ANIL K. RUSTGI, MD

T. Grier Miller Professor of
Medicine and Genetics
Chief, Division of Gastroenterology

Chief's CORNER

The gastroenterology team at the Perelman School of Medicine at the University of Pennsylvania School of Medicine and the University of Pennsylvania Health System is nationally recognized for clinical research and superlative care of its patients. I am pleased to announce the following honors and accomplishments of its faculty:

JONATHAN KATZ, MD, has been elected to the American Society of Clinical Investigation, a national society and honor for physician-scientists

BEN STANGER, MD, and ANDREW RHIM, MD, published a landmark paper on circulating pancreatic cells from pre-cancerous lesions:

Rhim AD, Mirek ET, Aiello NM, Maitra A, Bailey JM, McAllister F, Reichert M, Beatty GL, Rustgi AK, Vonderheide RH, Leach SD, Stanger BZ. EMT and Dissemination Precede Pancreatic Tumor Formation. Cell. 2012;148:349-361.

Columbia University and the UNIVERSITY OF PENNSYLVANIA have collaborated on the first animal model of Barrett's esophagus:

Quante M, Bhagat G, Abrams JA, Marache F, Good P, Lee MD, Lee Y, Friedman R, Asfaha S, Dubeykovskaya Z, Mahmood U, Figueiredo JL, Kitajewski J, Shawber C, Lightdale CJ, Rustgi AK, Wang TC. Bile Acid and Inflammation Activate Gastric Cardia Stem Cells in a Mouse Model of Barrett-Like Metaplasia. Cancer Cell. 2012;21:36-51.

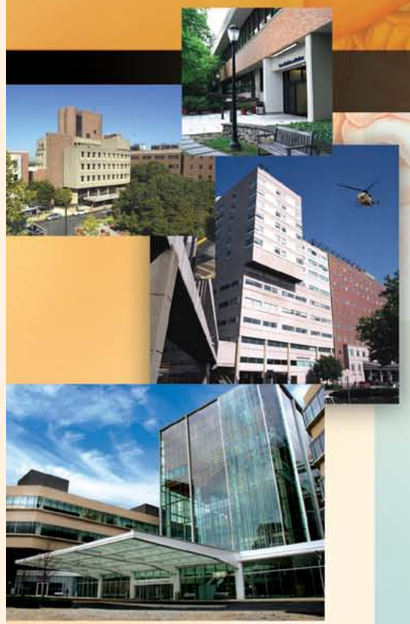
Penn Medicine
3600 Market Street
Suite 210
Philadelphia, PA 19104

Non-Profit Org.
U.S. Postage
PAID
Permit No. 2563
Phila., PA 19104

GASTROENTEROLOGY NEWSLETTER 2012

Penn Digestive & Liver Center
Penn Medicine

Spring 2012 | Volume 4 | Issue 7
Annual Research Issue



GASTROENTEROLOGY NEWSLETTER

RESEARCH IN GASTROINTESTINAL MEDICINE AT PENN

Penn Gastroenterology researchers are advancing the understanding of the intricate pathophysiology of gastrointestinal disease.

Gastrointestinal research has historically been an important focus at Penn Medicine. Thus, this issue of the Division of Gastroenterology Newsletter focuses on the Division's substantial contribution to the field of basic research and the rapid translation of that research into improved care at the Hospital of the University of Pennsylvania and other Penn facilities. The Division of Gastroenterology has realized dramatic growth in the past 15 years. With a cadre of new and established faculty recruited from among the nation's finest research institutions, and the many developed within Penn, a consequent increase in the number, complexity and diversity of research has occurred throughout the institution.

Funding for the division's research programs has seen significant increases, as well. National Institutes of Health (NIH) grants make up nearly 95 percent of the research portfolio at Penn. Total research is over $14 million/year. The GI division is currently home to one of only 16 National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) P30 GI/Liver Centers in the United States, several U01/U54 multiconsortia grants (imaging and translational medicine in GI cancers, intestinal stem cells, Barrett's esophagus, drug-induced liver injury, viral hepatitis B, liver transplantation) and a program project in esophageal cancer.

The division also receives a variety of training and lectureship grants to support the education of clinical investigators and physician scientists. The numerous educational initiatives under way at the division include a highly esteemed NIH undergraduate student scholar program; a "sabbatical" program that permits Penn medical students to devote a year of their education to research supported by the NIH; a GI pathophysiology module for medical students; and the division's renowned GI fellowship program. Supported by two NIH training grants, the GI fellowship program at Penn attracts the best medical residents in the country.

I hope you find this report, which highlights two of the many important research efforts within the Division of Gastroenterology at Penn, both informative and useful.

ANIL K. RUSTGI, MD

T. Grier Miller Professor of Medicine and Genetics
Chief of Gastroenterology

LOCATIONS

The Ruth and Raymond Perelman Center for Advanced Medicine
3400 Civic Center Boulevard
Philadelphia, PA 19104
215.349.8222

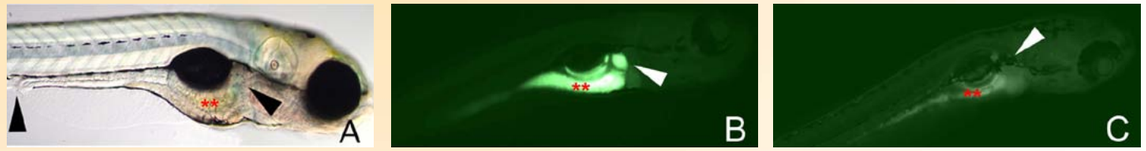
Penn Presbyterian Medical Center
38th and Market Streets
Philadelphia, PA 19104
215.662.8900

Penn Medicine Radnor*
250 King of Prussia Road
Radnor, PA 19087
610.902.1500

**A facility of the Hospital of the University of Pennsylvania*

Defining the Toxic Etiology of Biliary Atresia

In collaboration, the laboratories of Penn Gastroenterology researchers Rebecca Wells, MD, and Michael Pack, MD, have received NIH funding to identify toxins that cause biliary atresia.



Zebrafish assay for biliary abnormalities. A) Normal fish with arrowhead on right showing location of the gallbladder and asterisks showing the intestine. B) Normal fish showing uptake of fluorescent lipids into the gallbladder (white arrowhead). C) Toxin-treated fish showing lack of uptake of lipid into the gallbladder (location of gallbladder shown by white arrowhead). Photo courtesy of Michael Pack, MD.

Biliary atresia (BA) is the leading indication for liver transplantation in children. The condition is characterized by a progressive fibroinflammatory process that leads to the obliteration of all or part of the extrahepatic biliary tree, and manifests in the neonatal period. Surgery (i.e., Kasai portoenterostomy) in infancy is required in virtually all cases to permit bile drainage. Approximately two-thirds of children with the disease ultimately require a liver transplant due to overwhelming fibrosis (deposition of scar tissue).

Biliary atresia is uncommon (~ 1/15,000 live births) and has no known cause. Most researchers suspect it occurs in genetically susceptible patients who receive an environmental exposure (potentially a virus or toxin) late in gestation; however, no specific exposure has ever been identified, and the nature of the bile duct damage that leads to biliary atresia is not known.

This may soon change as a result of the combined efforts of Rebecca Wells, MD, and Michael Pack, MD, at the Perelman School of Medicine and their collaborator John Porter at the University of the Sciences. Dr. Wells' laboratory studies mechanisms of liver fibrosis, while Dr. Pack's laboratory uses zebrafish models to study the development of the biliary system.

Dr. Wells' interest in biliary atresia began in 2004 when Elizabeth Rand, MD, medical director of the liver transplant program at Children's Hospital of Philadelphia (CHOP), urged her to investigate the mechanism of fibrosis in this disease. Several years later, while gathering background material on the disease, Dr. Wells discovered that there had been a recent epidemic of biliary atresia in lambs in rural Australia. Similar to two previous epidemics decades before, this epidemic seemed to be associated with the ingestion of Australian pigweed (*Dysphania glomulifera*) by pregnant ewes grazed in unusual pastures during drought years. It appeared that the plants contained a toxin that affected the lambs' biliary system late in development, causing them to be born with biliary atresia. Dr. Wells contacted the Australian veterinarians who had diagnosed biliary atresia in the lambs, and arranged for them (in collaboration with botanists, rangers and veterinary pathologists) to collect samples of the plant. If the toxin could be purified and tested in animal studies, Dr. Wells reasoned, similar compounds of relevance to humans might be identified.

Both Dr. Wells and Dr. Pack are members of the Fred and Suzanne Biesecker Pediatric Liver Center at CHOP, which is dedicated to studying biliary atresia and liver development. Dr. Pack had developed an interest in biliary atresia through his membership in the Biesecker Center and his laboratory had previously developed assays to identify biliary damage in zebrafish.

To identify the molecular structure of the plant toxin, Dr. Wells and Dr. Pack contacted John Porter, PhD, an experienced natural products biologist at the University of the Sciences in Philadelphia. Dr. Porter agreed to fractionate the plant in an attempt to isolate a toxic fraction. The team was able to import the plant samples in 2008. For the next 16 months, Dr. Porter fractionated the plant and Dr. Pack tested the fractions to determine whether they caused biliary damage in zebrafish. Throughout this process, they received support from the Biesecker Center and the University of Pennsylvania NIDDK Center for Molecular Studies in Digestive and Liver Diseases.

In July 2009, Dr. Pack found that exposure to a highly purified fraction of the plant caused damage to the gallbladders and extrahepatic bile ducts of zebrafish, mimicking biliary atresia – a new animal model of the disease. In February 2012, Dr. Porter's team identified the structure of one of the toxins, a compound never before described. The laboratories of Dr. Wells and Dr. Pack are now seeking to determine the mechanism of action of the toxin, to find chemical mimetics to which humans might be exposed, and to use this information to develop potential therapies for biliary atresia. The collaborators have recently been awarded four years of funding from the NIH to further this line of research.

Diet, Genetic Factors, and the Gut Microbiome in Crohn's Disease

Frederic D. Bushman, PhD, James D. Lewis, MD, MSCE & Gary D. Wu, MD

With Frederic Bushman, PhD, of the Department of Microbiology at the Perelman School of Medicine, Gary Wu, MD, and James Lewis, MD, MSCE, of Penn Gastroenterology have initiated a series of studies to investigate the relationship between alterations in the human gut microbiome and the pathogenesis of Crohn's disease and colitis.

The large intestine is home to one of the most densely populated microbial communities on earth, with the number of bacteria exceeding host cells by more than ten-fold. The aggregate of all human gut bacteria, or microbiome, holds 100 fold more genes than those of its host. These microbiota provide a metabolic diversity that, among other benefits, aids in the digestion of foods and the development of the immune system. Alterations in the gut microbiome are associated with numerous diseases, however, including opportunistic infections such as *C. difficile colitis* and inflammatory conditions such as Crohn's disease.

Drs. Wu, Bushman and Lewis of Penn have previously examined the role of diet in modulating the gut microbiome composition.[1-2] More recently, they have initiated a series of studies, as part of the NIH Human Microbiome Project, to investigate the hypothesis that dietary therapy leads to consistent changes in the human gut microbiome that are associated with clinical response in Crohn's disease. The investigators are using deep sequencing to characterize the composition of the gut microbiome. Described below are two completed studies, FSM and COMBO, and a third study (known as PLEASE) that is ongoing.

Fecal Storage Methods (FSM)

This initial study was designed to systematically evaluate methods for surveying bacterial communities in human feces using 454/Roche pyrosequencing of 16S rRNA gene sequences, generating a total of 797,276 tags. Fecal samples from 10 individuals were analyzed and comparisons made of methods for fecal storage, DNA purification and sequence acquisition compared. These data were used to optimize protocols to collect, process and sequence bacterial 16S rDNA from fecal samples in subsequent studies.

Cross-sectional Study of Diet and Stool Microbiome Composition (COMBO)

Objectives: To evaluate the association between dietary intake, as determined by dietary questionnaire, and the composition of the gut microbiome in healthy subjects in the outpatient setting.

Methods: Diet inventories and 16S rDNA sequencing were used to characterize fecal samples from 98 individuals. Specific nutrients associated with variation in the gut microbiome for the 98 subjects were extracted, along with demographic factors. A controlled-feeding study of 10 subjects sequestered in a hospital environment was performed to compare high-fat/low-fiber and low-fat/high-fiber diets. For these subjects, stool samples were collected and DNA samples analyzed by 454/Roche pyrosequencing of 16S rDNA gene segments and, for selected samples, shotgun metagenomics.

Results: Fecal communities clustered into enterotypes distinguished primarily by levels of Bacteroides and Prevotella. Enterotypes were strongly associated with long-term diets, particularly protein and animal fat (*Bacteroides*) versus carbohydrates (*Prevotella*).

Results of the controlled feeding study showed that microbiome composition changed detectably within 24 hours of initiating a high-fat/low-fiber or low-fat/high-fiber diet, but that enterotype identity remained stable during the 10 days of sequestration. Thus, alternative enterotype states are associated with long-term diet.

Pediatric Longitudinal Study of Elemental Diet and Stool Microbiome Composition (PLEASE)

Currently under way, this study examines the effects of an elemental diet treatment on pediatric patients diagnosed with inflammatory bowel disease (IBD), particularly Crohn's disease. Elemental diet therapy is often effective in treating pediatric Crohn's disease. This study permits investigators to examine the microbiome changes associated with successful therapy, failed therapy and relapse. Longitudinal studies allow the investigators to specify microbial changes associated with successful or failed elemental diet therapy.

Reference

1. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, Sinha R, Gilroy E, Gupta K, Baldassano R, Nessel L, Li H, Bushman FD, Lewis JD. *Science.* 2011;334:105-108.

2. Wu, GD,* Lewis, JD,* Hoffmann C, Chen YY, Knight R, Bittinger K, Hwang J, Chen J, Berkowsky R, Nessel L, Li H, Bushman FD.* *BMC Microbiol.* 2010;10:206. *Joint corresponding authors.