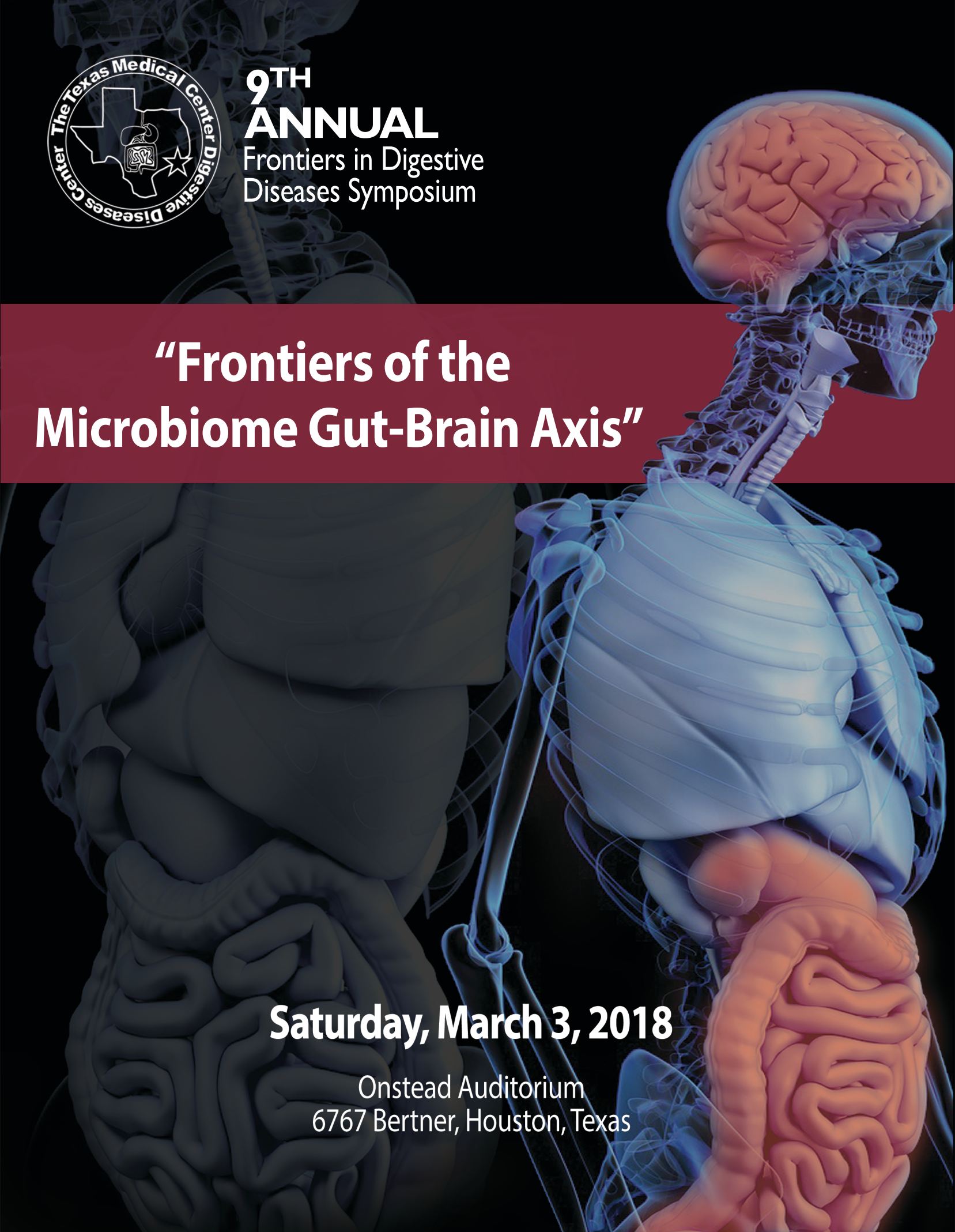


# 9TH ANNUAL Frontiers in Digestive Diseases Symposium

# "Frontiers of the Microbiome Gut-Brain Axis"

## Saturday, March 3, 2018

Onstead Auditorium
6767 Bertner, Houston, Texas

**Texas Medical Center Digestive Diseases Center**
**9th Annual Frontiers in Digestive Diseases Symposium:**
Frontiers of the Microbiome Gut-Brain Axis

# Saturday, March 3, 2018
# Onstead Auditorium, Houston, Texas 77030



## About Texas Medical Center Digestive Disease Center

The Texas Medical Center Digestive Diseases Center (DDC) facilitates cutting-edge digestive diseases research, promotes translational collaborative research between basic and clinical areas, develops new projects, nurtures new investigators, and provides GI educational activities. The DDC is a federally funded center (NIH P30DK056338) designed to serve basic and clinical scientists at institutions within the Texas Medical Center, including Baylor College of Medicine, The University of Texas Health Science Center at Houston and the MD Anderson Cancer Center.

The Director is Hashem B. El-Serag, M.D., M.P.H., Margaret M. and Albert B. Alkek Chair of the Department of Medicine, and professor of gastroenterology and hepatology at Baylor College of Medicine. Doug Burrin, Ph.D., director, of the Fellowship Research Training and professor of pediatrics at Baylor College of Medicine, is the co-director. Dr. James Versalovic, M.D., Ph.D., pathologist-in-chief, and head at Texas Children's Hospital and professor of pathology and immunology at Baylor College of Medicine, serves as associate director. Michelle Barton, Ph.D., professor of epigenetics and molecular carcinogenesis at The University of Texas MD Anderson Cancer Center and J. Marc Rhoads, M.D., gastroenterology division director and professor of pediatric gastroenterology at The University of Texas Health Science Center serve as assistant directors.

For more information, visit https://www.bcm.edu/research/centers/digestive-disease.

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# A G E N D A

| | |
|---|---|
| 7:30 -8:15 AM | **Breakfast** |
| 8:15 - 8:30 AM | **Welcome Remarks**<br>Hashem El-Serag, M.D., M.P.H.<br>Director, Texas Medical Center Digestive Diseases Center |
| 8:30 - 9:10 AM | **"Bacteria, brain and behavior in early life"**<br>John Bienenstock, CM, M.D. (Hon), FRCP, FRCPC<br>Distinguished University Professor, Pathology and Molecular Medicine<br>McMaster University |
| 9:10 - 9:50 AM | **"Brain Gut Communications – Unraveling the Role of the Microbiome"**<br>Emeran A. Mayer, M.D., Ph.D.<br>Director, UCLA Gail and Gerald Oppenheimer Family Center for Neurobiology of Stress<br>University of California, Los Angeles |
| 9:50 - 10:30 AM | " **In vivo modeling of the human intestine; looking at the big picture"**<br>Michael A. Helmrath, M.D., M.S.<br>Surgical Director, Surgical Weight Loss Program for Teens, Director of Surgical Research<br>Cincinnati Children's Hospital |
| 10:30 - 10:50 AM | **Coffee break** |
| 10:50 - 11:20 AM | **"The gut-microbiome in neurodevelopment disorders"**<br>Mauro Costa-Mattioli, Ph.D.<br>Associate Professor Neuroscience<br>Baylor College of Medicine |
| 11:20 - 12:00 PM | **"Serotonin as a Connection in the Brain-Gut Manifestations of Autism and Antenatal Antidepressant Exposure"**<br>Kara G. Margolis, M.D.<br>Associate Professor of Pediatrics<br>Columbia University |
| 12:00 -12:30 PM | **"Where the gut and brain collide: unraveling the mysteries of GI symptoms and behavior in autism spectrum disorder"**<br>Ruth Ann Luna, Ph.D.<br>Director of Medical Metagenomics, Texas Children's Microbiome Center<br>Texas Children's Hospital |
| 12:30 - 1:00 PM | **"Why Do Children with Irritable Bowel Syndrome have Pain?"**<br>Rob J. Shulman. M.D.<br>Professor of Pediatrics-Nutrition<br>Baylor College of Medicine |
| 1:00 - 2:00 PM | **Lunch / Poster Session** |
| 2:00 - 2:30 PM | **Poster Awards and Concluding Remarks** |

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Texas Children's Hospital®

**APPROVED CME ACTIVITY**
Directly provided by Texas Children's Hospital
Hosted by the Texas Medical Center Digestive Diseases Center
Saturday, March 3, 2018 | 8:30 am – 2:30pm | Onstead Auditorium

**"Bacteria, brain and behavior in early life"**
JOHN BIENENSTOCK, CM, M.D. (HON), FRCP, FRCPC, McMaster University

**"Brain Gut Communications – Unraveling the Role of the Microbiome"**
EMERAN A. MAYER, M.D., PH.D., University of California, Los Angeles

**" In vivo modeling of the human intestine; looking at the big picture"**
MICHAEL A. HELMRATH, M.D., M.S., Cincinnati Children's Hospital

**"The gut-microbiome in neurodevelopment disorders"**
MAURO COSTA-MATTIOLI, PH.D., Baylor College of Medicine

**"Using translational models to study gut-brain axis disease: Links between autism and depression"**
KARA G. MARGOLIS, M.D., Columbia University

**"Where the gut and brain collide: unraveling the mysteries of GI symptoms and behavior in autism spectrum disorder**
RUTH ANN LUNA, PH.D., Texas Children's Hospital

**"Why Do Children with Irritable Bowel Syndrome have Pain?"**
ROB J. SHULMAN. M.D., Baylor College of Medicine

**TARGET AUDIENCE**
Internal Audience, Physicians, Specialists Gastroenterology, Research in Digestive Diseases, Medical Students, Residents, Fellows, Any physician or researcher with interest in digestive diseases

**EDUCATIONAL OBJECTIVES**
At the conclusion of this live activity, participants should be better able to: 1. Define the availability of microbiome gut-brain axis research in the field of digestive diseases, 2. Apply best clinical practices concerning gut-brain axis in patients with digestive diseases, 3. Identify opportunities to apply microbiome gut-brain axis in the treatment of digestive diseases

**ACCREDITATION STATEMENT**
This live activity has been planned and implemented in accordance with the accreditation requirements and policies of the Texas Medical Association through the joint providership of Texas Children's Hospital and Texas Medical Center Digestive Disease Center. Texas Children's Hospital is accredited by the TMA to provide continuing medical education for physicians.

**CREDIT DESIGNATION**
Texas Children's Hospital designates this live activity for a maximum of *4.0 AMA PRA Category 1 Credit(s)™*. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

**DISCLOSURE**
Drs. Bienenstock, Mayer, Helmrath, Costa-Mattioli, Margolis, Luna, and Shulman have reported no relationships with proprietary entities related to the content of this activity. Persons involved in the planning of this activity have reported no relevant financial relationships with any commercial interest.

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## LIST OF ABSTRACTS

(Listed in alphabetical order by author.)

**Texas Medical Center Digestive Diseases Center**
**9th Annual Frontiers in Digestive Diseases Symposium:**
Frontiers of the Microbiome Gut-Brain Axis

| Page: | Name and Institution: | Abstract Title: |
|---|---|---|
| 25 | Shawn Gurwara, M.D.<br>*Baylor College of Medicine* | **P-22.** "One carbon metabolism nutrients in relation to mucosa-associated gut microbiome in healthy individuals" |
| 26 | Farnoosh Haghighi<br>*University of Houston* | **P-16.** "Clostridium difficile infection (CDI) patient guts are frequently colonized by caspofungin-resistant strains of Candida glabrata in Houston, Texas" |
| 27 | Anne Hall<br>*Baylor College of Medicine* | **P-17.** "Lactobacillus reuteri suppresses IL-8 production by intestinal epithelial cells via production of an antioxidant amino acid metabolite" |
| 28 | Irtiza Hasan, M.D.<br>*University of Houston* | **P-18.** "Antimicrobial susceptibility assessment of clinical & environmental Clostridium difficile isolates in relation to CRISPR-Cas" |
| 29 | Irtiza Hasan, M.D.<br>*University of Houston* | **P-18.** "Association of CRISPR-Cas and antimicrobial susceptibility of gut pathogen Clostridium difficile" |
| 30 | Beatrice Herrmann<br>*Baylor College of Medicine* | **P-20.** "Goblet cell ER stress is alleviated by Bifidobacterium dentium secreted products" |
| 31 | Feroz Hossain, Ph.D.<br>*University of Houston* | **P-21.** "Vancomycin-resistant Enterococcus (VRE) with vanA gene common in Clostridium difficile infected patient guts" |
| 32 | Umesh C. Karandikar, Ph.D.<br>*Baylor College of Medicine* | **P-23.** "A model to study cross-talk between the human intestinal epithelium and immune cells." |
| 33 | Mahesh Krishna<br>*Baylor College of Medicine* | **P-24.** "Household income and education influence pediatric inflammatory bowel diseases in the Houston Metro Area" |
| 34 | Juneyoung Lee, Ph.D.<br>*University of Texas Health Science Center* | **P-25.** "Reconstitution of Aged Mice with Young Microbiome Enhances Post-Stroke Recovery" |
| 35 | Stephanie L. LeValley, BSc<br>*Baylor College of Medicine* | **P-26.** "Cleavage of Glucagon-like peptide-1 by microbial gelatinases and its role in metabolic disease" |
| 36 | Jessica S. Lin<br>*Baylor College of Medicine* | **P-27.** "Helicobacter pylori antibiotic-resistance patterns among non-US born and US-born patients from an ethnically diverse population who failed first-line therapy" |
| 37 | Yuying Liu, Ph.D.<br>*University of Texas Health Science Center* | **P-28.** "Lactobacillus reuteri DSM 17938 modulates gut microbiota in newborn mice by boosting beneficial metabolites" |
| 38 | Mario Loeza-Cabrera<br>*Baylor College of Medicine* | **P-29.** "High-throughput functional genetics of the host landscapes that underlie development of gut dysbiosis using C. elegans" |
| 39 | Mauricio Lozano<br>*University of Houston* | **P-30.** "Gut pathogen, Clostridium difficile, are highly prevalent in community shoe swabs" |

## Role of CRISPR-Cas system in vancomycin-resistant Enterococcus spp.

Khurshida Begum, Feroz Hossain, Jacob K. McPherson, Julie Miranda, Jonathan Amadio, M. Jahangir Alam, and Kevin W Garey

University of Houston College of Pharmacy, Houston, TX

Background: In bacteria CRISPR-Cas (clustered regularly interspaced short palindromic repeats with CRISPR associated proteins CAS) system is an innate defense mechanism found in approximately 40% of sequenced prokaryotic bacteria genomes. The CRISPR-Cas inhibits plasmid and foreign DNA acquisition into prokaryote host which may include antimicrobial resistance gene acquisition. In this study we examined the occurrence of vancomycin-resistant genes and CRISPR1-Cas1 among Enterococcus species. We also assessed the protective role of CRISPR system in Enterococcus.

Methods: We analyzed 255 clinical Enterococcus samples (203 blood and 52 stool isolates) obtained from hospitalized patients in Texas Medical Center, Houston. All isolates were assessed for CRISPR1-Cas1 genes, Enterococcus species and vancomycin-resistant genes (vanA, vanB, vanC1, vanC2/C3) by PCR and gel electrophoresis. The function of CRISPR system in Enterococcus was examined by co-culturing vanA gene carrying plasmid with CRISPR positive and negative strains.

Results: The most abundant species were E. faecalis, (51%) followed by E. faecium (30%) and other Enterococcus spp. (19%). Prevalence of vanA gene was significantly higher in E. faecium (61%, 47/77) compare to E. faecalis (5%, 7/129) and other Enterococcus spp. (2%, 1/49). The occurrence of CRISPR1-Cas1 gene was higher in E. faecalis (48%, 58/129) followed by E. faecium (12%, 9/77) and other Enterococcus spp. (8%, 4/49). Prevalence of the vanA gene was inversely correlated with the prevalence of CRISPR1-Cas1 genes. CRISPR positive strains were less likely to acquire the vanA gene (2/6; 33%) compared to CRISPR negative strains (5/6; 83%).
Conclusion: An inverse relationship was found between presence of vanA gene and CRISPR1-Cas1 locus among clinical isolates of Enterococcus. Enterococcus strains with a CRISPR system were less likely to acquire the vanA gene in a co-culture model.

## Prevalences and mechanisms of chronic hepatic complications in urea cycle disorders

Lindsay C. Burrage[1,2], Simran Madan[1], Xiaohui Li[1], Ming-Ming Jiang[1], Racel Cela[1], Terry Bertin[1], Danielle Guffey[3], Charles Minard[3], Milton Finegold[2,4], Deeksha Bali[5], Sandesh Nagamani[1,2], Members of the Urea Cycle Disorders Consortium, Brendan H. Lee[1,2]

[1]Department of Molecular and Human Genetics, Baylor College of Medicine, [2]Texas Children's Hospital, [3]Dan L. Duncan Institute for Clinical and Translational Research, Baylor College of Medicine, [4]Departments of Pathology and Immunology, Baylor College of Medicine, [5]Department of Pediatrics, Duke University School of Medicine

*Background*: With early diagnosis and improved treatments to prevent hyperammonemia, individuals with urea cycle disorders (UCDs) are surviving longer. However, clinical studies have demonstrated that typical therapies do not ameliorate all long-term complications of these disorders. Chronic liver disease is one long-term complication that can occur independently of hyperammonemia. Chronic liver disease in UCDs includes hepatomegaly, chronic hepatocellular injury, hepatic fibrosis, cirrhosis, and hepatocellular carcinoma, yet the prevalences of these complications are unclear. Moreover, there are no surveillance protocols for these complications, and the pathogenesis of chronic liver disease in UCDs is unknown.

*Methods*: We used laboratory data from >600 subjects who are enrolled in the Longitudinal Study for Urea Cycle Disorders, a natural history study performed by the Urea Cycle Disorders Consortium, to determine the prevalences of chronic hepatocellular injury. In addition, we used regression analysis and mixed modeling to identify covariates associated with chronic hepatocellular injury. To complement our studies, we investigated the pathogenesis of chronic liver disease in a mouse model ($Asl^{Neo/Neo}$) for argininosuccinate lyase deficiency (ASLD).

*Results*: Using serum transaminase levels from baseline and longitudinal study visits, the overall prevalence of chronic hepatocellular injury in this cohort was 9%. The highest prevalences of chronic hepatocellular injury were observed in ASLD (33%) and arginase deficiency (23%). Given that there was a much larger cohort of subjects with ASLD, we performed further studies in this cohort (n=92). Four covariates (presence of at least one episode of hyperammonemia, use of any nitrogen-scavenging agent, use of sodium phenylbutyrate/glycerol phenylbutyrate or use of sodium benzoate) were associated with higher AST and ALT in subjects with ASLD. Similarly, the $Asl^{Neo/Neo}$ mouse has evidence of chronic hepatocellular injury with hepatomegaly and hepatic glycogen accumulation, which is observed in individuals with urea cycle disorders. Moreover, these hepatic injuries were corrected by a helper-dependent adenovirus expressing *Asl* using a liver specific (*ApoE*) promoter. In contrast, supplementation with nitrite did not reverse the hepatic phenotype suggesting that the nitric oxide deficiency observed in ASLD does not primarily contribute to this phenotype.

*Conclusions*: Although chronic hepatocellular injury may be observed in nearly all UCDs, the prevalence of this complication is highest in distal disorders of this pathway. In addition, the severity of urea cycle dysfunction appears to be associated with chronic hepatocellular injury in ASLD. The $Asl^{Neo/Neo}$ mouse model serves as a good model for studying chronic liver disease in UCDs, and gene replacement with *Asl* but not nitric oxide supplementation prevents injury in this model. Further studies of this model will provide novel insights into the relationship between the urea cycle and glycogen metabolism and may provide new avenues for research aimed at understanding these long-term hepatic complications.

## Study of Pancreatic Fibroblasts in Experimentally Induced Acute Pancreatitis via Lineage Tracing

J. Li[1], Y. Cao[1], B. Cheng[1], J.M. Bailey[2], M. Younes[3], and T.C. Ko[1]

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Introduction: As key mediators, collagen (Col) producing fibroblasts are reported to play a critical role in organ fibrosis including chronic pancreatitis, while their role in acute pancreatitis (AP) is unclear. To explore their role in AP, we employed an inducible Col1a2-creERT mouse that can mark Col1 producing fibroblasts in tissue stroma, and performed lineage tracing of Col1 producing pancreatic fibroblasts in normal and cerulein-induced AP pancreas.

Methods: Col1a2-cre;tdTom mice (bred by crossing Col1a2-creERT mice with tdTomato reporter mice, 3-6 wks old) were injected with tamoxifen (TAM, 1mg, 5days, ip) for induction of Cre recombination to illuminate Col1 producing fibroblasts with red fluorescence (tdTom+). Pancreata were harvested at 48h after TAM injection. In a separate set, mice received TAM as above, and then received cerulein (50μg/kg, 9x hourly, ip) for AP induction. PBS injection was used as control. Pancreatic tissue samples were harvested at 1, 24, and 48h after the last cerulein injection and were analyzed by linage tracing of tdTom+ cells, H&E staining, Sirius red staining, and fluorescence staining using FITC (green) conjugated PNA (an acinar marker), antibodies against CD31 (an endothelial marker), desmin (a pancreatic stellate cell (PSC) marker), or α-smooth muscle actin (α-SMA, a myofibroblast marker). tdTom+ and PNA+ cells were isolated by fluorescence-activated cell sorting for RNA extraction and then qPCR.

Results: Upon TAM injection in Col1a2-cre;tdTom mice, 8% of total pancreatic cells were labeled as tdTom+ that surrounded acini, ducts, and blood vessels. tdTom+ cells did not co-localize with PNA or CD31, while 50% of tdTom+ cells were co-labeled with desmin. Col1a2 and desmin expression in tdTom+ cells were confirmed by qPCR. AP induction was validated by increased histopathological scores ($p<0.05$). Sirius red staining did not detect fibrosis in AP. Compared to control, AP increased tdTom+ cells by 12, 13, 18% at 1, 24, and 48 h respectively ($p<0.05$, n=3-4/group/time points). There is no significant difference between the time points. Furthermore, 80% of the tdTom+ cells were co-labeled with α-SMA in AP 48h pancreas, but not in the control.

Conclusions: PSCs constitute a major cellular source of Col1 producing pancreatic fibroblasts. Pancreatic fibroblasts are proliferated and activated in cerulein-induced AP. How these cells impacts AP induction and resolution warrants further investigation.

## Study of Sex-Dependent Responses to Experimentally Induced Chronic Pancreatic Injury and Recovery in Mouse Model

T.F. Obafemi[1], K. Liu[1], B. Cheng[1], P. Yu[1], J. Li[1], M. Younes[2], T.C. Ko[1], Y. Cao[1],

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Introduction: A higher incidence of chronic pancreatitis (CP) in males has been reported in human studies. Whether CP is reversible and whether sex factor influences CP recovery, remains unclear. Cerulein, a cholecystokinin analogue that induces CP, is a commonly used CP mouse model. Using the cerulein CP model for a simulated period of CP injury and recovery, we tested our hypothesis that sex-dependent responses would occur during CP injury and recovery.

Methods: Adult C57BL/6 mice were administered cerulein (n=3-6/sex/group, 50µg/kg, 5x hourly/day, 3 days/week, ip) for 4 weeks, then allowed a 4-week recovery period. Normal saline was injected as control. Body weight was recorded weekly. Pancreata were harvested, either 4 days (injury group) or 4 weeks (recovery group) after the last injection and weighed. Pancreatic paraffin sections were stained for H&E and parenchymal acinar injury was scored. Fibrosis was assessed by Sirius Red staining for extracellular collagen deposition. Macrophage infiltration was evaluated by CD68 immunohistochemistry.

Results: From week 3-4 and 2-6, male and female mice injected with cerulein weighed less than their time matched controls, respectively ($p<0.05$). Four days after CP induction in injury groups, compared to the control groups, pancreatic injury was shown (respective to males, females) by decreased pancreas weight/body weight ratio (41, 39 %), increased acinar injury score (3, 3), increased fibrosis (11, 16 % of area), and increased macrophage infiltration (8, 23 cells/field). Both males and females displayed similar responses on acinar injury and fibrosis, while females exhibited a 3-fold greater macrophage infiltration than males ($p<0.05$). Four weeks after CP induction in recovery groups, pancreatic recovery occurred (respective to males, females) with a marginal recovery of pancreas weight/body weight ratio (52, 54 %), recovery of acinar injury (0.33, 0), and partial recovery of fibrosis (8, 10 %). Similar recovery responses for these parameters were observed in both males and females. A full reversal of macrophage infiltration was observed in males and females. Notably, the time-matched controls for the recovery mice, possessed higher baseline of macrophage infiltration than CP injury group.

Conclusion: Male and female mice gained less body weight with induction of cerulein, but both showed recovery to normal weight after withdrawal of insult. Cerulein-induced acinar injury is reversible, while fibrosis is partially reversible. Both male and female mice demonstrate comparable responses in CP injury and recovery, except for macrophage infiltration. Ultimately, understanding of CP recovery may provide insight and guidance for future translational studies.

## Rotavirus Infection Induces Intercellular Calcium Waves Through Purinergic Signaling

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Rotavirus (RV) causes severe diarrheal disease in children worldwide. RV infects the intestinal epithelial cells at the villous tips of the small intestine. Despite this localized infection pattern, RV infection causes life-threatening fluid loss potentially through the release of signaling molecules, such as the RV enterotoxin NSP4 protein, to facilitate widespread gastrointestinal disease. However the spread of signaling molecule(s) from an infected to uninfected cells has not been directly observed and the molecular details of these processes remain poorly defined. Critically, RV increases cytosolic calcium in infected cells and is necessary for RV replication and activation of secretory pathways in GI epithelium. Thus, characterizing RV-induced calcium signaling at the molecular level is needed to understand RV pathogenesis. We conducted live cell fluorescence imaging in cell lines and human intestinal enteroids (HIEs) as a biologically relevant model of the human small intestine to measure calcium signaling dynamics and chloride channel activation during RV infection.

We first engineered African green monkey kidney MA104 cells and HIEs to stably express GFP-based genetically encoded calcium indicators to measure calcium dynamics throughout RV infection using live cell epifluorescence microscopy. We found that RV significantly increases the number and magnitude of calcium transients. Furthermore, we observed that single RV-infected cells triggered long-distance intercellular calcium waves that encompassed surrounding uninfected cells in both MA104 cells and both duodenum and jejunum HIEs. Treatment with the ectoNTPase apyrase or purinergic receptor inhibitors blocked these intercellular waves and reduced mucin secretion and RV replication. Serotonin, which activates the enteric nervous system to cause diarrhea and vomiting in RV infection, was decreased in RV-infected HIE monolayers with apyrase and purinergic inhibitor treatments. These results indicate that extracellular ATP/ADP signaling elicits calcium waves and is an important mediator of calcium dynamics during RV infection.

Overall, our results demonstrate that RV infected cells release extracellular ATP/ADP to cause calcium signaling in neighboring, uninfected cells. This pathway is important not only for RV replication, but also serotonin and mucin secretion, and thus purinergic signaling is a therapeutic target for developing life-saving anti-diarrheal drugs. Furthermore, we are the first to show that viruses can exploit purinergic signaling to generate long-distance calcium waves that encompass uninfected cells and thereby **potentially amplify the pathophysiological signaling important for diarrhea.**

## Limiting the toxicity of chemotherapy by enhancing regeneration of intestinal stem cells

Min-Shan Chen[1], Yuan-Hung Lo[1], Joann Butkus[2], Noah Shroyer[1,2]

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Introduction. Chemotherapy is the backbone treatment for most malignancies. Typical cytotoxic chemotherapy drugs, such as doxorubicin, a topoisomerase II inhibitor, also harm normal cells that divide rapidly, such as the gastrointestinal lining, and cause morbidity and mortality that limits medical treatment. There is a need to reduce chemotherapy toxicity and thus provide a therapeutic benefit and improve overall quality of life of cancer patients. Intestinal stem cells are selectively killed by chemotherapy drugs, which trigger a poorly characterized activation of quiescent stem cells and/or progenitors for crypt regeneration. Growth Factor-Independent 1 (GFI1) is a zinc finger transcriptional repressor implicated in the differentiation of secretory precursors into goblet and Paneth cells in the intestinal epithelium. Here, we hypothesize that following injury, increasing reversion of Gfi1$^+$ secretory cells into stem cells will improve intestinal epithelium regeneration and mitigate chemo-induced injury.

Methods. In our injury model, mice were treated with 20 mg/kg Doxorubicin through intraperitoneal injection leading to Lgr5+ stem cell injury and crypt loss. Gfi1 reporter mice (Gfi1cre; *ROSA26*$^{floxSTOP\text{-}YFP}$) mice and intestinal organoids were used to investigate intestinal stem cell reversion and repair mechanism following injury.

Results. Under homeostatic conditions, Gfi1-lineal cells were secretory Paneth and Goblet cells, which were not part of the stem cell pool. After injury, we found that Gfi1$^+$ secretory cells can re-enter the cell cycle and give rise to all cell lineages of the intestinal epithelium, indicating that Gfi1-lineal cells revert to become active stem cells and repopulate the stem cell pool following tissue injury. The extent of reversion was generally correlated with the severity of injury as assessed by dysmorphic crypts. To identify potential boosters of intestinal regeneration, we generated three-dimensional organoid cultures from Gfi1 reporter mice which provide a convenient and physiologically relevant model to identify key pathways regulating stem cell injury and regeneration caused by chemotherapy drugs. Our results demonstrated that PI3kinase/AKT activation using PTEN inhibitor improved cell survival, and elevated WNT signaling using high R-spondin treatment increased efficiency of Gfi1$^+$ cell reversion upon injury. High R-spondin treatment and PTEN inhibition combined to enhance both survival and reversion upon injury. Additionally, we used AKT inhibitor to suppress AKT activity. AKT inhibition prevented survival of organoids after injury despite R-spondin or PTEN inhibitor co-treatment, indicating that AKT is epistatic to WNT signaling, and activation of AKT after injury is critical for intestinal epithelium regeneration.

Conclusions. These findings indicate that Gfi1$^+$ secretory cells display plasticity and can reacquire stemness upon tissue damage. Moreover, PI3kinase/AKT and WNT are key regulators of cell survival and stem cell reversion after severe tissue injury. Currently, we are testing Rspondin and PTEN inhibitor treatment *in vivo*. Our studies in intestinal stem cells will improve our current understanding of injury-induced regeneration and identify potential therapeutic strategies to mitigate the effects of chemo-induced normal tissue injury and improve cancer chemotherapy.

## Role of glutaredoxin 3 in iron homeostasis

Ninghui Cheng[1], Fan Zhao[2], Jimmonique Donelson[1], Han Yu[1], Qianxing Mo[3], Juan C. Marini[1], Trung C. Nguyen[1], Chunbao, Li[2], Kendal, D. Hirschi[1], Lawrence Chan[1], Jin Wang[4,5], and Paul A. Nakata[1]

USDA/ARS Children Nutrition Research Center, Department of Pediatrics[1], Dan L. Duncan Cancer Center[3], Pharmacology[4], and Center for Drug Discovery[6], Baylor College of Medicine, Houston, TX 77030, USA; College of Food Science and Technology[2], Nanjing Agricultural University, P.R. China

Background: Iron is an essential mineral nutrient that is tightly regulated through mechanisms involving iron regulatory genes, intracellular storage, and iron recycling. Dysregulation of these mechanisms often results in either excess tissue iron accumulation (overload) or iron deficiency (anemia). Many biochemical reactions associated with energy production, biosynthesis, replication, locomotion, and gene regulation utilize iron in some form. At the core of hundreds of enzymes, which are mostly involved in primary metabolism in the cell, iron is often found complexed with cysteine or sulphide as in the iron-sulfur (Fe-S) cluster. *In vitro* and *in vivo* biochemical analysis has established the role of monothiol glutaredoxins (Grxs) in Fe-S cluster biogenesis in both prokaryotes and eukaryotes. In both classes of organisms monothiol Grx dimers function and serve as carriers delivering intact Fe–S clusters to apoproteins and/or form [2Fe–2S] cluster–ligand complexes that mediate signaling events in the cell. In addition, recent studies have suggested that mammalian Grx3 may play a crucial role in iron homeostasis and hemoglobin maturation, but the underlying mechanism is still largely unknown.

Methods: To study the function of Grx3 in iron regulation *in vivo*, a mouse Grx3 conditional allele with two LoxP sites flanking exon2 was created and a liver specific Grx3 deficient (LKO) mouse strain was generated by crossing it with an Albumin-cre mouse strain. RNA-seq analysis was conducted to profile gene expression and western blot analysis was performed to study signaling pathways.

Results: Grx3 expression was found to increase in the livers of mice during development. Mice with liver specific deletion of Grx3 were viable and grew indistinguishably from their wild type littermates. Although measurements showed no differences in total body weight between the LKO and WT mice, the LKO mice were found to have livers larger than controls with higher concentrations of iron. Grx3 LKO mice also displayed impaired liver function at the age of 8 months. RNA-seq and q-PCR analysis revealed increased expression of iron homeostasis genes in LKO compared to controls. Disruption of Grx3 in the LKO mice was found to result in altered mitochondrial and nuclear Fe-S cluster assembly. Among the many iron regulatory proteins present in the liver, ferritin H accumulation was increased in the LKO mice compared to controls. Interestingly, the accumulation of hepatic ferritin H was correlated to a down-regulation of the autophagy pathway (Ferritinophagy). Furthermore, our data suggests that Grx3-mediated ferritinophagy may be dependent on cellular iron status.

Conclusions: These findings support the hypothesis that Grx3 is an important factor in regulating iron homeostasis in hepatocytes by controlling cellular storage protein turnover through a mechanism related to the autophagy pathway.

## The Role of Homeobox Genes in Conferring Intestinal Regional Identity

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Homeobox (HOX) genes are known for their role in anterior-posterior patterning during development. As in other tissues, HOX genes are expressed in a spatial collinear fashion along the gastrointestinal tract. Ectopic expression of hoxd13, a posterior HOX gene, causes partial distalization of the developing avian proximal gut, implicating HOX genes in intestinal patterning and regional identity. Our unpublished data, in accord with others, shows that the crypt compartment, which houses stem cells necessary for intestinal epithelial renewal, retains intestinal regional-specific identity.

The objective of this project is to determine the requirement and sufficiency of HOX genes to maintain and establish intestinal regional identity. Here, three-dimensional human intestinal enteroids (HIEs) are used as a model to study the establishment and maintenance of intestinal regional identity. Additionally, embryonic stem cell-derived intestinal organoids (HIOs) are being used as a mode of human intestinal development to test the role of HOX genes in the initial establishment of intestinal regional identity. RNA-seq and ChIP-seq data revealed an enriched expression of several HOX genes in human ileal crypts compared to jejunal crypts.

Furthermore, qPCR analysis indicates that mid-cluster HOX9 paralogs (HOXA9, HOXB9, HOXC9 and HOXD9) are generally markers of ileal and colonic stem cells. Currently, the manipulation of HOX gene expression in HIEs and HIOs is being performed in order to elucidate the role of HOX expression in conferring and maintaining intestinal regional identity. Ultimately, this knowledge is an important step towards creating regionally specified segments of intestine for transplantation into patients who have lost bowel.

**Metabolic plasticity of *Clostridium difficilie* ribotype 078 strains confers a fitness advantage.**

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*Clostridium difficile* is the most frequent cause of nosocomial acquired infections is the U.S. and Europe. At the turn of the century there was a significant increase in *C. difficile* infection in North America, which has become a global problem. These recent outbreaks have been primarily associated with two hypervirulent ribotypes, 027 (RT027) or 078 (RT078). Previously, we have shown that these ribotypes independently evolved the ability to utilize a common food additive, trehalose, at much lower concentrations than other *C. difficile* ribotypes. RT078 strains have two unique attributes: predominance as a colonizer of livestock, and the presence of a novel four gene insertion that is only present in very closely related *C. difficile* ribotypes.

This four-gene insertion includes a second trehalase, *treA2*, the cognate repressor, *treR2,* a PTS transporter component, *ptsT*, as well as a putative debranching enzyme, *treX*. To assess if PtsT was a trehalose specific transporter, we performed growth experiments in minimal medium supplemented with a variety of carbon sources, including simple sugars and complex polysaccharides. These experiments revealed that PtsT allows CD630 (a non RT078 strain) to grow on low concentrations of trehalose and contributes to growth on polysaccharides such as inulin and arabinogalactan in a PtsT dependent manner. We hypothesize that this genetic element contributes to utilization of complex carbohydrates in livestock feed as well as the recently increased human dietary components (trehalose, inulin, and arabinogalactan). We predict that this metabolic plasticity promotes animal carriage and has significantly contributed to pathogenicity and disease severity in humans. We have undertaken experiments to determine if this genetic element promotes growth on starches found in the top 5 animal feed components: corn, wheat, oat, sorghum, and rice. We are investigating the impact of these substrates on *C. difficile* fitness and pathogenicity *in vitro* and *in vivo*.

We anticipate these studies to provide important information about the metabolic efficiency and virulence traits of C. *difficile* epidemic RT 078 strains which may inform patient dietary guidelines during *C. difficile* infection.

## Diet-induced evolution of the human microbiome: the impact of dietary sugars on gut health

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Host diet acts as a strong selective pressure on the gut microbiome. Adaptation can occur through taxonomic shifts in the composition of the microbiome and through adaptation of individual microbes producing strain variation, both of which can have profound effects on host-microbe interactions. During the 20th century, Americans increased their consumption of simple sugars in the diet, including trehalose and high fructose corn syrup. How gut microbes evolve in the presence of these carbohydrates and what affect adapted strains have on host physiology and metabolism is unknown.

Here I propose to identify the alleles selected for in a microbial population exposed to trehalose or high fructose corn syrup, the phenotypic effect these alleles have on interactions with the host gut, and to demonstrate these alleles are causative for these phenotypes. To this end, I will experimentally evolve a defined community of gut microbes in bioreactors in the presence of trehalose or high fructose corn syrup for 6 weeks. Sampling will be taken over the course of the experiment and mutations and genome rearrangements arising in the population will be determined by metagenomics and population level allele determination. The populations will be assessed for their effect on host gut phenotypes including barrier function, immune modulation, and gut hormone regulation using human intestinal enteroids. Machine learning and association analysis will be used to determine which of the mutations in the microbial community is likely responsible for the observed host phenotypes. Identified microbial strains will be retested using the enteroid model. This research will directly address how changes in dietary sugar affect human health via evolution of the gut microbiome.

*Supported by a training fellowship from the Gulf Coast Consortia, on the NLM Training Program in Biomedical Informatics

## The role of the mevalonate pathway in intestinal lipid absorption

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Cardiovascular disease (CVD) is the number one cause of death within the United States (U.S.) regardless of race or gender. Hypercholesterolemia is one of the leading causes of CVD in the U.S., and 39.7% of American adults have high cholesterol. Statins are the front-line therapy for lowering plasma cholesterol, and act by inhibiting 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (Hmgcr), the rate limiting enzyme in the mevalonate pathway. While statins are widely prescribed, the physiological importance and regulation of the mevalonate pathway in non-hepatic tissues is poorly understood. Next to the liver, the intestine is believed to be one of the primary sites of *de novo* cholesterol synthesis in humans, and an important organ that mediates the absorption and secretion of dietary lipids into the bloodstream. However, the specific role of Hmgcr and the mevalonate pathway in the intestine has not been directly investigated. We hypothesize that Hmgcr activity is required for proper lipid absorption by the intestine. We have bred mice harboring floxed alleles for *Hmgcr* with the *Villin-Cre* transgene to specifically knock out *Hmgcr* in the small intestine and colon. Preliminary data has revealed that these mice display significantly lower body weights beginning at time of weaning, which gradually recover by 12 weeks of age. The *Hmgcr* intestinal knockout mice also display aberrant crypt morphology, suggesting a compensatory regenerative response to inhibition of this pathway. Studying the effects of *Hmgcr* deletion in the intestine will help clarify the importance of *de novo* cholesterol synthesis in intestinal function and lipid absorption, leading to improved personalized medicine for hypercholesterolemia and CVD.

## Examining the role of gut dysbiosis in neuroinflammation and hypertension in a model of obstructive sleep apnea

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Obstructive sleep apnea (OSA), characterized by repeated closure of the upper airway during sleep, is a significant clinical problem and an independent risk factor for systemic hypertension. The importance of a healthy gut microbiota, and detriment of a dysbiotic microbiota, on host physiology is becoming increasingly evident. We have previously demonstrated that gut dysbiosis is associated with the development of hypertension in a rat model of OSA. Transplanting the dysbiotic microbiota from a hypertensive OSA rat into a normotensive rat induces hypertension, demonstrating a causal relationship between gut dysbiosis and hypertension. The mechanisms linking gut dysbiosis to hypertension are unknown. We tested the hypothesis that OSA-induced gut dysbiosis impairs gut barrier function, causing systemic inflammation and neuroinflammation, which is linked to hypertension. Using an in vivo model of OSA, we exposed high fat fed rats to 2 weeks of sham or OSA (60 apneas/hr). Relative to sham rats, OSA decreased the number of goblet cells/crypt in the cecum ($n=6$, $p<0.05$) and reduced the mucosal layer thickness ($n=3$, $p<0.05$). OSA reduced gut barrier function and 16S rRNA signatures for several genera were elevated in peripheral tissues of OSA, versus sham, rats. Flow cytometric analysis using whole brain revealed a significant increase in the number of activated microglia following OSA ($n=4$-$6$, $p<0.05$). Short chain fatty acids play a key role in maintaining gut barrier integrity and regulating immune responses. We found that OSA significantly reduced the acetate concentration in the cecum ($n=5$, $p<0.05$). To increase short chain fatty acid production in the gut, rats were treated with a prebiotic (diet enriched with 20% resistant starch), or probiotic (Clostridium butyricum; $10^9$ CFU by gavage every three days) during the 2 weeks of sham or OSA. Pre- and probiotics each prevented the OSA-induced decrease in cecal acetate concentration and loss of goblet cells ($n=5$-$6$, $p<0.05$ for each). Additionally, pre- and probiotics reduced 16S rRNA signatures for several genera in peripheral tissues, and prevented OSA-induced activation of brain microglia ($n=6$-$8$, $p<0.05$). Importantly, both the pre- and probiotic successfully prevented the development of hypertension in OSA rats. To test the direct effects of acetate, we chronically infused 20µmol/(kg-min) acetate into the cecum during the 2 weeks of sham or OSA. Cecal acetate infusion prevented OSA-induced hypertension. These data demonstrate a causal role for gut dysbiosis in the development of hypertension, which involves reduced acetate production, gut barrier disruption, and neuroinflammation. Our data suggest that manipulation of the gut microbiota may serve as a novel therapy in the prevention of hypertension.

Funding Information
This project was funded by NINDS R01NS080531 (RMB), AHA 16SDG29970000 (DJD), and by Public Health Service grant DK56338, which funds the Texas Medical Center Digestive Diseases Center
(DJD).

## *Bifidobacterium dentium* modulates the intestinal serotonergic system and species-typical behavior

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**Background:** The metabolite and neurotransmitter serotonin (5-HT) is a key molecule in both the gastrointestinal tract (GIT) and brain. 5-HT is predominantly synthesized by the enterochromaffin (EC) cells in the intestine via tryptophan hydroxylase (TPH). Within the gut, 5-HT concentrations are controlled by uptake via the transporter SERT and it can interact with multiple cell types to regulate gastrointestinal function. Recent work has identified an essential role for a complex microbiota in regulation of 5-HT. Germ-free mice have altered turnover and levels of 5-HT and exhibit aberrant behavior compared to mice with a gut microbiota (specific pathogen free (SPF)). To date, few studies have identified single bacterial species that are able to directly regulate the serotonergic system. *Bifidobacterium* species are short chain fatty acid (SCFA) secreting commensals that contribute in various ways to host health. We hypothesize that *Bifidobacterium dentium*-derived acetate stimulates 5-HT production by ECs. We also speculate that increased 5-HT release corresponds with increased 5-HT receptor and SERT expression in both the gut and brain resulting in behavioral alterations. **Methods & Results**: Germ-free mice were mono-associated with a model *Bifidobacterium* species (*B. dentium*) for 17 days. *B. dentium* mono-associated mice exhibited increased cecal and fecal acetate levels compared with germ-free controls, with no changes in other SCFAs. Increased acetate in *B. dentium* mono-associated mice corresponded with increased luminal 5-HT concentrations in the ileum and colon, as well as increased plasma 5-HT as measured by mass spectrometry (MS/MS). These results were also reflected in increased ileum and colon *tph-1* expression. *B. dentium* mice exhibited increased colonic 5-HT receptor 2a and 4 mRNA, as well as increased SERT mRNA and protein. To determine if *B. dentium*-produced acetate could stimulate 5-HT release from epithelial ECs, enteroid cultures were utilized. Mouse ileal enteroids microinjected with *B. dentium* conditioned media (CM) and acetate resulted in a dose-dependent release of 5-HT. Additionally, human jejunal enteroids overexpressing neurogenin 3 (NGN3) were used, which drives overexpression of enteroendocrine cells. Incubation with *B. dentium* CM and acetate stimulated enhanced 5-HT release in NGN3 human enteroids. Microinjection of *B. dentium* CM, but not acetate, upregulated epithelial SERT in mouse ileal enteroids. Finally, we assessed whether changes to the serotonergic system were also occurring in the central nervous system (CNS) based on colonization status. Although no changes were observed between groups in the concentrations of whole brain 5-HT, *in situ* hybridization revealed increased hippocampal 5-HT receptor 2a in *B. dentium* mono-associated mice. Physiological processes mediated by this receptor include anxiety-like behavior. Our behavior studies indicate that anxiety-like behavior is also modulated by *B. dentium* colonization. **Conclusions:** These data demonstrate that the commensal microbe *B. dentium* is capable of regulating key components of the serotonergic system in multiple host tissues.

## *Lactobacillus reuteri* modulates dendritic cell maturation and expression of IL-10

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**Background:** Dendritic cells (DCs) are antigen-presenting cells located in the skin, mucosa and lymphoid tissues. Their main function is to process antigens and present them to T cells to promote immunity to foreign antigens and tolerance to self-antigens. They also secrete cytokines to regulate immune responses. DCs may be targets for modulation by gut microbes, including probiotics. *Lactobacillus* species have been shown to extensively modulate the host immune system. Our lab has previously shown that *Lactobacillus reuteri* ATTC 6475 is able to ameliorate a TNBS model of colitis. However, the role of *L. reuteri* bacterial components on dendritic cells (DCs) remains uncharacterized. We hypothesized that *L. reuteri* affects DC maturation. **Methods & Results**: To assess the effects of *L. reuteri* on DC maturation, bone marrow-derived mouse dendritic cells were exposed to commensal *L. reuteri* 6475 UV-irradiated bacteria or bacterial secreted products (condition media). LPS at 1 µg/ml was included as a positive control in all experiments. Cell surface maturation markers CD40 and CD83 were examined with DC specific markers CD11c and MHC class II by flow cytometry. CD40 signaling induces changes in DCs, which make them more effective antigen presenting cells (APCs) and these changes are typically associated with upregulation of co-stimulatory molecules such as CD83. *L. reuteri* conditioned media, and to a greater extent *L. reuteri* bacteria, increased the $CD40^{high}$, MHC $II^{high}$ and $CD83^{high}$, MHC $II^{high}$ populations. By qPCR, *L. reuteri* conditioned media was found to upregulate CCL22, a T-cell chemoattractant, CCR7, the MIP-3-β chemokine receptor, and Gadd45b, a mediator of the DC stress-responsive MTK1/MEKK4 MAPKKK. In confirmation of our flow data, CD83 was also upregulated in *L. reuteri* treated groups by qPCR. Incubation of *L. reuteri* conditioned and UV-irradiated bacteria resulted in production of the anti-inflammatory cytokine IL-10 by qPCR and ELISA. To confirm the ability of *L. reuteri* to stimulate IL-10 production in a complex system, mouse ileum enteroids were co-cultured with mouse bone-marrow derived DCs. Enteroids microinjected with *L. reuteri* UV-irradiated bacteria stimulated IL-10 production, indicating potential cross-talk between the gut epithelium and DCs. **Conclusions**: *L. reuteri* is capable of eliciting DC maturation and IL-10 production, which may contribute to activation and polarization of naive T cells to ameliorate intestinal information. This may represent a crucial mechanism for maintaining intestinal immune homeostasis.

## Development of a microbial biosensor for inflammation-associated microenvironments

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Fecal calprotectin assays have become a widely-used diagnostic test for the detection and monitoring of inflammatory bowel disease (IBD). However, these tests are only administered between one and four times per year for the average remissive patient, and as a result, can miss an oncoming symptomatic flare. The fecal calprotectin assay also requires patient-clinician contact for each test, which results in low compliance among the IBD patient subpopulation. To address these gaps in IBD maintenance, we are currently developing a microbial calprotectin biosensor that would be used by IBD patients at-home. These microbial biosensors would be taken orally, and upon detection of a positive calprotectin signal within the gastrointestinal (GI) tract, would release a dye that alters fecal pigment and alerts patients to the presence of elevated fecal calprotectin.

Calprotectin is a neutrophil-source antimicrobial peptide that chelates free metals, resulting in a bacteriostatic effect on nearby microbes. Thus, to build an IBD-specific microbial biosensor, we identified calprotectin-sensitive bacterial gene promoters using an RNA-seq transcriptomic-based approached, and have constructed sensors by coupling these regulatory sites with green fluorescent protein. The raw promoter constructs were cloned into *Lactobacillus reuteri* LJO1 and *Escherichia coli Nissle* 1917 due to these microbes ability to traverse the GI tract. Function was then verified in a series of *in vitro* and *ex vivo* studies. The *E. coli Nissle* biosensors co-cultured with sub-clinical levels calprotectin exhibited a 1.6 to 3.0-fold increase in GFP expression, while the L. reuteri biosensors had a 1.3 to 4.5-fold increase. Biosensor activity was sensitive to metal chelation, as addition of the synthetic metal chelator TPEN turned on GFP expression between 2.5 to 5.6-fold amongst the sensors. Sensors were also co-cultured in fecal slurries obtained from healthy subjects as well as IBD patients. Both *E. coli Nissle* and *L. reuteri* biosensors displayed higher GFP output when cultured in IBD fecal slurry containing >110 ug/mL compared to sensors cultured in healthy fecal samples. Future directions will focus on increasing the human cohort sample size, and verifying function using *in vivo* models.

Successful completion of a microbial biosensor for intestinal inflammation will represent a non-invasive, at-home method of disease monitoring for the IBD patient population. This will allow those with IBD to have reduced clinician contact, yet still have the autonomy and foreknowledge to contact their doctor should they observe an alteration in fecal pigment. The earlier warning provided to the patient by the biosensor may reduce surgery and hospitalizations by allowing clinicians to intervene with the disease course before a symptomatic flare becomes problematic.

## Diacylglycerol kinase and histamine mediated signals between *Lactobacillus reuteri* and mammalian intestinal epithelium

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*Lactobacillus reuteri* ATCC PTA 6475, probiotic bacterium, with an intact *hdc* gene cluster is known to synthesize and secrete histamine, and can suppress inflammation in mammalian systems via specific activation of the type 2 histamine receptor (H2R). However, it is unclear if *L. reuteri*-derived histamine also modulates activity of pro-inflammatory H1 receptors in the intestinal epithelium. In this work, we identified a soluble secreted isoform of diacylglycerol kinase (Dgk) from *L. reuteri* 6475. DGKs belong to a distinct, conserved family of intracellular lipid kinases that phosphorylate diacylglycerol (DAG), catalyzing conversion of DAG to phosphatidic acid (PA). This reaction may diminish DAG quantities in the cell membrane possibly modifying host intracellular signaling downstream of DAG. Histamine binding to H1R can cause phosphorylation of PKC via DAG activation. We found that *L. reuteri* 6475 suppressed basal levels of the pro-inflammatory cytokines IL-6, IL-1, Eotaxin (eosinophilic chemoattractant proteins) and G-CSF in the luminal mucosa and in blood plasma. *L. reuteri* lacking Dgk could not suppress the aforementioned pro-inflammatory biomarkers. In addition, we demonstrated that histamine synthesized by *L. reuteri* 6475 activates both H1 and H2 receptors, but Dgk synthesized by the bacterium suppresses H1R downstream signaling. Inhibition of signaling downstream of H1R was supported by diminished PKC phosphorylation in the intestines of wild-type (WT) *L. reuteri* treated but not in *ΔdgkA* mutant treated germ-free (GF) mice. In addition, we also report suppression of $CD11b^{+}Gr1^{+}$ $Ly6G^{hi}$ immature myeloid cells (IMCs) after WT *L. reuteri* treatment. The proportion was consistent with in vivo experiments in our mouse model, PKC phosphorylation was reduced in human epithelial cells after treating the cells with *L. reuteri* derived conditioned media. *L. reuteri* suppressed immune responses by direct effects of the metabolite, histamine, and a secreted bacterial enzyme, diacylglycerol kinase, which converts a membrane lipid signal to an inactive form. Additionally, 1% DSS treated conventional BALB/c mice treated with $10^9$ cells of WT *L. reuteri* showed reduced proinflammatory $CD11b^{+}F4/80^{+}MHCII^{hi}$ Mϕ compared to the mice that received *ΔdgkA L. reuteri* in the intestinal mucosa. Also, $CD4^{+}CD25^{+}Foxp3^{+}$ T-reg cells were increased with WT *L. reuteri* treatment in intestinal mucosa. These findings provide a deeper mechanistic understanding of intestinal immunomodulatory probiotics and microbiome: host interaction.

## One carbon metabolism nutrients in relation to mucosa-associated gut microbiome in healthy individuals.

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**Background:** Folate plays an important role in one-carbon metabolism and nucleotide synthesis, and therefore in colorectal carcinogenesis. Folate, methionine, betaine, and choline are dietary methyl donors, whereas vitamins B2, B6, and B12 act as cofactors in one-carbon metabolism. **Aims:** We examined the association between dietary intake of methyl donors and one carbon metabolism cofactor nutrients and the composition of gut microbiota. **Methods:** A total of 21 men (age 50-75 years old, 71% white) were included in this cross-sectional study. Study participants underwent a screening colonoscopy and were found to have grossly normal appearing colon mucosa. We obtained a total of 98 snap frozen colonic mucosa biopsies of the rectum, sigmoid, descending, transverse, ascending, and cecum from these individuals. Microbial DNA was extracted from colonic mucosa and subsequently amplified and sequenced for 16S rRNA V4 region using the Illumina MiSeq platform. We utilized a BLOCK Food Frequency Questionnaire to estimate daily consumption of foods and nutrients (energy-adjusted). We used UPARSE and SILVA database for operational taxonomic unit (OTU) classification. We compared alpha-diversity (observed OTU and Shannon index), beta-diversity (Weighted UniFrac principal coordinates analysis), and relative abundance of bacterial phylum and genus by low or high consumption of dietary folate, methionine, betaine, choline, vitamins B2, 6 and 12 using the median consumption as the cutoff point for each nutrient. Reported *P* values were adjusted for multiple testing using false discover rate (FDR). FDR *P*-values < 0.001 indicated statistical significance. **Results:** Dietary consumptions of methionine, betaine, and choline were not significantly associated with gut microbiota richness or evenness. However, higher consumptions of vitamins B2, B6, and B12 were associated with higher alpha diversity and evenness (Shannon index $P < 0.0001$). The bacterial composition significantly differed by high or low consumption of total folate, methionine, betaine, vitamins B2, B6, and B12 ($P = 0.001$). At the phylum level, individuals who had higher dietary consumption of methionine, betaine, and choline had significantly lower abundance of Firmicutes (FDR $P < 0.0005$); individuals who had higher consumption of vitamins B2, B6, and B12 had higher abundance of Verrucomicrobia (FDR $P < 0.0001$) compared with individuals who had lower consumption of those nutrients respectively. At the genus level, higher consumption of vitamins B2 and B12 was associated with increased abundance of genus *Akkermansia* and *Faecalibacterium* (FDR *P* value < 0.001) compared with lower consumption. **Conclusion:** Consumption of dietary nutrients related to one-carbon metabolism are associated with abundant beneficial bacteria as well as increased diversity, and therefore may influence mucosa-associated gut microbiome composition. Future studies are warranted to investigate the role of gut microbiota in DNA methylation and other epigenetic events.

## *Clostridium difficile* infection (CDI) patient guts are frequently colonized by caspofungin-resistant strains of *Candida glabrata* in Houston, Texas

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Background: *Candida glabrata* is the second most common cause of invasive candidiasis in the United States. The echinocandin class of antifungals, including caspofungin has become the preferred therapy for invasive candidiasis due to *C. glabrata* and other species demonstrating decreased azole susceptibility. Caspofungin resistance has been uncommon, but reports suggest that the incidence is increasing, particularly among *C. glabrata* isolates. The dysbiosis associated with *Clostridium difficile* allows for overgrowth of *Candida* spp. However, the prevalence of *C. glabrata* in stool of CDI patients is not well studied. Therefore, our objectives were to investigate the incidence of potentially pathogenic species of *C. glabrata* in stool samples of CDI patients.

Method: We collected 1,241 CDI patient stool samples from two large hospitals in Houston, Texas and enrich the samples in brain heart infusion (BHI) broth at 37C for 48-72 hours and then sub-cultured onto selective HardyChrom *Candida* agar and incubated at 37C for 48 to 72 hours. Characteristic *Candida* colonies were stocked in cryovials and kept at -80C for further analyses. Isolates were then identified by multiplex PCR. *C. glabrata* isolates were screened for caspofungin resistance on Muller-Hinton agar (with 8.0 ug/ml)

Result: Overall, 14.8% (184/1241) samples were culture positive for *Candida* spp. The predominant species was *C. glabrata* (9.2 %) followed by *C. albicans* (2.3%), *C. tropicalis* (1.6%), *C. parapsilosis* (1.2%), *C. krusei* (0.6%) or not speciated (6.9%). The majority of C. glabrata isolates (70.2%; 80/114) were caspofungin resistant.

Conclusion: The result of this study showed that colonization of *C. glabrata* is common in patients with CDI and could be a source of antifungal-resistant pathogens.

## *Lactobacillus reuteri* suppresses IL-8 production by IL-1β-stimulated intestinal epithelial cells via a reactive oxygen species (ROS)-dependent mechanism

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**Background & Hypothesis:** Inflammatory bowel diseases are chronic pathologies of the intestine characterized by increased cytokine production and immune cell infiltration that result in damage to the epithelial tissue. In particular, elevated mucosal and serum concentrations of the neutrophil chemoattractant interleukin-8 (IL-8), and subsequent accumulation of neutrophils in inflamed tissue are often correlated with disease severity. IL-8 expression is under control of the NFκB promoter, and its secretion by intestinal epithelial cells is driven by interleukin-1β (IL-1β) and oxidative stress. Previously, our group has shown that the human commensal *Lactobacillus reuteri* ATCC PTA 6475 reduces inflammation and tissue damage in a mouse model of acute colitis and reduces tumor burden in a mouse model of inflammation-associated colorectal cancer. In both models, *L. reuteri* administration reduced expression of keratinocyte chemoattractant (KC), the mouse homologue of IL-8. However, in an intact animal, it is difficult to decipher the specific cross-talk between *L. reuteri* and epithelial cells. Given the proximity of *L. reuteri* to host epithelium, we speculate that bacteria-epithelial communication may have broader implications for intestinal homeostasis. *L. reuteri* can produce several antioxidant metabolites (*e.g.* glutathione, γ-glutamyl-cysteine), and other Lactobacillus species reduce production of reactive oxygen species (ROS) by mammalian cells. Thus, we propose that *L. reuteri* secretes factor(s) that can reduce IL-8 secretion by human intestinal epithelial cells via modulation of ROS metabolism. **Methods & Results:** For studies with human intestinal epithelial cells (HT-29), *L. reuteri* conditioned media was generated by sterile filtration of semi-defined medium (LDM3) cultured with bacteria. HT-29 cells were stimulated with IL-1β to induce IL-8 production and immediately treated with 1, 5, 10, 25, 50, 75, or 100%-strength conditioned media. IL-8 in the supernatants was measured by ELISA. We observed dose-dependent IL-8 suppression, with IL-1β alone eliciting a 100-fold increase in IL-8, and co-treatment with IL-1β and 100% conditioned media providing complete suppression. We assessed NFκB activity by transfecting HT-29 cells with a luciferase reporter. IL-1β alone increased promoter activity ~100-fold, while co-treatment with 50% conditioned media reduced activity to ~35-fold above baseline. In order to examine the potential role of ROS-intermediates, we stimulated IL-8 production in HT-29 cells by addition of hydrogen peroxide. In this model, we also observed dose-dependent suppression of IL-8. IL-8 gene expression (*CXCL8*) was examined in peroxide-stimulated cells treated with 50% conditioned media. Peroxide treatment alone increased IL-8 expression 3.5-fold, while co-treatment with 50% conditioned media resulted in a 4-fold reduction in expression. We also examined IL-8 output in the more physiologically-relevant model of human jejunal enteroid monolayers. For these studies, conditioned media was generated by incubating *L. reuteri* in Advanced DMEM. Enteroids were deprived of key media antioxidants, including N-acetylcysteine, catalase, superoxide dismutase, and glutathione, which allowed them to produce IL-8 in response to IL-1β and peroxide. We observed 100-fold induction of IL-8 with peroxide and 2-fold induction with a low dose of IL-1β. IL-8 output was returned to baseline or lower upon *L. reuteri* conditioned media treatment. **Conclusion:** Together, these data suggest that *L. reuteri* can suppress IL-8 secretion in human intestinal epithelial cells via a ROS-dependent mechanism. These studies provide new insights for both probiotic and pharmacologic treatment strategies for IBD.

## Antimicrobial susceptibility assessment of clinical & environmental *Clostridium difficile* isolates in relation to CRISPR-Cas

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**Background**: Clostridium difficile is the leading cause of hospital acquired infection in the US and worldwide tagged with an urgent threat level for the development of drug resistance. Antibiotic resistance coupled with emergence of hypervirulent drug resistant strains harbors the potential for pandemic outbreak. Mutant selection and acquisition of resistant genes are one of the common mechanisms for the development of antibiotic resistance. This current study highlights the CRISPR Cas associated adaptive immune system and aims to assess the differences in antibiotic susceptibility patterns in C. difficile based on the presence or absence of CRISPR Cas system stratified by source of isolation.

**Methods:** Previously isolated and ribotyped C. difficile isolates were CRISPR typed based on the presence of Cas1 and Cas2 genes which are the markers for the presence of functional CRISPR Cas system. A total of 93 Cas positive and 113 Cas negative isolates was tested in this study. 48-hour MIC was determined from the overnight culture of each C. difficile strains using broth microdilution method for 8 clinical antibiotics (vancomycin, metronidazole, fidaxomicin, ridinilazole, cefepime, meropenem, clindamycin and levofloxacin). MIC 50, MIC 90 and geomean of MICs were calculated for each antibiotic. 48-hour MIC from a standard strain was used to compare the antibiotic susceptibility pattern.

**Results:** No marked difference in the MIC 50, MIC 90 and geomean of the MICs for the antibiotics was observed between the Cas positive and Cas negative strains but in general the Cas negative isolates were slightly less susceptible than the Cas positive samples. Presence of both Cas1 and Cas2 genes were found to be a determinant of the functional status of the CRISPR Cas system. A significant association was found between the antibiotic susceptibility pattern and presence of CRISPR Cas system ($p<0.001$). The odds of reduced susceptibility to at least one antibiotic was found to be 3.65 times higher in the Cas negative strains as compared to the Cas positive strains. There was no significant difference in the MICs by ribotype and source of isolation.

**Conclusion:** Functional CRISPR Cas system might have the potential to protect against development of antibiotic resistance in commonly used clinical antibiotics.

## Association of CRISPR-Cas and antimicrobial susceptibility of gut pathogen *Clostridium difficile*

Irtiza Hasan, Tasnuva Rashid, Khurshida Begum, M. Jahangir Alam, Kevin W. Garey

University of Houston College of Pharmacy, Houston, TX

**Background**: *Clostridium difficile* is the leading cause of hospital-acquired infection in the United States and worldwide, tagged with an urgent threat level for the development of drug resistance. Antibiotic resistance coupled with the emergence of hypervirulent drug resistant strains harbors the potential for a pandemic outbreak. Mutant selection and acquisition of resistant genes are some of the common mechanisms for the development of antibiotic resistance. This current study highlights the CRISPR-Cas associated adaptive immune system and aims to assess the differences in antibiotic susceptibility patterns in *C. difficile* based on the presence or absence of CRISPR Cas system stratified by source of isolation.

**Methods:** Previously isolated and ribotyped *C. difficile* isolates were CRISPR typed based on the presence of Cas1 and Cas2 genes which are the markers for the presence of functional CRISPR Cas system. A total of 93 Cas positive and 113 Cas negative isolates was tested in this study. 48-hour MIC was determined from the overnight culture of each *C. difficile* strains using broth microdilution method for 7 clinical antibiotics (vancomycin, metronidazole, fidaxomicin, cefepime, meropenem, clindamycin, and levofloxacin). MIC 50, MIC 90 and geomean of MICs were calculated for each antibiotic. 48-hour MIC from a standard strain was used to compare the antibiotic susceptibility pattern.

**Results:** No marked difference in the MIC50, MIC90 and geomean of the MICs for the antibiotics were observed between the Cas positive and Cas negative strains. But in general, the Cas negative isolates were slightly less susceptible than the Cas positive strains. The presence of both Cas1 and Cas2 genes were found to be a determinant of the functional status of the CRISPR
Cas system. A significant association was found between the antibiotic susceptibility pattern and presence of CRISPR Cas system ($p<0.001$). The odds of reduced susceptibility to at least one antibiotic was found to be 3.65 times higher in the Cas negative strains as compared to the Cas positive strains. There was no significant difference in the MICs by ribotype and source of isolation.

**Conclusion:** Functional CRISPR Cas system might have the potential to protect against the development of antibiotic resistance in commonly used clinical antibiotics.

## Goblet cell ER stress is alleviated by *Bifidobacterium dentium* secreted products

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**Background:** Endoplasmic reticulum (ER) stress is induced by the increase of protein misfolding in the ER, thereby compromising protein production and secretion. This can lead to decreases in the mucus barrier, particularly the protein Muc2, which normally protects the intestinal epithelium. As result, ER stress in intestinal goblet cells has been linked with inflammatory bowel disease (IBD). *Bifidobacterium* has been postulated to have many beneficial effects on its host which includes the promotion of mucin from goblet cells. However, little work has been done in regards to the effects of *Bifidobacterium* on goblet cell ER stress and its role in upkeep of the mucosal barrier. We hypothesized that secreted factors from *Bifidobacterium dentium* would downregulate ER stress genes and modulate the unfolded protein response (UPR) to promote Muc2 secretion. **Methods**: *B. dentium* was grown in MRS and fluorescently tagged using CFDA-SE. Fluorescent bacteria were allowed to adhere to T84 cells for 1 hour at 37°C, washed, and then imaged on the Nikon Eclipse microscope. To generate *B. dentium* secreted products or "conditioned media", *B. dentium* was grown in MRS and sub-cultured into a fully defined minimal media termed LDM4. This "conditioned media" was used for all subsequent experiments. Additionally, *B. dentium* bacteria from the LDM4 cultures were UV-irradiated. The mucin-producing cell line T84 were grown as monolayers and treated with the glycosylation inhibitor tunicamycin, which stimulates ER stress. Muc2 secretion was then measured by alcian blue staining of the supernatant and Muc2 expression was examined by qPCR. ER stress genes were also examined by qPCR. Cell viability was also measured at 24h hours post tunicamycin treatment *via* resazurin spectrophotometry assays and trypan blue staining. **Results**: We demonstrate that CFDA-SE fluorescent *B. dentium* binds and adheres to the mucus layer of mucin-producing colonic epithelial cells. Through qPCR analysis we revealed an upregulation of Muc2 gene expression in goblet cells treated with *B. dentium* conditioned media, in line with a modulation of Muc2 by mucin-adhering *B. dentium*. Through stimulation of cells with N-glycosylation inhibitor tunicamycin, we show an induction of UPR response genes, such as CHOP and sXBP1, which are attenuated upon treatment with *B. dentium* conditioned media. Furthermore, tunicamycin was seen to suppress Muc2 secretion. Muc2 secretion was subsequently recovered with *B. dentium* conditioned media co-treatment. Additionally, trans-epithelial barrier integrity was monitored with tunicamycin stimulation and upon treatment with conditioned media. **Conclusions**: Collectively this data demonstrate modulation by *B. dentium* secreted compounds to regulate the UPR response and Muc2 synthesis and secretion. These results could demonstrate a role of *B. dentium* in attenuation of ER stress after pathogenic disruption of the mucus membrane or in cases of inflammatory bowel disease. Indeed, this secretion of Muc2 and repression of ER stress genes by *B. dentium* could benefit the commensal microbes by sustaining the mucus layer adjacent to the epithelium. Consequently, this study demonstrates a link between ER Stress and mucosal barrier disruption while suggesting a potential therapeutic purpose of *B. dentium* in colitis and IBD.

## Vancomycin-resistant *Enterococcus* (VRE) with *vanA* gene common in *Clostridium difficile* infected patient guts

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**Background:** Gut colonization and overgrowth of vancomycin-resistant *Enterococcus* (VRE) is common in patients with *C. difficile* infection (CDI). However, rates of VRE overgrowth and susceptibility is not well understood. The objectives of the study were to isolate and characterize VRE strains from stool samples obtained from patients with CDI.

**Methods:** A total 219 stool samples were collected from patients with CDI hospitalized at three hospitals in Houston, TX as part of our state-wide multidrug resistant organisms surveillance. Samples were enriched in brain heart infusion broth (BHI) and sub-cultured onto selective enterococcus agar plate with 6ug/ml vancomycin. *Enterococcus* isolates were identified and characterized for vancomycin resistance genes (*vanA, vanB, vanC1, vanC2/3*) using a multiplex PCR. Vancomycin minimum inhibitory concentration (MIC) were determined by micro-dilution method using BHI broth at 37C.

**Results:** Overall, 54 of 219 samples (24.6%) grew *vanA* gene positive *Enterococcus faecium* strains (range from the three hospitals: 20.2%-28.8%). No *vanA* gene detected among the *E. faecalis* isolates (18 isolates tested). Nine other *Enterococcus* isolates were positive for *vanA* gene (4.1% samples). Thirty nine other *Enterococcus* spp isolates (17.8% samples) were also positive for *vanC1* gene. Minimum inhibitory concentration (MIC) of 63 *vanA* gene positive *E. faecium* isolates were highly resistant to vancomycin (MIC range: 250 to >2000 mg/L).

**Conclusion:** We found a high incidence of potentially pathogenic vancomycin-resistant *E. faecium* in patient's stool samples from three different Houston area hospitals.

**A model to study cross-talk between the human intestinal epithelium and immune cells.**

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Cross talk between the intestinal epithelium and immune cells plays a crucial role in determining the innate immune response to pathogens as well as influences the absorptive and secretory functions of the epithelium. While human intestinal enteroids (HIEs) are an excellent, physiological model to study the innate immune response of the human intestinal epithelium, the absence of immune cells in HIEs precludes the ability to study the role of crosstalk between the intestinal epithelium and immune cells. Intestinal macrophages play major roles in diverse functions including regulating immune response and gut homeostasis. Further, different functions of macrophages are carried out by specialized subtypes such as pro-inflammatory (M1), and anti-inflammatory (M2) macrophages, to name a few. Here we confirm the development of an HIE-macrophage coculture system[1], which will allow us to model and analyze the interactions between HIEs and immune cells, and further includes different subtypes of macrophages. Establishing a HIE-immune cell coculture will allow us to accurately identify and evaluate the molecular players that regulate cross talk in the intestinal epithelium during infection and homeostasis.

## Household Income and Education Influence Pediatric Inflammatory Bowel Diseases in the Houston Metro Area

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**Background:** The incidence of pediatric inflammatory bowel diseases (PIBDs: Crohn's disease [CD], ulcerative colitis [UC], and indeterminate colitis [IC]) is on the rise both in developed and developing countries adopting the "Westernized" environment. Yet, the critical risk factors for the pathogenesis of IBD or PIBD secondary to such environmental changes are not well understood. Demographic characteristics of PIBD may improve our understanding of their developmental origins and aid in prevention. The diverse racial and ethnic population of the Houston Metro area is an ideal location for investigating such demographics. Within this area, Texas Children's Hospital (TCH) is the major center capturing over 80% of PIBD cases. Prior work on PIBD demographics indicated that African American children present later and more commonly with CD while Hispanics suffer from UC or IC more often. Studies on young adult IBD patients indicated that persons with IBD were more likely to have higher income and attain a post-secondary school degree or receive a diploma more commonly than controls. **Methods:** We conducted a geography focused study on 488 PIBD patients diagnosed between 2010 and 2015 at TCH. The cases occurred within region 12 of the Texas Commission on Environmental Quality (TCEQ), which includes 13 counties around Houston. An annual incidence map was created by ZIP code of residence at diagnosis by using the American Community Survey 5-year estimate of population between 2011 and 2015 from the U.S. Census Bureau and utilizing the ArcGIS program. We also examined the correlation between ZIP code based demographic variables and PIBD incidence. Statistical analyses were performed on GraphPad Prism V7. **Results:** The PIBD population included 272 Non-Hispanic Whites (55.7%), 84 Hispanics (17.2%), 78 African-Americans (16.0%), 28 Asians (5.7%), and 26 Unknown or Other (5.3%). There were 266 cases of CD (54.5%), 147 cases of UC (30.1%), and 75 cases of IC (15.4%). Chi-squared based testing revealed that Hispanic children were more likely to be diagnosed with UC ($p<0.01$) and IC ($p<0.03$) compared to other races/ethnicities. In respect to ZIP code based demographics, a significant positive correlation ($r=0.35$, $p<0.0001$) between household income and PIBD incidence was observed, similar to UC ($r=0.23$, $p<0.0001$) and CD ($r=0.22$, $p=0.0004$). Additionally, ZIP codes with majority college educated adults had a higher incidence of PIBD than ZIP codes with majority high school educated adults ($p<0.0001$). Mean household income, however, similarly correlated with adult education. Pediatric UC cases were more common in ZIP codes where the majority of adults were high school educated ($p = 0.0036$). On the contrary, pediatric CD cases were more common in ZIP codes where the majority of adults were college educated ($p<0.0001$). **Conclusions:** Our findings lend further support for Hispanic children more commonly presenting with UC and IC in the Southern USA. A significant positive correlation between household income and PIBD incidence was observed. Adult education differentially correlated with CD and UC. We speculate that household income and/or adult education related dietary differences may be important in the developmental origins of PIBD in large metro areas, such as Houston. Based on our murine model studies, we hypothesize that rapid food composition change may be a common PIBD risk attribute of the demographic variables identified (household income has been associated with dietary diversity). Higher income and higher education in families may increase risk for pediatric CD, while higher income but lower education may increase the risk of pediatric UC. These conclusions can focus further research towards the nutritional origins of IBD and may guide the generation of diet based prevention against this highly morbid disease group.

## Reconstitution of Aged Mice with Young Microbiome Enhances Post-Stroke Recovery

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**Introduction:** Age is the most important non-modifiable risk factor for stroke. With increasing life expectancy, the prevalence of stroke will continue to increase. Stroke is the leading cause of adult disability, and the elderly have higher mortality and poorer recovery than younger patients. Currently, there was no treatments available to improve functional recovery after stroke. Emerging evidence suggests that the composition of gut microbiota changes with age and stroke. Interestingly, in our recent studies we have found that transplantation of young biome into aged mice reduces mortality and improves functional recovery if given prior to an induced stroke. However, as stroke is an unpredictable event, we wanted to assess the potential of biome reconstitution **after** an ischemic injury as a potential protective strategy. We hypothesized that transplantation of a young microbiome and production of beneficial bacterial metabolites would enhance post-stroke recovery in aged mice, even when performed several days after injury. **Methods:** Aged C57BL/6 male mice (18-20 months) were subjected to a 60 minute middle cerebral artery occlusion and assigned to two groups given: 1) young fecal microbiome (young FMT) or 2) aged fecal microbiome (aged FMT). To further elucidate the role of short chain fatty acids (SCFAs), we performed a separate cohort of aged mice that are gavaged with: 1) vehicle or 2) a cocktail of SCFA producers with inulin (substrate). Behavioral outcomes were examined for 2 weeks after stroke. Changes in both brain and the gastrointestinal tract were further examined by flow cytometry and quantitative real-time PCR. **Results:** Aged mice that received young FMT had improved post-stroke recovery in 1) the open field when measured for spontaneous locomotor activity (n=5-7, $P<0.05$); 2) decreased immobility in the tail suspension test (n=5-7, $P<0.05$), and 3) improved cognitive performance in the novel object recognition test (n=5-7, $P<0.05$). To determine the mechanisms underlying these beneficial effects on recovery, we examined alterations in T cells that are associated with microbiota or inflammation in aged mice. Our results showed that aged mice given young FMT had a significant increase in regulatory T ($T_{reg}$) cells in the gut and decreased pro-inflammatory γδ T cells in the brain compared to aged mice that received aged microbiome (n=5-7, $P<0.05$). These results indicate that young FMT can shape T cell activity and alter homeostasis in both the gut and brain. Since FMT can directly affect the intestinal epithelium, we then examined several key genes in the intestinal epithelial cells after FMT. Aged animals receiving young FMT had enhanced gene expression of mucins, an important barrier defense mechanism. Fecal SCFAs were higher in aged mice receiving young FMT compared to aged mice receiving aged FMT. Additionally, aged animals transplanted with selective SCFA-producing bacteria also had improved functional outcomes. **Conclusion:** This is the first study to show the therapeutic potential of young microbiome and microbial products given after stroke in aged mice. The benefit of young microbiome appears to be mediated by improved gut homeostasis, mucin production, and reduction in inflammation both in the CNS and systemically. Although SCFA production appears to be a key contributor to recovery, future studies are needed to further develop and refine therapeutic strategies to improve recovery in stroke patients.

## Cleavage of Glucagon-like peptide-1 by microbial gelatinases and its role in metabolic disease

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Commonly referred to as “twin epidemics,” Type 2 Diabetes Mellitus (T2DM) and obesity are two hallmarks of metabolic disease and current global public health concerns, burdening 422 and 650 million individuals worldwide, respectively (WHO, 2014 & 2016). The gastrointestinal hormone glucagon-like peptide-1 (GLP-1), stimulated by nutrient ingestion, is currently targeted as a therapeutic agent for T2DM due to the many relevant consequences of its release from enteroendocrine cells: increasing insulin biosynthesis, insulin secretion, and insulin sensitivity, while decreasing glucose production, glucagon secretion, appetite, and gastric emptying. Scientific work over the past decade has established connections between the gastrointestinal (GI) microbiota and host metabolism. In particular, research has indicated that the microbiota is capable of modulating the presence of various GI hormones, including GLP-1, but the mechanisms responsible for these effects remain unclear. The goal of the current work is to understand the method(s) by which individual members of the microbiota may be contributing to the development of T2DM and weight gain, and to harness this knowledge for diagnostic and therapeutic purposes. Identification of GLP-1 modulatory strains was accomplished by an in vitro screening pipeline using thousands of bacterial strains isolated from healthy human fecal and breast milk samples on the GLP-1 producing NCI H716 cell line, followed by measurement of GLP-1 release by ELISA. Screenings resulted in the identification of several bacterial species capable of decreasing GLP-1 levels, including *Enterococcus faecalis, Clostridium perfringens*, and *Bacillus subtilis*. Based on cell-free assays using genetic mutants of *E. faecalis*, we hypothesize that Gelatinase (GelE) is cleaving GLP-1, thereby decreasing functional GLP-1. Ongoing studies include purifying GelE to confirm the observed GLP-1 inhibitory activity, as well as employing a Caco-2 transwell model to demonstrate relevance of GLP-1 inhibition in the GI tract. Ultimately, we hope to characterize this novel host-microbe interaction and understand its implications in the development of T2DM and obesity.

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# Helicobacter pylori antibiotic-resistance patterns among non-US born and US-born patients from an ethnically diverse population who failed first-line therapy

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**Introduction:** *Helicobacter pylori* treatment failure is becoming more common as antibiotic resistance increases worldwide. Reasons for treatment failure include prior antibiotic exposure for other infections, poor patient compliance, and being born in a location with high rates of resistant strains. The aim of our study is to determine antibiotic resistance among patients who have failed at least one treatment regimen and to relate these results to the patient's country of origin. **Methods:** This is a case series of 55 patients who had failed at least one regimen from 2009 through 2016 at a single large academic center in Houston which sees an ethnically diverse population of patients. We collected gastric biopsies from 55 patients undergoing esophagogastroduodenoscopy. The Epsilometer test was used to determine the minimum inhibitory concentrations of amoxicillin, clarithromycin, metronidazole, levofloxacin, and tetracycline, and culture analyses to detect *H pylori* were performed. Patients were stratified based on continent of origin and US-born (2nd generation African, Latino, African American, and White) and non-US-born (African, East Asian, Latino, and Middle Eastern). **Results:** The prevalence of resistance to clarithromycin was 82% (45/55), to metronidazole 82% (45/55), to levofloxacin 71% (39/55), and to amoxicillin 7% (4/54). Only 1 sample was resistant to tetracycline. Multi-drug resistance was seen in 89.1% of patients. Resistance to more than 3 antibiotics was present in 50% of the samples. Non-US born strains had significantly higher rates of clarithromycin and metronidazole resistance ($p<0.05$), and US-born strains had higher rates of resistance to levofloxacin. When stratified by continent, patients from Asia had higher resistance rates to clarithromycin, metronidazole, and amoxicillin, compared to North America and Africa. Patients from Latin America exhibited higher rates of dual clarithromycin and metronidazole resistance. Of the 55 patients, we were able to obtain prior treatments for 45 patients, and 33% of the 45 patients had triple therapy with clarithromycin, metronidazole and PPI, compared to 67% who had quadruple therapy with clarithromycin, metronidazole, PPI, and amoxicillin. Patients previously treated with quadruple therapy exhibited higher rates of resistance to amoxicillin ($p<0.05$) whereas no statistically significant difference was found for the other antibiotics tested. **Conclusion:** In our cohort, non-US born strains of H. pylori have a higher prevalence of dual resistance to clarithromycin and metronidazole, especially those from Latin America, while US-born strains had higher resistance rates to levofloxacin. These data suggest that retreatment with H. pylori in both US and non-US born should be with tetracycline, PPI, metronidazole, and bismuth. US-born patients are likely to fail quinolone salvage therapy. Further studies will require larger sample sizes to better characterize definitive country-specific associations and local resistance patterns.

Table 1. Antibiotic-resistance patterns of *H pylori* Strains isolated in patients who failed at least one treatment regiment during 2009 to 2016

| | Total N=55 | | US-Born N=28 | | Non-US Born N=27 | | P-Value |
|---|---|---|---|---|---|---|---|
| All susceptible | 1 | 1.8% | 0 | 0.0% | 1 | 3.7% | 0.309 |
| Resistant to One | 5 | 9.1% | 2 | 7.1% | 3 | 11.1% | 0.609 |
| Resistant to Two | 20 | 36.4% | 9 | 32.1% | 11 | 40.7% | 0.511 |
| Resistant to Three | 29 | 52.7% | 17 | 60.7% | 12 | 44.4% | 0.230 |
| Any clarithromycin resistance | 45 | 81.8% | 23 | 82.1% | 22 | 81.5% | 0.950 |
| Any metronidazole resistance | 45 | 81.8% | 22 | 78.6% | 23 | 85.2% | 0.528 |
| Any amoxicillin resistance* | 4 | 7.4% | 1 | 3.6% | 3 | 11.5% | 0.289 |
| Any levofloxacin resistance | 39 | 70.9% | 25 | 89.3% | 14 | 51.9% | 0.003 |
| Any tetracycline resistance** | 1 | 2.9% | 1 | 7.7% | 0 | 0.0% | 0.193 |
| Clarithromycin + metronidazole | 10 | 18.2% | 1 | 3.6% | 9 | 33.3% | 0.005 |
| Clarithromycin + levofloxacin | 7 | 12.7% | 6 | 21.4% | 1 | 3.7% | 0.051 |
| Metronidazole + levofloxacin | 3 | 5.5% | 2 | 7.1% | 1 | 3.7% | 0.582 |
| Clarithromycin + metronidazole + levofloxacin | 25 | 45.5% | 15 | 53.6% | 10 | 37.0% | 0.221 |

*Total sample size of 54, Non-US Born sample size 26
**Total sample size of 35, Non-US Born sample size 22, US-Born sample size 13

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# *Lactobacillus reuteri* DSM 17938 modulates gut microbiota in newborn mice by boosting beneficial metabolites

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**BACKGROUND:** Early administration of *Lactobacillus reuteri* DSM 17938 (LR) prevents necrotizing enterocolitis and Treg-deficiency-associated autoimmunity in mice. In humans, LR reduces crying time in breastfed infants with colic, severity of acute diarrheal illnesses in infants and toddlers, and pain severity in older children with functional abdominal pain. In healthy breastfed newborns with evolving microbial colonization, it is unclear whether early administration of LR can modulate gut microbiota and their metabolites in such a way as to promote homeostasis in the normal developing gut. **OBJECTIVES:** To analyze the effect of early oral feeding LR on gut microbiota and metabolites in plasma and stool, and the mucosal T cell subsets in healthy dam-fed mouse pups. **METHODS:** C57BL/6J male mice received LR ($10^7$ CFU/day, daily) by gavage at 8 days of life for 2 weeks before weaning. The composition of the stool microbiota was analyzed using high-throughput sequencing analysis of PCR-amplified 16s rRNA genes. Bacterial diversity and species composition were assessed using the QIIME. Immune cells isolated from the mesenteric lymph nodes and intestines were stained with T cell markers analyzed by flow cytometry. Metabolomics data in plasma and stool were analyzed by Metabolon, Inc. We used in-house program developed for integrated analysis.
**RESULTS:** LR induced a shift of the microbial composition, marked by a reduced number of observed species and an increased relative abundance of phyla *Firmicutes* and *Bacteroidetes*. The relative abundances of genus *Parabacteroides (p_Bacteroidetes)* and *Anaerotruncus (p_Firmicutes)* increased. *Parabacteroides* species have been reported to produce bacteriocins which protect against the invasion of pathogens. Gavage feeding LR to healthy breastfed mice unexpectedly exerted a major impact on the plasma and stool metabolomics. The upregulated metabolites participate in several major networks associated with amino acid metabolism, including arginine/citrulline and polyamine metabolism. The identified pathways have been shown to be beneficial for intestinal restitution and barrier function. Key augmented metabolites were involved in the urea, tricarboxylic acid, and methionine cycles. The most remarkable increases were in the levels of N-acetylornithine and N-acetylglycine, two metabolites which correlated with *Parabacteroides*, *Lactobacillus*, *Anaerotruncus*, and *Clostridium*. LR also affected lipid metabolism, by lowering the levels of many lipids in plasma and upregulating secondary bile acid levels in stool. In addition, LR increased the % of $CD62L^+$T cells in the intestinal mucosa. CD62L (L-selectin) is highly expressed in naïve T cells, which utilize CD62L expression to facilitate immune surveillance by programming T cells. **CONCLUSIONS:** Early oral administration of LR to healthy breastfed mice led to changes which would be predicted to be beneficial to overall general health, and promote immune surveillance.

This study was supported, in part, by BioGaia AB (Stockholm, Sweden), and NIH R01 AT007083.

## High-throughput functional genetics of the host landscapes that underlie development of gut dysbiosis using *C. elegans*

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Animals have evolved intimate symbiotic relationships with a consortium of gut microbes that functionally extend the host genome and influence host health. When well-tended, this microbiome confers great benefit to its host. Under states of persistent imbalance the microbiome can contribute to a variety of dystrophies, including inflammatory bowel disease (IBD) and the like. Many studies of IBD in humans and mice have shown that pathologic development of the disease is dependent on the microbiome and involves a complex interplay of host genetic risk factors. These structural imbalances in the microbiome (so called dysbiosis) are often at odds with the patient's immune system and result in frequent infections, hospitalizations that lead to heavy use of immunosuppressive medications and antibiotics, which may predispose them to colonization with antimicrobial-resistant organisms. Indeed, blooms of inflammation-tolerant, antibiotic-resistant Proteobacteria like *E. coli* frequent the microbiome of IBD patients. Despite the incredible impact of IBD, there is still much to be understood about the interactions between host and microbial risk factors in the pathophysiology. Part of the challenge comes from the feasibility and throughput of current whole organismal models to rapid genetic screening systems. To overcome these challenges, we have developed the genetically robust and high-throughput amenable nematode *Caenorhabditis elegans* as a model of intestinal dysbiosis. *C. elegans* exhibits conserved innate immune pathways, endocrine regulation of intestinal function and is colonized by a similar Proteobacteria-rich microbiome as observed in patients with dysbiosis. They are also easy to make 'germ-free', so microbiome composition can be completely and reproducibly controlled. Using this system, we have developed genetic lines that model key aspects of IBD pathophysiology, including impaired gut motility, bacterial overgrowth and/or over-activation of epithelial immunity. As a first test of the system, we demonstrate that *E. coli* isolated from IBD patients are better able to 3 and proliferate in the genetic compared to *E. coli* of control patients. They also differentially engage pathways involved in regulation of gut motility. Comparative genomic analyses of these 12 strains have helped to identify conserved microbial mediators of gut function in other systems that can now be functionally examined in this system. In order to examine the functional impact IBD genetic risk factors, we tested the impact of 136 RNAi knockdowns of their *C. elegans* orthologs on *E. coli* colonization levels in either wild-type or dysbiotic animals. For the wild-type background, under 10% of the knockdowns exacerbated colonization, compared to over 35% the already genetically susceptible dysbiotic animals. Interestingly, another 30% of the clones lead to amelioration of colonization levels in dysbiotic animals specifically, including genes involved in autophagy, lipid metabolism, cell death and stress resistance. Further, specific isoforms of autophagy genes appear to have opposite impacts on colonization levels. Examinations of the impact of these candidate genes on aspects of disease (motility and immune activation) are ongoing. Together, these studies highlight the utility of the *C. elegans* system for dissecting the combinatorial and complex nature of microbial and host genetic risk factors that interact influence the pathophysiology and severity of dysbiosis in IBD. Such an understanding is critical in order to develop effective microbiome-mediated or -directed intervention in the treatment of IBD and related diseases.

## Gut pathogen, *Clostridium difficile,* are highly prevalent in community shoe swabs

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Background: Environmental surfaces can be frequently contaminated with animal and bird fecal materials in any geographical locations. Spores of *C. difficile* can survive for months on fecally contaminated material. Shoe bottoms are in frequent contact with these materials and thus, can be contaminated frequently with *C. difficile* spores. To investigate shoes as source of *C. difficile* contamination on a worldwide basis, we collected shoe bottom swab samples from two unique geographic locations and cultured to isolate and characterize the *C. difficile* bacterium.

Method: As part of a hospital-wide surveillance effort, we collected shoe-bottom swabs samples from Mumbai, India (n=187) and Nuevo Laredo, Mexico (n=65). Samples were assessed for *C. difficile* using anaerobic enrichment culture and molecular methods. Suspected colonies from cycloserine cefoxitin fructose agar (CCFA) plates were identified and characterized by PCR (tpi, *tcdA, tcdB, cdtA, cdtB*) and strain typed using fluorescent PCR ribotyping.

Result: A total 101 of 187 (54.0%) shoe-bottom swab samples from India, and 22 of 65 (33.8%) from Mexico were culture positive for *C. difficile* of which 36.4% (India) and 16.9% (Mexico) samples were toxigenic (*tcdA* and *tcdB*) *C. difficile.* A total of 20 distinct ribotypes were identified from 72 *C. difficile* isolates tested from India. A total of 11 distinct ribotypes were identified from 20 isolates from Mexico. Predominant ribotypes were F106, F014-020, F005, and F002.

Conclusion: We have found a high prevalence of toxigenic *C. difficile* with diverse ribotypes from shoe bottom swabs in two geographical locations. Shoe bottom swabs may be a unique method to perform active surveillance in developing countries.

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Frontiers of the Microbiome Gut-Brain Axis

## Neonatal colonization of the gnotobiotic mouse intestine with human "infant-type" *Bifidobacterium* species modulates synapse-related gene expression, microglial maturation, and adult behavior

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**Background**: Colonization of the intestinal microbiota coincides with the organization of fundamental neural circuitry in the brain during early postnatal development. Microbe-mediated modulation of neural circuit patterning in the brain via the microbiome-gut-brain axis during neurodevelopment may have significant long-term implications that we are only beginning to appreciate. In humans, *Bifidobacterium* species (spp.) are detectable within the first week after birth, and are a predominant genus of the infant intestinal microbiota. These features, in addition to their ability to affect the gut-brain axis in adult rodent models, make *Bifidobacterium* spp. attractive models of the early-life gut microbiota. The central hypothesis of this research is that the early-life microbiota supports the functional organization of neural circuitry during postnatal development via modulation of synapse-related gene expression and microglial activation, resulting in long-term effects on adult behavior. **Methods:** Gnotobiotic mice were colonized at the neonatal stage with a simplified model of the human infant gut microbiota consisting of four "infant-type" *Bifidobacterium* species (BIF). Germ-free mice (GF) and mice neonatally-colonized with a complex, conventional murine microbiota (CONV) were used for comparison. Colonization patterns were confirmed and characterized by 16S ribosomal RNA (rRNA) gene sequencing. Postnatal expression of synapse-related genes in the cortex, hippocampus, and cerebellum was profiled via qPCR arrays at postnatal days 4, 10, and 20 in all three groups of mice. At the same timepoints, microglial maturation was characterized via flow cytometry and immunostaining, and synaptic functionality was assessed by immunostaining and *in vivo* electrophysiology. At 6-7 weeks of age, mice from each cohort were removed from the gnotobiotic isolators and underwent a battery of behavioral testing. **Results:** GF mice displayed an upregulation of synapse-promoting genes in the CNS, especially at P4. Expression of these genes was significantly down-regulated by both neonatal conventionalization and *Bifidobacterium*-colonization. Additionally, CONV and BIF mice had increased numbers of activated microglia relative to GF animals during early postnatal development (P4 and P10). During late postnatal stages (P20), GF animals also displayed abnormally decreased percentages of mature microglia. At the P20 timepoint, germ-free animals were shown to have increased synaptic density as measured by immunostaining and densiometric image analysis. In addition, we recorded from Purkinje cells in the cerebella of P20 mice to assess differences in functional synaptic output, and found that GF mice had a statistically significant decrease in firing rate relative to BIF mice. As adults, our behavior test data demonstrate that the early presence of a complex gut microbiome, or human "infant-type" *Bifidobacterium* species, affects long-term behavior (especially anxiety and memory), and shows that *Bifidobacterium*-colonization can selectively recapitulate the results observed when mice are colonized with the complex microbiota. **Conclusions:** We propose that postnatal microbial colonization promotes network refinement and functional organization of neural circuitry by down-regulating early expression of synapse-promoting genes, and by promoting the early activation of microglia during a critical window of synaptic refinement. Conversely, the lack of microbe-mediated control of gene expression and decreased microglial activation in GF animals ultimately results in deficits in circuit connectivity and a lack of synchronization of neuronal activity which leads to the aberrant behavioral phenotypes observed in adult GF mice. Importantly, selection of key species, like the four *Bifidobacterium* species employed here, has the capacity to reverse the deficits observed in GF mice when colonized at the neonatal stage.

**Texas Medical Center Digestive Diseases Center**
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# Statins Use and Overall Survival in Pancreatic Cancer Patients: A Systematic Review and Meta-analysis

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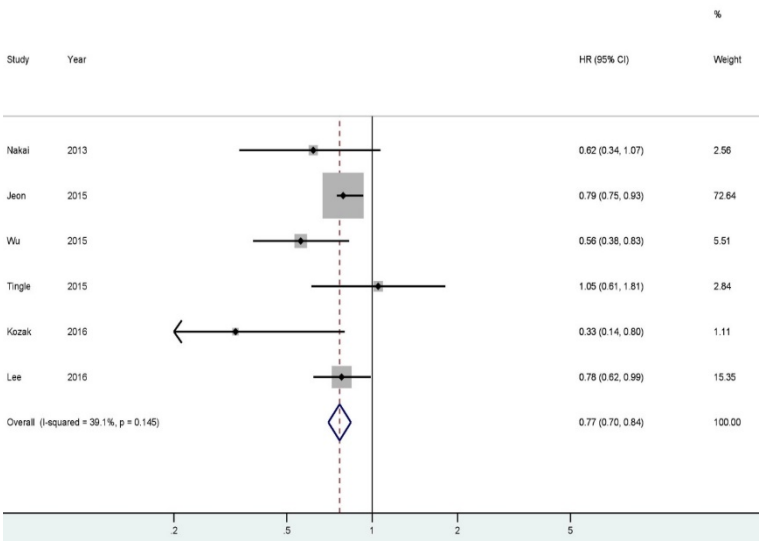
**Background:** Pancreatic cancer (PanC) is fatal cancer with poor five-year survival. It is projected to be the second leading cause of cancer-related deaths in the U.S. by 2020. Some experimental and clinical studies suggest antitumor properties of statins. The potential role of statins as a treatment to help improve overall survival (OS) of PanC patients is not established. We performed a systematic review and meta-analysis to examine the association between statin use and OS in PanC patients. **Methods**: We searched PubMed and Embase databases to identify original clinical trials and epidemiological studies evaluating the association of statin use and OS within PanC patient cohorts published from 9/1987 (FDA approval date) to 6/2016. Two investigators performed searches and abstractions from full papers published in English. We pooled results when there were ≥2 studies with comparable relative risk measures. We also estimated pooled risk estimates stratified by timing of statin use (prior to vs. after diagnosis only) and cancer stage (early vs. advanced). **Results:** We identified 8 eligible studies; 7 retrospective cohort studies and 1 randomized clinical trial (RCT), 4 were performed in the U.S with total PanC cohort sample sizes from 114-7813 (prevalence statin use: 7-51%), with 100% of study participants with pancreatic ductal adenocarcinoma. Two studies did not report relevant relative risk estimates including the single RCT performed in Korea (n=114 total PanC cases, 51% randomized statins post-diagnosis) which reported no OS benefit in crude analysis (median OS=6.6 vs. 8.9 months in statin vs. placebo, p=0.74). Pooled analysis of the 6 cohort studies reporting hazards ratios (HRs) that adjusted for variables like age, sex, treatment and cancer characteristics demonstrated modest significant prolonged OS in PanC patients ever using statins vs. PanC patients never using statins ($HR_{adj}$=0.77, 95% CI 0.70-0.84, p<0.05). (*Figure 1*) Subgroup analysis based on statin timing suggested similar OS benefit if statins use started before vs. started only after diagnosis in PanC patients ($HR_{adj}$=0.71, 95% CI 0.50-1.02, p=0.06 from n= 4 studies statins started pre-diagnosis vs. $HR_{adj}$=0.79, 95% CI 0.67-0.93, p<0.05 from n=2 studies for statins started after, respectively). Analysis by stage demonstrated potentially stronger OS benefit from statins in PanC patients with early stage disease ($HR_{adj}$=0.51, 95% CI 0.36-0.73, p<0.05 from n=2 studies for early stage vs. $HR_{adj}$=0.79, 95% CI 0.72-0.87, p<0.05 from n=4 studies for advanced stage, respectively). **Conclusion:** Our novel pooled analysis of cohort studies suggests statin use may improve OS in PanC patients especially in those with early disease. However, additional large prospective studies that examine type, dose, duration and timing of statin use are needed to determine their potential OS benefit.



## Validation of International Classification of Disease, 10th Version (ICD-10) codes for cirrhosis and its complications.

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**BACKGROUND:** Cirrhosis is a common problem and its prevalence is likely to increase over the next decade due to the aging of hepatitis C cohort and the rise in nonalcoholic fatty liver disease. Cirrhosis is also the most important risk factor for hepatocellular carcinoma (HCC). Administrative databases can be valuable sources of information for epidemiological, health services and outcomes research in cirrhosis. Previous studies found the International Classification of Diseases (ICD), 9th revision diagnostic codes for cirrhosis to be highly predictive of the presence of cirrhosis and its complications in medical records. With the transition from ICD-9 to ICD-10 codes, it is important to examine the validity of ICD-10 codes for cirrhosis and its complications.

**OBJECTIVE:** We sought to examine the accuracy of the ICD-10 codes for cirrhosis and its related complications (i.e. ascites, varices and hepatocellular carcinoma (HCC)) in identifying patients with these conditions in the medical records.

**METHODS**: We used the national Department of Veterans Affairs (VA) Corporate Data Warehouse administrative and clinical database to identify a random sample of 300 patients with at least one ICD-10 code from an inpatient or outpatient encounter for cirrhosis or any of its complication during FY2016 (Table of ICD-10 codes displayed on poster). A trained clinician abstracted the electronic medical record using a standardized, detailed abstraction form to determine the presence or absence of the key conditions, while blinded to the database coding. Clinical diagnoses were identified from progress notes, pathology, endoscopy, radiology, or laboratory reports. We calculated the positive predictive value (PPV i.e. the probability of having the clinical diagnosis of cirrhosis or one of its complications (as identified in medical records) among those with a corresponding ICD-10 code. We also calculated the negative predictive value (NPV), i.e., the probability of not having the clinical diagnosis among those who did not have a corresponding ICD-10 code.

**RESULTS:** The PPV for any cirrhosis ICD-10 code (with or without any complication codes) ranged from 90.1% to 93.4%. A total of 82, 48 and 55 patients had ICD-10 codes of varices, ascites and HCC. The PPV for varices, ascites, HCC were 90.2%, 87.5%, and 98.2% respectively. The corresponding NPVs were 71.7%, 71.7%, and 98.1% respectively.

**CONCLUSION:** The cirrhosis ICD-10 codes demonstrated excellent PPV (>90%) and NPV ranging from 72% to 98%. Our data show that ICD-10 codes can be reliably used to identify patients with cirrhosis in epidemiological, health services and outcomes research.

## Intestinal pathogen *Clostridium difficile* are highly prevalent in community soil environs

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Background: *C. difficile* spores can survive and disseminate in soil environs and act as a reservoir for human and animal colonization/infections. Although likely ubiquitous worldwide, the ecology and prevalence of *C. difficile* spores in soil as related to human health is poorly understood. The objective of this study was to isolate and characterize *C. difficile* from the top- and sub-soil.

Method**:** As part of a Texas state-wide surveillance effort, we collected top-soil (1 square foot of soil surface swab) and sub-soil (1 gram of soil collected from 1 inch below the surface) samples (total n=234; top n=117; sub n=117) from the same location in pairs. Samples were assessed for *C. difficile* using anaerobic enrichment culture and molecular methods. Suspected colonies of *C. difficile* from cycloserine cefoxitin fructose agar (CCFA) plates were isolated and then characterized by multiplex PCR (*tcdA, tcdB, cdtA, cdtB, and tpi* genes). PCR ribotyping was then performed to determine *C. difficile* strain genotypes.

Result: A total 79 of 234 (30%) of soil samples were culture positive for *C. difficile*, of which 52/234 (20%) were toxigenic. We found that 50% (55/117) of top-soil samples contained *C. difficile,* of which 30% (35/117) of top-soil samples contained toxigenic *C. difficile*. Sub-soil samples contained 20% (24/117) *C. difficile,* and 10% (17/117) of sub-soil samples contained toxigenic *C. difficile.* We successfully ribotyped 71 isolates of *C. difficile* and found 16 different ribotypes from the top-soil and 11 ribotypes from the sub-soil. Ribotypes F106, F014-020, and FP310 were the three (out of 16) dominating ribotypes in top-soil samples. F106, F002, and FP310 were the three (out of 11) dominating ribotypes of *C. difficile* in sub-soil samples.

Conclusion: We identified a high prevalence of toxigenic *C. difficile* with diverse ribotypes from surface and sub-soil terrains in Houston, Texas. Our preliminary findings suggest that fecal contamination on top-soil might be a reservoir for toxigenic *C. difficile.*

## Proinflammatory sulfur reducing bacteria are more abundant in colonic biopsies of patients with microscopic colitis compared to healthy controls.

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**Background:** Microscopic colitis (MC), a subtype of inflammatory bowel disease, is a chronic conduction of unknown etiology. Recent evidence has linked MC with intriguing changes in the stool microbiota, which may be linked to disease pathogenesis. The composition of the mucosal microbiome in patients with MC remains unclear.

**Methods:** We performed a cross-sectional study comparing colonic tissue samples from patients with MC to those of healthy controls at the Michael E DeBakey VA Medical Center. We included adults older than 18 who underwent a colonoscopy with biopsies to evaluate chronic diarrhea. Cases were defined by histology consistent with MC and controls by the absence of histologic disease. We conducted structured chart review to exclude other gastrointestinal diseases and obtain demographic (age, sex, race) and clinical (duration of symptoms and concurrent medications) information for cases and controls. We extracted bacterial DNA from formalin fixed paraffin embedded tissue samples, and sequenced the v4 region of the 16S rRNA gene. Operational Taxonomic Unit (OTU) clustering was performed using UPARSE, and OTUs were assigned using the SILVA database. Statistical analysis was performed in QIIME and LEfSe. Comparisons with FDR adjusted p values of less than 0.05 were considered statistically significant.

**Results**: We included 20 MC patients and 20 controls with mean ages of 62 and 54, respectively. Most cases were White (95%), 60% had symptoms for greater than 12 months, and 50% were taking PPIs and NSAIDs at the time of their diagnosis. Compared to controls, MC patients had a significant increase in the proinflammatory sulfur reducing bacterial family Desulfovibrionales. The Coriobacteriaceae family, abundant in the healthy gut, was significantly decreased in MC cases. There was also an increase of the genus *Actinomyces* in MC patients on PPI and an increase in the class Bacilli among those taking NSAIDs.

**Discussion**: Patients with MC have an increase in the proinflammatory family Desulfovibrionales. *Actinomyces* and Bacilli were associated with medications (PPI and NSAIDs) known to increase the risk of MC. Our findings may have important implications for understanding the pathogenesis of MC.

## Human gut pathogen *Clostridium difficile* on park trees leaves and wild birds in Houston, Texas

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Background: Spores of toxigenic *C. difficile* can survive on many different environmental surfaces for many months and can be transmitted into human and animal intestines though oral-fecal routes. Tree leaves are frequently contaminated with bird feces which could possibly be contaminated with *C. difficile* spores. Dried feces could be then disseminated though air and colonize or infect humans and other animals. Therefore, our objectives were to collect visible bird fecal contaminated three leaves from parks and isolate and characterize C. difficile as potential sources of infections.

Method: As part of a Texas state-wide surveillance effort, we collected tree leaves with bird feces (n=164) from 9 parks in and around Houston, Texas. Samples were assessed for *C. difficile* using anaerobic enrichment culture using brain heart infusion broth (with bile salt) and molecular methods. Suspected colonies from cycloserine cefoxitin fructose agar (CCFA) plates were identified and characterized by multiplex PCR (for *tcdA, tcdB, cdtA, cdtB, and tpi* genes) and genotyped using fluorescent PCR ribotyping.

Result: A total 54 of 164 (32.9%) samples were culture positive for *C. difficile* of which 44 of 164 (26.8%) samples were toxigenic *C. difficile.* A total of 7 distinct ribotypes (F002, F106, F014-020, F015, FP310, FP415, and F027) were identified from 51 *C. difficile* isolates tested.

Conclusion: We identified a high prevalence of toxigenic *C. difficile* with diverse ribotypes from bird feces contaminated tree leaves. Our findings suggest that bird feces might be a significant source of toxigenic *C. difficile*.

## In vivo imaging of murine colitis and colonic pathology: Colonoscopy and high frequency-micro ultrasound

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Inflammatory bowel disease (IBD) is a refractory disease, characterized by periods of remission and relapse, and is a major risk factor for colon cancer. Murine models of experimental colitis, either genetically-engineered or chemically induced, are essential tools in today's research in human IBD. However, most of the traditional methods to evaluate murine experimental colitis require euthanasia. In order to obtain more accurate and statistically powerful data from longitudinal studies, while reducing the number of animals for a study, a noninvasive, reproducible, and inexpensive method is needed.

Mouse models of acute and chronic colitis and inflammation-associated colon tumors were assessed by colonoscopy as well as high-frequency micro-ultrasound to test the feasibility of these modalities. Both colonoscopic evaluation and ultrasound assessment were performed in vivo prior to the traditional visual scoring on mouse necropsy samples and histopathological assessments. Colonoscopy provided superior images of mucosal inflammation and tumors compared to traditional terminal visual evaluation. However, colonoscope cannot pass through tumors obstructing more than 80% of the colonic lumen and the transverse colon was not accessible due to the acute angle of the splenic flexure. B-mode ultrasound provided quantitative data of mucosal thickening throughout the colon and 3D-mode ultrasound revealed tumor volumes in most areas of the colon. Colonoscopic scores and mucosal thickening measured by ultrasound both corresponded to visual and histological assessments. Mucosal biopsy was successfully performed to analyze gene expression changes during inflammation. Furthermore, molecular ultrasound imaging using micro-bubbles conjugated with an antibody against P-selectin was performed to assess severity of DSS-induced inflammation in the mouse colon and correlated with immunofluorescence in colonic tissue sections obtained after euthanasia.

We propose that concurrent use of colonoscopy and ultrasound is a reliable and inexpensive method for longitudinal studies of the mammalian colon and such concurrent imaging strategies will advance our understanding of intestinal pathology using animal models.

## Bacterial production of folates alters expression of folate receptors and transporters in human Enterocytes

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The human intestinal microbiome consists of complex, adaptive ecosystems that are uniquely attuned to host conditions. This reciprocal relationship is achieved, in part, through the production of diverse bacterial metabolites which are required for human health. Folates are essential vitamins required by humans for DNA synthesis and epigenetic regulation. The typical American does not consume enough folate to reach the daily recommended intake of 400 ug/day, however folic acid deficiency is rare due to supplementation and the ubiquity of folate production by the microbiome. This study characterizes folate production and secretion by a representative bacterial cohort of the human microbiome and evaluates the effects of those folates on human enterocytes. Bacterial folate synthesis was assessed during exponential and stationary phase growth through 1) the evaluation of the expression of select folate synthesis genes, 2) quantification of total folate production by chemiluminescent analysis and 3) analysis of the folate polyglutamylation by MALDI-MS. We identified increased expression of key folate synthesis genes in exponential phase, and increased folate polyglutamylation during late stationary phase. Together these findings suggest that while there may be more folate production during exponential phase, folates may be converted to more stable forms during stationary phase. We found that folates secreted by *Lactobacillus reuteri* altered expression of folate receptor genes in human enterocyte cell lines and human enteroid monolayers. By delineating the mechanisms regulating the production and secretion of molecules beneficial to the host, such as folate (vitamin B9), we can better understand the holometabolome and contributions of gut microbes to vitamin metabolism.

## A role for the gut microbiome in cerebral small vessel disease

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The importance of healthy gut microbiota on host physiology is becoming increasingly evident. Recent studies suggest that alterations to the microbiota can affect organs such as the brain that are beyond the GI tract. We hypothesize that gut dysbiosis contributes to the development of cerebral small vessel disease (CSVD), an inflammatory disease accompanied by remodeling of small cerebral arteries and arterioles, blood-brain barrier (BBB) breakdown, and neuroinflammation. Furthermore, CSVD is most often, but not always, associated with hypertension. We have recently determined that the microbiota of the spontaneously hypertensive stroke prone rat (SHRSP; a model of CSVD) is significantly different than that of WKY controls. If our hypothesis is valid, then creating a gut microbiome in a WKY rat that resembles one from an SHRSP rat should produce CSVD phenotypes. Conversely, creating a gut microbiome in a CSVD rat that resembles one from a healthy strain should abolish or attenuate the onset of hypertension. Given that the gut microbiota in pups is strongly affected by the microbiota of the nursing mother, we cross-fostered rat pups on different strains. WKY and SHRSP pups were removed from their birthing mother at 2-3 days of age and fostered on non-biological mothers of the same or opposite strain. Systolic blood pressure (SBP) was measured using tail cuff plethysmography. At 20 weeks of age, rats were euthanized, tissues collected, and gene expression for inflammatory markers were measured. Feces was collected after each BP measurement to evaluate gut microbiota using 16s rRNA sequencing. Over the range of 8 to 20 weeks, SBP was decreased by 19 mm Hg in SHRSP fostered on WKY mothers compared to SHRSP fostered on SHRSP mothers ($P=0.001$, 2 X repeated measures ANOVA). Alternatively, SBP increased 7 mmHg in WKY rats fostered on SHRSP mothers compared to WKY rats fostered on WKY mothers ($p<0.05$). SHRSP rats nursed by SHRSP mothers had increased expression of Il-1α and Il-6 in mucosa of the ileum compared to SHRSPs nursed by WKYs ($p<.01$). Furthermore, IgG extravasation into brain parenchyma, a measure of BBB disruption, was less in SHRSP nursed on WKY mothers compared to SHRSP controls ($p<.05$). Relatively bacteria abundance was determined, often to the genus level, by sequencing the bacterial 16s rRNA gene in feces. Principle coordinate analysis of the fecal bacteria demonstrated that cross-fostering can lead to alterations in the gut microbiota. In summary blood pressure, the inflammatory state, and the onset of CSVD can be influenced by the gut microbiome. Maintaining a healthy gut microbiota effects the physiological state of organ system distant from the GI tract. These findings support the idea that the gut is a possible therapeutic target to treat CSVD and other related disease states.

## Need for enterotype stratified microbiome analysis

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Reproducibility is a big challenge in the microbiome field. Usual practice is to recruit control and disease subjects and to treat each group homogenous. Essentially the assumption is that there is one universal control group and that the recruited sample group would reflect that universal group. However, it is also known that there are identifiable distinct enterotypes with their inner variations. Without enterotype stratification it is hard to distinguish between sought after control-case variations and variations originating from differences in enterotypes and different enterotype distributions in the controls and cases. Here, we report the results of traditional homogenous assumed and enterotype stratified analyses of a small pilot gut microbiome study.

Our study included 14 healthy controls (HC), 13 Multiple Sclerosis patients (MS), and 13 Neuro Behcet Disease patients (NBD) from the Istanbul metropolitan area. Microbiome abundance - subject based network analyses of these groups individually, showed that there were Prevotella and Bacteroides dominated subgroups in each one of them. There were 12, 5, and 5 Prevotella and accordingly 2, 8, and 8 Bacteroides dominated subjects in HC, MS, and NBD. Traditional analysis showed that Prevotella and Bacteroides were the most abundant and differentiating between health and disease states. In particular it indicated that Prevotella was different between HC and both MS and NBD, while Bacteroides was different only between HC and NBD. However, these differences were not there when the analysis was done after stratification with the Prevotella dominated subjects alone, although Prevotella was present in all and Bacteroides was present in 8 out of 12 in HC, 2 out of 5 in MS, and 4 out of 5 NBD subjects.

## Impact of Bariatric Surgery on Liver FunctioniIn Children with Non-Alcoholic Fatty Liver Disease

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**Background**: Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide, with an increasing incidence in children. This study assessed the impact of bariatric surgery on liver enzymes in the first year following treatment.

**Materials and Methods:** We performed a retrospective review of children ages 14-19 with non-alcoholic fatty liver disease who underwent bariatric surgery at a single tertiary care pediatric institution from 2011-2016. Data collected included demographics, type of bariatric surgery, grade of NAFLD, liver function, and metabolic markers. Liver function was compared by NAFLD Activity Score (NAS)

**Results:** 76 children were included, (28.9% male, mean age 16.7), of those, 60 children were diagnosed with NAFLD via biopsy. Mean NAS was 1.5. Ultrasound sensitivity and specificity were found to be 62% and 93% respectively. No comorbidities or abnormal lab values were found to have a significant association with the diagnosis of NAFLD. Metabolic syndrome was found to have association with high NAS (≥3), but no other comorbidity or abnormal lab value had this association. Children with a high NAS were significantly more likely to have elevated ALT levels at pre-op ($p<0.02$), and 12 months post-op ($p=0.02$). There was no significant association between high NAS and percentage decrease in ALT following surgery.

**Conclusions:** Elevated ALT was found to be significantly more common in patients with more severe NAFLD. However, reduction of ALT was not significantly different in those patients with higher NAFLD activity scores. Although weight loss as treatment for NAFLD has been shown to be effective in adults, it may not be possible to monitor the efficacy of treatment through reduction in ALT alone. Ultrasound was found to have low sensitivity and high specificity, in agreement with previous studies. Future investigation into non-invasive diagnostic techniques and their clinical usefulness for diagnosis and monitoring is merited.

## Engineering of *Lactobacillus reuteri* as a Biotherapeutic Delivery System

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Our ability to engineer biologically relevant microorganisms to fight diseases of the gastrointestinal tract (GIT) is exponentially increasing. *Lactobacillus reuteri* 6475 (*LR*) is a probiotic strain with desirable features for therapeutic delivery purposes due to its adaptation to the human GIT and remarkable safety profile. Our goal is to develop a robust platform for efficient delivery of therapeutic proteins to the GIT by engineering LR to precisely secrete proteins with therapeutic purposes such the human cytokine interleukin-22 (IL-22). This cytokine has proven to confer colonization resistance against enteric pathogens in mice and significantly improve wound healing in murine diabetic models. IL-22 delivered by LR is being explored as a therapeutic tool in response to diseases such as graft versus host disease and wound-healing disorders.

First, to achieve precise control of protein production, we designed and developed libraries of gene expression tools in LR, generating a ribosomal binding site (RBS) (1000 fold increase) and promoter library (10,000 fold increase) for the expression of green fluorescent protein. Next, to generate a strain secreting active IL-22 we explored using several signal peptides in order to improve signal peptide cleavage and reduce its proteolysis, which yielded LR strain producing up to 2µg/ml of IL-22. The bioactivity of IL-22 has been confirmed *in vitro* through the induction of IL-10 in colo205 colonic cells, mouse enteroids as well as *in vivo* by stimulating expression of intestinal Reg3γ in mice. In conclusion, we have been able to generate an *L. reuteri* that produces active IL-22, and we are currently working on evaluating its therapeutic value in a number of disease models.

**Durability of vedolizumab treatment in pediatric inflammatory bowel diseases following anti-tumor necrosis factorα (anti-TNF) failure: a single center experience**

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**Background:** Vedolizumab is an anti-α4β7 integrin antibody with gut-selective anti-inflammatory activity that has been used successfully in the treatment of adult onset inflammatory bowel diseases (IBDs: Crohn's disease [CD] and ulcerative colitis [UC]) usually after failure of anti-TNF therapy. Its application in the pediatric IBD population is increasing with evolving data on effectiveness, but limited knowledge on durability beyond 6 months of treatment. We aimed to describe the effectiveness and durability of vedolizumab in pediatric IBD patients at our center.

**Methods:** We conducted a retrospective study of pediatric patients treated with vedolizumab at Texas Children's Hospital from September 2015 to January 2018. Data on demographics, prior and concomitant treatments, and disease activity was obtained at week 14, 26, and 52 of therapy. Primary outcome was week 26 and 52 steroid and other biologic free remission (quiescent or mild disease activity by physician global assessment).

**Results:** Twenty four (24) patients had 14 week outcomes on maintenance therapy with at least 4 infusions of vedolizumab received. Seventeen (17) had remission, out of whom 13 (54%) were steroid free, and 9 (37.5%) were steroid and other biologic agent free.

Nineteen (19) patients had 26 week outcomes. Seven (7=36.8%) were discontinued from drug by this time due to treatment failure (4) or progressive disease requiring surgical intervention (3). Ten (10=52.6%) had remission, out of whom 7 (36.8%) were steroid free, and 5 (26.3%) were steroid and other biologic agent free.

Seventeen (17) patients had 52 weeks outcomes, 9 (52.9%) remained on vedolizumab. Eight (8=47%) were discontinued from drug by this time due to treatment failure (5), or surgery (3). Six (6=35.3%) had remission, all of whom were steroid free, but only 2 (11.8%) were steroid and other biologic agent free. None of the patients were on monotherapy with vedolizumab by this length of therapy.

There was no significant ($p>0.1$) difference in respect to gender or IBD subtype in any of these outcomes.

**Conclusions:** Vedolizumab is a useful therapeutic modality in pediatric IBD patients, but with declining efficiency, especially as monotherapy in this real-life single center cohort. Our findings emphasize the need for novel preventative and therapeutic measures to combat this highly morbid disease group.

## Determination of the Circulating Factors Involved in Small Bowel Adaptation Following Intestinal Resection in Human Intestinal Enteroids

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**Background and Significance:** Intestinal failure is defined as having a small bowel and/or colon with an inadequate ability to absorb nutrients for proper growth and development. Following bowel resection, we know that the remaining portion of bowel undergoes "adaptation," an innate process whereby the bowel attempts to regain its original functionality. Intestinal adaptation is manifested by bowel dilation and elongation, villous lengthening subsequent to enterocyte proliferation, and increased crypt depth. Studying small bowel adaptation in intestinal failure patients is significant because there are many pediatric patients who are unable to achieve enteral autonomy via innate adaptation alone. In these patients, short bowel syndrome is a clinically relevant medical process with a large health care burden. Even more so, current therapies to enhance the innate adaption process are insufficient. Yet, the molecular mechanisms involved in the intestinal adaptation process are largely unknown.

**Innovation:** The development of small bowel human intestinal enteroids (HIEs) provides a unique medium in which to study bowel adaptation. Intestinal enteroids are generated from human tissue (i.e. from endoscopic biopsies) and consists of intestinal epithelium. Enteroids provide an efficient system to measure adaptation under many conditions. The growth period to obtain sufficient material for a single experiment is about 1-2 weeks. In addition, they provided us with the ability to test biological variability.

**Hypothesis:** A protein in the circulating plasma of piglets, who have under gone mid-small bowel resection, will increase the proliferation of intestinal epithelium and expand the stem cell pool within the crypt base of human duodenal enteroids.

**Methods:** Duodenal enteroids will be developed from endoscopically obtained duodenal tissue (accessed from our enteroid bank). Piglet plasma will be isolated from pigs that have undergone either mid small bowel resection or sham operation. Subsequently, 10% plasma will be applied to the enteroid cell culture and undergo an incubation for 48 hours. Cell proliferation analysis will happen via flow cytometry (following EdU and Dapi staining).

**Results:** Increasing pig plasma concentrations on the enteroid cell culture decreases cell proliferation under either the small bowel resection or sham operation condition. Preliminary data suggests that there is no statistical difference in cell proliferation between the enteroids that have been treated with plasma taken from small bowel resected pigs and the enteroids that have been treated with plasma taken from sham operated pigs ($p= 0.53$ in HMGM and $p=0.96$ in differentiation, respectively).

**Conclusions and Future Directions**: The current system is unable to detect a difference between the two conditions of interest. The next approaches will include: growth factor enhancement, an attempt to re-apply piglet plasma, containing only the isolated protein(s) of interest, to enteroid cell culture and/or a trial of human intestinal organoids to capture the epithelial effect that may be mediated by intestinal mesenchyme.

## Calicivirus NS1/2: A New Candidate Viroporin

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Viroporins are virus-encoded ion channels that function to disrupt host intracellular ion levels. Viroporins from different virus families' share little amino acid sequence homology, but have signature structural motifs, including: the ability to oligomerize, an amphipathic alpha-helical domain (AAH), and a cluster of basic amino acids [lysine /arginine]. Viroporins from enteric viruses, such as rotavirus (RV) NSP4 and enterovirus (EV) 2B, form calcium-conducting channels in the endoplasmic reticulum (ER) to release calcium stores. Caliciviruses are nonenveloped positive sense RNA viruses, many of which cause diarrheal disease in a variety of animals, including humans. Tulane virus (TV) is a recently discovered rhesus enteric calicivirus that is closely related to human noroviruses (HuNoV). TV replicates efficiently in cultured monkey kidney cells and is therefore an excellent surrogate for HuNoV studies of cellular pathophysiology. Like RV and EV, we found that TV decreases ER calcium and increases cytosolic calcium levels. Thus, we hypothesize that TV expresses a viroporin analogous to NSP4 and 2B. The TV positional homolog of the EV 2B viroporin is the NS1/2 protein, and we identified the signature viroporin motifs in the C-terminal region of NS1/2. Next, we used an *in vitro* bacterial lysis assay to analyze NS1/2 for viroporin activity. Wild-type NS1/2 induced robust cell lysis, similar to NSP4, but deletion of the putative viroporin domain completely abolished viroporin activity. We found that NS1/2 is an oligomeric integral membrane protein, but this requires an upstream transmembrane domain and not the viroporin domain. Finally, mutation of the clustered lysine residues to glutamic acid significantly decreased NS1/2 viroporin activity, but mutations that decreased the amphipathicity of the AAH had no significant effect. In conclusion, TV NS1/2 has viroporin activity, and deletion or mutation of the viroporin domain disrupts this activity establishing NS1/2 as the first candidate viroporin in the *Caliciviridae* family.

**Texas Medical Center Digestive Diseases Center**
**9th Annual Frontiers in Digestive Diseases Symposium:**
Frontiers of the Microbiome Gut-Brain Axis

## Mechanisms of coagulopathy in early postnatal malnutrition

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**Background**: Malnutrition affects 320,000 live births in the United States each year. Malnourished small-for-gestational-age (SGA) versus appropriate-for-gestational-age neonates are coagulopathic, with elevated international normalized ratio (INR) and increased risk of hemorrhage, including intraventricular hemorrhage that can lead to death or long-term disability including cerebral palsy. Although vitamin K deficiency can cause coagulopathy, vitamin supplementation fails to normalize INR in SGA neonates, in infants with acute malnutrition, and in chronically malnourished children. Why coagulopathy occurs in malnutrition is unknown, although recent evidence implicates the nutrient-sensing nuclear receptors, farnesoid X receptor (FXR) and peroxisome proliferator-activated receptor (PPAR)α, which competitively bind DNA regulatory elements with opposite transcriptional outputs. In the fed state circulating bile acids activate FXR to *suppress* gluconeogenesis and autophagy, while in the fasted state products of lipolysis activate PPARα to *stimulate* fatty acid oxidation and autophagy. Infants with mutations in the gene encoding FXR have vitamin K-refractory coagulopathy, with suppressed production of FXR-dependent genes including fibrinogen. We hypothesized that altered PPARα/FXR signaling mediates coagulopathy in malnutrition.

**Methods**: We modeled early postnatal malnutrition by administering low-protein, low-fat chow or isocaloric control chow to C57BL/6 wild type, $FXR^{-/-}$, or $PPAR\alpha^{-/-}$ mice from 2-7 weeks of life. We assessed coagulation indices in plasma, and gene expression and nuclear receptor binding by RNA-seq and ChIP-qPCR, respectively, in whole livers and in primary mouse hepatocytes.

**Results**: Compared to control mice, malnourished mice are 30% underweight and have decreased plasma fibrinogen with 1.3-fold increased INR, mimicking the coagulopathy of SGA neonates, acutely malnourished infants, and children with chronic malnutrition. FXR target genes were repressed as expected, given our recent report demonstrating decreased bile acids in malnourished mice. RNA-seq revealed strong PPARα activation, with induction of the known PPARα targets and decreased fibrinogen mRNA in wild type and $FXR^{-/-}$ (but not $PPAR\alpha^{-/-}$) malnourished mice and in hepatocytes treated with the PPARα agonist GW7647. At the fibrinogen promoter we identified a site of co-localization between PPARα and nuclear corepressors, suggesting the possibility of corepressor recruitment by activated PPARα. When activated, FXR and its steroid response coactivators occupy the same site, suggesting potential competition between PPARα and FXR. Indeed, ChIP-qPCR confirmed strong enrichment of PPARα and multiple corepressors at a PPAR-response element located near the transcriptional start site of fibrinogen in malnourished versus control livers.

**Conclusion**: Our data support a novel mechanism of transcriptional regulation of coagulation factor synthesis by the nutrient-sensing nuclear receptors PPARα and FXR and their co-regulator proteins.

## Reductions in oral intake perturb the intestinal microbiome and compromise the colonic mucus barrier

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**Background**: Reduced oral intake (ROI) is common in allogeneic hematopoietic cell transplantation (allo HCT) recipients following conditioning and with intestinal graft-versus-host disease (GVHD). Here we examine the interplay between nutrition, intestinal bacteria and colonic mucus.

**Methods**: Mice were subjected to ROI for 7 days (2 grams of chow per mouse per day, or ~ 50% reduction in oral intake) with unlimited water. Intestinal bacterial composition was assayed by 16S deep sequencing. Mucolytic activity of fecal samples or *Akkermansia muciniphila* (ATCC) was evaluated using porcine gastric mucin (Sigma) and a colorimetric assay that quantifies polysaccharides (Periodic acid-Schiff method, PAS). In 8 allo HCT patients with a 30% or greater reduction in oral intake from pre-HCT (day -8 to day -4) to post-HCT (day +4 to day +10), fecal samples were collected and evaluated with their consent. Colonic mucus layer thickness in mice was measured by PAS staining of histological samples. Supplemental sugars were introduced to the drinking water of mice (2g/L).

**Results**: In mice we found that after ROI, the intestinal bacterial composition was perturbed with a pronounced increase in *Akkermansia muciniphila*, an intestinal commensal that degrades mucins as a carbohydrate source. ROI also led to increased mucolytic function in fecal samples. Histologically, the colonic mucus was thinned following ROI. Increases in mucolytic function were seen in 4 of 8 patients undergoing allo HCT who developed mucositis and nausea.

We asked how ROI could favor mucolytic bacteria. Hypothesizing that ROI reduced bacterial fermentation, we evaluated the pH of the colonic lumen. We found that ROI led to a higher pH in the colonic lumen. Also, *Akkermansia muciniphila* gains the ability to degrade mucins *in vitro* when the pH rises from 5.5 to 6.0.

Finally, we asked if supplementation of sugars to mice undergoing ROI could prevent thinning of the colonic mucus layer. We found that 4 different sugars in the drinking water of mice all prevented, to varying degrees, the thinning of the colonic mucus.

**Conclusions**: Reduced oral intake in mice and many patients leads to increased mucolytic activity of the intestinal microbiome and may be an important clinical contributor to impaired intestinal barrier function during allo HCT. A strategy of low-dose oral supplementation with sugars could help suppress mucolytic activity of the intestinal microbiome.

## Risk of bias in systematic reviews of existing systematic reviews and meta-analyses of probiotic use in irritable bowel syndrome

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**Background**: There are discrepancies between systematic reviews of probiotics for treatment of irritable bowel syndrome (IBS).

**Purpose**: To use the Review Of Bias In Systematic reviews (ROBIS) instrument to better understand discrepancies between reviews.

**Data Sources**: Systematic review of Embase, PubMed, and Cochrane Database of Systematic Reviews from inception to November 6, 2017 for systematic reviews with or without meta-analyses of randomized controlled trials assessing probiotics as treatment for IBS.
**Study Selection**: 13 of 1363 articles met inclusion criteria.

**Data Extraction**: Two independent reviewers extracted data to standardized data extraction forms and applied ROBIS.

**Data Synthesis**: Most (11 of 13, 84.6%) rated as having “high” overall risk of bias due to multiple errors in the conduct of the reviews. ROBIS helped to elicit key methodologic contributors to bias using signaling questions within each of four domains: study eligibility criteria (9 of 13, 69.2% with inappropriate restrictions in eligibility criteria, 13 of 13 lacked a priori study design), study identification and selection (7 of 13, 53.8% with difficult to replicate search strategies), data collection and study appraisal (9 of 13, 69.2% without at least two independent reviewers to perform bias assessments), and data synthesis and findings. Two studies rated as “low” overall risk of bias demonstrated numbers needed to treat of 7 (95% CI 4-12.5) and 4 (95% CI 3-12.5) for global improvement of symptoms or abdominal pain.

**Limitations**: ROBIS is a complex instrument, requiring time to become acquainted with and with some degree of subjectivity to ratings made.

**Conclusions**: Bias is common in systematic reviews. Use of ROBIS can highlight low risk of bias reviews, which in the case of IBS pointed to treatment benefit of probiotics.

## Association between Cheese Consumption and Colorectal Adenoma: A Hospital-Based Case-Control Study

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**Background:** Colorectal cancer (CRC) is the third most common cancer diagnosis among men and women in the U.S. It is estimated that one-third of the population will develop a slow growing, non-malignant precursor colorectal adenoma (CRA) by age 60. Increased dairy and calcium intake have shown a slight reduction in CRA and CRC risk, as has fiber. While evidence is limited, diets high in saturated fat have been positively associated with CRC. Cheese is a dairy product high in both calcium and saturated fat, containing no fiber. The amount of cheese consumed by Americans is rising rapidly and further study to understand the association between cheese consumption and the development of precancerous colorectal adenoma is warranted.
**Objectives:** To compare whether adult diets high in cheese consumption resulted in a significantly higher risk of CRA, or advanced CRA, than in those who consumed little to no cheese. Additionally this study sought to examine gender, race, alcohol and tobacco use, BMI, polyp history and specific food types as confounding factors.
**Methods:** Study participants were 50-79 year old men and women from an endoscopy clinic-based case-control study at the Michael E. DeBakey VA Medical Center (MEDVAMC) who underwent screening colonoscopy between June 2013 and May 2017. Data on diet over the past year was gathered from self-administered semi-quantitative Block Food Frequency Questionnaire (FFQ), lifestyle data was collected via a questionnaire administered by research coordinators, and data on size and pathology of excised adenoma was obtained from medical record review. Cases had one or more CRA removed during colonoscopy while controls were polyp free. Advanced CRA were >1cm and/or with villous component and/or severe dysplasia. Demographic and lifestyle factors of cases (advanced and all) and controls were compared with Student's t or chi-square test. Odds ratios (OR) for advanced CRA and all CRA and their 95% confidence intervals were calculated using unconditional logistic regression models. IRB approval of this and of the parent study (H30941) was through Baylor College of Medicine and MEDVAMC.
**Results:** A total of 88 cases with advanced CRA, 137 cases with non-advanced CRA and 105 controls with no CRA completed the FFQ. The study participants were predominately male (>90%) and non-Hispanic (>80%). Smoking status was significantly different between groups ($P<0.05$) with advanced cases and all cases more likely than controls to be current smokers ($P=0.0008$, $P=0.01$). No other significant demographic or lifestyle differences were found between cases and controls. The OR (95%CI) for all CRA cases, when adjusted for age and other confounders was 0.54 (0.28-1.04) for moderate consumption of cheese, and 0.67 (0.33-1.37) for high consumption. For advanced cases compared to controls, the OR was 0.43(0.18-1.03) for moderate consumption and 0.42(0.17-1.05) for high consumption of cheese.
**Conclusion:** Consumption of cheese was found to be associated with a statistically non-significant decrease risk of CRA in a veteran population. Daily intake of moderate amount of cheese provided greater risk reduction than did higher intake, except in the case of advanced CRA, in which risk reduction was slightly greater with high consumption. Further studies, with larger sample size and more detailed modeling, are needed to confirm this association.

## Targeting *Clostridium difficile* infection through optimized delivery of precursor-directed probiotic *Lactobacillus reuteri* biofilms on glycerol-containing dextranomer microspheres

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Integrated antibiotic and probiotic therapy has the potential to decrease the incidence and severity of *Clostridium difficile* infection (CDI). The probiotic *Lactobacillus reuteri* is potentially an ideal candidate for adjunct therapy, given the intrinsic resistance of *L. reuteri* to clinically relevant CDI antibiotics (vancomycin, metronidazole and fidaxomicin) coupled with the ability to convert glycerol to the antimicrobial compound, reuterin. Co-delivery of *L. reuteri* and glycerol interferes with *C. difficile* growth in an antibiotic-treated human-fecal microbial community *in vitro* without significantly affecting the overall microbial community composition. However, glycerol provided in the growth media is consumed by microbial community members other than *L. reuteri* which impacts community structure, decreases efficiency of reuterin production by *L. reuteri* and is not optimal for *in vivo* administration. To optimize clinical efficacy of co-delivering glycerol and *L. reuteri* we have utilized a semi-permeable dextranomer microsphere delivery system that provides diffusible luminal substrates to biofilm-associated bacteria. Previous studies showed luminal sucrose enhanced *L. reuteri* adherence to microspheres. We found time-course reuterin production to be similar when adhered as a biofilm to microspheres containing glycerol and sucrose. Additionally, this formulation inhibited *C. difficile* growth as efficiently as planktonic cells *in vitro*, and more efficiently than planktonic cells *ex vivo* with cecal content from antibiotic treated mice. These experiments further confirm these microspheres as viable means for delivering precursor-directed *L. reuteri* targeting *C. difficile* in feces and support the continued development of this coordinated probiotic targeted delivery system in the fight of CDI.

## Biosensor Development to Study Calicivirus Pathophysiology

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Enteric viruses in the *Caliciviridae* family cause acute gastroenteritis (AGE) in humans and animals, though the molecular mechanisms of calicivirus (CV)-induced AGE remain unknown. Two barriers must be overcome to facilitate identification of cellular pathways important for CV pathogenesis. First, we need a CV model system that has robust replication in cell culture, and second, we need new experimental tools to mark live CV-infected cells so cellular dysfunction can be directly associated with CV infection and virus-infected cells can be isolated for subsequent characterization. My project addresses the first barrier by studying Tulane virus (TV), a member of the new rhesus enteric calicivirus (ReCV) genus, which cultivates in several monkey kidney cell lines. To address the second barrier, I am designing a fluorescent biosensor that activates upon cleavage by the TV-encoded cysteine protease NS6. This biosensor will be stably expressed in cell lines and therefore upon infection the TV-infected cells will be marked by increased fluorescence and will enable tracking of TV infection by live cell microscopy.

The TV NS6 biosensor utilizes a protease-activated 'dark GFP' (PA-dGFP), which has been used previously to make biosensors for caspase 3 & 7, and the rhinovirus 3C protease. These biosensors juxtapose GFP to a protease cleavage site and quenching peptide, preventing GFP maturation. dGFP fluorescence is activated by protease cleavage to release the quenching peptide, allowing GFP to mature. I will insert a TV protease cleavage sequence into the linker and optimize this sequence for optimal cleavage efficiency and signal-to-noise ratio using site-directed mutagenesis. I will screen for the best sequence using co-expression of PA-dGFP with NS6 in *E. coli* and validate the top hits in mammalian cells expressing NS6 alone or infected with TV. Ultimately, I will combine the NS6 biosensor with fluorescent reporters for calcium signaling, such as GCaMP6s and YFP-STIM1, to monitor in real-time TV-induced exploitation of host signaling.

## Surveillance-indication for colonoscopy is associated with early stage colorectal cancer among patients with inflammatory bowel disease

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**Background:** Inflammatory bowel disease (IBD) is associated with an increased risk of colorectal cancer (CRC). Current guidelines recommend colonoscopy surveillance programs for patients with significant colonic disease; however, there is a paucity of data showing the benefit of surveillance colonoscopy among IBD patients.

**Aim**:The aim of this study was to explore the association between indication of colonoscopy and CRC stage at diagnosis in patients with IBD.

**Methods**: Using the national VA administrative databases, we performed a retrospective study of patients with IBD diagnosed with CRC using a previously validated algorithm of administrative codes, and further manually verified all cases of CRC by chart review. Patients with complete CRC staging performed in the VA and whose CRC-diagnosing colonoscopy was performed in the VA with available endoscopy and pathology reports were included in the study. CRC-diagnosing colonoscopy was classified as either “surveillance” or “diagnostic” based on reported indication on the endoscopy report. Statistical analyses were performed comparing the indication for colonoscopy (surveillance vs diagnostic) and stage of CRC at diagnosis using the student’s t-test.

**Results**: A total of 712 patients with IBD and CRC were identified and confirmed on chart review, of whom 193 patients had complete staging data and full endoscopy and pathology reports of their CRC-diagnosing colonoscopy available and were included in the analyses. 49% of CRC were diagnosed on surveillance colonoscopy, while 51% were diagnosed on diagnostic colonoscopy. The most common indications for diagnostic colonoscopy were bleeding (41%), anemia (28%), and abnormal CT findings (16%). Patients diagnosed with CRC on surveillance colonoscopy were more likely to have carcinoma-in-situ (13.8% vs. 4.3%, p=0.018) or stage 1 CRC (29.8% vs. 13.8%, p= 0.0049) compared to patients diagnosed on diagnostic colonoscopy.
Finally, patients diagnosed by surveillance colonoscopy were less likely to have stage 4 CRC (16% vs. 32%, p=0.018).

**Conclusion**: Among patients with IBD-associated CRC, those diagnosed on surveillance colonoscopy were more likely to have early-stage CRC compared to patients diagnosed on diagnostic colonoscopy. These findings suggest a benefit in surveillance colonoscopy for IBD patients, however further study is required to explore the effect of surveillance on CRC-associated mortality and other CRC and IBD outcomes.

## Prevalence of Barrett's esophagus in esophageal adenocarcinoma: a systematic review and meta-analysis

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**Background:** Esophageal adenocarcinoma (EAC) diagnosed by Barrett's esophagus (BE) surveillance is found at an earlier stage and more likely to receive curative treatment, but the proportion of patients with EAC diagnosed this way is unclear. The last meta-analysis in 2002 reported that only 4.7% of EAC patients had a diagnosis of BE prior to their cancer diagnosis. However, the meta-analysis only included esophagectomy studies, excluding non-resected EAC cases. It also did not account for prevalent BE diagnosed at the same time as EAC, which represents missed BE screening, and did not include cases of gastroesophageal junction adenocarcinoma (EGJAC). We conducted a systematic review to estimate the prevalence of prior as well as concurrent BE at the time of EAC or EGJAC diagnosis.
**Methods:** We searched PubMed and Embase for relevant articles from 1966 to 7/1/2016 to identify studies that examined the prevalence of prior BE diagnosis or concurrent BE at the time of EAC and/or EGJAC diagnosis. We defined concurrent as BE found on histopathology at the time of cancer diagnosis. We used random effects models to estimate pooled prevalence rates, both overall and in subgroups of patients based on study type defined as single/multi-center or population-based study. We also report between-study heterogeneity using Higgins $I^2$.
**Results:** We identified and reviewed 5873 potential studies. A total of 57 studies, including 17,373 patients with EAC and 1,558 with EGJAC met the eligibility criteria. The pooled prevalence rate of prior BE diagnosis in patients with EAC was 10.4% (95% CI: 8.3-12.7%;$I^2$=86%) (Figure 1). Most studies defined prior BE diagnosis as occurring ≥6 months prior to cancer diagnosis. The prevalence of prior BE in EAC patients was higher in single/multi-center studies that included many early EAC stage esophagectomy studies (7 studies; prevalence=14.5%, 95% CI 7.6-22.9%) compared with population-based cancer registry studies (5 studies; prevalence=8.4%, 95% CI 7.2-9.7%). In 32 studies, the prevalence of concurrent BE in EAC was 50.8% (95% CI 41.3-60.2%;$I^2$=96%). Single/multi-center studies had a higher prevalence of concurrent BE (53.0%, 95% CI 43.3-62.6%) than population-based studies (21.3%, 95% CI 16.5-26.6%) (Figure 2). Among patients with EGJAC, the prevalence of prior BE diagnosis was 8.5% (95% CI 2.8-16.5%;$I^2$=84%) in 4 studies and concurrent BE was 24.3% (95% CI 14.1-36.0%;$I^2$=95%) in 15 studies.
**Conclusions:** Our meta-analysis suggests that ~10% of EAC patients had a prior diagnosis of BE at least 6 months prior to their cancer diagnosis, with modestly higher prevalence (14%) in early stage EAC likely attributed to active BE surveillance. Concurrent BE was found in at least 51% of EAC at the time of cancer diagnosis and represents missed opportunity of BE screening.

## Biliary Tumors in Children: A Comparison of Cholangiocarcinoma and Biliary Rhabdomyosarcoma

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**Background:** Cholangiocarcinoma (CCA) and biliary rhabdomyosarcoma (RMS) are rare malignancies in the pediatric population, and thus are topics of limited research. An association with comorbidities such as primary sclerosing cholangitis (PSC), inflammatory bowel disease (IBD), HIV, biliary atresia, and choledochal cysts may increase a child's risk of developing CCA. Certain genetic mutations such as Recklinghausen disease, Li-Fraumeni syndrome, Costello syndrome, Noonan syndrome, cardio-facio-cutaneous syndrome, and Beckwith-Wiedemann syndrome have shown an increased incidence of RMS. Complete surgical resection is the only potential cure for CCA, thus making early diagnosis essential to survival. Protocols are in place to guide treatment of biliary RMS, but there is currently no definitive cure.

**Objective:** The objective of this study was to analyze literature and the data contained within the Surveillance, Epidemiology, and End Results Program (SEER) and the Healthcare Cost and Utilization Project (HCUP)-Kids' Inpatient Database (KID) databases to describe the incidence, clinical characteristics, and outcomes of CCA and biliary RMS within the pediatric population. The databases focused on patient demographics, risk factors, presentation, diagnosis, and treatment options for both types of biliary tumors. Understanding these factors will help providers recognize the signs and symptoms of these cancers and learn the best treatment options for them.

**Methods:** This was a retrospective study of pediatric patients diagnosed with biliary tract malignancies using literature and hospital database systems. Cases of pediatric biliary malignancies were identified using the SEER and HCUP-KID. The SEER registry includes data from to 18 US cancer registries collected from 1973-2013, representing 28% of the US population. Data from SEER was used to identify patient demographics, treatment methods, and survival outcomes for patients with CCA and biliary RMS. The HCUP-KID includes three million pediatric discharges per year from more than 4,100 U.S. community hospitals in 44 states. HCUP-KID data from 2003, 2006, 2009, and 2012 were analyzed using SPSS software to identify secondary diagnoses.

**Results:** Twenty-two cases of CCA and 30 cases of biliary RMS were identified in the literature. SEER identified 15 cases of CCA and 13 cases of biliary RMS. Males are affected by both cancers at a 2:1 ratio. Caucasians are affected by both diseases with Hispanics at equal risk of biliary RMS. The median age of diagnosis for CCA is 17, and 3 for biliary RMS. CCA is usually intrahepatic, while biliary RMS is extrahepatic. Surgery is the mainstay of treatment for CCA. Neither differences in tumor stage nor treatment regimen are associated with a better outcome for biliary RMS. HCUP-KID identified 23 cases of biliary malignancy with a median age at diagnosis of five years. Twelve patients were male and the majority Caucasian (n=13). Twelve tumors were intrahepatic and 11 extrahepatic. Nine patients had at least one underlying gastrointestinal comorbidity, with cholangitis most prevalent (n=4).

**Conclusions:** Both CCA and biliary RMS are extremely rare in the pediatric population. There are no clear risk factors for developing biliary RMS, so further research should be conducted on the influence of genetic disorders or comorbidities. For individuals with PSC, it may be beneficial to develop screening guidelines in hopes of having an earlier diagnosis. Surgical resection can cure CCA, making earlier diagnosis prudent for survival. More needs to be discovered about the risk factors for biliary RMS before guidelines can be created to screen for it. As more is learned about the disease, the treatment regimen can be adjusted to allow for the best chance of survival for these individuals.

## Neurogenin-3 human intestinal enteroids as a novel model of gastrointestinal hormone and peptide secretion

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Enteroendocrine cells (EECs) of the gastrointestinal tract (GIT) are a class of specialized epithelial cells that produce hormones and molecules for the maintenance of intestinal and general host homeostasis. EECs are responsible for the production of molecules such as gastric inhibitory peptide, glucagon-like peptide-1, pancreatic peptide YY, cholecystokinin, serotonin, and histamine. The process by which the secretion of these molecules is governed is still not fully understood and current cell culture models to study EECs are substandard. Human intestinal enteroids (HIEs), which may provide a more suitable model, are derived from intestinal biopsies and form a polarized epithelium containing all the cell types present in the GIT, including EECs. Unfortunately, as in the human GIT, EECs constitute only 0.5-1% of the cells present in HIEs, rendering their study challenging. The goal of the presented work is to provide a better model for EEC studies by transducing HIEs with neurogenin-3 (NGN3), a transcription factor responsible for driving the differentiation of intestinal stem cells into EECs, with the hope of increasing their counts. To generate this model, HIEs were derived from the jejunum of adult patients. Tetracycline (tet)-inducible-NGN3-HIEs were created by lentivirus transduction followed by selection. The tet-NGN3-HIEs showed stable expression of the lentivirus construct in culture, as demonstrated by immunofluorescence (IF) staining for chromogranin A (ChrA). Differentiation conditions, including various doxycycline concentrations and differentiation time, were optimized to induce NGN3 expression in flat HIE monolayers, transwell monolayers, and in three-dimensional format. EEC population counts were monitored using qPCR and IF staining for ChrA. With doxycycline concentrations ranging from 0.01 to 1 µg/mL, HIEs were obtained with <1% and up to approximately 40% ChrA-positive cells, respectively, demonstrating the establishment of a "tunable" model. We also quantified, by IF and qPCR, the presence of the various GIT cell subtypes, including enterocytes, stem cells, EECs, Paneth cells, and goblet cells. In addition, we investigated the tet-NGN3-HIEs for response to physiological stimuli, specifically with exposure to the enteric pathogen rotavirus and the neurotransmitter norepinephrine, with serotonin release as a model biological response. Both rotavirus infection and norepinephrine exposure significantly increased serotonin release in the tet-NGN3-HIEs compared to control treated tet-NGN3-HIEs. Serotonin levels in treated but non-NGN3-induced HIEs were significantly lower. Based on this work, the newly-developed NGN3-HIEs provide a flexible and tunable in vitro model for future investigations.

## Discovery of a novel host-microbial peptide interaction and its therapeutic potential for metabolic disease

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Early work in germ-free rodents highlighted the gut microbiota's importance in metabolic disease, including Type II Diabetes Mellitus (T2DM) and obesity. Studies also demonstrated that the gut microbiota could modulate gut hormone secretion, including glucagon-like peptide-1 (GLP-1). GLP-1 is an incretin hormone secreted by enteroendocrine L cells that line the gastrointestinal epithelium. GLP-1 has important functions including promoting insulin secretion, insulin sensitivity, and β-cell mass, while inhibiting gastric emptying and appetite. The objective of this work is to elucidate how the microbiota can modulate GLP-1 secretion, with the goal to develop a metabolic disease therapeutic. Over 1500 human-derived microbial strains were isolated from fecal, breast milk, and colon and intestinal biopsy samples obtained from healthy individuals. In vitro screening for GLP-1 modulation was performed by incubating bacterial cell-free supernatants with NCI H716 human L cells. Approximately 45 strains capable of increasing GLP-1 levels, measured by ELISA, were discovered. Interestingly, all the positive strains were identified as *Staphylococcus epidermidis* by 16S rRNA sequencing. Non-GLP-1 stimulatory *S. epidermidis* strains were also identified. Mass spectrometry analysis identified a 3 kDa peptide, subsequently termed GLP-1 stimulating peptide (GspA), present in the GLP-1 positive but absent in the GLP-1 neutral *S. epidermidis*. Studies in NCI H716 cells and human intestinal enteroids demonstrated that GspA alone is sufficient to enhance GLP-1 secretion. When administered in high-fat fed mice, GspA-producing *S. epidermidis* significantly reduced markers associated with obesity and T2DM, including adiposity and hyperinsulinemia. Ongoing studies aim to characterize the peptide and the mechanism(s) of GLP-1 modulation. Ultimately, this work may lead to the development of a microbial peptide-based therapeutic for obesity and T2DM.

## Environmental Exposures Target Epigenomic Plasticity to Increase Fatty Liver Disease Risk

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Early life exposure to endocrine-disrupting chemicals (EDCs) can lead to obesity and metabolic syndrome in adulthood. Although this is thought to occur via developmental reprogramming of the epigenome, the molecular mechanisms underlying this developmental reprogramming are not well defined. The liver plays a central role in whole body fat metabolism and obesity, and is a target for environmental exposures that contribute to development of non-alcoholic fatty liver disease (NAFLD). We observed that rats exposed postnatally to the EDC bisphenol A (BPA), when fed a high fat diet (HFD) as adults, had increased liver weight, increased serum triglycerides, increased serum LDL/VLDL, and increased serum free cholesterol. These data are consistent with the hypothesis that BPA had developmentally reprogramed the liver, making exposed rats prone to a NAFLD phenotype.

To test this hypothesis, we combined RNA-seq and ChIP-seq analyses in liver tissue from vehicle- and BPA-exposed animals. RNA-seq identified a unique cassette of genes that were induced in response to HFD only in the livers of BPA-reprogrammed rats. Of these reprogrammed genes, the top pathways identified by gene set enrichment analysis (GSEA) were involved in fatty acid and/or lipid metabolism and are known to play a role in NAFLD. We have previously shown that acute, neonatal exposure to EDCs can reprogram the epigenome of developing tissues by increasing the activity of mixed lineage leukemia (MLL), the histone methyltransferase in the COMPASS complex responsible for methylation of histone H3 at lysine 4 (H3K4), an active chromatin mark. To determine the underlying mechanism responsible for the observed change in response to HFD, we performed ChIP-seq for histone modifications associated with active (H3K4me3, H3K4me1, H3K27ac) and repressive (H3K27me3) histone marks. The epigenome of a subset of reprogrammed genes exhibited new H3K4me1, H3K4me3 and/or H3K27ac marks at their promoters. Importantly, these novel, active chromatin marks 1) appeared in the neonatal liver in response to BPA; 2) persisted into adulthood; and 3) were not associated with any change in gene expression in the absence to HFD (i.e. their persistence was not a *consequence* of altered gene expression). Therefore, the neonatal BPA exposure resulted in an altered epigenetic landscape that persisted into adulthood and preceded the increase in gene expression seen in the reprogrammed rats in response to HFD.

H3K4me1 and H3K27ac marks are generally associated with active enhancer regions, and their presence at promoters is indicative of so-called “super promoters”. Together, these data suggest that early life EDC exposure modulates the activity of the COMPASS complex to reprogram the developing epigenome and generate new “super promoters” that are responsive to later life stimuli, such as HFD, resulting in a liver phenotype that is prone to NAFLD and metabolic disease.

## Targeting cyclin-dependent kinase 9 enhances radiosensitization and identifies Axl as a novel downstream target in esophageal adenocarcinoma

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**Background & Aims**: Improvements in patient outcome with advanced esophageal adenocarcinoma (EAC) are limited. There is an urgent need for novel radiosensitizing targeted therapy with anticancer efficacies. Cyclin-dependent kinase 9 (CDK9) transcriptionally regulates several proteins and cellular processes common to radiation induced injury. We investigated the role of CDK9 inhibition in radiosensitizing esophageal adenocarcinoma (EAC).

**Methods**: Sensitization to a novel highly specific CDK 9 inhibitor, BAY1143572 was assessed by MTS assay, and flow cytometry. Synergistic/additive effects between CDK9 inhibitors and radiation were evaluated by clonogenic, and 53BP1 foci assays *in vitro* and in multiple xenograft models including a highly refractory patient derived xenograft (PDX) model. CDK9-dependent targets were determined and validated by reverse phase protein array (RPPA), WB, IHC, and qPCR.

**Results**: Both CDK9 inhibitors demonstrated synergy/additive effects with radiation and prolonged DNA damage in 2 EAC cell lines. BAY1143572 plus radiation significantly reduced FLO1 (80%) and SKGT4 (98%) xenografts compared to control while BAY1143572 alone retarded FLO-1 (48%) and SKGT4 (84%) tumors compared to vehicle. Combination treatment reduced PDX tumor volumes compared to radiation (67%, p=0.016) and BAY1143572 alone (48%, p=0.062). Proteomic profiling and WB identified Axl as a downstream target of CDK9. *In vitro*, high dose of each treatment reduced Axl mRNA and protein levels and together enhanced this reduction (83% compared to control mRNA, p=0.02). Axl protein expression in xenografts with BAY1143572 and radiation treatment was significantly lower than that in only radiation-treated tumors ($p$=0.003).

**Conclusions**: Targeting CDK9 significantly enhances the effects of radiation in EAC and Axl is a downstream target of CDK9. These data suggest that CDK9 inhibitors as adjunct can enhance clinical efficacy of radiotherapy.

**Context of my work:** Improving sensitization to radiation in esophageal adenocarcinoma (EAC) is one of the priorities recognized by National Cancer Institute- Gastrointestinal (GI) steering committee. Further no **targeted agent** has demonstrated promising efficacy as a radiosensitizer in EAC.

## 16S rRNA Deep sequencing technique and applications

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**Background:** Carl Woese was one of the first scientists to find that the genes that code for the 16S rRNA have very slow rates of evolution and can be used to reconstruct phylogenies[1]. Within the bacterial 16S gene there are 9 hypervariable regions that differ in length and primary sequence, and may be recognizably similar only in very closely related bacterial species[2]. By combining 16S-based taxonomical classification with deep sequencing techniques, characterization of bacterial abundances in complex mixtures is now technically streamlined and relatively inexpensive. Characterization of commensal bacteria, from locations including the intestinal tract, skin, mouth, and other locations, has in recent years yielded novel insights into health and disease, including the care of cancer patients. One of the goals of our laboratory is to provide 16S sequencing capabilities in-house to the institution.

**Methods:** Major steps in 16S sequencing include DNA purification, PCR amplification of a region of the 16S gene, post PCR clean-up, DNA quantification, and sample pooling, sequencing, and sequence analysis. Microbiome samples are lysed using a combination of mechanical disruption, heat, and lysis buffers. DNA is isolated using commercially available reagents (Qiagen) which allow for removal of PCR inhibitors and DNA purification. Using the highly conserved sequences of the 16S gene that are found between the hypervariable regions, published universal primers can be selected to amplify sections of the 16S rRNA gene that allows identification via mapping to established 16S databases. The V4 region (~250 bp) is a popular segment of the 16S rRNA commonly utilized by the microbiome field, given its variability and short size, making it amenable to Illumina-platform based sequencing. The region is amplified by PCR using primers that include Illumina adapters and unique barcodes, to allow direct loading to the flowcell without requirements of ligation, as well as multiplexing. Amplification is validated by gel electrophoresis and quantified using the Agilent TapeStation. Up to (and over) 500 samples can be pooled and the library is then sequenced on an Illumina Miseq platform to obtain paired-end reads[3]. These are then de-multiplexed and clustered into Operational Taxonomic Units (OTUs). Readouts can include alpha-diversity, beta-diversity (distance) measures, as well as relative abundances of different bacterial taxonomic subsets.

**Results/Future Directions:** We have in addition two potential strategies to improve upon existing approaches for 16S rRNA deep sequencing. One is a spike-in approach which has already been reported by two groups [4; 5], which allows for absolute quantification of bacteria, in addition to relative abundance quantification. To do this, we will spike-in DNA purified from *Yersinia ruckeri*, a bacterium found in river trout that is absent from both human and mouse samples, and compare numbers of sequences with a series of standards of a plasmid with a 16S gene insert mixed with *Yersinia ruckeri* DNA at varying ratios. We have also cultivated a mock community of 20 cultured bacterial isolates that are commonly found in the large intestine of humans which will help validate sequencing findings and allow quantification of PCR amplification-based artifacts.

## Lnc RNA PVT1 Overexpression Predicts Unfavorable Prognosis and Serve as a Therapeutic Target in Esophageal Adenocarcinoma

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**Background:** Long non-coding RNA PVT1 has been emerged as an oncogene in many tumor types. However, the role of the PVT1 in Barrett's progression and esophageal adenocarcinoma (EAC) remains unclear. The aim of this study is to investigate the role and functional mechanism of PVT1 in EAC progression and evaluate whether targeting PVT1 could be an effective strategy in curtailing EAC. **Methods:** PVT1 expression was measured by quantitative PCR (qPCR) in 37 normal tissue, 56 Barrett's epithelium and 101 EAC tissues. Statistically analysis were performed to determine the association of PVT1 expression and EAC disease states (staging, metastasis and survival). PVT1 Antisense oligonucleotides (ASOs) was performed to knockdown PVT1, and serial functional studies-proliferation, invasion, colony formation and tumor sphere assay *in vitro* and tumor growth *in vivo* were used to demonstrate the role of PVT1 in EAC malignancy. **Results:** PVT1 is highly amplified in several cohort of EAC tissues from TCGA. PVT1 expression is significantly up-regulated in EAC tissues compared with paired Barrett's epithelium and normal counterparts. The higher expression of PVT1 is significantly correlated with poorer differentiation, lymph node metastasis and poorer prognosis. In EAC cell lines JHESO and OE19, effective knockdown of PVT1 using PVT1 ASOs resulted in significantly decreased cell proliferation, invasion, colony formation along with decreased PVT1 level. Interestingly, PVT1 ASOs suppressed cancer stem cells (CSCs) properties by reducing tumor sphere formation and reducing the fraction of ALDH1+ cells. Remarkably, PVT1 inhibition by ASOs in combination with docetaxel demonstrated greater efficacy in inhibiting EAC cell growth and tumor sphere formation. Mechanistically, there is mutual regulation of PVT1 and hippo coactivator YAP1 in EAC cells. Inhibition of PVT1 by PVT1 ASOs suppressed YAP1 expression and transcription; while knockout YAP1 in EAC cells significantly suppressed PVT1 expression in several EAC cell lines indicating a positive regulation loop between these two oncogenes. Moreover, PVT1-targeting ASOs treatment markedly decreased tumor growth *in vivo* CDX mouse model. **Conclusions:** Our results provide strong evidence for an oncogenic role of PVT1 in progression of EAC. Thus targeting PVT1 using PVT1 ASOs could be novel therapeutic strategy for EAC. **Keywords:** long noncoding RNA, PVT1, antisense oligonucleotides, esophageal adenocarcinoma

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The authors have no conflict of interest to declare.

## Overexpression of Shh and Gli1 Contributes to Poor Prognosis and Peritoneal Metastases in Gastric Adenocarcinoma

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**Background:** The prognosis of gastric adenocarcinoma (GAC) patients is very poor. Understanding of molecular biology is limited. Hedgehog (Hh) signaling plays an important role in many tumor types and expression of Shh/Gli-1, two major molecules in Hh pathway has been documented in GAC. However, their clinical impact on GAC patients particularly in peritoneal metastasis remains elusive.
**Methods:** Expression of Gli1 and Shh were examined using IHC in tissue microarrays containing more than 500 cases of GAC tissues with clinical annotation. The prognostic variables were determined using univariate and multivariate Cox regression analyses. GAC cell lines, patient-derived peritoneal metastatic cells and novel PDX metastatic model were used to determine the functional role of Shh/Gli-1 *in vitro* and *in vivo*. Genetic knockout Gli-1 using LentiCRISPR/Cas9 and Hh inhibitor GDC0449 as well as BET inhibitor were used to test their antitumor activities in GAC cell line and patient-derived cells. Cell proliferation, colony formation, invasion, tumor sphere assays and immunofluorescence were performed to evaluate their functionality and effects of targeted therapy.
**Results:** Both Gli1 and Shh expression are significantly overexpressed in GAC tissue. Among 519 GAC cases, 80.76% and 87.02% were positive for nuclear Gli-1 and cytoplasmic Shh expression respectively, while the strong nuclear expression rate for Gli-1 is 69.56% and 50.10% for Shh. In the univariate Cox analysis, the overall survival was shorter for patients with high Gli-1 (p=0.018) or high Shh expression (p=0.038). In the multivariate cox analysis for both markers, only Gli-1 remained as an independently prognostic for short survival. We also observed high Gli-1 nuclear expression correlated with the presence of lymph node metastasis (p=0.032). Interestingly, Gli-1 was significantly upregulated in mouse PDX-ascites cells compared to primary mice tumors. Genetic knockdown Gli-1 or pharmacologically inhibition of Gli-1 by GDC0449 Hh inhibitor or BET inhibitor JQ1 decreased Gli-1 and restored E-cadherin expression and significantly suppressed malignant cell properties and reduced population of cancer stem cells (ALDH1+ or CD133+) in patients' derived metastatic cells.
**Conclusions:** These findings indicate that overexpression of Gli1 and Shh plays an important role in progression of GAC. Targeting Gli1/Hh signaling may provide novel therapeutic strategies for GAC patients.
**Keywords:** Hh pathway, Gli1, Shh, Gastric cancer, metastasis

**Texas Medical Center Digestive Diseases Center**
**9th Annual Frontiers in Digestive Diseases Symposium:**
Frontiers of the Microbiome Gut-Brain Axis

# Galectin-3 Mediates Tumor Cells and Stroma Interactions by Activating Pancreatic Stellate Cells to Produce Cytokines via integrin Signaling

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**Background & Aims:** Pancreatic ductal adenocarcinoma (PDAC) is characterized by activated pancreatic stellate cells (PSCs), abundance of extracellular matrix (ECM), and production of cytokines/chemokines. Galectin-3 (GAL3), a β-galactoside–specific lectin, contributes to PDAC development but its effects on the stroma and cytokine production are unclear.
**Methods:** The effect of recombinant human GAL3 (rGAL3) on activation of PSC, production of cytokines and ECM proteins were determined by proliferation, invasion, cytokine array, and quantitative PCR. Co-culture of PDAC cells with *GAL3* genetic alterations with PSCs were assessed. Production of interleukin 8 (IL8) and activities of nuclear factor (NF)-□B were determined by ELISA and luciferase reporter analyses. Effects of inhibitors of NF-□B and integrin linked kinase (ILK) were studied on pathways activated by rGAL3. **Results:** Analysis of GEO database and our dataset revealed significantly higher levels of GAL3, IL8, and other cytokines in PDAC than in non-tumor tissues. Production of IL8, GMCSF, CXCL1, and CCL2 increased in PSCs exposed to rGAL3 compared to control. Co-cultured PSCs with PDAC cells that express varying levels of GAL3 led to GAL3 dose-dependent proliferation and invasion of PSCs. GAL3 stimulated transcription of *IL8* through integrin subunit beta 1 (ITGB1) on PSCs, which activated NF-□B through ILK. Inhibitors of ILK or NF-□B or a neutralizing antibody against ITGB1 blocked transcription and production of IL8 from PSCs induced by rGAL3. GAL3 inhibitor significantly reduced growth and metastases of orthotopic tumors by co-implantation of PDAC and PSC cells in mice.
**Conclusion:** GAL3 stimulated PSC cells via ITGB1 signaling to ILK and subsequent activation of NF-□B to produce inflammatory cytokines. Inhibition of this pathway reduced growth and metastases of orthotopic pancreatic tumors in mice. **Key Words**: Tumor microenvironment, Galectin-3, pancreatic stellate cells, cytokines, mouse model

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There is no conflict interest in this study for all authors

**Texas Medical Center Digestive Diseases Center**
**9th Annual Frontiers in Digestive Diseases Symposium:**
Frontiers of the Microbiome Gut-Brain Axis

## Relevance of *clostridium difficile* ribotype 014-20 in cancer patients with diarrhea

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**Background:** Cancer patients have an increased risk of *Clostridium difficile* infection (CDI) due to frequent health care contact, chemotherapy, antibiotics, and immunosuppression. Ribotype 014-20 is emerging in US hospitals. The role for two-step diagnostic testing and the impact of this ribotype on CDI outcomes are not well defined in cancer patients.

**Materials/methods:** Demographic and clinical data were collected from 147 cancer patients with CDI. CDI was identified by PCR followed by EIA for A/B toxins. Fluorescent PCR ribotyping was performed on fecal isolates and divided into 3 groups: G1: 014-020, G2: 002, 027, 078-126, 244 and G3 with the remainder. Patient's demographics, risk factors and clinical outcomes were stratified by ribotype group and fecal EIA toxin. Outcomes were compared by using ANOVA, Fisher's exact test and the Mantel-Haenszel Chi-squared test.

**Results:** Baseline clinical characteristics were similar between the 3 groups. Most were white (69.8%), >50 years old, presented primary infection (89%) with severe CDI (55%), active malignancy (85%) and were on chemotherapy (66%). The most common ribotypes found were F014-020 (24.2%), F002 (12.1%) and F106 (11.4%). Stools were $PCR^+/EIA^+$ in 50 (34%) cases; 11 of 35 (31%) in G1, 13 of 28 (46%) in G2, and 26 of 84 (31%) in G3; P=NS. Overall patients G1 had fewer complications (17%), vs. G2 (50%) and G3 (42%) P=0.009. The sustained clinical responses, treatment failure and mortality were similar in the three groups regardless of EIA results. Recurrences were identified in 32 cases (22%), were more likely to occur following an $EIA^+$ episode (18/50 vs. 14/97 P=0.006) and were less common in G1 (27%), than G2 (46%) and G3 (35%), P=0.004. Of interest, patients in G3 and $EIA^-$ were more likely to have bacteremia [15/58 (26%)] when compared to G2 [2/15 (13%)] and G1 [2/22 (8%)], P=0.02.

**Conclusions:** When compared to patients infected with other ribotypes, patients with ribotype 014-020 experienced similar rates of response to therapy but fewer complications including bacteremia and when EIA+ was found in their stools, were less likely to recur within 12 weeks after treatment. Determining *C. difficile* ribotypes in cancer patients with CDI is relevant.

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## Microbiomic profiling of early-onset colorectal cancer

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**Background**: Although the incidence of colorectal cancer (CRC) is declining among individuals ≥50 years old, it is rising among younger individuals, particularly those between the ages of 20 and 39. Growing evidence suggests that intestinal dysbiosis may promote CRC development. Studies have compared the microbiota of CRC patients and healthy controls, but none has focused on subjects younger than 50.

**Methods:** To identify organisms that are more or less abundant in the mucosa-associated microbiome of microsatellite stable, sporadic young-onset CRC, we performed a pilot study comparing colonic tissue samples from CRC patients <50 at diagnosis (cases), CRC patients ≥50 at diagnosis (CRC controls), and individuals <50 without CRC (non-CRC controls). Manual chart review was performed to extract demographic and clinical data, and corresponding formalin fixed paraffin embedded (FFPE) tissue samples from index colonoscopy were obtained from the Human Tissue Repository at Baylor College of Medicine. Bacterial DNA was extracted, and the v4 region of the 16S rRNA gene was sequenced. Operational Taxonomic Unit (OTU) clustering was performed, and OTUs were assigned using the SILVA rRNA database. Statistical analysis was performed using QIIME, with FDR-adjusted p-values <0.05 considered statistically significant.

**Results**: We included 26 samples for DNA sequencing, yielding an average of 10,676 classifiable reads per sample. One sample from each group was excluded after rarefaction analysis. Median ages at diagnosis for cases (n=6), CRC controls (n=14), and non-CRC controls (n=3) were 27, 69, and 37, respectively. The majority (67%) of case tumors were located in the sigmoid or rectum. Alpha and beta diversity analysis showed no significant differences in richness, evenness, or ordination between cases and control groups, and non-parametric analysis with FDR correction revealed no statistically significant phylogenetic differences. However, the mean relative abundance of *Fusobacterium* increased nearly 7-fold in cases compared to non-CRC controls, and the mean relative abundance of *Prevotella* increased nearly 7-fold and 9-fold in cases compared to CRC and non-CRC controls, respectively. There was also a nearly 10-fold decrease in the mean relative abundance of *Escherichia/Shigella* in cases compared to both control groups.

**Conclusion**: In this small pilot study, there were no statistically significant differences between the microbiota of young CRC patients and the microbiota of either control group. However, microbiomic profiling using FFPE samples was feasible, and the mean relative abundance of certain bacteria previously associated with CRC risk was numerically higher in cases compared to control groups. Further prospective, well-powered studies are needed to characterize the microbiome of patients with sporadic, early-onset CRC and its role in tumorigenesis.

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This event is supported by in part by a grant from the National Institute Diabetes and Digestive and Kidney Diseases through its Silvio O. Conte Digestive Diseases Research Core Center program. (P30 DK056338)

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CME Activity Sponsored by Texas Children's Hospital