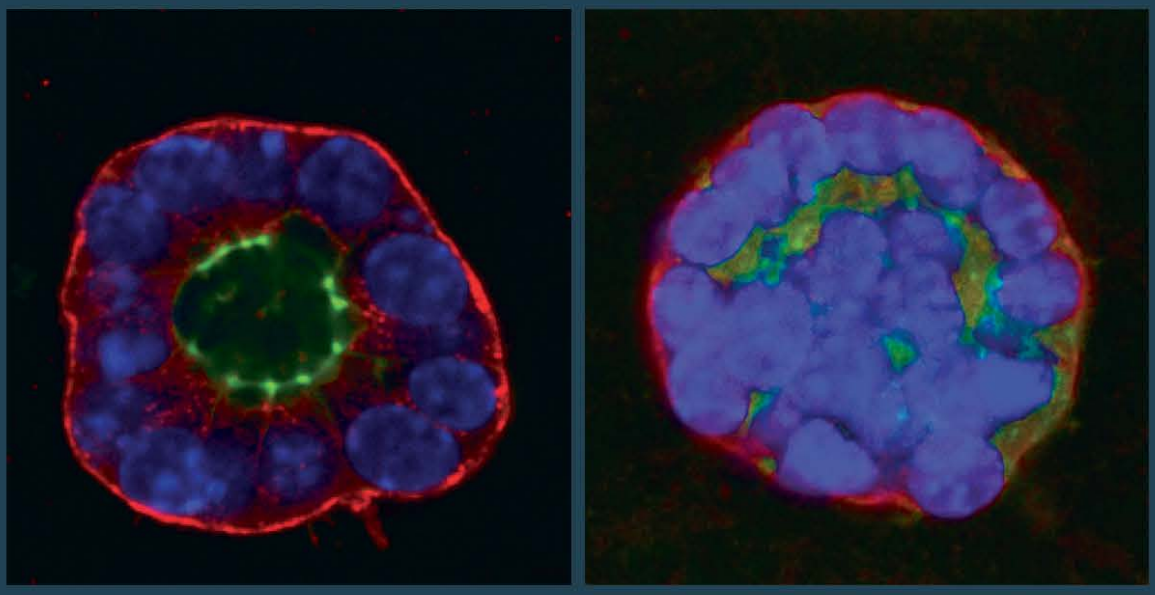

Biliary atresia is the leading cause for liver transplants in children. It occurs when, for reasons unclear, the bile ducts that drain the liver become inflamed and scarred, which causes a back-up of bile into the liver, resulting in jaundice and liver failure. Research described in this chapter probes the mysterious origins of this pediatric disease by examining the possible role of environmental factors. The images above show cross sections through spheroids in cell culture of mouse bile duct cells treated with either a harmless fluid (left) or a newly discovered plant-based toxin called biliatresone (right). The toxin disrupts the usually hollow opening in the center of the bile duct and the overall arrangement of the cells. This toxin's ability to interfere with the normal architecture of the bile duct could contribute to the bile duct obstruction, back-up of bile into the liver, and resulting liver damage seen in diseases such as biliary atresia.

Images courtesy of Dr. Rebecca G. Wells, University of Pennsylvania School of Medicine. From: Lorent K, Gong W, Koo KA, Waisbourd-Zinman O, Karjoo S, Zhao X, Sealy I, Kettleborough RN, Stemple DL, Windsor PA, Whittaker SJ, Porter JR, Wells RG, and Pack M. Identification of a plant isoflavonoid that causes biliary atresia, Sci Transl Med 7: 286ra67, 2015. Reprinted with permission from AAAS.

Digestive Diseases and Nutrition

Digestive diseases are among the leading causes of doctor visits, hospitalizations, and disability in the United States each year. These conditions span a wide spectrum of disorders that affect the gastrointestinal (GI) tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. The latest concerted effort to address the burden of all digestive diseases combining multiple big data sources estimated a total of 72 million ambulatory care visits to physicians' offices and to hospital emergency and outpatient departments with a primary diagnosis of digestive diseases in the United States.[1] In addition, 4.6 million hospitalizations with a primary diagnosis of digestive diseases and 13.5 million hospitalizations with a primary or secondary diagnosis of digestive diseases were reported.[1] More recently, a study focusing specifically on the clinical and economic burden of emergency department visits reported 15.1 million emergency department visits with a primary diagnosis of digestive diseases and a total charge of $27.9 billion in 2007.[2]

Some digestive diseases are common and others quite rare. Yet collectively, they strike individuals across the lifespan, exacting a significant toll on public health in terms of their effects on quality of life, years lost due to premature death, and costs associated with hospitalization and pharmaceutical and surgical interventions. NIDDK-supported scientists are vigorously pursuing research with the ultimate goal of reducing the public health burden associated with digestive diseases. Such efforts aim to better understand how widespread these diseases are across the United States and in specific population groups, to identify their causes and how they progress, and to test new interventions for prevention and treatment, including drugs, surgery, and behavior modification.

Inflammatory bowel diseases (IBD), which include Crohn's disease and ulcerative colitis, are marked by damaging inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. These diseases often strike early in life, with a peak age of onset in adolescence or young adulthood. Treatment frequently requires prolonged use of multiple drugs and may require surgery, including removal of the affected region of the intestine. Scientists are investigating the complex interactions among the genetic, environmental, immune, microbial, and cellular factors that contribute to, or protect against, the development of IBD. The continued discovery of predisposing genetic variations, potential autoimmune and microbial influences, and new methods to repair damaged intestinal tissue will help catalyze the design of novel therapeutic strategies. Research on controlling intestinal inflammation has potential benefits not only for patients with IBD, but also for those at risk of developing colorectal cancer.

Diseases of the stomach and intestines include some of the most common digestive diseases, such as

[1] *Everhart JE and Ruhl CE. Gastroenterology 136: 376-386, 2009.*

[2] *Myer PA, et al. Am J Gastroenterol 108: 1496-1507, 2013.*

peptic ulcer disease, which is typically caused by an infection with the bacterium *Helicobacter pylori* or use of non-steroidal anti-inflammatory drugs. Stomach and intestinal disorders also include functional bowel disorders, which result in symptoms of abdominal pain and altered bowel habits. For example, irritable bowel syndrome (IBS) causes pain and constipation or diarrhea. IBS more frequently affects women, who may display a different range of symptoms and respond differently from men to pharmacologic treatments for the disease. While diet and stress contribute to this disorder, its underlying causes are unknown. Gastroesophageal reflux disease, in which stomach acids rise up into the esophagus, is a common functional bowel disorder that can lead to a condition known as Barrett's esophagus. This condition, in which cells lining the esophagus turn into an intestinal type of cell, is associated with a heightened risk of esophageal cancer—one of the cancer types still on the rise in the United States. Gastroparesis, another type of functional bowel disorder, is characterized by delayed emptying of food from the stomach, resulting in nausea, vomiting, and abdominal discomfort. While many cases of gastroparesis are of unknown origin, a common cause is diabetes, which is thought to damage nerves leading to the stomach and controlling movement of food. Fecal incontinence, or impaired bowel control, is another bowel disorder that poses a major public health burden. Although fecal incontinence is more common in older adults, it can affect people of any age. Because it is difficult to talk about, many people suffer without seeking professional treatment for this surprisingly prevalent condition. Researchers thus aim both to examine barriers in addressing fecal incontinence and to develop improved treatment strategies.

Some digestive diseases can be triggered by the body's reaction to certain foods. For example, in individuals with celiac disease, the immune system reacts to the protein gluten—a component of wheat, barley, and rye—and damages the small intestine. This damage interferes with the ability of the intestine to absorb nutrients from foods and can result in chronic diarrhea, bloating, anemia, and, in children, slower growth and short stature. The only current treatment for celiac disease is maintenance of a strict gluten-free diet, which is difficult for many people. Diagnosis of celiac disease can be challenging, due to the non-specific and often minimal symptoms in people with the disorder. Recent and continued advances in the understanding of genes that predispose individuals to develop celiac disease may contribute to improved diagnosis in the future through genetic-based screening.

The microbes that inhabit the GI tract are important factors in maintaining or tipping the balance between digestive health and disease. These bacteria and viruses can affect long-term health and nutritional status in some surprising ways, depending on their interactions with each other, with intestinal cells, and with nutrients ingested by their human host. Scientists are gaining insights into the ways these GI microbes influence the development and function of the digestive tract and other systems throughout the body, such as those with immune and metabolic functions, as well as how the composition of the GI microbial community changes with factors such as age, geography, diet, and antibiotic usage.

The exocrine pancreas, which secretes enzymes required for digestion, is vulnerable to disorders such as acute and chronic pancreatitis and their complications. Common causes of pancreatitis include gallstones, heavy alcohol use, inherited genetic factors, and drugs. In all forms of pancreatitis, digestive enzymes attack the pancreas from within, causing inflammation, loss of function, and severe pain. Research has elucidated genetic and other factors contributing to pancreatitis that may lead to ways to treat or prevent this disorder.

The liver is an organ within the digestive system that performs many critical metabolic functions, including processing and distribution of nutrients such as fats. When the liver is functionally compromised by disease, serious adverse effects on health can occur, which sometimes leads to complete liver failure. Some liver diseases primarily affect children, such as biliary atresia (a progressive inflammatory liver disease), while others generally affect adults, such as a form of nonalcoholic fatty liver disease (NAFLD) or nonalcoholic steatohepatitis (NASH). In recent years, however, NAFLD has been increasingly diagnosed in children in the United States as well, concurrent with rising overweight and obesity. Some forms of liver disease are caused by viral infection, as in most cases of hepatitis, or by genetic mutations such as alpha-1-antitrypsin deficiency; others arise from diverse factors such as autoimmune reactions, drug toxicity, and other triggers, some of which are unknown. Many liver diseases, such as chronic hepatitis B and C, place individuals at elevated risk for developing liver cancer. A healthy liver is necessary for life, and the only treatment for end-stage liver disease is a liver transplant. Because the number of livers available from deceased donors is limited, research is critical to identify liver disease early, find methods to preserve liver function in people with liver disease, and develop and further study new treatment options, including experimental, cell-based approaches to liver regeneration.

The number of Americans who are overweight or obese has risen dramatically in recent decades and is now at epidemic levels. Obesity is associated with numerous diseases, including type 2 diabetes, heart disease, and cancer. Multiple factors contribute to obesity. As scientists elucidate the molecular, genetic, microbial, and environmental factors that influence appetite, metabolism, and energy storage, they are identifying potential avenues for the development of new intervention strategies to promote safe, long-term weight loss. In addition to new pharmacologic interventions for obesity that may arise from research, existing bariatric surgical techniques are being evaluated for their long-term impacts on weight loss, obesity-associated disease, and well-being. Investigators are also continuing research to help people achieve healthy lifestyles that include physical activity and improved diet. *(Additional information on NIDDK-supported research endeavors focusing on obesity is provided in the Obesity chapter.)*

Other nutrition-related disorders under investigation involve specific, inherited alterations in nutrient metabolism. NIDDK-supported research has enhanced knowledge of how these nutritional disorders develop and how they can best be treated.

GUT MICROBES IN HEALTH AND DISEASE

Gut Microbial Communities Shaped by Human Genetic Factors: A collaborative research group studying a large population of twins in the United Kingdom has shown that human genetic factors shape the composition of the gut microbial community, and some gut microbes may in turn affect the metabolism of their human "hosts." The first members of the gut microbiome, the collection of all microbes present in the gut and/or their genetic material, are acquired from the maternal "environment" at birth, or possibly even earlier in the womb. Similar gut microbiomes in related adults are often attributed largely to a shared environment, including common diets. However, previous studies also hinted at the possibility that other determinants of an individual's unique gut microbiome over time may lie in one's own human genome. Researchers based at institutions in the United States and the United Kingdom set out to analyze a sufficiently large number of people to test this idea, using the TwinsUK

study. They collected fecal samples from 416 pairs of identical or fraternal twins. By sequencing a portion of the microbial genetic material from each of those samples, they were able to identify and quantify specific gut microbial species present in the twins' samples. Pairs of genetically identical twins had more similar gut microbiomes than pairs of fraternal twins did. Within the identical twin pairs, winning the prize for the group of gut microbes whose abundance was most closely tied to human genetic influences were members of the *Christensenellaceae* family—a family of bacteria that has only been described since 2012. The varying abundance of these bacteria in different twin pairs may actually have a larger impact on their gut microbial communities, as they tended to coincide with certain other gut microbes. The researchers also noticed that the *Christensenellaceae* family microbes were more abundant in lean study participants than in those who were obese. They tested whether these microbes might protect against weight gain by introducing microbes from obese participants into mice that had been raised up to that point in a sterile environment (free of microbes). To some of the mice they also introduced bacteria from one species of *Christensenellaceae*. After 3 weeks, the mice that received *Christensenellaceae* family microbes along with the obese donor's gut microbes were leaner than those that received only the obese donor's microbes. These findings point to members of this microbial family as important contributors to human metabolism, with potential effects on weight gain, and show that their abundance, and in some cases presence or absence, is strongly affected by one's genes. This may be one way in which genes affect human metabolic health: by influencing the capacity to host beneficial microorganisms. Future studies will explore which human genes influence the components of the gut microbiome. Such knowledge could inform future health-promoting interventions, potentially suggesting ways to adjust gut microbial levels depending on an individual's genetic background.

Goodrich JK, Waters JL, Poole AC,...Ley RE. Human genetics shape the gut microbiome. Cell 159: 789-799, 2014.

Harmful Gut Bacterial Effects Behind Persistent Childhood Undernutrition: By collecting samples of fecal bacteria from severely undernourished infants and children in Malawi and testing these in mice, researchers have identified a group of bacteria that take hold in the gut during nutrient deficiency and damage the intestinal lining, thwarting the body's ability to absorb available nutrients in the diet and to fend off disease. Childhood undernutrition is a large, intractable problem and the leading cause of childhood mortality worldwide. Research has shown that it is driven not only by limited access to nutritious food, but could also be exacerbated by other factors, including the gut microbial community. Studies of severely undernourished children living in Malawi have found changes in their gut microbes that persist despite interventions to provide adequate nourishment. Recently, the same researchers continued this line of inquiry by delving deeper into identifying the core group of gut bacterial strains harbored by undernourished infants and children and uncovering the ways in which they compromise future nutrition and health.

The team of American, Malawian, and Finnish researchers used samples collected from two studies conducted in male and female children living in Malawi: one of twin pairs and another of single-born children. They transplanted fecal microbes from a pair of Malawian twins—one with persistent undernutrition and the other without—into separate groups of germ-free, male mice fed either a nutrient-deficient diet similar to what the children ate before a nutritional intervention or a standard, nutrient-rich diet. Their aim was to identify

gut microbes that were particularly abundant in the undernourished twin, compared to the healthy twin, and that interact with the immune system, possibly contributing to adverse effects such as weight loss. They focused on microbes that bound to the molecule immunoglobulin A (IgA), which is secreted by activated immune cells directly into the gut and is altered in malnutrition. Using a technique to pull out IgA-bound bacteria and sequence their genes, they identified a collection of IgA-activating gut bacteria present in large numbers in the undernourished twin compared to the healthy twin. They found a certain bacterial family called *Enterobacteriaceae*, which includes *Escherichia coli*, was especially abundant in the undernourished twin's IgA-activating gut microbial community. By giving the IgA-activating gut microbes obtained from either the undernourished or healthy twin to another set of germ-free mice on the nutrition-deficient diet, they saw a stark demonstration of the microbes' powerful effects—half the mice given the microbes from the undernourished twin died within 5 days while all the mice receiving microbes from the healthy twin survived despite their equally poor diet. However, the mice given the undernourished twin's microbes could also be saved by either feeding them a nutritious diet or giving them a few key bacterial species found in the healthy twin's gut. Looking more closely at how bacteria present in the undernourished twin's gut could compromise health, the scientists found that the *Enterobacteriaceae* family bacteria, in combination with others, activated inflammatory molecules, damaged the structural integrity of the intestine—thereby impairing nutrient absorption and weakening this barrier to infection—and increased weight loss in the mice. Applying the knowledge they gained from the animal studies, they then designed studies to seek out these bacteria in the human samples from young Malawian twins and single-born children. They found the same *Enterobacteriaceae* family bacteria to be enriched in the undernourished twins and single-born children, but the children's ability to launch an immune response with IgA offered some protection against the growth stunting caused by their heavy burden of pathogenic bacteria.

This study shows how harmful gut bacteria interact in a powerful way with diet and the host immune and digestive systems to affect overall health. These bacteria can also be passed on, limiting the resilience of others living nearby under the same dietary restrictions. Because the mouse studies were done in male animals only, additional research will be needed to determine if results are applicable to both females and males. These findings may be used to identify individuals at risk for persistent childhood undernutrition and design more effective therapeutic and preventive strategies for ameliorating this global problem in the future.

Kau AL, Planer JD, Liu J,...Gordon JI. Functional characterization of IgA-targeted bacterial taxa from undernourished Malawian children that produce diet-dependent enteropathy. Sci Transl Med 7: 276ra24, 2015.

Viruses Can Cover for Helpful Bacteria in the Gut's Microbial Community: Recent research in mice has shed new light on the role of viruses in the gut, suggesting that a particular type of virus can confer some of the same benefits to its host as do gut bacteria. The community of microorganisms living in the digestive tract (collectively called the gut microflora or gut microbiome) performs many functions beneficial to its host. Some of these functions, like digestion of food, are well-known, but recent research has shown that the gut microbiome can also play a significant role in host immunity, physiology, and metabolism. These findings have sparked concerns about possible health effects of widespread antibiotic use on the gut microbiome, because antibiotics not only kill pathogenic bacteria,

but also reduce levels of normal gut bacteria. In addition to bacteria, the intestinal community also contains other types of microorganisms, including enteric viruses (viruses that preferentially infect the intestinal tract). Although some viruses are harmful, others appear to inhabit the intestines peacefully. Enteric viruses' role as part of the gut microbiome has been largely unstudied, as challenges in detecting and characterizing these viruses have been overcome only recently.

To learn more about the roles these viruses play in the gut, researchers studied the effect of a common type of mouse enteric virus called murine norovirus (MNV) in germ-free (GF) mice. Due to their lack of gut bacteria, GF mice have intestinal and immunological abnormalities that can be corrected by adding back some types of gut bacteria. To determine if a virus could also reduce these abnormalities, researchers used MNV to infect GF mice, who then passed the infection to their offspring. GF mice inoculated with MNV and their offspring both exhibited a partial reversal of the typical abnormalities seen in GF mice: their intestinal tissues more closely resembled those of conventional mice. MNV also reversed some of the irregularities in their immune systems, in some cases returning certain immune factors and immune cell types to near-normal levels. Thus, the mice's reactions to MNV were in some ways similar to their reactions to normal intestinal bacteria. To see if MNV could protect mice against some of the harmful effects of antibiotics, the researchers treated normal mice with antibiotics for 2 weeks, inoculated these antibiotic-treated mice with either MNV or gut bacteria, and observed the resulting intestinal and immunological changes. Both the viral and bacterial treatments reduced antibiotic-induced intestinal abnormalities, though the two treatments had slightly different effects on the immune system and on the genes activated. Antibiotic treatment can also leave the gut susceptible to damage, due to its destruction of the normal gut microbiome. MNV, however, was able to protect the guts of antibiotic-treated mice from further damage or death caused by either a chemical agent used to mimic human inflammatory bowel disease or by pathogenic bacteria. These results demonstrated for the first time that a virus in the mouse intestinal tract could have beneficial effects on intestinal physiology, immune function, and disease protection similar to those granted by gut bacteria. Further research is needed to determine if human enteric viruses have similar functions and to define further the roles viruses play in the mammalian gut microbiome.

Kernbauer E, Ding Y, and Cadwell K. An enteric virus can replace the beneficial function of commensal bacteria. Nature 516: 94-98, 2014.

EARLY NUTRITION AND GASTROINTESTINAL DISEASE

Understanding Breast Milk's Protective Effects Against Deadly Gastrointestinal Disease in Newborns: Studies in newborn mice have uncovered how breast milk could protect against a sometimes lethal form of gastrointestinal disease in newborns called necrotizing enterocolitis or "NEC." NEC is the most common and deadly form of gastrointestinal (GI) disease affecting premature infants, causing destruction and permanent loss of entire portions of the intestine, which can lead to lifetime dependency on artificial nutritional support. The mechanism by which NEC develops involves an interaction between intestinal bacteria and the intestinal lining in which a pro-inflammatory immune reaction is launched, including activation of a receptor, called Toll-like receptor 4 (TLR4), that recognizes toxic molecules on the surfaces of intestinal bacteria. Breast milk prevents NEC development, but the basis of its protective effect has not been understood fully.

Researchers studied both rodent intestinal cells in culture and newborn mice given breast milk from lactating mice under various conditions to get at the underlying reasons for the milk's NEC-protective properties. Pretreating intestinal cells in culture with breast milk reduced the activation of pro-inflammatory factors such as TLR4 within cells when in the presence of the bacterial toxin. Heating the breast milk at a high temperature, which inactivates proteins, abolished this effect. Based on this and previous studies, the scientists suspected a protein in breast milk called epidermal growth factor (EGF) might be involved. By treating the cells with breast milk depleted of its EGF, then adding the growth factor back in again, they confirmed their suspicions that EGF in breast milk plays a role in tamping down the intestinal inflammatory process by inhibiting TLR4. Studies in mice confirmed that these findings held true in a whole animal. Newborn mice whose cells were genetically engineered to light up when they expressed a key inflammatory molecule activated by TLR4 were injected with the bacterial toxin and then given breast milk or saline. The group given breast milk showed reduced inflammation and pro-inflammatory factors such as TLR4 in their intestines compared to those given saline. The essential role of EGF in breast milk's protective effects was also replicated in the mice. In the mice fed breast milk but either treated with an EGF inhibitor, given breast milk depleted of its EGF, or genetically engineered to remove the receptor needed for EGF response in the intestine, the milk's protective effects were abolished. The milk was no longer able to protect the intestine by inhibiting inflammatory molecules produced in response to bacterial proteins, supporting intestinal cell replication, or protecting against cell death.

These studies illuminate breast milk's direct beneficial effects on the cells that line the inside of the intestine by guarding them against inappropriate inflammatory responses and cell death in the presence of gut bacteria. Additional studies will be needed in humans to show whether breast milk works similarly, through the action of EGF, to protect newborn girls and boys from deadly GI disease.

Good M, Sodhi CP, Egan CE,...Hackam DJ. Breast milk protects against the development of necrotizing enterocolitis through inhibition of Toll-like receptor 4 in the intestinal epithelium via *activation of the epidermal growth factor receptor. Mucosal Immunol 8: 1166-1179, 2015.*

Workshop on Biomarkers of Pancreatic Disease

On July 22, 2015, the NIDDK, the National Institute of Biomedical Imaging and Bioengineering (NIBIB), and the National Pancreas Foundation sponsored a workshop to address research gaps and opportunities in the development of new biomarkers of pancreatic disease. Biomarkers, or indicators in the body that can signal the presence of a disease, risk factors, or other health conditions, are extremely important as screening and diagnosis tools. Accurate and sensitive biomarkers would have important implications for the treatment of pancreatic diseases, which are typically diagnosed at advanced stages due to the difficulties in detecting the early stages of the diseases. Screening for early biomarkers would greatly expand the opportunity for early intervention to improve health outcomes. The workshop, titled "Advances in Biomedical Imaging, Bioengineering, and Related Technologies for the Development of Biomarkers of Pancreatic Disease," focused on recently developed, noninvasive approaches to the diagnosis of chronic pancreatitis and the detection of pancreatic cancer. The workshop was convened to build on recent research advances that are offering new approaches to diagnosing the presence and stage of pancreatic diseases at an early time point in their course, which would allow early treatment and possibly the ability to monitor response to therapy.

The workshop highlighted some of the exciting new imaging advances that are expanding the ability to detect early stage pancreatic disease. For example, the participants discussed the current imaging techniques that are used to diagnose chronic pancreatitis, such as computed tomography (CT) and magnetic resonance imaging (MRI), noting that they do not yet have adequate sensitivity to diagnose early disease. They also discussed noninvasive imaging techniques that hold promise, such as magnetic resonance elastography (MRE), as well as future research directions to pursue.

The workshop participants also discussed the limitations to current imaging techniques used to diagnose pancreatic cancer, including the

inability to detect the cancer until it has spread to other parts of the body (metastasized), contributing to poor survival rates. As a potential biomarker of early pancreatic cancer, they discussed the promise of using cells or exosomes (small, fluid-filled sacs) that are shed by the primary tumor into the bloodstream. With further research, it may be possible to use these circulating cells or exosomes to detect pancreatic cancer with a blood test before the tumor itself is detectable by other diagnostic techniques.

To propel research progress in this area, the NIDDK, with support from the National Cancer Institute (NCI), has recently funded a multi-center consortium to pursue clinical research on pancreatic diseases, including chronic pancreatitis, acute recurring pancreatitis, pancreatic cancer, and the type 3c diabetes that may result from these diseases.

A major conclusion of the workshop was that future research is needed to refine the new sophisticated technical methods that were discussed and to validate them in large-scale studies of patients at risk for the development of pancreatic disease. This will help clinicians diagnose these pancreatic diseases at early stages when intervention can reverse the disease or improve the outcome of treatments. Because of new and emerging technologies, this goal is now more attainable than ever before.

RISK FACTORS FOR INFLAMMATORY BOWEL DISEASE

Digging Deeper for the Genetic Roots of Inflammatory Bowel Disease: A recent study of more than 32,000 men and women with Crohn's disease or ulcerative colitis has shed light on the genetic underpinnings of these debilitating diseases. Inflammatory bowel disease (IBD) results from chronic inflammation in the gut and is characterized by symptoms such as diarrhea, intense abdominal pain, and weight loss. The two most common forms of IBD—Crohn's disease and ulcerative colitis—are thought to be caused by a complex interplay between genetic and environmental factors. Genome-wide association studies have identified over 160 areas of the human genome that contain IBD risk factors, although it is not clear which specific genes are important for the initiation and development of IBD. Among the candidates are genes encoding the human leukocyte antigen (HLA) molecules that play important roles in immunity. While several researchers have examined the HLA genes in search of genetic variants that would confer susceptibility to IBD, the studies were limited by small sample sizes, so it was unclear if any known HLA variants could contribute to the development of IBD.

To address this, an international group of researchers compared the genomes of people living in 15 countries across Europe, North America, and Australia, including 18,405 people with Crohn's disease, 14,308 people with ulcerative colitis, and 34,241 people without these diseases. The scientists employed a powerful technique, called high-density single nucleotide polymorphism typing, to identify variants of the HLA genes that were more common in people with IBD than in people without the disease. While the group found that most of these genetic variants were either associated with Crohn's disease or ulcerative colitis, the researchers found that a certain variant, HLA-DRB1*01:03, was highly associated with both diseases, suggesting that it may be involved in the development of both forms of IBD. The researchers also found that the HLA genes in people with ulcerative colitis, but not Crohn's disease, were less likely to be heterozygous—in other words, people with ulcerative colitis were more likely to have two copies of the same HLA variant. This suggests that having two different variants for each HLA gene might protect people from developing ulcerative colitis to some degree. These findings point to specific similarities and differences in immune-related molecules between the two most common forms of IBD, and they point to specific genetic traits that could be important in the development of these diseases.

*Goyette P, Boucher G, Mallon D,...Rioux JD. High-density mapping of the MHC identifies a shared role for HLA-DRB1*01:03 in inflammatory bowel diseases and heterozygous advantage in ulcerative colitis. Nat Genet 47: 172-179, 2015.*

Viruses in Gut Linked to Inflammatory Bowel Disease: New research points to viruses inhabiting the gut as possible culprits in inflammatory bowel disease (IBD). IBD, which includes Crohn's disease and ulcerative colitis, is a group of debilitating conditions caused by inflammation in the gut, leading to cramps, diarrhea, and bleeding. There is ample evidence that this inflammation could be caused by a combination of genetic factors and an improper immune reaction to the community of bacteria that reside in the gut. Many studies have explored the connection between gut bacteria and IBD; in fact, bacterial diversity is lower in patients with IBD, although it is not clear what causes this change. In addition to trillions of bacteria, the gut is also home to a diverse population of viruses; both bacteria and viruses are members of the community

of microbes known as the "microbiome." Most of these viruses are bacteriophages, which are viruses that infect bacteria, including those found in the gut, and insert genes into the bacterial DNA. The close relationship between gut bacteriophages and bacteria raises the possibility that there could also be a relationship between these resident viruses and IBD, although this connection is only beginning to be explored.

To examine the possible link between gut viruses and IBD, fecal samples from men and women with IBD in Chicago, Los Angeles, and the United Kingdom were examined for viral genetic material. Importantly, to control for potential environmental effects, the IBD samples were compared to samples from healthy volunteers living in the same area—sometimes even the same household. The most abundant viruses identified in all samples were members of two groups of bacteriophages called *Microviridae* and *Caudovirales*. The healthy participants had similar numbers of members from these two viral groups. However, in the participants with Crohn's disease or ulcerative colitis, the *Caudovirales* viruses were not only more abundant than the *Microviridae*, but there were also more types of *Caudovirales* viruses. In other words, the *Caudovirales* group of viruses appeared to have expanded and diversified in the volunteers with IBD. Even though these bacteriophages have a close relationship with bacteria and rely on them to reproduce, the *Caudovirales* diversification did not appear to be simply due to an increase in bacterial diversity, because bacterial diversity was lower in the IBD participants than in healthy individuals.

These results introduce a new twist to the complicated understanding of IBD. The exact role gut viruses may play in this disease—or in any other diseases and conditions in which gut bacteria have been found to play a role, such as diabetes, obesity, metabolic diseases, and cancer—remains to be determined. Nonetheless, the discovery of this link between gut viruses and IBD could open the door to designing better treatments or preventative measures in the future.

Norman JM, Handley SA, Baldridge MT,...Virgin HW. Disease-specific alterations in the enteric virome in inflammatory bowel disease. Cell 160: 447-460, 2015.

INSIGHTS INTO GUT DEVELOPMENT AND ACTIVITIES

Gut Sensory Cells Can Signal Directly to the Nervous System: New research in mice has shown that sensory cells in the gut are closely connected to nerves, enabling the gut to transmit signals about ingested nutrients directly to the nervous system. The lining of the gut is dotted with biological sensors called enteroendocrine cells that respond to dietary intake. For example, these cells can detect chemicals such as ingested fats and sugars. They can also contribute to satiety, or the feeling of fullness after a meal, by sending signals to reduce appetite. The prevailing model has been that these cells respond to ingested nutrients by releasing hormones that diffuse into surrounding tissue and the bloodstream. The hormones eventually affect the nervous system, leading to regulation of glucose (sugar) levels, pancreatic secretions, or the movement of intestinal contents. While some elements of this hormonal process have been identified, it was unclear whether the enteroendocrine cells might use another means to contact the nervous system.

A team of scientists recently uncovered a new, more efficient method by which enteroendocrine cells transmit signals to nerves. Looking at the intestinal tracts of mice, the researchers found

that many enteroendocrine cells were extending arm-like projections to directly interact with nerves. To understand this connection in more detail, the scientists filmed enteroendocrine and nerve cells together under a microscope. They observed that a nerve cell would extend a nerve fiber toward an enteroendocrine cell, which would then respond by sending its own projection toward the nerve cell until the two cells were connected. The scientists also found that many of the typical components involved in transmitting and receiving neural signals are present in enteroendocrine cells. This suggests that these cells not only have the ability to send signals from the gut to the nervous system, but also to receive signals from nerves that may in turn affect gut function. As a final test of this enteroendocrine-nerve cell circuit, the scientists infected enteroendocrine cells in mice with modified rabies viruses that can each further spread to only one additional cell. The viruses spread to adjacent nerve cells, suggesting that the enteroendocrine cells and nerve cells are intimately associated.

Compared to hormonal diffusion, this type of direct cell-to-cell communication allows a more rapid and localized response to nutrients in the gut, although some viruses may take advantage of this connection as an easy route to infect nerve cells. Future research may explore whether this connection occurs in humans, and what role it may have in nutrient sensing, satiety, and, potentially, infection.

Bohórquez DV, Shahid RA, Erdmann A,...Liddle RA. Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. J Clin Invest 125: 782-786, 2015.

Animal Models Provide Important Clues to Gut Vessel Development: A recent study in animal models has uncovered details of how arteries and lymphatic vessels develop in the gut before birth. The gastrointestinal tract is enveloped by an intricate network of vessels, including arteries that supply the gut with oxygen and nutrients, and lymphatics that contribute to immunity and help absorb and transport fats. These lifelines travel through a thin, double layer of tissue attached to the stomach and intestines called the dorsal mesentery (DMe). During embryonic development, the DMe expands while the young gastrointestinal tract, which begins as a single and relatively straight tube, performs complicated maneuvers, rotating and looping to create the adult stomach and gut. These elaborate contortions are largely driven by the activity of a protein called Pitx2 exclusively in the left side of the DMe. Meanwhile, the blood and lymphatic vessels must mature in the expanding DMe, and their growth must occur in step with the movements of the developing gastrointestinal tract. Little is known about how this coordination takes place or how these vessels develop, although mistakes may result in vessel strangulation and embryonic lethality.

Researchers sought to uncover details of gut vessel development by examining artery and lymphatic vessel formation in the DMe of embryonic chickens, quail, and mice, which have gastrointestinal tracts similar to those in humans. They found that artery development coordinated with gut rotation by occurring exclusively in the left side of the DMe, and, like gut rotation, this was driven by Pitx2. Initially, small, temporary arteries, or cords, were produced, and then the cords converged to create a larger, permanent artery. This entire process was stimulated by Pitx2, because adding Pitx2 to the right half of the DMe resulted in the growth of arteries on that side, in addition to the left. The scientists found that Pitx2 accomplished this, in part, by stimulating the production of another protein involved in artery growth called Cxcl12. Blocking Cxcl12 prevented the transient cords from developing. However, adding Cxcl12 to the DMe in the absence of Pitx2 did not stimulate cord growth,

suggesting that there are additional signals activated by Pitx2 that are essential for cord growth other than Cxcl12. The scientists also found that, unlike peripheral lymphatic vessels that run along veins, the gut lymphatic vessels developed exclusively alongside the new arteries, which suggested that arteries are required for gut lymphatic vessels to form. In fact, when artery development was blocked in the DMe, the lymphatic vessels failed to develop. These results link Pitx2 and Cxcl12 to the growth of arteries—and ultimately to lymphatic vessels—in the left side of the DMe. Studies such as these are beginning to decipher the complicated steps involved in vessel development in the embryonic gut.

Mahadevan A, Welsh IC, Sivakumar A,...Kurpios NA. The left-right Pitx2 *pathway drives organ-specific arterial and lymphatic development in the intestine. Dev Cell 31: 690-706, 2014.*

Human Intestinal Model from Stem Cells Grown in Culture and Transplanted into Mice: A research group has built a better model for studying the human small intestine by growing intestinal tissue from human stem cells and then successfully transplanting it into the mouse kidney capsule, where it performs digestive functions and responds to the physiological environment. To date, scientists have had limited options available for studying the human intestine under physiological conditions similar to the human body. Mouse models and human cells in laboratory culture fall short in terms of simulating what happens to the human intestine inside the body. By building on a previous advance that created three-dimensional mini-intestines, or "organoids," from human stem cells, researchers have now produced a more accurate model for studying the human intestine. First, they grew human stem cells in laboratory cell culture with a special mix of growth factors for about 5 weeks to allow them time to form hollow, intestine-like organoids. They were able to use different types of human stem cells to form the organoids, including embryonic stem cells approved for NIH research and "induced pluripotent stem cells" made from an adult human cell type called a fibroblast. The organoids were then transplanted into an immune-deficient breed of mice, which would not reject the human tissue, by placing them under the kidney capsule, a layer of connective tissue covering each kidney. In more than 130 transplant procedures performed, over 90 percent were successful. Transplants were allowed to grow and mature inside the mice for 6 weeks before they were collected. After collection, the transplants were seen to have grown 50- to 100-fold and exhibited signs of mature human intestinal tissue, particularly similar to the small intestine, including a diversity of differentiated intestinal cell types, appropriate intestinal structures and layers, and functions such as maintaining the intestinal barrier, producing digestive enzymes, and absorbing nutrients. The transplanted tissue's maturation outstripped that of tissue grown for the same time period inside a culture dish. The researchers also tested the advantages of this model for studying human intestinal responses in a physiological (whole-body) system. Surgical removal of a portion of the intestine is known to stimulate some compensatory growth of the remaining intestine through factors present in the circulation. The researchers either surgically removed part of the intestine or performed a sham surgery in mice that had previously been transplanted with the human intestinal organoids for 6 weeks, then examined the effect on the transplant. The mice with the intestinal surgery had more robust growth in the transplanted intestinal organoids than the transplants in mice receiving the sham surgery, highlighting the transplanted intestinal organoids' responsiveness to physiological signals. This study has created the first functional, *in vivo* model of human small intestine generated from stem cells. This unique model can be used to study more complex contributors to human intestinal function and disease than was possible before. In the future, the technology could be used for further research toward

a goal of generating personalized human intestinal tissue, using organoids from induced pluripotent stem cells in particular, as a treatment for diseases such as short bowel syndrome.

Watson CL, Mahe MM, Múnera J,...Helmrath MA. An in vivo *model of human small intestine using pluripotent stem cells. Nat Med 20: 1310-1314, 2014.*

INTESTINAL CANCER GENETICS

Identification of Genetic Mutation May Lead to Better Screening for Carcinoid Tumors, a Type of Intestinal Cancer: A new hereditary mutation has been found to be associated with a rare but serious type of intestinal cancer, providing a potential screening target for the disease. Small intestinal carcinoids are a form of cancer that develops in the lining of the small intestine, originating from a type of cell that produces hormones. Because the carcinoid tumors are difficult to diagnose, they often go unnoticed until they reach an advanced stage when they could produce large amounts of hormone and cause symptoms such as abdominal pain, bloating, diarrhea, a rapid heartbeat, and difficulty breathing. At this stage, the cancer is difficult to treat, and most patients are faced with a poor prognosis. While most cases of intestinal carcinoids appear to be sporadic, or random, there are some that seem clustered within families, suggesting there might also be hereditary genetic factors that could be used as markers for screening. Early detection of carcinoids in someone with a family history of the disease would greatly improve the chances for a successful outcome; however, it has been difficult to identify specific mutations linked to the disease.

A group of scientists working in the NIDDK's Intramural Research Program sought to identify genetic mutations that could increase the likelihood of a person developing intestinal carcinoid tumors. The scientists studied 181 men and women in 33 families with histories of carcinoids. They found that, unlike random cases of intestinal carcinoids that typically develop from one tumor, familial cases are more likely to develop multiple tumors in several places at once, strongly suggesting an underlying genetic mutation is involved. To identify this mutation, the scientists focused on one of the families and carefully compared the genomes of its members. The researchers were eventually able to narrow their search down to a disruptive mutation in a single gene, *IPMK*, present in the cells of all members of this family with carcinoids but not in unaffected family members. In tests of cell growth in the laboratory under certain conditions, cells with the *IPMK* mutation survived better than normal cells, which may explain how they can grow excessively into a tumor.

This study provides evidence that mutations in the *IPMK* gene could be involved in familial cases of small intestinal carcinoids, which has important implications for screening. In fact, 17 members of the studied family who had not yet been diagnosed with carcinoids were found to have the mutation in *IPMK*, which means they could choose to undergo regular, careful screening to detect the disease in its early stages, when treatments would be more successful. None of the other 32 families in this study carried this same *IPMK* mutation, however, which means there are likely other hereditary factors yet to be identified. Nonetheless, the discovery of the link between a specific genetic mutation and familial small intestinal carcinoids is an important step towards improving screening for and successfully treating this disease.

Sei Y, Zhao X, Forbes J,...Wank SA. A hereditary form of small intestinal carcinoid associated with a germline mutation in inositol polyphosphate multikinase. Gastroenterology 149: 67-78, 2015.

SYMPTOM REPORTING IN IRRITABLE BOWEL SYNDROME

"Total Recall"? Variations in Memory of Pain and Other Irritable Bowel Syndrome Symptoms Over Time: New results concerning how people with irritable bowel syndrome (IBS) recall their symptoms could help inform both clinical research and clinical practice. IBS is a disorder of the digestive tract that disproportionately affects women. Symptoms include abdominal pain, diarrhea, and constipation. Despite these symptoms, people with IBS do not have corresponding signs of damage or disease in the digestive tract. Because a simple test or "marker" for IBS isn't currently available, both health care providers and scientists have relied upon patients' self-report of symptoms—typically, their recall of symptoms for a preceding week—to guide clinical care and the interpretation of clinical research study results, respectively. However, how accurately this recall reflects the IBS symptoms people have experienced over several days has not been established.

To test the accuracy of self-reports, researchers assessed symptom recall in persons (mostly women) diagnosed with moderate to severe IBS who were enrolled in an IBS treatment clinical trial. They asked participants to rate and record every day for 7 days information about four discrete symptoms: abdominal pain (average and worst), typical stool consistency (on a standard seven point scale), number of bowel movements, and feelings of urgency to defecate (average and worst). Participants were to enter this information at home at the end of each day into an electronic diary, which was time-stamped to ensure that entries were made on the symptom day, or "real time." Then, on day 8, participants returned to the clinic to fill out a clinical questionnaire assessing how well they recalled symptoms that they had had for the past 7 days. Using data from 177 participants, the researchers found that, as a group, the average recall on day 8 reflected certain, although not all, symptoms as recorded in the daily diaries. Recall of stool frequency, worst intensity of pain and urgency, and days of urgency corresponded well with diary entries, whereas other symptoms tended to be over-reported (*e.g.*, average intensities of pain and urgency and days of abdominal pain). In contrast, when responses were analyzed at the individual level—as they would be in a doctor's office, for example—the researchers found that, for a significant number of participants, day 8 recall did not correspond well at all with the real-time entries, including those that corresponded well on the group level. The difference in accuracy observed for the group versus individual responses could apparently be explained by the balancing out of retrospective over- and under-reporting of symptoms—and also indicated how group level results could easily mask individual level responses.

These results have important implications for both clinical practice and research. They suggest that both health care providers and researchers will need to consider when they need to accurately capture discrete symptom experiences and changes for individuals, as these can be masked on a group level (in a research situation) or subject to significant recall bias over time (in a clinical situation) when using the 1 week symptom recall approach. These and other considerations arising from this study are important both for optimizing care and for assessing efficacy of treatments for IBS symptoms.

Lackner JM, Jaccard J, Keefer L,...Brenner D; Representing the IBSOS Research Group. The accuracy of patient-reported measures for GI symptoms: a comparison of real time and retrospective reports. Neurogastroenterol Motil 26: 1802-1811, 2014.

UNDERSTANDING AND TREATING LIVER DISEASE

Plant Toxins Linked to Biliary Atresia in Newborn Animals: Studies in cell and animal models have led to the discovery of a plant toxin that causes changes that resemble the important pediatric liver disease called biliary atresia. Biliary atresia is a disease of the bile ducts that affects newborns and invariably leads to liver failure. The disease is fatal if not treated with surgery in the newborn period or liver transplantation thereafter. In biliary atresia, the bile ducts that drain the liver and deliver bile acids to the intestine become inflamed and scarred, which causes a back-up of bile into the liver, resulting in jaundice and liver failure. Although a rare disease, biliary atresia is still the most common form of severe liver disease in children and is the leading cause for pediatric liver transplantation. Its causes are not known, but both inherited and environmental factors seem to play a role. Clustering of biliary atresia cases within some geographic areas and time periods suggests that an environmental component, such as an infectious agent or toxin, may contribute to the disease.

An insight into a possible environmental factor came when Australian scientists identified a disease of newborn sheep that resembled biliary atresia. Strikingly, outbreaks of this condition occurred in Australian lambs during the immediate period following severe droughts. They subsequently found that pregnant sheep in searching for food would consume plant species that were growing on land that was usually under water. Analysis of the plants eaten by the Australian sheep herds during a recent drought pointed to a species of the genus *Dysphania*. Scientists in the United States imported samples of these plants from Australia and analyzed their components, isolating and examining 95 distinct fractions. Each of these components was then tested for its effects on the larvae of a small translucent fish called the zebrafish, a "model system" that can be used to identify substances and genes that cause injury or disease states. In one of the plant extracts, they identified a toxin that caused a similar damage to the bile ducts of zebrafish larvae as was described in the newborn sheep. They named this toxin "biliatresone" and showed that in high doses it caused defects in the formation of both the gallbladder and bile ducts of zebrafish larvae. They also found that larvae with a certain genetic mutation were more sensitive to gallbladder and bile duct injury from biliatresone. The region of the zebrafish genome that contained this mutation was sequenced and was found to be similar to regions in the human genome that have been associated with increased susceptibility to biliary atresia in humans. Moving to a mammal model closer to humans, the researchers analyzed cells in culture taken from newborn mouse bile ducts that had been exposed to biliatresone. The exposure reduced the number of hair-like projections called cilia on the cell surface, which perform essential functions, including sensing fluid flow and detecting molecules such as bile acids. In a final experiment, the group used a mouse bile duct cell culture system in which the duct cells form spherical-shaped structures with hollow centers, similar to true bile ducts. Exposure to biliatresone disrupted their hollow centers and proper orientation of the cells, which could obstruct bile ducts in the whole animal and contribute to diseases such as biliary atresia.

These findings of a newly identified plant-derived chemical that is toxic specifically for bile duct cells, particularly in genetically susceptible animals, suggests that this or other similar chemicals in the environment might serve as a trigger for biliary atresia in young humans. Even normal bacteria in the human gastrointestinal tract may produce

compounds similar to biliasterone by metabolizing nutrients found in the human diet, such as soy, beets, and chard. This research has also identified pathways that are critically altered in the course of biliary atresia and focused attention on regions of the human genome containing genes that appear to play a central role in this disease. These insights bring new light to our understanding of biliary atresia and point to directions for future research into means of prevention and treatment of this most important and fatal newborn liver disease.

Lorent K, Gong W, Koo KA,...Pack M. Identification of a plant isoflavonoid that causes biliary atresia. Sci Transl Med 7: 286ra67, 2015.

Promise and Caution Mark Results from New Drug Trial for Fatty Liver Disease: A clinical trial testing a new drug designed to treat a severe form of nonalcoholic fatty liver disease (NAFLD) has shown its promise for reducing disease, but also prompted questions concerning its long-term safety. NAFLD is a form of chronic liver disease that is on the rise in the United States and around the world, in parallel with obesity rates. Its more severe form, nonalcoholic steatohepatitis (NASH), can lead to cirrhosis, liver failure, and liver cancer. End-stage liver disease from NASH has become an increasingly common cause for liver transplantation, both in the United States and abroad. Lifestyle changes and weight loss can improve liver injury in NASH, but they are only partially effective and are challenging to maintain in the long term. No treatment has been approved specifically for NAFLD/NASH, although vitamin E and the diabetes drug pioglitazone have shown some benefit in clinical studies.

The Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment (FLINT) trial was conducted as part of the NIDDK's Nonalcoholic Steatohepatitis Clinical Research Network, with support from an industry partner. This clinical trial set out to test the drug obeticholic acid (OCA) as a potential treatment for severe NAFLD. OCA is a synthetic form of a bile acid that binds to a factor in cells called the farnesoid X nuclear receptor, which plays a role in the liver's metabolism of glucose (sugar) and lipids (cholesterol and fats). At eight clinical centers across the United States, adults with NASH confirmed by liver biopsy were recruited to participate. Study participants were assigned to groups receiving either OCA or placebo once a day for 72 weeks, with return visits at weeks 2 and 4, then every 12 weeks thereafter. After completing the 72-week treatment phase of the trial, participants underwent a liver biopsy and, after stopping treatment, came in for a 24-week follow-up visit. At each visit, blood samples and body measurements were taken, and patients received recommendations about healthy eating and exercise. The main outcome of interest was whether OCA decreased the degree of liver injury, as assessed by study pathologists looking at the liver biopsy samples under the microscope and assigning an "NAFLD activity score." An interim analysis conducted before all the participants had completed the trial showed a significant benefit from OCA in terms of improvement in the NAFLD activity score. However, this analysis also flagged some potential problems in the form of increased total cholesterol and LDL cholesterol levels, as well as decreased HDL cholesterol—all of which are typically hallmarks of increased cardiovascular disease risk. The interim results of the trial prompted the study's data safety and monitoring board to recommend halting the end-of-study liver biopsies, which can pose some risks, and stopping treatment of the remaining 64 patients, though the trial was continued to collect the 24-week follow-up data on all participants. In the final analysis, OCA

reduced NAFLD activity and the scarring of the liver (fibrosis) caused by NASH, but did not result in complete resolution of NASH at a rate higher than occurred with placebo, the equivalent of no treatment other than lifestyle recommendations.

This trial showed the promise of a once-a-day drug that targets a bile acid receptor, the farnesoid X nuclear receptor, for reducing harm from this common form of liver disease. The degree of improvement with this approach and its safety when administered long-term remain issues. Larger, longer-term studies are now underway that will carefully evaluate the safety of this drug and its effects on reducing NAFLD.

Neuschwander-Tetri BA, Loomba R, Sanyal AJ,...Doo E; for the NASH Clinical Research Network. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet 385: 956-965, 2015.

Liver Injury from Herbal and Dietary Supplements Workshop

On May 4-5, 2015, the NIDDK and the American Association for the Study of Liver Diseases co-sponsored a workshop on "Liver Injury from Herbal and Dietary Supplements," together with federal partners at the Centers for Disease Control and Prevention, the U.S. Food and Drug Administration, the U.S. Department of Agriculture, the U.S. Government Accountability Office, and the NIH's National Center for Complementary and Integrative Health, the NIH Office of Dietary Supplements, and the National Institute of Environmental Health Sciences. The purpose of this workshop was to identify research opportunities for better understanding the causes, mechanisms, clinical features, and outcomes of liver injury associated with some herbal or dietary supplements, as well as finding better ways to treat or prevent this type of liver injury.

Use of herbal and dietary supplements—including vitamins, minerals, herbs, and other bioactive compounds found in foods and plants—is high in American adults and children. Cases of acute liver injury resulting from these supplements, though rare, are on the increase in this country and others. This form of liver injury can be severe and may even lead to acute liver failure or the need for a liver transplant. It is often challenging to diagnose as there is no specific diagnostic test available and the liver injury is unpredictable, can mimic acute viral hepatitis, and is sometimes difficult to trace to a single compound consumed by a supplement user. Consumers of herbal and dietary supplements often neglect to report taking the supplements, and the supplements themselves are often poorly labeled or even mislabeled in terms of the actual ingredients and dosages they contain.

The workshop, held on the NIH campus in Bethesda, Maryland, brought together an international group of federal, academic, and industry experts from a diversity of disciplines needed to address this issue, including the study of herbal and dietary supplements, biochemical analysis techniques,

liver disease, population disease patterns, and surveillance of adverse events.

Reports of national data on herbal and dietary supplement-related liver injury were presented from the perspectives of the United States, Iceland, Europe and Latin America, India, and China. For example, the workshop included a presentation of findings from the ongoing Drug-Induced Liver Injury Network sponsored by the NIDDK. This Network's studies have shown that herbal and dietary supplements in the United States account for an increasing proportion of cases of liver injury from medications and now rank second only to antimicrobial drugs as a cause of liver injury. They also characterized the types of liver injury caused by commonly used supplements, such as those taken for bodybuilding and weight loss.[1] Other presentations focused on developing new techniques to aid the diagnosis of specific causes of herbal and dietary supplement-related liver injury by profiling the chemical compounds contained in the supplements and screening them in cell-based toxicity assays.

Additional major themes discussed during the workshop included distinctions drawn between: 1) harmful, illegal adulterants added to some herbal and dietary supplements, such as anabolic steroids, and the generally safe contents of most vitamins and minerals, and 2) the nature of herbal supplements used in countries like the United States, with common mixtures of several purified compounds, which may derive from the stated plant species or from a species that appears very similar, versus places like China, with the use in Traditional Chinese Medicine of more unrefined plant materials from well-identified species.

The workshop closed with comments on future directions to guide research efforts in this important area. The event organizers plan to share the information discussed in the workshop with the wider research community and public through publishing a summary of the workshop in the scientific literature.

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[1] *Navarro VJ, et al. Liver injury from herbals and dietary supplements in the U.S. Drug-Induced Liver Injury Network. Hepatology. 60: 1399-1408, 2014.*

NEW DIRECTIONS IN HEPATITIS TREATMENT

Over-the-Counter Antihistamine May Be Effective in Treating Hepatitis C: In a recent study, researchers in the NIDDK's Intramural Research Program screened a large number of over-the-counter drugs to pick out one that also has activity against hepatitis C in cell and animal models. They found the drug with the greatest activity was chlorcyclizine, a commonly used antihistamine that is typically prescribed to treat symptoms of the common cold or hay fever allergy.

Hepatitis C can lead to damaging liver disease, including cirrhosis, liver failure, and liver cancer. For years, standard therapy for hepatitis C was a combination of the antiviral drugs peginterferon and ribavirin, but in recent years, combination treatments with drugs specifically targeting the virus have made therapy more effective and tolerable, although at a high cost. Opportunities remain to find effective and affordable treatments for hepatitis C among existing drugs approved by the U.S. Food and Drug Administration (FDA) for other uses. A team of NIDDK scientists, in collaboration with staff of the NIH National Center for Advancing Translational Sciences, employed a system to rapidly screen large numbers of compounds against the hepatitis C virus (HCV). They grew human liver cells infected with HCV in multiple wells on small plates; in each, they added a dose of one of approximately 3,800 active FDA-approved drugs. The virus was tagged with a chemical that lights up when viral genes are activated. Among the many compounds tested, a common antihistamine drug first approved in 1940, called chlorcyclizine, was particularly potent at suppressing HCV activity. The effect of the antihistamine also held true in a more standard cell model of HCV infection using a liver cancer cell line. The drug was found to inhibit multiple subtypes or "genotypes" of HCV that infect people and affect treatment response. Combining the antihistamine with drugs approved for treating hepatitis C led to additive effects inhibiting HCV. To evaluate the antihistamine's unique pharmacologic properties for treating HCV, including its metabolism and distribution throughout the body, the group tested it in mice injected with the drug. The drug showed long-lasting effects, and it was found in high amounts in the liver, where it would need to concentrate to fight off the HCV infection. The team also found large amounts of the drug in brain, a possible problem as antihistamines are known to cause drowsiness and sedation. To test how effective the drug was against HCV in an animal model designed to mimic human infection, they transplanted human liver cells into mice, infected them with two different genotypes of HCV, and treated them with the drug for several weeks. The drug was found to lower viral levels of both strains of HCV in a rapid and reversible manner. This research showed the potent antiviral activity of an affordable, over-the-counter drug in cell and mouse models of hepatitis C. Because this drug is already FDA-approved for long-term human use, it is known to be relatively safe. Studies are now underway in humans with hepatitis C with close monitoring for side effects and careful analysis of the effects on HCV levels and liver damage. Future research will explore ways to biochemically modify the drug to reduce levels in the brain and increase the activity against HCV, thus adding to its efficacy and reducing side effects.

He S, Lin B, Chu V,...Liang TJ. Repurposing of the antihistamine chlorcyclizine and related compounds for treatment of hepatitis C virus infection. Sci Transl Med 7: 282ra49, 2015.

Clinical Trial Shows Promising Results of New Chronic Hepatitis D Treatment: A pilot clinical trial conducted by scientists in the NIDDK Intramural Research Program provides the first evidence that a drug called lonafarnib may be safe and effective as the only dedicated treatment available for

chronic hepatitis D. Chronic hepatitis D is found throughout the world and often causes severe liver disease. The hepatitis D virus (HDV) is unique in that it cannot cause infection on its own, but requires help infecting its host from the hepatitis B virus and, therefore, is found only in persons who also have hepatitis B. Peginterferon has been the standard therapy for chronic hepatitis D, but the response rate is quite low and side effects are frequent, dose-limiting, and sometimes severe. While many advances have been made in the therapy of chronic hepatitis B and C, there has been no progress in antiviral therapy of hepatitis D. Scientists in the NIDDK Intramural Research Program, in collaboration with an international group of investigators and the drug sponsor, performed a pilot, phase 2a clinical trial to test the safety, tolerability, and effectiveness of the drug lonafarnib in treating chronic hepatitis D. Lonafarnib inhibits an important biochemical process that modifies proteins and is essential for HDV replication in liver cells. In this first human trial, 14 men and women with chronic hepatitis D were given one of two oral dose levels of lonafarnib or a placebo for 4 weeks and monitored during treatment and then for 6 months afterwards. In those who received lonafarnib, HDV levels decreased during treatment compared to the placebo group; the effect was more pronounced in those given the higher dose of the drug. The drug was well-tolerated, with no trial participant choosing to discontinue treatment due to an adverse event, though gastrointestinal side effects, such as diarrhea and nausea, were common. This clinical trial shows the promise of lonafarnib as a potentially groundbreaking new type of therapy for chronic hepatitis D. Studies of longer courses of treatment (6 months) using the higher dose of lonafarnib are now under way. Future studies will explore long-term therapy, dose adjustment, and combination with drugs to increase the antiviral activity and reduce side effects of treatment.

Koh C, Canini L, Dahari H,...Heller T. Oral prenylation inhibition with lonafarnib in chronic hepatitis D infection: a proof-of-concept randomised, double-blind, placebo-controlled phase 2A trial. Lancet Infect Dis 15: 1167-1174, 2015.

Hepatitis C: From Non-A, Non-B Hepatitis to a Cure

The story of hepatitis C from discovery to cure is very much like the plot of a good mystery novel. It begins with a puzzling who-done-it, followed by a lengthy hunt for the suspect, and, finally, rigorous efforts to subdue the perpetrator. Many of these efforts were spearheaded by the NIDDK, and, although the narrative is not quite finished, the battle against hepatitis C is evolving into one of the biggest modern success stories in scientific research.

An Unknown Culprit

Hepatitis, or inflammation of the liver, has long been a part of human history. The symptoms are unfortunately familiar to many: abdominal pain, tiredness, jaundice (the yellowing of skin and eyes), and, in many serious cases, liver failure and death. It wasn't until the twentieth century that scientists discovered that most cases of hepatitis were caused by viruses that infect cells in the liver. Eventually, researchers divided viral hepatitis cases into two distinct diseases based on their characteristics; both diseases were potentially serious, but they differed in how they spread and made people sick. "Hepatitis A" was spread by person-to-person contact or through contaminated food or water, had a short incubation period, and resulted in an acute (temporary yet serious) illness. "Hepatitis B" was spread through blood and other bodily fluids, had a longer incubation period, and could lead to a chronic (long-lasting) infection. Because many cases of hepatitis seemed to be coming from blood transfusions, the identification of the viruses, particularly the blood-borne agent that causes hepatitis B, became imperative. If the virus was known, the blood supply could be screened to prevent spread of the disease.

A major protein from the hepatitis B virus was discovered in 1963 by scientists at the NIDDK (then called the National Institute of Arthritis and Metabolic Diseases), which eventually allowed for testing of the blood supply. However, screening for the hepatitis B virus and exclusion of infectious donors resulted in a decrease of only 25 to 50 percent in post-transfusion hepatitis cases. It was assumed that the remaining cases were either caused by the hepatitis A virus, or by the hepatitis B virus that may have slipped through the screening process. By the mid-1970s, however, investigators at the NIH in the Hepatitis Branch of the Laboratory of Infectious Diseases of the National Institute of Allergy and Infectious Diseases (NIAID) had identified the hepatitis A virus, and, in collaboration with the NIH Clinical Center's Division of Transfusion Medicine, they showed that the remaining hepatitis cases were neither hepatitis A nor hepatitis B. Something else was damaging the liver, and the signs were pointing to a third virus. Like hepatitis B, this newly

STORY OF DISCOVERY

identified disease could be contracted *via* infected blood and could result in a chronic infection and liver cirrhosis (scarring). However, the chance of chronic disease in adults was much higher than with hepatitis B. Also, unlike hepatitis B, people with this disease rarely experienced acute symptoms, which could mean that the disease could slip into a chronic state before an individual had any obvious signs that he or she was even infected. For the next 15 years, the stealthy culprit behind this disease was unknown, and thus the disease was simply called non-A, non-B hepatitis.

Interfering with Non-A, Non-B Hepatitis

While scientists hunted for the mysterious agent behind non-A, non-B hepatitis, they also concentrated efforts on its treatment. Because the virus was still unknown, the first drugs to be tested were those that had been shown to be effective against a broad range of viruses. Hepatitis B patients were responding with some success to a chemical called interferon alpha (interferon), a naturally occurring substance produced by immune cells in response to viral infections or other environmental stresses. Usually administered *via* injection, interferon produces an antiviral state inside cells that "interferes" with virus replication—hence its name—and protects the cells against infection. Because interferon acts as a general defense mechanism against a variety of viruses, it was logical to try using it as a tool against the unidentified virus that caused non-A, non-B hepatitis.

In 1984, scientists in the NIDDK Intramural Research Program led a pilot study of interferon in 10 patients at the NIH Clinical Center in Bethesda, Maryland. The patients were given daily doses for 16 weeks, and their liver health was monitored by testing their blood for a marker of liver damage. The results of the trial were immediate and dramatic: most of the patients showed evidence of a healthier liver after a month of treatment. The patients relapsed when the interferon treatment was stopped after 4 months; however, once the treatment was restarted, their liver health again improved and stayed normal even after the dose was gradually lowered and then stopped after a full year. Some of the patients had only minimal responses to interferon therapy, and others responded but then relapsed, but, in the end, half the patients in the trial showed no signs of liver infection in follow-ups that were eventually extended for 10 to 25 years. These were the first patients to be cured from the disease that would eventually be known as hepatitis C.

Despite these initial results, larger clinical trials tempered expectations with interferon. The outcomes of the studies varied greatly from patient to patient, but treatment with interferon alone generally had a low success rate, measured as the rate of sustained virologic response (SVR). Patients achieving SVR have no detectable virus for at least 24 weeks after discontinuing the treatment—which means there is a very high probability that the treatment was successful and the patient will not relapse. Treating with interferon alone typically yielded SVR rates of less than 20 percent. Combining interferon with other antiviral drugs showed promise, however. One of these drugs, ribavirin, had first been tested by NIDDK intramural researchers as a stand-alone therapy, but it had only a modest and temporary effect on virus levels.

STORY OF DISCOVERY

However, later studies showed that a combination of interferon and ribavirin was superior to interferon alone, showing SVR rates of 30 to 40 percent. Another improvement came when scientists chemically modified interferon to make it last longer in the body. With SVR rates of 55 percent, this "pegylated" interferon (peginterferon), combined with ribavirin, became the standard of care for hepatitis C patients.

The results of these studies also made it clear that more research was needed. While interferon-based therapy was typically successful for over half of patients, it was usually accompanied by side effects such as fever, fatigue, muscle aches, and depression that often limited the dose and duration of the treatments. Nevertheless, these initial trials delivered important insights into how the virus responds to (or resists) therapy and provided important clues about the virus' biology and resilience. This information would prove to be useful when designing therapies based on more effective treatments, and there was a huge development right around the corner that would bring those treatments within reach.

The Discovery of the Hepatitis C Virus

The non-A, non-B hepatitis virus was identified in 1989 by scientists at a California biotechnology company called Chiron who were collaborating with investigators at the Centers for Disease Control and Prevention (CDC). The research confirmed that this was a new virus—now officially called the hepatitis C virus, or HCV. This was a landmark advance in medicine that allowed for development of tests to detect HCV, which were rapidly applied to screen blood donations. Over the next few years, as the testing improved, HCV was effectively eliminated from the blood transfusion supply. The identification of HCV also led to further studies, undertaken by NIAID- and NIDDK-funded researchers and others, to determine its molecular structure. This was crucial for the design of drugs that would specifically interact with components of the virus and inhibit its replication. The identification of the virus also allowed for a more accurate diagnosis and a better sense of its prevalence; in fact, it was eventually determined that HCV was the most common cause of chronic hepatitis, cirrhosis, and liver cancer in the Western world.

Applying new direct tests for the presence of HCV showed that interferon therapy lowered the level of virus in the blood; importantly, patients who had a clinical response to treatment and did not relapse also became HCV negative and were cured of their chronic viral infection. Tests for HCV RNA (the virus' genetic material) in blood were key to future progress in treatment, because they demonstrated that a sustained loss of the HCV RNA—for 12 weeks after stopping treatment—was a reliable end point for treatment. Achievement of SVR became the benchmark end point for clinical trials of new treatments, and the criteria for approval of a new therapy was that it yielded a better SVR rate than peginterferon with ribavirin.

Studying HCV's genetic makeup revealed that the virus has several genotypes, or genetic varieties, and these determine how effectively the virus responds to therapy. For example, genotype 1 is the most common genotype worldwide, but clinical trials found that it was more resistant to

interferon-based therapy than other genotypes. The identification of different genotypes meant researchers were able to better predict and tailor therapies, and it provided one explanation for why some clinical trial participants had better outcomes with peginterferon than others. Another important consequence of identifying HCV was that researchers were now able to analyze the molecular components of the virus and determine which ones could be ideal targets for drugs. These potential targets included an HCV enzyme called a polymerase that is crucial for the replication of the virus' genetic material; an enzyme called a protease that the virus uses to process its components before assembly; and a protein called NS5A, which appears to have several important roles in virus replication, including regulating the cell's response to interferon.

While scientists were working towards characterizing HCV, they were also making strides in its treatment. A huge step toward drug design occurred in 2005, when three different groups of investigators, including NIDDK intramural researchers, were able to grow the virus in cells in the laboratory. This allowed for the study of the HCV life cycle and the identification of essential viral components. These studies then led to the development of the first therapies that were specifically designed to block HCV replication by directly targeting parts of the virus. While broadly antiviral therapies like interferon and ribavirin were somewhat effective, the side effects made the treatments difficult to tolerate. If a drug could be designed to target HCV specifically, the effects might be more limited to the cells that were infected with the virus, greatly limiting "friendly fire" damage to other parts of the body.

Zeroing in on the Hepatitis C Virus

The era of direct-acting antivirals (DAAs) that specifically target HCV began in 2011 with the U.S. Food and Drug Administration (FDA) approval of the first protease inhibitors. These drugs—telaprevir and boceprevir, along with several similar drugs approved later—targeted the HCV protease that is critical for viral replication. When used in conjunction with peginterferon and ribavirin, protease inhibitors yielded SVR rates of up to 75 percent. However, this triple therapy was accompanied by additional side effects to those already present with peginterferon and ribavirin. Nevertheless, the success of HCV-specific protease inhibitors showed that the virus had vulnerabilities that could be exploited by a well-designed and properly administered drug.

More new anti-HCV drugs were developed and tested over the next several years. These new drugs included sofosbuvir and dasabuvir, which interfered with the activity of the HCV polymerase, an enzyme that is responsible for the viral replication. Members of a second class of drugs, ledipasvir and daclatasvir, targeted the NS5A region of the virus, which makes a structural protein critical for viral replication. Many of these drugs were initially tested in conjunction with peginterferon and ribavirin, or in combination with a protease inhibitor. Generally, the results were SVR rates of at least 80 percent.

With the success of DAA therapies, it soon became apparent that when several of them were used in combination, interferon was no longer necessary. This was a crucial step in the progress of hepatitis C therapy, because eliminating the need for peginterferon avoided the many distressing

side effects that accompanied interferon-based therapy. These all-oral regimens also opened up the possibility of treatment in individuals in whom peginterferon could not be safely administered. Perhaps the most successful DAA combination was that of sofosbuvir and ledipasvir; with these two drugs, the SVR rates soared to 99 to 100 percent. Furthermore, this combination was successful with just 8 to 12 weeks of treatment. After years of painstaking research, there was a *bona fide* cure for hepatitis C that worked for nearly everyone.

The Future of Hepatitis C Therapy

With such high rates of success with current treatments, it may seem like the hepatitis C story is in its final chapters, but it is not over yet. A vaccine against hepatitis C would cause the prevalence of the disease to plummet, but efforts to produce a vaccine, while still under way, have not yet been successful. While hepatitis A and B have vaccines, the hepatitis C virus is more variable than either of these viruses, which, along with other factors, complicates vaccine development efforts. Additionally, the current drugs show great promise, but the costs of the more successful FDA-approved DAA treatments are extremely high, which present a significant obstacle to many with the disease. But the research has come a long way. From the early investigations into a mysterious new virus, to the identification of the culprit, and the rigorous work to develop an effective treatment—the story of hepatitis C is definitely a thriller.

Dr. David Brenner—Mapping the Origins and Fates of Cells Underlying Liver Fibrosis

Dr. David Brenner is the Vice Chancellor for Health Sciences, Dean of the School of Medicine, and Distinguished Professor of Medicine at the University of California, San Diego (UCSD). Dr. Brenner earned his B.S. from Yale University, and his M.D. from the Yale University School of Medicine. Following his medical internship and residency, at the Yale-New Haven Medical Center, he completed fellowship training as a research associate in the Genetics and Biochemistry Branch of what is now the NIDDK and in gastroenterology at UCSD. He later joined the medical school faculty at UCSD and served as a physician in the Veterans Affairs San Diego Healthcare System. He was then appointed as Professor and Chief of the Division of Digestive Diseases and Nutrition at the University of North Carolina at Chapel Hill, before moving to the Columbia University College of Physicians and Surgeons, where he was Samuel Bard Professor, chair of the Department of Medicine, and physician-in-chief of New York Presbyterian Hospital at Columbia. Currently, Dr. Brenner leads the UCSD School of Medicine, Skaggs School of Pharmacy and Pharmaceutical Sciences, UCSD Medical Center, and UCSD Medical Group. Dr. Brenner is a member of several professional societies and has served on numerous editorial boards, including his tenure as editor-in-chief of the journal Gastroenterology *from 2001 to 2006. Dr. Brenner is a leader in the field of gastroenterological research, specializing in diseases of the liver. Dr. Brenner presented his laboratory's recent research findings at the May 2015 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, of which he is a member. The following are highlights from his presentation.*

For all its versatility in performing many essential physiological functions within the body, the liver has a limited repertoire when it comes to its response to injury. Whether from a viral infection or toxic insult, including an excess of alcohol or even fat, the liver responds with a similar routine of inflammation then scarring (also known as "fibrosis"), which involves the laying down of extracellular matrix proteins as part of an overly aggressive healing process. Fibrosis is followed by an advanced stage of damage called cirrhosis, which often results in the need for liver transplantation. Dr. Brenner's group has focused on identifying the key cellular contributors to liver fibrosis from different causes using animal and cell models and on understanding their significance for human disease.

SCIENTIFIC PRESENTATION

Liver Fibrosis: One Process with Many Questions

Dr. Brenner noted how, for many years, hepatitis C viral infection was the most studied liver disease for understanding the process of fibrosis. Now, with highly effective antiviral therapies for hepatitis C, researchers have shifted their attention to nonalcoholic fatty liver disease (NAFLD), a form of liver disease that is on the rise in the United States and elsewhere, in tandem with increasing rates of obesity and insulin resistance. NAFLD begins with fat accumulation instead of viral infection, but then, in those individuals in whom it progresses, goes through the same stages as hepatitis C of inflammation, fibrosis, and cirrhosis. In its more severe form of nonalcoholic steatohepatitis, the disease can result in liver cancer, liver failure, and need for a liver transplant.

Dr. Brenner and his lab have pursued their inquiry from a variety of angles to arrive at fundamental truths underlying the complex process of fibrosis. For example, is it the liver's own cells or cells from some other location that are responsible for driving this harmful process of liver fibrosis that damages the organ in response to an injury? Where do the cells originate from? And after they have played their part in the fibrotic process, where do they go, and how do they affect a person's health or disease susceptibility in the future?

In Pursuit of the Cellular Source of Fibrosis

As Dr. Brenner pointed out in his presentation, these questions surrounding the cell type(s) responsible for liver fibrosis were the subject of considerable controversy in the field for many years. Cells called myofibroblasts were found to be active in the liver only during injury and to disappear once the injurious agent was removed, along with regression of disease. However, scientists still disagreed about the source of these cells. There were three potential sources: transformation of resident epithelial cells into these myofibroblasts; recruitment of circulating bone marrow cells, which include cells that might develop into scar-forming cells; and activation of resident myofibroblast cells in the liver.

Scientists, including those in Dr. Brenner's lab and others, have utilized a wide range of animal models of liver injury and other technologies at their disposal over the years, such as cell-specific markers and cell fate mapping to address these questions. Some models have been used for decades, such as ones using the chemical carbon tetrachloride or bile duct ligation, while others are relatively recent dietary models meant to mimic the conditions of NAFLD.

To trace the origins of cells causing liver fibrosis, they genetically marked certain cells in mice so that those cells—and their descendants—would glow yellow. Using this strategy of "cell fate mapping," they were able to rule out the resident epithelial cells in the liver as a source of the fibrosis-promoting cells. Dr. Brenner's group also disproved the idea that bone marrow-derived cells were a major source of the liver fibrosis. To do this, the group started with a genetically modified mouse model that glowed green where

the fibrotic molecule collagen was produced. They then performed a bone marrow transplant from that mouse into a non-modified mouse and observed whether the transplanted bone marrow cells made their way into the liver to lay down collagen. Analyzing the green glow, they concluded that only a small percentage of the liver fibrosis was due to these transplanted bone marrow cells.

After these findings, the research community turned its attention to other resident cells in the liver as a source for the activated myofibroblasts during fibrosis. One camp pointed a finger toward the star-shaped liver cells called hepatic stellate cells, while the other suspected cells called fibroblasts. Later, more sophisticated cellular sorting methods allowed scientists to accurately identify the cells involved. As it turned out, both sides were correct, depending on the type of liver injury induced in the animal model.

Again using the mouse model that glowed green where the fibrotic molecule collagen was produced, Dr. Brenner's group was able to find tell-tale signs of hepatic stellate cells due to their high amounts of vitamin A. This study showed that the hepatic stellate cells were activated into myofibroblasts and contributed to fibrosis characterized by liver cell death in response to chemical injury by carbon tetrachloride, which mimics hepatotoxic injury to cells in the liver called hepatocytes. In another study, they subjected the same mouse model to bile duct ligation, mimicking conditions of bile duct obstruction in humans and injury of the cells lining the bile duct from a back-up of bile acids. They observed that fibroblasts surrounding the portal vein became activated into myofibroblasts, contributing to fibrosis in this scenario. Through profiling the genes activated in these cells in mice, the scientists discovered new markers unique to these activated cells that were once portal fibroblasts, which they also found in samples from patients with biliary cirrhosis.

Charting a Path To Protect Against Future Liver Fibrosis

Fortunately, in the face of multiple potential causes of liver injury and fibrosis, the liver is capable of recovering from fibrosis once the harmful agent is removed or suppressed. Dr. Brenner pointed to treatment responses, such as those from antiviral drugs for hepatitis C and bariatric surgery for NASH, and to removal of carbon tetrachloride or alcohol in mouse models as key examples of this phenomenon. But, Dr. Brenner and his group were interested in probing further into the fate of those cells that had once been activated during liver fibrosis in the recovered organ—did the cells disappear or remain in a new form?

In the mice whose liver myofibroblasts glowed yellow when they were activated and producing collagen, they saw that at 1 month after recovering from the carbon tetrachloride chemical injury, virtually no activated cells remained. Using an assay to quantify the number of cells that underwent a form of cellular suicide called apoptosis, they found that some of the once-activated myofibroblasts died off.

SCIENTIFIC PRESENTATION

Dr. Brenner and his research group wanted to find out what happened to the remainder of once-activated, fibrosis-promoting cells. To do this, they used cell mapping techniques in a mouse model genetically engineered to light up any remaining liver cells that had once produced collagen. They found that about half of the once-activated myofibroblasts persisted. By characterizing the genes turned on in these inactivated myofibroblasts compared to never-activated liver cells, they found that the inactivated cells showed some signs of reverting to a dormant state, but other indications that they were primed to react more aggressively to any future insult. Furthermore, when the group treated mice repeatedly with the carbon tetrachloride chemical, their livers fared worse in terms of developing severe fibrosis upon the second insult compared to animals only treated once. And when they transplanted either the inactivated cells or the never-activated cells into young mice, then induced experimental fibrosis, mice with the once-activated cells showed more severe fibrosis. Dr. Brenner and his team have recently begun investigating the factors that help the once-activated liver cells escape death and persist in the liver after recovery from fibrosis.

This major observation of Dr. Brenner's lab described in his presentation—of "sleeper cells" that, once active in fibrosis, silently remain in the liver after recovery with the ability to quickly reactivate in response to another fibrotic trigger in the future—has important clinical implications. People who have recovered from liver fibrosis may have livers that appear normal, but they are likely more susceptible to developing severe fibrosis in the future in response to liver injury, due to the cellular "memory" of their inactivated hepatic stellate cells or portal fibroblasts. These findings can inform future research focused on identifying targets for treatments that might someday allow people with a history of liver fibrosis to make a more complete recovery and be protected against developing severe liver disease.

PATIENT PROFILE

Surgical Procedure for Chronic Pancreatitis Transforms Young Person's World from Pain into Promise

Sydney

Fifteen-year-old Sydney is an active, academically high-achieving, and caring teenager living the full life of a tenth grader with school activities, playing her favorite sport of soccer, and pursuing her interests in medicine and theater production.

But just a few years ago, her life was dramatically different—marked by frequent attacks of abdominal pain so severe that they put her in the hospital for weeks at a time and kept her from going to school or engaging in any of the other typical activities of children her age. Her struggles with pancreatitis and her entire family's journey with managing the disease have not been easy. With the help of research on genetic factors underlying this disease, however, much of which was performed by scientists with National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) support, and a surgical procedure called "total pancreatectomy-islet autotransplantation," the future is bright for this young person who is eager to give back to the world of medicine and other children dealing with serious illnesses.

Living with Pancreatitis

The pancreas is an organ located behind the stomach that has many important functions. Specialized cells in the pancreas called islet cells produce hormones such as insulin and glucagon that are released into the blood to regulate the level of sugar (glucose) in the blood. The pancreas also produces fluid that is released through ducts into the intestine and contains enzymes and bicarbonate that are necessary for digestion of food. Usually, these powerful digestive enzymes are inactive until they exit the pancreas and enter the intestine. In cases of pancreatitis, however, digestive enzymes are activated prematurely while still inside the pancreas, resulting in damage and inflammation, and outward symptoms of abdominal pain, nausea, and vomiting. The acute form of the

PATIENT PROFILE

disease, often caused by gallstones as they pass through the duct connecting the pancreas and gallbladder to the intestine, resolves after a few days of treatment. However, chronic pancreatitis is marked by frequent attacks of debilitating abdominal pain that become worse over time, causing more long-term damage and preventing those affected from engaging in everyday activities such as attending school or work.

Her pancreatitis symptoms started when she was 8 years old. "I just started having really bad stomach pain.... I would go in and out of the hospital to figure out what was wrong, and for the longest time they didn't know what was going on," says Sydney.

In Sydney's case, her pancreatitis symptoms started when she was 8 years old. "I just started having really bad stomach pain," she says. "I would go in and out of the hospital to figure out what was wrong, and for the longest time they didn't know what was going on."

She would be in the hospital for about 1 to 2 weeks at a time, and for as long as 7 weeks on one occasion, to manage the pain. During this time, she was unable to eat, but received intravenous (IV) fluids while her pancreas recovered from the attack. The disease took a heavy toll not only on Sydney, but on her whole family.

"As far as the pain, you know seeing my daughter was the most difficult part," says Sydney's mother, LaKindra. Sydney shares that feeling, saying "it was hard to see my parents see me in pain and then have two little brothers that needed help too."

Typically, pancreatitis is diagnosed through a combination of medical history, physical examination, blood tests for elevated levels of digestive enzymes, and imaging tests showing the pancreas, gallbladder, and surrounding ducts.

Pancreatitis can be hereditary. A few years after her symptoms of recurrent acute pancreatitis first started, the disease had progressed to chronic pancreatitis, and her doctors decided to do genetic testing to determine if Sydney's was a hereditary form of the disease. Chronic pancreatitis is rare in children, and in approximately 50 to 70 percent of cases, it is associated with a genetic mutation. People with hereditary forms of pancreatitis also have a 40 to 70 percent chance of developing pancreatic cancer later in life.

In the years preceding Sydney's diagnosis, NIDDK-supported science had advanced knowledge of the genetic factors underlying hereditary pancreatitis. In 1996, scientists reported the groundbreaking discovery of the first genetic mutation associated with hereditary pancreatitis, in a gene coding for the protein trypsinogen, an inactive precursor form of the digestive enzyme trypsin. Additional mutations associated with hereditary pancreatitis have since been discovered in the trypsinogen gene, in other genes that affect trypsinogen/trypsin, and in genes that have other functions.

PATIENT PROFILE

For example, mutations in the *serine protease inhibitor Kazal type 1* or "*SPINK1*" gene, which encodes a protein that inhibits trypsin, were identified in people with pancreatitis. Mutations in the gene associated with cystic fibrosis—called *CFTR*—were linked to pancreatitis. Depending on the nature and number of mutations, pancreatitis sometimes occurs in people with cystic fibrosis and sometimes in those who do not have cystic fibrosis, but have a *CFTR* mutation. *CFTR* encodes a protein that helps enzyme precursors like trypsinogen leave the pancreas and enter the small intestine.

Sydney's doctors sequenced her DNA and found that she carried two of the genetic mutations that had been discovered a few years before as risk factors for pancreatitis: in the *SPINK1* and *CFTR* genes. This knowledge gave Sydney's family and doctors more information to go on in understanding her disease. But because hereditary pancreatitis in children is so rare, it was difficult for Sydney's family to find information that would help them decide with their health care team about the best course of treatment for her. Also, options tailored to the needs of pediatric patients were limited.

As Sydney's father, Robert, says, it was "almost impossible" to find information about treatments for people with chronic pancreatitis who had the same kind of genetic mutations as Sydney. "It was almost a word-of-mouth kind of thing trying to get your hands on any data so that you could try to see exactly what other possible treatments have been tried on other patients with like symptoms," he says.

Treatment for chronic pancreatitis typically focuses on relieving pain and managing any complications, such as blocked ducts within the pancreas. For example, a technique called endoscopic retrograde cholangiopancreatography (or "ERCP") allows doctors to view the pancreas, gallbladder, and surrounding ducts, as well as treat any narrowing or blockage of the ducts using small plastic tubes called stents. Another procedure called a celiac plexus block involves injection of a local anesthetic directly into a group of nerves leading to the pancreas. Pancreatic enzymes are also usually prescribed to be taken with meals so that the pancreas does not have to work so hard in assisting digestion.

In Sydney's case, her pain was managed through a combination of ERCP, celiac plexus blocks, and taking two powerful narcotic pain relievers. But, with the concern about possible addiction from long-term narcotic pain reliever use, her parents sought alternatives.

"There is practically no one that supports pediatric patients for pain management, so literally we had to work with doctors that did not specialize with pediatric patients," says LaKindra.

They worked with the staff at the nearby University of Chicago Medicine Comer Children's Hospital, where Sydney tried different approaches to manage her pain, including more holistic approaches such as aromatherapy and guided imagery. The family also sought out acupuncture services outside the hospital for Sydney to help with the pain.

PATIENT PROFILE

Unfortunately, by 2011, all the pain management techniques had stopped working for her. Then, at only 11 years old, she was hospitalized six times that year and was in school only part time. Also, for several months during that year, she was unable to eat, receiving her nourishment exclusively through total parenteral nutrition or "TPN" *via* an IV tube delivering a high-calorie mix of nutrients directly into her bloodstream. Her family decided it was time to pursue another option with the potential to relieve Sydney's pain more permanently.

Choosing a Life Without Pain

Sydney's family credits the talented health care professionals they encountered near their home in Chicago with correctly characterizing her disease and quickly putting them on a path to a long-term solution.

"The stars were all aligned and God was in our favor because we had doctors who were more than willing to say 'I don't know what's going on, but let me direct you to someone else,'" says LaKindra.

From their local community hospital, they were sent to a physician at the University of Chicago who had seen another pediatric patient with chronic pancreatitis. This patient had undergone a procedure called a total pancreatectomy-islet autotransplantation (TP-IAT) at the University of Minnesota Masonic Children's Hospital.

In the TP-IAT procedure, the pancreas is surgically removed and its insulin-producing islet cells, which regulate blood sugar, are collected and infused back into the body through a large vein going into the liver, where the cells are able to implant and function. This surgery serves the dual purpose of removing the source of pain and increased cancer risk while preserving some insulin production, so as not to cause diabetes. The TP-IAT procedure had been used in adults since 1977 and in children since 1996. The surgery is more successful, in terms of achieving independence from insulin medications for diabetes, the earlier it is done after a diagnosis of pancreatitis and when performed in young patients under 12. Currently, about 15 U.S. medical centers perform the TP-IAT procedure. The NIDDK has supported some of the clinical research on TP-IAT use for treating chronic pancreatitis in adults and children.

Sydney's family weighed the pros and cons of having the procedure, including Sydney being free of the pancreatic attacks and less likely to develop pancreatic cancer later, but also the serious risks of any surgical procedure and also the possibility of developing diabetes if the transplanted islets did not produce enough insulin.

"It was a group decision...we all felt really on the same page that 'hey, we really didn't have an option.' The alternative just was unacceptable," says Sydney's father, Robert.

"It was a group decision. We included Sydney in it, but leading up to that we all felt really on the same page that 'hey, we really didn't have an option.' The alternative just was unacceptable," says Robert.

PATIENT PROFILE

They were helped through this difficult decision process by other families coping with the disease. "It's really a close-knit community of people kind of relying on each other and forming support groups, so that was really instrumental in strengthening us and helping us make it through," he says.

Sydney's family traveled with her to Minneapolis to stay in a hotel close to the University of Minnesota and prepare for the TP-IAT surgery. The family was hopeful that the surgery would help her, but also unsure of what the outcome might be. "It was a lot of anticipation," says Sydney.

On November 14, 2011—which coincidentally was the same day as the annual World Diabetes Day—Sydney had the surgery performed by a surgical team at the University of Minnesota, including her surgeon, endocrinologist, pediatric gastroenterologist, and others.

> ***"All kids that have this surgery, there are so few of us that we all share this bond and we connect instantly," says Sydney.***

"I remember us praying before I went in to surgery," recalls Sydney. "And I remember my mom coming in, in like this bunny surgical suit and making me laugh." LaKindra accompanied Sydney into the surgery room initially; then the family waited anxiously outside over the next 14 hours while the surgery took place.

The Long Road to Recovery

Sydney's recovery in the following months after the TP-IAT surgery was slow and full of challenges. Beginning immediately upon returning to her hospital room after her surgery, now without a pancreas, Sydney's recovery would require many interventions in the weeks and months that followed, to help her maintain her blood sugar, manage pain, and help with some difficulty she experienced breathing.

"Everything had to be sustained by a lot of pumps, a lot of medications, managing her blood sugar, managing her pain, managing her breathing… she doesn't remember any of that, but it was quite painful for her as well," recalls Robert.

Nutrition was also a major challenge during this time. Sydney remained on the IV TPN for a few months following the surgery. For weeks, Sydney had to have her blood sugar monitored around the clock until the transplanted islet cells showed signs that they were functioning normally in their new home inside the liver. Until then she was considered to have "brittle diabetes," a condition in which blood sugar levels fluctuate unpredictably. Even after beginning to eat by mouth again, she was taking both short- and long-acting forms of insulin, in addition to pancreatic enzymes, with her meals, and closely counting her carbohydrates, to provide intensive control of her blood sugar while her islet cells recovered.

The family stayed at a hotel nearby after Sydney was released from the hospital and then at the Ronald McDonald House for about a month while

PATIENT PROFILE

she fully recovered. During that time, they had to continue managing Sydney's blood sugar and pain, with help from nurses who came by on a regular basis.

Sydney also experienced a few complications from the surgery, including internal bleeding and infection with the bacterium *Clostridium difficile*, which put her back in the hospital after returning home to Chicago. And for a long time after the surgery, Sydney experienced pain and itching in the scar covering her surgical site.

But a bright spot from that difficult recovery period came when, while in the hospital, Sydney was able to meet another child who went through the procedure around the same time. The two became "inseparable" afterwards and remain good friends.

"Now I'm totally normal," says Sydney. "I'm doing whatever a normal teenager does ... everything I didn't get to do from that period that I was sick."

"We share this special bond…all kids that have this surgery, there are so few of us that we all share this bond and we connect instantly," says Sydney. The family also offers support to others going through the procedure.

A New Quality of Life

Now, 4 years after the TP-IAT surgery, Sydney is back in school full time and studying diligently, even attending a math and science camp during her summer break. She no longer experiences abdominal pain, takes minimal insulin medication and lower doses of the pancreatic enzymes with her meals, and only has to check her blood sugar after exercising. She also continues to take special care through her diet, including daily vitamin supplements.

"Now I'm totally normal," she says. "I'm doing whatever a normal teenager does—I go to school, I do my sports, my clubs, and just try and do everything I didn't get to do from that period that I was sick."

"We believe this is what the surgery has given her, that quality of life," adds LaKindra. Though in Sydney's case, the decision to have the surgery also meant a lifelong commitment to managing her blood sugar—a tradeoff they willingly accepted to give Sydney a life free of chronic pain. Sydney, her family, and her health care team take care to preserve the function of the islet cells transplanted into her liver, which cannot replicate like islet cells within the pancreas.

"She has a set number of islet cells for the rest of her life," says Robert, adding "There aren't any long-term data right now," for children undergoing the surgery and islet cell transplantation. Sydney returns to the University of Minnesota each year for a check-up with her doctors and sees her local doctors every 6 months to make sure everything is still on track.

PATIENT PROFILE

Sydney's experience with pancreatitis and the TP-IAT surgical procedure has strengthened her interest in science and the medical profession. "Now that I've experienced what some kids have to go through when you're in a situation where they don't have that much information for kids having this, it just touched my heart and I was like 'you know, I could really make a difference, I could really help,'" she says.

For someone who has overcome formidable health challenges at such a young age and even found inspiration in them, anything is possible.

Hope Through Research

The NIDDK has supported research related to pancreatitis conducted by individual investigator-led teams, as well as larger, multi-center studies, such as the North American Pancreatic Study Group, which in 2012 performed the first genome-wide association study of pancreatitis, discovering additional genetic factors involved in the disease. The Institute has also sponsored workshops in recent years to foster new ideas and collaborations that can further advance pancreatitis research. For example, a 2013 workshop co-sponsored by the NIDDK and the National Cancer Institute (NCI) focused on pancreatitis, diabetes, and pancreatic cancer; a 2014 workshop focused on opportunities for research on TP-IAT use as a treatment for chronic pancreatitis; and a 2015 workshop focused on the development of biomarkers to facilitate early diagnosis of pancreatic disease.

Also in 2015, a new Consortium for the Study of Chronic Pancreatitis, Diabetes and Pancreatic Cancer was established with support from the NIDDK and the NCI. The Consortium's work will include conducting studies of people with chronic pancreatitis to improve understanding of disease processes and related outcomes such as diabetes and pancreatic cancer development. These efforts offer hope through research to advance knowledge of pancreatic disease and improve its management.

Sydney's experience with pancreatitis and the TP-IAT surgical procedure has strengthened her interest in science and the medical profession. "Now that I've experienced what some kids have to go through...it just touched my heart and I was like 'you know, I could really make a difference, I could really help,'" she says.