  
Methods

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**P26. Yingfei Ma<sup>1</sup>, Liying Yang<sup>1</sup>, Carlos W. Nossa<sup>2</sup>, Yu Chen<sup>1</sup>, Yongzhao Shao<sup>1</sup>, Michael Poles<sup>1</sup>, Fritz Francois<sup>1</sup>, Morris Traube<sup>1</sup>, Ulas Karaoz<sup>3</sup>, Shibu Yooseph<sup>4</sup>, Patrick S. Yachimski<sup>5</sup>, Eoin L. Brodie<sup>3</sup>, Karen E. Nelson<sup>4</sup>, Zhiheng Pei\*<sup>1,6</sup>.**

### Novel Insights of Human Papillomavirus Infection Revealed by Metagenomic Analysis

<sup>1</sup>New York University, School of Medicine, New York, NY; <sup>2</sup>Rice University, Houston, TX; <sup>3</sup>Lawrence Berkeley National Laboratory, Berkeley, CA; <sup>4</sup>J. Craig Venter Institute, Rockville, MD; <sup>5</sup>Vanderbilt University School of Medicine, Nashville, TN; <sup>6</sup>The Department of Veterans Affairs New York Harbor Healthcare System, New York, NY

**Purpose/Hypothesis:** The objective of this study was to profile human papillomavirus (HPV) exposure or infection through metagenomic analysis of the whole genomic shotgun sequencing data generated from human microbiome projects.

**Background/Significance:** HPV is the cause of a number of benign and malignant neoplasms in humans but knowledge about HPV infections is mainly obtained by assays designed to detect high/low risk HPV types associated with cervical cancer. Metagenomic analysis could provide a broader and less biased insight into HPV infection in organs outside the reproductive system.

**Microbiomes:** Of the 748 samples from 103 healthy human subjects of northern America, 65 were from the vagina of 41 subjects, 118 from the skin of 75 subjects, 411 from the mouth of 90 subjects, and 154 from the gut of 98 subjects, respectively. Esophageal samples were from 50 subjects with normal esophagus (N), reflux esophagitis (RE), Barrett's esophagus (BE), or esophageal adenocarcinoma (EA).

**Methods:** A HPV database was constructed with genomes of 148 prototypes of HPVs downloaded from the database of Papilloma Virus Episteme. A sequence was assigned to HPV and a specific HPV type based on BLASTN and TBLASTX with defined thresholds.

Phylogenetic trees based on L1 genes were constructed.

**Results:** Overall, we detected 109 HPV types plus some that were unclassified. HPV prevalence in this healthy human cohort was 68.9% (71/103). By organ, HPV prevalence was highest in the skin (61.3%), followed by the vagina (41.5%), mouth (30%), and gut (17.3%). HPV types varied with organs in abundance and prevalence. Dominant HPV types accounted for 71% of assigned reads in the vagina (types 34, 53, 45, 52), 74.5% reads in the skin (types 75, 80, 50, 5, 151, 17 and 9), 91% reads in the mouth (types 32 and 144), and 90% reads in the gut (type 47). Most (12/18) high/low risk HPV types were found only in vaginal samples.

Phylogenetically, the HPV types detected in the skin mainly belonged to genera beta and gamma while those in the vagina to genus alpha. The HPV types detected in the mouth and gut were grouped with either the skin or vaginal HPV types. In the esophagus,

HPV sequences were detected in 2 of 21 subjects with BE (type 49) and 1 of 12 subjects with EA (type 21).

**Conclusions:** In conclusion, metagenomic analysis can detect a broader spectrum of HPV types than current PCR-based HPV detection methods. The organ tropism of the HPV types as revealed by the phylogenetic analysis suggests that the high HPV prevalence probably reflects true infection rather than transitory exposure.

**Significance:** HPV infections invisible to current HPV detection methods affect a significant proportion of healthy human subjects. Study of these “invisible” HPV infections may provide new insight into the role of HPV in human diseases as suggested by the detection of HPV in esophageal diseases.

**Major past and future challenges to progress:** A broad spectrum, cost-effective HPV assay is needed to adequately evaluate the role of HPV in human diseases outside of the female reproductive system.

**Keywords:**

HPV

Metagenomic analysis

Human Microbiome

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**P27. Yoshiyuki Mishima<sup>1</sup>, Chang Soo B. Eun<sup>1</sup>, Maureen Bower<sup>2</sup>, Ryan B. Sartor\*<sup>1</sup>.**

**Impact of a simplified human microbiota consortium on a gnotobiotic murine model of colitis and intestinal mucosal homeostasis**

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**Background:** A dysbiosis of intestinal microbial composition is associated with human inflammatory bowel diseases (IBD). However, little is known which of the complex human microbiota preferentially cause/sustain intestinal inflammation in genetically susceptible hosts and the mechanisms of their interaction with the host. Aim: Examine how a simplified human microbiota (SIHUMI) consortium,

composed of 7 human-derived IBD- related enteric bacteria (*E. coli* LF82 (human ileal CD isolate), *Bacteroides vulgatus*, *Enterococcus faecalis* OG1RF, *Ruminococcus gnavus*, *Bifidobacterium infantis*, *Lactobacillus plantarum*, and *Faecalibacterium prausnitzii*), influence murine experimental colitis.

**Hypothesis:** SIHUMI preferentially induce colitis in genetically susceptible mice and each individual bacterial species causes different host immune responses on different genetic backgrounds. In addition, B cells are multi-functional immune cells that decrease the threat of bacterial invasion by antibody-production and antigen-presentation. Recently we reported that B cells regulate T cell activation/differentiation and ameliorate T cell-mediated colitis through IL-10 secretion. Thus, we evaluated B cell IL-10 induction by individual members of SIHUMI.

**Methods:** Germ-free (GF) wild-type (WT) and IL-10<sup>-/-</sup> 129S6/SvEv (129) and C57BL/6 (B6) mice were colonized with SIHUMI and maintained in gnotobiotic conditions. Bacterial quantification of feces, ileal and colonic contents and tissues were performed at different times using 16S rRNA selective quantitative PCR. Colonic segments were scored histologically, and IFN-γ, IL-12p40, and IL-17 levels were measured in supernatants of unstimulated colonic tissue explants and mesenteric lymph node (MLN) cells stimulated by lysates of individual or aggregate bacterial species. In parallel, isolated GF or SPF splenic B cells from IL-10<sup>+/EGFP</sup> reporter B6 mice were cultured with individual SIHUMI lysate for 72 hours. GFP and IL-12p40 were analyzed by intra-cellular staining of flow cytometry (FACS), and supernatant levels of IL-10 and IL-12p40 were assessed by ELISA.

**Results:** Relative bacterial species abundance changed over time and differed between 129 and B6 mice, WT and IL-10<sup>-/-</sup> mice, luminal vs. mucosal, and ileal vs. colonic or fecal samples. SIHUMI induced colitis in all IL-10<sup>-/-</sup> mice with more aggressive colitis and MLN cell activation in 129 than B6 strains. *E. coli* and *R. gnavus* lysates induced predominant ex-vivo MLN TH1 and TH17 responses, even though *E. coli* and *R. gnavus* mucosal concentrations were low. FACS showed that *R. gnavus* preferentially induced higher IL-12p40-

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secreting B cells and relatively fewer IL-10-producing B cells than other bacterial species. B cells from GF mice responded to SHIHUMI stimulation more vigorously than SPF B cells.

**Conclusions:** SIHUMI induced colitis in GF 129 and B6 IL-10<sup>-/-</sup> mice. Relative concentrations of individual SIHUMI species are determined by host genotype, presence of inflammation and anatomical location. Human enteric bacterial species differentially stimulate bacterial antigen-specific Th1 and Th17 immune responses in SIHUMI-colonized gnotobiotic IL-10<sup>-/-</sup> mice independent of luminal and mucosal bacterial concentrations. Our humanized gnotobiotic model with SIHUMI is an important resource for clinically relevant studies to elucidate the mechanisms by which IBD-associated innate immunity genes regulate composition, spatial relationships and function of intestinal microbiota and to investigate the impact of microbial factors on host immune responses and chronic inflammation in IBD.

### Keywords:

Host/microbiome interaction  
Immune system  
Disease association

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**P28. Aimee M. Moore**<sup>1</sup>, Sara Ahmadi<sup>2</sup>, Sanket Patel<sup>2</sup>, Phillip I. Tarr<sup>3</sup>, Barbara B. Warner<sup>1</sup>, Gautam Dantas<sup>\*2</sup>.

### The Pediatric Fecal Resistome Is Established in Early Infancy

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**Purpose/Hypothesis:** To test the hypothesis that amoxicillin exposure alters the development of infant fecal resistomes.

**Background:** Pediatric fecal microbiota harbor antibiotic resistance genes (resistomes). Factors influencing resistome development in early life are unknown.

**Microbiomes:** Fecal microbiomes were sampled in this

study.

**Methods:** Fecal samples were collected from three sets of healthy, vaginally-delivered, formula-fed twins and their mothers, who were enrolled in a large fecal microbiome study. One twin pair was concordant and one was discordant for 10 days of amoxicillin at 8 months; a third pair had no antibiotic exposure. Metagenomic DNA was extracted from maternal stools collected at delivery and twin stools at 1 month, 6-7 months (~30 days after solid food introduction), and 11 months. Fecal DNA was used to construct metagenomic libraries in an Escherichia coli host, which were screened for resistance to 17 antibiotics. Resistance-conferring fragments were PCR-amplified, barcoded, and sequenced (Illumina platform). Sequences were assembled and annotated using the PARFuMS (Parallel Annotation and Reassembly of Functional Metagenomic Selections) computational pipeline.

**Results:** Antibiotic resistance was found in all libraries. There was no difference in resistance phenotype between amoxicillin-exposed and amoxicillin-naïve twins. Infants' resistome phenotypes at later time points exhibited 80-86% concordance with their own baseline samples (resistance or lack thereof measured at both time points) and with the baseline samples of their sibling. Infant resistome concordance with their own baseline samples did not diminish with time. Concordance with maternal samples was 65-75%. This difference in self concordance vs. maternal concordance was statistically significant. Most maternal-infant discordance was attributable to differences in beta-lactam resistance. In every family infants had beta-lactam resistance not found in the mother, primarily to broad-spectrum drugs (meropenem, cefepime, cefotaxime, piperacillin-tazobactam, piperacillin, aztreonam). There was one mother with aztreonam resistance not found in her children. Sequence analysis revealed distinct non-overlapping resistance gene populations in infants vs. their mothers. PCR validation of these findings is ongoing.

**Conclusions:** Although there was no apparent effect of amoxicillin exposure on the resistome, our finding that neonatal fecal resistomes differ from their mothers' at

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1 month of age suggests resistome establishment in the first weeks of life. Infant resistomes were stable over the first year, unlike known patterns of gut microbiome development. Infants harbored broader-spectrum beta-lactam resistance than their mothers, suggesting that contrary to expectation, the maternal resistome may not be the primary determinant of neonatal resistomes. The differences between infant and maternal resistome phenotypes are likely attributable to distinct populations of resistance genes in infants and mothers.

**Significance:** The first weeks of life may represent a critical period for fecal resistome establishment. Understanding modifiable clinical factors influencing resistome establishment could have important public health implications.

**Major Past and Future Challenges to Progress:** Longitudinal analysis of resistome development is complex, and will require development of new computational tools. Larger-scale studies may be necessary to isolate the effects of specific clinical variables on infant resistomes.

### Keywords:

Host/microbiome interaction  
Clinical applications  
Antibiotic resistance

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**P29. Teruaki Nakatsuji<sup>1,2</sup>, Hsin-I Chiang<sup>3</sup>, Shangi B. Jiang<sup>1</sup>, Karsten Zengler<sup>4</sup>, Aimee M. Two<sup>5</sup>, Faiza Shafiq<sup>5</sup>, Tissa Hata<sup>5</sup>, Richard L. Gallo\*<sup>1,2</sup>.**

### The Microbiome Extends to Subepidermal Compartments of Human Skin: Correlation with Skin Barrier Function

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Commensal microbes on the skin surface influence the behavior of cells below the epidermis. We hypothesized that bacteria or their products exist below the surface epithelium and thus permit physical interaction between microbes and dermal cells. To test this, we employed multiple independent staining techniques for bacteria. Gram-staining visualized structures that appeared to be bacteria in locations where they were expected such as the stratum corneum, hair follicles and eccrine glands of normal human facial skin. Using specific antibodies, bacterial antigens, such as *S. epidermidis*, *Pseudomonas spp*, lipoteichionic acid or lipopolysaccharide, were routinely detected outside of appendageal structures identified by keratin 14 staining. To further support immunostaining approaches by another independent technique, we next performed *in situ* hybridization for 16S rRNA with an oligonucleotide probe EUB338. Bacterial 16S rRNA was detectable in dermal adipose tissue. By qPCR with universal 16S rRNA primers and genus- or species-specific primer/probes, bacterial DNAs were consistently detectable within the dermis and dermal adipose of normal human facial skin, obtained with laser-capture microdissection. Pyrosequencing of 16S rRNA gene from dermis and dermal adipose tissue identified bacterial 16S rRNA reflective of a diverse and partially distinct microbial community in each skin compartment. These results show the microbiota extends within the dermis, therefore enabling physical contact between bacteria and various cells below the basement membrane. We further assumed that skin barrier controls the balance between surface and dermal microbe communities. Thus, we explored microbial communities in the dermis of skin disorders associated with skin barrier defect, such as atopic dermatitis (AD). Abundant bacterial 16S rRNA gene was detected by qPCR in the epidermis (146-fold) and dermis (32.2-fold) of lesional AD skin in comparison to those of non-lesional skin. Pyrosequence of 16S rRNA gene revealed altered microbial community in the dermal compartment of lesional skin as well as surface community. Our results suggest that altered microbial communities in the dermal compartments directly

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account for inflammatory reaction of immune systems in AD.

### Keywords:

Host/microbiome interaction  
Atopic dermatitis  
Immune system

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**P30. Daniel J. Nasko<sup>1</sup>, Shawn W. Polson<sup>1</sup>, Bing Ma<sup>2</sup>, Jacques Ravel<sup>2</sup>, K. Eric Wommack\*<sup>3</sup>.**

### Assessing CRISPR Spacer Composition in the Vaginal Microbiome

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Bacterial vaginosis (BV) is a disease that appears to be linked to the ecology of the vaginal microbiome. BV is characterized by sudden elevations in vaginal pH, reductions in Lactobacillus sp., and increases in populations of strict anaerobes within the vaginal microbiome. No single bacterial taxon, or subset of taxa, are unique to the disease state. Viruses are ubiquitous and abundant within microbial communities, including human microbiomes. It is therefore possible that bacteriophage, as bacterial predators, contribute to the underlying changes occurring in the vaginal microbiome during BV. Examining bacterial CRISPR loci, we investigated the possibility of active bacteriophage infection processes prior to, during, and after BV infection. Clustered regularly interspaced short palindromic repeat (CRISPR) loci are an acquired immunity system found in Bacteria and Archaea, functioning to arrest infection from exogenous nucleic acid - namely viruses. The adaptive nature of the CRISPR system, centers on spacers, short nucleotide sequences incorporated into the locus from previously infecting phages. Spacers act to eliminate exogenous nucleic acid through an RNA interference mechanism. Despite increasingly detailed mechanistic information on CRIPSR immunity, the

impact CRISPRs on natural viral communities is largely unknown. In particular, little is known about the identity of phage genes targeted by spacer sequences. Fortunately, the characteristic repeats of the CRISPR locus make it possible to identify spacers within genomes. However, existing CRISPR finding tools performed poorly when applied to microbial metagenome data. In particular, existing tools have high false positive rates, thus, we created CASC (CASC Ain't Simply CRT) a program that predicts and validates CRISPR spacers, drastically reducing the false positive rate from metagenome data.

Shotgun metagenomic, 16S community profiles and metatranscriptomic libraries were constructed from samples before, during and after BV in a sub-set of subjects from a larger study. These data provided information on microbial composition and was the input for CRISPR identification using CASC.

CASC found 4,640 unique spacers in 680,000 contigs. Vaginal metagenomes contained a higher frequency of CRISPR spacers and paired metatranscriptomes showed that spacer transcripts appeared to have been up-regulated in patients experiencing BV. Ninety-eight percent of the unique spacers had a significant hit to a known virus genome, a surprisingly high hit rate. Interestingly, 257 of these spacers hit conserved regions of genes within the Lactobacillus Phage Lv-1 genome. That Lv-1 infects a Lactobacillus host is significant given the prevalence of Lactobacilli within the healthy vaginal microbiome. In particular, several spacers were homologous to conserved regions of the protease-scaffold major head protein, a gene critical in phage capsid assembly. The high proportion of spacer hits implies that this gene is a preferred target for CRISPR-mediated immunity. The extent to which spacers from vaginal bacterial populations target genes critical to the lytic cycle in other phages is unknown, a knowledge gap stemming from the lack of vaginal bacteriophage sequence data. Thus, an important scientific challenge is to gain a better understanding of the genetic content of bacteriophages and other DNA viruses within the vaginal microbiome.

### Keywords:

Ecology

Reproductive health

Bioinformatics/computational tools

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**P31. Julia Oh<sup>1</sup>, Alexandra F. Freeman<sup>2</sup>, NISC Comparative Sequencing Program<sup>3</sup>, Morgan Park<sup>3</sup>, Robert Sokolic<sup>1</sup>, Fabio Candotti<sup>1</sup>, Steven M. Holland<sup>2</sup>, Julia A. Segre<sup>1</sup>, Heidi H. Kong<sup>4</sup>.**

**The Altered Landscape of the Human Skin Microbiome in Patients with Primary Immunodeficiencies**

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**Background/Significance:** Although landmark studies have shown that microbiota activate and educate the immune system, the extent to which the immune system shapes the microbiome and contributes to disease is incompletely characterized. Primary immunodeficiency (PID) patients are vulnerable to recurrent bacterial and fungal infections and provide a unique opportunity to address this issue. To gain insights into the potential influence of host immunity on the composition of the human skin microbiome, we examined alterations of skin microbiomes of three rare monogenic PID populations: Hyper IgE (STAT3 deficient), Wiskott-Aldrich (WAS), & Dederator of Cytokinesis 8 (DOCK8) immunodeficiency syndromes. While the specific immunologic defects differ, a hallmark presentation of all three syndromes is eczema, similar to classical atopic dermatitis (AD).

**Objective:** Our goal was to examine alterations in skin microbial communities in human immunodeficiencies as a basis for understanding the forces exerted by both the ecological niche and immune selection.

**Microbiomes/Methods:** Using 170,167 full-length Sanger, 6,850,352 V1-V3, and 1,283,635 ITS1 sequences, we compared the bacterial and fungal skin microbiome amongst 41 PID, 13 AD patients, and 49 healthy controls at four geographically distinct skin sites covering the major microenvironments (moist, dry, sebaceous, nares) of the skin, representing two

sites of disease predilection, one control site, and one site of pathogen carriage.

**Results:** PID was associated with colonization by microbial species and community structures not previously observed in healthy or AD control skin. Although we identified unique taxa (e.g., Clostridium species, *Serratia marcescens*), these PID skin-associated microbial species remained restricted to phyla typically observed in human microbiomes. Despite the maintenance of a phylum barrier in the human skin notwithstanding the absence of certain immune cell populations, we observed increased ecological permissiveness in the PID skin to altered microbial population structures beyond colonization by unique taxa. We identified decreased body site specificity and temporal stability of the skin microbiomes in PID patients as compared to controls. Disease severity and selected laboratory metadata were positively correlated with prevalence of *Staphylococcus*, *Corynebacterium*, and other less abundant taxa. Elevated fungal diversity and the increased representation of opportunistic *Candida* and *Aspergillus* fungi also supported increased permissiveness of PID skin, suggesting also that the skin may serve as a reservoir for recurrent fungal infections observed in these patients.

**Conclusions/Significance:** This study examines differences in microbial colonization and community stability in PID skin and informs our understanding of host-microbiome interactions. Primary immunodeficient patients' skin is colonized with unusual bacteria and distinct microbial population structures, documenting a bi-directional dialogue between skin commensals and the host organism.

**Major Challenges:** Host studies quantitating bacterial and fungal load remain difficult in the skin, requiring invasive techniques. The extent to which bacterial and fungal populations interact remain unknown. Genetic manipulations in humans to impute directionality to causation are not feasible. Mouse models of STAT3-deficiency or AD remain imperfect analogues for human studies.

**Keywords:**

Skin

## HUMAN MICROBIOME SCIENCE: Vision for the Future

Bethesda, Maryland | July 24 – 26, 2013

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Immune system  
Disease association

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**P32. Matthew C. Ross<sup>1</sup>, Matthew Wong<sup>1</sup>, Ginger Metcalf<sup>2</sup>, Donna Muzny<sup>2</sup>, Richard Gibbs<sup>2</sup>, Richard Lloyd<sup>1</sup>, Alberto Pugilese<sup>3</sup>, Jeffrey Krischer<sup>4</sup>, Joseph Petrosino<sup>1</sup>.**

### Viral Metagenomics for Etiologic Agent Discovery

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Viruses are thought to outnumber bacteria by 10 fold. They inhabit all known ecosystems, including extreme environments. Yet until recently, our understanding of viruses was dependent on culturing them in host cells and isolating enough virions to characterize and manipulate them. Many of the developments in microbiology over the last ten years have been due to our ability to sequence the microbe under study. Advances in NextGen and Third Generation sequencing have enabled the field of virology to expand past cultivatable pathogens and commensals, to identify and characterize novel viruses and those unable to be grown. To date, there are complete reference genomes available for over 2700 viruses representing 84 viral families that are helping drive viral research. There is significant evidence that the gastrointestinal virus community and the bacterial community are interdependent and perturbation in either effect human health. Recent studies have suggested that enteric viruses may influence and even exploit the human microbiota, which aids in viral replication. Directly, viral metagenomics and characterization of communities may provide fingerprints for overall health or disease states. And unlike bacterial community structure, viotypes are retained over time with remarkable genetic stability and intrapersonal diversity. This has a significant impact on identifying causative viral pathogens in disease. For example, a

recent study identified Cardioviruses, a virus group that has been linked to type 1 diabetes (T1D), at high frequencies in south Asian children using metagenomic methods. Moreover, many novel viruses have been discovered and characterized with metagenomics, which has important implications for diseases of unknown etiology.

The work described here represents projects utilizing metagenomics to investigate possible viral etiologies for T1D. These include collaborations with The Environmental Determinants of Diabetes in the Young (TEDDY) and the Network for Pancreatic Organ Donors with Diabetes (nPOD) looking into possible triggers for the development of T1D. Development of T1D is widely recognized to involve both genetic and environmental factors. Among environmental factors, viral infections, particularly with common Human Enterovirus B species (HEV-B), have been closely correlated with T1D. We have elected to utilize high-throughput sequencing (Illumina) of randomly amplified DNA/cDNA to evaluate viral populations in clinical samples. We have shown this method to be sensitive and it is culture-independent. We are beginning to process more than 19,000 samples, made up of both stool and plasma that we are receiving from the TEDDY group. These represent longitudinal sampling of children at high risk for development of T1D. We are also sequencing numerous pancreatic tissue sections from type 1 diabetics suspected of containing virus in collaboration with nPOD.

To aid in analysis we have compiled our own custom viral database consisting of viral and phage sequences downloaded from the NCBI, JCVI, CAMERA and similar repositories. We are mining this sequence data using a combination of BLAST, Bowtie and similar algorithms on both reads and assembled contigs. We hope that the combination of deep sequencing and longitudinal sampling will shed light on relevant microbiome/virome changes that occur during, and may be contributing to, the development of T1D.

#### Keywords:

Diabetes  
Disease association  
Methods

## HUMAN MICROBIOME SCIENCE: Vision for the Future

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**P33. Jyoti Shankar\***<sup>1</sup>, Stephanie Mounaud<sup>1</sup>, Norma V. Solis<sup>2</sup>, Sebastian L. Szpakowski<sup>1</sup>, Hong Liu<sup>2</sup>, Suman Pakala<sup>1</sup>, Lilliana Losada<sup>1</sup>, William C. Nierman<sup>1</sup>, Scott G. Filler<sup>3,2</sup>, Karen E. Nelson<sup>1</sup>.

### Differential Effect of Antibiotics on the Gastrointestinal Bacterial and Fungal Microbiomes and their Influence on Colonization with *Candida*.

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<sup>3</sup>David Geffen School of Medicine, University of California at Los Angeles, Torrance, CA

**Purpose:** The gastrointestinal fungal and bacterial flora interact with each other and with the local host immune response. Our objective was to analyze differences in the effects of antibacterial antibiotics on the bacterial microbiome, cytokine response and local fungal colonization when challenged with *Candida albicans*.

**Background:** Vancomycin and combinations of penicillin, streptomycin and gentamicin (PSG) are some of the most commonly used antibiotics in clinical settings. In immunosuppressed (either treatment-related or pathologically induced) or dysbiotic populations, ordinarily commensal fungal members of the intestinal tract such as *C. albicans* have the potential to become opportunistic pathogens leading to local colonization and systemic infections. It is possible that antibiotic-induced changes in bacterial members occupying the same ecological niche as the fungal agents encourage colonization. Given the increasing prevalence of immunocompromised states in current patient populations, studying factors that promote opportunistic fungal pathogenesis is becoming more relevant than ever before.

**Microbiomes:** We sampled microbiota from three gastrointestinal sites (ileal luminal, ileal mucosal, fecal) in C57BL/6 mice.

**Methods:** Mice were treated orally with either vancomycin or combination PSG, followed by a challenge with *C. albicans* by gavage. To measure the effects of antibiotics and *C. albicans* on the gut immune response, we sampled sections of the ileum to

determine levels of a panel of cytokines and measured *Candida* colony-forming units by culturing fecal samples. We profiled the bacterial microbiome using 454 pyrosequencing of 16S markers. We processed and assigned these 16S sequences to taxonomic categories using a custom pipeline that incorporates mothur and the RDP classifier. To identify microbiome taxa that drive differences between the effects of vancomycin and PSG under challenge with *Candida*, we employed the elastic net regularized logistic regression. This multivariate statistical learning method incorporates k-fold cross-validation to achieve a balance between model complexity and correlational structure within microbiome taxa.

**Results:** Mice treated with either vancomycin or PSG showed a marked reduction in gut Th17 response. While mice treated with vancomycin had only transient GI colonization with *C. albicans*, those treated with PSG showed sustained, high level colonization. Mice treated with vancomycin had increased Parasutterella, Anaeroplasma, and members of Enterobacteriaceae and Firmicutes, whereas those treated with PSG had increased Parabacteroides.

**Conclusions:** Antibiotic-induced differences in the GI microbiome likely influenced susceptibility of the mice to *C. albicans* colonization.

**Significance:** Our experiments suggest that in the long-term, it may be possible to design antibiotic regimens with targeted probiotics that take into account immune status-based predisposition to fungal colonization.

**Past/future challenges:** The initial studies in this field have primarily utilized univariate approaches to compare specific taxa proportions and have used multiple-hypothesis testing frameworks. These have lead to either many false-positives or excessively stringent criteria in the context of high-dimensional datasets. An ongoing challenge in this field is to move towards multivariate, statistical learning techniques that work with noisy data. Another challenge for researchers in the fields of fungal pathogenesis is the sparsity of current ITS databases for fungal taxonomy assignment. Thus assessment of the fungal mycobiome lags behind its bacterial counterpart.

**Keywords:**

Host/microbiome interaction  
Antibiotics  
Methods

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**P34. Baochen Shi<sup>1</sup>, Michaela Chang<sup>2</sup>, John Martin<sup>3</sup>, Makedonka Mitreva<sup>3</sup>, Renate Lux<sup>2</sup>, Perry Klokkevold<sup>2</sup>, Erica Sodergren<sup>3</sup>, George Weinstock<sup>3</sup>, Susan Kinder Haake<sup>2</sup>, Huiying Li\*<sup>1</sup>.**

**Dynamic Changes in the Periodontitis Metagenome**

<sup>1</sup>David Geffen School of Medicine, UCLA, Los Angeles, CA; <sup>2</sup>School of Dentistry and Dental Research Institute, UCLA, Los Angeles, CA; <sup>3</sup>The Genome Institute at Washington University in St. Louis, St Louis, MO

Chronic periodontitis is the widespread local infectious disease mediated by tooth-borne microbial communities. It is prevalent in the US population, affecting 35% or more of dentate adults. Although investigations have correlated specific bacterial species in the periodontal microbiota with states of disease and health, the functional capability encoded in the genomes of the microbes at health and disease states needs to be defined. Given the large diversity and dynamics of the oral microbiota, there is a tremendous amount of genomic information in the periodontal microbiome to be explored. To define microbial factors contributing to periodontal health and disease, we characterized the changes in the subgingival microbiota from periodontitis patients before and after treatment using a metagenomic approach. Twelve systemically healthy adults with chronic periodontitis were sampled longitudinally at two tooth sites on average before and after treatment. In total, 48 subgingival plaque samples were analyzed using whole genome shotgun sequencing (WGS). We analyzed the taxonomic compositions of the subgingival microbiome using two different approaches: 1) based on the microbial 16S/18S rDNA sequences extracted from the WGS data; 2) based on the WGS sequences mapped against a database of microbial reference genomes. The community

compositions inferred from the two methods were consistent with each other. In addition, we analyzed eight samples using traditional 16S rDNA clone library method, which yielded comparable results. Consistent with previous microbiome studies, we found that the periodontal communities within the same individuals were more similar to each other than between individuals, and different sites from the same individuals were more similar at the diseased state than at the resolved state. By comparing the longitudinally paired samples, we found that the diversity of the periodontal microbiome decreased significantly after treatment and the community composition shifted substantially. The genera detected can be clustered into two groups - healthy-associated and disease-associated. The relative abundances of *Streptococcus* and *Actinomyces* were increased after treatment, while the relative abundances of *Porphyromonas*, *Treponema*, *Tannerella* and *Synergistetes* were decreased. These findings are in agreement with previous studies on the microorganisms associated with periodontitis. Additionally, with the advantage of deep sequencing, we revealed potential pathogens that were not discovered previously. Lastly, the large-scale and longitudinal WGS data allowed us to determine the dynamic shifts in the functional potentials of the microbiome by identifying the representative functional groups. The periodontal microbiome at the resolved state was enriched in genes involved in carbohydrate metabolism. In contrast, the microbiome at the diseased state was enriched in genes involved in energy metabolism. By analyzing clinical samples longitudinally, our study revealed the dynamic changes in the periodontal microbiome and their associations with health and disease states at both taxonomic composition level and functional level. Major past challenge in this study was the large amount of WGS sequencing data to be analyzed at the functional level. In our opinion, this will remain to be a challenge in the future, as well as the functional validation and application of the findings from the study in clinical practice.

**Keywords:**

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Disease association

Host/microbiome interaction

Bioinformatics/computational tools

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**P35. Yang Song<sup>1</sup>, Shashank Garg<sup>2</sup>, Cynthia Maddox<sup>1</sup>, Mohit Girotra<sup>2</sup>, Anand Dutta<sup>2</sup>, W. Florian Fricke<sup>1</sup>.**

### **Microbiota Changes and Colonization During Treatment of Clostridium Difficile Infection With Fecal Microbiota Transplantation**

<sup>1</sup>Institute for Genome Sciences, Baltimore, MD; <sup>2</sup>Sinai Hospital of Baltimore, Baltimore, MD

*Clostridium difficile* infection (CDI) is associated with increasing clinical and economic burdens. Fecal microbiota transplantation (FMT) has been used in multiple small studies to treat CDI with success rates of >95%. However, short- and long-term effects of FMT on the CDI patient microbiota remain a concern, as increasing evidence points at a role of the gut microbiota for various inflammatory and metabolic health problems, including inflammatory bowel diseases and obesity. Furthermore, the mechanism of action for FMT remains largely unknown, hampering the development of alternative treatments, e.g. with controlled, in vitro-assembled mock microbiota. Using a subset of 14 patient/donor pairs from 25 cases of CDI patients successfully treated with FMT at Sinai Hospital (Baltimore, MD), we performed fecal microbiota analysis by 16S rRNA gene amplicon sequencing to shed light on two clinically important questions: (i) What are the characteristic microbiota changes associated with CDI and FMT treatment? (ii) Does FMT lead to stable colonization of the transplanted microbiota in the patient? Using the automated CloVR\_16S analysis pipeline we identified significant microbiota changes within the phylum Firmicutes associated with CDI, namely a shift from *Streptococcaceae* or *Lactobacillaceae* (class: Bacilli) in CDI patients to *Lachnospiraceae* (class: Clostridia) in donors and post-FMT CDI patients. The abundance ratio between *Streptococcaceae* and *Lachnospiraceae*

shifted from 6:1 to 1:2 between pre-FMT and post-FMT samples, compared to 1:17 in healthy donor samples. The mean relative abundance of *Lachnospiraceae* significantly increased from 3.8% to 30% within 2 weeks after FMT and remained stable afterwards. In several cases individual donor species (i.e. operational taxonomic units; OTUs), which were not present in pre-FMT patient or any sample from other patient/donor pairs, were identified in post-FMT patient samples up to two months later, suggesting at least occasional and transient integration of transplanted microbiota members in CDI patients. Overall, our results provide evidence that the balance of potentially pathogenic (*Streptococcaceae*) and beneficial (*Lachnospiraceae*) members of the Firmicutes may be critical for determining CDI-associated disease and risk states. Future work addressing taxonomic and functional microbiota changes on the individual strain level, using metagenomic and metatranscriptomic deep sequencing, will be needed in order to confirm and expand the presented results.

#### **Keywords:**

Host/microbiome interaction  
Clinical applications  
Disease association

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**P36. Karla M. Stucker<sup>1</sup>, Martin N. Nyaga<sup>2</sup>, Manolito Torralba<sup>3</sup>, Mapaseka L. Seheri<sup>2</sup>, Asmik Akopov<sup>1</sup>, Nadia Fedorova<sup>1</sup>, Rebecca A. Halpin<sup>1</sup>, Timothy B. Stockwell<sup>4</sup>, Nonkululeko B. Magagula<sup>2</sup>, Ina Peenze<sup>2</sup>, Philip Venter<sup>5</sup>, David E. Wentworth<sup>1</sup>, M. Jeffrey Mphahlele\*<sup>2</sup>, Karen E. Nelson<sup>3</sup>.**

### **Sequencing the Enteric Microbiome to Understand Pediatric Diarrheal Disease in South Africa**

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*<sup>5</sup>Department of Medical Sciences, University of Limpopo, Sovenga, Polokwane, South Africa*

**Purpose/Hypothesis:** This study aims to advance our understanding of pediatric diarrheal diseases in Africa by using metagenomic approaches to evaluate enteric community relationships among rotaviruses, other diarrheal pathogens, and commensal microbiota. We hypothesize that rotavirus infection has a direct impact on the gut microbiome and that the rotaviral genotype is likely to correlate with specific alteration of the host microbiota. We also anticipate that the rotaviral vaccination campaign in South Africa has altered pediatric enteric microbial communities.

**Background/Significance:** The WHO estimates that 1.5 million children under 5 years of age die annually from diarrheal diseases, with the majority of these deaths occurring in developing countries. Rotaviruses from multiple genotypes contribute to roughly 500,000 pediatric deaths annually. This study uses microbiome/metagenomic approaches to advance our understanding of the enteric microbial populations that contribute to pediatric diarrhea in sub-Saharan Africa.

**Microbiomes:** Ultimately, this study will analyze the microbiomes and rotaviral diversity of ~300 archival stool samples collected between 1998 and 2010 from children under the age of 5 years, spanning pre- and post-rotaviral-vaccination periods in several African countries. Here, we present data from an initial 20 samples where we have completed rotaviral genome sequencing and partial 16S ribosomal DNA (rDNA) gene sequencing for microbiome analysis.

**Methods:** Bacterial microbiomes were profiled using 454 pyrosequencing of a 16S rDNA marker, and segmented double-stranded RNA rotaviral genomes were amplified as full-segment amplicons and sequenced using multiple next-generation sequencing technologies. Rotaviral genomes were assembled using CLC Bio reference-based assembly software and genotypes were assigned using RotaCv2.0 software. The 16S rDNA gene sequences were processed through a JCVI in-house taxonomic-assignment pipeline that employs MOTHUR and the RDP classifier. Analyses of

the 16S data were performed at multiple taxonomic levels, and the Wilcoxon-rank-sum test was used to test for differences in the distribution of operational taxonomic units between samples grouped by rotaviral genotype and vaccination status.

**Results:** The samples show an increased proportion of Burkholderiaceae and Enterococcaceae for non-G1P[8] rotaviral genotypes and a higher proportion of Pseudomonadaceae for G1P[8] samples. Among G1P[8]-positive samples, there is a trend for a higher proportion of Enterobacteriaceae from vaccinated individuals and an increased proportion of Streptococcaceae from non-vaccinated individuals.

**Conclusions:** Our sequence analyses of 20 specimens suggest that enteric bacterial communities vary by rotaviral genotype and may be changing with the introduction of rotaviral vaccines in South Africa.

**Significance:** These preliminary data demonstrate the need for a deeper understanding of the complex interactions among enteric microbial populations in cases of pediatric diarrhea. This study will advance our knowledge in this area by analyzing samples from sub-Saharan Africa, a region where childhood mortality due to diarrheal diseases remains high.

### **Major past and future challenges to progress:**

Challenges include 1) determining statistically appropriate analysis methods for comparing two host microbiome populations, 2) appropriately interpreting data from small study sample sizes, 3) capturing and identifying the true metagenomic population in a sample, including low abundance species, and 4) determining when the presence of a microbial genome reflects a functional role in host health and disease.

### **Keywords:**

Disease association

Vaccine

Evolution

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**P37. Casey M. Theriot<sup>1</sup>, Mark Koenigsknecht<sup>2</sup>, Paul Carlson, Jr.<sup>2</sup>, Gabrielle Hatton<sup>1</sup>, Adam Nelson<sup>1</sup>, Bo Li<sup>3</sup>, Gary Huffnagle<sup>1</sup>, Jun Li<sup>3</sup>, Vincent Young<sup>1</sup>.**

**Antibiotic-mediated Shifts in the Gut Microbiome and Metabolome Leads to Susceptibility to Clostridium difficile Infection**

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**Background:** *Clostridium difficile* is the leading cause of antibiotic-associated colitis worldwide. Antibiotics disrupt the indigenous gastrointestinal microbiota, reducing intrinsic resistance to *C. difficile* colonization. The colonic microbiota plays a major functional role in supporting colonic health. We sought to define the structural and functional changes that are associated with decreased colonization resistance against *C. difficile* in the murine gut microbiome and metabolome.

**Methods:** C57BL/6 mice were treated with the broad-spectrum antibiotic cefoperazone and tested for susceptibility to *C. difficile* infection (CDI) at different time points, before antibiotic treatment and at 2 days and 6 weeks following antibiotic treatment. To define the gastrointestinal tract environment, cecal content from mice that were susceptible and resistant to *C. difficile* colonization were analyzed by 454-pyrosequencing and mass spectrometry (GC- and LC-MS).

**Results:** The intestine of antibiotic-treated mice susceptible to *C. difficile* infection was characterized by a change in the gut microbial community composition accompanied by dramatic shifts in the gut metabolome. Levels of free fatty acids, secondary bile acids and dipeptides were decreased while glucose utilization and availability of sugars increased, which reflects the diminished metabolic activity of the gut microbiome. Targeted functional analysis demonstrated that *C. difficile* can utilize many of the metabolites that increased in the murine gut after antibiotics, including the primary bile acid taurocholate for germination, and carbon sources mannitol, fructose, sorbitol, raffinose and stachyose for growth. By determining whether the intestinal ecosystem

would support the colonization of *C. difficile*, we determined that at least two distinct community structures had complete colonization resistance against the pathogen. Although these two resistant community structures had observable differences with regards to the total metabolome, they were indistinguishable with respect to primary and secondary bile acids, carbohydrates, and fatty acids.

**Conclusions:** This study indicates that multiple community structures of the microbiome can share a similar function, as suggested that individuals may possess one of several enterotypes, distinguished by dominant microbiome members, but all have normal gastrointestinal health. We determined that the ability to metabolize bile acids and ferment carbohydrates into SCFAs could be carried out both by a community with an equal abundance of Firmicutes and Bacteroidetes as well as one that dominated by Firmicutes alone. These results underscore the importance of looking beyond microbiome community structure and measuring functional aspects when determining the relationship between the microbiome and human health and disease. Our in vitro data further supports the concept that the changes immediately following antibiotic administration were sufficient to substantially induce germination and growth, thus could act as a key mechanistic link between antibiotic use and *C. difficile* susceptibility.

**Keywords:**

Gastrointestinal tract

Metabolome

Disease association

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**P38. Michal Toborek<sup>1</sup>, Jeong June Choi<sup>1</sup>, Sung Yong Eum<sup>1</sup>, Evadnie Rampersaud<sup>2</sup>, Maria T. Abreu<sup>2</sup>, Sylvia Daunert<sup>1</sup>.**

**Exercise Attenuates Changes in the Gut Microbiome Induced by Polychlorinated Biphenyls**

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<sup>2</sup>*University of Miami School of Medicine, Miami, FL*

**Purpose/hypothesis:** The present study was designed based on the recent evidence implicating the role of the gut microbiome in risk assessment of environmental chemicals. We examined whether exposure to polychlorinated biphenyls (PCBs) affects the abundance and composition of the gut microbiome. In addition, there is an emerging interest in the role of behavioral factors in modulating toxicity of environmental pollutants. While the role of nutrition has been explored, the impact of exercise on the health effects of toxicants is not known. Because exercise can influence the outcomes of disorders known to be associated with alterations of the gut microbiome, we hypothesized that physical activity may affect the composition of the gut microbiota and thus influence the impact of environmental toxicants.

**Background/Significance:** The gut microbiome is a dynamic bacterial community that interacts with the host and closely relates to human health by regulating energy metabolism and immune functions. Despite its diverse effects on human health, the influence of microbiota on the toxicity of environmental pollutants and its role in risk assessment are largely unknown. It was recently suggested that preabsorptive metabolism can modify toxicity of environmental pollutants, influencing their health effects. The most compelling evidence illustrating this phenomenon was obtained in studies on biotransformation of heavy metals by the gut microbiome. However, the effects of microbiota in modulation of toxicity of persistent organic pollutants, such as PCBs, are unknown.

**Microbiomes:** We studied the gut microbiome of C57BL/6 mice.

**Methods:** Mice exercised voluntarily for 5 weeks, followed by the exposure to a mixture of

environmentally relevant PCB congeners (PCB153, PCB138 and PCB180; total PCB dose, 150 µmol/kg) for 48 h. The microbiome was assessed by determination of 16S rRNA.

**Results:** Oral exposure to PCBs significantly altered the abundance of the gut microbiome in mice primarily by decreasing the levels of Proteobacteria. The activity level correlated with a substantial shift in abundance, biodiversity, and microbiome composition.

Importantly, exercise attenuated PCB-induced changes in the gut microbiome.

**Conclusions and significance:** This study provides the first evidence that oral exposure to PCBs can induce substantial changes in the gut microbiome, which may then influence their systemic toxicity. Importantly, these changes can be attenuated by behavioral factors, such as voluntary exercise.

**Major past and future challenges to progress:** The major challenges resulting from this study is to evaluate the host-microbiome interactions. Of special interest is how the behavioral factors of the host (namely, exercise) can influence the abundance and taxonomic diversity of the gut bacteria. Regarding our data on the effect of PCBs, a fascinating problem is the role of the microbiota in risk assessment.

**Keywords:**

Host/microbiome interaction

Environmental toxicants

Exercise

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**P39. Luke K. Ursell**, Adam Robins-Pianka, Will Van Treuren, William A. Walters, Gaddy Bergmann, Noah Fierer, Rob Knight, \*

**Microbial Communities of the Human Gastrointestinal Tract Demonstrate Individualized Responses to Low Molecular Weight Substrate Perturbations**

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**Background:** The microbial communities of the human gastrointestinal tract (GIT) are powerful determinants of health, and understanding how these communities may shift in response to changes in dietary and pharmaceutical intake is important for designing new therapeutic treatments. It has been previously shown in soils that shared bacterial groups (e.g. Betaproteobacteria) increase in abundance across different soil types in response to substrate addition (1). However, the GIT of humans demonstrates high interpersonal variation in terms of community membership and structure, and it is currently unknown whether these highly varied communities will share patterns of community dynamics.

**Hypothesis/Microbiomes:** We sought to address whether similar bacterial groups from different human fecal samples show concordance in response to substrate addition as is common in soils.

**Methods:** Four human fecal samples were incubated with ten different substrates or water controls at room temperature for 24 hours. After incubation, 16S rRNA was extracted, sequenced, and processed in QIIME. The sequences were also compared against the Integrated Microbial Genes database at 99% identity, and the KEGG Orthology groups and pathways of the hits were identified.

**Results:** Fecal samples obtained from humans did not demonstrate concordance in changes at the community level with the addition of low molecular weight substrates, including amylopectin, casein, chitin, citric acid, DNA, glucosamine, glucose, glutamine, glycogen, and oleic acid. Beta diversity analysis revealed that control groups from one individual could be indistinguishable from the

treatment group of others. Also, the samples do not converge towards a shared microbial community when receiving the same substrate. In PCoA space the magnitude of change between water controls and treatment communities is similar across individuals, suggesting that there might be an upper limit to the change the community can tolerate on these treatments. Analysis of shifts in individual taxa showed that some substrates, including amylopectin, glucose, and glycogen, significantly altered the community composition at the phylum level when compared against water controls. However, chitin resulted in significant changes at the species level, and not at higher taxonomic levels. KO analysis revealed that some substrates significantly changed pathways at Level 1, while others changed at the most specific levels. Interestingly, beta diversity plots on pathways, using Bray-Curtis, Pearson, or Euclidean distance metrics, revealed that the interpersonal differences found in the 16S data could only be recaptured at Level 4 pathways.

**Conclusions:** Our results show that microbial communities of distinct GITs behave individually in response to substrate addition. The universal response of specific bacterial groups, as seen in soils, is not recaptured.

**Significance:** Changes seen in one individual in response to a pharmaceutical treatment are likely not applicable to other individuals. This observation, which is pertinent to all researchers studying human microbial communities, underscores the importance of personalized culture collections for testing individualized responses to treatments.

**Major Challenges:** The major challenges moving forward with human microbial studies include assessing the taxonomic, functional, and community-wide factors that control community dynamics and metabolic responses to perturbation resulting from natural dietary inputs or xenobiotic drugs.

**Keywords:**

Host/microbiome interaction  
Ecology  
Clinical applications

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**P40. Arvind Venkataraman, Thomas Schmidt\***

**Niche vs. Neutrality in microbial communities**

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Managing or restoring microbiomes can be guided by delineating species present as a result of active environmental selection (niche-driven) and dispersal and random growth/loss processes (neutrally distributed). However, discriminating the relative contributions of selection and neutrality is challenging. Here, we adapted a neutral model of community ecology to identify the “environmentally-selected” and “neutrally-distributed” species in two diverse microbiomes - healthy human lungs and soils. 16S surveys from the oral cavity, upper respiratory tract, bronchoalveolar lavages, and soils were curated using mothur. Resulting operational taxonomic units (OTUs; 97% similarity) were analyzed with our adaptation of a neutral model with custom scripts in the R-language.

We benchmarked our neutral model by testing it with soil microbiomes where there were two well-defined expectations based on current studies. First, the agricultural soil microbiome would not appear to be derived from forest soils via neutral processes. Second, as the microbiome recovers from a press disturbance (i.e., long-term agriculture), the neutral model will transition from being a poor fit (agriculture vs. deciduous forest) to being a good fit (successional vs. deciduous forests). Indeed, the model conformed to both these expectations.

Subsequently, the neutral model was used to interrogate the lung microbiome in healthy humans in two steps: i) test whether dispersal from the upper respiratory tract rather than active environmental selection, could explain the microbial community composition in the lungs, and ii) identify bacterial groups that deviate significantly from the predictions of a neutral distribution, since these are likely the niche-driven members of the lung microbiome. The neutral model considering the mouth as a source of microbes explained much of the lung microbial community (Spearman’s rank correlation coefficient

between model prediction and empirical observation = 0.84,  $p < 0.01$ ) suggesting that stochastic dispersal from the mouth is largely responsible for the lung microbial community composition. However, importantly we also identified OTUs that were disproportionately represented in the lung compared to the neutral model predictions. For example, representatives of *Ralstonia* sp., and *Methylobacterium* sp., appear to be enriched in the lungs of healthy humans. Our results provide a methodology to describe the “neutrally-distributed” and “environmentally-selected” species in a microbiome. We anticipate that such a description will facilitate monitoring and restoring microbiomes. For example, our identification of certain bacterial groups as being niche/neutrally distributed in healthy human lungs can be used to supervise the lung microbiome (e.g., in lung transplants) and as a baseline for comparing diseased lungs (e.g., chronic obstructive pulmonary disorder, cystic fibrosis).

Despite its widespread recognition, studies describing use of the neutral model towards microbiome management are lacking. Identifying factors responsible for niche creation and neutral distribution of species in microbiomes is the next challenge. This could be achieved by applying the concept of non-synonymous to synonymous mutations ( $dN/dS$ ) to shotgun metagenomes. A  $dN/dS \approx 1$  indicates no selective pressure (i.e., functional equivalence or neutrality with respect to these genes), whereas a value  $< 1$  is evidence of purifying selection, and a  $dN/dS > 1$  suggests positive selection (both indicating niche creation and maintenance).

**Keywords:**

Ecology  
Host/microbiome interaction  
Methods

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**P41. Marius Vital<sup>1</sup>, Qiong Wang<sup>1</sup>, Adina Chuang-Howe<sup>2</sup>, Christopher R. Penton<sup>1</sup>, Vincent Young<sup>3</sup>, Dionysios Antonopoulos<sup>2</sup>, Thomas M. Schmidt<sup>1</sup>, Mitchell L. Sogin<sup>4</sup>, Hilary G. Morrison<sup>4</sup>, Laura H. Raffals<sup>5</sup>, Eugene B. Chang<sup>6</sup>, James Cole<sup>1</sup>, James M. Tiedje\*<sup>1</sup>.**

### **Investigating the role of butyrate-producing bacterial communities in the development of Ulcerative Colitis**

<sup>1</sup>*Michigan State University, East Lansing, MI;* <sup>2</sup>*Argonne National Laboratory, Argonne, IL;* <sup>3</sup>*University of Michigan, Ann Arbor, MI;* <sup>4</sup>*Marine Biological Laboratory, Woods Hole, MA;* <sup>5</sup>*Mayo Clinic, Rochester, MN;* <sup>6</sup>*University of Chicago Medical Center, Chicago, IL*

Ulcerative colitis (UC) is a chronic inflammatory disorder affecting the colon. Imbalances of the microbiota are believed to be an important environmental risk factor contributing to this disease. Because it is difficult to capture individuals just before the onset of UC, it has been challenging to identify causative associations between the microbial flora and development of disease. Pouchitis, an inflammatory condition in UC patients who underwent total proctocolectomy with ileal pouch anal anastomosis (IPAA), is thought to mirror UC pathogenesis and, hence, provides an opportunity to establish direct influences of the microbiota for UC development. In the healthy colon, beneficial butyrate-producing bacteria have been found to be present at high concentrations (10 % - 20 %), which promotes epithelial integrity by providing the primary energy source for colonocytes. It is postulated that dysbiosis of these bacteria plays a key role in the development of UC. Since butyrate producers form a functional rather than a monophyletic group, accurate detection based on conventional taxonomic markers such as 16S rRNA genes has been limited. We therefore developed methods using 454 pyrotag sequencing and quantitative PCR (qPCR) that directly target the two terminal genes in the main pathways for butyrate synthesis: butyryl-CoA:acetate CoA-transferase (but) and butyrate kinase (buk). Furthermore, for metagenomic analysis, a gene catalogue consisting of 22 genes comprising all known butyrate-producing pathways was established from publicly available

sequenced genomes. In an initial study we investigated the establishment of butyrate-producing communities in the pouch of selected IPAA patients over a two-month period. We found that communities were distinct between patients and the results further indicate that the formation of a 'healthy-type' butyrate-producing community characterized by abundant but genes mainly linked to *Roseburia* sp. / *Eubacterium rectale* and *Faecalibacterium prausnitzii* is one key to preventing the development of pouchitis. qPCR analysis from a broad follow-up study, where 16 IPAA patients (8 developed pouchitis) were sampled over a two-year period, supports our initial findings. 454 pyrotag sequencing as well as metagenomic analysis for those samples is in progress. Future directions include the integration of (meta)genomic information with additional global analysis tools such as transcriptomics/proteomics and ecosystem parameters (e.g. pH, nutrient availability) in order to reveal specific key markers enabling accurate and fast predictions on functional activity (butyrate synthesis) of microbiomes.

#### **Keywords:**

Disease association  
Ecology  
Methods

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**P42. Anastasia N. Vlasova\*, Kuldeep S. Chattha, Sukumar Kandasamy, Zhe Liu, Malak Esseili, Gireesh Rajashekara, Linda J. Saif\*.**

### **Lactobacilli and Bifidobacteria promote immune homeostasis and modulate innate immune responses to human rotavirus vaccine and challenge in neonatal gnotobiotic pigs**

*The Ohio State University, Wooster, OH*

**Goal:** To determine if/how probiotic colonization modulates innate immunity to enteric vaccines and infections, thereby alleviating diarrhea.

**Background:** Human rotavirus (HRV) is a leading cause

of childhood diarrhea globally. Because current attenuated HRV (AttHRV) oral vaccines lack efficacy in impoverished countries, alternative affordable strategies are critical to reduce diarrheal disease. Probiotics/commensals promote immunomaturation conferring beneficial health effects through undefined mechanisms. Neonatal gnotobiotic (Gn) pigs are outbred like humans; they resemble infants in their physiology, anatomy and development of mucosal immunity; and they are microbially sterile and susceptible to HRV diarrhea.

**Microbiomes:** Gram-positive commensal microbes, lactobacilli and bifidobacteria are dominant species in the gut of breastfed infants.

**Methods:** Effects of co-colonization with Lactobacillus rhamnosus GG (LGG) and Bifidobacterium lactis Bb12 (Bb12) on vaccination with AttHRV and challenge with virulent HRV (VirHRV) were assessed in 4 groups of Gn piglets: Vac+Pro (vaccinated/colonized), Vac (vaccinated), Pro (colonized) and Control (non-colonized, non-vaccinated). Subsets of piglets were euthanized pre- and post-VirHRV challenge to assess diarrhea, fecal HRV shedding and dendritic cell/innate immune responses.

**Results:** Post-challenge, Vac+Pro and Vac groups were completely protected from diarrhea; protection rates against HRV shedding were 100% and 83%, respectively. Diarrhea and HRV shedding were reduced in Pro compared to Control pigs following VirHRV challenge. Diarrhea scores and virus shedding were significantly higher in Controls, compared to all other groups, coincident with significantly higher serum interferon-alpha levels post-challenge. LGG and Bb12 colonization ± vaccine promoted immunomaturation as reflected by increased frequencies of CD4, SWC3a, CD11R1, MHCII expressing mononuclear cells (MNCs) and conventional dendritic cells in intestinal tissues and blood post-challenge. Toll-like receptors (TLR) recognize specific microbial associated molecular patterns and play a multifaceted role in induction of innate immune responses. TLR2 and TLR4 recognize bacterial peptidoglycans and lipopolysaccharides, respectively. Colonization decreased frequencies of TLR2 and TLR4 expressing MNCs from vaccinated pigs (Vac+Pro) pre-challenge and increased frequencies of

TLR3 expressing MNCs from Pro pigs post-challenge indicating that probiotics exert anti-inflammatory (TLR2 and 4 down-regulation) and antiviral (TLR3 up-regulation by HRV dsRNA) actions via TLR signaling. Colonization of unvaccinated pigs (Pro) increased frequencies of intestinal and systemic apoptotic MNCs pre-challenge, thereby regulating immune hyperreactivity and tolerance. However, frequencies were decreased in intestinal and systemic tissues post-challenge, moderating HRV-induced apoptosis. Additionally, post-challenge, Vac+Pro and Pro groups had significantly decreased MNC proliferation, suggesting that probiotics control excessive lymphoproliferative reactions upon VirHRV challenge.

**Conclusions:** In Gn pigs, selected probiotics contribute to immunomaturation, regulate immune homeostasis and modulate vaccine and virulent HRV effects, thereby moderating diarrhea.

**Significance:** Probiotics moderate HRV diarrhea, likely through activation of immunoregulatory mechanisms. They contribute to maturation of the neonatal immune system to enhance immunity to HRV vaccine.

**Major past and future challenges to progress:**

Probiotic effects vary with species, strains, doses and interactions with one another or host microflora. Their mechanisms of action differed systemically and locally and in the context of attenuated vaccine or enteropathogenic infection. Understanding these factors will expand application of probiotics as immunostimulatory (vaccine adjuvants) or immunoregulatory (moderate infections) mediators.

**Keywords:**

Host/microbiome interaction  
Immune system  
Probiotics

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**P43. Ana Y.L. Wang<sup>1</sup>, Chris J. Vickers<sup>1</sup>, John R. Yates,  
III<sup>2</sup>, Andrew I. Su<sup>1</sup>, Dennis W. Wolan<sup>1</sup>.**

### Chemical-based Metaproteomics of the Healthy Human Distal Gut Microbiome

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The human gut microbiome is indispensable in maintaining health, and alterations in the bacterial communities have been associated with several disease states, including diabetes, colorectal cancer, and inflammatory bowel diseases. Significant insights into the abundance and diversity of bacterial species in both healthy and diseased individuals have been provided with DNA deep sequencing and 16s rRNA metagenomic approaches. The goal of this project is to develop and employ a complementary chemical biological proteomic method to directly and quantitatively identify bacterial proteins and/or classes of enzymatic families at the host:microbiome interface. We have established a universal methodology whereby bacterial proteins with conserved functionalities are covalently modified with small molecule activity-based probes (ABPs), isolated from the complex proteome, and subjected to multidimensional protein identification technology (MudPIT) for identification. As an initial proof-of-principle, we have employed several biotinylated ABPs that modify nucleophilic serine and cysteine residues for enrichment of bacterial proteins within healthy human fecal samples. ABP-susceptible proteins were isolated with streptavidin-conjugated beads via the probe's biotin tag, trypsinized to generate mass spectrometry (MS)-compatible peptides, and subjected to MudPIT data collection and analysis. We compared our MS data to a comprehensive library composed of NCBI RefSeq proteins from every sequenced genome. This method allows us to identify proteins from all organisms of the gut flora including fungi and archaea, providing information inaccessible by metagenomics alone.

Our metaproteomic method yielded results consistent with data previously generated with metagenomic

studies and suggests an abundance of bacteria from the Firmicutes and Bacteroidetes phyla. Significantly, a large diversity of Proteobacteria species was identified that have not been previously reported. KEGG pathway analyses revealed the majority of ABP-labeled bacterial proteins are involved in lipid and carbohydrate metabolism, and 16% of proteins are currently categorized as hypothetical in the NCBI RefSeq databases. We are actively addressing bioinformatic challenges to build a pipeline to automate the analysis of our MS data against the large proteomic NCBI libraries. Future studies will focus on using our methods to quantitate similarities and differences of the bacterial proteins secreted from the microbiomes of healthy and diseased individuals as well as building our arsenal of ABPs to interrogate a wide range of enzyme classes. Our chemical-based proteomic methods provide a novel and universal addition to the metagenomic and transcriptomic techniques currently applied to gain insights into the bacteria that comprise human microbiomes.

#### Keywords:

Proteomics  
Activity-based Probes  
Methods

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**P44. Sida Wang\*, Jihyang Park, Vincent B. Young, Xiaoxia Lin, Mark A. Burns.**

### Microfluidic droplet enabled co-cultivation and characterization of microbial communities

*University of Michigan, Ann Arbor, MI*

**Purpose/hypothesis:** To elucidate microbe-microbe-host interactions in complex microbial communities including the human microbiome, we have been developing a microfluidic droplet platform for high-throughput co-cultivation and characterization of symbiotic mixed cultures. This is based on the hypothesis that microbes that require symbiotic partners in their native communities can be co-

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cultured when they are co-localized in aqueous droplets and provided the appropriate condition; furthermore, the resulted co-cultures reflect the underlying positive and negative interactions.

**Background:** Microbial interactions in natural microbiota are crucial for the sustenance of the communities, but the precise nature of these interactions remain largely unknown. Conventional pure culture-oriented cultivation does not account for these interactions, which severely limits its utility in studying “unculturable” microorganisms from synergistic communities.

**Microbiomes:** We have applied the microfluidic droplet technology to the following systems: i) a synthetic microbial community consisting of cross-feeding *Escherichia coli* strains, as a model system for symbiosis; ii) murine fecal microbiota; and iii) human gut microbiota.

**Methods:** We have developed prototype microfluidic devices for i) robust generation of nanoliter aqueous droplets dispersed in a continuous oil phase at a frequency of 500 droplets/second; ii) stable droplet incubation on devices (up to 1,400 droplets on one device) or in micro-centrifuge tubes (up to a few million droplets simultaneously); and iii) generation of linear oxygen gradients from anaerobic to aerobic conditions for droplet cultivation. We are further extending this technology to perform Fluorescence in-situ Hybridization (FISH) in droplets.

**Results:** We first tested our microfluidic droplet technology with a synthetic model system consisting of cross-feeding *E. coli* mutants. With on-chip cultivation, our device was able to detect a pair-wise symbiotic relationship when one partner accounted for as low as 1% of the total population or each symbiont was about 3% of the artificial community. Next, we combined droplet co-cultivation with oxygen gradient generation to study murine fecal microbial samples. Various species, including obligate micro-aerobes, were successfully cultivated. Finally, we are currently exploiting our technology to cultivate human gut microbiota for isolation of strains on the HMP “most wanted” list.

**Conclusions:** Our work has demonstrated that droplet-enabled co-cultivation can effectively decompose

complex microbial communities into subsets of symbiotic members and thus facilitate the elucidation of underlying microbial interactions.

**Significance:** Our technology enables high-throughput co-cultivation and characterization of synergistic microbial communities previously thought uncultivable in laboratory settings. We can utilize this to elucidate interactions between members of various human microbiomes and with their host, which are crucial for the maintenance of these complex communities and have important implications for the diagnosis and treatment of related diseases.

**Challenges:** First, to implement the general conceptual framework, we are required to both develop new microfluidic technologies and integrate many existing ones. Second, it is essential for us to collaborate with biologists to identify specific questions to address and to develop the technology accordingly. Finally, working with human samples has been challenging. For instance, we had to upgrade our laboratory to BSL-2 and are constrained by the limited number of samples (including host fluid) available to our experiment.

### Keywords:

Microfluidics  
Co-cultivation  
Microbial Interaction

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**P51. Christina G. Warinner<sup>1</sup>, Raul Y. Tito<sup>1</sup>, Joao F. Matias Rodrigues<sup>2</sup>, Rounak Vyas<sup>2</sup>, Camilla Speller<sup>3</sup>, Christian Trachsel<sup>4</sup>, Jonas Grossmann<sup>4</sup>, Hans U. Luder<sup>5</sup>, Natallia Shved<sup>6</sup>, Christian von Mering<sup>2</sup>, Frank Rühli<sup>7</sup>, Enrico Cappellini<sup>8</sup>, Cecil M. Lewis\*, Jr.<sup>1</sup>.**

### **Extending Oral Microbiome Research into the Human Evolutionary Past**

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Fossilized dental calculus (mineralized plaque) and fossilized feces (coprolites) have recently been shown to preserve a rich biomolecular record, potentially extending the investigation of oral and gut microbiomes into the human evolutionary past. The objective of this study is to outline a robust protocol for the recovery and validation of endogenous biomolecules from ancient oral and gut microbiome samples.

Current studies of human microbiome diversity focus largely on contemporary, metropolitan populations in North America and Europe. Recent investigations of rural, non-Western populations have found important differences between Western and non-Western microbiomes, raising questions about the relationship between microbiome composition and factors such as diet, hygiene, antibiotic exposure, and lifestyle. Direct investigation of ancient microbiomes provides an additional path to investigating ancestral states of human microbiomes and the potentially medically-relevant influences of modernization. However, recovery of authentic microbiome data from ancient sources is currently hindered by the dual challenges of substrate preservation and microbial environmental contamination, and until recently, there have been few tools to identify and quantify post-depositional microbial alteration and contamination in ancient samples.

Using currently available laboratory and bioinformatic tools, we present a novel protocol for targeted 16S rRNA sequence data analysis and contamination management that allows 1) identification of well-preserved ancient samples, 2) sensitive detection of contamination artifacts, and 3) if necessary, removal of contaminant DNA sequences. We test this protocol on recently published ancient oral and gut microbiome data (n=48 samples), demonstrating our protocol to be an effective method for identifying and screening out low quality samples and data.

Next, focusing on a subset of high quality Medieval dental calculus samples, we then apply high-throughput whole genome (Illumina HiSeq) and proteome (LTQ-Orbitrap Velos) sequencing and demonstrate that high resolution taxonomic and functional information can be recovered from well-preserved ancient samples, including evidence of periodontal pathogenesis. Specifically, we characterize: (i) ~2,700 microbial taxa, (ii) 45 opportunistic pathogens implicated in oral, respiratory, and cardiovascular disease, (iii) the three “red complex” periodontal pathogens, with 5.7x genome coverage of *Tannerella forsythia*, and (iv) 239 bacterial and 43 human proteins, including bacterial virulence and host immune factors related to periodontal disease. We conclude that direct investigation of ancient human oral and gut microbiomes presents a unique opportunity to investigate microbiome evolution by placing human diseases of microbial dysbiosis into biogeographical and temporal context. Future challenges include testing this protocol on ancient skin microbiome samples to determine if endogenous microbiome data can be recovered from additional body sites.

**Keywords:**

Evolution  
Immune system  
Methods

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**P45. Alexa Weingarden<sup>\*1</sup>, Aleh Bobr<sup>2</sup>, Yuwei Lu<sup>3</sup>, Valerie Nelson<sup>4</sup>, Matthew Hamilton<sup>5</sup>, Chi Chen<sup>3</sup>, Michael Sadowsky<sup>6</sup>, Alexander Khoruts<sup>7</sup>.**

**Bile Acid Metabolism Changes Following Fecal Microbiota Transplantation for Clostridium difficile Infection**

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Clostridium difficile infection (CDI) is currently estimated to be among the most common nosocomial infections in the US, affecting more than half a million people annually in care facilities and many more in the community. Unfortunately this diarrheal disease responds poorly to recommended antibiotic therapy, since 20-30% of patients experience recurrent disease once treatment is stopped. A powerful emerging treatment for recurrent CDI is fecal microbiota transplantation (FMT), where fecal bacteria from a healthy donor are transplanted into the patient's colon, restoring the microbiota and curing the patient of clinical disease in ~90% of cases. However, it is not yet understood how restoration of the colonic microbiota suppresses the growth of C. difficile and cures patients. To investigate the mechanism behind FMT, we examined the urine metabolites of 17 patients with recurrent CDI before and after FMT, and analyzed the fecal microbiota and fecal metabolites of a subset of these patients.

17 patients with multiply recurrent CDI were treated with FMT using frozen, processed material from a standard donor as previously described (Hamilton et al. 2012). Urine samples were collected before and 1-5 times after FMT, from 1-182 days after the procedure. Samples were also collected from the two donors whose fecal material was used to treat patients. Samples were mixed in 50% aqueous acetonitrile, centrifuged to remove protein and particulates, and analyzed on a Waters Acquity ultra-performance liquid

chromatography system and SYNAPT QTOF mass spectrometer (Milford, MA). Selected fecal samples from before and approximately 7 days after FMT, as well as donor samples, were collected from 14 patients and DNA was extracted. The V6 region of the bacterial 16S rRNA gene was amplified via PCR and sequenced on the Illumina platform, followed by analysis using mothur and Fast UniFrac. Metabolites from four sets of patient fecal samples were also extracted and analyzed as described for urine samples.

Fecal microbial communities were substantially different before and after transplant by principal coordinate analysis, while urine and fecal metabolite compositions were distinct before and after transplant by partial least squares-discriminant analysis. Urine glycdeoxycholic acid, a conjugate of the secondary bile acid deoxycholic acid, was increased in urine following FMT in our patients. Similarly, the secondary bile acids deoxycholic acid and lithocholic acid were increased in fecal samples following FMT, while primary bile acids cholic acid and chenodeoxycholic acid were decreased. Because some secondary bile acids, including lithocholic acid, inhibit C. difficile germination in vitro, while some primary bile acids, including taurocholic acid, the taurine-conjugated form of cholic acid secreted by the liver, induce germination, these changes in bile acids may reflect a mechanism whereby restoration of normal gut microbial ecology following FMT treat CDI.

These results imply that the metabolism of primary to secondary bile acids by fecal bacteria is an important mechanism whereby the intestinal microbiota protect against pathogens. However, many challenges remain for future work, including identifying the organisms responsible for these metabolic changes and investigating whether other mechanisms also contribute to the suppression of CDI.

**Keywords:**

Disease association  
Biomarkers  
Ecology

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**P46. Laura S. Weyrich<sup>1</sup>, Keith Dobney<sup>2</sup>, Alan Cooper<sup>1</sup>**

### From Neanderthals to Chimpanzees: Origins of the Oral Microbiome

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<sup>2</sup>*University of Aberdeen, Aberdeen, United Kingdom*

Microorganisms and the human body have co-evolved for thousands of years. However, we know little about how these microbial communities originated or how much they vary within and between different homininae species (e.g. Chimpanzees, Gorillas, Neanderthals, and humans). Further, little is known about how variation in these microbial communities may have impacted overall health, immune resistance, mental stability and development, or even extinction events within this subfamily. To address these questions, we sequenced DNA preserved in calcified dental plaque (calculus) from Neanderthals, non-human primates, and ancient and modern humans. Using both 16S rRNA amplicon and shotgun sequencing approaches, we were able to compare the oral microbiomes of ancient hominids with modern humans and non-human primates. Data was analysed using both QIIME and MEGAN5, allowing us to investigate bacterial, fungal, and viral diversity, as well as complete functional analysis through time. Using ancient DNA, we were able to characterise bacterial communities in ancient and extinct homininae species and identify pathogenic bacterial species from up to 30,000 years ago. These results highlight differences and similarities in the microbial communities among homininae species and provide data on how microorganisms may have contributed to the delineation of these species. Further, we can begin to map the origin of the oral microbiome and track the origination of specific microbial species within these diverse communities through time.

#### **Keywords:**

Evolution

Ecology

Bioinformatics/computational tools

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**P47. Katrine Whiteson<sup>1</sup>, Simone Meinardo<sup>2</sup>, Yan Wei**

**Lim<sup>1</sup>, Rob Schmieder<sup>1</sup>, Donald Blake<sup>3</sup>, Doug Conrad<sup>4</sup>,**

**Forest Rohwer<sup>1</sup>.**

### Breath gasses as biomarkers in Cystic Fibrosis

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*Diego, CA*

**Background/Significance:** Long-term microbial infections in the lungs of Cystic Fibrosis (CF) patients are complex, individual, and difficult to correlate with patient condition or response to treatment. Gasses found in the breath of CF patients may enable detection of the presence of specific microbial metabolism in a particular microbial community and disease state.

**Purpose/Hypothesis:** Our goal is to find molecules in breath samples that are specific to microbial metabolism in the polymicrobial infections of CF patients. Ultimately, we would like to unveil mechanisms that explain how bacteria persist in the CF lung, and what drives periods of worsened symptoms. This information could be used to diagnose and treat infection specifically.

**Microbiomes:** Microbial and viral metagenomes along with transcriptomes from 6 CF patients at 2-4 timepoints each were sequenced from induced sputum samples.

**Methods:** Using the Gas Chromatography and Mass Spectrometry methods established in the Rowland-Blake lab at UCI, we have analyzed triplicate breath samples from a CF patient and gender matched healthy control in a longitudinal study including seven approximately monthly timepoints. We also conducted a cross-sectional study of seven CF patients thought to possess distinct microbial profiles according to clinical culture data. Total microbial DNA was sequenced from simultaneous induced sputum samples.

**Results:** We find elevated levels of 2,3-butanedione in the CF patient compared to the healthy controls during clinically stable periods, but not in the sample taken during i.v. antibiotic treatment. 2,3-butanedione is a toxic fermentation product specific to a subset of

bacteria during low pH, low O<sub>2</sub> conditions. The cross-sectional study also found elevated 2,3-butanedione in 5 of the 7 CF patients, excluding one patient undergoing i.v. antibiotic therapy and another who recently completed antibiotic treatment. We find hits to *Streptococcus* spp. genes involved in 2,3-butanedione metabolism and catabolism, along with *Pseudomonas* spp. genes for phenazine production and Fe(II) transport in our CF microbial metagenomes.

**Conclusions:** Observation of elevated 2,3-butanedione in the breath of CF patients is evidence for active *Streptococcus* spp. metabolism in the lung, as opposed to the mouth, where both healthy and CF patients harbor *Streptococcus* spp. as part of the oral community.

**Significance:** Volatile molecules detected in the breath of patients with lung infections including CF may enable taxonomic identification of the microbes driving the infection, and also indicate their active metabolism. Volatile molecules mediate synergism between bacteria in the biogeochemical circumstances unique to an individual lung, and may be an important driver of microbial growth. Earlier and more specific diagnosis and treatment of periods of worsening symptoms known as exacerbations in CF may be possible using breath tests.

**Major past and future challenges to progress:** A lack of basic information about the local chemistry of the environment where CF microbes live, along with most internal human environments, is a major challenge. We also lack spatial information about where the microbes live and how they are distributed throughout the lung. These are both difficult to obtain through the most accessible sample, induced sputum.

**Keywords:**

Biomarkers  
Disease association  
Ecology

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**P48. Kristine M. Wylie\***<sup>1,2</sup>, Jenna Goeckner<sup>2</sup>, Maria Canella<sup>1</sup>, Sheila Mason<sup>1</sup>, Erica Sodergren<sup>2</sup>, Richard Buller<sup>1</sup>, George M. Weinstock<sup>2</sup>, Gregory A. Storch<sup>1</sup>.  
**The Human Virome in Immunocompromised and Immunocompetent Children**

<sup>1</sup>Washington University School of Medicine, St. Louis, MO; <sup>2</sup>Washington University School of Medicine, St. Louis, MO

Children are exposed to many viruses, and these early exposures can have important long-term effects, such as the development of immunity against the virus or development of disease due to effects such as chronic inflammation. Within the first three years of life, common viruses such as herpesviruses, adenoviruses, and anelloviruses establish persistent infections that may be carried throughout life. Children are also infected with pathogenic viruses that cause acute infections that are typically cleared, such as enteroviruses, parechoviruses, and rhinoviruses. The goals of this study are to (a) characterize the virome in immunocompetent and immunocompromised children and (b) to determine whether viruses are more common in children with unexplained fever compared with afebrile children, which would suggest that viruses cause many of these fevers. We have expanded our previous study of the role of viruses in children with fever to include nasopharyngeal swabs (NP), plasma samples, and stool samples from immunocompetent children with fever, immunocompetent children who were afebrile, and immunocompromised children with and without fever. DNA and RNA viruses were assessed using an extensive panel of PCR assays and high-throughput sequencing analysis. Viruses were identified in the sequence data set based on nucleotide and amino acid sequence similarity to reference genomes using RTG map and MulticoreWare mblastx software. Groups of subjects were compared using Fisher's exact test. Contigs were assembled using IDBA-UD software. Sequence variation was assessed using LoFreq software. In this expanded data set, viruses are more common in samples from immunocompetent children with fever compared with samples from immunocompetent

children without fever, suggesting viruses may cause many unexplained fevers. The difference is most marked in plasma samples. Children with fever are also more likely to carry multiple viruses. Stool samples from immunocompromised children were evaluated, and the prominent groups of viruses were the same as those found in immunocompetent children. These viruses included adenoviruses and noroviruses, among others. We are currently examining the data to determine whether different viral subtypes or higher levels of the viruses are associated with samples from the immunocompromised children compared with immunocompetent controls. In general, PCR assays were more sensitive, although the sensitivity of sequencing was improved by increasing the depth of sequencing or enriching for viral sequences using sequence capture. Sequencing allowed the detection of viruses not included in the PCR panel, such as a kobuvirus and papillomaviruses. Sequencing was also advantageous in that it provided more information about the viral genome, including viral subtypes and variation, both between and within samples. These analyses begin to give us an understanding of the scope of the virome, which will help us understand its effects on health and disease. Past challenges include (1) finding sequence alignment software that can align rapidly enough to deal with millions of sequences in a reasonable time frame but are sensitive enough to detect virus sequences that vary from the closest reference genome and (2) avoiding false positives. Current challenges include finding good methods to enrich microbial signals in the assays.

**Keywords:**

Virome  
Pediatrics  
Fever

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**P49. Roger Zoh**<sup>1</sup>, Bani Mallick<sup>1</sup>, Raymond J. Carroll<sup>1</sup>, Johanna W. Lampe<sup>2</sup>, Meredith AJ Hullar<sup>2</sup>, Robert S. Chapkin<sup>3</sup>, Ivan I. Ivanov<sup>4</sup>

**Probabilistic Correlation Analysis of the Metagenome and Host Transcriptome: A Tale of Two Non-Normal Data Sets**

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Many of the questions of interest in biological studies can effectively be addressed by correlation estimation; for example, the effect of diet or treatment on the gut metagenome and host transcriptome in di\_erent samples or biological replicates. Various correlation estimation techniques such as sample correlation, Spearman correlation, among others are often used to estimate correlation. The sample correlation, for example, is known to be a consistent estimator of correlation in the presence of normal data. Unfortunately, gene expression data as measured by current sequencing technologies, before any normalization step, are integer type data for which sample correlation estimates are inappropriate and in some cases lead to poor or overly inated estimates of correlation. For example, estimates of correlation between two features with low counts across samples will always tend to be very close to zero, even when the features are in fact highly correlated. Thus, sample correlation estimates have the potential to cause scientists to overlook very important biological questions and lead to wasted efforts. We propose a model-based approach to correlation estimation between two non-normal data sets. Our model takes into consideration the non-Gaussianity of next generation sequencing gene expression data and suggests that correlations estimated at the natural parameter level are more meaningful than correlations estimated directly on the gene expression data sets. We demonstrate, through a simulation study, that our approach outperforms other standard approaches

(sample correlation, Spearman correlation, etc.) in the mean-squared error sense when estimating true high positive and negative correlations. Data used in the simulation were generated from our proposed model and other accepted next generation sequencing models. We propose that analysis of the multivariate structure underlying the microbiome and host transcriptome over time will provide richer and fuller information content compared to analyses focusing on single data sets (e.g., only host transcriptome data, or only microbiome data) and only single variables (e.g., gene by gene differential expression testing).

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**Keywords:**

Correlation  
Gene expression  
Natural parameter  
Poisson  
Next-generation sequencing

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**Sponsor Abstracts****P52 – Qiagen****Identification of antibiotic resistance genes in *Klebsiella pneumoniae* isolates and metagenomic samples using real-time PCR arrays**

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Treatment of bacterial infections has become more difficult due to increasing rates of antibiotic resistance, highlighting the importance of prevention and surveillance. As effective surveillance activities are vital in determining the measures needed to control antibiotic resistance, new and rapid laboratory methods are necessary to facilitate this important effort. Real-time PCR methods have proven effective for the detection of antibiotic resistance genes and PCR array technology allows the detection of a large number of genes in a single PCR run. Therefore, in this study, an antibiotic resistance gene identification PCR array was developed that allows for rapid screening of a range of antibiotic resistance genes present in a sample. The PCR array contains 5' hydrolysis probe assays (primer and dual labeled probe sets) that uniquely target 87 antibiotic resistance genes. All assays exhibited low-end sensitivity between 5–80 copies and a linear dynamic range of at least 5 orders of magnitude. A pilot research study was performed on a collection of *Klebsiella pneumoniae* isolates to identify the diversity of resistance genes. The PCR results revealed that the SHV antibiotic resistance gene was present in all 14 *K. pneumoniae* isolates tested and 10 (71.4%) of the isolates were positive for wild-type SHV-156G/238G240E. One (7.1%) sample harbored the SHV-156D mutation and three (21.4%) isolates harbored the SHV-238S240E variant. To verify the results from the PCR array, a subset of the antibiotic resistance genes were analyzed by pyrosequencing. Results of pyrosequencing confirmed the presence of KPC, SHV-156G, SHV-156D, SHV-238G240E, SHV-238S240E, tetA, tetB,

CTX-M-1/2 groups, AAC(6)-lb-cr, and aadA1 in the *K. pneumoniae* isolates. Since the gut is known to act as a reservoir for antibiotic resistance genes, a small-scale research study was performed on five stool samples isolated from healthy human adults using the antibiotic resistance gene identification PCR array. All five samples had ermB and mefA, and three of the samples were positive for tetA. In conclusion, PCR arrays can be effective and reliable tools for profiling antibiotic resistance genes from both isolates and metagenomic samples.

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**P53 - Roche****800+ Base 16S and 18S rRNA Gene Sequencing using the GS FLX+ and GS Junior Systems**

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The Roche 454 GS FLX+ and GS Junior System feature the unique combination of long reads, exceptional accuracy, and high-throughput, making the platforms well suited for characterizing complex microbial communities. With the launch of v2.9 software, long read sequencing of amplicons up to 800+ base pairs is now available on the GS FLX+ System. Here we describe the use of an acyclic nucleotide flow pattern, Flow Pattern B, for highly accurate targeted sequencing of long amplicons. The technique is particularly promising for metagenomics studies targeting 16S and 18S rRNA genes by allowing full coverage of more variable regions in a single uni-directional amplicon. The high quality results enable investigators to generate accurate diversity and abundance profiles and classify organisms down to the genus and species level, as demonstrated in several early customer access results. In addition to GS FLX+ System data, preliminary internal development results are shown for extra-long read sequencing on the GS Junior System, to become available in 2014.

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**P54 - Metabolon****Metabolomic Profiling of Gut Microflora Activity**

Metabolon scientists

The gut microflora consists of microorganisms that live in the digestive tract of animals and are most typically associated with those bacteria that reside in the intestines. This set of microorganisms is the largest reservoir of human flora with other reservoirs being skin, vagina, anterior nares of the nose, and oral cavity. Most of the microflora consists of bacteria, and although aerobic bacteria live in some segments of the intestine such as the cecum, nearly all of the gut microbiota are anaerobes. The contribution of gut microflora to the global metabolism of mammals cannot be ignored due to the estimated numbers of microorganisms to inhabit this niche. It is estimated that 500 to 1,000 different species of bacteria live in the gut containing origins in 50 different phyla with Bacteroidetes and Firmicutes playing the dominant roles in the population. The relationship between these microorganisms and mammals is symbiotic (mutually beneficial) and commensal (non-infectious) due to the contribution of these microorganisms to several biological processes. The metabolomic contributions of these microorganisms to host biology play crucial roles in disease progression, tissue and organ maturation, and absorption of dietary compounds into the bloodstream and are reflected through the metabolism of amino acids, bile acids/sterols, benzoate-containing molecules, carbohydrates, and flavonoids/anti-oxidants. The metabolic activities performed by these bacteria are equal to that of a virtual organ. The following are research areas in which gut microflora metabolism plays a central role: Disease, Pharmacology/Toxicology, Nutrition, Probiotics, Prebiotics

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