



As described in this chapter, researchers have developed a groundbreaking new mouse model of celiac disease that, in the presence of dietary gluten, mimics the immune system features and gluten-dependent intestinal damage seen in people with this disease. Celiac disease is an autoimmune reaction in the small intestine that is triggered by consuming gluten, a protein found mainly in foods containing wheat, barley, and rye. Shown here are cross-sectional images of the small intestine in the new mouse model, which is the most accurate animal model for celiac disease to date. These mice have a healthy small intestine when fed a gluten-free diet (far left image), with the wall of the small intestine lined with fingerlike structures, called villi, that project into the intestinal space and help the gut absorb materials by increasing its internal surface area. However, the villi become damaged (middle image) when mice eat gluten for 30 days. This damage is reversed (far right image) when mice that were given gluten are put back on a gluten-free diet. This mouse model could be a useful research resource for gaining new insights into the underlying causes of celiac disease and for discovering and testing new therapies.

Images provided by Drs. Valérie Abadie and Bana Jabri, University of Chicago. Reprinted by permission from Springer Nature: [Nature](#); 11-15, gluten and HLA-DQ8 drive tissue destruction in coeliac disease; Abadie V, Kim SM, Lejeune T,...Jabri B; 578: 600-604. Copyright 2020.

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 2016 national data sources estimated that digestive disease is the primary diagnosis in a total of 66.4 million ambulatory care visits to physicians' offices and hospital emergency and outpatient departments in the United States each year.¹ Similarly, analyses with 2016 national inpatient samples identified 4.1 million hospitalizations with a primary diagnosis of digestive diseases and 15.9 million hospitalizations with a primary or secondary diagnosis of digestive diseases.² In addition, analyses focusing specifically on the clinical and economic burden of emergency department visits identified 19.2 million emergency department visits with a primary diagnosis of digestive diseases and costs totaling \$94.9 billion in 2016.³

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

Inflammatory bowel diseases (IBD), which include Crohn's disease and ulcerative colitis, are marked by damaging inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. These diseases often strike early in life, with a peak age of onset in adolescence or young adulthood. Treatment frequently requires prolonged use of multiple drugs and may require surgery, including removal of the affected region of the intestine. Scientists are investigating the complex interactions among the genetic, environmental, immune, microbial, and

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

¹ National Ambulatory Medical Care Survey (NAMCS) and National Hospital Ambulatory Medical Care Survey (NHAMCS), U.S. Centers for Disease Control and Prevention; available at: www.cdc.gov/nchs/ahcd/index.htm.

² Healthcare Cost and Utilization Project (H-CUP) National Inpatient Sample (NIS), Agency for Healthcare Research and Quality; available at: www.hcup-us.ahrq.gov/nisoverview.jsp.

³ Healthcare Cost and Utilization Project (H-CUP) Nationwide Emergency Department Sample (NEDS), Agency for Healthcare Research and Quality; available at: www.hcup-us.ahrq.gov/nedsoverview.jsp.

drugs. Stomach and intestinal disorders also include functional gastrointestinal disorders, which can cause 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 gastrointestinal disorder that can lead to a condition known as Barrett's esophagus. This condition, in which cells lining the esophagus turn into an intestinal type of cell, is associated with a heightened risk of esophageal cancer—one of the cancer types still on the rise in the United States. Gastroparesis, another type of functional gastrointestinal 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. Fecal incontinence, or impaired bowel control, is a 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. Scientists continue to strive for a deeper understanding of the causes of gastrointestinal disorders, which will lead to improvements in diagnosis and management for patients with these conditions.

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 ingestion of gluten—a protein 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, viruses, and other microorganisms 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. Disruptions in this microbial ecosystem are associated with diseases such as IBD or infections by the harmful bacterium *Clostridium difficile*. 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 some medicines. 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 nonalcoholic steatohepatitis (NASH). In recent years,

however, NAFLD has been increasingly diagnosed in children in the United States as well, concurrent with rising overweight and obesity. Some forms of liver disease are caused by viral infection, as in most cases of hepatitis, or by genetic mutations such as alpha-1 antitrypsin deficiency; others arise from diverse factors such as autoimmune reactions, drug toxicity, 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. Additionally, a unique web-based health information program on drug-induced liver disease called LiverTox, jointly sponsored by the NIDDK and the National Library of Medicine, provides concise, up-to-date information on more than a thousand medications and supplements and their potential to harm the liver (available at: www.ncbi.nlm.nih.gov/books/NBK547852).

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, behavioral, 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. Investigators also conduct basic, clinical, and translational research on the requirements, bioavailability, and metabolism of nutrients and other dietary components in order to understand dietary needs in health and disease. The NIDDK and its Office of Nutrition Research have played a leading role in the NIH Nutrition Research Task Force, chaired by the NIDDK Director and co-chaired by 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. The Task Force was established to coordinate and accelerate progress in nutrition research across the NIH, including the development of the first NIH-wide strategic plan for nutrition research (see feature later in this chapter).

GUT MICROBIOME AND NUTRITION

Studies Show Role of Human Gut Microbiome in Nutrient Absorption: Researchers in the NIDDK's intramural research program, partnering with scientists at academic institutions in the United States and Germany, found that changes in people's gut microbiomes, due to diet or antibiotic use, directly altered nutrient absorption, backing up results from animal models and indirect associations in prior human studies. Decades of research, mainly in animal models, have illustrated how influential gut microbes can be in supplementing individuals' metabolic machinery and determining how much nutrition is extracted from the diet. Human studies in this area have produced similar but indirect associations. For example, one study showed that underfeeding (ingesting less calories than required to maintain current weight) remodeled the microbiome and reduced nutrient absorption, but it was unclear if the effects on nutrient absorption were directly caused by changes in the microbiome.

Scientists wished to test whether there was a causal relationship between human gut microbes and nutrient absorption in a controlled feeding

study in which the participants' microbiomes were altered by diet or antibiotics. This study design provided accurate assessment of dietary intake, with adult men and women, some of whom had obesity or impaired glucose tolerance, staying at the NIH Clinical Research Unit at the Phoenix Indian Medical Center in Arizona throughout the study period of 31 days. During this time, they were given prepared meals, which were closely monitored to ensure 95 percent of foods were consumed, and had samples taken. For the first phase of the study, all participants were overfed and underfed the same foods for 3 days each, in random order, with a weight-maintaining diet provided in between. In the second phase, they were fed a weight-maintaining diet and given either the oral antibiotic vancomycin or a placebo pill. The scientists measured nutrient absorption by monitoring the calories passed in stool and urine samples. They also tracked the activity and composition of the gut microbiome by measuring plasma biomarkers of host and microbial metabolism and the number and types of gut bacteria present in the stool. Both underfeeding and antibiotics reduced nutrient absorption (*i.e.*, more nutrients were lost in the stool). In the case of underfeeding, the scientists attributed this to lower nutrient availability for gut microbial metabolism. These short-term dietary changes altered the composition of gut microbes slightly, and antibiotic treatment changed it more dramatically, with a loss of diversity in gut microbial species, which may have hampered the metabolic capacity of the remaining gut microbes. In fact, a marker for microbial metabolism was lower in the people who were underfed or treated with the antibiotic, pointing to reduced nutrient metabolism by the microbiome under these conditions.

This study offers high-quality evidence for a direct role of gut microbes in the amount of nutrients a given person can extract from their food, including the impact of environmental factors such as diet and antibiotic use. These findings also bolster the validity of animal models in which similar results were found. Additional, larger human studies are needed to determine the potential therapeutic implications of this research.

Basolo A, Hohenadel M, Ang QY,...Krakoff J. Effects of underfeeding and oral vancomycin on gut microbiome and nutrient absorption in humans. Nat Med 26: 589-598, 2020.

TOWARD PREDICTING EFFECTIVE TREATMENTS FOR INFLAMMATORY BOWEL DISEASE

A Cellular Signature Reveals Why Current Crohn's Disease Therapy Does Not Work for Some People:

Scientists have identified a tell-tale combination of cells in people with Crohn's disease who do not respond to one of its most effective treatments, shedding light on the nature of the disease and revealing potential new targets for therapy. Crohn's disease, a form of inflammatory bowel disease characterized by chronic inflammation in parts of the small intestine, can cause debilitating flares of diarrhea and abdominal pain, leading to malnutrition and weight loss. Treating Crohn's disease is challenging because the response to therapy varies greatly from person to person, and no single treatment works for everyone. Well-tolerated anti-inflammatory medications, for example, do not work in many people with Crohn's disease. Stronger medications that block a major component of the inflammatory response called tumor necrosis factor (TNF) have been approved to treat Crohn's disease and are effective for many whose health did not improve with the milder anti-inflammatory drugs. However, for unclear reasons, a substantial portion of people do not respond to TNF-blocking drugs and must eventually resort to more drastic approaches to control the inflammation, which usually means surgically removing the affected areas.

In a recent study, scientists applied state-of-the-art technology to determine why some people do not benefit from certain Crohn's disease treatments. They started by isolating and identifying single cells from intestinal lesions of 11 men and women with Crohn's disease. Comparing these cells with cells from healthy intestinal tissue from the same patients, the researchers found a specific combination of cells—including activated immune cells and cells that make up connective tissue—in the lesions from several of the study participants. When the researchers looked for this cellular "signature" in a larger, well-characterized group of male and female children with Crohn's disease, they found it was more likely present in those whose symptoms did not respond to anti-TNF therapy. Analysis of how the cells communicate with each other showed that this particular cellular combination does not rely solely upon TNF to maintain gut inflammation, which may explain why TNF-blocking drugs are ineffective.

Importantly, this analysis also identified several other molecular targets for other potential therapies that, if used in combination with TNF-blocking drugs, might be able to prevent inflammation in people with Crohn's disease who do not respond to anti-TNF medications alone.

This study builds upon recent findings showing that there are probably at least several distinct types of Crohn's disease, which could explain why treatment responses vary so widely from person to person. The results also show that the cellular makeup of the inflammatory lesions could affect how well different Crohn's disease medications will work. Biological signatures such as this could help health care providers predict which therapies would be most effective, and they could provide the basis for new treatments for people with this disease.

Martin JC, Chang C, Boschetti G,...Kenigsberg E. Single-cell analysis of Crohn's disease lesions identifies a pathogenic cellular module associated with resistance to anti-TNF therapy. *Cell* 178: 1493-1508.e20, 2019.

A Common Fungus Sets the Stage for Successful Fecal Microbiota Transplantation in People with Ulcerative Colitis: A recent study found that high levels of a common fungus in the gut could signal whether a microbe-based treatment would be successful for people with ulcerative colitis. Changes or disruptions in the gut's microbiome—the community of bacteria, viruses, and fungi that naturally inhabit the intestines—have been implicated in inflammatory bowel diseases like ulcerative colitis. One treatment that researchers are investigating is fecal microbiota transplantation (FMT), whereby a sample containing gut microbes from a healthy donor is introduced into a person with colitis to help reestablish a more functional gut microbiome. While FMT has proven to be a successful therapy for people with *Clostridioides difficile* (*C. diff*) bacterial infections, with over 90 percent of people cured after a single treatment, FMT is less successful for people with ulcerative colitis: in a recent clinical trial, less than half the participants with colitis experienced remission after FMT. Knowing why the treatment works only in some people is important, because it would allow health care providers to predict which individuals with ulcerative colitis would benefit from FMT as opposed to another treatment strategy. One possibility is that FMT's success may be dependent upon the makeup of the recipient's microbiome. For example,

FMT is less likely to succeed as a *C. diff* treatment for people who have high levels of *Candida*, a type of fungus found in the guts of nearly everyone. *Candida* is an opportunistic pathogen that can exacerbate inflammation when the immune system is weakened or the microbiome is disrupted. Moreover, high levels of *Candida* could determine FMT outcomes by affecting the levels of other microbial members of the gut. Thus, like in people with *C. diff*, high levels of *Candida* may also play an important role in determining the outcomes of FMT in people with ulcerative colitis.

To determine whether gut microbes such as *Candida* may be affecting FMT for people with ulcerative colitis, researchers studied the microbiomes of 24 men and women who had received FMT as a trial treatment for the disease. Unlike in people with *C. diff* infections, the study participants who had higher levels of *Candida* before FMT were more likely to have improved colitis symptoms and clinical features following treatment. After FMT, levels of *Candida*—and the immune response against *Candida*—were lower in these people compared to those who received a placebo. This raises the possibility that introducing gut microbes from healthy donors suppresses the overgrowth of *Candida*—and the inflammation caused by it—in people with ulcerative colitis. The researchers also found that study participants who had higher pre-FMT levels of *Candida* were more likely to have higher pre-FMT levels of certain gut bacteria that have been linked to successful FMT outcomes for ulcerative colitis. This suggests that a high level of *Candida* may create a permissive environment for FMT in people with ulcerative colitis by encouraging the growth of specific gut bacteria in the microbiome. Overall, the results of this study hint of an intricate relationship between *Candida* and other members of the microbiome, whereby high levels of *Candida* in people with ulcerative colitis make the microbiome more receptive to FMT. In turn, FMT results in reduced levels of *Candida* and the inflammation associated with it. In this manner, *Candida* levels could be a promising marker to predict whether FMT may be effective for people with ulcerative colitis.

Leonardi I, Paramsothy S, Doron I,...Iliev ID. Fungal trans-kingdom dynamics linked to responsiveness to fecal microbiota transplantation (FMT) therapy in ulcerative colitis. *Cell Host Microbe* 27: 823-829. e3, 2020.

NEW MOUSE MODEL OF CELIAC DISEASE

New Mouse Model Mimics Celiac Disease in People:

Researchers have developed a new mouse model that mimics the immune system features and gluten-dependent intestinal damage seen in people with celiac disease, providing a new research tool for discovering and testing therapies. Celiac disease is an autoimmune reaction in the gut that is triggered by consuming gluten, a protein found mainly in foods containing wheat, barley, and rye. This autoimmune reaction—where the body targets its own cells—damages the small intestine, interfering with the intestine's ability to absorb nutrients from foods. This can result in chronic diarrhea, bloating, anemia, and, in children, slower growth and short stature. The only treatment for celiac disease is a strict gluten-free diet, which is difficult for many people. Scientists are trying to identify non-dietary treatments for celiac disease, but research has been hampered by the lack of animal models that accurately mimic the human form of the disease.

In new research, scientists used their understanding of human celiac disease to genetically engineer a new model of the disease in female and male mice. In people, the presence of certain genetic variants in the human leukocyte antigen (HLA) region of the genome—which is responsible for regulating the immune system—increases the likelihood of developing celiac disease. Additionally, people with the disease have high levels of an immune system-produced protein called interleukin-15 (IL-15) in their small intestines. The researchers engineered a mouse model with similar characteristics. Just like in people, the mice developed damage to their small intestines after eating gluten for 30 days, and the damage was reversed when the animals were no longer fed gluten. The animals' immune systems also produced some of the same types of antibodies that are commonly used to diagnose celiac disease in people, and the mice had similar gene activity in the presence and absence of gluten as observed in people with the disease, suggesting that the mouse model could be useful for testing therapies before they are tried in humans. Further experiments suggested that the model could be useful for drug discovery. For example, the model gave insights into the critical role for IL-15 in the disease. The researchers also found that they could prevent small intestine damage by treating gluten-fed mice with a drug that inhibits a protein known to be involved in the underlying cause of celiac disease. Thus, this new

animal model that replicates features of human celiac disease upon introduction of gluten could be a useful research tool not only for increasing understanding of the underlying biology of celiac disease, but also for identifying new therapeutic targets and testing novel prevention and treatment strategies before they are tested in people.

Abadie V, Kim SM, Lejeune T,...Jabri B. IL-15, gluten and HLA-DQ8 drive tissue destruction in coeliac disease. Nature 578: 600-604, 2020.

CLINICAL RESEARCH ON BILIARY ATRESIA

Testing a New Screening Approach for Early Diagnosis of Biliary Atresia:

Researchers have found that a two-step newborn screening approach measuring bilirubin levels could identify those with biliary atresia—progress toward a goal of more successfully treating babies earlier in the course of disease. Biliary atresia is a rare and serious progressive liver disease diagnosed in infancy that leads to a back-up of bile into the liver. The disease must be treated with a surgery to restore bile drainage called hepatoportoenterostomy or HPE (also referred to as the Kasai procedure) or a liver transplant. Children who undergo HPE before 30 days of age have the best chance of delaying or preventing the need for a liver transplant, but HPE often occurs after that window because biliary atresia is difficult to detect in its early stages. Thus, an important research goal is to identify ways to diagnose biliary atresia soon after birth to facilitate early treatment and improve health outcomes.

In new research, scientists used knowledge that infants with biliary atresia have elevated levels of bilirubin (a major component of bile) at birth to design and test a 2-stage screening strategy in over 124,000 newborn girls and boys. In the first stage, they analyzed data on babies' bilirubin levels in blood measured shortly after birth, which was collected as part of routine newborn tests at the 14 south Texas hospitals where the research was conducted. If the newborns had high bilirubin levels in stage 1, they underwent repeat bilirubin testing at or before their routine 2-week well-child doctor's visit. Infants who tested positive in stage 2 were referred for additional tests to see if they had biliary atresia. The results showed that the screening approach successfully identified all seven children who were

subsequently diagnosed with biliary atresia in the study population. However, it also identified another 112 children who did not have the disease—so-called false positives. Tests on those children showed that a few of them had other conditions that benefitted from early detection and treatment, but about half were not diagnosed with any disease or condition. The researchers also conducted a second, smaller study of 43 infants with biliary atresia to compare clinical outcomes of babies who underwent HPE either before or after the screening strategy was implemented. They found that children in the screening group underwent HPE at a younger age, suggesting that the screening approach may facilitate earlier treatment.

This research demonstrates promising progress toward newborn screening for biliary atresia, but the scientists noted several limitations that would have to be overcome before such a screening approach could be recommended on a national scale. For example, screening only occurred at South Texas hospitals, potentially limiting the applicability of the study's results to other populations. Also, the outcomes study included a relatively small number of children, so it is unknown if similar results would be observed in a larger study. Additional research could help to address these limitations, as well as to study the cost-effectiveness of implementing a population-wide screening approach, as has been done in other countries, and identify ways to reduce the number of false positives. Such research could accelerate further progress toward longer-term goals of early diagnosis and treatment of biliary atresia to improve the health of children.

Harpavat S, Garcia-Prats JA, Anaya C, ...Shneider BL. Diagnostic yield of newborn screening for biliary atresia using direct or conjugated bilirubin measurements. JAMA 323: 1141-1150, 2020.

Gene Activity Signature Predicts Survival in Young Children with Biliary Atresia: Researchers identified a unique signature of gene activity levels at diagnosis that predicts survival in young children with biliary atresia—knowledge that could help determine disease progression and inform new treatment approaches. Biliary atresia is a rare and serious liver disease that occurs during the first few months of life in which bile ducts that drain from the liver to deliver bile acids to the intestine become inflamed and scarred, leading to a back-up of bile into the liver. To restore bile drainage, children with this

disease must be treated with a surgery called hepatoportoenterostomy or HPE (also referred to as the Kasai procedure), but even after this surgery, the liver can develop progressive damage requiring a liver transplant. Because the clinical course of biliary atresia is variable, it would be extremely valuable to have biological markers that predict disease progression, to inform the development of new and personalized treatment approaches.

Toward this goal, researchers studied liver biopsy tissue from girls and boys with biliary atresia, including those enrolled in the NIDDK's Childhood Liver Disease Research Network (ChiLDRen). By comparing the gene activity patterns of children who survived until age 2 after the HPE surgery without the need for a liver transplant to those who did not, they identified a set of 14 genes with activity levels at diagnosis that predicted transplant-free survival at 2 years of age. Further experiments suggested that the 14-gene activity pattern coupled with total bilirubin level (a major component of bile) measured 3 months after surgery was an even better predictor of 2-year survival without a liver transplant than the gene activity signature alone. Researchers next explored differences in overall gene activity in the children and how those differences may contribute to disease progression, finding increased activity of genes associated with liver injury and scarring in the children with lower transplant-free survival. They also found increased activity of genes involved in metabolism of glutathione (an antioxidant produced in the liver) in the children who survived without a transplant, suggesting that restoration of cellular glutathione may help protect against biliary atresia-associated liver damage. Investigating this concept further, studies of mouse models of biliary atresia showed reduced bile duct obstruction and improved survival when the animals were treated with N-acetylcysteine (NAC), a drug that restores cellular glutathione and was shown to treat acute liver failure in children and adults by other NIDDK-sponsored studies.

This study suggests that the newly identified 14-gene activity signature could be a useful biological marker to predict how biliary atresia will progress in young children after surgery. It also shows that restoring cellular glutathione may be a possible approach to decrease progressive liver damage caused by the disease. Further research could help determine if using NAC or another

approach to increase cellular glutathione in children with biliary atresia could halt liver damage and improve overall health.

Luo Z, Shivakumar P, Mourya R, Gutta S, and Bezerra JA. *Gene expression signatures associated with survival times of pediatric patients with biliary atresia identify potential therapeutic agents. Gastroenterology* 157: 1138-1152.e14, 2019.

DIETARY CONTRIBUTORS TO NONALCOHOLIC FATTY LIVER DISEASE

How High Fructose Intake May Trigger

Fatty Liver Disease: A team of researchers supported by the NIDDK, as well as other NIH Institutes, discovered that consuming high amounts of fructose may promote nonalcoholic fatty liver disease by damaging the intestinal barrier, which leads to inflammation and effects on the liver. Fructose is a common type of sugar in the American diet, including its processed form called high-fructose corn syrup that is used to sweeten a variety of foods. Studies have linked excessive consumption of high-fructose corn syrup and other added sugars to health problems like obesity, diabetes, heart disease, and nonalcoholic fatty liver disease (NAFLD), in which too much fat is stored in liver cells. Fatty liver disease can lead to liver inflammation and liver damage, resulting in a more aggressive disease called nonalcoholic steatohepatitis (NASH) that can progress to scarring of the liver (cirrhosis), liver cancer, and liver failure. Scientists have been unsure how high fructose consumption might contribute to NAFLD.

A research team set out to explore fructose's role in NAFLD. The researchers gave male mice either a high-fructose diet or a control diet with equivalent calories from corn starch, which breaks down into glucose, the sugar cells use for energy. Within a few months, mice on the high-fructose diet developed fatty livers and had greater rates of liver tumors than mice on the control diet. Mice bred to be prone to develop NASH showed clinical signs of the disease on the high-fructose diet. The team found that mice fed the high-fructose diet for long periods showed not only liver inflammation, but also deterioration of their intestinal barrier, which normally prevents bacteria and toxins in the gut from leaking into the bloodstream. Mice fed a high-fructose diet also had higher circulating levels of endotoxins—toxins

released from certain bacteria when they die. The team discovered that leaked endotoxins prompted immune cells in the liver called macrophages to react and increase the production of cell signaling proteins like tumor necrosis factor (TNF) that can cause inflammation. Further experiments showed that these signaling proteins boosted enzymes that convert fructose into fatty deposits in the liver. Restoring the mice's intestinal barrier prevented this fatty buildup in the liver. Using drugs and genetic manipulations, the team was able to stop the gut barrier deterioration from excessive fructose intake and prevent the onset of severe fatty liver disease and liver tumors. Experiments in human liver cells showed that a similar cellular process could take place in people: adding TNF to the human liver cells increased the conversion of fructose into fat.

Overall, this study points to a pathway in which high fructose levels could trigger a breakdown in the intestinal barrier and leakage of gut microbial products into the liver, thereby exacerbating inflammation and boosting the conversion of fructose into fatty deposits. The findings from this study could lead to new ways to treat and prevent NAFLD, which affects an increasingly large percentage of the U.S. population. For example, future studies could test agents that restore the integrity of the intestinal barrier in people at risk for NAFLD.

Todoric J, Di Caro G, Reibe S,...Karin M. *Fructose stimulated de novo lipogenesis is promoted by inflammation. Nat Metab* 2: 1034-1045, 2020.

(Information adapted from original article by Ms. Erin Bryant, published on September 15, 2020, in *NIH Research Matters*.)

NEW INSIGHTS INTO BASIC LIVER CELL BIOLOGY

Insights into How Immune Cell Recruits Are Programmed To Defend the Liver: Researchers working with a mouse model have gained insights into the genetic and cellular factors that drive the transformation of circulating immune cells into Kupffer cells, part of the liver's "special forces" of immune cells. This research focuses on a type of immune cell called the macrophage—derived from the Latin for "large" and "thing that devours"—that protects the body by engulfing pathogens and

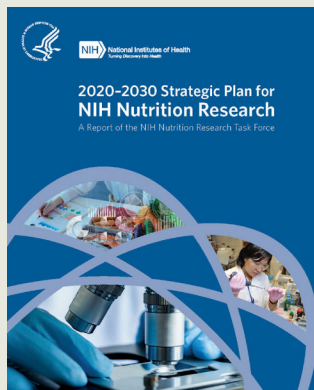
cellular debris. The Kupffer cell is a special type of macrophage residing in blood vessels within the liver. During early life, Kupffer cells develop in the liver, where they perform important functions lifelong, including clearing cell debris and toxins produced by gut bacteria, as well as playing a role in iron metabolism. Kupffer cells also play critical roles in some liver diseases and therefore could be targets for therapy, but little is known about the factors involved in the formation and maintenance of these unique liver defense cells.

Researchers aimed to address this knowledge gap by using a mouse model in which these cells can be experimentally depleted. They studied both female and male mice over a 2-week period, analyzing cells from blood and liver samples using techniques that show gene activation. In the first 12 hours after the mouse Kupffer cells were depleted, circulating monocytes (white blood cells in the immune system that can turn into more specialized immune cells as needed) had already started to colonize the liver. Within 24 hours of residing in the liver environment, the monocytes showed signs of a transformation, including activation of over half of the roughly 300 genes unique to Kupffer cells. A combination of

sequential signals emanating from surrounding liver cells, including those lining blood vessels (called liver sinusoidal endothelial cells) and underlying liver cells called hepatocytes, were important for transforming the monocytes and maintaining their new identities as Kupffer-like cells. In the first step of the process, monocytes migrating to the liver interacted with sinusoidal cells that produced a protein called DLL4, setting off a cascade of gene activation needed to transform the cells. The second step involved signals such as transforming growth factor-beta and desmosterol released by the surrounding liver cells that further fine-tuned the gene activation program to resemble a Kupffer-like cell. These findings offer insights into the developmental program triggered in circulating immune cells—and within cells in the local tissue environment that sense a need to replace lost defenses—to form the Kupffer cells that protect the liver. Future directions for this research include plans to explore how Kupffer cells function in a disease context, such as a form of severe fatty liver disease called nonalcoholic steatohepatitis.

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Strategic Plan for NIH Nutrition Research



In May 2020, the NIH released its first agency-wide strategic plan for nutrition research. The *2020-2030 Strategic Plan for NIH Nutrition Research* presents a bold vision for advancing NIH-supported nutrition research over the next decade to

answer fundamental questions, such as “what should we eat to stay healthy?” The *Strategic Plan* provides a framework to answer this and other important questions in nutrition research, to advance the field of nutrition science as a whole, and ultimately to promote health and reduce the burden of diet-related disease.

The *Strategic Plan* is structured around a core vision of advancing “precision nutrition” research to address the impacts of diet and nutrition on individuals, with the goal of moving research closer to enabling personalized dietary recommendations for what, when, why, and how to eat. Through its strategic goals and associated objectives, the *Strategic Plan* identifies current research challenges and opportunities to advance nutrition science. The *Strategic Plan* also identifies cross-cutting research areas that underpin successful future activities across the field of nutrition research: addressing minority health and health disparities; the health of women; rigor and reproducibility; data science, systems science, and artificial intelligence; and training the scientific workforce.

The *Strategic Plan* was developed by the NIH Nutrition Research Task Force with broad stakeholder input, described below. The Task Force was established by the NIH Director, Dr. Francis Collins, and chaired by

Dr. Griffin 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 Bianchi, Director of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development. The NIDDK Office of Nutrition Research provided support for the strategic planning process, with the Office’s Director, Dr. Christopher Lynch, serving as Executive Secretary of the Task Force. The Task Force was assisted in this effort by other NIH staff who represented the NIH’s many Institutes, Centers, and Offices that support nutrition research.

The Task Force sought extensive stakeholder input throughout the development of the *Strategic Plan*. Activities included a “crowdsourcing” website to solicit ideas from researchers and members of the public; a Thought Leaders Panel of federal and external nutrition experts who provided their input on research opportunities; and online posting of the draft research plan for public comment. Throughout the planning process, the Task Force convened regularly to discuss research priorities and progress.

Implementation of the *Strategic Plan* is already under way, being led by a number of Implementation Working Groups with NIDDK staff participation. For example, plans for a large study supported by the NIH Common Fund will answer key questions about precision nutrition by studying participants in the NIH *All of Us* cohort. The NIH invited public input on this study through a Request for Information notice in 2020 and a workshop on precision nutrition research in January 2021. In the years ahead, the NIH will implement the recommendations made in the *Strategic Plan* to advance nutrition research, while remaining responsive to emerging opportunities and the ever-changing scientific landscape.

For additional information on the *2020-2030 Strategic Plan for NIH Nutrition Research*, please visit: www.niddk.nih.gov/about-niddk/strategic-plans-reports/strategic-plan-nih-nutrition-research.

Consortium Advancing Childhood Pancreatitis Research

A collection of recent advances enhances understanding of pancreatitis development and management in children—the latest in a long line of important contributions to the field over the past decade made by participants and investigators in the International Study Group of Pediatric Pancreatitis: In Search for a Cure (INSPPIRE) study.

Pancreatitis, an inflammation of the pancreas, has been more commonly diagnosed in children in recent years. Yet, pediatric pancreatitis is still frequently missed on initial evaluation, often delaying diagnosis. It can occur in acute (short-term), acute recurrent (two or more acute episodes), or chronic (long-lasting) forms. While all three forms could lead to complications, acute recurrent and chronic pancreatitis, in particular, can increase risk for diabetes and pancreatic cancer. Since 2010, the NIDDK has supported the INSPPIRE study—the first and largest multi-center group dedicated to studying acute recurrent and chronic forms of pancreatitis in children. This study grew out of a collective effort by an international group of investigators at sites throughout the United States, Canada, Israel, and Australia. This multi-center approach powers research by gathering a sufficient number of participants for studying the relatively rare disease of pediatric pancreatitis. Currently in its second iteration as INSPPIRE 2, it is now part of the Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer co-sponsored by the NIDDK and the National Cancer Institute, and features an even larger and more diverse study population of children and adolescents with pancreatitis.

In a recent report from the INSPPIRE study, researchers analyzed data from 479 children across INSPPIRE study sites and identified regional differences in the clinical presentation or management of pediatric pancreatitis that could affect disease outcomes. For example, participants at U.S. versus non-U.S. sites more frequently had the chronic form of pancreatitis, and therapies were more frequently utilized. Within the United States, the greatest disease

burden—based on measures of pain, emergency room (ER) visits/hospitalizations, and missed school days—was observed in western and midwestern study sites; also variants in the gene *PRSS1*, which are risk factors for pancreatitis, were more common at midwestern study sites, and gallstones were more frequently found at southern study sites. These regional differences may be due to the distribution of genetic risk factors and patterns created by different clinical practices and referrals. These study findings can inform a more streamlined and personalized approach to improve outcomes in children with pancreatitis.

Another study of INSPPIRE participants aimed to find out whether diabetes, a disease that commonly develops in adults with pancreatitis, similarly affects children with pancreatitis. Following 397 children with pancreatitis over 5 years, they found that 24 (or about 6 percent) were diagnosed with diabetes—a 30-fold higher rate than in the general population of children. The children diagnosed with diabetes tended to be older when they first developed pancreatitis and were more likely to have elevated blood lipids or autoimmune disease, which are known risk factors for diabetes. They also had signs of atrophy in the pancreas, indicating more advanced disease. These results may be helpful in the future for monitoring children with pancreatitis, in order to identify individuals at risk for developing diabetes.

In addition to their original research, investigators have conducted reviews of the medical literature on pediatric pancreatitis, much of it generated by the INSPPIRE study, to provide up-to-date information for physicians caring for these children. One analysis, conducted by INSPPIRE investigators and using diagnostic criteria developed in the INSPPIRE study, reviewed available medical literature to assess the extent to which a rare cause of pancreatitis in adults, called functional pancreatic sphincter dysfunction (FPSD), can contribute to pediatric pancreatitis. FPSD is one form of functional sphincter dysfunction, a condition of reduced flow of bile or pancreatic fluid from the liver and pancreas through a muscular valve

and into the small intestine, causing backup of these digestive fluids and associated abdominal pain and pancreatitis. Surveying the literature, including both adult and pediatric studies, the group developed a consensus on expert recommendations, based on available evidence, for approaches to diagnosis and treatment in cases in which FPSD is suspected as the cause of pediatric pancreatitis. For example, they generated recommendations for when to consider the use of invasive diagnostic and surgical treatments. These include endoscopic retrograde cholangiopancreatography—an invasive diagnostic procedure used to visualize the pancreatic and bile ducts with potential risks as well as benefits—and an associated surgical therapy called endoscopic sphincterotomy. While many gaps in knowledge remain and clinical care options are limited, these recommendations specific to children with these conditions give their health care providers more evidence-based tools to optimize care.

Turning to available treatments for pediatric pancreatitis, opioids are often used to control the severe abdominal pain often associated with the disease. But their use comes with concerns about addiction, particularly in light of the opioid overdose epidemic in the United States and elsewhere. INSPPIRE investigators surveyed 427 children/parent participants and their physicians about patient patterns of opioid use for pain management. Nearly one in five children with pancreatitis in the study reported daily or weekly opioid use, based on self-reports. Factors associated with more frequent opioid use included an older age at the time of diagnosis, *PRSS1* genetic risk factor, insufficient production of pancreatic enzymes that help digest food, antidepressant use, and constant pain that impaired daily functioning. Rates of frequent opioid use were also higher in participants living in the western and midwestern regions of the United States. These findings can help identify children with pancreatitis who may be at risk of frequent opioid use for pain management, so that other medical or psychological treatments can be considered early on.

One potential therapeutic alternative is the first psychological intervention to be tested for management of pancreatitis-related pain. In this pioneering clinical trial, researchers at INSPPIRE study sites will test an intervention based on cognitive behavioral therapy (CBT) to reduce pancreatitis-related pain. Investigators and staff

aim to enroll 260 young people ages 10 to 18 with pancreatitis in the trial of this web-based intervention, compared to a web-based educational program. The web-based CBT intervention includes relaxation and pain coping strategies for the youths, as well as behavioral and communication techniques for their parents, with weekly assignments for both to practice their new cognitive and behavioral skills. The researchers will test whether the intervention is effective over 2 to 3 months for reducing abdominal pain and improving health-related quality of life, as well as whether its effects are retained 6 months after the intervention. If this web-based psychological intervention is found to be effective, it would represent an economical, convenient, and potentially wide-reaching option for helping to manage pancreatitis-related pain.

The INSPPIRE study will continue to pursue these promising new directions toward better understanding, diagnosing, and optimally managing pancreatitis in children and adolescents. This study is leading the way in filling long-standing gaps in knowledge about how this disease manifests in children, and how best to diagnose and treat them for achieving the highest quality of life in the many years they have ahead.

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Recent Advances from the Gastroparesis Clinical Research Consortium: Toward Better Understanding, Diagnosis, and Treatment

After food is swallowed, muscles in the stomach wall grind it into smaller pieces and push it into the small intestine to continue digestion. In people with gastroparesis, however, these muscles work poorly—or not at all—and the stomach takes too long to empty its contents. This slower movement is called “delayed gastric emptying,” and it can cause chronic nausea, vomiting, and abdominal pain, often leading to malnutrition, dehydration, and other serious complications. People with gastroparesis typically need to adhere to strict, low-portion diets that are low in fat and fiber, and at times they may need to avoid solid foods altogether.

Developing treatments for gastroparesis has been challenging, largely because the underlying causes are unclear. For unknown reasons, women are significantly more likely to develop the disorder than men. Diabetes, surgery, and other conditions are known to cause gastroparesis in some people, but idiopathic gastroparesis—in which the cause is not identified—is more common. In 2006, the NIDDK established the Gastroparesis Clinical Research Consortium to accelerate research on the causes and progression of this disorder and to explore new approaches for treatment. The Consortium is made up of several clinical research centers across the country, allowing researchers to share techniques and tools and, importantly, to assemble the Consortium’s most valuable resource: a broad spectrum of hundreds of people with gastroparesis who volunteered to be a part of the Gastroparesis Registry. The Registry is the largest clinical and physiologic data repository for gastroparesis in the world, containing a large body of information accessible to qualified researchers, such

as detailed test results, samples, and questionnaires. Women make up the majority of participants, reflecting the higher incidence of gastroparesis in this group. The information collected in the Registry is used by researchers to link symptoms, severity, and treatment responses to patient characteristics—a critical step toward understanding the causes, progression, and outcomes of the disorder. Consortium scientists can also access the Registry when recruiting people for clinical trials, which could benefit Registry participants who are eager to try new therapies. Over the years, the Consortium has undertaken several such trials to test treatments for gastroparesis. This Gastroparesis Registry includes only adult participants, but the Consortium recently built upon its success to establish the first national pediatric registry (the Pediatric Gastroparesis Registry, or PGpR) for children and adolescents with gastroparesis. Enrollments for both registries are ongoing.

Recent studies from the Consortium have leveraged information from the adult Registry to help improve diagnosis of gastroparesis, which can be challenging because many of its symptoms are not specific to the disorder and could be mistakenly attributed to something else, like indigestion. Consortium researchers are trying to help with diagnosis by identifying groups of people who are at higher risk. For example, the Consortium recently studied people in the Registry who have both diabetes and symptoms of gastroparesis, and they found that those with delayed gastric emptying had a higher number of diabetic complications than those who showed normal gastric emptying. Retinopathy

(damage to blood vessels in the eye) was particularly associated with delayed gastric emptying, which suggests that people with diabetic retinopathy are also more likely to have gastroparesis. However, gastroparesis can occur in people who do not have complications of diabetes.

Consortium researchers are also using information from the Registry to investigate the underlying causes of gastroparesis, including why the stomach is not working properly. One possibility is that there could be defects in the activity of the nerves that envelope the stomach and control its muscles. Specifically, the researchers wanted to know whether the sympathetic and parasympathetic parts of the nervous system, which both control involuntary actions such as heart rate and stomach muscle activity, could be important in gastroparesis. While both sympathetic and parasympathetic systems are always active at some level, they exist in balance: the sympathetic component is more active during periods of stress, slowing the stomach's activity, while the parasympathetic system counters the sympathetic system and is more active during rest. The researchers found that this delicate balance is disrupted in many people with gastroparesis, with the parasympathetic system less able to offset the effects of the sympathetic responses, even when the body is at rest. Studies such as this, which identify the key mechanisms underlying gastroparesis, could identify potential new avenues for treatment.

Other recent studies from the Consortium focused on symptom management. For example, one study found that approximately 12 percent of people with gastroparesis participating in the Registry acknowledged that they used cannabis for symptom relief. Registry participants who were experiencing severe nausea and abdominal pain were more likely to use cannabis and likely to report that it helps ease their symptoms. Research on cannabis for various health conditions is in its early stages, but these results should raise health care providers' awareness that people with gastroparesis may be using cannabis to manage their symptoms, and that U.S. Food and Drug Administration (FDA)-approved synthetic products related to cannabis might be effective therapeutic agents. In fact, a portion of Registry participants were using at least one of these approved products, and they too were likely to report that it helps them manage their symptoms.

The Consortium also sought to determine whether a procedure called gastric electrical stimulation (GES) is effective for treating symptoms of gastroparesis. GES involves implanting an electronic device in the abdomen to deliver mild electrical impulses to the stomach's nerves and muscles. The device has been controversial because evidence on its effectiveness has been slow to accumulate, primarily because the procedure is expensive and requires additional visits and maintenance, making clinical trials difficult. Again using information from the Registry, researchers gathered information on people who underwent GES and determined that the procedure was effective for treating nausea in the participants with more severe gastroparesis. However, larger clinical trials would be necessary to test its efficacy more fully and to identify which people with gastroparesis would most likely benefit from this procedure.

These studies are only a recent sampling of research endeavors from the Consortium. Future years will see the Consortium continuing to undertake clinical studies to further understand, diagnose, and treat gastroparesis. The disorder can be devastating, but this research gives strong hope that the lives of people coping with gastroparesis will continue to improve.

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STORY OF DISCOVERY

Porphyrias Research

Decades of scientific research discoveries have advanced understanding of and care for a group of potentially severe and debilitating disorders called porphyrias. These advances include the identification of genetic factors underlying many of its forms, development of animal models for exploring these genetic factors and other contributors, and continuing findings from collaborative, large-scale clinical research on these rare disorders. In recent years, this research progress has led to new treatments, meaning that some forms of these disorders—recognized since ancient times—are now treatable. One major driver of this progress has been the NIDDK and its support of research on liver and hematologic (blood) diseases, particularly the Porphyrias Consortium, as well as many additional research studies conducted by investigators across the United States.

A RARE AND VARIED SET OF DISORDERS

Derived from the Greek word for purple, porphyrias were so named for the unusual reddish-purple color of urine when exposed to sunlight in samples from people with this condition. Some have speculated that major historical figures such as King George III may have had a form of this disorder. Porphyrias result from an overabundance of heme precursors originating in the liver or bone marrow, where red blood cells are produced. Heme is the red, iron-rich component of the hemoglobin proteins used by red blood cells to deliver oxygen to cells throughout the body, and heme is also found in liver proteins called cytochromes that break down hormones, medications, and other chemicals. The heme precursors, called porphyrins, are essential for heme production, but they can also be toxic to tissue in high concentrations.

Porphyrias are typically inherited, due to a genetic variant in any one of several enzymes within the

multi-step pathway that transforms biochemicals into heme. The altered enzymatic function causes buildup of porphyrins in the liver or bone marrow. This results in the various forms of porphyria, which can be divided into two broad categories: “acute” and “cutaneous.” The acute forms include acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), variegate porphyria (VP), and aminolevulinic acid dehydratase deficiency porphyria (ADP). These are marked mainly by sudden attacks of stomach pain that can be severe and persist for many days, as well as pain in the chest, limbs, or back; nausea, vomiting, or constipation; urinary retention; confusion, hallucinations, or seizures; and muscle weakness. Some acute forms disproportionately affect certain groups of people; for example, AIP is more common in women of reproductive age, with severe abdominal pain attacks related to hormonal changes during the menstrual cycle. The cutaneous forms of porphyria, which include hepatoerythropoietic porphyria (HEP), congenital erythropoietic porphyria (CEP), erythropoietic protoporphyria (EPP) or X-linked protoporphyria (XLP), and porphyria cutanea tarda (PCT), are associated with skin issues, such as blistering, itching, swelling, scarring, pain, or redness upon exposure to sunlight. Each of these disorders can profoundly compromise quality of life, often beginning in early childhood, due to pain or the need to avoid the sun or wear sun-protective clothing over the entire body and face, to avoid potentially disfiguring tissue damage. PCT is the most common form of the disorder found in the United States, and can be triggered by factors such as excessive iron accumulation from the diet, heavy alcohol use, taking certain drugs containing estrogen, smoking, and as a complication of infection with the hepatitis C or HIV viruses.

Diagnosing porphyrias is based on clinical symptoms and high levels of porphyrins in the blood, stool, or urine, but can be challenging because these disorders are rare and the symptoms overlap with

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other conditions, which often results in missed or incorrect diagnoses. Treatment depends on the specific form of porphyria, but may involve use of heme-based or glucose treatments that reduce porphyrin production in the liver; liver transplantation; avoiding sunlight as much as possible; removal of some blood (therapeutic phlebotomy); and medications, including pain relievers. Genetic testing may also be used to confirm a particular inherited form of porphyria and determine appropriate care, for individuals and their potentially affected family members.

RESEARCH ADVANCES IN UNDERSTANDING AND MANAGING PORPHYRIAS

NIDDK-sponsored efforts helped lay the groundwork for understanding the underlying mechanisms and clinical symptoms of porphyrias and developing diagnostic tests and treatments. In the 1980s, researchers capitalized on the unique fluorescent properties of porphyrins to identify diagnostic plasma markers for forms of porphyria such as VP. Also during this time, researchers documented responses in individual patients to treatments for CEP, such as oral activated charcoal to lower porphyrin levels or continuous blood transfusions to suppress red cell production. Building on findings supported by others to develop a form of heme called hematin as a therapy for acute porphyrias, and its U.S. Food and Drug Administration (FDA) approval in 1983, the NIDDK sponsored continuing research in the 1990s to further develop this treatment.

NIDDK research in the 1980s to 1990s helped to test the use of liver transplantation to treat people with some forms of porphyria, sometimes in combination with a pre-treatment to lower blood porphyrin levels, such as hematin or plasmapheresis (a procedure involving removal, treatment, and reinfusion of blood plasma). Research at this time also aimed to understand the burden of porphyrias in the population, such as studies in the 1990s

documenting mortality in people with AIP and characterizing PCT and its risk factors, including hereditary hemochromatosis gene mutations and hepatitis C infection. Advances in recent decades demonstrated the influence of factors, such as nutrition, alcohol use, and smoking, on heme biosynthesis and their impacts on different forms of porphyria.

In parallel with, and supportive of, these advancing clinical efforts, NIDDK-supported researchers helped map out the intricate pathway of heme biosynthesis, identifying the key enzymes that catalyze reactions at multiple steps to convert porphyrins into heme. Scientists supported by the Institute also identified and sequenced some of the genes that encode enzymes of the heme synthetic pathway, and helped to define numerous genetic mutations associated with different forms of porphyria. For example, in the 1980s, scientists purified the enzyme that is impaired in CEP and isolated the gene encoding it. A study in 1990 identified the gene coding for the enzyme at the first step in the heme biosynthetic pathway, delta-aminolevulinic acid synthase 1 (ALAS1)—mutations in which are responsible for some of the acute hepatic porphyrias—from human liver samples. Studies in the 1980s and 1990s also characterized and purified the enzyme underlying PCT and HEP, including multi-generational family genetic studies that shed light on relationships between genetic traits, enzyme activity, and clinical symptoms. In the 1990s to 2000s, through several studies in animal models and people, researchers identified the genetic mutations responsible for the symptoms of EPP in the final enzyme within the heme biosynthetic pathway that result in reduced enzymatic activity. Throughout the 1990s, researchers continued to identify mutated forms of genes coding for enzymes responsible for other forms of porphyria, such as ADP and HCP. Later studies provided additional insights into these enzymes, including their structures—key information for understanding their functioning and role in disease processes. In 2005, the NIDDK initiated support for a Center for Excellence in Molecular Hematology that enabled additional discoveries

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about the molecular mechanisms in the porphyrias, including clues to the underlying pathways involved in PCT. Further genetic discoveries, related to EPP and XLP, were made by the Porphyrias Consortium, described below.

Scientists have utilized this explosion of new knowledge about the genetic factors underlying each of the porphyrias to develop unique animal models in which to study disease pathways and processes, as well as to test new treatments. For example, in 1998, researchers developed a zebrafish model with a mutated form of the gene associated with HEP in humans, followed soon after by mouse models with the genetic mutations and symptoms present in PCT, VP, and CEP. In 2010, a research group used a mouse model with a genetic mutation causing AIP to successfully test a corrective gene therapy approach using a viral vector to target cells in the liver. A more recent mouse model of an AIP subtype that causes early-onset neurological impairment revealed mechanisms behind this severe form of the disorder. Researchers developed mouse models with distinct genetic mutations associated with different severities of human CEP to further understanding of these disorder subtypes.

THE PORPHYRIAS CONSORTIUM

In 2009, building upon these decades of basic and clinical research, the NIH's Rare Diseases Clinical Research Network announced the establishment of the Porphyrias Consortium. The Consortium is supported by the NIDDK and the NIH's National Center for Advancing Translational Sciences, in collaboration with the American Porphyria Foundation. The present-day Consortium includes six centers in the United States and other satellite sites, which bring together scientists, patient advocates, and industry to address the current challenges in porphyria research by conducting collaborative, large-scale clinical studies on these rare disorders. The Consortium's multiple centers, covering several geographical areas, help researchers recruit enough

participants with these rare disorders for robust studies. The Consortium also focuses on training the next generation of clinicians and investigators in porphyria care and research, and provides information on porphyrias for patients and their families, health care professionals, and the public.

The Consortium has conducted several studies aimed at understanding the mechanisms and disease course of the multiple forms of porphyria and developing new approaches to their diagnosis, treatment, and prevention. In 2014, the Consortium reported on early observational findings in their U.S.-based study cohort, describing the key clinical and genetic features and current approaches to clinical care for acute porphyrias—including AIP, HCP, and VP—and documenting an average delayed diagnosis of more than a decade. In 2015, researchers reported variability in how different clinical laboratories were measuring red blood cell protoporphyrin, a molecule derived from porphyrin during heme production, that can lead to missed diagnoses, particularly for the EPP and XLP forms of porphyria. Results of a Consortium study in 2017 helped to identify the genetic mutations present in people with EPP and XLP and showed that higher levels of red blood cell protoporphyrin were associated with increased disease severity and risk of liver dysfunction. In addition to these original studies, the Consortium has also issued recommendations for the diagnosis, treatment, and counseling of patients with acute porphyrias that originate in the liver, to ensure optimal health outcomes.

PROGRESS TOWARD NEW TREATMENTS

The decades of research advances on the porphyrias and the ongoing efforts of investigators involved in the Porphyrias Consortium have helped develop breakthrough treatments for two forms of porphyria—AIP and EPP. NIDDK-supported studies mapping out the biological pathways involved in these disorders provided the foundation for this work.

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In the case of AIP, a disease marked by severe abdominal pain attacks and fatigue, the main treatment since the 1970s has been intravenous infusions with hematin, containing heme extracted from red blood cells. However, this treatment proved relatively slow-acting and came with side effects such as inflammation in the veins and iron overload. In recent years, the NIDDK and other NIH Institutes supported research on a cutting-edge approach to therapeutics called RNA interference (RNAi) using small interfering RNAs (siRNAs)—short strings of genetic material that effectively turn off genes that produce disease-causing proteins. Research in a mouse model supported through the Porphyrias Consortium showed that this approach could be used to target ALAS1, an overactive enzyme in AIP: an infusion of the siRNA inhibiting this enzyme effectively prevented and treated the attacks associated with AIP. The NIDDK also supported early pre-clinical research to develop an injection-based form of the treatment and assays that monitored response. This important work paved the way for testing this therapeutic in humans as part of clinical trials sponsored by a pharmaceutical company, with the participation of many scientists and centers within the Porphyrias Consortium. In November 2019, the FDA approved this new therapeutic for AIP in the form of a drug called givosiran (Givlaari®), which is already improving quality of life for people with AIP.

The Porphyrias Consortium also worked closely with another pharmaceutical company on a clinical trial to evaluate a treatment for EPP, called afamelanotide (Scenesse®). Afamelanotide binds to a receptor in skin cells, stimulating them to produce a pigment that protects against sunlight. In clinical trials conducted by the pharmaceutical company in the United States and Europe, with additional support from the NIDDK and the NIH's National Heart, Lung, and Blood Institute, Consortium investigators worked closely with the company to evaluate the safety and efficacy of an implant under the skin containing afamelanotide for treating EPP. These trials found afamelanotide increased the amount of pain-free time people with EPP could

spend in sunlight, as well as their overall quality of life. The treatment was approved by the FDA in October 2019—the first agent available to help people with EPP experience pain-free sun exposure.

FUTURE DIRECTIONS

Building on the past progress made possible by the Porphyrias Consortium and other NIDDK-supported investigators, the Institute is committed to continuing support for research to advance understanding of the porphyrias and discover better ways to manage these disorders. The Consortium is conducting ongoing clinical studies on natural history, diagnosis, and treatments for all forms of porphyria. The Consortium's active studies include a longitudinal study to characterize the long-term course and outcomes of several forms of porphyria. Ongoing clinical trials will determine the safety and efficacy of new therapies. Recent efforts by the Consortium include characterizing the natural history and clinical management of acute hepatic porphyrias in people with recurrent attacks, evaluating methods for measuring quality of life in people with AIP, and joining together with the European Porphyria Network to establish an international database with diagnostic information on genetic variants linked to all forms of porphyria. In addition to these Consortium-based activities, the NIDDK also continues to support investigator-initiated porphyria research, such as studies in cell and animal models to decipher specific mechanisms of cell injury caused by an overabundance of porphyrins. Together, these research efforts will continue to advance our understanding of porphyrias, and help lead the way to new treatments, with the overarching goal of improving the lives of people with these disorders.

For more information on the Porphyrias Consortium, please visit:
www.rarediseasesnetwork.org/cms/porphyrias.

Developing New Diagnostic and Treatment Options for Children with Biliary Atresia

Biliary atresia is a rare but severe liver disease that begins in infancy. This disease is marked by inflammation and scarring of the bile ducts, the tubes that carry bile from the liver to the gallbladder and intestines to aid digestion. The resulting back-up of bile in the liver leads to jaundice, liver damage, and if untreated, liver failure and need for liver transplantation.

NIDDK-supported research has focused on efforts to improve the understanding of biliary atresia and other pediatric liver diseases and to advance care of children with these conditions. Toward this goal, the NIDDK established the Childhood Liver Disease Research Network (ChiLDRen) in 2009 by combining the NIDDK-supported Biliary Atresia Research Consortium (BARC) and the NIDDK-supported Cholestatic Liver Disease Consortium (CLiC). This Network is a collaborative team of doctors, scientists, research coordinators, medical facilities, patients and their families, and patient support organizations at sites 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. As such, ChiLDRen continues to conduct clinical trials, facilitate the discovery of underlying causes of disease, and search for new diagnostic and treatment options for children with rare liver diseases.

ABOUT BILIARY ATRESIA

Typically, the first sign of biliary atresia is yellowing of the skin and whites of the eyes, called jaundice, which results from the buildup of bile in the body. Bile contains a reddish-yellow substance called bilirubin. Infants often have jaundice from other causes in the first 2 weeks of life, so it is not easy to identify biliary atresia in newborn infants based on this symptom alone. Jaundice that lasts beyond 3 weeks of age may be the first sign of biliary atresia. Infants with biliary atresia may also have pale yellow, gray, or white stools if bilirubin is not reaching the intestines.

Diagnoses can be delayed because biliary atresia is just one of many possible causes of blocked bile flow in newborns. If not caught early and treated with a surgery called hepatportoenterostomy or HPE (also referred to as the Kasai procedure), the disease is potentially fatal and an early liver transplant may be required. During the HPE procedure, the damaged bile ducts outside the liver are removed and a loop of the infant's small intestine is used to connect the liver directly to the small intestine. This allows bile to flow directly from the liver to the small intestine. The procedure is not curative, but may slow or, in some cases, prevent the development of cirrhosis (liver damage and scarring) and liver failure. In children with biliary atresia, cirrhosis may cause complications such as portal hypertension, which is increased blood pressure in the portal vein, a blood vessel that carries blood from the intestines to the liver. As a consequence of portal hypertension, children with biliary atresia may have an enlarged spleen—setting up a condition in which platelets are sequestered in the spleen and

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are not readily available in circulating blood to stop bleeding. Even after surgery, many children with biliary atresia develop severe disease requiring liver transplantation by the time they reach adulthood.

While the causes of biliary atresia are not fully understood, studies indicate that genetic, environmental, and infectious factors may play a role. Researchers funded by the NIDDK through ChiLDReN and other grants have set about to uncover how biliary atresia develops and progresses, as well as how to improve management of this disease.

ChiLDReN

To better understand the causes of biliary atresia and develop improved treatments, ChiLDReN has conducted research related to diagnosis and disease progression, and currently has several ongoing clinical studies.

For example, researchers recently reported that a protein called matrix metalloproteinase-7 (MMP-7) may be a potentially useful clinical biomarker for diagnosing biliary atresia. MMP-7 was found to be at high levels in blood from infants with biliary atresia compared with other liver diseases, especially when combined with another marker of impaired bile flow called γ -glutamyltranspeptidase. Scientists have also identified *PKD1L1* gene variants in some infants with biliary atresia with splenic malformation (BASM), a syndrome occurring in about 10 percent of patients with biliary atresia. The gene variants code for a protein called PKD1L1 found in bile ducts. PKD1L1 is important for key biological processes, including proper positioning of internal organs during embryonic development, underscoring its possible role in BASM syndrome. Furthermore, investigators have described a set of 14 genes with activity levels at diagnosis that predicted transplant-free survival at 2 years of age in contrast to those who require liver transplantation or succumb to the disease. This finding suggests that the newly identified 14-gene activity signature could be a useful biological marker

to predict how biliary atresia will progress in young children after the Kasai procedure (see research advance earlier in this chapter).

In two ongoing clinical research studies conducted by ChiLDReN—*A Prospective Database of Infants with Cholestasis* (PROBE) and *Biliary Atresia Study in Infants and Children* (BASIC)—clinicians are collecting clinical information and biological samples from infants, children, and young adults with biliary atresia. These types of prospective, observational studies will allow researchers to identify potential causative factors that lead to disease onset and follow the natural history of disease progression. In a third clinical research study called *FibroScan™ in Pediatric Cholestatic Liver Disease* (FORCE), researchers are evaluating the use of noninvasive monitoring of liver fibrosis (scar tissue formation in cirrhosis) as part of the clinical management of children with liver disease marked by bile duct blockage. While liver biopsy is often used in the initial diagnostic evaluation of children with liver disease, subsequent surveillance by liver biopsy is rarely performed in children because of its inherent invasiveness and risks. A noninvasive means of assessing fibrosis throughout the liver would be highly desirable and clinically useful in children with biliary atresia.

HOPE THROUGH RESEARCH

The NIDDK continues to support research into biliary atresia through ChiLDReN and several investigator-initiated research projects. For example, investigator-initiated studies include those that seek to identify the biological basis of clinical outcomes, map disease pathways, and identify new therapeutic agents for biliary atresia. Researchers are also trying to improve early diagnosis of the disease, such as recent research showing that a two-step screening approach measuring newborns' bilirubin levels could identify those with biliary atresia—progress toward a goal of more successfully treating babies earlier in the course of disease (see research advance earlier in this chapter).

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EMILIA'S STORY



Left to right: Lucia (mother), Emilia, Mateo (brother), and Marco (father)

Nine-year-old Emilia is an extremely talented young girl living in Colorado. She has many interests but is especially passionate about theater. “I am a theater person!” she exclaims. Her specialty is singing and dancing in musical theater productions. “One of my favorite songs is Welcome to Wonderland,” she shares, which is from the musical Alice in Wonderland. Most recently, she was cast in a production of The Jungle Book as the narrator and a singer. Emilia’s other interests include art, volleyball, basketball, swimming, skiing, and hockey. Clearly, she has not let a diagnosis of a serious liver disease called biliary atresia keep her down.

In addition to being busy with theater and other activities, in 2019, when she was only 7 years old, Emilia was selected to represent Children’s Hospital Colorado as an Ambassador—a great honor. In this role, she shared her story as someone living with biliary atresia, and participated in community fundraising events. For example, Emilia participated in a fashion show preceding a Colorado Avalanche hockey game and served as a host at the 2019 Children’s Hospital Colorado Gala. She was also asked to participate in an in-person interview with a Denver Bronco football player, and now as a result, Emilia says, “I have my medal as a junior reporter.” In describing all of her outreach activities, Emilia’s bright personality shines through.

Emilia also enjoys doing outdoor activities with her mother Lucia, father Marco, and younger brother Mateo. The family takes part in bike rides, walks, hikes, and swimming, whenever possible. Although they strive to maintain an active lifestyle, Lucia and Marco are well aware that Emilia has a serious liver disease and have to carefully plan the family’s activities accordingly. “She’s been pretty healthy and it’s just [a job for] us as parents to be aware and more cautious in everything she does. It’s hard for us, [and hard] for her to understand,” says Lucia. “But still, she’s a very happy girl.”

“She’s been pretty healthy and it’s just [a job for] us as parents to be aware and more cautious in everything she does,” says Emilia’s mother, Lucia, explaining how the family avoids activities that may increase her risk of an unwanted bleeding event due to her biliary atresia.

DIAGNOSIS AND EARLY TREATMENT

During her 6-week-old checkup, the pediatrician noted that Emilia looked jaundiced, and lab test results indicated that she had elevated levels of bilirubin. The pediatrician subsequently made an appointment for Marco and Lucia to bring their baby daughter to see Dr. Ronald Sokol, a pediatric gastroenterologist at Children’s Hospital Colorado. During this initial visit with Dr. Sokol, a liver biopsy was conducted that confirmed that Emilia had biliary atresia. Three days later at approximately 55 days of age, Emilia underwent the Kasai procedure. It was “a big surgery ... we were extremely nervous,” recalls Marco. Thankfully, “it went pretty smoothly,” says Lucia. After a week in the hospital, Emilia was discharged and sent home. Lucia remembers those early days after the surgery: “We took care of her at home and she was ... pretty

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happy.” The only complication of the surgery was the build-up of fluids in her abdomen, but after a few months, the excess fluids resolved.

“One of the hopes that we have is that soon, hopefully, there is a medicine that can reverse or stop the cirrhosis of the liver,” says Marco, describing how studies such as those conducted by the Childhood Liver Disease Research Network (ChiLDReN) may be able to help his daughter Emilia.

From the time she was discharged from the hospital following the surgery, Emilia has taken a broad-spectrum antibiotic every day to prevent an infection called cholangitis. Cholangitis arises when bacteria normally residing in the intestine enter the liver and cause infection. Despite the daily use of the antibiotic, she has had two episodes of cholangitis, which required high-dose antibiotic treatment in the hospital to combat the infections.

MAINTAINING LIVER FUNCTION AND OVERALL HEALTH

To maintain her liver function and overall health, Emilia and her parents take several proactive measures. Her liver function is monitored twice a year during her visits with Dr. Sokol. Marco recalls, “We kept her in good hands, and we followed the instructions from Dr. Sokol—and she’s been thriving health-wise.” Lab results indicate that her liver function has been fairly stable over time. In addition to liver function tests, her platelet levels are also measured. Because of her portal hypertension, Emilia has an enlarged spleen that “soaks up” more platelets from the blood than normal, making those blood cells unavailable to help stop episodes of bleeding. As a result, Marco and Lucia also keep a close eye on their daughter for any wound that isn’t healing as it should. Emilia refrains from activities that would increase her

risk of an unwanted bleeding event. Additionally, because children with biliary atresia often have nutritional deficiencies, she takes vitamin supplements daily along with a compound called ursodiol that aids in fat absorption. She also takes probiotic supplements. And, looking to the future, Emilia and her family participate in research.

When asked how likely he would be to recommend clinical research participation to others whose children have liver disease, Emilia’s father, Marco, says: “Yes, participate. It’s basically how improvement and progress in medical studies goes on.... It’s progress toward the future.”

PARTICIPATING IN CLINICAL RESEARCH STUDIES

Given the diagnosis of a serious liver disease, it was an easy decision for Lucia and Marco to enroll Emilia as a baby in A *Prospective Database of Infants with Cholestasis* (PROBE) clinical research study conducted by the NIDDK-supported Childhood Liver Disease Research Network (ChiLDReN). Logistically, her participation in the clinical study is easy and convenient. Dr. Sokol serves as both Emilia’s liver specialist and as the principal investigator for the PROBE site at Children’s Hospital Colorado. Blood is drawn during her twice-a-year visits with Dr. Sokol, and a small portion is used by researchers in the PROBE clinical research study to understand how the disease progresses over time. Emilia has also been participating in ChiLDReN’s *FibroScan™ in Pediatric Cholestatic Liver Disease* (FORCE) clinical study for approximately 4 years, which seeks to determine whether noninvasive monitoring of liver fibrosis is as reliable and informative a tool in children as it is in adults. This portion of the visit is performed quickly and utilizes an ultrasound device to measure scarring in the liver.

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The family is happy to make these contributions to research that may not only one day improve Emilia's health, but also the health of other children living with biliary atresia or other serious liver diseases. "One of the hopes that we have is that soon, hopefully, there is a medicine that can reverse or stop the cirrhosis of the liver," says Marco. Additionally, Marco would encourage others to volunteer for research studies if the

opportunity arises. "Yes, participate," he says. "It's basically how improvement and progress in medical studies goes on.... It's progress toward the future." As for Emilia's future, she can't wait for the next opportunity to participate in a musical theater production where she will no doubt steal the show with her vibrant energy, passion, and talent.