

Many people in the United States are genetically susceptible to celiac disease, an autoimmune disorder that wreaks havoc in the small intestine due to an improper immune reaction to dietary gluten. Yet, despite the high genetic prevalence, only a small fraction of these people actually develops the disease, leading scientists to search for additional genetic or environmental factors that are involved. Research described in this chapter implicates a common virus—which typically does not cause disease in healthy people—as a possible trigger for celiac disease. The researchers found that this virus, a type of reovirus similar to the one shown in the image above, provokes the immune system to launch an attack on gluten in genetically susceptible mice. The researchers also found that people with celiac disease had higher levels of reovirus antibodies, raising the possibility that reoviruses may play a role in celiac disease in humans as well.

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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 that digestive disease is the primary diagnosis in a total of 72 million ambulatory care visits to physicians' offices and hospital emergency and outpatient departments in the United States each year.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 costs totaling \$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 determine 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 other 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 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. Scientists are working to understand the

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

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

causes of functional bowel disorders, which will lead to improvements in diagnosis and management for patients with these conditions.

Gastroparesis, another type of functional bowel disorder, is characterized by delayed emptying of food from the stomach, resulting in nausea, vomiting, and abdominal discomfort. Most cases of gastroparesis are of unknown origin, which makes it difficult to treat. Most current therapies are directed toward helping people manage this chronic condition so they can be as comfortable and active as possible. The NIDDK's Gastroparesis Clinical Research Consortium is fueling research on the causes and progression of gastroparesis and exploring new approaches to treat the disorder.

Fecal incontinence, or impaired bowel control, is a 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. Recent and continued research advances in the understanding of genes and environmental triggers that are involved in the development of celiac disease may contribute to improved diagnosis and new ways to treat this condition in the future.

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. Advanced pancreatitis can be debilitating and may lead to cancer or diabetes, but because pancreatitis is difficult to detect in its early stages, many cases are advanced by the time they are diagnosed. 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 its more severe form, nonalcoholic steatohepatitis. 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, bile duct obstruction, 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, sometimes a healthy living person will donate part of his or her liver, most often to a family member who is recommended for a liver transplant. The living donor's liver eventually regenerates and grows back to normal size, as does the part of the liver that is donated.

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. The NIDDK's Office of Nutrition Research is overseeing a process to develop the first NIH-wide strategic plan for nutrition research, including providing support for activities of the NIH Nutrition Research Task Force, which is chaired by the NIDDK Director and co-chaired by the directors of the National Heart, Lung, and Blood Institute, the National Cancer Institute, and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development. Staff from these and a number of other NIH components participate on the Task Force.

GUT MICROBIOME AND HEALTH

Molecules Produced by Gut Bacteria Extend

“Healthspan”: Researchers have discovered that a class of molecules produced by friendly gut bacteria could extend the time that worms, flies, and mice remain healthy during their lives—with potential

implications for human health. Advances in research and health care have contributed to people living longer, particularly those in developed countries. However, associated with this longer lifespan is a decrease in “healthspan,” which is the length of time that a person is healthy and free of age-related illnesses. Finding ways to increase healthspan could reduce frailty and health care costs, while improving people’s quality of life. Thus, researchers were interested in identifying biological pathways that regulate healthspan independently of lifespan.

Gut microbiome composition changes with aging, but it is not known if the microbiome plays a role in regulating healthspan. To examine a potential link, researchers focused on a class of molecules, called indoles, produced by *Escherichia coli* (*E. coli*) and other bacteria found in the gut. (These bacteria include harmless types of *E. coli*.) They built on their previous research showing that indoles secreted by *E. coli* protected the roundworm *Caenorhabditis elegans* (*C. elegans*) from damage induced by stress, which is one component of healthspan. In new research, scientists examined the effect of indoles on other aspects of healthspan in the same animal model. To do this, they fed *C. elegans* strains of *E. coli* that either did or did not make indoles and measured markers of healthspan, such as motility, reproduction, and how well the worms swallow. They found that the presence of indoles did not have a significant effect on maximal lifespan, but did extend the healthspan of *C. elegans*. The scientists also studied whether indoles had a similar role in other animal models. Adult fruit flies lacking their own gut bacteria had greater ability to climb and were more resistant to heat stress when colonized with an *E. coli* strain that produced indoles compared to a strain that did not. Additionally, both young and old mice that had indole-producing *E. coli* in their guts showed improved measures of healthspan over a 3-month period. Together, these results suggest that indoles play a similar role in promoting healthy aging across different animal models. Further experiments showed that indoles exert these effects via cellular components called aryl hydrocarbon receptors, which are on the cell surface and bind to indoles and other small molecules to activate certain genes. The scientists showed that indoles regulated the activity of genes in *C. elegans* that were associated with healthspan but not lifespan, and exposure to indoles

also shifted gene activity profiles in aged worms to reflect those of younger worms.

This research has identified a new role for gut bacteria: promoting healthy aging via the production of indoles. If the same results are observed in people, indoles or similar molecules represent possible therapeutics to increase healthspan. The researchers note that plants, such as kale and broccoli, also produce indoles. However, additional research would be needed to determine whether these sources of indoles would be potent enough for inducing the effects seen in this research study before the results can be translated into human therapy.

Sonowal R, Swimm A, Sahoo A,...Kalman D. *Indoles from commensal bacteria extend healthspan. Proc Natl Acad Sci USA 114: E7506-E7515, doi: 10.1073/pnas.1706464114, 2017.*

Seasonal Variability in the Gut Microbiome of a Hunter-gatherer Population: Researchers have observed seasonal variability in the gut microbiome of a traditional hunter-gatherer population living in the African country of Tanzania, providing new insights into how diet and modernization may affect bacteria in the gut. The gut microbiome is the collection of all microbes present in the gut and/or their genetic material. It is known that people who eat a diet that is relatively low in fiber and high in fat and simple sugars, as is common in industrialized countries, have a less diverse gut bacterial community than people who eat a more traditional diet such as that of hunter-gatherers who forage for food. In other words, some bacterial groups in the gut microbiome of people eating traditional diets are missing in those consuming a more industrialized diet. A less diverse microbiome is associated with a variety of health conditions, such as obesity and inflammatory bowel disease, so exactly which bacterial groups are missing from industrialized societies and how microbiome diversity could be restored are intriguing questions for researchers.

In new research to help shed light on these questions, scientists studied microbiomes from people who eat a more traditional diet, the Hadza hunter-gatherers in Tanzania, and compared their microbiomes to microbiomes from people in other countries, including those in industrialized areas with a more modern diet. The Hadza's traditional diet, which consists of

food available in the wild, varies with local seasonal conditions. For example, berries and honey are more available during the wet season, and hunting, (*i.e.*, meat consumption,) is more prevalent in the dry season. Researchers collected fecal samples from 188 Hadza people over a year. When the researchers compared the microbiome profile of the Hadza to 18 other populations across 16 countries, they confirmed previous research findings showing that gut microbial diversity relates to modernization: more traditional groups, like the Hadza, had the most diversity in their gut microbiomes. But the researchers uncovered an important clue when they looked at the Hadza's gut microbiomes over different times throughout the year. They found that it differed in the wet and dry seasons, with some bacterial groups becoming undetectable in one season and then reappearing in another. In fact, when these bacterial groups disappeared seasonally, the Hadza microbiome profile was increasingly similar to those of people from industrialized countries. In other words, the bacterial groups that are most susceptible to the observed seasonal cycling in the Hadza are rare or absent in people living in industrialized countries.

These findings support the idea that the gut microbiome can fluctuate rather quickly with changes in diet. Also, a shift from a traditional diet to one more typical of industrialized populations could at least partially explain the loss of gut microbial diversity seen in modern societies. Further studies are needed to directly link these differences in gut microbial diversity to human health. However, understanding exactly what dietary changes could restore gut microbial diversity could help guide strategies to modify the gut microbiome for potential therapeutic purposes.

Smits SA, Leach J, Sonnenburg ED,...Sonnenburg JL. *Seasonal cycling in the gut microbiome of the Hadza hunter-gatherers of Tanzania. Science 357: 802-806, doi: 10.1126/science.aan4834, 2017.*

Elucidating Complex Interactions Between Human Gut Microbes, Diet, and Response: Research is illuminating the multiple levels of complex interactions that contribute to how human gut microbes affect health: at the level of communities of people with different diets, within an individual's gut microbial community, and among metabolites produced by these gut microbes.

One research group explored the larger social context of how a community of people and their dietary habits can affect an individual's gut microbial community and response to changes in diet. They used genetic sequencing of bacterial DNA in fecal samples from adult Americans—either from those following a typical American diet or a diet restricted in calories but with sufficient nutrients—to identify unique gut microbial communities associated with these diets. Gut microbial diversity, which is a marker of digestive health, was enriched in the individuals who consumed the calorie-restricted diet. To model how microbial exchange among a close community of individuals affects response to diet, the scientists collected fecal microbes from people in the two diet groups and transplanted these into male mice that had been raised in a sterile environment to be “germ-free.” The mice colonized with microbes from the people consuming a typical American diet were then housed with mice that harbored microbes from people on a calorie-restricted diet, and all the mice were given a calorie-restricted diet. The researchers found that the mice originally colonized with the American-diet microbes developed a more diverse gut microbial community, resembling that of their cage-mates. These animals also showed metabolic changes in their use of dietary components such as glucose. Because mice share gut microbes more easily than humans, further human studies are needed. However, these findings show the potentially profound effects of the gut microbes present in people who come into contact with one another and how this exchange may enhance response to dietary interventions.

Another group of researchers, many of whom also worked on the previous study, focused on interactions within the gut microbial community in the context of malnutrition, a leading cause of childhood mortality worldwide. Fecal samples had been collected for an earlier study from two, 2-year-old children living in low-income households in Dhaka, Bangladesh. One child was stunted in growth, underweight, and harbored a pathogenic strain of the microbial species *Bacteroides fragilis* that causes diarrhea, while the other child showed normal growth and had harmless strains of *B. fragilis* bacteria. The researchers transplanted the children's fecal microbes into adult, male, germ-free mice whose food was similar to the children's diets. Mice transplanted with the underweight child's microbes suffered significant weight loss within a few weeks while

those transplanted with the healthy child's microbes maintained their weight. The scientists then isolated the microbial strains present in the children's fecal samples and created custom microbial communities for transplantation that contained the pathogenic strain of *B. fragilis*, the harmless strain, or a mixture of the two—along with other microbes from each fecal sample. They found that the pathogenic *B. fragilis* from the stunted donor caused weight loss in mice when in the context of its original microbial community, but not when transplanted with the healthy donor's microbial community containing harmless *B. fragilis* strains, in addition to other microbes. The weight loss associated with the growth-stunted donor's microbial community was passed down between generations, from pregnant mice harboring the microbes to their offspring. The scientists also showed how nutrient metabolism and immune function in the host mice were adversely affected by the presence of microbes from the stunted donor. These studies reveal the importance of microbial community context in influencing how the total burden of microbes harbored by young children can affect metabolism and growth, immune function, and disease.

A third study, from a separate research group mining data from the NIH Human Microbiome Project, focused on the biologically active small molecules produced by resident gut microbes and how they might affect health or disease in their human hosts. Using computational analysis, they searched the genomes of human gut microbes to identify bacterial gene clusters that are present in samples from a majority of people and are unique to their intestinal niche, but were of unknown function at the time. They put these gene clusters into two common types of gut bacteria, turned on the genes, and then purified and analyzed the molecules that were produced as a consequence. They found that the active component produced by the gene clusters was a group of molecules called peptide aldehydes, which can affect human host cells by inhibiting enzymes that play important roles in antimicrobial defense. The findings suggest that the peptide aldehydes produced by gut microbes may help human hosts to tolerate “friendly” bacterial species.

This trio of studies provides a snapshot of the vibrant research efforts on the gut microbial community that

are taking place at many levels simultaneously—from the social to the microbial to the molecular. Studies such as these are helping to improve understanding of the complex mechanisms by which gut microbes affect human health. This work can serve as a basis for developing future dietary and other interventions that are more effective by virtue of being tailored to individuals and their microbes, specifically by considering the impacts of diet, social interactions, and the totality of the gut microbial community and its metabolic output.

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Wagner VE, Dey N, Guruge J,...Gordon JI. Effects of a gut pathobiont in a gnotobiotic mouse model of childhood undernutrition. *Sci Transl Med* 8: 366ra164, doi: 10.1126/scitranslmed.aah4669, 2016.

Guo CJ, Chang FY, Wyche TP,...Fischbach MA. Discovery of reactive microbiota-derived metabolites that inhibit host proteases. *Cell* 168: 517-526, doi: 10.1016/j.cell.2016.12.021, 2017.

Immune Molecule Defends Against Bacterial

Infection by Withholding Iron: An international group of researchers has found, in a mouse model, one way in which the body thwarts infection by pathogenic bacteria: an immune molecule called IL-22 activates proteins in the blood to limit availability of iron, which bacteria need. In humans and other animals, most of the body's iron is concentrated in structures called heme groups within hemoglobin inside red blood cells, which carry out the vital function of ferrying oxygen from the lungs to sites around the body. Iron is also an essential nutrient for microorganisms, and the body naturally limits bacterial access to the nutrient as a defense against infection in a process called "nutritional immunity." Some pathogens in the circulation circumvent the scarcity of free iron in the blood by attacking red blood cells, causing them to release their iron-rich hemoglobin and heme groups.

Researchers set out to discover whether nutritional immunity involved limiting bacterial access to the major iron stores contained in the heme groups and blood cell hemoglobin. They focused their efforts on one immune system molecule in particular called interleukin-22 (IL-22), which is known for its role in

protecting against bacterial infection. Using a mouse model, they tested how the presence or absence of IL-22 affected infection with two pathogenic bacteria—*Citrobacter rodentium*, a major pathogen in mice, and *Escherichia coli*, a leading cause of blood infection in humans. Mice that had been genetically altered to lack IL-22 were much more likely than normal mice to die from *C. rodentium* infection due to greater pathogen burden in the blood. While analyzing changes in blood protein levels during infection, the scientists noticed that the infected mice without IL-22 had much lower amounts of two proteins in the blood, called hemopexin and haptoglobin. These proteins are produced mainly by the liver and bind to free heme and hemoglobin, respectively, that are released during an infection, thereby limiting their toxicity to other host cells. The IL-22-deficient mice also had more free hemoglobin in the blood after infection, indicating more activity by the bacteria in attacking blood cells. Replacing IL-22 in the deficient mice with an intravenous infusion helped their blood proteins fight the infection in the animals and in cell culture by boosting levels of the heme-binding hemopexin. Similar results were found when the human pathogen *E. coli* was used instead of *C. rodentium*.

This work shows how the immune molecule IL-22 helps protect animals against bacterial infection in the blood through raising hemopexin levels and limiting the availability of heme. Further studies may seek to understand how these bacterial pathogens are able to access the iron contained within heme. Future applications of nutritional immunity may capitalize on this knowledge to develop new treatments for bacterial infections in the blood using IL-22, hemopexin, or another protein that limits heme availability.

Sakamoto K, Kim YG, Hara H,...Núñez G. IL-22 controls iron-dependent nutritional immunity against systemic bacterial infections.

Sci Immunol 2: eaai8371 doi: 10.1126/sciimmunol.aai8371, 2017.

AUTOIMMUNE DISORDERS OF THE GUT AND LIVER

Uncovering the Genetic Basis for Primary Sclerosing

Cholangitis: In the largest study of its kind, an international group of researchers revealed several areas of the human genome that convey risk for developing primary sclerosing cholangitis (PSC), a disease that can

lead to liver damage, also shedding light on this disease's relationship to inflammatory bowel disease (IBD). In PSC, a network of tubes, called biliary ducts, become inflamed. These tubes carry bile from the liver, where it is made, to the small intestine, where it helps digest fats and certain vitamins. As the bile ducts become inflamed and then scarred, they eventually become blocked. Unable to exit the liver, the accumulating bile has devastating effects on the liver tissue, causing scarring, cirrhosis, and, ultimately, liver failure. The reasons underlying the bile duct inflammation are not completely understood, although genes appear to play a role, as people who have relatives with PSC are more prone to develop the disease themselves. But finding genes that could be involved has been difficult because PSC is relatively rare: it is diagnosed in about 1 in 100,000 people per year in the United States. Roughly three-quarters of the people with PSC also have a form of IBD, most often ulcerative colitis, raising the possibility that there are shared genetic factors between these two diseases.

Recently, a group of researchers studied the DNA of about 4,800 people with PSC and compared it to the DNA of almost 20,000 healthy individuals. PSC is more common in men than women, but women do develop the disease, so the researchers studied both sexes. They identified four areas of the genome with variants—changes in the DNA—that are more common in people with the disease. One of the variants causes higher levels of a protein called UBASH3A, suggesting that this protein may have a role in PSC and could be a therapeutic target. The scientists also partnered with the International IBD Genetics Consortium, of which the NIDDK's IBD Genetics Consortium is a member, to compare the genomes of people with PSC to those who have IBD. The researchers found that genetic factors linked to PSC are more closely correlated with ulcerative colitis than Crohn's disease, another form of IBD. This could explain why a larger percentage of people with PSC have the ulcerative colitis form of IBD, rather than Crohn's disease. However, the genetic associations that they found were not enough to fully explain why so many people have both PSC and ulcerative colitis. This suggests that there may be other shared factors between these two diseases that have yet to be uncovered, such as environmental influences or rare genetic variants that are more difficult to detect. Further research could pinpoint the genes involved in PSC, resulting in potential therapeutic targets and improved screening methods to help diagnose and treat the disease.

Ji SG, Juran BD, Mucha S, ...Anderson CA. *Genome-wide association study of primary sclerosing cholangitis identifies new risk loci and quantifies the genetic relationship with inflammatory bowel disease.* *Nat Genet* 49: 269-273, doi: 10.1038/ng.3745, 2017.

Shedding Light on the Functional Genetic Architecture of Inflammatory Bowel Disease:

Recent findings have uncovered two genetic variants that increase the risk for inflammatory bowel disease (IBD) and also provided clues to their functional impacts, including interactions with the immune system and gut microbes. IBD is the general term for the diseases, including Crohn's disease and ulcerative colitis, that are characterized by chronic inflammation in the gut. This inflammation leads to recurring abdominal cramps, bleeding, and diarrhea. Effective treatments for IBD have been elusive, largely because the disease is a result of complicated interactions between multiple genetic and environmental factors, including an improper response to bacteria inhabiting the gut. To discover the genetic underpinnings of IBD, the NIDDK's IBD Genetics Consortium has enrolled thousands of patients and identified more than 200 regions of the human genome that are associated with risk of Crohn's disease or ulcerative colitis, yielding important new insights into the nature of IBD. Building upon these important first steps, the Consortium is now working together with international colleagues to identify and characterize specific genetic variants that are involved in IBD susceptibility. This is important because even if a genetic variant is rare, its linkage to IBD could give insight into how the disease develops. For example, several variants identified thus far appear to affect the immune system, which in turn could affect the gut's reaction to resident bacteria.

One group of scientists, including members of the NIDDK IBD Genetics Consortium, sought to discover new genetic variants that would have a strong effect on the likelihood of developing Crohn's disease. They focused on the Ashkenazi Jewish population, which has a higher prevalence of the disease than non-Jewish people of European ancestry. Analyzing DNA from a population of men and women of Ashkenazi Jewish descent—approximately 1,500 with Crohn's disease and 2,600 without the disease—the researchers found a rare variant of a gene called *CSF2RB* that was more common in the people with the disease. This finding was validated by examining the DNA of another Ashkenazi Jewish study population with approximately 1,500 people with Crohn's

disease and 7,000 healthy people. When this variant was introduced into human cells grown in the laboratory, it weakened the activation of a signaling pathway critical for restraining the immune response. Immune cells from Ashkenazi Jewish people with this variant had similar defects in this signaling pathway. These results suggest that this variant may confer IBD risk by failing to repress certain immune reactions.

Another research team with ties to the NIDDK IBD Genetics Consortium and other international consortia combed the genomes of over 10,000 people, both women and men, of non-Jewish European ancestry with IBD (Crohn's disease or ulcerative colitis) and over 5,000 people without IBD to find new genetic variants that are associated with the disease. They found a variant in a gene called *SLC39A8* that was more common in people with Crohn's disease compared to people without this disease (controls). When activated, the *SLC39A8* gene produces a protein within cells that transports zinc. Interestingly, this particular genetic variant has also been implicated in other aspects of health, including obesity. Knowing that both IBD and obesity are associated with changes in gut bacteria, and that zinc metabolism relates to immune function, the researchers thought that this variant may somehow be affecting the gut microbiome. Examining the microbiomes of over 300 people from another study, the scientists found that the *SLC39A8* variant was associated with an altered gut microbiome in both the controls and the people with IBD. These results point to differences in the gut microbiome—driven by variations in the human genome—that could eventually contribute to IBD or obesity.

Uncovering the roles of these genetic variants in IBD offers targets for developing potential new treatments and provides remarkable insight into how the disease develops. It also identifies genetic markers that could be used for screening to help individuals seek treatment before symptoms become severe.

Chuang LS, Villaverde N, Hui KY,...Cho JH. A frameshift in CSF2RB predominant among Ashkenazi Jews increases risk for Crohn's disease and reduces monocyte signaling via GM-CSF. Gastroenterology 151:710-723, doi: 10.1053/j.gastro.2016.06.045, 2016.

Li D, Achkar JP, Haritunians T,...Duerr RH. A pleiotropic missense variant in SLC39A8 is associated with Crohn's disease and human gut microbiome composition. Gastroenterology 151:724-732, doi: 10.1053/j.gastro.2016.06.051, 2016.

Uncovering Factors Linked to Celiac Disease:

Two recent studies have provided important insights into celiac disease, including its prevalence in different areas of the United States and the possibility that a viral infection may trigger the disease in genetically susceptible people. The immune system is constantly poised to attack foreign material in the body, but, importantly, it will refrain from attacking benign substances, such as the food we ingest or the body's own cells. In people with celiac disease, however, the immune system in the small intestine treats gluten—a protein naturally found in wheat, barley, and rye—as a foreign invader. The resulting immune response in the gut mistakenly identifies one of the body's own proteins as foreign, damaging the intestinal lining, interfering with nutrient absorption, and leading to bloating, diarrhea, and anemia. The two genetic variants that are known to convey risk for celiac disease are very common—up to one-third of the U.S. population carries one of them. Yet only a small fraction of these people will develop the disease, meaning other genetic or non-genetic factors are likely involved.

Some studies have sought to determine whether celiac disease is more common in some geographic regions than others, which could help pinpoint factors involved in the onset of the disease. In one such recent study, scientists combed through health data from 22,277 women, men, and children living in the United States who participated in a national health survey between 2009 and 2014. The survey included questionnaires, medical histories, and blood samples, allowing the researchers to determine the number of diagnosed celiac disease cases, as well as those that were previously undiagnosed but were detected in the serological tests performed during the survey. The researchers found that people living north of latitude 40° North (approximately the northern border of Kansas) were over five times as likely to have celiac disease as those living south of 35° North (approximately the southern border of Tennessee). People living in between these latitudes were also over three times as likely to have celiac disease as people living south of the 35° North line. The reasons for a higher frequency of celiac disease in northern states are not clear—genetic or environmental factors could be involved—but the trend does appear to be independent of race, ethnicity, socioeconomic status, and body mass index. The scientists also found that

participants who had previously undiagnosed celiac disease that was detected during the survey had lower levels of vitamin B-12 and folate in their blood, likely reflecting a deficiency in the uptake of these nutrients because of intestinal damage. This deficiency was not observed in participants with diagnosed celiac disease, underscoring the importance of diagnosing the disease and undergoing proper treatment (*i.e.*, avoiding gluten).

Other studies have hinted that viral infections may contribute to the onset of celiac disease, but, until recently, direct evidence of a role for viruses has been lacking. A new study found that infection with a common virus, called a reovirus, may trigger celiac disease in people who are genetically susceptible to developing the disorder. People are typically exposed to reoviruses throughout their lives, but infections tend to go unnoticed because the viruses are cleared by the immune system, and any symptoms are usually mild. Nonetheless, the researchers thought that the immune responses evoked by these infections might lead to gluten intolerance in genetically susceptible people. To test this idea, the scientists first infected mice with two types of reoviruses that were originally isolated from humans, and they examined the effects on the immune systems of the mice. Both types of reoviruses infiltrated the intestinal cells, where they activated genes such as those involved in antiviral immunity. But one of the reovirus types, called T1L, evoked a more robust immune response and also activated genes in areas of the gut involved in regulating immune tolerance to ingested food. These changes appeared to disrupt the immune system by stimulating attack pathways while blocking suppression pathways, effectively interfering with the immune system's ability to develop tolerance to certain dietary proteins. Next, the scientists sought to determine whether an immune reaction to T1L could lead to gluten intolerance in mice genetically modified to carry a human genetic variant that confers susceptibility to celiac disease. Like the experiments in the non-genetically modified mice, the scientists found that a T1L infection in these celiac disease-prone mice stimulated the immune system and prevented the mice from developing tolerance to ingested gluten. Lastly, the scientists examined plasma samples from women and men with celiac disease and found they had higher levels of antibodies to reoviruses than people without the disease, providing evidence linking celiac disease to immune

responses from reovirus infections. Similarly, people with high levels of reovirus antibodies were more likely to have celiac disease.

Taken together, the results from these studies provide insight into factors that could be involved in triggering celiac disease in people who are at genetic risk, offering leads on potential approaches to disease prevention. More work along these particular lines of research could shed light onto why celiac disease is more prevalent in northern areas of the United States and whether vaccination or other antiviral approaches may be effective in preventing the disease.

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UNDERSTANDING LIVER DISEASE

Mouse Models of Liver Disease Highlight Important Roles of Temperature and Sex: Recent studies with animal models of two different forms of liver disease demonstrate the importance of factors such as the sex of the animals and even the ambient temperature where they are housed in designing experiments that are relevant to human disease.

Nonalcoholic fatty liver disease (NAFLD) is a form of chronic liver disease in both women and men; it is associated with obesity and other metabolic disorders and is on the rise in the United States and around the world. In NAFLD, fat builds up in the liver, sometimes followed by more severe disease marked by liver inflammation and scarring that can lead to cirrhosis, liver failure, and liver cancer. But, despite its prevalence and potential severity, treatments for NAFLD are limited. Few animal models exist to study NAFLD, with the most prominent being a mouse model fed a high-fat diet to elicit NAFLD-like disease. These mice, however, show marked differences from their human counterparts with NAFLD, namely that the mice have less liver inflammation

and scarring, and also the female mice show no signs of NAFLD and therefore cannot be studied with this model. Researchers sought to improve the relevance of this animal model of NAFLD to humans by optimizing the environment of the mice and thereby altering their physiology. The researchers noted that mice in the laboratory are typically housed at temperatures adjusted for human comfort, but mouse metabolism functions more efficiently at warmer temperatures. With this in mind, the researchers raised the temperature of the room where the mice on the high-fat diet were housed to align more closely with mouse metabolism. They found that this simple change resulted in altered responses in the mice, most notably a more pronounced NAFLD-like disease, even in the female mice. A myriad of physiological changes were also apparent with the thermostat adjustment, compared to the usual temperature set for human comfort, including heightened inflammatory responses, greater intestinal permeability, and alterations in the gut microbes—all of which are features of human NAFLD. This study shows how changing the temperature under which mice are typically housed improves the utility of a mouse model for studying human liver disease, particularly in females. This finding could enable future studies to better understand disease processes underlying NAFLD as a foundation for developing improved therapeutic approaches.

In another type of liver disease, called primary sclerosing cholangitis (PSC), the ducts that drain the bile from the liver are damaged, with inflammation leading to scarring and blockage of the ducts over time and a back-up of bile into the liver. Liver cirrhosis and liver failure can result, requiring transplantation. Both men and women are susceptible to PSC, though the disease is more common in men. Animal models of this disease aid in the understanding of disease processes and in developing new approaches to treatment. One research group investigated a genetically modified mouse model that spontaneously develops features of PSC due to the absence of a key component of bile. Interestingly, the mice also display an unusual sex difference—the female mice develop more severe liver injury than the males. The researchers probed this model to uncover mechanisms underlying the disease, particularly in females compared to males. They identified the molecular pathways associated with the different stages of disease in the female mice compared to males. In particular, they noted that female mice had dramatically higher levels of a molecule called H19 produced in the bile duct cells

that regulates cell proliferation and differentiation into specific cell types. By reducing the levels of H19, the group was able to reduce liver injury in the female mice. In human samples from people with PSC, the researchers showed the same alterations in molecules such as H19 as in the PSC mouse model. These results highlight clinically relevant molecular factors involved in PSC that may also play a role in sex-related differences in disease progression. Factors such as H19 may represent a new target for the development of future therapies against diseases such as PSC.

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Studies Document Some Severe Outcomes After

Drug-induced Liver Injury: Three recent studies conducted by NIDDK's Drug-Induced Liver Injury Network (DILIN) have provided new insights into outcomes from this potentially severe form of liver injury, including the rate of fatal outcomes, frequency of bile duct damage and loss, and racial/ethnic disparities in disease severity. Drug-induced liver injury is a relatively rare, but potentially life-threatening type of liver disease that is a growing cause of death and of the need for liver transplantation in the United States. It can occur with use of over-the-counter or prescription drugs, as well as with herbal or dietary supplements. Diagnosis and prognosis is complicated by the varying patterns of clinical injury observed to the liver and to the ducts carrying bile away from the liver to the intestine, sometimes resulting in liver failure. The Network was formed in 2003 to understand how drugs or herbal/dietary supplements cause liver injury and to determine long-term outcomes following this injury. It includes several clinical sites, a data coordinating center, and a sample repository. Study participants are at least 2 years of age, though most are adults, of both sexes. A panel of experts reviews each case to determine whether the injury was likely caused by the drug or herbal/dietary supplement, as well as the severity of the injury.

As part of the prospective study conducted by the Network, researchers analyzed how frequently participants with drug-induced liver injury experienced what were considered “fatal outcomes”—either death or liver transplantation, without which the individual would have died—over the following 2 years. Experts reviewed the case details to assess whether the injury was a primary cause of death or life-saving transplantation, or if it was simply one contributing factor. Among their findings, they showed that fatal outcomes occurred in 9.8 percent of the study participants in the 2 years following liver injury. The liver injury was judged to directly cause the fatal outcome in 64 percent of these cases and to contribute to it in 14 percent of cases, both mostly occurring in individuals who were younger than 45 years of age or women. The investigators also tested three mathematical formulas used to predict mortality risk from liver disease based on clinical measures, such as liver enzymes, bilirubin, and creatinine. They were able to rank the relative value of these formulas, some of which proved more useful for predicting who might be at greater risk of dying after drug-induced liver injury.

Another Network-driven study focused on one particular pattern of injury from drug-induced liver injury in which the bile ducts are damaged or even lost in a condition called vanishing bile duct syndrome. They analyzed liver biopsies that had been obtained from some of the participants sometime in the decade following their injury and scored the biopsies for bile duct loss. During this time, 26 (7 percent) of the study participants who had liver biopsies showed signs of bile duct loss, from which seven individuals died and two others eventually required liver transplantation. Most of these individuals first presented with a type of clinical pattern marked by severe injury to the bile ducts and liver inflammation. The researchers identified some of the drugs or supplements most commonly associated with the bile duct injury, including specific antibiotics and herbal supplements. The degree of the bile duct loss was found to be the best predictor of poor outcomes, such as death or need for a liver transplantation. As there is currently no way to prevent or treat bile duct loss, these findings may help to identify those at risk at an earlier point in the disease process, before bile duct loss occurs, and to prompt the development of early interventions.

In a third analysis conducted by Network investigators, the focus was on defining any racial/ethnic differences in drug-induced liver injury, specifically in African Americans compared to Caucasians. The analysis was prompted by

evidence from other studies of adverse drug reactions, such as the NIDDK-supported Acute Liver Failure Study Group, which found that African Americans were more at risk for acute liver failure from drugs than Caucasians. In the Network’s study, the most frequent cause of drug-induced liver injury in African Americans was a two-drug antibiotic treatment containing trimethoprim and sulfamethoxazole, while another common two-antibiotic combination (clavulanic acid/amoxicillin) was the most frequent culprit in Caucasians. African Americans typically experienced more severe liver disease after drug-induced liver injury than Caucasians, which was more likely to result in outcomes such as death or need for liver transplantation. Severe skin reactions following drug-induced liver injury were also more common in African Americans. These findings may help improve care of groups at higher risk for liver injury from certain drugs by informing health care providers’ prescription choices and monitoring of those individuals who might be at higher risk for developing severe disease.

These results represent some of the major advances coming from the DILIN’s extensive studies of how liver injury caused by drugs and herbal or dietary supplements affects the U.S. population. Each of these studies’ findings points to new research questions that need to be addressed in drug-induced liver injury, including new approaches to managing potentially fatal outcomes such as bile duct loss and understanding why groups such as African Americans suffer worse outcomes or why the injury is more often a primary cause of death in those who are younger and female. Work towards answering these questions and others will be continued by DILIN’s investigators and study participants through a 5-year extension starting in 2018.

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LIVER TRANSPLANT OUTCOMES

Adherence to Immunosuppressive Medication Leads to Better Outcomes in Young Liver Transplant Recipients:

A study of children around the country who received liver transplants has found that a tool to measure their adherence to taking immunosuppressive medication can predict organ rejection later. Life-saving liver transplants rely on use of a scarce resource—organs from living or deceased donors—and they also generate high health-care costs. Efforts to improve outcomes after organ transplantation benefit the recipients' health, as well as optimize use of the transplanted organs. A leading factor in determining outcomes for organ transplant recipients in the long term, including whether the body rejects the transplant and recipient survival, is consistently taking immunosuppressive medications as prescribed. These medications are required life-long after transplant to avoid the body's rejection of the transplanted organ. Researchers developed a tool to gauge medication adherence in transplant recipients based on blood levels of the medication over time called the Medication Level Variability Index (MLVI). They tested it within a study of 400 children ages 1 to 17 who had received liver transplants at five centers throughout the United States and were then followed for 2 years after. Although there were no deaths or organ failures requiring re-transplantation during the study, liver biopsies from some participants showed signs of transplant rejection associated with lower medication adherence. For example, 53 percent of adolescents who had a higher MLVI score in the first year of the study, indicating lower adherence to medication, showed signs of transplant rejection in the following year. This study demonstrates that the MLVI is a useful tool for predicting which pediatric liver transplant recipients, particularly those who are adolescents, are at risk for eventual transplant rejection based on not taking their immunosuppressive medication as prescribed. Further clinical trials are needed to test behavioral interventions, based on MLVI score, to improve medication adherence. However, this knowledge could one day help health care providers to better monitor pediatric liver transplant recipients, especially adolescents, and to intervene before transplant rejection occurs.

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Donor Outcomes in the Years After Living Donor Liver Transplantation:

Two recent studies of people who donated part of their livers to others in need of a liver transplant have highlighted some of the ways in which this altruistic act can affect donors psychologically, socially, and financially in the first few years after the procedure. Living donor liver transplantation is the only treatment option available for those with end-stage liver disease who are unable to obtain an organ from the limited supply provided by deceased donors. Health care professionals aim to optimize this treatment so that it can continue to be offered as a life-saving therapy that best supports the health of both recipients and donors.

The NIDDK's Adult-to-Adult Liver Transplantation Cohort Study 2 (A2ALL-2) consortium conducted two studies at its nine centers in the United States and Canada to investigate potential burdens placed on donors, including those affecting their mental health, relationships, and finances. As part of these studies, 271 donors participated in phone surveys performed before donation and at 3, 6, 12, and 24 months after donation. They were asked about relationship changes, financial burdens, and mental well-being. They found that most donors' relationships with their family members or spouses/partners stayed the same or even improved, with nearly a third of survey respondents reporting better relationships than before the transplant. However, the majority of donors reported out-of-pocket medical or non-medical expenses related to the transplant, which 44 percent of donors considered burdensome. This financial burden was heaviest in the first few months after the transplant procedure and persisted for 1 to 2 years after. Lower income donors were at greater risk of incurring these burdensome costs. These findings support the need for programs to expand resources for donors, in order to reduce the financial burden and eliminate these disincentives to being a donor. A second study focused on mental well-being by evaluating donors for symptoms of conditions such as depression, anxiety, and alcohol abuse. The donors reported well-being comparable

to or better than the general population, with low rates of depression, alcohol abuse, or anxiety. Asked 2 years after their donation, nearly 95 percent of donors said they would donate again if they could. However, 5 to 10 percent of donors reported some type of impairment in mental well-being sometime during the 2 years following donation. The researchers identified factors that affect donors' well-being and their perceptions of self-worth and personal growth after donation. For example, some donors whose recipients passed away experienced guilt and feelings of responsibility. These results suggest that donors should be monitored post-donation to identify any individuals at risk for developing impaired mental well-being, so that they can receive proper care.

These studies provide a more complete picture of how living donor liver transplantation affects donors in a myriad of ways, adding to information

from previous studies of donors' physical health. This knowledge can help to identify programs and systems needed to offer additional support to donors and their families, as well as to fully inform them ahead of time so they know what to expect from the donation and recovery experience. Further research will be needed to assess longer-term impacts on the donors, since some effects may not be apparent until many years after the procedure.

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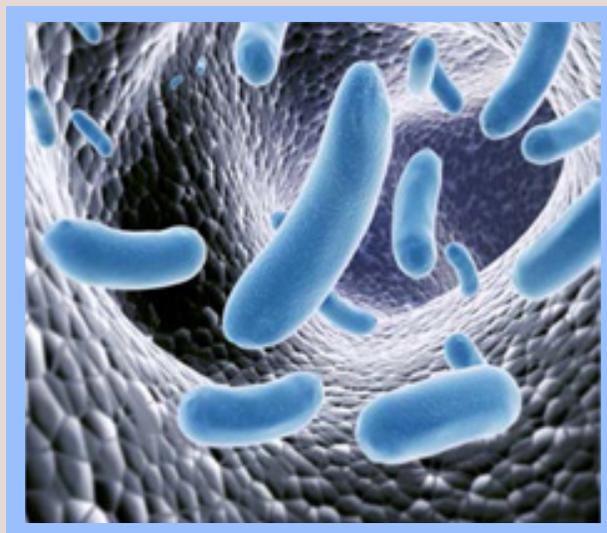
Workshop Highlights Best Practices for Studies of Diet and Intestinal Microbiome

On June 13-14, 2017, the NIDDK, in collaboration with the NIH Office of Dietary Supplements, Agricultural Research Service of the U.S. Department of Agriculture, and the International Life Sciences Institute of North America, held a workshop on the NIH campus in Bethesda, Maryland, to improve the rigor and reproducibility of research on diet and the intestinal microbiome.

The purpose of this workshop was to develop recommendations for identifying important dietary information that should be reported in these studies and experimental design factors that should be considered by researchers, particularly for clinical studies. The workshop stemmed from a growing awareness in the field that many studies of the intestinal microbiome—including *in vitro*, animal model, and human studies—were limited in their design or reporting in terms of dietary considerations.

Diet has profound effects on gut microbial composition, through delivering nutrients, antimicrobial components, or even bacterial strains. Workshop presenters illustrated this point through a wide array of research, including studies showing profound effects of a low-fiber diet, which promotes blooms of gut bacteria that degrade the protective mucus lining the intestine. The workshop aimed to identify ways to improve the quality and reproducibility of gut microbiome research by increasing focus on standardization and reporting of dietary factors.

The 2-day workshop brought together researchers from 12 different countries and from across federal agencies, universities, and industry to discuss best practices in studying the impact of diet on the intestinal microbiome. Presentations highlighted key issues in this area, including how to characterize effects of nutrients such as dietary fibers on the



microbiome, use of *in vitro* and animal models, and a focus on designing more informative human studies. For example, speakers highlighted the importance of such themes as considering the form and chemical structure of foods when designing diets for testing, use of “bioreactors” containing a controlled ecosystem of gut microbes to allow *in vitro* testing of diet, and ways to improve on dietary assessments used in human studies. Speakers also noted the coincidence of the workshop with the 10th anniversary year of the NIH’s Human Microbiome Project, which catalyzed much of the progress in microbiome-related research.

The workshop closed with a discussion of research gaps that should be addressed by future studies of diet and the gut microbiome. The event organizers plan to share the information discussed in the workshop with the wider research community and public through a summary of recommended best practices for experimental design and reporting of diet in gut microbiome studies to be published in the scientific literature.

New NIH Nutrition Research Task Force Guiding Nutrition Research Planning

On October 11, 2016, the NIH Director, Dr. Francis Collins, established the NIH Nutrition Research Task Force to guide the development of the first NIH-wide strategic plan for nutrition research. This plan will emphasize the identification of cross-cutting, innovative opportunities to advance nutrition research across a wide range of areas, from basic science to clinical experimental design to research training, over the next decade. The strategic plan will also highlight ways to complement and enhance ongoing research efforts across NIH to improve health and prevent or combat diseases and conditions affected by nutrition.

The Task Force is led by its Chair, Dr. Griffin P. Rodgers, NIDDK Director, with Co-chairs Dr. Gary Gibbons, Director of the National Heart, Lung, and Blood Institute; Dr. Norman Sharpless, Director of the National Cancer Institute; and Dr. Diana W. Bianchi, Director of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development.

The Office of Nutrition Research, housed within the NIDDK, has been spearheading the nutrition research strategic planning effort. The Office's Director, Dr. Christopher Lynch, serves as Executive Secretary of the Task Force. A writing group and senior leadership group staffed by representatives from across the NIH's many Institutes and Centers has also been convened to assist the Task Force in this effort.

This nutrition research strategic planning activity is being informed by broad stakeholder input throughout its development. In fall 2016, the Office

conducted a review of the scientific literature and compiled information on existing recommendations from the research community, including professional societies, academic researchers, nonprofit organizations, and federal agencies, to help prioritize areas of nutrition research in the strategic planning. In spring 2017, the Office also undertook a "crowdsourcing" effort to elicit ideas for the nutrition research planning effort from a broad swath of the research community and public through an online platform. Over 600 ideas were ultimately received, with a total of 4,400 people participating by either submitting ideas or voting on others' suggestions.

The Task Force assembled for its first meeting in June 2017 and featured presentations from a representative of the U.S. Food and Drug Administration on the importance of nutrition research across Federal agencies, and from an academic institution scientist on recommendations to improve nutritional assessment tools. Also in June 2017, the Task Force convened a Thought Leaders Panel of external experts in nutrition research for a day-long series of meetings to provide recommendations on priorities in nutrition research, including feedback on the ideas already received through crowdsourcing and existing strategic plans.

The Task Force's writing group and senior leadership group are helping to further prioritize these ideas and is drafting the research plan, which is anticipated to be released in draft form for public comment in Spring 2018. The final plan is expected to be released in October 2018.

Biliary Atresia Workshop Focuses on Clinical and Translational Advances

The NIDDK hosted a workshop on June 28, 2017, on the NIH campus in Bethesda, Maryland, to bring together researchers in biliary atresia for the purpose of gauging the state of clinical and translational science and identifying research priorities to advance understanding of disease processes.

Biliary atresia is a rare disease, but in children living in the United States, it is one of the most common severe liver diseases and reasons for liver transplantation. The disease affects children in early life, during the first 3 months after birth. In biliary atresia, the bile ducts that drain the liver and deliver bile acids to the intestine become inflamed and scarred, causing a back-up of bile into the liver and resulting in jaundice and liver failure. While the causes of biliary atresia are not fully understood, studies indicate that genetic, environmental, and inflammatory factors may play a role. If not caught early and treated with a surgery called the Kasai procedure or with a liver transplant, the disease is fatal. Even with the surgical procedure, a majority of children with biliary atresia develop disease requiring liver transplantation by adulthood. Diagnoses can be delayed due to biliary atresia being one of many possible causes of blocked bile flow in newborns. Some countries have improved early diagnosis through use of a card that parents can use to identify abnormally colored stools in infants with biliary atresia.

The NIDDK supports the Childhood Liver Disease Research Network (ChILDRen), a collaboration among clinical sites and research laboratories in the United States and Canada focused on improving the lives of children and families dealing with rare liver diseases such as biliary atresia. The Network's research constitutes the largest study of biliary atresia in the world, with past results shedding light on contributors to disease development and testing new treatment approaches. However, research on new treatments has not yet yielded significant improvements in altering the course of this liver disease and its associated complications.

Speakers and other workshop participants represented diverse viewpoints on biliary atresia from around the globe, including the United States, Canada, Germany, Italy, Spain, Belgium, Finland, the United Kingdom, Israel, and France. The workshop featured presentations on such important issues in disease development as defining the timeframe when the disease process leading to biliary atresia begins; multiple genetic factors and clinical variants of the disease; environmental exposures such as toxic plant components and viruses; and new approaches to therapy. For example, evidence now suggests that this form of biliary injury most often starts during development inside the mother's womb, followed by liver injury and disease progression after birth. This finding represents a paradigm shift in the field, with implications for defining the ideal window of opportunity to prevent or treat disease. Latest results were also presented from groundbreaking studies on the first environmental toxin identified, called biliatresone, found in recent years to cause a biliary atresia epidemic in Australian lambs born to mothers consuming a particular plant present during drought conditions. Such "natural experiments" in animals of environmental or dietary exposures related to biliary atresia, along with extensive follow-up testing in other animal and laboratory models, are providing insights into possible disease processes at work in human disease. Workshop participants also described new tools being used to uncover the mysteries of biliary atresia development, including unique animal models and systems such as spheroids or organoids composed of bile duct cells, mouse bile duct explants, and bile ducts on a chip.

Throughout the workshop, participants identified key research challenges, opportunities, and priorities for biliary atresia, such as efforts to define causes and disease processes, to promote earlier detection in the United States, to improve early treatment based on factors such as disease risk and severity, and to prevent disease progression after the Kasai procedure. The meeting organizers plan to prepare a summary of the workshop for dissemination to the wider research community.

Intestinal Stem Cells

Every 5 days, the inner lining of the gastrointestinal tract, called the intestinal epithelium, completely renews itself. Understanding exactly how it accomplishes this feat would help researchers develop new therapies to rejuvenate damaged intestinal tissue and explore new approaches to treating diseases such as colon cancer, where regulation of this process is lost. Studies over the past several decades have pointed to a relatively small number of cells that lie at the heart of intestinal epithelium renewal. The discovery of these cells—the intestinal stem cells, the ancestors to all other cells in the intestinal epithelium—has led to prolific research by the NIDDK's Intestinal Stem Cell Consortium (ISCC) into the complex dynamics that regulate the development and turnover of the intestinal lining, the most rapidly regenerating tissue in the body.

The Cellular Structure of the Intestine

At first glance, the intestinal epithelium may appear to be a simple barrier that separates the contents of the gut from the rest of the body, but research over the past century has revealed its extremely diverse and dynamic makeup.

The wall of the small intestine is lined with fingerlike structures, called villi, that project into the intestinal space (lumen) and help the gut absorb materials by increasing its internal surface area. The most numerous epithelial cells on the villi are enterocytes, which absorb water and nutrients from ingested food. Scattered among the enterocytes are goblet cells, which secrete protective mucus; enteroendocrine cells, which release hormones that regulate digestive functions such as appetite control and the muscle contractions that move food through the gut; and tuft cells, which play a role in sensing intestinal contents and initiating immune responses.

Interspersed among the villi in the small intestine are areas of lymphatic tissue called Peyer's patches, which play an important role in the immune system by preventing the growth of pathogenic bacteria. Specialized epithelial cells called microfold cells, or "M cells," coat the Peyer's patches and continuously sample the contents of the gut for signs of pathogenic microbes.

The intestinal wall is also dotted with pit-like structures called "crypts." Nestled at the bottom of the crypts are Paneth cells, which are epithelial cells that secrete antibacterial compounds. Researchers in the late 1800s noticed that crypts also had a distinct attribute: while the cells in the villi appeared dormant, many cells in the crypts seemed to be constantly dividing. This gave rise to the idea that all cells in the intestinal lining are, rather ironically, "born" in crypts. The cells would then migrate up into the villi, where eventually they would be shed from the villi tips into the intestinal lumen.

Using a mouse model, researchers in the 1940s not only found evidence that this conveyor belt-type process takes place, but that it happens in a matter of mere days. It also raised several important questions. How do the crypts produce such a diverse variety of intestinal cells, each with their own specialized role in digestion? And how is this process of cell production and death regulated, especially during injury and disease, so there will always be an appropriate number of cells to maintain a functional intestinal wall? By the 1960s, researchers were beginning to suspect that the underlying answers to these questions related to the existence of stem cells—a pool of ancestral cells that continuously duplicate themselves while also producing progeny that morph into all the various types of intestinal cells, all in a tightly regulated process called differentiation. They also realized that these stem cells, if they did exist, must lie somewhere in the intestinal crypts.

STORY OF DISCOVERY

Intestinal Stem Cells

In the 1960s and 70s, scientists zeroed in on a group of cells wedged among the Paneth cells at the bottom of intestinal crypts in mice. These small cells, called crypt base columnar (CBC) cells, were conspicuous because they were continuously dividing, unlike their larger Paneth cell neighbors. Labelling the CBC cells in mice with a radioactive compound, the scientists were able to track their progeny as they divided and moved up the walls of the crypts. The label was eventually found in several different types of intestinal cells in the villi, providing the first direct evidence that the CBC cells were stem cells that give rise to all other intestinal epithelial cells.

Another type of crypt cell that has characteristics of stem cells was discovered at around the same time as CBC cells. These cells were called “+4 cells” because they were typically found at a position about four cells above the bottom of the crypt. Unlike CBC cells, +4 cells were observed to divide slowly, or not at all. Later studies found that these +4 cells are able to undergo rapid divisions and take on CBC cell characteristics when the bottom of the crypts are damaged, suggesting that they act as a reserve to replace stem cells that are lost to disease or injury.

Above the +4 cells is a stretch of the crypt wall where the stem cell progeny rapidly divide as they migrate away from the crypt's base. It was believed that the cells start to differentiate in this area, called the transient amplifying zone, taking on the characteristics of enterocytes, goblet cells, or any one of the other intestinal cell types. This model gained more support when advances in technology allowed researchers to identify the different types of intestinal cells (including stem cells) using molecular markers that are specific for each cell type. This enabled scientists to identify progenitor cells—the stem cell progeny that are on their way to becoming specialized intestinal cells.

The intestinal stem cell model has helped researchers understand the basis of digestive diseases during

which the intestine is damaged and needs to heal (such as in celiac disease or inflammatory bowel disease) or in cases when regulation of cell proliferation goes awry (such as in colon cancer). Yet much work remains to further understand the many steps involved in the production of specialized intestinal cells and how this knowledge might be applied for therapeutic interventions.

The Intestinal Stem Cell Consortium

By the beginning of the 21st century, it was becoming clear that intestinal stem cells held great promise for understanding and treating digestive diseases. In 2009, the NIDDK formed the ISCC to grasp a better understanding of the biology of the intestinal stem cells during development, homeostasis, regeneration, and disease. The Consortium, consisting of a data coordinating center and nine study centers across the United States, enables participating researchers to share ideas and resources, including data, research materials, methods, and expertise. The immediate goals of the ISCC were and are to isolate, characterize, culture and, validate populations of intestinal stem cells; answer major questions in stem cell biology of the intestinal epithelium; and accelerate research by making information and resources available to the research community.

Since its inception, the ISCC has produced a wealth of information on the biology and therapeutic potential of intestinal stem cells. The ISCC's earlier years focused on the respective roles of active and quiescent stem cells, along with the genetic mechanisms that control differentiation of stem cells into specialized intestinal cells. Consortium members also identified molecular markers that are unique to stem cells and their various stages of differentiation. This was an extremely important step in intestinal stem cell research, as it gave researchers more tools to identify and track specific cell populations in the gut. The ISCC also began efforts to recapitulate intestinal development outside of an animal model, which would enable researchers to

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examine events surrounding crypt formation more closely and to test the possibility of using cultured cells for therapeutic purposes.

Based on these early successes, the NIDDK renewed support for the ISCC in 2014. Consortium members continue to be extremely productive, demonstrating the synergy and efficiency of the ISCC. The following examples are just several of the many advances that the Consortium has contributed to intestinal stem cell research.

Making Mini-intestines

While most of the pioneering research on intestinal stem cells was accomplished in mouse models, the identification and characterization of intestinal stem cells allowed researchers to separate them from the surrounding tissue and culture them outside of an animal. This presented scientists with new opportunities to study more closely the molecular changes that occur in these cells. It also allowed investigators to co-culture human intestinal stem cells to differentiate into various intestinal cell types in the laboratory, providing models to study types of human intestinal cells that are otherwise not easily accessible. For example, one group of ISCC scientists cultured stem cells from human intestinal crypts and induced them to differentiate into M cells. These cells even behaved like functional M cells: they took in pathogenic bacteria, much like they would in an intestine when they are delivering pathogens to the immune system in an underlying Peyer's patch. This provided an important model system for studying how these cells protect the intestine from pathogenic microbes in the gut.

The ISCC has also used human pluripotent stem cells (PSCs) to create microscopic three-dimensional models of the intestine. PSCs are stem cells that can differentiate into many other different types of cells in the body, including cells that act as intestinal stem cells. The studies used induced pluripotent stem cells (which are derived from cells that were not originally stem cells but were induced to be pluripotent in the

laboratory) and embryonic stem cell lines, used within NIH guidelines for human stem cell research. For example, the ISCC has cultured PSC-derived intestinal stem cells in a three-dimensional setting, allowing them to proliferate and differentiate, resulting in a conglomeration of cells that look and behave like a miniature portion of a human intestine (e.g., they contained functional villi-like and crypt-like structures). The ISCC has used these cellular arrangements, called "organoids," as models of the human small intestine, and, more recently, of the human colon.

These laboratory-grown organoids can be used to study human intestinal and colonic diseases in a laboratory setting. For example, ISCC researchers recently succeeded in generating intestinal organoids containing functional nerve cells. The scientists then used these organoids as a model of a functional enteric nervous system—the mesh-like arrangement of nerves that governs the function of the gastrointestinal tract. They applied this model to study the molecular events in human diseases that involve the enteric system, such as Hirschsprung's disease, where stool moves slowly (or stops completely) because the nerves near the end of the colon do not function properly.

Mini-intestines may also eventually be used to grow tissue to replace damaged intestinal tissue. In fact, ISCC researchers recently demonstrated that PSC-derived intestinal stem cells can be induced to differentiate into tissue that resembles different parts of the intestine, such as the ileum (the lower end of the small intestine) and duodenum (the section of the small intestine closest to the stomach). This is important from a therapeutic standpoint because distinct regions of the intestine have different functional roles in digestion.

Studies on Stem Cell Renewal and Plasticity

The ISCC has also concentrated efforts on understanding how intestinal stem cell proliferation and differentiation are regulated. Many studies have focused on the intestinal stem cell "niche," or the environment in and surrounding the crypt that

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produces signals controlling when cells multiply and which type of mature epithelial cell they will become.

One of the most important of these signals is a family of molecules called Wnt proteins. Wnt proteins maintain the intestinal stem cell niche by helping to stimulate proliferation of intestinal stem cells at the bottom of the crypt and the partially differentiated cells in the transient amplifying zone. The ISCC has uncovered several key features of Wnt signaling that may be developed for therapeutic purposes. For example, a recent study by Consortium scientists showed that Wnt proteins are not required for cell proliferation in the early stages of intestinal development in the mouse (*i.e.*, before villi are formed in the mouse embryo). This would be important to consider when developing therapies because tissue repair following injury is believed to rely on such embryonic pathways. Another recent study by ISCC researchers found that Wnt proteins are secreted by immune cells called macrophages in the connective tissue surrounding crypts in mice, and this is critical for intestinal cell proliferation and tissue repair following radiation-induced injury. Another ISCC study uncovered the surprising finding that Wnt proteins alone do not drive stem-cell proliferation in adult mice. Rather, they prime the stem cells for proliferation by making the stem cells more receptive to additional signaling proteins called R-spondins. The researchers found that R-spondins directly stimulate proliferation, which should be taken into consideration when developing therapeutics that aim to boost growth in the intestinal epithelium.

To gain a better understanding of the stages of intestinal epithelial cell differentiation, the ISCC has taken advantage of state-of-the-art techniques such as single-cell RNA sequencing, which allows researchers to compare gene activation in individual cells from mouse intestinal crypts. Scientists used this technique to detect progenitor cells that have begun to differentiate into enteroendocrine cells. These early enteroendocrine cells show significant plasticity—that is, they are able to revert back to an intestinal stem cell state when there is an injury. Single-cell RNA sequencing also

allowed researchers to discover what appears to be one of the earliest stages of differentiation of intestinal stem cells, when they are simultaneously expressing genes for stem cells, secretory cells (such as goblet and enteroendocrine cells), and enterocytes. This provided valuable insight into how and when stem cells “decide” to become mature intestinal cells. Another study sought to provide a better characterization of the +4 cells that lie between the stem cell compartment and the transient amplifying zone. The researchers found that +4 cells have actually started down the path toward differentiation into secretory cells, although they can revert back to stem cells when the original supply of stem cells is lost. They also found that this shift between +4 cells and stem cells is at least partially driven by changes in the three-dimensional structure of the cells’ DNA, which controls the activity of genes required for the transition.

These advances greatly expand the understanding of how the intestinal epithelium develops into such an active, multifunctional, and critical component of the body. This helps set the stage for future studies that focus on treating damaged (or maintaining healthy) intestinal tissue.

The Future of Intestinal Stem-cell Research

The great progress in understanding the development and turnover of the intestinal epithelium has opened many doors that could lead to new therapies for treating digestive diseases. As scientists continue to investigate the intricate steps involved in the proliferation and differentiation of intestinal cells, efforts are underway to apply this knowledge toward ways to protect and heal the gastrointestinal tract. This is reflected in the ISCC’s long-term goals: to contribute to the greater understanding of stem cell biology and to lay the ground work for therapeutic manipulation of the intestinal epithelium. The coming years may eventually see intestinal stem cell-based therapies for a wide range of gastrointestinal diseases like inflammatory bowel disease, genetic disorders, disease-causing infections, radiation injury, and colon cancer.

Ronetta: Finding Strength Within the Turmoil of Gastroparesis



Ronetta and her family. Left to right: Ronetta, her daughter Zion, her son Harvey, and her husband Jarvis.

Ronetta was 37 years old when, in the spring of 2015, her stomach just seemed to shut down. She had recently undergone a medical procedure—unrelated to her stomach—and was expecting to feel a bit off. But after several weeks she continued to be held captive by constant nausea and relentless vomiting. Soon she was unable to eat meals without getting sick. “It was ongoing,” she says. “I was nauseous 100 percent of the time, vomiting 100 percent of the time.... No relief at all.”

The inability to nourish her body took an enormous toll on Ronetta, a successful owner of a mental health counseling business and, with her husband, a parent to two young children. She began to lose weight and energy at an alarming pace. To make the matter worse, her doctors struggled to find a cause. “No one knew what was wrong with me,” she says. Eventually her gastroenterologist discovered that food wasn’t moving down and out of her stomach properly after she ate. She was given a few different medications to try, including one that could help food move

through her stomach. But they didn’t work, or they produced dangerous side effects. By then it had been 8 months since she had started experiencing the symptoms, and she had lost 100 pounds. “My doctor basically said, ‘I don’t know what else to do,’” she recalls. “And I just kept losing weight, wasting away.”

She was referred to Dr. Kenneth Koch, a gastroenterologist at Wake Forest University, which is a 2-hour drive from her home. It was Dr. Koch who, after a battery of tests, was finally able to give her a definitive diagnosis: gastroparesis, a chronic, relatively uncommon, and poorly understood disorder that slows or stops the movement of food from the stomach to the small intestine.

Ronetta was relieved to have an answer at last, and soon thereafter she signed up for an NIDDK-sponsored gastroparesis research study. But she then faced another daunting challenge: how to find the strength to cope with such a debilitating disorder. Yet, through the turmoil of constant nausea, vomiting, and pain, Ronetta was resolute: “I’m a fighter,” she says. “And I refuse to let anything beat me.”

Living with Gastroparesis: “It’s Complicated”

Normally, the muscles of the stomach contract to break up food and move it through the gastrointestinal tract. This, along with the release of hormones and enzymes, allows for the digestion of food. But in people with gastroparesis, the stomach muscles stop working normally, causing food either to move too slowly from the stomach to the small

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intestine or to stop moving altogether. As a result, people with gastroparesis can experience long-term nausea, vomiting, bloating, abdominal pain, and early satiety (the feeling of fullness after just a few bites of food). At the very least, this disorder makes eating a normal-sized meal extremely difficult without getting sick. In extreme cases, it could prevent eating completely. In fact, Ronetta's gastroparesis is severe enough that at times she needs to take in nourishment through a tube as a substitute for eating—a process called intravenous feeding. In Ronetta's case, the tube is implanted in her chest.

Ronetta's bouts of nausea forced her to cut back on time spent at her counseling business. Her long-term clients noticed the changes in her physical appearance as she lost weight.

"I could see the fright in their eyes when they would see me at appointments," she recalls, "because time after time, I'm getting smaller and smaller." She says several of them tended to feel sorry and anxious for her, which interfered with their counseling sessions. "Their feelings of 'you don't deserve this' became a distraction for them," she says.

While her professional life was upended, the effects on her family were even more heartbreaking. "This is the first time I've actually seen my parents age," she says, adding that her parents haven't taken a vacation since her symptoms started. Ronetta's son was only 2 years old when she got sick, and he still approaches his mother's illness with an innocence that is a testimony to his young age. "He doesn't remember a well mommy," she explains. "Now he just prays that God will make Mommy big and strong so that we could get a dog and that Mommy can run and play outside."

Her daughter, on the other hand, is several years older and can remember when her mother

was healthier. Although dancing is her coping mechanism, she would still become worried when Ronetta would go to Wake Forest for treatment, so Ronetta explained to her what gastroparesis was and which procedures she was undertaking. "I decided it would be easier for her to tell her exactly what was going on. I would show her pictures, diagrams, and YouTube videos, if I could find them," she says. "And she'd feel so much better, knowing exactly what [the doctors] would be doing."

Still, missing out on precious time with her family has been extremely difficult for Ronetta. "It's hard to sit back and watch your children grow up, and you

can't be a part of it," she says. "It's almost like watching a movie, and you're in the audience instead of participating." There

are times when she would promise her children that they could all go somewhere together, but then she would suddenly get sick. "In a matter of minutes, something I ate would become a hard ball in my stomach," she says. "I can't hardly move because I'm bent over [with pain]. And then, all of a sudden, I'm throwing up...and I have to break their hearts because I can't go where I said I was going to go." Those are the horrible moments, she says, adding that she has trained herself to live in the present, instead of exhaustingly trying to make up for lost time after the pain and nausea subside. "All I could do is live in the here and now," she says.

Ronetta's friends would desperately try to understand what she was going through, but she has learned that the symptoms can be very difficult to describe. Many people with little or no experience with gastroparesis tend to think, understandably, that the symptoms are similar to that of an upset stomach, like how someone feels after overindulging in a big or overly rich meal. But Ronetta says common indigestion is very mild

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compared to the unyielding nausea she constantly feels. She doesn't like invoking pity from others, so she uses a bit of humor when explaining how she lives with the turmoil of gastroparesis. "I stopped saying what it was," she says. "And I would make a joke out of it. You know how on Facebook, if someone's relationship is bad, they say 'It's complicated'? Well, that's kind of what my answer is to this. It's complicated."

Learning To Cope with Gastroparesis

Getting treatment at Wake Forest during the years following her diagnosis has helped Ronetta to begin managing her gastroparesis. She takes prescription anti-nausea medicines several times a day. "And with that, there's not a whole lot of vomiting," she says. "There's always the nausea, but I'm more functional."

While the medication has eased her symptoms, they never completely went away, and she has sudden, debilitating relapses—or "flares"—that could last several hours. "[The symptoms] would be lying in the background, but would become more prominent when I ate the wrong thing. Or sometimes I wouldn't know what the wrong thing was—I would have a flare that would render me just helpless.... I can't move, I can't eat anything. And that's how it can be still."

About 2 years after the onset of her gastroparesis, she underwent a procedure, called a pyloroplasty, in which the opening between the stomach and the small intestine is surgically widened to allow food to pass through more easily. But the result was somewhat disappointing—she said some symptoms got better, but others got worse. "I had unrealistic expectations," she says. "I think I had it in my mind that it was going to solve everything, thinking, 'This is it.... I'm going to go back to a normal life.'" But she still experiences major flares, sometimes out of the blue, and at other times

when she strays from the pescatarian diet she has adopted. If she eats something greasy, or even a meat that isn't lean enough, she gets sick. "Or sometimes it doesn't matter what I eat at all," she says. "It could just be one of those days.... And for whatever reason, I will be just doubled over in pain from my stomach."

In addition to watching which foods she eats, Ronetta has had to be careful about the size of her portions at each meal. "And that's one of the things that will mess me up—if I eat a larger portion than I should," she says. "And it may be because I'm simply enjoying what I eat." It has gotten to the point where, at times, she will avoid eating anything at all, because she will be worried that she will get sick. Not only does this sap her energy, but it also makes her social life difficult.

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"You don't want to go to dinner at other people's houses because you don't know how sensitive you're going to be

to the food," she says. Usually people will try to serve her food that she could eat, but they could innocently overlook something that could upset her stomach. "People don't mean to, but you never know what's inside their food and what stuff it's cooked in," she says. "We're in the South, so they'll say, 'I'll make green beans.' And then you look, and there's this big ham hock in the middle of it."

Ronetta says that eating out can be difficult, adding that people who don't know her well "can get really offended when I say I'm coming to something and then I don't show up, and they have no idea what's going on with my body." Not only is it hard for her to eat with people other than family and close friends who have a better understanding of her condition, but the unpredictability of her flares also makes it difficult to leave home in the first place. "I could be dressed and ready to go, and then all of sudden, I get sick, and I'm lying on the bed, and my husband is taking off my shoes," she says.

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With such debilitating episodes, Ronetta also needed to adjust her life as a professional counselor and business owner. “Gastroparesis taught me more about business,” she says. “I realized that too much about my business was in my head and not on paper,” which made it harder to train someone else how to do things. She also convinced herself to delegate more to others—something that she wasn’t used to doing. “I had a lot of pride, which was to my detriment,” she says. “I’ve always been so ambitious and such a go-getter, so it was really hard to hand the reins over and say, ‘I can’t do this anymore.’” She was also able to use some of her own counseling training—including anxiety-relieving techniques—to help her cope with the physical and mental pain. But she says the best technique she used was seeking counseling for herself. “I needed to take the time to process all the feelings that were going on,” she says, “and all the physical changes, the changes with my business, the changes with my marriage, the changes with my children, and with all of my social relationships.”

“I’ve always been so ambitious and such a go-getter, so it was really hard to hand the reins over and say, ‘I can’t do this anymore.’”

Hope in Research

Like most people with gastroparesis, Ronetta was diagnosed with an “idiopathic” form of the disorder, which means that the cause is unknown. This presents a major obstacle for treatment—if doctors don’t know what causes it, it’s extremely difficult to develop therapies to fix it. Current treatments, which aren’t always successful, include ways to coax the stomach to empty faster, such as medications that make the stomach muscles contract, or surgery or injections that help to open the valve between the stomach and small intestine. Other medications may focus on treating symptoms like nausea and abdominal pain.

There is still much to learn about gastroparesis, including why it seems to affect women more often than men. But progress toward understanding the disorder has been slow, partly because researchers have struggled to recruit enough participants to conduct effective clinical trials. Also, scientists at a single research center have a limited number of relevant clinical and research techniques available to them.

To try to overcome these obstacles, the NIDDK established the Gastroparesis Clinical Research Consortium to accelerate research on the causes and progression of gastroparesis and to explore new approaches to treat the disorder. The Consortium is made up of several clinical research centers across the country, including at Wake Forest where Ronetta receives treatment. This network of clinical centers allows researchers to share techniques and tools and to recruit a broad spectrum of patients from many regions to participate in large clinical studies.

After Ronetta was diagnosed with gastroparesis, she agreed to participate in the Gastroparesis Registry, one of the most important undertakings of the Consortium. Established in 2007, the initial goal of the Gastroparesis Registry was to enroll a sufficiently large number of patients to clarify the clinical features of the condition, which tend to be variable. Over several years, the Consortium gathered detailed test results and samples from hundreds of patients, assembling the largest clinical and physiologic data repository for gastroparesis in the world. The information collected in the Gastroparesis Registry is used to link symptoms, severity, and treatment responses to patient characteristics—an extremely important step toward understanding the disorder.

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Building upon its success, the Gastroparesis Registry was expanded in 2012 to recruit more participants, and researchers are now using its rich dataset to study the causes, progression, and outcomes of gastroparesis. Scientists can also access the Gastroparesis Registry when recruiting people for clinical trials, which could benefit registry participants who are eager to try new therapies. Ronetta's motivation for joining the Gastroparesis Registry was altruistic: "Anything I can do to help someone else," she says. "It helps bring meaning to all of this."

A Network of Support

Scientists are continuing to explore new therapies as they learn more about gastroparesis. In the meantime, Ronetta still experiences harsh and unpredictable flares, but the medications have helped to make them somewhat more tolerable and less frequent. She still wears the feeding tube embedded in her chest—she needs to be prepared to take in nourishing fluids, particularly in the warmer months when she is more prone to dehydration.

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She feels lucky to have a very close support network in her family. She says her daughter tries to keep an overly watchful eye on her: "She'll say, 'Mommy, are you supposed to be doing that? Mommy, is [your feeding tube] supposed to be connected? Mommy, I haven't seen you do your fluid.'" Ronetta's husband, whom she calls "amazing," took a second job when she was forced to cut down on the number of clients she sees. She tries to make sure he doesn't become overwhelmed himself, "because he literally has to bring home the bacon, fry it up in a pan, and feed it to the kids," she says.

Despite the severe disruptions to her personal and professional lives, Ronetta perseveres. "I fight to win," she says, citing her desire to be healthy so she can be involved in her children's lives. She has a keen

awareness of her inner strength ("I feel like I'm a ninja warrior," she says), and it gives her hope. "The days I

can't control, I can't control," she says. "But either way, if I'm vomiting, if I'm doubled over in pain, I tell my kids: 'It's OK, the doctors are going to make Mommy better. Keep praying.' And so I fight on."