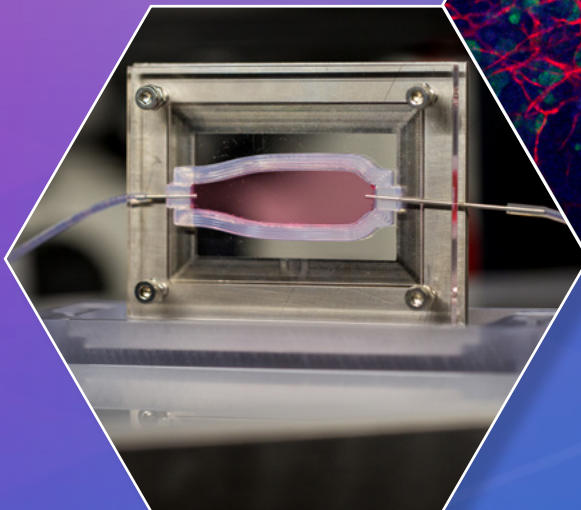
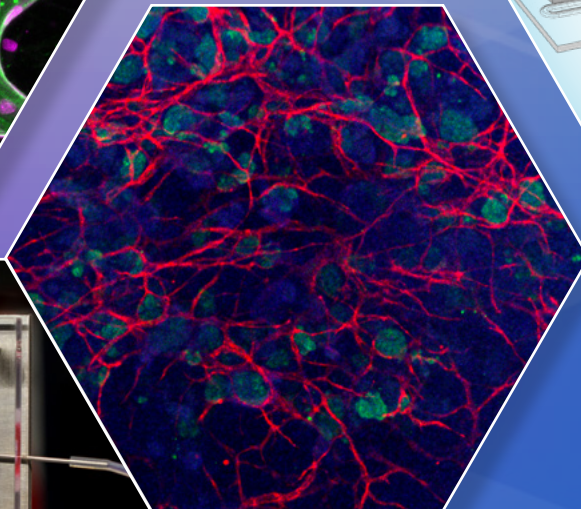
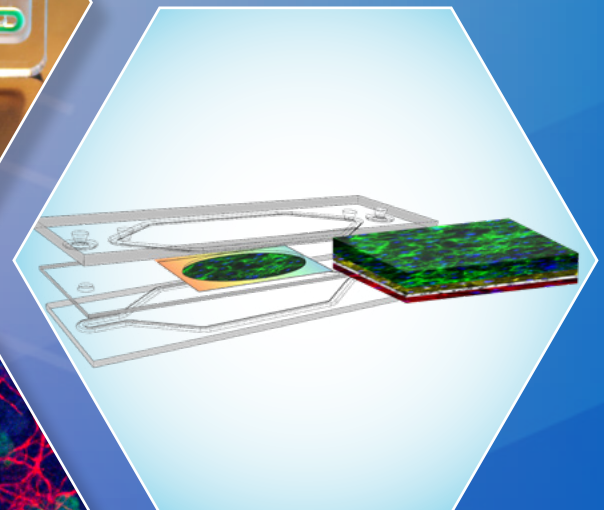
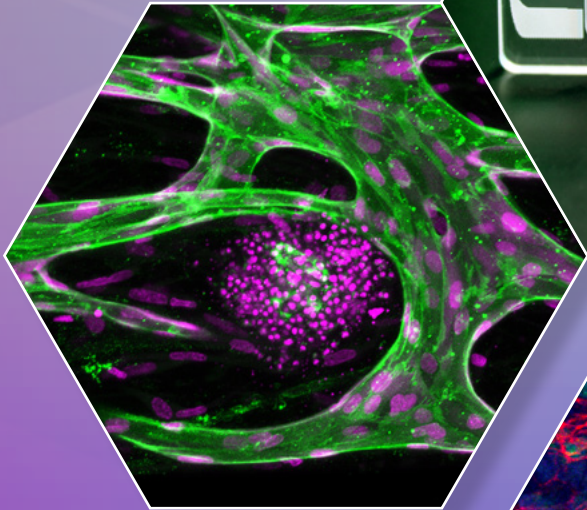
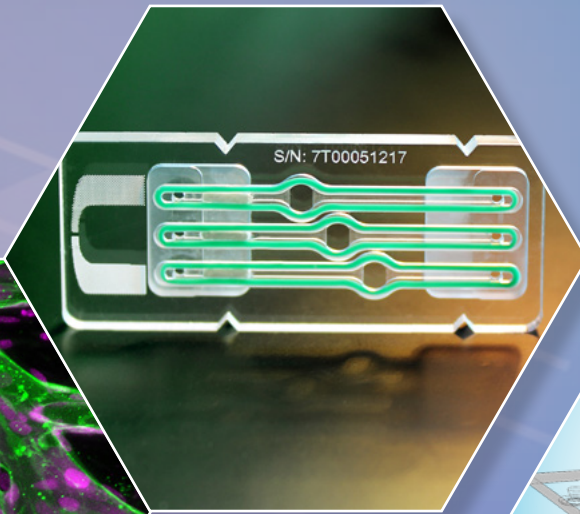


# NIDDK

## Recent Advances & Emerging Opportunities

January 2020

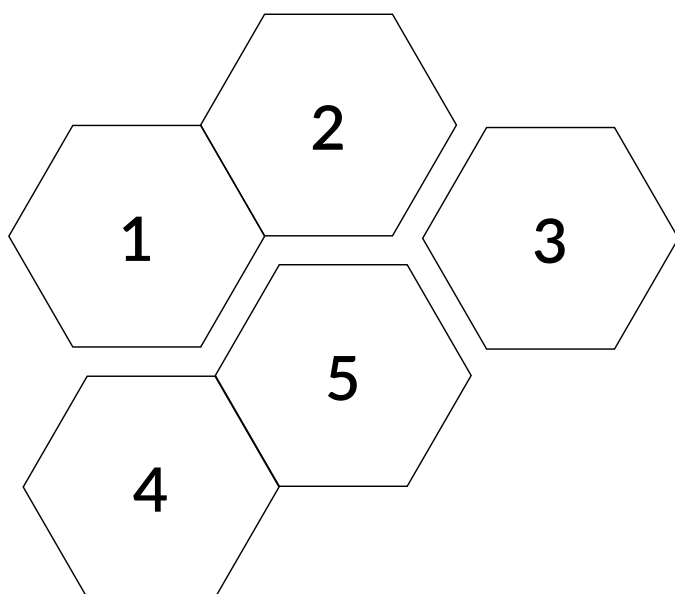


U.S. Department of Health and Human Services  
National Institutes of Health  
National Institute of Diabetes & Digestive & Kidney Diseases



National Institute of  
Diabetes and Digestive  
and Kidney Diseases





The NIDDK is supporting research to develop three-dimensional chips—about the size of a thumb drive—that replicate the structure and function of human organs/tissues in a laboratory setting, such as the islet, liver, and kidney chips shown on the cover. Such chips, by modeling human health and disease, could advance knowledge of human biology, act as platforms to validate biological markers of disease, and play a central role in drug development, screening, and testing to speed progress toward new therapies.

**Islet chip (1-2):** Researchers studying type 1 diabetes are developing chips with clusters of insulin-producing beta cells from the pancreas, called islets, that incorporate or mimic diverse elements that support islets in the body, such as blood vessels. This is shown in the magnified image of cells (1), with islets (central sphere) surrounded by blood vessels (green). In developing these islet microenvironments, housed on physical chips (2), researchers are also incorporating immune cells involved in the type 1 diabetes autoimmune attack. Through these efforts, researchers are modeling the complex cellular interactions involved in human type 1 diabetes.

**Liver chip (3):** The liver is a multicellular organ with many vital functions, including drug detoxification, and liver injury is the most common reason that new drugs fail during development. Scientists are developing chips, such as the one shown here, with complex structural features similar to human liver. This chip contains layers of multiple liver cell types and connective tissue (see close-up of cell layers in the image with the chip) and mimics physiological conditions through a flow of fluid and oxygen, with the gradient of varying oxygen levels shown as a lightly colored rainbow in the rectangle within the middle chip layer. Chips like this one may offer a more precise predictor of toxicity in human cells prior to clinical trials than is possible with other cell and animal models.

**Kidney chip (4-5):** Kidneys are complex organs in which blood vessels intertwine with other structures to filter extra water and waste products out of the blood and make urine. Researchers recreated some of the conditions under which kidneys normally develop inside the body to help kidney organoids—engineered aggregates of kidney cells—mature properly. Kidney organoids housed on a 3D (three-dimensional) chip and exposed to a high rate of fluid flow (chip image [4]) become well-vascularized with a network of new blood vessels (close-up cell image [5], with blood vessels in red surrounding kidney organoids) and exhibit more mature structures, akin to those observed in human kidneys. These 3D kidney organoid-on-chip models are providing new opportunities to study kidney development and disease.

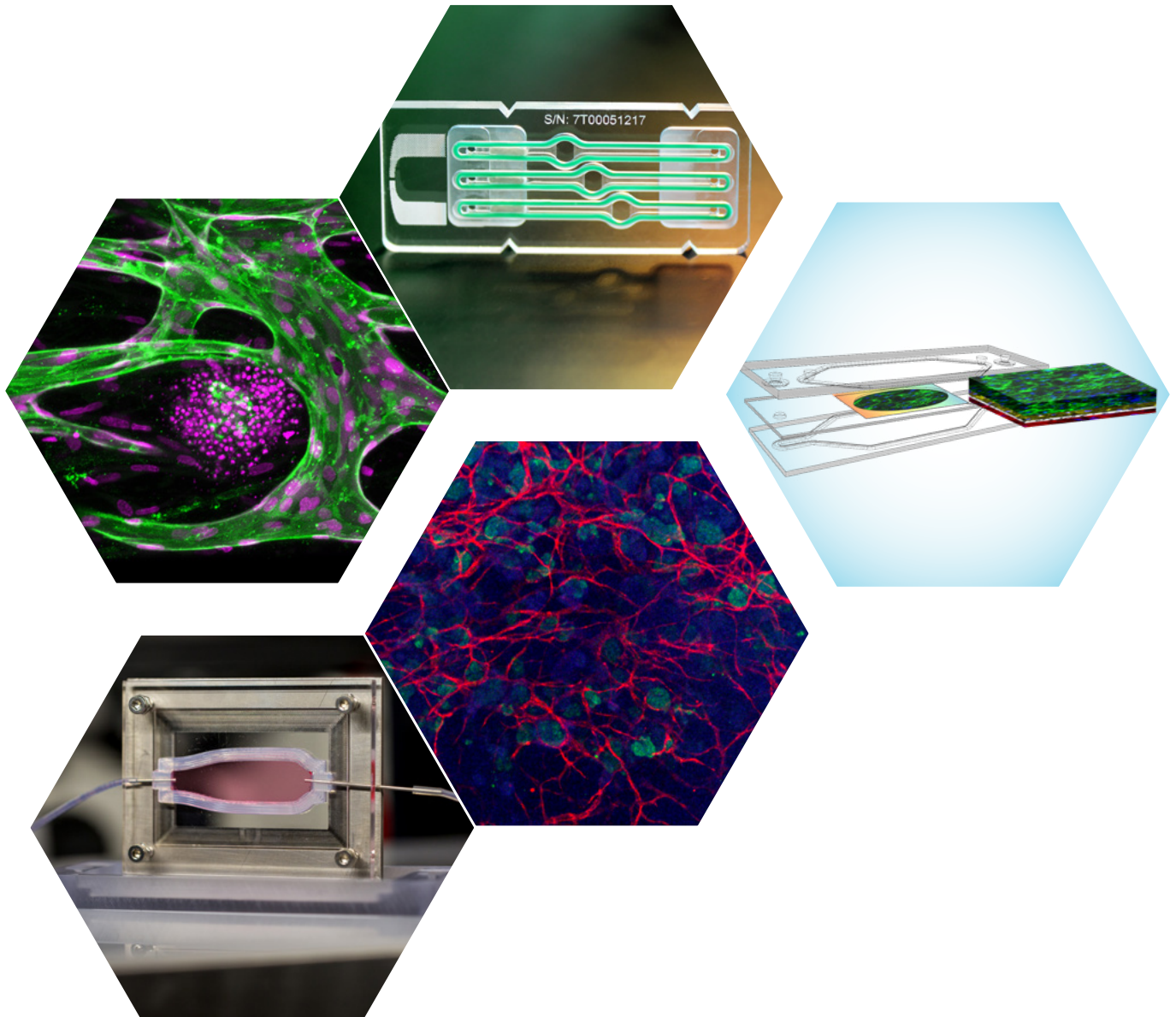
**Image credits:**

1. Hugh Bender, Ph.D., in the laboratory of Christopher Hughes, Ph.D., University of California, Irvine.
2. Dr. Ashu Agarwal Laboratory at the University of Miami, and Dr. Cherie Stabler Laboratory at the University of Florida.
3. D. Lansing Taylor, Ph.D., and Albert Gough, Ph.D., University of Pittsburgh.
- 4-5. Kimberly A. Homan\*, Navin Gupta†, Jennifer A. Lewis\* and Ryuji Morizane†; \*Harvard University and †Brigham and Women's Hospital.

# NIDDK

## Recent Advances & Emerging Opportunities

January 2020



U.S. Department of Health and Human Services  
National Institutes of Health  
National Institute of Diabetes & Digestive & Kidney Diseases

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ACKNOWLEDGMENTS

# Message from the Director



As the Director of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), I am pleased to present this annual report highlighting the research efforts and programs supported by the Institute. The NIDDK has a broad research responsibility that includes some of the most common, debilitating, and costly conditions affecting Americans. These conditions include diabetes and other endocrine and metabolic diseases; liver disease and other digestive diseases and conditions, such as inflammatory bowel disease and irritable bowel syndrome; nutritional disorders; obesity; kidney diseases, such as polycystic kidney disease and glomerular disease; urologic diseases and conditions, such as interstitial cystitis/bladder pain syndrome, prostatitis, and urinary tract infection; and blood diseases.

The 20<sup>th</sup> edition of this report highlights recent NIDDK-supported scientific advances, such as:

- Demonstration that treatment with a drug targeting the immune system can prevent onset of clinical type 1 diabetes in high-risk individuals for at least 2 years;
- New details on development of children's gut microbiomes;
- Finding that pre-pregnancy dietary supplementation with folate may reduce risk of gestational diabetes;

- Progress in therapeutic approaches for cystic fibrosis;
- Finding that people consumed more calories per day and gained weight on an ultra-processed diet;
- Demonstration that teens with severe obesity who underwent Roux-en-Y gastric bypass surgery were significantly more likely to have remission of both type 2 diabetes and high blood pressure compared to adults who had the same procedure;
- Identification of patient characteristics that can predict how well children with ulcerative colitis will respond to treatment, moving toward a more personalized approach to treat this disease;
- Development of remarkable liver organoids—miniature livers in a laboratory dish—to advance research on human fatty liver disease;
- Determination that intensive blood pressure control does not lead to kidney injury in people who do not have chronic kidney disease;
- New information about the frequency, intensity, and duration of urological chronic pelvic pain syndrome symptom exacerbations (“flares”); and
- Demonstration of the importance of fibrosis in lower urinary tract symptoms.

In addition to reporting on recent advances, this publication traces the multi-step path to research achievements through several “Stories of Discovery” and “Scientific Presentations.” These essays illustrate how major new discoveries that have greatly advanced biomedical science and are benefitting human health often emerge from incremental insights gained from research investments spanning many years and even multiple research disciplines.

This report also includes personal stories of those who have given time and effort to participate in



NIDDK-sponsored clinical research or whose lives have been transformed by biomedical research. A mother and daughter describe their dedication to participating in clinical research to prevent or delay clinical type 1 diabetes in those at high risk for the disease. A teenager with pancreatitis shares her experience undergoing a surgical procedure that removed her pancreas and also transplanted back her own healthy pancreatic insulin-producing cells to stave off diabetes. An avid cyclist describes how he was sidelined by a harrowing brush with drug-induced liver injury, and how he is participating in a national research network that is working toward better ways to detect, prevent, and manage the disease. Five women share their perspectives and experiences as participants in special focus groups designed to inform a larger research effort on improving bladder health among women and girls in the United States.

The NIDDK continues efforts to ensure that knowledge gained from its research is disseminated to health care providers, patients, and the public. We develop science-based information on diseases and disorders within the NIDDK mission and distribute it through our information and education programs and our website. I invite you to visit us at [www.niddk.nih.gov](http://www.niddk.nih.gov). Health information, news, and scientific advances related to NIDDK research are also available on our Twitter feed: [@NIDDKgov](https://twitter.com/NIDDKgov).

This report reflects only a fraction of the immense body of NIDDK-funded research across the country, performed by basic scientists, clinical investigators, and patient volunteers. Moving forward, we remain committed to supporting these important areas of research and translating scientific discoveries into improvements in the health and quality of life of all people.

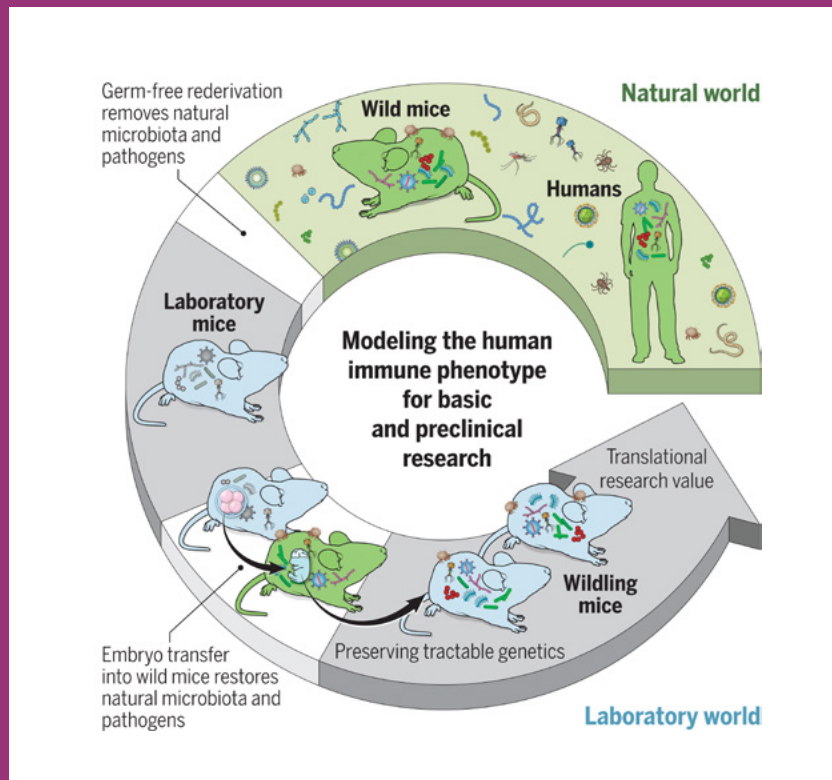
The efforts featured in this publication reflect the core mission of the NIDDK, including the Director's guiding principles:

- Maintain a vigorous investigator-initiated research portfolio
- Support pivotal clinical studies and trials
- Promote a steady and diverse pool of talented new investigators
- Foster exceptional research training and mentoring opportunities
- Ensure knowledge dissemination through outreach and communications

More information on how the NIDDK's activities support these core values can be found in the "NIDDK Funding Trends and Support of Guiding Principles" section at the end of this report and on our website at [www.niddk.nih.gov](http://www.niddk.nih.gov).

**Griffin P. Rodgers, M.D., M.A.C.P.**

Director  
National Institute of Diabetes and Digestive and Kidney Diseases  
National Institutes of Health  
U.S. Department of Health and Human Services



Mice are often studied in the lab as stand-ins for humans in basic research and early-stage studies of potential treatments. However, traditional lab mice vary greatly across different labs and in comparison to their free-living, wild cousins in terms of the microbes they carry, which can have profound effects on immune responses. A group of NIDDK intramural researchers and scientists from other NIH Institutes and universities have pioneered the development of a new mouse model, called the “wildling mouse.” This mouse is the genetic offspring of conventional lab mice, but carries the microbes of its surrogate mother, a wild mouse, at all body sites. Compared to standard lab mouse microbiota, the wild mouse microbiota have increased resilience towards environmental challenges. With an immune state and response to immune-modulatory drugs more similar to that of humans than conventional laboratory mice, the wildling mouse may be a better translational model for multiple human diseases and for pre-clinical testing of drugs.

Image courtesy of Dr. Barbara Rehermann, NIDDK, and Ms. Nathalie Cary, AAAS. From Rosshart SP, Herz J, Vassallo BG,...Rehermann B. Laboratory mice born to wild mice have natural microbiota and model human immune responses. *Science* 365: pii: eaaw4361, 2019. Reprinted with permission from AAAS.

# Cross-Cutting Science

*Medical advances are not usually achieved in great, intuitive leaps. More often, new prevention strategies, treatments, and cures result from a long, gradual accumulation of knowledge from years of scientific research. Insights into the fundamental biologic building blocks and processes of an organism—its genes, the proteins they encode, the inner workings of cells, and the ways cells communicate with each other—can have broad and far-reaching implications. Indeed, many significant advances in our knowledge of disease and disease treatment can be traced to laboratory studies whose relevance to health could not have been fully known or appreciated at the time they were conducted.*

With the development of innovative scientific technologies and the emergence of new scientific disciplines as talented and creative research teams tackle ever more complex challenges, new opportunities to make exciting discoveries arise each day. Described in this chapter are several recent studies as well as features on the Institute's 70<sup>th</sup> Anniversary and on some up-and-coming scientific investigators who have received special presidential recognition. The insights gained through research described here can be expected to further scientific progress in many research areas; for today's discoveries may hold the seeds of tomorrow's cures.

## IMPROVING ANIMAL MODELS FOR RESEARCH ON HUMAN HEALTH AND DISEASE

**Where the “Wildlings” Are—Lab Mice with Wild Microbes Better Model Human Immune Responses:** NIDDK's intramural scientists, together with researchers from other NIH Institutes and universities, have developed laboratory mice that better reflect the natural complexity of mammals and the microbes that co-evolved within them, and serve as an improved model of human physiology. Scientists using mice as stand-ins for humans in early-stage drug development studies have come to appreciate the importance of having a more complex system that better reflects how mammals exist in their “natural” state, colonized by a unique mix of co-evolved microbes, including bacteria, viruses, fungi, protozoans, and other organisms that make up their “microbiome.” Traditional lab mice allow researchers to control for genetic background and

other factors in their studies, but carry microbes that vary greatly across labs and when compared to wild mammals. This variation may have contributed to past failures to translate successful pre-clinical drug studies into humans.

To address this, a group of NIDDK intramural researchers and scientists from other NIH Institutes and universities created the so-called “wildling” mouse. They started with conventional female lab mice raised in a sterile environment, and transferred fertilized embryos from these mice into wild mice. The offspring—the wildling mice—are therefore the genetic offspring of conventional lab mice but have been exposed to the natural microbes of their surrogate (wild) mothers. The scientists catalogued bacteria, fungi, and viruses present in wildling, wild, and conventional lab mice in parts of the body where microbes are usually found, including the gut. The microbe communities were more similar in the wildling and wild mice than in conventional lab mice. A similar result was seen when looking at the immune landscape—the types and numbers of different immune cells—at various sites in the body important to an immune defense. The wildling mice microbiomes were also more stable than laboratory mice microbiomes and showed greater resilience to challenges such as antibiotic treatment and a high-fat diet. In a final coup de grâce, the group demonstrated how wildling mice may better model human disease and treatment response by testing two drugs with a history of beneficial effects on the immune system in conventional laboratory mice, but adverse immune effects when tested in humans.

In the wildling mice, these drugs provoked a similar immune response as in humans. Thus, wildling mice may be an improved animal model for research that is reproducible and predictive of human response, saving time and money, and sparing clinical trial participants from adverse effects of potentially harmful drugs.

Rosshart SP, Herz J, Vassallo BG,...Rehermann B. Laboratory mice born to wild mice have natural microbiota and model human immune responses. *Science* 365: pii: eaaw4361, 2019.

## RESEARCH ON NEURAL SLEEP SIGNATURES

**New Technique Illuminates Sleep Similarities Between Humans and Zebrafish, with Implications for Better Understanding Human Sleep:** Researchers recently identified several neural activity signatures in the zebrafish brain that suggest certain sleep states in these fish are similar to human sleep and that they could thus be used for studying the role of sleep in health and disease. In humans, appropriate sleep patterns support emotional and physical well-being and quality of life, and chronic sleep deficiency has been linked to obesity, diabetes, kidney disease, and other health problems. How exactly sleep contributes to good health, however, is still unclear, and more research in this area could yield important insights. Zebrafish share neural and biochemical similarities with other vertebrates, including humans, that make them a potentially good model for studying sleep and sleep-related disorders. Zebrafish are also uniquely useful in biological research, partially because their larvae are transparent, allowing use of advanced visualization techniques to answer scientific questions in living animals. However, measuring the neural signatures of sleep in zebrafish has been technically challenging, and it was unknown whether or not they organize their sleep cycle similar to humans and other vertebrate animals.

To explore this question, researchers sought to determine whether the zebrafish brain undergoes sleep states found in people and other vertebrates, such as the slow-wave and rapid eye movement (REM) states. To do this, the scientists developed a technique they called fluorescence polysomnography to visualize the brain and muscle activity, eye movement, and heart rate of living fish during sleep. This technique allowed the researchers to analyze not just the behavioral characteristics of sleep such as muscle relaxation and decrease in heart rate, but also the neural signatures of sleep in the zebrafish brain. One state they observed, which they named “slow

bursting sleep,” shared features with slow-wave sleep, while another state, named “propagating wave sleep,” was similar to REM sleep. This new visualization technique also enabled the researchers to study and identify cellular and molecular regulators of zebrafish sleep, such as a hormone that controls the onset of propagating wave sleep.

Given the zebrafish’s place in the vertebrate family tree, these results suggest that common mechanisms of sleep are shared among animals as disparate as zebrafish and humans. These findings suggest that zebrafish, and the novel imaging techniques possible in this model organism, could be powerful new tools for research to increase understanding of human sleep and its impacts on health and disease.

Leung LC, Wang GX, Madeline R,...Mourrain P. Neural signatures of sleep in zebrafish. *Nature* 571: 198-204, 2019.

## VISUALIZING DNA REPLICATION MACHINERY

**Determining the Architecture of DNA Replication Machinery:** Scientists in NIDDK’s Intramural Research Program have determined the three-dimensional structure of a protein complex that copies DNA—a fundamental molecular process required by all biological organisms. In order for a cell to divide, its DNA must be replicated to form two identical copies—one copy for each of the two cells resulting from the division. The process of DNA replication is a highly ordered, complex orchestration of many proteins serving different functions to achieve complete fidelity. The two intertwined strands that make up each DNA molecule must be unwound, and the sequence of each strand read as a template for synthesizing a new partner strand, to generate two double-stranded copies of the original DNA. However, researchers had not previously been able to visualize the complete structure of the genome replication protein machine, also known as the “replisome,” as it fully engages all critical portions of its DNA target. Therefore, important questions about the inner workings of the protein machinery have remained unanswered.

In recent research, NIDDK scientists used a method called cryo-electron microscopy to visualize the structure of a relatively simple replisome complex, originating from a virus called bacteriophage T7. They mixed isolated proteins, DNA pieces, and other molecules necessary for DNA replication together in conditions that promoted the formation of replisomes.

By quickly freezing the assembled replisomes, thus capturing the complexes in their natural states, the scientists could use a powerful electron microscope to visualize their three-dimensional structures at atomic resolution. They found that six “helicase” proteins encircled one separated DNA strand and moved along the strand sequentially in a “hand-over-hand” motion to continue unwinding the DNA. They showed that the helicase proteins served as a central organizer of the replisome, providing a focal point for the positioning of “polymerase”—the protein that actually synthesizes new DNA strands based on the original DNA sequence. The structures also revealed that the DNA molecule bends in a specific manner as the replisome advances. These findings define core replisome operating principles that are found in all organisms even though specific components of the replication machinery differ, shedding important light on a critical, foundational molecular process.

Gao Y, Cui Y, Fox T,...Yang W. Structures and operating principles of the replisome. *Science* 363: pii: eaav7003, 2019.

## RESEARCH ON DNA EDITING TECHNOLOGIES

**Determining the Fidelity of DNA Editors:** Scientists calculated the rate of anticipated and unanticipated mutations generated by use of an emerging DNA editing technology. Many human diseases result from the change of the smallest element of the genetic code—a “single base”—and therefore considerable effort has been made to develop tools for altering single bases as a means of treating disease. Recently, several new technologies have emerged, including “base editing” which directly converts a target base into a different base. As with any technology that alters DNA, however, it is critical to understand how accurate (i.e., was the targeted base edited correctly?) and precise (i.e., were other edits introduced?) the changes are.

Researchers in this study examined how well several base editors worked for changing two different kinds of DNA bases, cytosine and adenine, at specific places in the mouse genome. For this, they generated their own test DNA edits and also analyzed DNA editing done by others, for a total of 430 base edits examined. They found that the adenine base editor tested was both accurate and precise, but that the cytosine base editors, in general, were less faithful. However, two of the five cytosine base editors tested resulted in higher frequencies of

the intended genomic changes and caused fewer unintended changes than did the other three. This study furthers the potential of base editors over other genetic editing technologies that routinely introduce unintended edits. These results provide scientists with important information to consider when deciding which technology to choose for research and, potentially, for future clinical applications.

Lee HK, Willi M, Miller SM,...Hennighausen L. Targeting fidelity of adenine and cytosine base editors in mouse embryos. *Nat Commun* 9: 4804, 2018.

## UNDERSTANDING GENETIC UNDERPINNINGS OF A RARE DISEASE

**An Unusual Cause of a Rare Disease—Finding a Needle in an Expanded Haystack:** Researchers have discovered that a rare disease can be caused by an unusual genetic mechanism. Roughly 2 percent of human DNA contains genes that code for proteins, which are critical to all cellular structure and function. Thus, much disease research, especially for rare genetic diseases, has focused on sequencing only this subset of a person’s genome—collectively called the “exome”—as many diseases are rooted in variants found in the protein-encoding genes, and narrowing the search this way can save a great deal of time. However, the remaining 98 percent of the genome does include some vital stretches of DNA. For example, certain regions that do not encode proteins themselves can have a large impact on how much protein gets produced by nearby genes or may determine which specific organs and cell types produce them. Exome sequencing alone can therefore miss critical variations in such regulatory DNA regions.

In the current study, researchers studying rare diseases wished to determine why three unrelated young children from different parts of Europe and Canada had experienced similar developmental delay and neurological problems. The first clue came with the discovery that each child had much higher than normal blood levels of glutamine, one of the amino acid building blocks of proteins. Exome sequencing did not provide a clear explanation, but one finding of the analysis did stand out: two of the children were found to have a rare variant in one of their two copies of the gene for the protein glutaminase, an enzyme that is needed to break glutamine down into other amino acids. However, each of these children also had an apparently normal version of the gene as well, and in the third child, both copies of the gene were normal. Intriguingly, the scientists then discovered



that, despite having at least one normal glutaminase gene, all three children had extremely low levels of glutaminase activity. Reasoning that non-coding variation might be playing a role, they sequenced the whole area around the glutaminase gene. In the great majority of people, an area adjacent to the gene has between 8 and 20 repeats of 3 of the 4 chemical bases that make up DNA—designated by the letters G, C, and A—in that order. In dramatic contrast, the normal glutaminase genes in the three children were accompanied by a much larger than normal number of the GCA repeats, ranging from 680 in 1 child to about 1,500 in another. These additional repeats had

the effect of preventing cells from properly producing glutaminase. Unfortunately, there is currently no way to treat glutaminase deficiency, but this research provides a compelling case for expanding the search when exome sequencing alone is not enough to identify genetic causes of disease. Thus, this research may spur discovery in a range of other genetic diseases.

*van Kuilenburg ABP, Tarailo-Graovac M, Richmond PA,...van Karnebeek CDM. Glutaminase deficiency caused by short tandem repeat expansion in GLS. N Engl J Med 380: 1433-1441, 2019.*

# NIDDK Celebrates Its 70<sup>th</sup> Anniversary



National Institute of  
Diabetes and Digestive  
and Kidney Diseases



In 2020, the NIDDK celebrates 70 years since its founding in August 1950 (see “History of the NIDDK” inset). Over the course of its history, the Institute that is known today as the National Institute of Diabetes and Digestive and Kidney Diseases is proud to have supported and conducted research on many of the Nation’s most serious chronic diseases. Affecting people of all ages and racial and ethnic groups, the diseases within the NIDDK research mission encompass some of the most common, costly, and disabling conditions, as well as less prevalent but nonetheless debilitating diseases, affecting Americans today: endocrine and metabolic diseases and disorders such as diabetes and obesity, digestive diseases such as nonalcoholic fatty liver disease and inflammatory bowel disease, chronic kidney disease and kidney failure, urologic diseases and conditions such as interstitial cystitis/ bladder pain syndrome and benign prostate enlargement, and blood diseases such as anemias.

The research advances made possible through 70 years of NIDDK support have saved lives, improved quality of life, and laid the foundation for future progress. The Institute has supported

a number of winners of the world’s greatest scientific honors. Many have won the Nobel Prize in Physiology or Medicine, and others have received the Nobel Prize in Chemistry. These include extramural scientists at universities and other research institutions across the country who have been supported by the NIDDK (Institute grantees), as well as scientists within the Institute’s Intramural Research Program.

As part of activities to mark its 70<sup>th</sup> anniversary, starting in the summer of 2020, the NIDDK will highlight the research accomplishments supported over the past seven decades, and how they inform the Institute’s current activities and vision for the future. These communications will be highlighted on the NIDDK website and in social media, and additional communications will take place through such venues as the “Healthy Moments” radio broadcast featuring the NIDDK Director. During the 70<sup>th</sup> anniversary year, the NIDDK will also undertake the development of its first-ever, Institute-wide strategic plan, which will complement disease-specific planning efforts and help guide research planning across its mission.

*History of the NIDDK:* On August 15, 1950, President Harry S. Truman signed into law the Omnibus Medical Research Act, establishing the National Institute of Arthritis and Metabolic Diseases (NIAMD)—which would become today’s NIDDK. The new Institute incorporated the laboratories of the Experimental Biology and Medicine Institute and expanded to include clinical investigation in rheumatic diseases, diabetes, and a number of metabolic, endocrine, and gastrointestinal diseases. That same year, the NIAMD Council held its first meeting and recommended approval of NIAMD’s first grants. Over the years, the NIAMD evolved into the National Institute of Arthritis, Metabolism, and Digestive Diseases (in 1972) and the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases (in 1981). In 1986, the Institute’s Division of Arthritis, Musculoskeletal and Skin Diseases became the core of a new, independent Institute. The NIDDK then acquired its current name—the National Institute of Diabetes and Digestive and Kidney Diseases.

# NIDDK-supported Scientists Receive Presidential Award

Scientists supported by the NIDDK were among the recipients of the Presidential Early Career Award for Scientists and Engineers (PECASE) in 2019. The PECASE is awarded to scientists and engineers who, while early in their research careers, have pursued innovative research and shown outstanding scientific leadership. Current NIDDK extramural grantees who received the PECASE include Melena Bellin, M.D.; Zachary Knight, Ph.D.; Sandeep Mallipattu, M.D.; and Jason Wertheim, M.D., Ph.D. NIDDK Intramural Research Program scientist Katherine McJunkin, Ph.D., was also a PECASE recipient.



Dr. Bellin, an Associate Professor in the Departments of Pediatrics and Surgery at the University of Minnesota Medical School, received a PECASE award in recognition of her work on optimizing a surgical treatment for severe

chronic pancreatitis, called total pancreatectomy-islet autotransplantation, in which the pancreas is removed but patients are given back their insulin-producing pancreatic islets to reduce risk of diabetes. Determining which patient and disease characteristics are associated with better resolution of pain and health-related quality of life with this procedure, optimal timing for performing it, and improved methods for preserving the islets may lead to new strategies to address severe chronic pancreatitis. (See the Diabetes, Endocrinology, and Metabolic Diseases chapter for a profile of an individual who underwent this procedure.)



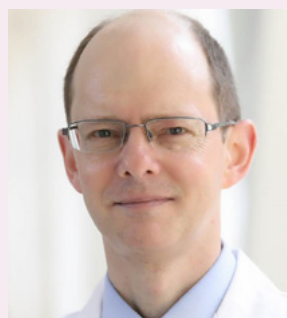
Dr. Knight, an Associate Professor in the Department of Physiology at the University of California, San Francisco, received a PECASE award in recognition of his work on neural mechanisms in the brain that control

hunger, thirst, and thermoregulation. One facet of his research indicates that certain pathways regulating food intake are affected by the mere presence of food even before it is eaten; new knowledge about how the brain regulates behavior in response to food and other cues can help pave the way to new approaches to prevent or treat obesity.



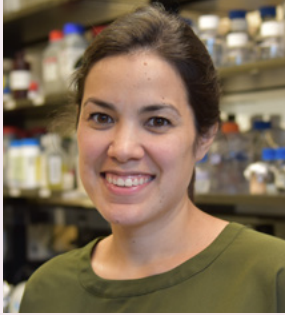
Dr. Mallipattu, Chief of the Division of Nephrology and Hypertension and the DCI-Martin R. Liebowitz Associate Professor of Medicine at Stony Brook University, received a PECASE award in recognition of his work

on mechanisms that regulate the proliferation and differentiation of certain cells in the kidney, especially as they relate to the progression of chronic kidney disease in people with diabetes. These efforts, combined with the ability to grow kidney cells in the laboratory into three-dimensional structures, could someday lead to being able to build a functional kidney—a potential treatment approach for kidney injury and disease.



Dr. Wertheim, Vice Chair for Research, Department of Surgery, and an Associate Professor in the Feinberg School of Medicine and the McCormick School of Engineering, Northwestern University, received a PECASE award

in recognition of his work on how injured tissues and organs heal, regenerate, and repair, with a focus on bioengineering approaches tailored to treat liver and kidney damage and disease. This research could help to develop new tissues and regenerative approaches as future treatments for chronic organ failure.

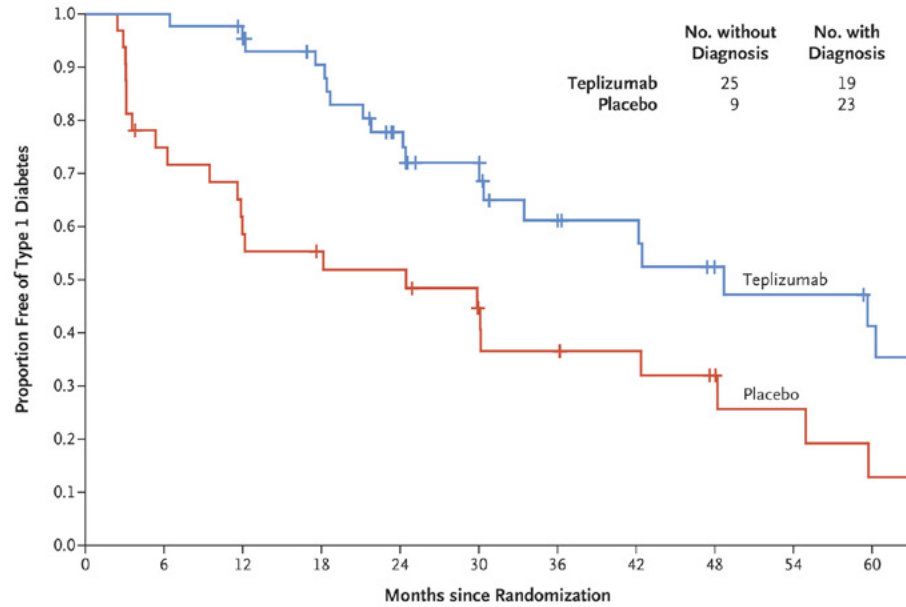
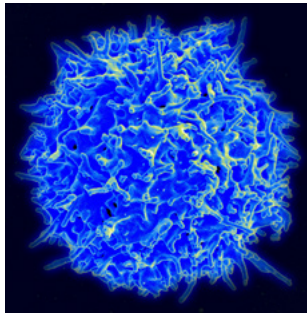


Dr. McKunkin, Acting Chief, Section on Regulatory RNAs, Laboratory of Cellular and Developmental Biology at NIDDK, received a PECASE award in recognition of her work on the role of microRNAs in embryonic

development and how these critical molecules—and hence, the biological pathways they help govern in cells—are themselves regulated over time. This research could ultimately lead

to a better understanding of normal human development and of how various diseases arise.

In addition to these investigators, other scientists supported by the NIH and other federal agencies also received the PECASE for their scientific achievements. The PECASE is the most prestigious award given by the U.S. government to scientists at the outset of their independent research careers. These awards support the continued professional development of awardees, promote careers, foster innovation in science and technology, and recognize the scientific missions of participating agencies.



As described in this chapter, a clinical trial conducted by NIDDK's Type 1 Diabetes TrialNet has demonstrated that early preventive treatment with a drug called teplizumab slowed the progression to clinical type 1 diabetes in high-risk individuals without diabetes. Teplizumab targets T cells of the immune system (depicted in left image), which are known to play a role in the type 1 diabetes autoimmune attack. Data from the clinical trial are illustrated in the graph, which shows that over time, more people in the teplizumab treatment group (blue line) were free of type 1 diabetes (*i.e.*, were not diagnosed with the disease) compared to those in the placebo group (red line). This exciting discovery provides the first evidence that clinical type 1 diabetes can be delayed with early preventive treatment.

Graph courtesy of Dr. Kevan Herold, Yale University. From *The New England Journal of Medicine*, Herold KC, Bundy BN, Long SA,...Greenbaum CJ; Type 1 Diabetes TrialNet Study Group, An anti-CD3 antibody, teplizumab, in relatives at risk for type 1 diabetes, 381: 603-613. Copyright © 2019 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society. T cell image courtesy of the National Institute of Allergy and Infectious Diseases/National Institutes of Health.



# Diabetes, Endocrinology, and Metabolic Diseases

*NIDDK support of basic and clinical research in the areas of diabetes, endocrinology, and metabolic diseases spans a vast and diverse range of diseases and conditions, including diabetes, osteoporosis, cystic fibrosis, and obesity. Together, these diseases and conditions affect many millions of Americans and can profoundly decrease quality of life. Many of these diseases are complex—an interplay between genetic and environmental factors contributes to disease development.*

Not only is diabetes chronic and relentless, but its slow accumulation of insults to the body can rob a person of the ability to see, hear, feel, think, and walk. In addition to increasing the risk for complications of vision loss, kidney failure, and amputation, diabetes doubles risk for heart disease, many forms of cancer, some forms of dementia, hearing loss, erectile dysfunction, urinary incontinence, and many other common diseases.<sup>1</sup> NIDDK is vigorously pursuing research to combat diabetes and its associated health consequences.

Diabetes is a debilitating disease that affects an estimated 30.3 million people in the United States—or 9.4 percent of the total population—and is the seventh leading cause of death.<sup>2</sup> Although overall rates of diabetes-related complications have declined substantially in recent years, disease burden remains significant as the number of people with diabetes is still very high.<sup>1</sup> Diabetes can affect many parts of the body and is associated with serious complications, such as heart disease and stroke, blindness, kidney failure, and lower-limb amputation. In addition to these human costs, the estimated total financial cost for diagnosed diabetes in the United States in 2017—including costs of medical care, disability, and premature death—was \$327 billion.<sup>3</sup> Effective therapy can prevent or delay diabetic complications, but nearly one-quarter of Americans with diabetes are undiagnosed and therefore not receiving therapy.<sup>2</sup>

Diabetes is characterized by the body's inability to produce and/or respond appropriately to insulin, a hormone that is necessary for the body to absorb and use glucose (sugar) as a cellular fuel. These defects result in persistent elevation of blood glucose levels and other metabolic abnormalities, which in turn lead to the development of disease complications. The most common forms of diabetes are type 1 diabetes, in which the body loses its ability to produce insulin, and type 2 diabetes, in which the

body becomes resistant to insulin signaling, with subsequent impaired insulin production. In addition, a significant proportion of pregnant women each year are diagnosed with gestational diabetes, a form of diabetes that develops during pregnancy, but in many cases may resolve after pregnancy. However, women who develop gestational diabetes are at greater risk of developing type 2 diabetes later in life. Untreated, any form of diabetes during pregnancy increases the risk of serious complications for the mother and baby before, during, and after delivery.

Type 1 diabetes, formerly known as juvenile diabetes, affects approximately 5 percent of diagnosed diabetes cases in adults, and the majority of diagnosed cases in children and youth.<sup>2</sup> It most often develops during childhood but may appear at any age. Type 1 diabetes is an autoimmune disease in which the immune system launches a misguided attack and destroys the insulin-producing  $\beta$  (beta) cells of the pancreas. If left untreated, type 1 diabetes results in death: without insulin, glucose is not transported from the bloodstream into the body's cells, where it is needed.

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<sup>1</sup> *Diabetes in America*, 3<sup>rd</sup> ed. Cowie CC, et al., Eds. Bethesda, MD, National Institutes of Health, NIH Pub No. 17-1468, 2018.

<sup>2</sup> Centers for Disease Control and Prevention. *National Diabetes Statistics Report, 2017*. Atlanta, GA: Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, 2017.

<sup>3</sup> American Diabetes Association. *Diabetes Care* 41: 917-928, 2018.

This disruption of the body's metabolism causes a biochemical chain reaction that can result in a life-threatening condition called diabetic ketoacidosis (DKA). DKA can be deadly if it is not aggressively treated with insulin. Thus, people with type 1 diabetes require lifelong insulin administration—in the form of multiple daily injections or via an insulin pump—to regulate their blood glucose levels. The NIDDK's landmark Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) study demonstrated that keeping blood glucose levels as near to normal as safely possible reduced the risk of eye, kidney, nerve, and heart complications associated with type 1 diabetes. However, despite vigilance in disease management and current technologies to test blood glucose levels and administer insulin, it is still not possible for people with type 1 diabetes to manage blood glucose levels as well as functional pancreatic  $\beta$  cells do. Thus, researchers are actively seeking new methods to improve blood glucose monitoring and insulin delivery. In this regard, NIDDK-supported research has contributed to the development or testing of new diabetes management technologies recently approved by the U.S. Food and Drug Administration, including the first commercial "hybrid artificial pancreas" device that automatically links glucose monitoring and insulin delivery, and next-generation continuous glucose monitors, including the first fully implantable device. Researchers are also working to develop  $\beta$  cell replacement therapies, such as islet transplantation, to cure type 1 diabetes.

Type 2 diabetes is the most common form of the disease, accounting for about 90 to 95 percent of diagnosed diabetes cases in U.S. adults.<sup>2</sup> The risk for developing type 2 diabetes is associated with older age, obesity, family history of diabetes, history of gestational diabetes, impaired glucose metabolism, physical inactivity, and race/ethnicity.<sup>3</sup> Type 2 diabetes occurs at higher rates among racial and ethnic minority populations in the United States, including African Americans, Hispanic and Latino Americans, American Indians, some Asian Americans, and Native Hawaiians and Pacific Islanders.<sup>2</sup> Gestational diabetes is also a risk factor: about half of women with gestational diabetes will develop type 2 diabetes within 5 to 10 years after giving birth.<sup>4</sup>

In people with type 2 diabetes, cells in muscle, fat, and liver tissue do not properly respond to insulin. As a result, the pancreas initially produces more insulin to compensate. Gradually, however, the

pancreatic  $\beta$  cells lose their ability to secrete enough insulin to restore balance, and the timing of insulin secretion becomes abnormal, causing blood glucose levels to rise. Treatment approaches for managing glucose levels include diet, exercise, and oral and injected medications, with insulin often required as the disease progresses. There are also an estimated 84 million U.S. adults who have a condition called "prediabetes," in which blood glucose levels are higher than normal but not as high as in diabetes.<sup>2</sup> This population is at elevated risk of developing type 2 diabetes. Fortunately, the NIDDK-supported Diabetes Prevention Program (DPP) clinical trial has shown that people with prediabetes can dramatically reduce their risk of developing type 2 diabetes with diet and exercise changes designed to achieve a 7 percent reduction in body weight. To a more limited degree, the safe and well-tolerated drug metformin can also help prevent or delay type 2 diabetes. Moreover, follow-up research has shown that the benefits of reduced diabetes risk from weight loss or metformin can persist for at least 15 years.

Type 2 diabetes was previously called "adult-onset" diabetes because it is predominantly diagnosed in older individuals. However, this form of diabetes is increasingly being diagnosed in children and adolescents, and in this population it disproportionately affects youth from racial and ethnic minority populations in the United States. Believed to be related to increasing rates of pediatric obesity, this trend is alarming for many reasons. For example, results from the NIDDK-supported Treatment Options for type 2 Diabetes in Adolescents and Youth (TODAY) clinical trial and the Restoring Insulin Secretion (RISE) Pediatric Medication Study showed that the disease may be more aggressive and difficult to treat in youth compared to adults. This is worrisome because the onset and severity of disease complications correlate with diabetes duration and management of blood glucose levels, so those with early disease onset are at especially high risk for developing complications. In addition, increasing rates of type 2 diabetes in girls may lead to more women who enter pregnancy with diabetes, and maternal diabetes during pregnancy—either onset of type 2 diabetes before pregnancy or the development of gestational diabetes during pregnancy—confers an increased risk of type 2 diabetes in offspring. Thus, the rising rates of diabetes and prediabetes in young

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<sup>4</sup> Kim C, et al. *Diabetes Care* 25: 1862-1868, 2002.

women could contribute to a cycle of ever-growing rates of diabetes. Therefore, the advent of type 2 diabetes in youth has the potential to worsen the enormous health burden that diabetes already places on the United States.

The NIDDK is supporting research to better understand metabolism and the mechanisms that lead to the development and progression of diabetes and the many other endocrine and metabolic diseases within the NIDDK's mission; such research will ultimately spur the design of potential new intervention strategies. In parallel, based on knowledge from past scientific research investments, the NIDDK is vigorously pursuing studies of prevention and treatment approaches for these diseases.

## RESEARCH ON TYPE 1 DIABETES

### Drug Delays Type 1 Diabetes in People at High Risk:

Results from a recent clinical trial have demonstrated that a treatment targeting the immune system slowed the progression to clinical type 1 diabetes in high-risk individuals. Currently, there is no way to prevent type 1 diabetes, which is caused by an autoimmune attack that destroys the insulin-producing  $\beta$  (beta) cells in the pancreas. Previous research demonstrated that treatment with an anti-CD3 monoclonal antibody (teplizumab) slows  $\beta$  cell loss in people with recent-onset clinical type 1 diabetes; teplizumab targets immune system cells (T cells) that are known to play a role in the autoimmune attack. However, the drug had never been tested in people without clinical disease to see if it could also slow  $\beta$  cell loss earlier in the course of type 1 diabetes and thus prevent clinical disease onset.

To address this gap in knowledge, Type 1 Diabetes TrialNet conducted a trial in which they enrolled 76 mostly White, female and male participants aged 8 to 49 years who were relatives of people with type 1 diabetes; over 70 percent of the participants were 18 years old or younger. The participants did not have clinical type 1 diabetes, but were at high risk for developing it because they had at least two types of diabetes-related autoantibodies (proteins made by the immune system) and abnormal glucose (sugar) tolerance. Participants were randomly assigned to receive either a 14-day course of teplizumab or placebo (no medicine), administered intravenously. The results were striking: during the trial, 72 percent of people in the placebo group developed clinical

type 1 diabetes, compared to only 43 percent of the teplizumab group. The median time for people in the placebo group to develop clinical disease was just over 24 months, compared to 48 months in the treatment group. The study is the first to show that clinical type 1 diabetes can be delayed by 2 or more years among people who are at high risk.

The researchers noted that the study had limitations, including the small number of participants, their lack of ethnic diversity, and that all participants were relatives of people with type 1 diabetes, potentially limiting the ability to translate the study to a broader population of people who do not have relatives with the disease but do have other risk factors. Future research could help to address these limitations, as well as shed light on teplizumab's mechanism of action and long-term effects, to build on this exciting finding that clinical type 1 diabetes can be delayed with early preventive treatment.

*Herold KC, Bundy BN, Long SA,...Greenbaum CJ; Type 1 Diabetes TrialNet Study Group. An anti-CD3 antibody, teplizumab, in relatives at risk for type 1 diabetes. *N Engl J Med* 381: 603-613, 2019.*

### Type 1 Diabetes Study Provides New Details on Development of Children's Gut Microbiome:

Researchers investigating possible causes of type 1 diabetes have published new details about how environmental factors such as breastfeeding affect the microbes in the gut (*i.e.*, the gut "microbiome") as children age. The Environmental Determinants of Diabetes in the Young (TEDDY) is an international study investigating what environmental factors might trigger or protect against type 1 diabetes. TEDDY is following almost 6,000 children at high genetic risk of developing type 1 diabetes from their birth through age 15. One factor of interest to TEDDY researchers is the gut microbiome. Previous studies suggested that crosstalk between the immune system and gut microbes in infancy and childhood can be linked to immune-related diseases later in life, such as the autoimmune attack on pancreatic beta cells that occurs during type 1 diabetes. TEDDY scientists are interested in whether or not changes in the gut microbiome affect a child's risk for developing type 1 diabetes. To investigate this, they analyzed donated stool samples from hundreds of girls and boys participating in TEDDY, identifying the bacteria in the children's gut microbiomes and tracking how the types and abundance of these bacteria changed as the children grew. Information on the children's health, diet, geographical location, and other environmental

exposures was then used to explore whether these factors may have affected the gut microbiome and how those effects correlated with the children's type 1 diabetes status.

From this research, one of the largest-ever clinical microbiome studies in infants and children, TEDDY scientists identified three distinct phases of gut microbiome development: a developmental phase (3-14 months of age), a transitional phase (15-30 months of age) where the microbiome diversifies, and a stable phase (31-46 months of age) where the microbiome's composition is largely established. Within these phases, researchers found considerable personal variability between gut microbiomes. Breastfeeding was the most important factor associated with how the gut microbiome developed in the first years of life. For example, the microbiome of infants not receiving breast milk moved more quickly through the developmental phases compared to children receiving any amount of breast milk, even if supplemented with formula or solid foods. Some of the other factors that affected the gut microbiome included probiotic and antibiotic use, geographical location, and having siblings and/or furry pets. Finally, researchers found a possible beneficial effect on risk for type 1 diabetes from bacteria that produce short-chain fatty acid molecules. These molecules are often made during fermentation of indigestible carbohydrates like fiber, and future research will be needed to determine whether these molecules or the bacteria that produce them protect against type 1 diabetes.

The TEDDY cohort is largely White and non-Hispanic, so further work will be needed to determine if these discoveries are applicable to all children. However, these new findings could help inform the development of strategies or therapies to support the development of a healthy gut microbiome in children. They also demonstrate how the ambitious TEDDY study is already expanding our knowledge of child development and the human microbiome, while continuing its search for causative and protective factors for type 1 diabetes.

*Stewart CJ, Ajami NJ, O'Brien JL,...Petrosino JF. Temporal development of the gut microbiome in early childhood from the TEDDY study. [Nature](#) 562: 583-588, 2018.*

*Vatanen T, Franzosa EA, Schwager R,...Xavier RJ. The human gut microbiome in early-onset type 1 diabetes from the TEDDY study. [Nature](#) 562: 589-594, 2018.*

### **Novel Imaging Technology Sheds Light on How**

**Type 1 Diabetes Progresses:** Two studies have used a sophisticated novel imaging technology to visualize the pancreas and gain new insights into how type 1 diabetes progresses—knowledge that could inform strategies to prevent or halt the disease. Type 1 diabetes is an autoimmune disease in which the immune system destroys the insulin-producing  $\beta$  (beta) cells found in clusters called islets in the pancreas. In two recent studies, researchers used a novel technology, called imaging mass cytometry, to analyze pancreases from female and male organ donors with type 1 diabetes of varying disease duration, as well as female and male donors without the disease. The technology allowed the scientists to measure simultaneously over 30 cellular markers and visualize at a single-cell level not only  $\beta$  cells and other pancreatic cell types, but also immune cells involved in the autoimmune attack. This novel technology enabled an unprecedented look not only at the numbers and characteristics of various cell types in the pancreas, but also at how cells interacted with each other, and provides an exciting resource for future studies.

Both studies confirmed that there were significant differences in the number and types of cells found in islets from different individuals. The studies also confirmed that  $\beta$  cell numbers were reduced in pancreases from donors with type 1 diabetes compared to those without disease, although there were differences observed among the donors. For instance, a surprising finding from one of the studies was that two donors with new-onset disease had a similar proportion of  $\beta$  cells in their pancreases as those without disease. This finding suggests that even at type 1 diabetes onset when people are showing clinical symptoms and need to take insulin to lower their blood glucose (sugar) levels, their pancreases may still have high numbers of  $\beta$  cells. Further experiments also suggested that, as type 1 diabetes progresses, the  $\beta$  cells go through an altered state, in which they display fewer characteristic features of  $\beta$  cells, before they are destroyed by the immune system.

The technology also enabled scientists in both studies to begin exploring the immune cell environment that plays a role in type 1 diabetes—looking at many different immune cell types at the same time and cataloging the number and timing of immune cell interactions with pancreatic cells.

For example, one finding was that certain types of immune cells (T cells) were abundant in people with new-onset type 1 diabetes and less abundant in people with long-standing disease, suggesting that the immune system attack is maximal around the time of disease onset and that immune cells leave islets after  $\beta$  cells are destroyed.

By using imaging mass cytometry, these research groups not only painted a new and more vivid picture of type 1 diabetes disease progression, but also demonstrated the promise of this technology to garner new knowledge about the type 1 diabetes disease process. Results from these and future studies could help to inform the development of new therapies to prevent or treat the disease.

*Damond N, Engler S, Zanotelli VRT,...Bodenmiller B. A map of human type 1 diabetes progression by imaging mass cytometry. Cell Metab 29: 755-768.e5, 2019.*

*Wang YJ, Traum D, Schug J,...Kaestner KH. Multiplexed in situ imaging mass cytometry analysis of the human endocrine pancreas and immune system in type 1 diabetes. Cell Metab 29: 769-783.e4, 2019.*

## RESEARCH ON TYPE 2 DIABETES

### Study Shows Vitamin D Supplementation Has Little or No Effect on Type 2 Diabetes Prevention:

In the largest study to directly examine if daily vitamin D supplementation helps prevent or delay type 2 diabetes in people at high risk for the disease, scientists found no meaningful difference in the rate of type 2 diabetes development between those taking the vitamin D supplement and those taking a placebo pill daily. Previous observational studies reported an association between low levels of vitamin D and increased risk for type 2 diabetes, and smaller studies found that vitamin D could improve the function of beta cells, which produce insulin. To determine whether vitamin D supplementation could prevent type 2 diabetes, the Vitamin D and Type 2 Diabetes (D2d) study enrolled 2,423 adults and was conducted at 22 sites across the United States. All participants had blood glucose (sugar) levels higher than normal but not high enough to be diagnosed with type 2 diabetes, and the group included a range of physical characteristics, including sex, age, and body mass index (a measure of weight relative to height), as well as racial and ethnic diversity, to ensure that the study findings could be widely applicable to people at high risk for

developing type 2 diabetes. All study participants had their blood levels of vitamin D measured at the start of the study. At that time, about 80 percent of the participants had vitamin D levels that were considered sufficient at the time.

The study assigned participants randomly to take either 4,000 International Units (IUs) of the D3 (cholecalciferol) form of vitamin D—greater than the average daily recommended intake of 600 to 800 IUs a day, but within limits deemed appropriate for clinical research at the time—or a placebo pill daily. The study screened participants every 3 to 6 months for an average of 2.5 years to determine if they had developed diabetes. At the end of the study, 293 of the 1,211 participants (24.2 percent) in the vitamin D group developed diabetes compared to 323 out of the 1,212 (26.7 percent) in the placebo group. Although a lower proportion of those taking vitamin D developed diabetes, the difference was too small to reach statistical significance. The researchers found no meaningful differences between the two groups regardless of age, sex, race or ethnicity, and they saw no difference in the number and frequency of predicted side effects when they compared the two groups. With the rising numbers of people at high risk for type 2 diabetes, efforts will continue to search for new ways to prevent the disease.

*Pittas AG, Dawson-Hughes B, Sheehan P,...Staten M; for the D2d Research Group. Vitamin D supplementation and prevention of type 2 diabetes. N Engl J Med 381: 520-530, 2019.*

### Study in African Populations Leads to Identification of Type 2 Diabetes Risk Gene:

In a genetic study with participants from sub-Saharan Africa, researchers discovered that variants of a gene not previously known to be associated with type 2 diabetes affect risk for the disease, and also found evidence for how the normal version of the gene may promote metabolic health. To date, genome-wide association studies (GWAS) have identified over 400 genetic regions that may affect risk for type 2 diabetes. However, the majority of GWAS have included at least some people of European descent, and little research has been done focusing exclusively on people of African origin, even though evidence suggests Africans have elevated genetic risk for type 2 diabetes.

In this new study with more than 5,000 female and male participants from Nigeria, Ghana, and Kenya,



variants in or near the gene *ZRANB3* were found to be associated with risk for the disease (as were variants of some genes previously associated with type 2 diabetes through studies in other parts of the world). To determine whether variations in *ZRANB3* itself were responsible for differences in diabetes risk—rather than differences in some other, nearby gene—the scientists sought to learn whether *ZRANB3* may have a role in the body's insulin-producing  $\beta$  (beta) cells. For this analysis, they used—as an experimental model—a strain of zebrafish that produce a fluorescent dye in their  $\beta$  cells so that the cells are comparatively easy to detect and count in the small, partially transparent fish. Targeted elimination of the zebrafish version of *ZRANB3* led to fish developing with about 30 percent fewer  $\beta$  cells, without causing any other obvious effects. Detailed analysis of data from these experiments suggests that initially a normal number of  $\beta$  cells may have been forming, but that some of them died during later development. Another set of experiments showed that the protein encoded by the *ZRANB3* gene—designated ZRANB3—may play an important role in mature  $\beta$  cells, as well:  $\beta$  cells isolated from a mouse pancreas did not secrete as much insulin as they should in response to high glucose (sugar) levels if they lacked the protein. Taken together, these results suggest that the ZRANB3 protein plays key roles in  $\beta$  cell biology and may one day be the target of therapies that help people with diabetes, be they from Africa or elsewhere.

Adeyemo AA, Zaghoul NA, Chen G,...Rotimi CN. *ZRANB3* is an African-specific type 2 diabetes locus associated with beta-cell mass and insulin response. *Nat Commun* 10: 3195, 2019.

### Predicting an Individual's Response to a Diabetes

**Drug:** Scientists demonstrated that genetic variation predicts individual responsiveness to the antidiabetic drug rosiglitazone. Rosiglitazone reverses insulin resistance in type 2 diabetes, but its use is limited due to its significant side effects, which can include an increased risk of heart attack and stroke. Additionally, rosiglitazone's beneficial insulin-sensitizing effect is seen in most but not all people, and scientists have been seeking to understand the reasons for these differential responses. With such knowledge, it might one day be possible to develop diagnostic tests to identify who will respond best to the drug and/or have the fewest side effects. In this study, a group of researchers developed a strategy—using stem-cell generated cells to identify human genetic variation—to study the differential response

to rosiglitazone and, in doing so, revealed genetic predictors of an adverse response to the drug.

The researchers generated adipocyte (fat) cell lines from tissue samples from five women who had obesity, treated the cell lines with rosiglitazone, and identified genes that were “turned on” in each of the cell lines in response to the treatment. Interestingly, each cell line showed a unique signature of genes that were turned on. For example, 87 genes that were activated in 4 of the lines remained inactive in the fifth; on the other hand, 399 of the genes that were activated by rosiglitazone in 1 of the cell lines remained inactive in the other 4. Because rosiglitazone is known to activate a protein called PPAR $\gamma$ , which binds to DNA to turn genes on, the scientists examined whether genetic differences between the participants affected PPAR $\gamma$  binding in these cell lines. They found specific genetic differences that not only altered PPAR $\gamma$  binding sites, but also accounted for corresponding differences in the responsiveness to rosiglitazone.

One genetic variation was of particular interest to the scientists because it was near a PPAR $\gamma$ -regulated gene whose protein is known to affect cholesterol levels. Treatment with rosiglitazone commonly leads to higher total cholesterol and low-density lipoprotein cholesterol, which may contribute to heart attacks and strokes among people taking the drug, but it is not well understood how rosiglitazone affects cholesterol levels. The scientists found that having a specific genetic variant (designated “C”) correlated with PPAR $\gamma$  responsiveness to rosiglitazone in that the nearby cholesterol-affecting gene was turned on, while it was not turned on in individuals with a different variant, “A.” The researchers went on to show that these genetic variants also affected cholesterol levels. People with the C variant had higher levels of total and low-density lipoprotein cholesterol in response to rosiglitazone while people who had the A variant near both copies of the cholesterol-affecting gene (cells have two copies of this gene) received the benefits of rosiglitazone treatment (lower blood glucose levels), but much less of the drug's cholesterol-elevating side effect. This result suggests it may one day be possible for clinicians to identify people who may benefit from rosiglitazone without the adverse effects on their cholesterol levels. The study also presents an approach to identify how human genetic variation determines response to a drug and may be an important tool for understanding drug responses in other diseases.

Hu W, Jiang C, Guan D,...Lazar MA. Patient adipose stem cell-derived adipocytes reveal genetic variation that predicts antidiabetic drug response. *Cell Stem Cell* 24: 299-308.e6, 2019.

## RESEARCH ON DIABETES COMPLICATIONS

**Long-term Type 1 Diabetes Study Reveals Immune System Links Between Blood Glucose Management and Heart Health:** Two recent studies have found new connections between blood glucose (sugar) management and heart health, which may explain the increased risk of cardiovascular diseases (heart disease and stroke) in those with type 1 diabetes. Previous insights about type 1 diabetes and cardiovascular health have come from the landmark Diabetes Control and Complications Trial (DCCT) and its follow-up, the Epidemiology of Diabetes Interventions and Complications (EDIC) study. DCCT/EDIC demonstrated that early and intensive blood glucose control lowered the risk of cardiovascular diseases and cardiovascular-related deaths, though exactly how blood glucose levels affect cardiovascular health was not fully understood.

In new research, scientists further studied the health of DCCT/EDIC participants to examine how blood glucose management influenced these people's risk of cardiovascular disease. In particular, researchers wondered if that risk was mediated through known cardiovascular factors or if there were unknown, diabetes-specific mechanisms at play, as well. The DCCT/EDIC research group investigated this question by analyzing whether or not an increased risk of heart problems could be accounted for by factors such as blood pressure, pulse rate, cholesterol levels, and/or measures of kidney function. Their analyses demonstrated that although having higher blood glucose levels over time was associated with many traditional risk factors for cardiovascular disease, these associations could not completely explain the increased risk seen in some DCCT-EDIC participants. This raised the question of what other factors were mediating the effect of high blood glucose levels on heart health.

Another research group hypothesized that the link between blood glucose levels and the heart is mediated by the immune system. By analyzing biological samples from a subset of DCCT participants, as well as samples from people with type 2 diabetes, scientists found signs of cardiac

autoimmunity—i.e., the presence of at least two cardiac autoantibody types—in people who had type 1 diabetes and elevated blood glucose levels (measured as an HbA1c greater than 9 percent). These autoantibodies were not found in people with type 2 diabetes who had similar blood glucose levels. People with type 1 diabetes and cardiac autoimmunity also had a higher risk of both accelerated atherosclerosis and cardiovascular events. Since cardiac autoantibodies developed decades before the cardiovascular complications, such autoantibodies might be useful as early biomarkers of cardiovascular disease risk specifically in people with type 1 diabetes. This study also suggested a new role for autoimmune mechanisms, possibly mediated by inflammation, in the development of cardiovascular complications of type 1 diabetes.

These results break new ground in the study of type 1 diabetes complications, identifying a novel cardiovascular disease pathway specific to type 1 diabetes and further emphasizing the importance of keeping blood glucose levels within a healthy range. More study is needed to clarify how exactly blood glucose levels affect heart health. Such studies could also lead to new insights into cardiovascular disease itself and to methods to detect, prevent, or treat cardiovascular complications in people with type 1 diabetes.

Sousa GR, Pober D, Galderisi A,...Lipes MA. Glycemic control, cardiac autoimmunity, and long-term risk of cardiovascular disease in type 1 diabetes mellitus. *Circulation* 139: 730-743, 2019.

Bebu I, Braffett BH, Orchard TJ, Lorenzi GM, and Lachin JM; DCCT/EDIC Research Group. Mediation of the effect of glycemia on the risk of CVD outcomes in type 1 diabetes: The DCCT/EDIC study. *Diabetes Care* 42: 1284-1289, 2019.

## GESTATIONAL DIABETES RESEARCH

**Folate Supplements May Help Reduce Risk of Gestational Diabetes:** New findings from a long-term study in thousands of women suggest that pre-pregnancy dietary supplementation with folate, a B vitamin, can reduce risk of developing gestational diabetes. Gestational diabetes (GDM) is a form of diabetes currently diagnosed during the late second or the third trimester of pregnancy and affects approximately 7 percent of pregnancies in the United States. GDM increases health risks for mothers and their babies both during pregnancy and delivery (e.g., high birth weight babies, delivery complications) and later on in life (e.g., greater risk of type 2 diabetes in

mothers and obesity and/or type 2 diabetes in the child). Research on GDM aims to better understand why and how it occurs and to try to find ways to prevent it. In the present study, researchers examined whether or not risk of developing GDM correlated with the self-reported folate intake—both from food sources and from folate dietary supplements—in over 14,000 women who became pregnant while enrolled in the Nurses' Health Study II over a 10-year period. After controlling for known risk factors for GDM, they found that, when consumed as a dietary supplement, folate was associated with decreased risk of GDM. Moreover, the GDM risk fell as the level of supplementation increased from adequate—i.e., the U.S. recommended daily allowance level—to somewhat higher levels. In contrast, folate from food sources was not found to reduce risk, an observation the researchers attributed to the body being able to use a lower proportion of the folate in food than it can from the synthetic version (folic acid) found in supplements. Therefore, dietary supplementation could represent a simple, cost-effective approach to GDM prevention. Future studies should help to extend these encouraging findings about folate and GDM risk and determine whether there is a safe and effective optimal supplementation dose that has a significant impact on GDM prevention in women.

Li M, Li S, Chavarro JE, ... Zhang C. *Prepregnancy habitual intakes of total, supplemental, and food folate and risk of gestational diabetes mellitus: A prospective cohort study.* *Diabetes Care* 42: 1034-1041, 2019.

## METABOLIC REGULATORS OF HEALTH AND DISEASE

**Metabolism, Memory, and the Role of Insulin in the Brain:** Gaining new insights into the link between diabetes and higher risk of dementia, scientists discovered, in research in mice, that insulin and the related hormone IGF-1 act in multiple parts of the brain to regulate blood glucose (sugar) levels, memory, and other vital mind and body processes. Insulin and IGF-1 transmit critical biological signals, and prior research showed that impaired insulin and IGF-1 signaling in the brain is associated with diabetes, obesity, and potentially increased risk for Alzheimer's disease and other cognitive problems. But much remains unknown.

To identify areas of the brain important for insulin and IGF-1 control of metabolism and cognitive functions, the researchers generated two groups

of mice with reduced signaling by these hormones in specific regions of the brain—the hippocampus and central amygdala, respectively—and examined the effects. They found that, in mice with insulin/IGF-1 signaling deficiencies in either of these brain regions, blood glucose levels rose above normal. Mice with these deficiencies in the central amygdala also could not maintain normal body temperature in a cold environment. From additional experiments, they determined that this effect was likely due to disrupted signaling along nerves that connect the brain to brown fat tissue, which generates heat. Investigating other effects, the researchers found that, compared to normal mice, those with insulin and IGF-1 signaling deficiencies in either of these brain regions did not differentiate between new and familiar objects, and they displayed anxiety-like behaviors. Mice with signaling deficiencies in the hippocampus were also much slower in learning to navigate their way through a maze and had more trouble remembering the route later. In their experiments, the researchers used only male mice to explore the role of insulin/IGF-1 signaling in the hippocampus, and only female mice in studies of the central amygdala. Thus, it is not yet clear whether some of the metabolic and cognitive effects reflect distinct functions of the two brain regions, differences between males and females, or both.

The results from this research in mice illuminate critical roles of the hormones insulin and IGF-1 in multiple areas of the brain and yield new insights into the connections between insulin action, metabolism, learning and memory, and anxiety-like behavior. These findings may lead to new ideas for therapies in humans, not only for diabetes and obesity, but also for Alzheimer's disease and other dementias.

Soto M, Cai W, Konishi M, and Kahn CR. *Insulin signaling in the hippocampus and amygdala regulates metabolism and neurobehavior.* *Proc Natl Acad Sci USA* 116: 6379-6384, 2019.

## STRIDES IN THE TREATMENT OF CYSTIC FIBROSIS

It has been over 30 years since the discovery of *CFTR*—the gene that is mutated in people with cystic fibrosis (CF)—and 7 years since the U.S. Food and Drug Administration (FDA) approved the first drug capable of rescuing *CFTR* protein function in people with certain CF-causing mutations. This medication, called ivacaftor, is a “potentiator,”

meaning it enables certain mutant versions of the CFTR protein to fulfill their critical function as a channel that allows chloride ions to travel in and out of various cells, greatly reducing the burden of the disease. Unfortunately, ivacaftor cannot repair damage caused before treatment begins; and, at least by itself, it is only helpful for the 5 percent or so of people with CF that have a version of the protein (resulting from mutations in the gene such as one designated *G551D*) that, although inactive without the drug, is stable and reaches the cell surface where it is supposed to reside. However, research has continued to improve functional rescue of various forms of the CFTR protein and thereby continues to improve the health and quality of life for people with CF. For example, the FDA has recently approved the use of “corrector” drugs that help other mutant forms of the CFTR protein reach the cell surface. These can be combined with ivacaftor to improve CFTR function in people who have CF and have one or two copies of  $\Delta F508$ , the most common disease-causing CFTR mutation. The improvements from these treatment regimens are modest, though clinically meaningful, so work has continued to improve upon this therapeutic approach. In the major recent advances described below, researchers examined whether it might be possible to maximize the clinical value of existing therapies like ivacaftor by starting treatment before birth; tested new, triple drug combinations in clinical trials with participants who have the  $\Delta F508$  mutation; and developed new candidate corrector drugs that could potentially raise CFTR channel levels in the majority of people with CF to normal or near-normal levels.

**In Utero Treatment May Promote Healthy Development:** New research shows that treatment during pregnancy can prevent or reduce developmental complications of CF in an animal model of the disease. While ivacaftor treatment for people with the *CFTR-G551D* mutation greatly improves patient health and quality of life, CFTR activity appears to be important during embryonic development, even before a baby takes his or her first breath—and thus before treatment begins. For example, infants with CF may be born with intestinal blockages so severe that they are life-threatening. Even in cases where this does not happen, defects in the intestines and pancreas can interfere with proper absorption of nutrients, slowing the baby’s growth. In addition, men with CF are almost invariably infertile, because the vas deferens and epididymis—ducts that carry sperm from the testes—do not develop properly during gestation. Although ivacaftor is FDA-approved for infants as young as 1 year old who have CF and at least one copy of *G551D*, prevention of

these digestive and reproductive consequences of the disease might require treatment to begin earlier—potentially even before birth.

To test this hypothesis, researchers utilized a ferret model of CF, which is much more prone to these developmental issues than are mouse or rat CF models. Ferrets born with *CFTR-G551D* have a very high frequency of serious intestinal blockages; those that survive infancy grow much more slowly than normal due to difficulty absorbing nutrients; and male ferrets with the mutation are sterile. However, the researchers found that if the mothers were treated with ivacaftor during pregnancy, both the digestive system and the male reproductive tract of the offspring developed much more normally. It remains to be determined whether treatment with ivacaftor or other small molecule drugs can safely and effectively promote healthy embryonic and infantile development in people with CF, but these results suggest that such treatments may one day allow much healthier development in children born with the disease and may help advance knowledge about the role of CFTR function during development.

Sun X, Yi Y, Yan Z,...Engelhardt JF. In utero and postnatal VX-770 administration rescues multiorgan disease in a ferret model of cystic fibrosis. *Sci Transl Med* 11: pii: eaau7531, 2019.

**Triple Combination Therapies Show Promise for People with the Most Common CF-causing Mutation:** Combinations of recently developed small molecule drugs have shown great promise for significantly improving treatment of people who have CF and have one or two copies of the  $\Delta F508$  CFTR mutation. About 90 percent of people with CF have at least one copy of  $\Delta F508$ , and half have two copies, one from each parent. The effects of the  $\Delta F508$  mutation on CFTR function are profound. Not only does the mutation inactivate the chloride channel, it also has two other serious consequences: it interferes with the protein’s biosynthesis, greatly reducing the amount that reaches the cell membrane, where it is needed; and it also renders the protein highly unstable, so that the small amount of CFTR protein that reaches its cellular destination is rapidly degraded. Thus, restoring robust CFTR function in people with  $\Delta F508$  will not only require an improvement in the protein’s function, it will also require both an increase in the amount of the mutant protein that cells produce and an improvement in the protein’s stability once it reaches the cell surface. To date, no single medication has been identified that is capable of meeting all of these needs.



Previous research had shown that the corrector drugs lumacaftor and tezacaftor are each capable of stabilizing  $\Delta F508$ -CFTR during biosynthesis so that a significant amount of the protein reaches the cell surface. That alone is not enough to provide clinical benefit, since the protein remains inactive; but when combined with the potentiator ivacaftor, either corrector can provide a modest but measurable improvement in  $\Delta F508$ -CFTR function. That was an important achievement, but it remained critical to further increase the amount of the mutant protein on the cell surface in order to improve clinical outcomes. A pharmaceutical company therefore developed new  $\Delta F508$ -stabilizing drugs that work by different mechanisms from tezacaftor and lumacaftor. They identified two such agents and designated them VX-445 and VX-659. In new research led by the pharmaceutical company but with additional support from the NIDDK, they tested the ability of each of these to supplement tezacaftor-ivacaftor. In lab-cultured human airway cells producing only  $\Delta F508$ -CFTR or  $\Delta F508$ -CFTR along with a rarer, minimally functional mutant CFTR protein, both of the tested three-drug combinations significantly increased the net amount of the protein through improved biosynthesis, stability, or both, and increased the flow of chloride ions through the cell membrane compared to the two-drug combinations.

The researchers also reported clinical trial results of these triple combination therapies in men and women with CF who had at least one copy of  $\Delta F508$ -CFTR. The two 4-week trials involved similar numbers of participants—122 for VX-445 and 117 for VX-659—and yielded very similar results. The resulting triple combination therapies significantly boosted CFTR function, based on improvements in measures of respiratory function and quality of life, among other tests. For example, in people with two copies of  $\Delta F508$ -CFTR who had been taking a combination of tezacaftor and ivacaftor at the beginning of the trial, a measure of respiratory function improved 9.7 percent with the addition of VX-659, and 11 percent when VX-445 was the third added drug. People with one copy of  $\Delta F508$ -CFTR and one copy of a different minimally functioning CF mutation—none of whom had been taking tezacaftor-ivacaftor to begin with—had an average improvement of 13.3 percent or 13.8 percent in the same test when they began taking the VX-659 or VX-445 triple combination therapies, respectively. Although these improvements may

appear small, their expected health benefit is potentially significant. Importantly, neither of the three-drug combinations appears to have caused serious side effects. Based in part on these results, the FDA approved the VX-445-tezacaftor-ivacaftor triple combination for treating CF in people ages 12 and over with at least one copy of the  $\Delta F508$ -CFTR variant. Further research will be needed to determine the long-term impact of this therapy on patient health, but the findings described here suggest it will provide a significant improvement in health for the majority of people with CF.

*Davies JC, Moskowitz SM, Brown C,...Rowe SM; VX16-659-101 Study Group. VX-659-tezacaftor-ivacaftor in patients with cystic fibrosis and one or two Phe508del alleles. N Engl J Med 379: 1599-1611, 2018.*

*Keating D, Marigowda G, Burr L,...Taylor-Cousar JL; VX16-445-001 Study Group. VX-445-tezacaftor-ivacaftor in patients with cystic fibrosis and one or two Phe508del alleles. N Engl J Med 379: 1612-1620, 2018.*

### Improving Combination Therapy for Cystic Fibrosis:

Researchers have developed new candidate medications that correct distinct structural defects of  $\Delta F508$ -CFTR, the most common CF-causing mutation, an approach that could potentially lead to improved therapies for the great majority of people with the disease. Like many proteins, CFTR contains multiple functional elements, called “domains.” Two such CFTR domains bind to a key, channel-activating cofactor called a nucleotide, so these structural elements are referred to as nucleotide-binding domains 1 and 2 (NBD1 and NBD2). Two other structural elements are the parts of the protein that cross the cell membrane (where CFTR must be located to allow the flow of chloride ions) and are thus called membrane spanning domains 1 and 2 (MSD1 and MSD2). The  $\Delta F508$ -CFTR mutation, while located in NBD1, not only destabilizes that key structural element, it also destabilizes NBD2 and the interactions of both NBDs with the MSDs. The two FDA-approved CFTR-corrector drugs, lumacaftor and tezacaftor, both work by similar means, stabilizing the interactions of NBD1 with MSD1 and MSD2. The researchers considered the possibility that restoring full or nearly full function of  $\Delta F508$ -CFTR would also require stabilizing the NBDs themselves, as well as the interactions of NBD2 with the MSDs.

The scientists screened 600,000 different chemical compounds in human cell lines and employed a variety of techniques to identify new CFTR correctors that would act on different parts of the



protein than do the existing FDA-approved agents. For example, they determined which of them allowed more of the protein to accumulate in the cell membrane of cells expressing  $\Delta F508$ -CFTR that were already being treated with lumacaftor: the idea is that if the candidate drug works by a different mechanism from lumacaftor, the actions of the two together would be expected to yield significantly greater stabilization of CFTR than will either medicine by itself. They followed up with tests to determine which of the key domains of CFTR these compounds bound to, in order to separate them into groups that act on distinct portions of the protein. With such approaches, they provisionally assigned them into three classes: I, compounds like lumacaftor that stabilize the interactions of NBD1 with the MSDs; II, compounds that stabilize NBD2 or its interactions with the MSDs; and III, compounds that specifically stabilize NBD1.

While each of these compounds on its own stabilized  $\Delta F508$ -CFTR just a little bit, when they combined correctors of different classes the researchers observed a synergistic increase in stability of the mutant protein. For example,

if a class I and a class II corrector each improved stability by 5 percent on its own, combining them together yielded substantially more than 10 percent improvement. And indeed, by treating cells simultaneously with all three classes of corrector, the scientists were able to make  $\Delta F508$ -CFTR roughly as stable as normal, healthy forms of CFTR. Importantly, even without addition of a potentiator like ivacaftor, these new triple corrector combinations also yielded near-normal CFTR function in cultured cells, and restored chloride channel function in mice that have  $\Delta F508$ -CFTR in place of their normal CFTR gene. Further, the triple correctors also appeared to be effective in treating a variety of other, rarer CF-causing mutations. Clinical trials would be needed to determine whether any of these combinations is safe and effective in people with CF. If one or more of them is, it could lead to a dramatic improvement in health for people with the disease.

*Veit G, Xu H, Dreano E,...Lukacs GL. Structure-guided combination therapy to potentially improve the function of mutant CFTRs. Nat Med 24: 1732-1742, 2018.*

# Pregnancy, Metabolism, and the Short- and Long-term Health of Women and Their Children

Pregnancy can be a time of great joy, but also acts as a stress test on a woman's body. Important physical changes that occur to support pregnancy can also unmask or exacerbate risk of metabolic problems and other conditions in the mother. These, in turn, can have lasting adverse impacts both on a woman's own health and the health of her offspring. For example, one set of changes affects how women are able to metabolize glucose (sugar), the body's major source of energy. In some women, these changes can cause their blood glucose levels to rise to such a degree that they are diagnosed with and treated for a condition called gestational diabetes (GDM), a form of diabetes that is diagnosed during pregnancy and is not clearly identified as either preexisting type 1 or type 2 diabetes. GDM is known to confer short- and long-term health risks to mothers and children. Now, through a long-term study involving thousands of women and children, researchers have found that elevated maternal blood glucose levels even below those meeting traditional GDM diagnostic criteria increase the risk of future type 2 diabetes in mothers and impaired glucose metabolism and greater excess fat in children ages 10 to 14 years post-delivery. These and other findings have prompted new NIDDK-supported research efforts with the ultimate goal of promoting healthier outcomes for women and their children both during and post-pregnancy.

## GESTATIONAL DIABETES

GDM is typically diagnosed just before or during the third trimester of pregnancy; approximately 7 percent of U.S. pregnancies are affected. GDM increases near-term health risks for mothers and babies, including high birth weight babies and delivery complications. Controlling maternal blood glucose levels through lifestyle change (modifications to diet and exercise) and/or with injections of the hormone insulin, if needed, can mitigate some of these risks.

Traditional approaches to diagnosing GDM in the United States include a screening test 24 to 28 weeks into pregnancy, during which women are given a sugary drink and then tested for blood glucose levels to see if they are at risk. Women identified as at-risk then go through further testing to see if their blood glucose levels exceed certain threshold values for diabetes. GDM, as diagnosed using these traditional criteria, is not only associated with near-term health risks, but also with longer-term health problems. For the mother, GDM confers a greater risk of developing type 2 diabetes post-pregnancy. For the children of an affected pregnancy, GDM increases the likelihood of developing obesity or type 2 diabetes.

## RISKS FROM ELEVATED GLUCOSE DURING PREGNANCY EVEN BELOW TRADITIONALLY DEFINED GDM LEVELS

In 2008, the landmark NIH-funded Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study reported findings of health problems associated with glucose levels during pregnancy that were above normal (hyperglycemia), but not high enough to be considered diabetes. Studying a racially and ethnically diverse international cohort of over 23,000 pregnant women and their babies, the HAPO researchers found that elevated maternal blood glucose levels below those diagnostic of GDM were associated with increased risk of multiple adverse outcomes for the mother and child. These included high birth weight, low blood glucose in the baby at birth, and need for caesarean delivery. Strikingly, the HAPO researchers observed that even modestly elevated maternal blood glucose levels were associated with risks, which increased with maternal blood glucose levels in a linear fashion for most outcomes. Because HAPO only included women whose glucose levels were not high enough for a diagnosis of GDM at the time, they were not considered to have this disease and thus were not treated for it.

Primarily as a result of HAPO, alternative criteria for diagnosing GDM were proposed and adopted by a number of organizations around the world; these criteria include lower blood glucose level threshold values for a GDM diagnosis. However, these criteria are not widely used in the United States, largely due to findings from an expert panel NIH convened in 2013 regarding GDM diagnosis. The findings pointed out gaps in knowledge about how best to treat women diagnosed with the alternative criteria, and the absence of evidence about long-term outcomes and benefits for these women with or without treatment—as well as the potential for short-term harms, such as the additional stress a woman can experience when diagnosed with GDM. Thus, a critical question regarding maternal blood glucose levels that are elevated but do not meet the traditionally defined criteria for GDM is this: what are the long-term impacts on the health of women and their children?

## FINDINGS FROM THE HAPO FOLLOW-UP STUDY

Researchers with the HAPO Follow-up Study (HAPO FUS) sought to address this question. Recognizing the enormously valuable information that could be gained from further study of HAPO participants, HAPO FUS researchers, with funding from NIDDK and additional support from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, recruited a subset of the original HAPO mother-child pairs and performed comprehensive metabolic tests 10 to 14 years post-delivery to help answer questions about the long-term impacts of these maternal glucose levels. Participating mothers were tested for type 2 diabetes and prediabetes and were also asked to fill out health-related questionnaires. The researchers measured the children's height, weight, waist circumference, and body fat (adiposity), and also tested them for blood glucose levels and other measures related to diabetes. A total of 4,967 women and 4,832 children participated in HAPO FUS.

The HAPO FUS researchers recently published several major study results. They first studied participating mothers whose pregnancies during

HAPO met, in retrospect, the alternate criteria for GDM, although they did not meet the traditional criteria for GDM. This group included about 14 percent of the women. Over half of these women developed type 2 diabetes or prediabetes in the 10 to 14 years post-delivery, versus only 20.1 percent of the women who had lower glucose levels. Moreover, even after adjusting for many known risk factors for type 2 diabetes, women who retrospectively met the alternative criteria for GDM were over five times more likely to develop diabetes than women who had lower glucose levels.

When the researchers examined participating children, they found that the likelihood of having any excess weight, at the level of either overweight or obesity, did not differ significantly between the children of mothers whose glucose levels met the alternate GDM criteria and the other children. However, narrowing their focus to just higher levels of excess weight in the children, they found that the likelihood of having obesity was significantly greater among children of affected pregnancies. (For these analyses, the researchers also took into account each mother's body mass index, BMI—a measure of weight relative to height—during pregnancy, as that, too, can influence the risk of excess weight in her offspring.) Furthermore, the researchers found evidence that children from pregnancies meeting the alternate GDM criteria were more likely than the other children to have developed insulin resistance, another risk factor for type 2 diabetes. No difference was seen between girls and boys in this regard.

Having examined the impact of maternal glucose levels meeting the alternative GDM criteria on risk for metabolic problems in the children, the HAPO FUS researchers then asked whether there was a direct correlation between maternal blood glucose levels—including those below levels meeting the alternative GDM criteria—and measures of childhood adiposity years later. After taking into account maternal BMI during pregnancy, they observed increasing risk of childhood adiposity with increasing maternal glucose levels during pregnancy. The overall outcome was largely similar for boys and girls.

## FUTURE DIRECTIONS

The HAPO FUS findings are important as they demonstrate that elevated maternal blood glucose levels below those traditionally used to diagnose GDM are associated with long-term health risks for mothers and children. However, large gaps in knowledge remain—for example, how maternal blood glucose levels change across the entire course of pregnancy is unknown. It is also unclear whether screening for GDM earlier in pregnancy than the late second to third trimester, treating GDM at an earlier stage of pregnancy, or providing treatment for lower (but still elevated) maternal blood glucose levels would result in health benefits for mothers and children. As other NIDDK-supported studies have demonstrated the devastating impact of type 2 diabetes in youth, closing these knowledge gaps could have critical ramifications for the health of future generations.

To begin to address these questions that are so important to both diabetes care and prevention, the NIDDK is cultivating new research in this area, starting with a newly funded pregnancy research

consortium. This clinical consortium will employ cutting-edge technology to ascertain the “profile” of blood glucose levels in women across the span of pregnancy, beginning in the first trimester. Such information could help lay the foundation for future clinical studies and trials evaluating new approaches to GDM screening, diagnosis, and intervention, with the ultimate goal of improving the health of women and their children.

Lowe WL Jr, Lowe LP, Kuang A,...Metzger BE; HAPO Follow-up Study Cooperative Research Group. Maternal glucose levels during pregnancy and childhood adiposity in the Hyperglycemia and Adverse Pregnancy Outcome Follow-up Study. *Diabetologia* 62: 598-610, 2019.

Lowe WL Jr, Scholtens DM, Kuang A,...Metzger BE; HAPO Follow-up Study Cooperative Research Group. Hyperglycemia and Adverse Pregnancy Outcome Follow-up Study (HAPO FUS): Maternal gestational diabetes mellitus and childhood glucose metabolism. *Diabetes Care* 42: 372-380, 2019.

Lowe WL Jr, Scholtens DM, Lowe LP,...Metzger BE; HAPO Follow-up Study Cooperative Research Group. Association of gestational diabetes with maternal disorders of glucose metabolism and childhood adiposity. *JAMA* 320: 1005-1016, 2018.

# NIDDK Director Testifies on Type 1 Diabetes Research

On July 10, 2019, NIDDK Director Dr. Griffin P. Rodgers testified about progress and future directions in type 1 diabetes research before the Senate Special Committee on Aging, which is led by Chairman Susan Collins (R-Maine) and Ranking Member Bob Casey (D-Pennsylvania). The hearing, entitled “Redefining Reality: How the Special Diabetes Program Is Changing the Lives of Americans with Type 1 Diabetes,” was held in conjunction with the Children’s Congress, an event sponsored every 2 years by JDRF to highlight the value and progress of type 1 diabetes research for children and adults living with this disease. Testifying with Dr. Rodgers were Dr. Aaron Kowalski, JDRF President and CEO; actor Mr. Victor Garber; and JDRF Children’s Congress delegates Ruby Anderson, age 9, and Adriana Richard, age 16.

In his testimony, Dr. Rodgers described research made possible by the Special Statutory Funding Program for Type 1 Diabetes Research (Special Diabetes Program), which the NIDDK administers on behalf of the Secretary of the Department of Health and Human Services. Examples of research progress include:

- Findings from a clinical trial conducted by NIDDK’s Type 1 Diabetes TrialNet showing that treatment with a drug targeting the immune system can prevent onset of clinical type 1 diabetes in high-risk individuals for at least 2 years (see advance in this chapter);
- Progress toward the development of new diabetes management technologies, including artificial pancreas devices that automate blood glucose (sugar) sensing and insulin administration;
- Results reported by the NIDDK’s Human Islet Research Network using novel technologies to visualize—at the same time—individual insulin-producing beta cells and other pancreatic cell types, as well as immune cells involved in the type 1 diabetes autoimmune attack (see advance in this chapter); and

- Findings from NIDDK’s The Environmental Determinants of Diabetes in the Young (TEDDY) study that are providing new insights on childhood health and development, as well as environmental factors that may contribute to type 1 diabetes in children (see advance in this chapter).

To solicit input on new, emerging, and innovative research for the treatment and prevention of type 1 diabetes and its complications that could be supported by the Special Diabetes Program, the NIDDK convened a planning meeting in May 2019, held under the auspices of the statutory Diabetes Mellitus Interagency Coordinating Committee. At



Shown at the dais (left to right): Sen. Kyrsten Sinema (D-AZ), Sen. Jeanne Shaheen (D-NH), Sen. Bob Casey (D-PA, Ranking Member), and Sen. Susan Collins (R-ME, Committee Chair). Photo credit: Camera1@nycphoto.com



NIDDK Director Dr. Griffin P. Rodgers testifying before the Senate Special Committee on Aging. Also shown at the table (left to right): JDRF President and CEO Dr. Aaron Kowalski, actor Victor Garber, and Children’s Congress delegates Ruby Anderson and Adriana Richard. Photo credit: Camera1@nycphoto.com



the meeting, a panel of external scientific experts and a lay representative provided input on potential new research initiatives proposed by the NIDDK, other institutes at NIH, and the Centers for Disease Control and Prevention; they also provided input on the continuations of ongoing programs.

Guided by that input, diabetes research strategic plans, and input that the NIDDK receives at venues such as scientific conferences and workshops, the

Institute is identifying the most compelling areas of current research opportunity to pursue with Special Diabetes Program funds, to continue the Program's exceptional track record of supporting cutting-edge research to improve the health and well-being of people with type 1 diabetes and its complications.

# STORY OF DISCOVERY

## Progress on the Pathway to Prevention of Type 1 Diabetes

Type 1 diabetes is a devastating illness where the body's ability to produce the pancreas-derived hormone insulin is lost, requiring people with the disease to administer insulin daily for survival. Even with this burdensome treatment, people with type 1 diabetes are at risk for life-threatening complications. Research shows that the incidence of type 1 diabetes is on the rise in the United States, so identifying ways to prevent type 1 diabetes in those at risk is critical, in parallel with efforts to cure the disease in those who have been diagnosed.

The story of type 1 diabetes—which is still being written as key questions and challenges remain—involves geneticists, epidemiologists, molecular and cellular biologists, immunologists, endocrinologists, bioengineers, researchers in other fields, and patient participants. This multifaceted and collaborative approach has resulted in valuable new knowledge that is moving us closer to a long-standing goal of type 1 diabetes prevention.

### IDENTIFYING THOSE AT RISK TO DEVELOP TYPE 1 DIABETES

Preventing type 1 diabetes requires not only a successful therapy, but also the ability to identify those who are at risk of developing the disease. But answering the deceptively simple question of “who is at risk?” required a multi-pronged research approach.

One of the first steps was to understand the disease better. Early on, scientists searched for a toxin or infectious agent that caused type 1 diabetes. However, some observed that people with type 1 diabetes sometimes had other disorders related to abnormal hormone levels or function (endocrine disorders), particularly those associated with autoimmunity, leading scientists, after decades of studying the disease, to hypothesize that

type 1 diabetes was an autoimmune disease. Autoimmune diseases result when a person's immune system does not properly distinguish between “self” and “non-self” and inappropriately targets and attacks the body's own organs, tissues, and cells. One component of an immune attack is antibodies, produced by an immune cell type called B lymphocytes. Self-directed antibodies are called “autoantibodies,” and their presence in the blood can indicate an autoimmune process.

In the early 1970s, researchers found that, by using blood from people with multiple autoimmune endocrine disorders, including type 1 diabetes, they could detect a specific autoantibody response to insulin-producing pancreatic islet tissue. Later research demonstrated that antibodies that react with islet cells could be found in the majority of people with newly diagnosed type 1 diabetes. Further research has identified more than four different autoantibodies specifically enriched in people with type 1 diabetes. One of the earliest autoantibodies to appear, most commonly in younger children, is directed against insulin.

Early studies of families with type 1 diabetes in the 1970s led to the observation that the disease often appeared in siblings, indicating that there could be a genetic component to the disease. NIDDK-supported scientists and others soon discovered that human leukocyte antigen (HLA) gene alleles (variant forms of a gene required for the function of another immune cell type—T cells) were associated with type 1 diabetes. With the use of modern tools for genetic analysis, we now know that HLA accounts for approximately 50 percent of the heritability of type 1 diabetes. Additionally, NIDDK-supported researchers and others have since identified more than 50 other genetic loci that contribute to type 1 diabetes susceptibility, accounting for nearly 90 percent of genetic risk

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in the Caucasian population, which is most affected by type 1 diabetes. Many of these genes are known to be involved in the immune response, further strengthening the understanding of type 1 diabetes as an autoimmune disease. These exciting findings set the stage for efforts to identify those at risk to develop type 1 diabetes and to test immune-modulating therapies to prevent the disease.

## A WINDOW OF OPPORTUNITY TO PREVENT TYPE 1 DIABETES

Until the discovery of autoantibodies, it was generally assumed that type 1 diabetes had an acute onset whose first clinical symptoms were the sudden appearance of metabolic abnormalities as a result of the loss of insulin in previously healthy people. Prevention would be difficult in such a disease, as there would be no warning before the clinical appearance of the disease and identifying at-risk individuals would not be possible. Not only did the revelation that type 1 diabetes was an autoimmune disease mean that autoantibodies could possibly be used to identify those at risk before the manifestation of clinical symptoms, but it also suggested that a window of opportunity for prevention might exist. Destruction of the insulin-producing  $\beta$  (beta) cells (which are in the pancreatic islet cell clusters) by an errant immune attack might happen over time, rather than immediately, and perhaps this destruction could be delayed or stopped altogether, preserving the precious remaining  $\beta$  cells.

NIDDK-supported scientists and others spent the 1980s studying cohorts of people that had these autoantibodies in their blood but had not been clinically diagnosed with diabetes to determine whether the appearance of autoantibodies preceded loss of insulin and if they indicated the early stages of type 1 diabetes. In one study, NIDDK-supported investigators followed a set of triplets and a set of twins, each with one person with type 1 diabetes. These people were studied for nearly 2 decades. Over that time, one triplet and one twin—neither

of whom had diabetes at the start of the study—first developed autoantibodies and then onset of clinically overt disease, allowing scientists to document the slow, progressive loss of insulin before the onset of clinical diabetes.

In another study, NIDDK-supported scientists screened over 1,700 first-degree relatives (parents, siblings, and offspring) of people with type 1 diabetes for the presence of islet-cell autoantibodies. Only 16 of those screened had the autoantibodies, but 2 of those developed type 1 diabetes in the next 2 years, compared to 1 of the 1,700 without antibodies. In addition, the researchers examined the insulin response in 12 of the relatives with autoantibodies and found that 6 of these individuals had low insulin responses, an indicator of diminished  $\beta$  cell function. Results from these and similar studies contributed to the growing body of evidence that islet-cell autoantibodies were predictors of type 1 diabetes and that  $\beta$  cell destruction was not an immediate event. These studies also provided key information on how screening programs could be designed to identify people and assess their risk, setting the stage for trials to prevent type 1 diabetes.

## SETTING THE STAGE FOR A LARGE-SCALE PREVENTION TRIAL

For a first test of type 1 diabetes prevention, researchers turned to a familiar candidate: insulin. Studies in animal models, as well as small pilot studies in humans, suggested that insulin could delay type 1 diabetes development. It was thought that administering low-dose insulin to an at-risk person before the disease progressed could induce protective immunity that might slow or prevent the immune system's attack. In 1994, the NIDDK-supported Diabetes Prevention Trial-Type 1 Diabetes (DPT-1) began screening first- and second-degree relatives to identify eligible participants for a clinical trial to test this hypothesis. More than 84,000 people were screened; about 340 were found positive for autoantibodies, had more than an estimated 50 percent chance to develop

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type 1 diabetes in the next 5 years, and elected to participate in a study testing injectable insulin for prevention of type 1 diabetes. Participants were studied for an average of about 3.5 years, and this clinical trial concluded in 2001. DPT-1 also tested the effect of orally administered insulin in relatives who had an estimated 26 to 50 percent chance of developing type 1 diabetes in the next 5 years. Over 370 participants were studied for an average of 4.3 years in that trial, which concluded in 2003. Although both injectable and oral insulin were very safe, with negligible side effects, neither was found to delay or prevent type 1 diabetes.

Despite the negative results, the DPT-1 was a success in other ways. DPT-1 researchers estimated participant risk using the presence of islet-cell antibodies, insulin response to glucose tests, and the presence or absence of specific HLA alleles, validating these predictive tools and demonstrating that it was possible to identify a cohort of people at high risk for type 1 diabetes. DPT-1 also demonstrated that large type 1 diabetes prevention trials were feasible in at-risk family members of individuals with type 1 diabetes, establishing a path for future prevention trials, just in time for the emergence of new agents that would require testing.

## CREATING A NETWORK FOR PREVENTION TRIALS: TYPE 1 DIABETES TRIALNET

As the DPT-1 was concluding, the continued need for a network of investigators and sites to conduct trials of promising therapies to prevent type 1 diabetes became evident. These trials would require screening of large numbers of people to identify those who would be eligible to participate. Additionally, a coordinated and collaborative effort would accelerate progress in this field. Thus, in 2001, NIDDK launched Type 1 Diabetes TrialNet. Since its start nearly 2 decades ago, TrialNet has become an international network of clinical research centers, affiliate sites, a hub, and a coordinating center that involves hundreds of scientists and staff

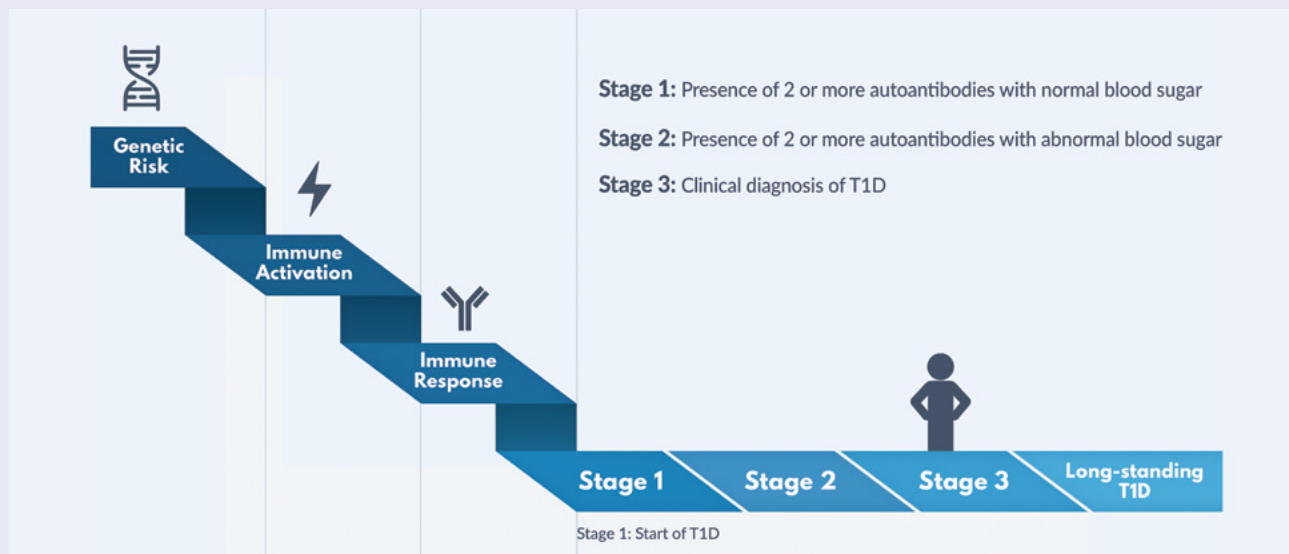
and, most importantly, thousands of participants. TrialNet has conducted multiple studies of agents to delay progression of type 1 diabetes in people with and at risk for the disease, as well as contributed key insights into understanding the type 1 diabetes disease process.

## REFINING RISK AND STAGING PROGRESSION OF TYPE 1 DIABETES

The ability to accurately assess those at risk for type 1 diabetes is critical to identify participants for prevention trials and to ensure that as many people as possible can benefit, if and when new prevention strategies are proven effective. To refine and quantify type 1 diabetes risk, NIDDK-supported researchers pooled data from multiple studies and, in 2013, reported that the majority of children who had multiple islet autoantibodies in their blood progressed to the disease over the next 15 years, suggesting that prevention studies focus on this high-risk population.

Data from DPT-1, TrialNet, and other studies revealed that progression to clinical type 1 diabetes proceeds through distinct stages prior to onset of symptoms. This formed the basis for a recommendation from TrialNet, JDRF, the Endocrine Society, and the American Diabetes Association for a type 1 diabetes staging classification in at-risk individuals. This staging provides a framework for the research and development of preventive therapies (see Figure 1): stage 1 is defined as the presence of two or more different types of islet autoantibodies with normal blood glucose (sugar) levels and is considered early type 1 diabetes; stage 2 diabetes is the presence of two or more autoantibodies but with abnormal blood glucose levels without symptoms; and stage 3 is when clinical diagnosis has been reached and symptoms of type 1 diabetes are usually present. TrialNet's prevention trials enroll individuals with pre-clinical (stage 1 and 2) type 1 diabetes, and TrialNet's new-onset trials enroll participants in early stage 3 diabetes.

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**Figure 1: Windows for Prevention of Type 1 Diabetes (T1D):** This graphic illustrates how type 1 diabetes progresses. Genetic risk, combined with an unknown environmental trigger(s), is followed by inappropriate activation of the immune system to attack the insulin-producing  $\beta$  cells. The appearance of more than one islet-cell autoantibody in a person's blood indicates that the immune system has been activated and the person has a high risk of development of type 1 diabetes. Stages 1 and 2 are considered the start of type 1 diabetes, even before the appearance of clinical symptoms and before the clinical diagnosis of diabetes is made—a window of opportunity for strategies to prevent onset of clinical disease. Research by Type 1 Diabetes TrialNet contributed to this new knowledge of type 1 diabetes staging, and TrialNet's goal is to test agents to prevent or delay the disease at all stages. (Graphic courtesy of Type 1 Diabetes TrialNet.)

The combination of these efforts led to the following understanding of type 1 diabetes disease risk: 35 percent of people in stage 1 and 70 percent of people in stage 2 will progress to clinical diabetes within 3 to 5 years of identification. The lifetime risk for developing clinical type 1 diabetes from stage 1 or 2 nears 100 percent. In the future, risk assessment could take into account an individual's genetic makeup and their environmental exposures to determine risk even before autoantibodies appear.

Of note, most new cases of type 1 diabetes occur in people who have no affected relatives. There is currently no way to identify these people other than by conducting population-wide genetic screening, which is not currently feasible. Therefore, for now, research has demonstrated that the most efficient way to identify people at risk for type 1 diabetes is to screen first- and second-degree relatives of people with the disease due to their 15-fold increased risk for developing the disease compared to the

general population. To date, TrialNet has screened more than 200,000 relatives and screens more than 15,000 annually to identify at-risk individuals for enrollment in trials. More than 7,000 people have enrolled in TrialNet's Pathway to Prevention Study (an observational study for relatives with autoantibodies) and/or have participated in a TrialNet trial.

## IDENTIFYING CANDIDATE THERAPIES TO TEST

One of the challenges of clinical trials is balancing the potential benefits against the risks. There is risk associated with introducing an agent, particularly one that modulates the immune system and may have serious side effects, into a healthy person—albeit one who will eventually develop clinical diabetes but has not yet done so—especially when the participants are children. Therefore, careful consideration is paramount in deciding which agents



# STORY OF DISCOVERY

are the most promising and should be tested in prevention trials. With that in mind, TrialNet looks for agents that have been tested for safety in animal models, in pilot studies in people, or have been tested (or even approved for use) in people with other autoimmune diseases or conditions before a larger prevention trial is considered.

One of the first agents to emerge as a possible candidate for therapy was an antibody known as anti-CD3, which modifies the function of T cells, but does not dramatically deplete them. In studies of mouse models of type 1 diabetes, anti-CD3 agents have consistently reversed diabetes at the onset of symptoms. In a small clinical trial funded by NIDDK and reported in 2002, scientists found that an anti-CD3 agent, teplizumab, preserved some insulin production after 1 year with no severe side effects in people recently diagnosed with type 1 diabetes. Following these results, multiple larger and longer studies confirmed that teplizumab treatment delayed the loss of insulin production, including one done by the National Institute of Allergy and Infectious Diseases' Immune Tolerance Network, an important TrialNet partner.

To continue to make progress towards a more effective, durable and safe therapy for type 1 diabetes, TrialNet has tested additional immune-modifying agents in people newly diagnosed with type 1 diabetes. For example, in 2009, TrialNet reported that rituximab, which is approved as a cancer therapy and destroys B lymphocytes, slowed disease progression for 6 to 9 months. Another TrialNet trial tested the drug abatacept, which acts on T cells and is an approved therapy for rheumatoid arthritis. The results showed that participants who received abatacept had higher insulin production than those who received placebo after 2 years. In 2018, TrialNet reported that another immune system suppressant, low-dose anti-thymocyte globulin (ATG), delayed the loss of insulin production and improved blood glucose control for up to 2 years. All of these drugs can have significant side effects and each had only temporary benefit, likely due in part to immune cell regeneration when the treatments

ended. However, these positive results showed that many immune-modulating therapies could slow the disease, indicating that these agents could be tested in prevention trials, where their effects could be more beneficial.

## DEMONSTRATION THAT TYPE 1 DIABETES CAN BE PREVENTED

Teplizumab, with several positive trials in people with newly diagnosed type 1 diabetes, was chosen by TrialNet as the first agent to test for disease prevention. TrialNet began a trial in 2011 and, in 2019, reported that teplizumab delayed onset of clinical type 1 diabetes in people at high risk (stage 2) for an average of 2 years (see advance and Patient Profile in this chapter). This exciting discovery provides the first evidence that the onset of clinical type 1 diabetes can be delayed with early preventive treatment. Participants are being followed to determine the durability of the effect, but these results have important implications for people, particularly youth. Treatment with teplizumab could give at-risk individuals 2 years free of type 1 diabetes and insulin administration; 2 years that they do not have to check blood glucose levels; and 2 more years of good health towards preventing or delaying diabetes complications. Based on TrialNet's results, the U.S. Food and Drug Administration gave teplizumab "Breakthrough Therapy Designation" to expedite its development and review.

## THE FUTURE FOR PREVENTION TRIALS OF TYPE 1 DIABETES

Much remains to be explored about teplizumab and other immune-modifying drugs so that more effective treatments can be designed. First and foremost, we need to understand more about the mechanisms of autoimmune pathogenesis and how individual people respond to therapies. From the beginning, TrialNet has engaged in mechanistic research, collecting blood samples from people

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enrolled in trials and analyzing them for the specific mechanistic effects of treatment. Building on these data, TrialNet has designed a new prevention trial that will combine two agents that showed benefit to newly diagnosed participants in previous trials and that affect complementary immune pathways. Alternative dosing regimens, testing agents even earlier in at-risk people (*i.e.*, stage 1), and other types of combination trials all present exciting opportunities to build on this advance. Additionally, TrialNet currently has two other single-agent prevention studies under way: one testing abatacept (see earlier) and one testing the drug hydroxychloroquine, both of which are already used to reduce symptoms and progression of other

autoimmune diseases. There are also many other promising therapies in TrialNet's pipeline, with even more expected in the future as new knowledge is uncovered by TrialNet's mechanistic work and through other NIDDK-supported research efforts focused on the underlying mechanisms of type 1 diabetes development.

With continued research, the goal of preventing type 1 diabetes—permanently and in anyone who could develop the disease—now seems possible after decades of contributions from countless scientists and, most importantly, the trial participants who never gave up hope.

# SCIENTIFIC PRESENTATION

## Dr. Jeff Pessin—The Link Between Body Fat Production, Feeding, and Fasting

*Jeff Pessin, Ph.D., is a Professor in both the Department of Medicine and the Department of Molecular Pharmacology at Albert Einstein College of Medicine. He is the Judy R. and Alfred A. Rosenberg Professorial Chair in Diabetes Research, and Director of the Diabetes Research Center at Albert Einstein College of Medicine. Dr. Pessin's research interests focus on three areas: the molecular mechanism of adipose (fat) tissue inflammation leading to fibrosis and programmed cell death; the regulation of insulin and nutrient signaling and the regulation of glucose and fat production in the liver; and the dysregulation of such signaling in states of insulin resistance. At the January 2019 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, Dr. Pessin presented results of his research.*

The body stores much of the excess energy (calories) from our diet as fat—usually in fat tissue, but also in the liver—and it synthesizes this body fat via a process called lipogenesis. Excessive lipogenesis can lead to fatty liver disease, which in turn can lead to more serious liver problems, and in some cases even to liver failure. Thus, it is important to understand the factors controlling this process.

The body increases lipogenesis when we consume more calories from food than we need, so the excess calories can be stored for later use; conversely, lipogenesis is inhibited during fasting, when the body must liberate energy from its storage depots rather than adding to them. Like many other processes in the body, lipogenesis begins with an increase in the quantities of the proteins that produce these fats within cells, and lipogenesis is turned off when cells reduce the amounts of those same proteins. Dr. Pessin described research on the molecular mechanisms that link these lipogenic (fat-generating) protein levels to the body's nutritional state—in this case, the amount of dietary energy available in the body.

A cell's first step in making more of any needed protein is to increase the “expression” of the gene

that encodes that protein. That is, the cell makes temporary copies of the gene, called transcripts, which are effectively instructions for assembling the protein. The signal to make these transcripts is the arrival of a protein called a transcription factor at the gene. Expression of the lipogenic genes is activated by arrival of the transcription factor SREBP-1c.

However, as is often the case with transcription factors, SREBP-1c does not scan the genome to locate the lipogenic genes on its own. A large group of proteins called the “mediator complex” must first bind to a nearby region of DNA. The mediator complex then binds SREBP-1c, bringing it close to the lipogenic genes and promoting their expression. During fasting, when lipogenesis is no longer needed, a component of the mediator complex called CDK8 modifies SREBP-1c in such a way that it is targeted for degradation. The effect is not to eliminate lipogenic proteins directly, but rather to reduce expression of their genes, so that more of these proteins are not made unnecessarily.

But what is the molecular link between nutritional state and CDK8 activity? Dr. Pessin presented recent research from his laboratory addressing that question. Working with mice, he and his colleagues found that feeding activates a group of proteins called mTORC1, which then tags CDK8 for destruction. Without CDK8 around, SREBP-1c can promote the expression of lipogenic genes and thus the production of body fat. During fasting, the process is reversed: mTORC1 is turned off, so CDK8 is available to target SREBP-1c for destruction... so lipogenesis is off during fasting. Dr. Pessin showed that inhibiting mTORC1 with a drug mimics fasting: even when the animal is feeding, lipogenic genes are not expressed.

In additional studies, Dr. Pessin showed that mTORC1 activity—and thus nutritional state—likely determines not only whether CDK8 is targeted for destruction, but also whether any remaining CDK8

# SCIENTIFIC PRESENTATION

continues to be associated with other proteins that comprise the large mediator complex. Thus, nutritional state affects lipogenic gene expression by determining not only how much CDK8 there is, but also whether it is close enough to SREBP-1c to affect its activity.

These findings extend our knowledge of how body fat production is controlled, which may one day lead to improved treatment for diseases such as fatty liver disease, in which control of lipogenesis goes awry.

# PATIENT PROFILE

## Claire: A Lifetime of Contributing to the Science of Type 1 Diabetes Prevention



*Left to right: Randall (father), Claire, Correne (mother), and Henry (brother)*

For Claire and her mother, Correne, type 1 diabetes has always been a part of their lives, even though neither has been diagnosed. Several of their close relatives have the disease, and when Claire was 4 years old, she and her family received the sobering news that she was at risk of developing type 1 diabetes as well. However, that knowledge has also enabled Claire, now age 13, to spend most of her life participating in clinical research, including a clinical trial aiming to halt type 1 diabetes before symptoms can occur.

"I think it's really important to contribute to science," Claire says, when asked what she'd tell others thinking of participating in a clinical trial. "Even though there are challenges ... they are all definitely worth it."

### LIVING IN THE SHADOW OF TYPE 1 DIABETES

Family members on both sides of Claire's family tree live with type 1 diabetes. "We just grew up with type 1 [diabetes] in our family," Correne says. At family gatherings, everyone knows what to do if someone with the disease has an episode of dangerously low blood glucose (sugar). For Correne,

a pediatrician, type 1 diabetes also affects her professional life: she diagnoses patients with the disease in her own practice. One New Year's Day, she even noticed that a visiting family member was abnormally thirsty at breakfast—an observation that led to a type 1 diabetes diagnosis. "Type 1 diabetes is so personal for our family because it just has had a huge impact on our lives," Correne says.

It was also family that brought Correne and Claire to NIDDK's Type 1 Diabetes TrialNet, an international clinical research network aimed at discovering ways to delay or prevent type 1 diabetes. When a family member was dropping her daughter off at diabetes camp, she noticed that TrialNet was recruiting participants from families of people with type 1 diabetes. She asked Correne if her family would be interested in enrolling. Correne's response was, "Sure! Anything we can do."

So began the summer tradition of what the family called "pokey parties," where Claire, her brother Henry, and other young children in their extended family would visit the diabetes camp to have their blood drawn so they could be screened for risk of type 1 diabetes. The "poke" was not popular with Claire, who was not a fan of needles, but there were prizes afterwards, and since the kids did it together, the sense of camaraderie helped.

The risk of developing type 1 diabetes can be determined through blood tests, like those Claire and Henry volunteered for. In particular, their blood samples were tested for the presence of diabetes autoantibodies. These autoantibodies are proteins produced by the body that indicate the immune system is attacking the insulin-producing beta cells of the pancreas, and their appearance in the blood means that the person has an increased risk of developing type 1 diabetes in the future. That risk



# PATIENT PROFILE

rises as the number of different types of diabetes autoantibodies rises.

When Claire was 4 years old, they got a phone call from TrialNet study staff. Claire had tested positive for one of the autoantibodies. With this sobering news, though, came a new opportunity: would they be interested in participating in a bit more testing and surveillance of Claire's health?

The family agreed, and so from age 4 until age 9, in addition to blood draws, every 6 months Claire took an oral glucose tolerance test that measured her body's ability to metabolize the sugar glucose. Claire dreaded these tests, because they required more needles: an intravenous (IV) line (or "straw shot" as she and her family took to calling it) in her arm. Participating in TrialNet, though, gave her and her family a front-row seat to how Claire's health was changing. By the time she was 9, Claire's blood tests indicated that she had four different diabetes autoantibodies, a sign that the autoimmune attack on her pancreas was well underway, and the results of her oral glucose tolerance tests indicated that her body's ability to manage her blood sugar was fluctuating.

***"Type 1 diabetes is so personal for our family because it just has had a huge impact on our lives," says Correne, whose daughter Claire participates in type 1 diabetes research.***

Claire remembers, when she first joined TrialNet, her mother telling her that she had "special blood" and that she might help find a cure for type 1 diabetes someday. As the number of diabetes autoantibodies in her blood rose, Claire says, "I didn't really understand what the antibodies were doing, but I knew that somehow it was related to the possibility that I would get this disease." Once she developed the fourth antibody, the possibility that she might be diagnosed with type 1 diabetes became more real to her.

Meanwhile, Claire's parents could only watch from the sidelines, because there weren't any treatments known to prevent type 1 diabetes or halt its progression. Once Claire had four autoantibodies in her blood, her risk of developing symptoms of type 1 diabetes in the next year or two was very high. Correne says, "At that point we had kind of resigned ourselves ... it's coming."

Then, her family received another call from TrialNet staff. TrialNet was recruiting for a clinical trial, and Claire might be eligible to enroll. Would the family be interested in learning more about a prevention trial? "Yes," Correne answered. "Absolutely."

## DISCUSSING RISKS AND HOPING FOR REWARDS

The prevention trial, Claire and her family learned, would be testing a drug called teplizumab (also known as "anti-CD3"). Teplizumab targets the immune system and had been shown previously to slow the loss of beta cells in people recently diagnosed with type 1 diabetes. Now researchers wanted to test whether the treatment could prevent clinical onset of type 1 diabetes. To investigate this, they were recruiting relatives of people with type 1 diabetes who did not yet have the disease themselves but who were at high risk of developing it...like Claire.

Taking part in the trial would be a big commitment. The study site at Yale University was hours away from their home, and the trial would involve an IV infusion daily for 14 days and additional trips for follow up. Also, there was a risk that the drug could cause short- or long-term side effects. Finally, due to the trial design (which would randomly assign participants to either inactive placebo or teplizumab treatment so the two could be compared), Claire might not receive the drug if she was in the placebo arm of the trial, something she and the research staff would not know until the end of the study. Claire

# PATIENT PROFILE

hoped she'd receive the treatment, and that it would help prevent type 1 diabetes, but there was no guarantee that either would be the case.

Whether or not to participate was a family decision. Claire's parents discussed the pros and cons from all angles. They and their extended family also gave then-9-year-old Claire information about the study, its goals, and its importance. "It was a really tough conversation, to kind of come to a consensus that we wanted to do this," Correne says. "We were excited that they were doing a prevention trial, but there was a lot of thought that went into making that decision [to enroll]."

In the end, the family agreed to participate. Correne explains, "We need randomized trials. We need good data ... to make sure that we're moving in the right direction for patients." Also, she says, "From a mom standpoint ... you'll do anything, you know? If you think that it may help."

## TAKING PART IN THE UPS AND DOWNS OF CLINICAL RESEARCH

In December of 2015, Correne and Claire traveled to New Haven, Connecticut, where they would stay for 2 weeks. Each day, Claire and Correne headed to the research hospital early in the morning, and the staff performed preliminary blood tests to ensure that Claire's organs were functioning normally and that she was still eligible for the trial. Then she received an infusion over an hour or two, and by lunch they were done for the day. Claire generally felt fine after the infusions, and they would often spend the rest of the day exploring New Haven. As time passed, Claire got used to the routine.

Correne praised the TrialNet research staff and nurses that they interacted with. "They were phenomenal. They were just outstanding." The nurses were both efficient and caring, always trying to accommodate the trial participants' preferences, answering any questions they had, and even suggesting things to do around town. "They went

above and beyond to take care of Claire and meet her needs," Correne says.

Despite the support, Correne says that accompanying Claire during the trial was an emotional experience. "It was just overwhelming ... you're sitting there, and you're just watching stuff go through this [IV] tube, and just worrying, you know? 'Am I doing the right thing?'"

Then, around day 11, Claire began feeling unwell. On day 13, after her morning blood tests, the TrialNet staff informed Claire that her liver function, though not alarming, was no longer within the range needed to continue in the trial. Though this change was not necessarily related to the experimental treatment she was receiving, she was not eligible to receive the final two planned infusions.

*Claire, a volunteer in the Type 1 Diabetes TrialNet network, encourages others to participate in clinical research. "Even if it doesn't end up benefitting them, it'll benefit others, and will definitely contribute to science."*

Correne and Claire were disappointed, and as they headed home, they wondered: had Claire been in the group receiving the experimental treatment after all? If so, did she get enough of the drug to have an effect?

## WAITING, AND HOPING FOR GOOD NEWS

Life resumed for Claire and Correne, and TrialNet study personnel continued to monitor Claire's health via regular blood tests and oral glucose tolerance tests. As the years passed and the trial's follow-up continued, no news was good news: despite her still having the diabetes autoantibodies that statistically give her a high chance of developing the disease, Claire did not show clinical symptoms of type 1 diabetes.

# PATIENT PROFILE



In the above images, Claire, then age 9, dramatized how she imagined her immune system attacking her pancreas (in red) and how the drug teplizumab (in green) would come to its rescue. Claire drew these images during her participation in a Type 1 Diabetes TrialNet study testing whether teplizumab could prevent clinical onset of type 1 diabetes. Images used with permission.

Then, the day that the teplizumab trial's results were published in June of 2019, Claire's family received the good news they had been waiting for: the study Claire had participated in had demonstrated that teplizumab could delay diagnosis of clinical type 1 diabetes by 2 or more years among people who were at high risk. Furthermore, Claire was part of the 57 percent of trial participants who received teplizumab and had not developed type 1 diabetes by the time the trial's results were analyzed. In comparison, only 28 percent of those who received placebo had not developed type 1 diabetes at the same timepoint. These results provided the very first evidence that clinical type 1 diabetes can be delayed with early preventive treatment. This breakthrough was only possible because of the dedication of clinical trial participants such as Claire. Their willingness to be randomly assigned to groups receiving either placebo or teplizumab treatment was critical, since participants in both groups were key to the trial's success.

The family was excited by these results and also by the confirmation that Claire had received teplizumab. Claire was happy that the results had been so positive: "I was just glad it worked." She believes that it's important to help move the science forward and feels that her participation in the teplizumab trial was a great way to do that. "All I had to do was get poked by needles," she says, a smile in her voice.

Claire has also signed up for a TrialNet follow-up study that will continue to monitor her health and the effects of the teplizumab treatment, including how long its effects last. She encourages others to participate in clinical research as well. "Even if it doesn't end up benefitting them, it'll benefit others, and will definitely contribute to science." Correne agrees: "I would do it again. And I would do more studies, or whatever they ask because ... there's such value in it."

***Despite her daughter Claire being still at risk for developing type 1 diabetes even after her participation in a Type 1 Diabetes TrialNet network study, Correne says, "Every day without diabetes? It's a gift."***

Both of them also continue to think about the future, and about others with type 1 diabetes. "It kills me when I have to make a new diagnosis of diabetes for my patients," Correne says. Given her family history, helping to find a new treatment or even a way to eliminate the disease would be a huge personal victory. Claire also looks forward to new ways to delay, prevent, and cure the disease, especially when she sees family members treating their type 1 diabetes.

# PATIENT PROFILE

## CHANGING WHAT YOU CAN

Correne and Claire understand, though, that even with the successes of the teplizumab trial, their struggle with type 1 diabetes may not be over. When they were interviewed for this article, 42 months from Claire's first teplizumab infusion, Claire was still type 1 diabetes-free. However, it's not clear if the treatment Claire received can halt type 1 diabetes progression permanently or if it has only delayed onset of the disease. To this day, when Claire sees family members injecting insulin or managing their insulin pumps, she says, her thoughts turn to the possibility that she might have to do the same, one day.

That, Correne and Claire say, was something they understood from the beginning: that there were no guarantees. When asked how they feel about how Claire might—despite the needles, travel, and other worries of the trial—still be diagnosed with type 1 diabetes, Correne takes a deep breath. “Every day without diabetes? It’s a gift.” She thinks

back to when Claire was 9, and they were expecting her to develop type 1 diabetes in the next year or two. “Where she’s at now, and the childhood that she’s gotten to enjoy.... It’s just a gift.”

Claire is now in eighth grade, her spare time often taken up by dancing, musical theater, and oil painting. She is thinking about studying anthropology and medical illustration in college, and she would like to attend Yale.

Her participation in TrialNet has made Claire a little more positive about things that she can't change. “I can't change the fact that I might get [type 1] diabetes,” she says, but “even a prevention that might wear off in a few years is still great.” And despite all the things she can't change, Claire continues to change what she can, for others and for herself. She and other clinical research volunteers make type 1 diabetes prevention trials possible, and through their efforts our understanding of how to prevent this disease is changing, one day at a time.

# PATIENT PROFILE

## Valentina: Overcoming Pancreatitis and Diabetes, All with a Positive Attitude



Valentina (2nd from left), her mother Sonia, her father Juan Pablo, and her sister Nicole

Seventeen-year-old Valentina is an extremely talented high school senior living in southern Florida. She has many interests and would like to attend a university in Florida to major in international studies. She is currently participating on the school cheerleading team, student government, and National Honor Society, and she volunteers for Relay for Life (American Cancer Society) and Best Buddies. However, since the fall of 2017, she has faced—and overcome—life-threatening medical issues related to chronic pancreatitis and its associated treatments, including a major surgery called total pancreatectomy-islet cell autotransplantation, or TP-IAT. “I was always positive,” Valentina states. “I never really focused on the bad things. I just kept pushing through. I think that’s one of the biggest things that helped me get better.” Her proud mother, Sonia, states simply: “It was just amazing how she overcame her disease!”

### AN OVERHEARD CONVERSATION LEADS TO A DIAGNOSIS

Valentina started having pancreatitis symptoms in second grade, but she, her mother, father Juan Pablo, and younger sister Nicole, now 13 years old, did not know it was pancreatitis at the time. “I would just have a lot of stomach aches,” Valentina remembers, “I would feel really sick occasionally. It got to the point where we would have to go to the hospital and they would just tell us that I had gastritis or that I was constipated.” Gastritis is a condition in which the stomach lining is inflamed, or swollen, and is often managed by reducing dietary acid intake. Valentina was told to follow a low-acid diet, which helped her. She remembers having flare-ups on rare occasion, but nothing too bad.

That all changed in October 2017 when she was in 10<sup>th</sup> grade and had a major flare-up. “We thought it was just going to pass away as it always does,” Valentina recalls, “but the flare-up lasted for a week, and it got to the point where I was vomiting blood. That’s when we were like ‘ok, we need to go to the hospital.’” At the hospital, the staff asked Valentina for her family history to help pinpoint the cause of her illness. By a stroke of luck, she had recently overheard her parents saying that her father may have pancreatitis based on a chance finding from a medical examination, although Valentina did not realize at the time that he had not been diagnosed, and he did not have symptoms. Thus, when asked about her family history, “I told them that my dad had pancreatitis,” she states. “Because of that reason ... they tested me for it and it came out positive.” The doctors told the family that they typically do not test children for pancreatitis because it is so rare, so it was fortunate that this conversation Valentina overheard—and decided to mention to the doctors—led to an accurate diagnosis of her illness so quickly after her severe symptoms started.



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## ABOUT PANCREATITIS

The pancreas is an organ located behind the stomach that has many important functions. Tiny clusters of cells in the pancreas, called islets, produce hormones such as insulin that regulate blood sugar (glucose) levels. The pancreas also produces fluid that is released through ducts into the intestine and contains enzymes that are necessary for digestion of food. Usually, these powerful digestive enzymes are inactive until they exit the pancreas and enter the small intestine. In cases of pancreatitis, however, digestive enzymes are activated prematurely while still inside the pancreas, resulting in damage and inflammation, and symptoms of abdominal pain, nausea, and vomiting. As Valentina explains, “My pancreas is actually digesting itself.” Chronic pancreatitis is rare in children and is often associated with a genetic mutation. People with genetic forms of pancreatitis have a higher risk of developing pancreatic cancer later in life.

After she was diagnosed, Valentina was admitted to the hospital for 5 days and then went home, but she was not there long. “She got very, very sick again, so we had to take her back to the hospital,” says her mom. At that time, the gastrointestinal doctor was on vacation, so there was no specialist available to help them. Sonia, speaking through tears, remembers how terrifying the experience was. “I couldn’t do anything for her. I was so worried because she couldn’t eat,” she says. “It was awful.... She was dying in the hospital. She was so, so sick, with a lot of pain.”

Not knowing what else to do, Sonia spent her nights searching the internet for a doctor who had expertise in pancreatitis and could help her daughter. She found a pancreatitis specialist at University of Florida (UF) Health, but the doctor was about 6 hours away and only treated adult patients. However, Sonia was adamant that Valentina desperately needed this doctor’s help, and her persistence paid off: the doctor made an exception and agreed to see Valentina very quickly. Reflecting on that experience, Sonia has an important message for other parents: “Be your own advocate.”

Sonia finding that doctor at UF Health changed the entire course of Valentina’s illness. It was there, in late November, about a month after her diagnosis, that Valentina underwent her first surgery to treat her pancreatitis: “Endoscopic retrograde cholangiopancreatography, or ERCP,” she explains. “What they did was remove what was blocking the pancreatic ducts, and placed stents to keep the ducts open and allow the enzymes to flow to the small intestine.” After that surgery, she was feeling better, and was even able to eat a little bit.

It was also there that Valentina found out that she had a genetic form of pancreatitis. She explained that the genetic mutation is in a gene called *PRSS1*. Because of the increased cancer risk associated with her form of pancreatitis, their doctor at UF Health recommended that Valentina have her pancreas removed via TP-IAT, and their doctor also suggested that Valentina be treated by TP-IAT experts at the Medical University of South Carolina (MUSC).

## UNDERGOING TP-IAT SURGERY

In the first stage of the TP-IAT procedure, the pancreas is surgically removed and the gastrointestinal tract is reconstructed. Removing the pancreas would result in lifelong diabetes because the organ contains the only source of insulin-producing beta cells in the body; without insulin, the body cannot regulate blood sugar levels, so the patient would become insulin dependent. However, in the second stage of TP-IAT—*islet autotransplantation*—the islets that contain the beta cells are collected from the patient’s pancreas and infused back into the portal vein of the liver. The islets then become lodged in blood vessels of the liver where they settle, grow, and begin producing insulin. Thus, the TP-IAT surgery serves the dual purpose of removing the source of severe pain and also eliminating the increased cancer risk while preserving some insulin production, reducing the risk of developing diabetes.

Additionally, because the islets are from the patient’s own pancreas, the body does not recognize them

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as “foreign” and mount an immune response to them. Thus, people undergoing TP-IAT do not need to take immune-suppressing medicines, which can have serious side effects. This is in contrast to islet transplantation alone, which is an experimental procedure that uses islets from deceased organ donors to treat some people with type 1 diabetes. Notably, people undergoing TP-IAT do require lifelong supplements to replace their pancreatic digestive enzymes.

When hearing about the possible TP-IAT surgery, Valentina didn’t have any reservations: “I immediately felt that this was something that I had to do.... I surprisingly wasn’t afraid.” Her parents, on the other hand, were not thrilled at the thought of their daughter undergoing major surgery to remove a vital organ. As Sonia explains, “Valentina was doing better with the stent. She was able to eat more, so we were kind of more relaxed.” Valentina remembers that her parents were also worried about the prospect that she could have diabetes for the rest of her life, so Sonia and her husband thought about waiting a year before considering the surgery. “But then Valentina started getting sick again, even with the stent, and we had to take her back to the hospital,” Sonia remembers. Because Valentina was barely able to eat, she lost 50 pounds during the course of her illness—weighing only 82 pounds at her lowest. She also had to stay in bed nearly all the time because she was so sick and weak. As a result of Valentina’s worsening health, the family decided to move forward with the TP-IAT evaluation to determine by the MUSC’s doctors if she was a candidate for this type of surgery.

For the evaluation, Valentina underwent 3 days of intensive testing and meetings with various doctors at MUSC. For example, “we had a meeting with an endocrinologist, a psychologist, and a nutritionist and they explained to us how a diabetes life would work,” recalls Valentina. Even though the hope was that the islet autotransplantation component of the surgery would reduce her risk of developing diabetes, there was still a possibility that her own transplanted islets would not function properly, or

not make enough insulin to regulate her blood sugar levels. Thus, it was possible that, after her surgery, she would have to manage diabetes by counting carbohydrates when she ate, monitoring her blood sugar levels, and administering insulin.

After the evaluation, the family returned to their home in Florida and soon found out that Valentina was a candidate for TP-IAT. The surgery was scheduled for January 2018—only 3 months after she was diagnosed with pancreatitis. Now the family was faced with the fact that they had to separate, as Valentina’s surgery was far from their home and her sister and father couldn’t come with her due to school and work. Her mom was able to obtain permission from work to take time off for Valentina’s surgery and for her recovery in South Carolina. Her father took care of her younger sister and provided all the financial support. The family rented a house near MUSC so that they could be near Valentina for the surgery and during the 1 month of recovery period.

***When hearing about the possible total pancreatectomy-islet cell autotransplantation (TP-IAT) surgery for pancreatitis, Valentina didn’t have any reservations: “I immediately felt that this was something that I had to do.... I surprisingly wasn’t afraid.”***

“The day of my surgery, I remember waking up very early. Honestly, it was very peaceful.... In the car ride there [to the hospital], I prayed the whole ride. It was kind of me saying I had an understanding that if it was time to go, that I was OK with it,” Valentina recalls, even though she was only 16 years old at the time. Her surgery lasted a total of 12 hours. During the first 6 hours, the surgeons removed her pancreas, along with nearby organs such as her spleen and gallbladder. After that, it took another 4 hours to isolate the pancreatic islets and 2 hours to transplant them

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into her liver. Throughout the procedure, Valentina was in excellent medical hands—both she and her mother raved about the MUSC doctors and nurses, who they said were outstanding, explained everything, and made Valentina feel comfortable.

## LEARNING TO MANAGE DIABETES

After the surgery, Valentina spent a few days in the intensive care unit before being transferred to another floor of the hospital. It was a difficult time. She was hooked up to numerous machines and had to take medicine to manage her severe post-operative pain. Even talking was difficult. Also, because it could take several months or longer to know whether Valentina's own transplanted islets were going to function properly, she had to be treated for diabetes. The nurses checked her blood sugar levels around the clock and administered insulin. However, Valentina's positive attitude shined through even under these most challenging circumstances: "She walked the second day after her surgery," beams her proud mother. "She did so great, walking with pain every single day, three or four times per day to recover fast to leave the hospital."

*"I am pain-free ever since recovering from my surgery," Valentina says.*

After Valentina was released from the hospital, the family stayed in South Carolina for a month to be close to the MUSC doctors. During that time, Valentina remembers that, "It was hard getting used to everything, especially the diabetes part. In the beginning, we were so lost ... with the counting carbs [carbohydrates] and understanding that a certain amount of carbs meant a certain amount of insulin." She said things only got harder over time. "At first it wasn't too hard," she states, "because after such a big surgery I wasn't eating very much. But ... when my appetite started going up, that's when we had the most difficulties."

## ACHIEVING INSULIN INDEPENDENCE

Coming home after a month in South Carolina, Valentina was still recovering from her surgery and managing her diabetes. Sonia had to return to work after taking 4 months off to care for her daughter, so the family hired a nurse to help Valentina during her long and challenging recovery. Valentina returned to school that summer, after missing nearly an entire school year. That transition was also difficult because she was not fully recovered from her surgery, got tired easily, and had to learn how to manage her diabetes while at school.

However, as time progressed, she made a welcome discovery: "We started noticing that my blood sugars were getting lower and lower every day." During one of her regular endocrinologist check-ups, the doctor told Valentina not to take insulin if her blood sugars were within a healthy range. "It got to the point that I didn't need any insulin," she states happily. As of November 2018, Valentina has been insulin independent. "Now, I only check my blood sugar when I'm feeling sick," she explains, which is roughly once every couple of weeks. If her blood sugar levels are elevated, she lays down or drinks water and that helps her levels come down without needing insulin. "We don't have insulin in the house anymore," says Sonia.

As Valentina notes, "My diabetes was obviously very different from most diabetes," since it resulted from the removal of her pancreas. Therefore, TP-IAT is not an option for treating other forms of diabetes, like type 1 or type 2 diabetes, because the underlying cause of the disease is different.

## LIVING A PAIN-FREE AND DIABETES-FREE LIFE

Her successful surgery and recovery have enabled Valentina to look toward the future, with her plan to pursue international studies or law when she begins college next year. At the time she was interviewed for this profile, it had been 1½ years since she

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underwent her TP-IAT surgery. “I am pain-free ever since recovering from my surgery,” she says. She has also recently been able to be more active, including cheerleading for her school, playing tennis and volleyball, and doing a lot of volunteer work.

***Valentina says that before her total pancreatectomy-islet cell autotransplantation (TP-IAT) surgery, which could cause diabetes, the doctors “told us there was a possibility that I wouldn’t be dependent on insulin, but I don’t think I really had high hopes for that.... For me to get past my expectations of where I was going to end up after the surgery is just mind-blowing.”***

One of the complications after her surgery is that her body cannot adequately absorb iron, so she has to get intravenous iron infusions. She also has to spend a lot of time keeping track of her diet to figure out what foods make her feel good and which ones make her feel unwell, and she takes enzyme replacement supplements at each meal, something she will continue to do for the rest of her life. And, even though she’s feeling much better physically, the entire experience has taken a mental toll on her. “I don’t really feel normal,” she explains. “Thinking about it mentally, I feel like a year of my life has been stolen from me, where I’ve been forced to become more mature and aware of everything.” However, Valentina’s positive attitude is still apparent: “But I think I’m appreciative of that, of what I know and what I’ve yet to learn.”

Additionally, Valentina says that before her TP-IAT surgery, the doctors “told us there was a possibility that I wouldn’t be dependent on insulin, but I don’t think I really had high hopes for that.... For me to get past my expectations of where I was going to end up after the surgery is just mind-blowing.” Valentina is grateful for the islet autotransplantation portion of her TP-IAT surgery—the extra 6 hours it took to

isolate and transplant her own islets has given her freedom from diabetes.

## HOPE THROUGH RESEARCH

NIDDK has supported much of the clinical research on use of TP-IAT for treating chronic pancreatitis in adults and children, and currently supports research to improve outcomes after TP-IAT surgery, including at MUSC and other sites. For example, MUSC researchers are testing a strategy to enhance survival and function of the transplanted islets in adults with chronic pancreatitis undergoing TP-IAT to further reduce the risk that people will develop diabetes after surgery.

NIDDK has also supported other research related to pancreatitis conducted by individual investigator-led teams, as well as larger, multi-center studies, such as the North American Pancreatic Study Group and, more recently, the Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer, that have led to important discoveries of genetic risk factors and other advances in understanding and managing pancreatitis in children and adults.

***“If we didn’t have this research, Valentina wouldn’t be here today,” her mother, Sonia, exclaims.***

Valentina and her mother value and appreciate the role of NIDDK-supported research in Valentina’s improved health. “If we didn’t have this research, Valentina wouldn’t be here today,” Sonia exclaims. “I’m so thankful to the Lord Jesus for the technology, the doctors, the researchers.” Valentina adds about the research progress: “I think it’s incredible.” Also incredible is the fact that Valentina, at such a young age, has faced formidable health issues with tremendous maturity and positivity—there is no question that her future is bright.

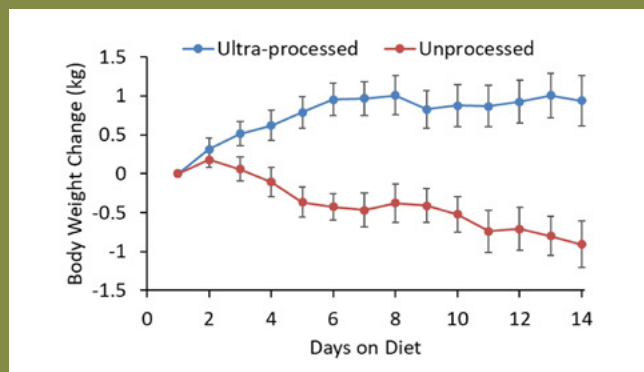




Ultra-processed Diet



Unprocessed Diet



In a recent study described in this chapter, researchers found that a diet of mainly ultra-processed foods—those with ingredients predominantly found in industrial food manufacturing—causes overeating and weight gain. For the study, they recruited 20 men and women to live at the NIH Clinical Center and eat a diet of ultra-processed foods and a diet of unprocessed foods for 2 weeks each, with all meals and snacks provided by the study staff. The ultra-processed and unprocessed meals presented to the participants had the same amounts of calories, sugar, fat, salt, and fiber; and the participants could eat as much or as little of each meal as they wanted. Examples of breakfasts, lunches, and dinners are pictured in this image, along with a graph showing the average changes in body weight. (To match the amount of fiber naturally found in unprocessed foods, many of the ultra-processed meals included fiber supplements dissolved in multiple beverages, shown in the photos.) On the ultra-processed diet, the study participants consumed 500 more calories per day and gained about 2 pounds (0.9 kilograms) on average, while the same individuals lost about 2 pounds during their time on the unprocessed diet. Further studies are needed to understand what aspects of the ultra-processed foods caused overeating and weight gain.

*Images courtesy of Dr. Kevin Hall, NIDDK. Reprinted from Cell Metabolism, 30, Hall KD, Ayuketah A, Brychta R,...Zhou M, Ultra-processed diets cause excess calorie intake and weight gain: An inpatient randomized controlled trial of ad libitum food intake, 67-77, Copyright 2019, with permission from Elsevier.*

# Obesity

*Obesity has risen to epidemic levels in the United States. Individuals who have obesity may suffer devastating health problems, face reduced life expectancy, and experience stigma and discrimination. Obesity is a strong risk factor for type 2 diabetes, fatty liver disease, and many other diseases and disorders within the NIDDK's mission. Nearly 40 percent of U.S. adults are considered to have obesity based on body mass index (BMI), a measure of weight relative to height.<sup>1</sup> More than 18 percent of children and adolescents also have obesity, and thus are at increased risk for developing serious diseases both during their youth and later in adulthood.<sup>1,2</sup> Obesity disproportionately affects people from certain racial and ethnic groups and those who are socioeconomically disadvantaged.*

The high prevalence of obesity in the United States is thought to result from the interaction of genetic susceptibility with behaviors and factors in the environment that promote increased caloric intake and sedentary lifestyles. Diet, activity, and aspects of our environment may also modify biologic factors in ways that promote obesity. Research is providing the foundation for actions to address this major public health problem by illuminating the causes and consequences of obesity, evaluating potential prevention and treatment strategies, and providing an evidence base to inform policy decisions.

The NIDDK supports a multi-dimensional research portfolio on obesity, spanning basic, clinical, and translational research. NIDDK-funded studies investigate a variety of approaches for preventing and treating obesity. These span behavioral and environmental interventions in families and in health care and other settings, using a variety of approaches and technologies; surgical interventions; and combinations of strategies. In parallel, NIDDK-supported investigations into the biologic processes associated with body weight have continued to spark new ideas for intervention approaches.

The NIDDK also continues to play a leading role in the NIH Obesity Research Task Force. The NIDDK Director co-chairs the Task Force along with the Directors of the National Heart, Lung, and Blood Institute and the Eunice Kennedy Shriver National Institute of Child Health and Human Development. The Task Force includes representatives from these and numerous other NIH Institutes, Centers, and Offices.

Highlights of recent advances from NIDDK-supported research on obesity are provided in this chapter.

## COMBATING CHILDHOOD OBESITY

**Responsive Parenting—An Early Start Toward Obesity Prevention:** In a recent clinical trial, researchers found that educating first-time moms on responsive parenting, with tips on infant feeding, sleep, play, and emotion, resulted in a modest improvement in body weight of the children through age 3 years. The researchers developed the intervention with the hope of setting children on a healthy growth trajectory starting early in life, because childhood obesity can lead to serious diseases during youth and later in adulthood, and rapid and excess weight gain at a young age increases risk for obesity.

The researchers recruited mothers soon after childbirth, randomly assigned them to either the responsive parenting intervention or a control intervention on home safety, and then tracked their children's growth for the next 3 years. In each intervention group, 116 mother-child pairs completed the full study; the participants were mainly white, middle-income families. Both

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<sup>1</sup> Hales CM, et al. 2017. CDC. National Center for Health Statistics Data Brief No. 288.

<sup>2</sup> For children and adolescents, obesity refers to a BMI at or greater than the 95<sup>th</sup> percentile on growth charts (which are based on previous national surveys).

interventions included home visits by research nurses, study participant visits to the research center, and other components. The responsive parenting intervention focused on responding to a child's needs in a prompt and age-appropriate way and provided a wealth of information helpful to new parents. This included tips on recognizing when infants are hungry or full, using alternatives to feeding to soothe infants who are fussy but not hungry, serving age-appropriate portion sizes of food, putting infants to bed and other sleep-related information, establishing routines, and interactive play. When the children were 1 year of age, those in the responsive parenting group were less likely to be overweight than those in the control group, a promising result reported previously. The research team has since found that, at age 3 years, children in the responsive parenting group had significantly healthier body weights than those in the control group, as assessed by "BMI z score," a measure of weight relative to height that also reflects how far a child's weight is from average. Although the effect was modest, it was within the range considered clinically meaningful. Interestingly, the intervention had a greater effect on girls' weight than on boys' weight, though the reasons are unclear. Research staff also monitored the children to see whether any were not gaining enough weight and found no significant differences between groups in this potential side effect.

This study thus provides hope that a parenting intervention, begun in infancy, may help reduce excess weight gain during childhood. To determine longer-term effects, the researchers plan to evaluate the children's weight and eating behaviors up to age 9. They also initiated a study of a similar intervention for obesity prevention in a different population, African Americans in low socioeconomic areas in the rural South, with the goal of improving the health of more children.

Paul IM, Savage JS, Anzman-Frasca S,...Birch LL. Effect of a responsive parenting educational intervention on childhood weight outcomes at 3 years of age: The INSIGHT randomized clinical trial. *JAMA* 320: 461-468, 2018.

## COMPARING BARIATRIC SURGERY IN TEENS AND ADULTS

**Age Is More Than Just a Number: Early Weight-loss Surgery May Lead to Better Health Outcomes:** Researchers have found that, despite similar weight loss, teens who underwent a form of bariatric surgery called Roux-en-Y gastric bypass were

significantly more likely to have remission of type 2 diabetes and high blood pressure compared to adults who underwent the same procedure.

Bariatric surgery can be an effective tool for treating severe obesity, leading to significant weight loss and improved health outcomes for both adolescents and adults. However, whether results vary depending on the age of the patient at the time of surgery was unclear. In this analysis that utilized data from two related but independent studies, researchers evaluated outcomes of gastric bypass surgery in 161 adolescents (ages 13-19) with severe obesity and compared them to outcomes in 396 adults (ages 25-50) who reported having obesity since they were teens and who underwent gastric bypass surgery during adulthood. Five years after surgery, the two groups had achieved significant weight loss that was similar in magnitude. While the number of participants with type 2 diabetes declined in both groups after surgery, adolescents were more likely to have their diabetes resolve. In fact, no teens in the group needed diabetes medication 5 years post-surgery. Moreover, among those participants with high blood pressure prior to surgery, teens were 50 percent more likely than adults to have their blood pressure return to normal and no longer require medication. Although teens had better health outcomes in certain areas compared to adults, they also experienced increased risks in other areas, including needing subsequent abdominal surgeries and exhibiting low iron and vitamin D levels, the latter of which could possibly be explained by nonadherence to vitamin supplements. Bariatric surgery risk is further highlighted by the fact that deaths occurred in both groups within 5 years post-surgery (3 teens, 7 adults). Two of the adolescent deaths were related to substance use, which merits attention given that a previous study had found an increased risk of substance use disorders following bariatric surgery in adults.

This study demonstrates that bariatric surgery at a younger age may provide significant health benefits like remission of type 2 diabetes and high blood pressure, in addition to substantial weight loss, potentially avoiding adverse effects of prolonged severe obesity into adulthood. While this study contributes important information about the reversal of obesity-related conditions post-surgery, the procedure is not without risk, and lifetime risk remains unknown. Future research can help to shed light on the best timing of bariatric surgery and the

most effective treatments for people with obesity and its related conditions.

Inge TH, Courcoulas AP, Jenkins TM,...Helmuth MA; Teen-LABS Consortium. Five-year outcomes of gastric bypass in adolescents as compared with adults. *N Engl J Med* 380: 2136-2145, 2019.

## RESEARCH TOWARD IMPROVING HEALTH IN PREGNANCY

### New Evidence-based Recommendations for Calorie Intake in Pregnant Women with Obesity:

Researchers have provided, for the first time, evidence-based recommendations for energy intake (caloric intake) in pregnant women with obesity, making this a pioneering study in its field that can potentially help improve obstetrical care.

Excess gestational weight gain occurs in two-thirds of pregnancies and can lead to metabolic impairments in the mother and increased risk for obesity in the child. There have been several trials to evaluate the effectiveness of lifestyle interventions for pregnant women with overweight/obesity, but only half have resulted in substantially reduced weight gain during pregnancy. Moreover, current recommendations for energy intake during pregnancy have been based only on studies in women without obesity or have been based on subjective, self-reported assessments, which are prone to recall bias. To enhance the understanding of caloric needs during pregnancy and characterize factors leading to excess gestational weight gain in women with obesity, researchers analyzed energy intake and energy expenditure (calories burned) in 54 pregnant women with obesity during the second and third trimesters using technologies and methods for rigorous, objective measurement. Applying the 2009 Institute of Medicine guidelines for gestational weight gain, 8 women from the study group gained the recommended amount of weight during the study period (approximately 4.5 kg or 9.9 lbs.) while 36 women gained an excess amount of weight (approximately 10.3 kg or 22.7 lbs.); 10 women experienced inadequate weight gain. The investigators determined that differences in weight gain were not related to differences in physical activity, physiological factors such as hormone activity, or factors such as diet quality. To understand other aspects of pregnancy-related weight gain, the researchers measured amounts of body fat in the women, as well as amounts of fat-free body tissues and fluid and calculated their

energy expenditure. The women who gained the recommended amount of weight had gained that weight in fluid and fat-free body mass (including the fetus and tissues such as the placenta), while actually losing a small amount of body fat. In those who gained excess weight, the extra weight was from increased fat tissue. The researchers' findings suggest that pregnant women with obesity should not consume extra calories during the second and third trimesters and that the energy needs of the fetus are met by mobilizing maternal fat mass to achieve healthy delivery of the infant. Importantly, these findings challenge the current recommendations for women with obesity, which advise consuming an additional 200-300 calories/day after the first trimester.

This study is unique in its use of objective methods to assess energy requirements in pregnant women with obesity and it has the potential to improve obstetrical patient care for better maternal and infant outcomes. However, it is limited by its small sample size, and evaluation of the longer-term effects on the children's development will be important. Future research could lead to the implementation of new, evidence-based recommendations for calorie intake in pregnant women with obesity.

Most J, Amant MS, Hsia DS,...Redman LM. Evidence-based recommendations for energy intake in pregnant women with obesity. *J Clin Invest* 130: 4682-4690, 2019.

## ULTRA-PROCESSED FOODS AND WEIGHT GAIN

### Diets of Ultra-processed Foods Cause Overeating and Weight Gain:

Comparing effects of a diet of ultra-processed food with a diet of unprocessed or minimally processed foods, researchers found that people consumed more calories per day and gained weight on the ultra-processed diet, while losing weight on the unprocessed food diet. Ultra-processed foods, as defined by a classification system called NOVA, are those with ingredients and additives predominantly found in industrial food manufacturing, such as hydrogenated oils, high-fructose corn syrup, flavoring agents, and emulsifiers. Past research had shown a correlation between ultra-processed foods and worse health, but it was not known whether ultra-processed foods actually caused people to eat too much and gain weight, or whether the correlation was the result of other factors. Thus, a team of NIDDK intramural

scientists and colleagues decided to test this with a small group of people in a rigorously controlled study.

The research team recruited volunteers, 10 men and 10 women, to live at the NIH Clinical Center for a month and eat a diet of ultra-processed foods and a diet of unprocessed foods for 2 weeks each. All meals and snacks were provided by the study staff, and the participants could eat as much or as little of each meal as they wanted. The researchers designed the ultra-processed and unprocessed meals to have the same amounts of calories, sugar, fat, salt, and fiber. The diets were also similarly palatable, as rated by the participants. (Examples of the meals are shown at the beginning of this chapter.) On the ultra-processed diet, the study participants ate an average of 500 more calories per day and gained about 2 pounds, while they lost about 2 pounds during their time on the unprocessed diet. They also ate faster (more calories per minute) when on the ultra-processed diet. What attributes of the ultra-processed foods might have caused these effects? Among the differences between the diets, the ultra-processed foods contained more saturated and other unhealthy fats; added sugars, rather than only sugars naturally occurring in foods; and industrially processed ingredients. There were also slight differences in amounts of protein between the two diets, which the researchers note could explain as much as half the difference in calorie intake. However, it is not yet clear whether these or other factors led to the effects of the ultra-processed diet.

The results of this study suggest that limiting consumption of ultra-processed foods could help prevent excess weight gain. However, ultra-processed foods are ubiquitous in the United States, inexpensive, highly convenient, have a long shelf-life, and do provide needed nutrients. By contrast, less-processed foods typically take more time and expense to prepare. Future research could lead to strategies for developing diets with the convenience of ultra-processed foods and the weight-related health benefits of unprocessed foods.

Hall KD, Ayuketah A, Brychta R,...Zhou M. *Ultra-processed diets cause excess calorie intake and weight gain: An inpatient randomized controlled trial of ad libitum food intake.* *Cell Metab* 30: 67-77, 2019.

## GUT MICROBIOME AND BODY WEIGHT

**Networking Gut Bacteria and Their Role in Body Weight:** A recent study has shown that women

with lean body types who eat high-fiber diets have complex, highly interactive bacterial networks in their gut microbiomes, and subsequent experiments in mice showed that these bacteria can impart resistance to obesity for several weeks on a high-fat diet. The human gastrointestinal tract is home to a thriving community of bacteria. Studies have shown that some of these bacteria are associated with leaner body types, suggesting that they may protect against obesity. One way that they might do this is by breaking down dietary fiber to produce short-chain fatty acids (SCFAs), which are molecules believed to play an important role in regulating metabolism. The overarching properties of the microbiome that may encourage SCFA production and protect against obesity are unclear, however. For example, little is known about how the many different types of bacteria in the gut interact with each other—one type of bacteria might rely upon another to survive while aggressively competing against other types of bacteria for space and nutrients. Recognizing relationships such as these would allow scientists to conceptualize an ecological network in the gut, wherein a type of bacteria is “connected” to another if it affects its ability to thrive. Scientists could then determine how changes to this microbial network affect health.

In a recent study, scientists sought to gain understanding of human gut microbial networks by analyzing the gut microbiomes of 50 women from rural Ghana and 50 African American women from an urban area of the United States. Roughly half the participants—some from each country—were women who had obesity, while the others had lean body types, allowing the researchers to compare not only the microbiomes between different geographical areas, but also between people with different body types. Sequencing the microbial genetic material from the participants’ microbiomes (obtained from fecal material), the scientists identified the types of bacteria that were inhabiting the women’s guts, along with their relative amounts. They then compared the microbiomes from all the women to gain an understanding of how the types of bacteria relate to each other—whether their quantities tend to increase or decrease in parallel from sample to sample, or if their abundances appear to be unrelated. They found that the Ghanaian women with lean and obese body types—all of whom tended to eat more starches and fiber-rich foods than the U.S. women—had more diverse microbiomes and



higher amounts of detectable SCFAs than their respective U.S. counterparts who ate diets higher in protein and lower in fiber. Also, the Ghanaian women with lean body types had microbiomes that formed the most densely interconnected bacterial networks compared to the rest of the study population; the researchers also estimated, with computer modeling, that the Ghanaian microbiomes were more stable and resistant to disruption. To determine how this affects health, the researchers inoculated male mice, which had been treated with antibiotics to deplete their native gut bacteria, with samples of the microbiomes from each group of women (those from the United States or Ghana, and with lean or obese body types). The mice that were given samples of the microbiome from a Ghanaian woman with a lean body type were significantly more resistant to weight gain when fed an obesity-inducing high-fat diet for 6 weeks, compared to the mice harboring the microbiomes from the other groups of women. These obesity-resistant mice also made higher levels of a molecule that interacts with SCFAs, suggesting that bacterial-derived SCFAs may be involved in preventing weight gain in these mice.

Rather than pointing to individual types of bacteria, this was the first study to implicate the characteristics of the entire microbiome network—including how the many types of bacteria in the microbiome relate to each other—in metabolic conditions like obesity. This could be important for future studies to determine the best ways to manipulate the microbiome to improve health.

*Dugas LR, Bernabé BP, Priyadarshini M,...Layden BT. Decreased microbial co-occurrence network stability and SCFA receptor level correlates with obesity in African-origin women. Sci Rep 8: 17135, 2018.*

## BRAIN DEVELOPMENT AND BODY WEIGHT

**Wired for Obesity: How Genes Involved in Brain Development Affect Body Weight:** Researchers have identified key genes that guide brain circuit development and link this process to body weight regulation. It is known that a part of the brain called the hypothalamus plays a critical role in body weight regulation. But, the exact molecules involved remain unclear.

In this study, scientists investigated the role of a group of molecules called semaphorins, which are

abundant in the hypothalamus during development and are released by brain cells, allowing them to communicate with other brain cells. The researchers first tested DNA samples from children and adults (male and female) and found that individuals with severe early-onset obesity had mutations in several genes involved in semaphorin signaling pathways. These mutations are rare, but, collectively, appeared more frequently in the people with severe early-onset obesity than in healthy individuals. To investigate the role of semaphorins further, they used a zebrafish model to test whether altered semaphorin signaling in the hypothalamus influenced body weight. They found that deletions in seven of the genes that code for semaphorins, or proteins they interact with, caused weight gain and/or an increased percentage of body fat. In addition, deletion in two other genes decreased zebrafish body fat percentage. These results suggest that disruption of semaphorin signaling pathways has an impact on energy balance, or the relationship between calories consumed and calories burned. Next, studying mouse hypothalamic cells in laboratory culture dishes with other cells that had normal or mutant semaphorin genes, they found that several semaphorin gene variants stunted the outgrowth of projections from the hypothalamic cells, preventing cells from growing properly and forming appropriate cellular connections. Lastly, the researchers genetically modified mice to deplete the protein “receptor” on the surface of hypothalamic brain cells on which semaphorins exert their effects. Hypothalamic cell cultures from the male mice that were studied showed that genetic loss of semaphorin action through loss of the receptor blocked the outgrowth of cell projections, suggesting a role of semaphorin signaling in the formation of connections between cells in the brain. Moreover, the genetically modified mice had significantly higher body weights and reduced energy expenditure (calorie burning) compared to their normal littermates.

Taken together, these results suggest that semaphorin signaling plays a role in the connections between different brain regions and the development of brain circuitry that governs body weight. Further studies on human semaphorin gene variants could inform our understanding of obesity in people and possibly lead to prevention and treatment strategies.

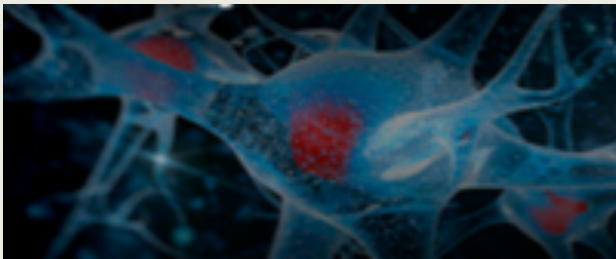
*van der Klaauw AA, Croizier S, Mendes de Oliveira E,...Farooqi IS. Human semaphorin 3 variants link melanocortin circuit development and energy balance. Cell 176: 729-742, 2019.*



# Beyond the Scale: Understanding Weight Gain—and Regain—from Studies of Body and Mind

NIDDK convened experts from across the country for three workshops in 2019 on challenges and opportunities in obesity research. An April workshop focused on novel technologies for brain imaging, to understand the brain's complex roles in obesity and diabetes. At a workshop in May, researchers explored challenges in measuring body fat and other tissues in infants and young children. At a June workshop, researchers presented studies of both body and mind that are illuminating why it is so difficult to maintain a healthier weight after weight loss.

**Neuroimaging and Modulation in Obesity and Diabetes Research 10<sup>th</sup> Anniversary Meeting, April 16-17, 2019:** Focusing on cutting-edge technologies for brain imaging and related studies, researchers at the workshop reviewed the progress over the past 10 years, new technologies on the horizon, and potential future directions. The broad range of topics included, for example, the roles of different parts of the brain in appetite suppression and motivation for eating, deep brain stimulation as a potential therapy for a particular type of overeating, obesity-associated impairments in memory and cognition, and brain imaging studies of appetite in children.

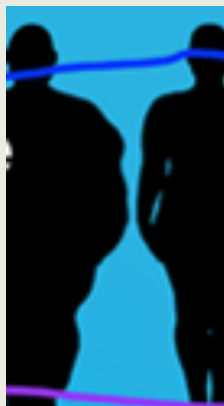


**Body Composition Measurements from Birth through 5 Years Workshop, May 30-31, 2019:** Because obesity often begins in childhood, it is critical to be able to measure body composition—the amounts of fat, other tissues, and water in the body—in very young children. Such measures are useful for understanding and predicting health risks and evaluating potential therapies. However, a simple scale does not give sufficient information, and

there are unique challenges to body composition measurements in very young children. For example, babies and toddlers typically cannot stay still long enough for accurate measures using technologies that measure fat and lean tissue. At this workshop, researchers discussed emerging technologies and research opportunities.



**The Physiology of the Weight Reduced State Workshop, June 3-4, 2019:** While many people can lose some excess weight over the short term, a majority have trouble maintaining that weight loss, even with persistent efforts. While some challenges come from living in an environment that promotes unhealthy eating and sedentary behavior, there are also physiological changes that occur after weight loss that lead to increased appetite and reduced calorie burning. At the workshop, researchers presented studies of molecular and cellular pathways in the brain and body that contribute to regain of lost weight;



potential strategies to reduce weight regain after lifestyle interventions, bariatric surgery, and weight-loss medications; and differences between individuals in weight loss and regain. The researchers also explored opportunities for future research that could lead to more effective and lasting obesity treatments.

# NIH Seminar Series: Nonalcoholic Fatty Liver Disease and Obesity



The liver is the largest internal organ and a true workhorse within the body. Among its many jobs are converting food into fuel, processing fat from the blood, and clearing harmful toxins. Due to its central role in metabolizing nutrients, this hard-working organ is also susceptible to a condition called nonalcoholic fatty liver disease (NAFLD). NAFLD is a condition in which excess fat is stored in the liver of people who drink little to no alcohol. It is rapidly becoming the most common form of chronic liver disease worldwide, due in part to the increasing prevalence of obesity, although not all people with overweight or obesity develop this condition. Approximately one-third of adults in the United States have NAFLD, and the majority of this group have a condition called simple fatty liver, in which fat has accumulated in the liver, but there are no signs of inflammation or damage.<sup>1</sup> However, in some people with NAFLD, the condition progresses to a more dangerous form called nonalcoholic steatohepatitis, or NASH, that is characterized by inflammation and liver cell damage that can lead to cirrhosis or liver cancer.

Given how common NAFLD is and that the only recommended therapy is weight loss through diet and exercise, the research community recognizes the need to accelerate development of new and innovative treatments for NAFLD and obesity and to close knowledge gaps with the goal of translation

into more effective patient care. To that end, four leading scientists highlighted their research on NAFLD and obesity at a September 2019 symposium at the NIH campus in Bethesda, Maryland. Dr. Averell Sherker from the NIDDK moderated a panel discussion following the presentations. The research presented was supported by NIDDK among other Institutes. The seminar was organized as part of the NIH Obesity Research Task Force Seminar Series.

Dr. Natalie Torok from Stanford University presented her research focusing on the molecular mechanisms behind the development of NASH and the role of factors such as age and diet in disease processes. Her group studies young and older mice fed either a typical chow diet or a diet that mimics a fast-food diet consumed by humans that can induce insulin resistance and/or liver disease. In this model, Dr. Torok's team found that specific molecules become activated in the livers of older mice on a fast-food diet; these molecules are known to mediate "oxidative stress" pathways that can cause cell and tissue damage. This discovery could lead to future therapies that specifically target these molecules and pathways.

Dr. Hashem El-Serag from the Baylor College of Medicine summarized his studies on the epidemiology of NAFLD in U.S. adults. Through research conducted using data from the national Veterans Affairs health care system, Dr. El-Serag and his team determined that the prevalence of NAFLD more than doubled from 2003-2011.<sup>2</sup> Dr. El-Serag went on to describe how NAFLD is more common in Hispanics and less common in African Americans. There are several established risk factors, including obesity, hypertension, metabolic syndrome, and type 2 diabetes—in fact, globally, NAFLD is estimated to affect more than half of people with type 2 diabetes.<sup>3</sup> Due to the rising prevalence of NAFLD, cirrhosis and liver cancer are also on the rise. Future research could help to ameliorate this trend.

<sup>1</sup> Spengler EK and Loomba R. *Mayo Clinic Proceedings* 90: 1233–1246, 2015.

<sup>2</sup> Kanwal F, et al. *Clin Gastroenterol Hepatol* 14: 301-308, 2016.

<sup>3</sup> Younossi ZM, et al. *J Hepatol* 71: 793-801, 2019.

NAFLD does not just affect adults, but also children. Research suggests that 10 percent of U.S. children ages 2-19 years have NAFLD; prevalence is higher in males.<sup>4</sup> Dr. Jeffrey Schwimmer from the University of California San Diego has focused his research on the prevalence, diagnosis, and treatment of this condition in children and teens. Like adults, obesity and type 2 diabetes are risk factors for children, and Dr. Schwimmer's research suggests early screening and diagnosis are crucial for better outcomes in adulthood. Moreover, his research indicates that diet plays a big role: a low-sugar diet compared to a standard diet resulted in significant improvement in liver disease in a group of adolescent boys. These findings are preliminary, and further research is necessary to evaluate long-term outcomes.

Dr. Yaron Rotman from the NIDDK Division of Intramural Research concluded the series of presentations by describing his research on current and emerging therapies to treat NAFLD. Lifestyle interventions through healthy diet and exercise remain the standard of care, although there is little consensus among health care providers about which diet to recommend for this condition.

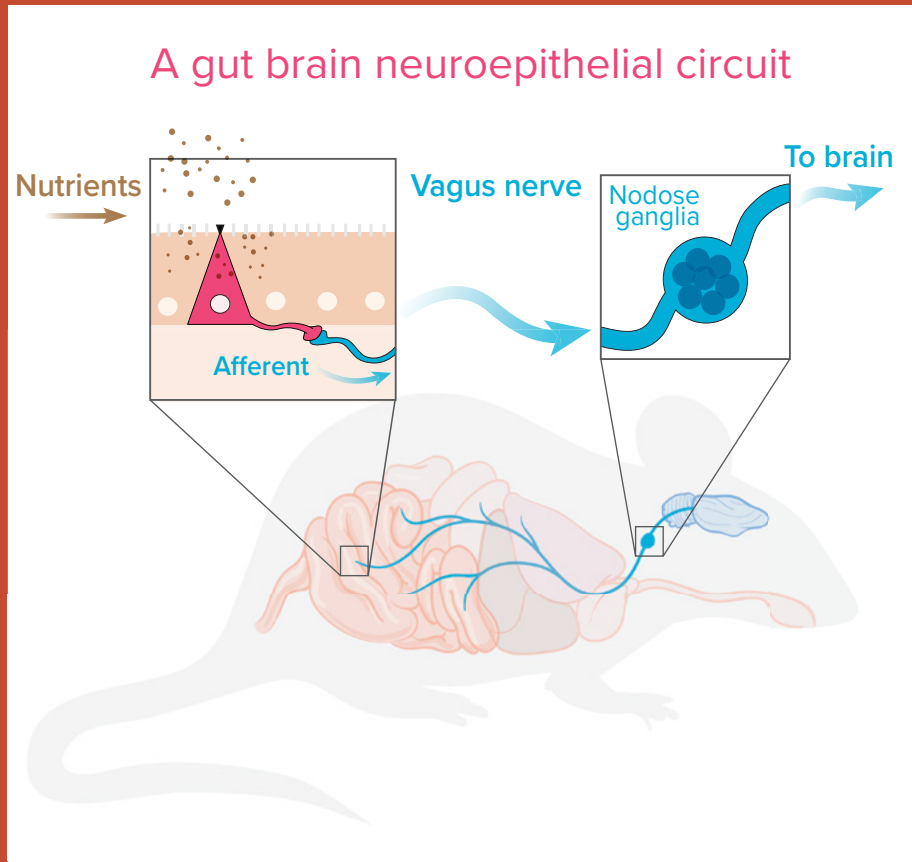
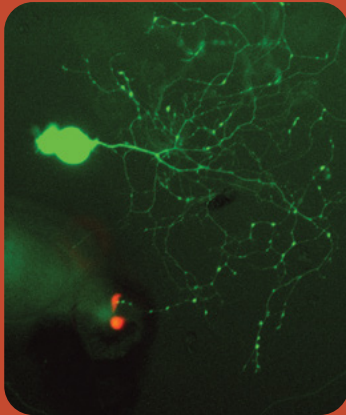
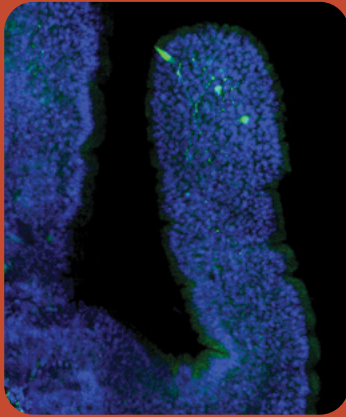
In fact, a 10 percent reduction in body weight can resolve NASH. For this reason, bariatric surgery is also an effective option. Dr. Rotman went on to describe several investigational pharmacological therapies, many of which target liver-specific proteins and pathways. While some treatments currently in development result in some positive outcomes, they also raise concerns such as insulin resistance, increased blood lipid profiles, and safety at effective doses. This remains an area of research that is ripe for innovation, and the "magic pill" could possibly involve a combination of medication and lifestyle changes tailored for an individual's needs—personalized medicine.

The seminar concluded with a discussion among participants of current challenges and opportunities in this field. Continued research in this important area can potentially reveal better ways to prevent, diagnose, and treat this increasingly common and chronic form of liver disease.

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<sup>4</sup> Schwimmer JB, et al. *Pediatrics* 118: 1388–1393. 2006.





The gut has long been known to communicate with the brain. The stomach and intestines can send information about hunger or feeling full, or even about the presence of microorganisms. However, scientists thought that this exchange of information occurred through a slow diffusion of hormones released by intestinal epithelial cells into the bloodstream. Given that circulating hormones take several minutes after food is consumed to reach their target and a person already has a sense of satiety, it seemed possible that a faster mechanism existed. Research described in this chapter using a mouse model shows that the gut has a dedicated sensory circuitry, like that of taste in the tongue. It is a direct neural connection between the gut and the brain that can exchange information in fractions of a second. Using a technique to detect cellular connections, the investigators were able to trace the signal of a fluorescently tagged novel subtype of intestinal cell, named neuropod by the investigators, all the way to cells, called neurons, in the brainstem. In the top left image, the specialized neuropod cells are shown connecting with neurons, both in green, set among other cells in the small intestine, shown in blue. This neural circuit was recreated in the laboratory in a cell culture system. In the bottom left image, a green label shows neurons from the brainstem connecting directly to red-labeled neuropod cells. The illustration on the right depicts the pathway of this circuit in a mouse after nutrient consumption. Further studies could provide insight into whether this direct gut-brain circuit can transmit more specific information such as caloric content of food.

*Images courtesy of Diego V. Bohórquez, Ph.D. and Melanie Maya Kaelberer, Ph.D., Duke University. From Kaelberer MM, Buchanan KL, Klein ME, Barth BB, Montoya MM, Shen X, and Bohórquez DV. A gut-brain neural circuit for nutrient sensory transduction. *Science* 361: pii: eaat5236, 2018. Reprinted with permission from AAAS.*



# Digestive Diseases and Nutrition

*Digestive diseases are among the leading causes of doctor visits, hospitalizations, and disability in the United States each year. These conditions span a wide spectrum of disorders that affect the gastrointestinal (GI) tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. The latest concerted effort to address the burden of all digestive diseases combining multiple 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.<sup>1</sup> 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.<sup>2</sup> 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.<sup>3</sup>*

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 drugs. Stomach and intestinal disorders also include functional bowel disorders, which result in symptoms of abdominal pain and altered bowel habits. For example, irritable bowel syndrome (IBS)

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<sup>1</sup> 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](http://www.cdc.gov/nchs/ahcd/index.htm).

<sup>2</sup> 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](http://www.hcup-us.ahrq.gov/nisoverview.jsp).

<sup>3</sup> 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](http://www.hcup-us.ahrq.gov/nedsoverview.jsp).



causes pain and constipation or diarrhea. IBS more frequently affects women, who may display a different range of symptoms and respond differently from men to pharmacologic treatments for the disease. While diet and stress contribute to this disorder, its underlying causes are unknown. Gastroesophageal reflux disease, in which stomach acids rise up into the esophagus, is a common functional bowel disorder that can lead to a condition known as Barrett's esophagus. This condition, in which cells lining the esophagus turn into an intestinal type of cell, is associated with a heightened risk of esophageal cancer—one of the cancer types still on the rise in the United States. Scientists are working to understand the causes of functional bowel disorders, which will lead to improvements in diagnosis and management for patients with these conditions. Fecal incontinence, or impaired bowel control, is a bowel disorder that poses a major public health burden. Although fecal incontinence is more common in older adults, it can affect people of any age. Because it is difficult to talk about, many people suffer without seeking professional treatment for this surprisingly prevalent condition. Researchers thus aim both to examine barriers in addressing fecal incontinence and to develop improved treatment strategies.

Gastroparesis, another type of functional bowel disorder, is characterized by delayed emptying of food from the stomach, resulting in nausea, vomiting, and abdominal discomfort. Most cases of gastroparesis are of unknown origin, which makes it difficult to treat. Most current therapies are directed toward helping people manage this chronic condition so they can be as comfortable and active as possible.

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 nonalcoholic steatohepatitis (NASH), a form of nonalcoholic fatty liver disease (NAFLD). 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 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 <http://livertox.nih.gov>).

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 plays 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 and has been involved in the development of the first NIH-wide strategic plan for nutrition research.

## GUT MICROBIOME AND NUTRITION

**Feeding the Microbiome To Help Malnourished Children:** Recent findings from an ongoing series of studies of malnourished children demonstrate that complementary foods (*i.e.*, foods given in addition to those consumed in the diet) that are designed to boost maturation of their gut microbiome can improve markers of normal growth, neural development, and immune function in these children. Research has shown that adequate recovery from severe childhood malnutrition requires more than access to healthy food (or breast milk in the case of the youngest children). Even when supplemented with life-saving, so-called “complementary” or “therapeutic” foods high in calories and protein, severely malnourished children do not recover completely and remain moderately malnourished in a way that can still cause stunting and other effects on health. Studies in resource-limited populations living in Bangladesh and Malawi have shown that malnutrition leaves a lasting impression on a child's developing gut microbiome—the collection of microbes and their genetic material—resulting in an underdeveloped, “immature” microbiome less capable of supporting human metabolism and growth. Building upon their past findings, scientists set out to identify a combination of food ingredients that would support maturation of the microbiome to encourage healthy growth and development of malnourished children. They measured health and metabolic indicators, as well as microbes present, in severely malnourished young girls and boys, ages 6 to 36 months, living in Bangladesh over the course of treatment with one of three types of “conventional” formulations of therapeutic foods, consisting of either rice

and lentils, chickpeas, or a commercially available peanut-based food. This initial study provided a baseline of information on how the gut microbiome typically changes during treatment with conventional complementary foods, as children made a partial recovery from severe to moderate malnutrition with continued immaturity in their microbiomes. In parallel, to identify potential complementary foods that could be more effective in improving the gut microbiome during malnutrition, they transplanted gut microbes from severely malnourished children into male mice raised in sterile conditions, to test microbial levels in response to 12 food ingredients found in the Bangladeshi diet. Based on these findings, they identified combinations of these 12 complementary food ingredients that, given together with a representative diet consumed by malnourished children, most improved the microbiome and growth markers in these mice and also in piglets similarly given microbes from the children. Coming full circle back to humans, the investigators then conducted a randomized controlled feeding study in children ages 12 to 18 months with moderate malnourishment. This study compared three complementary food combinations with the best results on the gut microbiome in the animal models. When the complementary foods were given in addition to the children's regular diet, one combination in particular—containing chickpea, soy, peanut, and banana—led to improved markers of growth, neural development, and immune function that more closely resembled those seen in healthy children. Through developing and testing complementary foods that are custom-designed to reverse microbiome immaturity caused by malnutrition, scientists hope to provide the means to more fully restore health to these children.

Gehrig JL, Venkatesh S, Chang HW,...Gordon JI. *Effects of microbiota-directed foods in gnotobiotic animals and undernourished children.* *Science* 365: pii: eaau4732, 2019.

### **Gut Feeling: A Direct Pathway for Gut-brain**

**Communication:** Researchers have identified for the first time a direct line of communication between the gut and the brain that allows for rapid signaling of sensory information about food intake. It has long been known that the stomach and intestines communicate with the brain. However, scientists thought that this exchange of information occurred through a slow diffusion of hormones released by specialized intestinal cells termed “enteroendocrine cells” into the bloodstream upon ingesting nutrients.

Recently, a team of investigators wondered if a faster conduit through the nervous system might exist, given that circulating hormones reach their peak several minutes after food is consumed and a person has already had a sense of feeling full.

To determine if a direct neural pathway exists, the researchers used a technique to detect cellular connections in mice with a modified, fluorescently tagged rabies virus, as rabies spreads through the body via connections between nerve cells (neurons) until it reaches the brain. They introduced this virus into the colons of the mice, and, remarkably, they were able to trace the fluorescent signal as the virus traveled from a novel subtype of intestinal enteroendocrine cell (named neuropod by the investigators) all the way to cells in the brainstem, called vagal neurons, indicating the presence of a direct circuit. To recreate the gut-brain connection in the lab, the researchers next grew these gut cells from mice in the same dish as vagal neurons. They saw the neurons' extensions crawl along the bottom of the dish to connect to the gut cells and begin communicating. Using a technique to measure the speed of the signal, they found that adding a sugar solution to the dish triggered a message to travel between gut and brain cells. The speed of communication was extremely fast—measured in milliseconds, much faster than the blink of an eye. When sugar was added to vagal neurons in the absence of gut cells, there was no measurable signal, suggesting that the message was being sent from the gut cells only in the presence of a food source. Because such rapid communication between the brain and other organs involving the five senses—smell, taste, touch, vision, and hearing—often occurs via a chemical messenger called glutamate, the researchers next investigated if glutamate was also responsible for delivering these fast signals from the gut to the brain. When they blocked the release of glutamate with a chemical agent in a dish containing vagal neurons and gut cells, the messages were silenced, suggesting that glutamate is the chemical messenger responsible for this communication; when they washed away the blocking agent, the signal was recovered. Thus, the researchers had discovered that certain gut cells connect with and speak the language of brain cells in rapid communication.

Taken together, these results lend new meaning to a “gut feeling” as a “sixth sense,” in terms of sensing and rapidly communicating information about the food we eat. Future research could

provide insight into whether this gut-brain system relays specific information about nutrients and caloric intake of food.

Kaelberer MM, Buchanan KL, Klein ME,...Bohórquez DV. A gut-brain neural circuit for nutrient sensory transduction. *Science* 361: pii: eaat5236, 2018.

## MICROBIAL FACTORS IN INFLAMMATORY BOWEL DISEASE

**Close Ties Between the Microbiome and Inflammatory Bowel Disease:** Two recent studies have identified changes caused by the community of microbes living in the gut that could contribute to inflammatory bowel disease (IBD), including microbial metabolic byproducts (substances produced by microbes) and the human immune system's response. One of the drivers of IBD (which includes Crohn's disease and ulcerative colitis) is thought to be an improper immune response to the gut microbiome, the trillions of microbes living in the gastrointestinal tract. Studies have implicated the microbiome as a key player in IBD, but the many and complex ways in which the microbiome affects the disease are not well understood. One complicating factor is that the disease is not active all the time—people typically experience periodic “flare-ups” of symptoms interspersed with latent periods—and the microbiome during an inactive period may be different from during a flare-up.

To better understand the interactions between the microbiome and its human host during IBD, the NIDDK supported studies through the NIH's Integrative Human Microbiome Project, which includes a multi-center study to understand how the human gut microbiome changes over time in adults and children with IBD. Researchers recruited 132 adult and pediatric volunteers, both female and male, either with IBD (Crohn's disease or ulcerative colitis) or without. Changes in the participants' guts and their microbiomes were tracked and catalogued through analysis of stool and other samples over 1 year, including microbiome composition, microbial metabolites, microbial and human gene activities, and immune response. The researchers found that, in the people with IBD, the composition of the microbiome changed significantly during a disease flare-up and then returned to an individual's initial, “baseline” composition when the flare subsided. There were corresponding changes in microbial metabolites during a flare-up, such as lower levels of short-chain fatty acids, which play important roles in digestive health and have been shown to protect against digestive diseases. Also, blood samples

from patients experiencing flare-ups showed higher levels of antibodies produced in response to microbial infections and inflammation, implicating the immune response as another key player in active IBD. These changes provide clues to the causes of IBD and could point to therapeutic targets or diagnostic markers to help predict flare-ups, which could be treated aggressively before symptoms become severe.

Another NIDDK-supported study delved further into the changes in the microbiome and the microbial metabolites in people with IBD. Researchers analyzed stool samples from 155 men and women with IBD (Crohn's disease or ulcerative colitis), or with no IBD, to identify differences in their microbiomes. They found that the microbial communities in people with IBD were less diverse than those in healthy people, confirming results from previous studies. Looking at over 8,000 different microbial metabolites, the researchers found higher levels of some metabolites in people with IBD, although a majority were depleted in the disease, reflecting the lower microbial diversity. By comparing these differences in metabolites with the differences in the microbiomes, the researchers were able to link specific metabolites to the types of gut bacteria that produce them, revealing new markers to help with diagnosis of IBD, and importantly, new targets for therapy.

The relationship between IBD and the gut microbiome is complex, but gaining a better understanding of it would present potential new targets for therapy. These studies and others represent pioneering work to more comprehensively characterize the metabolic and immune impacts of host-microbial interactions that contribute to disease development.

Lloyd-Price J, Arze C, Ananthakrishnan AN,...Huttenhower C. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* 569: 655-662, 2019.

Franzosa EA, Sirota-Madi A, Avila-Pacheco J,...Xavier RJ. Gut microbiome structure and metabolic activity in inflammatory bowel disease. *Nat Microbiol* 4: 293-305, 2019.

## PREDICTING EFFECTIVE TREATMENTS FOR INFLAMMATORY BOWEL DISEASE

**Predicting the Most Effective Treatment Approach for Pediatric Ulcerative Colitis:** Recent results from a multi-center clinical study identified several patient characteristics that can predict how well children with ulcerative colitis (UC) will respond to treatment, pointing toward a more personalized approach to

treating the disease. UC, a type of inflammatory bowel disease, is caused by a complex combination of genetic, microbial, and environmental factors that provoke chronic and painful inflammation in the lower gastrointestinal tract, resulting in diarrhea, cramping, and malnutrition. People with UC are typically treated with the non-steroidal anti-inflammatory drug mesalazine or corticosteroids, but many do not improve and eventually need to be treated with more potent drugs that suppress the immune response that causes inflammation (immunosuppressive drugs). Advance knowledge of which children with UC only respond to immunosuppressive treatments could enable them to be effectively treated and undergo remission as quickly as possible. However, predicting treatment responses to UC has been difficult due to the disease's variability from person to person. Determining the best treatment approach has been especially difficult for children with UC because most of the treatments are based upon results from adult studies.

To streamline treatment approaches for UC, particularly in children, the NIDDK supported the Predicting Response to Standardized Pediatric Colitis Therapy (PROTECT) study, which recruited several hundred boys and girls from 29 centers in the United States and Canada who were recently diagnosed with UC. The participants were initially given mesalazine or corticosteroids. After a year, only 38 percent of the participants were able to achieve corticosteroid-free remission—that is, they needed only mesalazine or no treatment at all. A majority of the participants required more intensive treatments, including immunosuppressive drugs, and several required surgeries to remove the colon. Importantly, several patient characteristics—such as high hemoglobin levels, clinical remission after 4 weeks, and the makeup of the gut microbial community—were associated with achieving corticosteroid-free remission, suggesting these characteristics can predict whether immunosuppressive drugs will be necessary. PROTECT researchers also identified certain genes that were more active in the participants who were responsive to corticosteroids, which opens the possibility of genetic screening to help determine which patients would be most likely to benefit from this treatment. Additionally, the researchers found that mitochondria—the tiny battery-like cellular components that supply energy—were less active in the colons of UC patients, such that boosting energy production in colonic cells might be another effective therapeutic approach.

The results from the PROTECT study suggest that the best treatment approaches for UC are those that are

tailored to individuals with the disease based upon their clinical, genetic, and microbial profiles. The study also presents a framework for additional clinical studies that will further move UC therapy toward more personalized, and ultimately more effective, approaches.

*Hyams JS, Davis Thomas S, Gotman N,...Denson LA. Clinical and biological predictors of response to standardised paediatric colitis therapy (PROTECT): A multicentre inception cohort study. Lancet 393: 1708-1720, 2019.*

*Haberman Y, Karns R, Dexheimer PJ,...Denson LA. Ulcerative colitis mucosal transcriptomes reveal mitochondriopathy and personalized mechanisms underlying disease severity and treatment response. Nat Commun 10: 38, 2019.*

## INTESTINAL REGENERATION

**Immune Cells Influence Balance of Intestinal Stem Cell Renewal and Maturation:** Scientists have discovered that intestinal stem cells interact with nearby immune cells in a bi-directional manner that affects both the renewal of this stem cell source and remodeling of the intestinal lining during infection. The intestinal lining is constantly regenerating (in humans about once a week) with its regenerative capacity depending upon resident stem cells. Within the intestine, stem cells must be able to mature into specific cell types to replace old or damaged cells, and they also must divide and maintain their numbers as a source of future cell lineages. The replenishment of mature intestinal cells is especially important during gut infections, when the intestinal lining may be severely damaged by the pathogen and the resulting inflammatory response. The scientific community has focused on discovering how the stem cells' environment of neighboring cell types and signals supports them in their important work maintaining the gut.

A team of investigators focused on investigating the role of immune cells residing in the intestine, including the chemical signals they release, called cytokines. They showed that intestinal stem cells from male and female mice produce a class of molecules, called "MHC II" molecules, typically only found on cells that signal to immune cells when detecting an infectious or "foreign" protein. Next, they mixed mouse intestinal stem cells in a dish with immune cells (from female mice) and a test protein that the immune cells could recognize. The stem cells used their MHC II molecules to present this protein to the immune cells, which became activated and secreted cytokines. To explore these interactions in a model more closely resembling the intestine, they turned to mouse intestinal organoids—miniature tubes of cells in culture that recapitulate some of the features of the intestine. Adding different types of the



immune cells, or the cytokines they produce, modified the balance between the number of stem cells present and how many of them matured into specific intestinal cell types within the organoids. To study the stem cells during infection, the researchers moved to a whole-organism model. They gave female and male mice either Salmonella bacteria or a parasitic worm, and found that interactions between intestinal stem cells and immune cells were important for stem cell maturation into distinct cell types that reconstitute the intestinal lining during infection.

These studies performed in a wide range of experimental models—from single cells to organoids to whole animals—demonstrate the importance of crosstalk between intestinal stem cells and neighboring immune cells for maintaining the stem cell pool and healthy intestinal lining in both healthy and infected states. More research is needed to understand how these interactions affect the balance between renewal of the stem cell pool and maturation into cell types that may be needed at a given time to perform distinct functions within the intestine.

*Biton M, Haber AL, Rogel N,...Xavier RJ. T helper cell cytokines modulate intestinal stem cell renewal and differentiation. Cell 175: 1307-1320.e22, 2018.*

## MODELING FATTY LIVER DISEASE

### Organoids Model Human Fatty Liver Disease:

Scientists have developed a remarkable model of human fatty liver disease using human cells to generate spherical “organoids”—miniature livers in a dish with complex cellular and structural features. Fatty liver disease, or steatosis, can in some people progress to steatohepatitis, a form of fatty liver disease marked by inflammation and damage, as well as fat accumulation. One form of this disease—nonalcoholic steatohepatitis—is becoming increasingly common in both adults and children in the United States and other countries along with the rise in obesity. However, not all individuals with obesity develop the disease, and nonalcoholic steatohepatitis can sometimes occur in people who do not have obesity. Despite many animal model studies of the disease, no approved drugs have been developed to treat steatohepatitis, and treatment guidelines consist of advising individuals who have overweight or obesity to lose weight. In these experiments, scientists used several stem cell lines capable of forming multiple cell types from healthy men and women and from women and girls with liver disease, to create human liver organoids with multiple liver cell types, similar

to the natural organ. With all these cell types, the organoids were functionally similar to human liver tissue in terms of their activation of genes, including those involved in fat metabolism. When treated with fatty acids to replicate high circulating levels of fats in the body due to excess fat tissue and/or a high-fat diet, the organoids showed many of the sequential features of human fatty liver disease. These features included fat accumulation, inflammation, and scar tissue formation, the last of which could be detected by measuring the stiffness of the organoids, simulating stiffness measurements of liver scarring in humans. Finally, organoids created from the cells of children with a genetic form of severe steatohepatitis, called Wolman disease, showed many features of the disease found in humans, and responded favorably to a protein called fibroblast growth factor 19, which is known to be produced by intestinal cells in response to a drug being tested as a fatty liver disease treatment. This research provides a new path forward to study human fatty liver disease in a more personalized way, using cells from individuals to create organoids, and may enable future discovery of effective treatments for this disease.

*Ouchi R, Togo S, Kimura M,...Takebe T. Modeling steatohepatitis in humans with pluripotent stem cell-derived organoids. Cell Metab 30: 374-384.e6, 2019.*

## PEDIATRIC LIVER DISEASE RESEARCH

### Study Identifies Gene Variants Associated with Biliary Atresia Splenic Malformation Syndrome in Children:

Researchers have identified gene variants present in infants with biliary atresia splenic malformation syndrome that may increase susceptibility to this severe and potentially deadly childhood liver disease. Biliary atresia is a serious liver disease that occurs during the first few months of life in which bile ducts that drain from the liver, delivering bile acids to the intestine, become inflamed and scarred, leading to a back-up of bile into the liver. This back-up can result in liver damage and, if not treated with surgery or liver transplantation, can lead to liver failure and death. Although a rare disease, biliary atresia remains the most common form of severe liver disease in children and the leading cause for pediatric liver transplantation. Some infants with biliary atresia have additional complications caused by improper positioning of some internal organs, such as the spleen, within the body; this condition is referred to as biliary atresia splenic malformation (BASM) syndrome. The causes of biliary atresia and the rarer BASM syndrome are not fully understood.



With support from the NIDDK, the National Center for Advancing Translational Sciences, and other sources, researchers set forth to identify genetic factors that might play a role in the development of BASM syndrome by studying infants enrolled in the NIDDK's Childhood Liver Disease Research Network (ChILDRen) and their parents. They sequenced portions of the genomes of the families and looked for genetic variants that likely disrupt biological functions previously implicated in biliary development and in organ positioning. In particular, they examined variants that might be associated with blockage of bile flow or dysfunction in the cilia (hair-like projections) that line the bile ducts and play a role in cell signaling. The investigators found variants of one gene in particular, called *PKD1L1*, in about 12 percent of the children studied with BASM. The protein produced by this gene is important for both ciliary signaling and proper positioning of internal organs during embryonic development, underscoring its possible role in BASM syndrome. Also, the researchers tested a tissue sample from one of the children with *PKD1L1* variants and found reduced activity of this gene within the bile ducts, meaning the functionality of the gene may be affected.

This study identifies *PKD1L1* as a new candidate gene in the development of BASM syndrome—and possibly some cases of biliary atresia without BASM syndrome, given the important role of this gene product in biliary function—in young children. Future studies in both humans and cell or animal models could explore the functions of this gene and the possible mechanisms by which these genetic variants might contribute to these rare diseases.

*Berauer JP, Mezina AI, Okou DT,...Karpen SJ; for the Childhood Liver Disease Research Network (ChILDRen). Identification of polycystic kidney disease 1 like 1 gene variants in children with biliary atresia splenic malformation syndrome. Hepatology 70: 899-910, 2019.*

## HEPATITIS B RESEARCH

### **Trials Test Combination Treatment for Chronic**

**Hepatitis B in Adults and Children:** Two clinical trials of a combination drug therapy—one in adults and another in children—found it was of limited benefit in treating chronic hepatitis B. Hepatitis B is a major health problem around the world and in the United States, particularly in people of Asian or African origin who emigrate from countries without the long-term universal vaccination and screening programs in this country. The chronic form of the disease can progress to cirrhosis and liver cancer, if not successfully treated. Infection with the hepatitis B virus (HBV)

often occurs at birth or in childhood. Unlike hepatitis C, no relatively short course of a drug or combination of drugs has been found to elicit a long-term response in people with chronic hepatitis B, for which the most effective drugs available need to be taken for years, decades, or even life-long.

The NIDDK's Hepatitis B Research Network is a multi-center study of both children and adults with hepatitis B at 28 sites throughout the United States and Canada, with a goal to better understand the natural history of hepatitis B and disease processes, and to test therapy approaches. The aim of these two Network trials, in adults and children, was to determine the safety and benefit of therapy with a limited course of a combination of drugs in the early phase of chronic HBV infection. In this phase, infected persons have no symptoms or abnormal liver tests, despite high levels of HBV in the bloodstream (called "immune tolerant" chronic hepatitis B). In both the adult and pediatric trials, 90 percent or more of participants were of Asian ancestry. The adult cohort had equal numbers of men and women, while 75 percent of the pediatric participants were girls. The treatment regimen was entecavir—a once-a-day, oral direct-acting antiviral drug—to which was added peginterferon, an immune-stimulating protein given weekly by injection. Entecavir was given alone for 8 weeks and then combined with peginterferon for the following 40 weeks. Researchers conducting the trials measured success by how well the combination therapy decreased levels of HBV DNA and proteins (called "HBeAg" and "HBsAg") in the blood, and whether the 48 weeks of treatment led to a permanent loss of the viral proteins and DNA, as measured 48 weeks after stopping treatment. With treatment, levels of HBeAg, HBsAg, and HBV DNA decreased in all patients. However, none of the 27 adult trial participants and only three of the 60 children (5 percent) experienced complete resolution of hepatitis B, losing both viral proteins and DNA and developing antibodies during the 48 weeks following treatment.

Thus, this particular combination treatment was found to be of limited benefit in adults and children at the early, mild stage of chronic hepatitis B infection. Yet, this study offers promising evidence that a complete response to therapy could be achievable in people with chronic hepatitis B. In particular, the dramatic and complete response seen in a small proportion of children suggests that combination therapy—using these drugs, together with another agent(s)—is likely to achieve a beneficial response in a high proportion of people with chronic hepatitis B.

Feld JJ, Terrault NA, Lin HS,...Lok AS; Hepatitis B Research Network. Entecavir and peginterferon alfa-2a in adults with hepatitis B e antigen-positive immune-tolerant chronic hepatitis B virus infection. *Hepatology* 69: 2338-2348, 2019.

Rosenthal P, Ling SC, Belle SH,...Schwarz KB; Hepatitis B Research Network (HBRN). Combination of entecavir/peginterferon alfa-2a in children with hepatitis B e antigen-positive immune tolerant chronic hepatitis B virus infection. *Hepatology* 69: 2326-2337, 2019.

## DRUG-INDUCED LIVER INJURY RESEARCH

### Genetic Variant Linked to Drug-induced Liver Injury:

In the largest, international drug-induced liver injury (DILI) genetic study to date, researchers found that persons with a genetic variant implicated in autoimmune diseases are at increased risk of liver injury triggered by drugs. DILI is one of the most common causes of acute liver failure in the United States and is one of the most frequent obstacles in the development and approval of new drugs. Yet DILI is difficult to prevent, predict, or treat because of its rarity, the lack of specific diagnostics, and the unidentified disease-causing characteristics unique to each individual. In the past 20 years, several human leukocyte antigen (HLA) genes, which are responsible for regulating the immune system, have been associated with DILI, which means that at least some cases may result from an improper immune response to the drug or its metabolites (breakdown products). But previous studies have included too few people with DILI to determine if there was a link to other types of genes.

In this study, a collaboration of NIDDK's Drug-Induced Liver Injury Network (DILIN) with the International DILI Consortium (iDILIC), researchers compared the genomes of over 2,000 individuals

with DILI and 12,000 people who did not have DILI. The large DILI cohort included men and women, children and adults, individuals of European, African-American, and Hispanic descent, and persons with liver injury from a wide variety of drugs, nutritional supplements, and herbal products. Through this analysis, the researchers found that a variant of a gene outside the HLA region called *PTPN22* was found to be more common in people with DILI than in the healthy controls. This genetic variant of *PTPN22* is also known to increase the risk for several autoimmune diseases such as psoriasis and rheumatoid arthritis—further implicating a role for the immune system in DILI. Importantly, this genetic variant was linked to liver damage caused by many drugs, unlike the genetic variants of HLA genes, which were usually linked to one specific drug. In addition, the *PTPN22* risk variant added to the risk of having liver injury from specific drugs in people with the known HLA risk variants. For example, the *PTPN22* variant almost doubled the risk of DILI in persons with liver injury caused by the antibiotic amoxicillin-clavulanate—the most common cause of DILI after acetaminophen in the United States.

These findings suggest that DILI is caused by an improper immune reaction to certain drugs or its metabolites that is ordinarily suppressed by *PTPN22*. Importantly, this study opens the possibility of developing therapies for DILI that focus on improving the activity of *PTPN22* and the related cellular pathways that curb immune responses.

Cirulli ET, Nicoletti P, Abramson K,...Watkins PB; Drug-Induced Liver Injury Network (DILIN) Investigators and International DILI Consortium (iDILIC). A missense variant in *PTPN22* is a risk factor for drug-induced liver injury. *Gastroenterology* 156: 1707-1716, 2019.

# Workshop Explores Ways To Improve Diagnosis and Treatment of Pancreatic Disease Through Precision Medicine



On July 24, 2019, the NIDDK, in collaboration with the National Pancreas Foundation, sponsored a workshop to understand the current status of precision medicine—the use of specific information about disease mechanisms and features to guide more targeted and effective medical care—in the diagnosis and management of pancreatic disease. An additional goal was to identify ways to apply precision medicine to tailor treatments for people with pancreatic disease. The workshop took place in conjunction with an annual meeting of pancreatitis researchers called PancreasFest in Pittsburgh, Pennsylvania.

Pancreatic diseases such as pancreatitis and pancreatic cancer are not easy to detect in their early stages, and by the time they are diagnosed they tend to be advanced and difficult to treat. These diseases also have complicated origins with contributing factors that vary from person to person, making it difficult to identify universal markers that could be used to make early diagnoses. Precision medicine approaches, which take into account genetic, environmental, and lifestyle factors, would be helpful for early diagnosis and treatment. For example, identifying genetic variations that are associated with pancreatitis could enable screening individuals to identify those at higher risk while also pinpointing potential targets for therapy. Precision medicine would also allow doctors and researchers to predict more accurately which treatment and prevention strategies will work in which groups of people.

The workshop included an introductory lecture on precision medicine in cancer treatment as an example of how it has been successful in that field and what aspects need to be further developed. The

rest of the workshop was divided into four sessions: (1) general considerations for analyzing large sets of data, including using artificial intelligence; (2) analyses of genes, proteins, and metabolic products to identify risk factors and markers for pancreatic disease; (3) advances in imaging techniques to assess the severity of disease; and (4) gaps, barriers, and needs that prevent applying precision medicine to tailor treatments for patients with pancreatic disease. Key areas identified for future efforts were: collaboration among institutions to generate and share large data sets; new ways to collect, interpret, and measure data to help understand disease mechanisms; new clinical trial designs to test and improve therapies; and a framework for measuring and assessing the value of precision medicine approaches to the health care system.

The workshop brought together people from the NIH and researchers throughout the nation. In general, the participants felt that precision medicine approaches can identify patients early in the course of their pancreatic disease and prevent progression to chronic or fatal illness.

The meeting organizers have developed a manuscript for publication in the scientific literature describing the workshop proceedings.<sup>1</sup> Recommendations from the workshop will help inform future NIDDK efforts to advance research, as a foundation for accelerating the development of new approaches to managing pancreatic disease.

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<sup>1</sup> Lowe ME, et al. *Pancreas* 48: 1250-1258, 2019.

# STORY OF DISCOVERY

## Pancreatitis in Children

A painful, chronic disease is hard enough for an adult to manage, but when a child faces such a disease, it proves even more difficult. Pancreatitis is one of these conditions, placing a significant burden on children and their families—physically, emotionally, and financially, as well as in terms of overall quality of life. For a child with pancreatitis, every aspect of their life is affected, including the ability to eat, be active, and go to school. Treatments are currently limited to supportive therapy for pain management and surgical procedures. Although relatively rare, pancreatitis in children is more common than was previously thought, and it can progress in a surprisingly short timeframe.

Over the years, NIDDK-supported research has made important strides toward better understanding pancreatitis in children and contributing to efforts to improve its diagnosis, treatment, and, ultimately, prevention.

### HOW PANCREATITIS AFFECTS CHILDREN

Pancreatitis is an inflammation of the pancreas, an organ located behind the stomach. The pancreas performs many important functions, including the secretion of insulin and other key hormones, as well as production of a fluid containing precursor forms of enzymes and bicarbonate that flows through ducts into the intestine, where the enzymes become activated and aid digestion of food. In pancreatitis, the digestive enzymes become activated too early—while still inside the pancreas. This causes inflammation and damage to the organ, leading to the symptoms of pancreatitis—the main one being abdominal pain that is often severe, as well as nausea and vomiting.

The disease can occur in three forms: acute, acute recurrent (two or more acute episodes), and chronic. Acute pancreatitis can progress to the chronic form, which carries with it an increased risk of pancreatic cancer. Once considered an uncommon disease in children, the incidence of pediatric acute pancreatitis

has increased over the last 10 to 20 years and currently affects approximately 1 in 10,000 children.<sup>1</sup> Chronic pediatric pancreatitis, in which children have diagnostic or functional evidence of irreversible pancreatic damage, is estimated to have an incidence of approximately 2 per 100,000 children per year.<sup>1</sup> Both acute recurrent and chronic forms of pediatric pancreatitis place a significant burden on children and their caregivers.

Risk factors for pancreatitis differ for children compared to adults. In children, the main risk factors are inherited genetic variants, followed next by obstructed ducts caused by congenital abnormalities or gallstones. In adults, risk factors include genetics and gallstones, but environmental factors such as alcohol and tobacco use are the most predominant. Although diabetes is another risk factor for pancreatitis, it is not as common in children as adults. An insufficient production of pancreatic enzymes is found in both children and adults with pancreatitis. Emergency room visits and hospitalizations are common in children and adults with the disease, as are missed days of school or work. Pain, whether it comes in discrete episodes or is constant, is frequently difficult to treat, leading to lost school time for children and increased utilization of health care and high medical costs.

There are currently no drugs that effectively halt progression of this potentially debilitating disease or that reverse the disease process. Treatment options to manage the severe, often unremitting pain typically accompanying chronic pancreatitis include opioids, which carry the risk of addiction. If traditional pain management fails, a child with pancreatitis may need a surgical procedure called a total pancreatectomy-islet autotransplantation (TP-IAT), in which the pancreas is surgically removed and its insulin-producing islet cells, which regulate blood glucose (sugar), are

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<sup>1</sup> Sellers ZM, et al. *Gastroenterol* 155: 469–478, 2018.

# STORY OF DISCOVERY

collected and infused into the liver, where the cells implant and function. (See the chapter on Diabetes, Endocrinology, and Metabolic Diseases for a profile on an individual who underwent TP-IAT for pancreatitis.)

This current state of knowledge of and care for children with pancreatitis is based, in part, on groundbreaking advances from studies conducted over the past several years with NIDDK support, including the following examples.

## EARLY CLUES TO PANCREATITIS DEVELOPMENT

A hereditary form of chronic pancreatitis, which affected multiple family members over generations, was first recognized in 1952, only a few years after the NIDDK was established. However, the discovery of the first genetic mutation associated with this disease would occur more than 40 years later. In 1996, NIDDK-supported scientists reported that a mutation in a gene called *PRSS1* was associated with hereditary pancreatitis; this gene codes for the protein trypsinogen, an inactive precursor of the digestive enzyme trypsin. This study and others identified a number of genetic variants associated with pancreatitis in the trypsinogen gene, in more genes that affect trypsinogen/trypsin, and in genes with additional functions. These discoveries were made possible by the availability of information on human gene sequences through such efforts as the NIH's Human Genome Project.

Around the same time of these discoveries by individual investigators, in 1996 the NIDDK funded the beginnings of the first large clinical cohort study of pancreatic disease in the United States, called the North American Pancreatic Study (NAPS) Group. Additionally, the NIDDK provided some support for a later study in European families on clinical and genetic characteristics of hereditary pancreatitis that associated different *PRSS1* mutations with age of symptom onset and disease progression, and showed a median age of 10 years for the onset of first symptoms of the disease in families with one of the mutations. These studies, together with a

subsequent larger cohort study in adults called the North American Pancreatitis Study 2, or "NAPS2," advanced knowledge of the numerous genetic and environmental factors playing a role in pancreatitis, including the first genome-wide association study of pancreatitis in 2012, which identified new genetic regions associated with the disease.

## INSPPIRE-D TO ADVANCE KNOWLEDGE OF PEDIATRIC PANCREATITIS

In 2009, a group of international investigators came together to form the first multi-center group dedicated to studying pancreatitis in children, which would come to be called the International Study Group of Pediatric Pancreatitis: In Search for a Cure (INSPPIRE) Consortium, established with support from the NIDDK. The Consortium's focus was the characterization of acute recurrent and chronic forms of pediatric pancreatitis, in terms of their global distribution, causes, disease processes, and outcomes at 14 sites throughout the United States, Canada, Israel, and Australia. This multi-center approach, assembling the largest cohort of children with pancreatitis to date, was necessary to have sufficient numbers of participants for studying the relatively rare disease of pediatric pancreatitis.

The work of the INSPPIRE investigators, together with study participants, proved extremely productive, yielding multiple research advances and publications. For example, one study presented a fuller picture of the significant burden placed on children with chronic pancreatitis by quantifying their experiences of severe, often constant pain, resulting in hospitalizations and missed school days. Another study revealed the steep economic cost of acute recurrent and chronic pancreatitis in children, due to repeated hospitalizations, tests, procedures, and medications. Other findings focused on better characterizing the genetic risk factors often at work in these forms of pediatric pancreatitis and how they influence disease onset and progression.

Following the success of the original INSPPIRE study group, and based on recommendations stemming from NIH workshops on pancreatitis



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research held in 2012 and 2013, the NIDDK and the National Cancer Institute co-sponsored the formation of the Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer in 2015. The INSPPIRE group became part of this larger Consortium as “INSPPIRE 2,” featuring an even larger and more diverse population of study participants.

Within the last few years, INSPPIRE 2 and the broader Consortium have made major contributions relevant to pediatric pancreatitis. Consortium studies of adult pancreatitis established standards for imaging procedures used to diagnose and assess disease severity as part of pancreatitis care. One analysis performed by the INSPPIRE 2 investigators better characterized risk factors and disease progression in children with pancreatitis, showing both commonalities and differences with adult patients. Other INSPPIRE 2 findings included the observation of the surprisingly short timeframe for children with acute recurrent pancreatitis to develop chronic pancreatitis, occurring over 2 to 4 years, with more rapid progression in those who were diagnosed later in childhood and who carried *PRSS1* genetic variants associated with the disease.

Another study conducted by an NIDDK grantee associated with the INSPPIRE program found that several genetic risk variants are likely to play a significant role in progression to acute recurrent and chronic disease after the first attack of pancreatitis in children. NIDDK-supported researchers also found that carriers of these pancreatitis-associated risk variants are at higher risk for developing pancreatic cancer later in life. These studies, reported in 2018 and 2019, could help researchers and clinicians develop better approaches to diagnose and treat children with pancreatitis.

## FUTURE DIRECTIONS IN PEDIATRIC PANCREATITIS RESEARCH

The NIDDK has been continuing its support for the “inspiring” work of the INSPPIRE 2 investigators and study participants, as well as others studying pancreatitis in children. In the future, INSPPIRE 2 researchers will continue the long-term cohort study to probe deeper into remaining questions, such as better understanding risk factors involved in pancreatic

disease progression, determining how chronic pancreatitis first develops, defining pancreatic enzyme insufficiency (a lack of digestive enzymes that hinders proper digestion of food), and improving treatment options. For example, one study is testing the first drug-free approach for pediatric pancreatitis—a web-based cognitive behavioral therapy intervention to manage pain without opioid exposure and improve quality of life in adolescents with chronic pancreatitis. Other ongoing INSPPIRE 2 investigator studies of pediatric pancreatitis are monitoring rates across sites, identifying the earliest diagnostic imaging evidence of disease, defining metabolic and skeletal complications, understanding why chronic pancreatitis more commonly affects girls, and understanding the contribution of drug-induced pancreatic diseases. The NIDDK also continues to support investigator-initiated research in this area, such as the recent development of the first pre-clinical mouse model to faithfully mimic human chronic pancreatitis—made possible through genetic alterations in the trypsinogen gene of mice—that can be used to inform the development of new treatments.

While providing ongoing support for research on pediatric pancreatitis, the NIDDK regularly sponsors scientific workshops that bring together leaders in the research community to discuss how to advance pancreatitis research. Past workshops have focused on research opportunities relating to such themes as research challenges in chronic pancreatitis, biomarkers of pancreatic disease, and optimizing use of the TP-IAT procedure. In 2018, the NIDDK, with additional support from the National Pancreas Foundation, sponsored a workshop focused on ways to accelerate the development of new treatments for pancreatitis. The workshop’s recommendations were shared widely with the scientific community through multiple publications in the scientific literature. Most recently, in 2019, the NIDDK sponsored a workshop on how precision medicine-related methods and technologies can be applied to new and more personalized ways to diagnose and manage pancreatitis and other forms of pancreatic disease. These workshop recommendations and additional sources of external stakeholder input will continue to inform the NIDDK’s efforts to reduce the burden of pediatric pancreatitis through research.



# PATIENT PROFILE

## Jeff: The Endurance To Overcome Drug-induced Liver Injury



Jeff

Jeff is an executive at a successful furniture company, is active in his church, and volunteers with youth. In his spare time, he skis and is a competitive mountain biker—a successful one, too, with a history of winning races in his age category. Not surprisingly, he tries to take good care of his body. Aside from a couple of treatable medical conditions, he is a healthy 60-year-old. He is also accustomed to training hard for races, but there was no way he could have prepared for his encounter with drug-induced liver injury.

In 2016, eager to rid himself of a lingering and headache-inducing sinus infection, Jeff started taking a 6-week antibiotic course prescribed by his allergist.

Four weeks later, he was experiencing nausea, headaches, and a low-grade fever. At that time, he was due to meet up with some friends for a 2-hour motorcycle ride from his hometown in Michigan to spend a few vacation days in a cottage at a ski resort. Despite feeling miserable, he made the trip with his wife riding on the seat behind him. At the cottage he became overwhelmed by cramps and vomiting, even briefly losing consciousness. He spent most of his remaining vacation confined to a bed. At that point, Jeff decided to stop taking the antibiotic. He wasn't fully aware of it at the time, but his liver was experiencing a severe reaction to the drug, and only after a harrowing trip to the emergency room and months of recovery would he begin to feel somewhat normal again. During that time, he would directly experience the agony and alarm that coincide with drug-induced liver injury. He would also join the NIDDK's Drug-Induced Liver Injury Network (DILIN), becoming part of a concerted, multi-site study to understand, manage, and prevent this potentially deadly disease.

### SIGNS OF A ROUGH ROAD AHEAD

Drug-induced liver injury occurs when a prescription drug, an over-the-counter drug, or a dietary or herbal supplement damages the liver. Some types of drug-induced liver injury, such as those caused by acetaminophen overdoses, are relatively easy to foresee and avoid because the type of injury tends to be similar among people and directly dependent on the amount of drug ingested. Others, such as Jeff's case, are called "idiosyncratic." They are relatively uncommon, with effects that are harder to predict and largely determined by a combination of factors unique to an individual, such as genetics and the condition being treated. This makes it difficult to know who will respond

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adversely to a given drug—even at dosages that are safe for most people.

Unsure of what was happening but knowing he needed medical treatment, Jeff decided to return home. When he and his wife got back, it was Father's Day, and his daughter and son-in-law came by for a visit. His son-in-law noticed that the whites of Jeff's eyes were tinged with yellow and his skin was darkened with a yellow-orange appearance. Jeff's daughter began to worry that there was something seriously wrong with his liver and urged him to seek treatment. He went to his family doctor, who ordered blood tests to detect markers for liver health, like bilirubin and the liver enzymes alanine aminotransferase (ALT) and alkaline phosphatase.

A high level of bilirubin, along with elevated levels of liver enzymes in the blood, are signs of liver damage, and Jeff's bilirubin level was almost six times the normal amount. Over the next few days, it continued to slowly rise, and there was seemingly nothing he could do to stop it. Jeff was starting to worry that something terrible was on the horizon. "As that was happening," he recalls, "I'm starting to wonder, how bad was this going to get?"

Jeff's doctor was beginning to suspect that drug-induced liver injury, triggered by the antibiotic, was the cause of Jeff's deteriorating health. The antibiotic that Jeff took was actually a combination of two drugs—amoxicillin and clavulanate—commonly called Augmentin®. Prescribed to treat mild-to-moderate bacterial infections, it is designed to be a double knock-out punch for bacteria: amoxicillin is an antibiotic derived from penicillin, and clavulanate targets bacteria that can degrade the amoxicillin before it can do its job. The combination is usually effective and safe and is one of the most frequently prescribed antibiotics. In a small percentage of people (about one in 2,500 people treated), it triggers an allergic response when it is broken down by the liver. The body appears to misread the breakdown products as unwelcome foreign invaders and sends powerful immune cells

streaming into the liver. The resulting immune reaction wreaks havoc on the vital organ, causing jaundice and, in severe cases, acute liver failure.

Jeff's doctor thought he should see a digestive disease specialist to keep a closer eye on his liver, so Jeff went to a gastroenterologist. In the meantime, his bilirubin level continued to climb.

*Jeff was starting to worry that something terrible was on the horizon: "I'm starting to wonder, how bad was this going to get?"*

## A RACE TO THE EMERGENCY ROOM

Jeff felt extremely weak and tired, but he pushed himself to continue working over the next several weeks. Then, shortly before the Fourth of July weekend, he got a call in the middle of a meeting: his test results were so alarming that his gastroenterologist urged Jeff to go to the emergency room right away. He remembers calling his wife, stopping by the house to grab some clothes, and then driving to the hospital, his mind racing. "All of a sudden, reality strikes me," he recalls. "What's going to happen here? Do I get a liver transplant? Do people die from this?"

Jeff's anxieties were not unfounded. Although most cases of idiosyncratic drug-induced liver injury resolve after the patient stops taking the drug or dietary supplement that triggered the disease, recovery is dependent upon a timely diagnosis, proper identification of the offending agent, and other factors such as genetics and the overall health of the liver. One complicating factor is that the injury from amoxicillin and clavulanate (and some other antibiotics) typically arises weeks after exposure to the drug, so symptoms may not manifest until 1-3 weeks after a short antibiotic course is completed. With rare exceptions, there are no current treatments that are effective in reversing this type of liver injury. The first and most important

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step in management is to stop taking the drug—and to not ever take it again (even if there are doses remaining in the prescribed course of antibiotics). This includes throwing away any leftover amounts of the drug and clearing it from the medicine cabinet as extra insurance that it is not used again. People who have experienced this type of liver injury should also make note of the drug so they can tell their doctors if they are ever prescribed it again. The liver has an amazing ability to heal itself and recover from damage, but if the damage is too great, liver failure occurs, and only a liver transplant will guarantee recovery. Drug-induced liver injury, although rare, is the major cause of death from acute liver failure in the United States and other developed countries of the world.

At the hospital, Jeff underwent hourly blood tests to monitor his liver. His bilirubin had soared to over 20 times the normal level—a surefire sign that his liver was under severe distress. “It was definitely a surreal feeling, realizing that I could die,” he remembers. His wife was sending out alerts and prayer requests to his friends and members of his family; his son and daughter-in-law, who live in Seattle, were trying to sort out whether to catch the next flight to Michigan.

***“I just remember realizing what was important in life at that point,” says Jeff when remembering his visit to the emergency room for a liver injury. “Your faith, your family, and your friends ... everything else didn’t matter.”***

Jeff and his family spent that night in the emergency room praying and waiting anxiously for each test result. “I just remember realizing what was important in life at that point,” he recalls. “Your faith, your family, and your friends ... everything else didn’t matter.”

On the second day, to everyone’s relief, Jeff’s bilirubin level finally began to ease downward. He spent a few more days at the hospital, weakened to

the point of barely being able to walk. After getting discharged, he sat at home on his deck, thankful, enjoying the Michigan summer weather and thinking, “I’m just happy to be here.”

## THE SLOW, UPHILL PATH TO RECOVERY

Soon after Jeff left the hospital, Dr. Robert Fontana, a liver specialist and principal investigator in the NIDDK’s multi-center DILIN program, contacted Jeff from the University of Michigan to see if he would be interested in joining the study. Jeff willingly agreed, even though he was still extremely fatigued from his episode in the emergency room. “I could hardly walk a hundred feet without becoming exhausted,” he recalls. “That’s pretty unusual for me, since I’m used to doing significant bike and ski races.”

He understood that joining the study would not only contribute to advancing medical knowledge but would also provide an opportunity for his recovery to progress more quickly and smoothly because it would be closely monitored with regular checkups. “It was an opportunity to have really good care and to be monitored on a regular basis,” he says. “I think that was the biggest thing, that they would be watching over me, trying to understand this better, and trying to help in the future.”

The NIDDK established DILIN in 2003 to collect and analyze cases of severe liver injury caused by prescription drugs, over-the-counter drugs, and alternative medicines, such as herbal and dietary supplements. Since that time, DILIN has collected data and specimens from more than 2,000 cases of liver toxicities due to these agents and made major contributions to understanding why certain medications and dietary supplements are more likely to damage the liver, why only some people are affected, and how the liver can heal itself after the injury. Answering these questions about the disease will continue to help researchers prevent and treat it. Genetic information from the Network’s participants, for example, is providing clues into how genetics could

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determine whether people react negatively to a drug or dietary supplement, even opening the possibility of screening patients before prescribing certain drugs to minimize the possibility of liver damage.

Jeff's participation in the DILIN study initially began with visiting the University of Michigan every 6 months for blood tests. At each visit he also underwent a specialized ultrasound procedure that detects the amount of scarring in the liver—a painless, noninvasive way to measure how well the liver is healing. His bilirubin level settled back into the normal range within 3 months after the trip to the emergency room, but other blood markers for liver health remained elevated, so he underwent a liver biopsy, which confirmed his liver was healing well. It was about a year after the initial injury when his liver markers came back close to the normal range—and that is where they remain 3 years after the injury, “not completely normal, but they’re close to normal,” Jeff says.

Jeff maintains a spreadsheet to keep track of his test results, and, with some trepidation, he still looks forward to getting his blood tested, which happens yearly now. “I want to know if I’m getting better,” he says. “But at the same time, I’m very anxious about it and concerned that it’s going to be moving backwards, so there’s definitely still some feelings that linger, like, ‘can this get worse?’”

In spite of his worries, 5 months after his liver injury Jeff was again racing in mountain bike competitions. Within a year, he was back to winning statewide races in his age group. “So, I was kind of back into full swing,” he admits modestly.

## SPOTTING THE FINISH LINE: OVERCOMING DRUG-INDUCED LIVER INJURY

DILIN continues to build upon its successes. The NIDDK renewed the Network for a new project period beginning in 2018 and included provisions for pilot studies that would lay the groundwork for

future clinical trials to treat severe drug-induced liver injury. The NIDDK also partners with the NIH’s National Library of Medicine on an online resource called “LiverTox” (<http://livertox.nih.gov>), which features sample cases of people with drug-induced liver injury based on the Network’s data, as well as a database summarizing liver injuries caused by drugs, including amoxicillin-clavulanate, and various herbal and dietary supplements. Meanwhile, Jeff continues to participate in the DILIN study and has adopted a new outlook. He admits that, like most people, he knew next to nothing about drug-induced liver injury before he was affected by it; now, after gaining firsthand knowledge, he wants to raise public awareness about it. He is thankful for everyone who supported him through his illness: his family and friends, his church, his gastroenterologist, and Dr. Fontana, who once jokingly called him “the healthiest sick person I know.”

*“It was an opportunity to have really good care and to be monitored on a regular basis,” Jeff says about joining the Drug-Induced Liver Injury Network study. “I think that was the biggest thing, that they would be watching over me, trying to understand this better, and trying to help in the future.”*

His experience has left an indelible impression on Jeff’s life, particularly with regards to his health. While there is no approved therapy for this type of liver injury, there are ways to manage it that focus on health maintenance and avoiding further injury, including stopping all medications except the most necessary, stopping alcohol consumption, paying careful attention to nutrition, and getting adequate rest. Once the injury resolves, it is possible to resume other medications and modest alcohol intake. “[The injury] is at the back of your mind,” he says. “For example, I always liked a glass of wine or beer now and then, but [shortly after the injury]

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***"I'm living my life to the fullest, because you don't know what's going to happen," says Jeff of his outlook after recovering from a potentially life-threatening liver injury.***

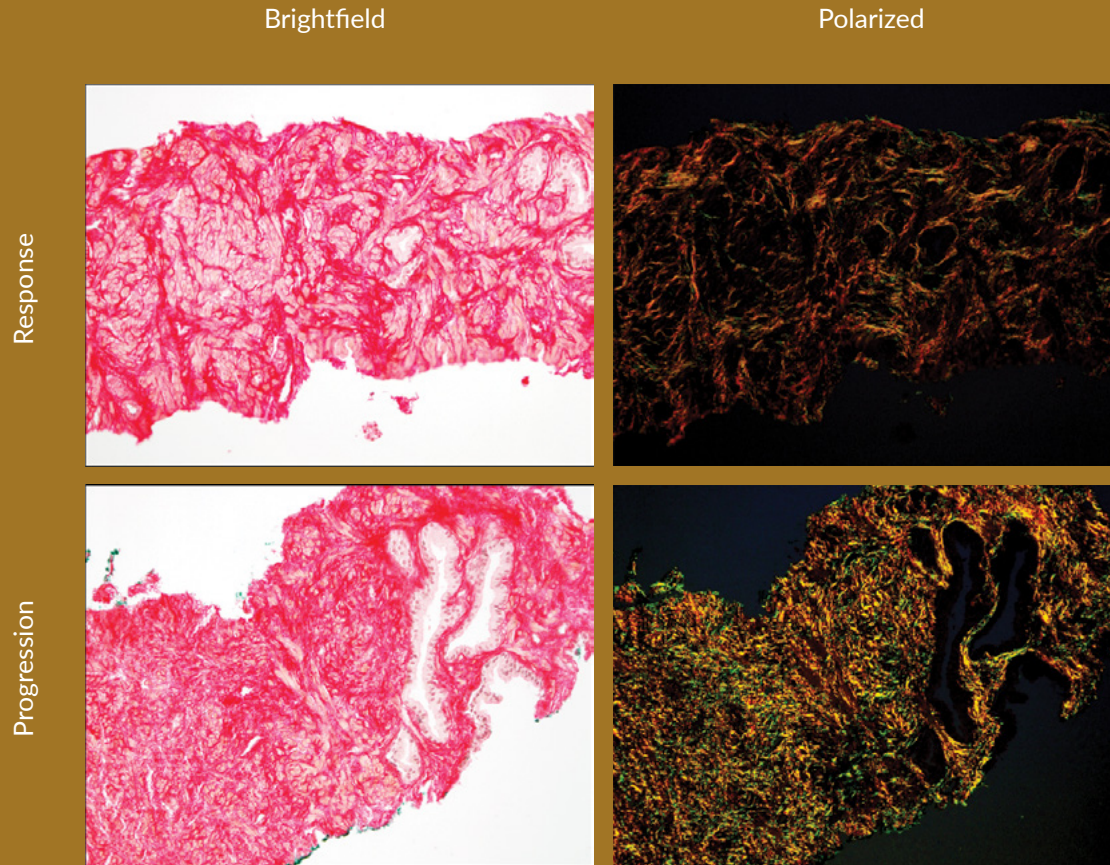
there was no alcohol. I didn't have any of that for at least a year. When things returned to normal, I would have just a little bit. So those kinds of things are always on your mind."

He also has a renewed desire to get the most out of life. "After the injury, I was looking at things

differently," he remembers. The next winter, when he and his family went on their annual skiing trip to the Canadian Rockies, he tried something new: heli-skiing. He and his son rode in a helicopter to a remote area at the top of a mountain, where they were deposited in fresh snow to ski down the untouched slope. "I said, 'OK, I'm just going to do this,' and it was great," he recalls, adding that this is an example of how liver injury affected the way he looks at things. "I'm living life to the fullest, because you don't know what's going to happen," he says. "Now I want to live life even more."







Benign prostatic hyperplasia (BPH), which is often associated with a collection of lower urinary tract symptoms (LUTS), affects men of all races and ethnic groups and can progress in severity over time. If untreated, BPH can lead to significant consequences, such as acute urinary retention, incontinence, and urinary tract infection. Medical and surgical interventions, however, do not achieve symptom relief for all men with lower urinary tract dysfunction or may not provide a durable response. Research described in this chapter implicates fibrosis in the process of BPH/LUTS in men; specifically, men for whom current drug treatments do not work have greater amounts of fibrosis in their prostates. In this figure, prostate samples were obtained from men treated for BPH with a combination of doxazosin and finasteride and analyzed with two different forms of microscopy. Upper panels: prostate tissue from men whose BPH responded to the treatment. Bottom panels: prostate tissue from men who did experience clinical progression to increasingly severe BPH symptoms.

*Images courtesy of William Ricke, Ph.D., University of Wisconsin-Madison.*

# Kidney, Urologic, and Hematologic Diseases

*Diseases of the kidneys, urologic system, and blood are among the most critical health problems in the United States. They affect millions of Americans, and their impact is felt across the lifespan. To improve our understanding of the causes of these diseases, and to identify potential new prevention and treatment strategies, the NIDDK supports basic and clinical research studies of the kidney and urinary tract and of the blood and blood-forming organs. The overall goal of the NIDDK's research programs is to improve the health of people who have or are at risk for kidney, urologic, and hematologic (blood) diseases.*

Normal, healthy kidneys filter about 200 quarts of blood each day, generating about 2 quarts of excess fluid, salts, and waste products that are excreted as urine. Loss of function of these organs, either for a short period of time or as a consequence of a gradual, long-term decline in kidney function, is a life-threatening condition.

It has been estimated that 37 million American adults have impaired kidney function—also called chronic kidney disease (CKD).<sup>1</sup> CKD has two main causes: high blood pressure and diabetes. The increases in obesity and type 2 diabetes in the United States in recent years—especially among children and adolescents—have grave implications for the Nation's health, as young people with these conditions are likely to face serious health complications at an earlier age than people who historically have developed these conditions later in life.

One feature common to kidney diseases arising from varying causes is the deposition of fibrotic scar tissue in the kidney. Research supported by the NIDDK has enhanced our understanding of the origin of this scar tissue, how it can impair kidney function, and how it might be prevented or treated. CKD, especially if undetected, can progress to irreversible kidney failure, a condition known as end-stage renal disease (ESRD). People with ESRD require dialysis or a kidney transplant to live. In 2016, over 726,000 patients received treatment for ESRD: over 511,000 received either hemodialysis or peritoneal dialysis, and over 215,000 were living with a kidney transplant.<sup>2</sup> Racial and ethnic minority populations in the United States, particularly African Americans, Hispanic and Latino Americans, and American Indians and Alaska Natives,

bear a disproportionate burden of CKD and ESRD. Compared to non-Hispanic Whites, ESRD prevalence in 2016 was about 3.7 times greater in African Americans, 1.3 times greater in Hispanics, 1.5 times greater in American Indians and Alaska Natives, 9.5 times greater in Native Hawaiians/Pacific Islanders, and 1.3 times greater in Asians.<sup>2</sup> In recent years, scientists supported by the NIDDK have uncovered important genetic clues that may play a role in some of the health disparities related to kidney disease susceptibility and progression in minority populations.

The Institute supports a significant body of research aimed at understanding the biology underlying CKD and developing treatment strategies. The NIDDK's chronic renal diseases program supports basic and clinical research on kidney development and disease, including the causes of kidney disease, the underlying mechanisms leading to progression of kidney disease to ESRD, and the identification and testing of possible strategies to prevent development or halt progression of kidney disease. In addition to research on kidney disease related to diabetes and high blood pressure, the NIDDK also supports studies of inherited diseases, such as polycystic kidney disease, congenital kidney disorders, and focal segmental glomerulosclerosis;

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<sup>1</sup> Centers for Disease Control and Prevention. *Chronic Kidney Disease in the United States, 2019*. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2019.

<sup>2</sup> United States Renal Data System. *2018 USRDS Annual Data Report: Epidemiology of kidney disease in the United States*. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2018.

and immune-related kidney diseases, such as IgA nephropathy and hemolytic uremic syndrome. The CKD Biomarkers Consortium (CKD BioCon) promotes the discovery and validation of novel biomarkers for CKD initiation, progression, and development of complications. A more complete understanding of biomarkers could allow physicians to detect kidney disease earlier and perhaps identify people at greater risk of progression, allowing them to tailor treatments to a specific individual. The Kidney Precision Medicine Project aims to obtain and evaluate human kidney biopsies from participants with acute kidney injury (AKI) or CKD for the purpose of creating a kidney tissue atlas, defining disease subgroups, and identifying critical cells, pathways, and targets for novel therapies.

Urologic diseases affect people of all ages, result in significant health care expenditures, and may lead to substantial disability and impaired quality of life. The NIDDK's urology research program supports basic and clinical research on the normal and abnormal development, structure, function, and injury repair of the genitourinary tract. Areas of interest include the causes of and treatments for urologic diseases and disorders such as benign prostatic hyperplasia, urinary incontinence, urinary tract infections, and urinary stone disease. To spur research in urinary stone disease, the Urinary Stone Disease Research Network (USDRN) is: a) conducting a randomized clinical trial to investigate the impact of increased fluid intake and increased urine output on the rate of recurrence of urinary stones in adults and children; b) conducting clinical research to understand and mitigate ureteral stent-related pain and symptoms; and c) providing data and collecting biological samples from the studies to create a resource for future researchers.

Other disorders of the genitourinary tract, such as interstitial cystitis/bladder pain syndrome (IC/BPS)—also known as IC/painful bladder syndrome (PBS)—in women and men and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) in men, are also important research topics of the NIDDK's urology program.

IC/BPS is a debilitating, chronic, and painful urologic disorder. Based on a recent large, national interview survey, it is estimated that among U.S. women 18 years old or older, 3.3 million (2.7 percent) have pelvic pain and other symptoms, such as urinary urgency or frequency, that are associated with IC/BPS.<sup>3</sup> Using a community-based epidemiologic

survey, researchers have estimated that among U.S. men ages 30 to 79 years old, 1.6 million (1.3 percent) have persistent urologic symptoms, such as pain with bladder filling and/or pain relieved by bladder emptying, that are associated with BPS.<sup>4</sup>

NIDDK-supported basic and clinical research on IC/BPS and on CP/CPPS is focused on elucidating the causes of these conditions, identifying important subsets of patients to aid diagnostic stratification, and improving treatment and interventions. One example of an ongoing study is the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network, which supports research designed to uncover the underlying causes of IC/BPS and CP/CPPS and to characterize the disease profiles in patients.

Based upon national public health surveys conducted over several years, it is estimated that about 54 percent of women (20 years and older) report urinary incontinence in the past 12 months. Urinary incontinence was self-reported by approximately 15 percent of men surveyed.<sup>5</sup> Many suffer in silence due to embarrassment and lack of knowledge about treatment options available. NIDDK-supported studies over the past several years have helped to advance knowledge about the efficacy of surgical treatment of urinary incontinence, as well as to provide new insights into non-surgical alternatives. As researchers continue to investigate treatment options, an equally important challenge is to identify and understand the important subgroups of patients with lower urinary tract symptoms (LUTS) through improved measurement of patient experiences of LUTS in men and women. To address this challenge, the NIDDK supports the multi-site Symptoms of Lower Urinary Tract Dysfunction Research Network (LURN). The NIDDK is also leading new efforts to explore whether it may be possible to prevent symptom onset and/or progression, thereby improving health. The NIDDK, in conjunction with the National Institute on Aging and the NIH Office of Research on Women's Health and Office of Behavioral and Social Sciences Research established the Prevention of Lower Urinary Tract Symptoms (PLUS) Research Consortium to develop the evidence base for normal

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<sup>3</sup> Berry SH, et al. *J Urol* 186: 540-544, 2011.

<sup>4</sup> Link CL, et al. *J Urol* 180: 599-606, 2008.

<sup>5</sup> *Urologic Diseases in America, 2018 Addendum*. NIDDK, NIH Publication Number 12-7865, 2018.

or healthy bladder function and to identify behavioral and other risk factors for conditions associated with lower urinary tract symptoms in women.

The NIDDK's hematology research program uses a broad approach to enhance understanding of the normal and abnormal function of blood cells and the blood-forming system. Research efforts include studies of a number of blood diseases, including sickle cell disease, the thalassemias, aplastic anemia, iron deficiency anemia, hemolytic anemias, thrombocytopenia, and the anemia of inflammation and of chronic diseases. To promote high-impact basic or pre-clinical research, the Institute supports the Stimulating Hematology Investigation: New Endeavors (SHINE) program and includes the following current research topic areas: regulation of blood (hematopoietic) stem cells, factors that play a role in the development of different types of blood cells, and red blood cell maturation. The Institute's SHINE II program seeks to further catalyze research in basic or pre-clinical, proof of principle research projects that are tightly focused and directed at validating novel concepts and approaches that promise to open up new pathways for discovery in benign hematology research. The NIDDK is also keenly interested in the basic biology of adult hematopoietic stem cells, which are used clinically in bone marrow transplants and may have broader application in gene therapy research.

## MODELING KIDNEY FUNCTION IN THE LABORATORY

**Streaming Fluid Across Kidney Organoids—Mini Kidney-like Structures—Grown on a Chip Drives Their Maturation:** Scientists found that streaming fluid across kidney organoids—engineered aggregates of kidney cells—prompts the organoids to develop blood vessels and to form natural tissue structures when grown on a chip, dramatically improving the extent to which they replicate normal kidney functions. Kidneys are highly complex organs in which systems of blood vessels intertwine with other structures to filter extra water and wastes out of the blood and make urine. Loss of kidney function can thus lead to build up of toxins in the blood and other problems, and total kidney failure is deadly without dialysis or a kidney transplant. Therefore, scientists have sought to develop treatments to repair, replace, or enhance lost kidney function. For many years, researchers have been improving

methods to use human stem cells in the laboratory to engineer kidney organoids, which are three-dimensional tissue constructs that can mimic kidney functions. However, it has been challenging to integrate blood vessels into growing organoids and coax stem cells to take on the required properties of kidney cells, and researchers have sought ways to overcome these technological hurdles to generate mature kidney organoids that fully replicate kidney function.

In a recent study, researchers attempted to recreate some of the environmental conditions under which kidneys normally develop in the body to see if these conditions would help organoids to mature properly. The scientists mounted organoids to small platforms, or “chips,” that can be modified to test various technological parameters. They reasoned that because developing kidneys normally are exposed to a flow of surrounding fluids, perhaps adding the stress of fluid flow to these chips could better mimic the natural environment. When the researchers grew chip-mounted kidney organoids in the presence of a high rate of fluid flow, they developed an array of blood vessels with varying diameters; by contrast, organoids exposed to low fluid rate or none at all had far fewer blood vessels. These blood vessels infiltrated the organoids and connected with internal tissue structures, as is required for normal kidney functions. The scientists found that under high-flow conditions, the developing blood vessels successfully transported fluids, and even assembled as networks connecting neighboring organoids. Organoids exposed to high flow also formed critical kidney tissue structures that closely resembled those found in normal kidneys. Together, these findings revealed that organoids grown on chips under high fluid flow conditions were far more physiologically mature than those under low or no flow.

The technological advances achieved in this study have boosted the ability of organoids on chips to mimic the natural physiological function of human kidneys. These conditions may help researchers utilize chips to test potential new drugs more quickly and accurately than has been possible. Improved kidney organoids also represent an important step toward the future development of functional, implantable structures that can enhance or replace lost kidney function in people.

*Homan KA, Gupta N, Kroll KT,...Morizane R. Flow-enhanced vascularization and maturation of kidney organoids in vitro. Nat Methods 16: 255-262, 2019.*



## RESEARCH TO PROMOTE KIDNEY CELL REGENERATION

### Key Regulators of Kidney Regeneration Identified:

Scientists have determined that two cellular signaling pathways dynamically control kidney regeneration in a fish model system. Zebrafish are useful research models for studying kidney development and disease because they are transparent, genetically tractable, and easy to manipulate in the laboratory. Unlike humans, zebrafish have the ability to regenerate kidney tissue in response to injury through the formation of new nephrons—the tiny filtration units in the kidney that remove waste products and excess fluid from the blood. Upon kidney injury, nephron progenitors, or stem cells, quietly residing in the kidney start expanding (*i.e.*, increasing in cell number) and migrate to tubules, where they form aggregates that develop into new nephrons that directly connect to the internal plumbing system. However, the cellular signals induced by kidney injury have been unknown. A pair of recent studies have identified key cellular signaling pathways—referred to as the Wnt and FGF signaling pathways—regulating new nephron formation during kidney regeneration.

In one study, researchers chemically induced kidney injury to promote new nephron formation in adult zebrafish. At the site of the injury, several Wnt signaling pathway-related genes were turned on specifically in nephron progenitor cells. Treatment with inhibitors of Wnt signaling blocked these genes from being turned on and far fewer nephron aggregates formed as a result, demonstrating that active Wnt signaling was required for new nephron formation. In addition, when the gene *fzd9b*, which encodes a critical Wnt signaling component, was genetically deleted in the setting of kidney injury, the numbers of nephron aggregates were significantly reduced when compared with normal injured zebrafish. These results define a clear role for the Wnt signaling pathway in new nephron formation.

Researchers in another study reported a similar series of zebrafish experiments demonstrating that the FGF signaling pathway is activated upon kidney injury. Blocking the FGF signaling pathway, with chemical inhibitors or genetic ablation, completely disrupted nephron aggregate formation following kidney injury. Further analysis showed that inhibiting FGF signaling prevented nephron progenitor cells from migrating to the proper location and aggregating to initiate new nephron

formation. Furthermore, beads soaked in FGF proteins and implanted into uninjured zebrafish kidneys attracted nephron progenitor cells to migrate to those sites. Taken together, these findings suggest that FGF signaling is critical for inducing new nephron formation in regenerating kidneys.

These studies identify two vital signaling pathways that control new nephron formation during zebrafish kidney regeneration. New nephron formation does not occur in adult humans; nephrons are only formed prior to or shortly after birth. However, research has shown that Wnt and FGF signaling pathways play important roles in human kidney development and disease, suggesting some commonalities between fish and mammals. These findings could provide the foundation for research to develop therapeutic strategies for kidney regeneration.

*Gallegos TF, Kamei CN, Rohly M, and Drummond IA. Fibroblast growth factor signaling mediates progenitor cell aggregation and nephron regeneration in the adult zebrafish kidney. Dev Biol 454: 44-51, 2019.*

*Kamei CN, Gallegos TF, Liu Y, Hukriede N, and Drummond IA. Wnt signaling mediates new nephron formation during zebrafish kidney regeneration. Development 146: pii: dev168294, 2019.*

## CLINICAL RESEARCH ON KIDNEY DISEASE

### Lowering Blood Pressure Does Not Lead to Kidney Damage:

In contrast to previous reports, scientists have determined, upon further research, that intensive blood pressure control does not lead to kidney injury in people who do not have chronic kidney disease (CKD). Elevated blood pressure is relatively common in the U.S. population and is a risk factor for heart disease, stroke, and CKD. The Systolic Blood Pressure Intervention Trial (SPRINT) was designed to test whether using medications to reduce systolic blood pressure to a lower goal than currently recommended would reduce cardiovascular disease in people with high blood pressure but not diabetes. (“Systolic” refers to the higher of the two numbers in a blood pressure reading; it measures the pressure in the arteries when the heart beats. “Diastolic” refers to the lower of the two numbers and measures the blood pressure when the heart rests between beats.)

SPRINT researchers previously reported that, among the subset of study participants who did not have CKD at the start of the trial, those who received an

intensive blood pressure control regimen were at a slightly higher risk of developing CKD than those who received standard care. They defined new-onset CKD as a minimum 30 percent reduction in the rate at which kidneys filter blood (filtration rate) to a level considered less than normal. This elevated CKD risk was generally outweighed by a reduced likelihood of cardiovascular events and death. However, because kidney filtration rates are dependent on blood pressure, the observed reduction in kidney function in these participants could simply reflect changes in blood flow, not necessarily underlying kidney damage. To explore this possibility, SPRINT researchers tested urine samples from study participants for the presence of several molecules known to be directly associated with various types of kidney damage (also known as “biomarkers” of kidney damage). Surprisingly, after 1 year of intensive blood pressure control, participants who developed CKD exhibited greater reductions in some urinary biomarkers of kidney damage than those who did not develop CKD. In addition, urine from study participants who did not receive intensive blood pressure control but nonetheless developed CKD had elevated kidney damage biomarkers compared with those who received the intensive blood pressure regimen. These results suggest that a blood flow effect, rather than a bona fide kidney injury, led to the misclassification of CKD in these study participants. Patients and clinicians are now empowered by this new information to seek more intensive blood pressure control to reduce the risk of mortality.

*Zhang WR, Craven TE, Malhotra R,...lx JH; for the SPRINT Research Group. Kidney damage biomarkers and incident chronic kidney disease during blood pressure reduction: A case-control study. Ann Intern Med 169: 610-618, 2018.*

### **Treatment of Depression for People with End-stage Kidney Disease Undergoing Hemodialysis:**

In a recent clinical trial testing therapies for depression in people undergoing hemodialysis for kidney failure, researchers found that an engagement interview had no effect on acceptance of depression treatment, while depression scores were modestly improved with the drug sertraline compared with cognitive behavioral therapy (CBT). Kidney disease can worsen over time and may lead to kidney failure. If less than 15 percent of the kidney is working normally, that’s considered kidney failure—also referred to as end-stage renal disease (ESRD). Hemodialysis is a treatment in which a machine

filters wastes and water from the blood, as the kidneys did when they were healthy; but it has limitations and does not totally replace the function of normal kidneys. Hemodialysis sessions usually last several hours each, on multiple days each week. A common condition associated with people on hemodialysis is depression. Depression is a serious mood disorder. It causes severe symptoms that affect how you feel, think, and handle daily activities, such as sleeping, eating, or working. Many people on hemodialysis do not receive treatment for depression, possibly because of their reluctance to accept a diagnosis for this condition and/or receive treatment. In addition, the effectiveness of antidepressant therapies in people undergoing hemodialysis has not been properly evaluated in clinical trials, making depression treatment in this patient population difficult.

A recent two-phase, randomized, controlled clinical trial assessed therapies for depression in women and men, ages 21 years and older, who had depression and ESRD and were receiving hemodialysis at any of 41 dialysis centers. The first phase of the trial investigated whether an engagement interview would increase the 184 participants’ willingness to accept the diagnosis of and treatment for depression. The engagement interview was conducted face to face by trained therapists while participants received their hemodialysis treatment. The participants in the engagement interview were also given a DVD to improve their understanding of depression and its treatment. The researchers’ findings indicated that the engagement interview had no effect on their acceptance of treatment for depression compared to a control group, which had a visit from a research team member during which they discussed the diagnosis of depression and associated treatment options. The 120 participants in the second phase of the trial were divided into 2 groups of 60 to receive either individual CBT or sertraline therapy. CBT teaches a person different ways of thinking, behaving, and reacting to situations. Participants in the CBT group were scheduled for 10 sessions of 60 minutes each over 12 weeks while undergoing hemodialysis. Sertraline is used to treat depression and is in a class of antidepressants called selective serotonin reuptake inhibitors. It works by increasing the amount of serotonin—a natural chemical in the brain that is implicated in the regulation of many behaviors, mental processes, and mood. After 12 weeks of treatment, depression scores were modestly better with sertraline treatment compared

to CBT. Sertraline use, however, was associated with mild to moderate adverse events related to the digestive, heart, and nervous systems.

These results provide a foundation on which health care providers and patients on hemodialysis can enter into the shared decision-making process to choose between CBT and sertraline to treat depression. It may also pave the way for potential future research on other depression treatments for people on hemodialysis.

Mehrotra R, Cukor D, Unruh M,...Hedayati SS. Comparative efficacy of therapies for treatment of depression for patients undergoing maintenance hemodialysis: A randomized clinical trial. *Ann Intern Med* 170: 369-379, 2019.

**Gene Sequencing Can Help Tailor Treatments for Young People with Kidney Failure:** Sequencing portions of the genome could help diagnose the underlying cause of chronic kidney disease (CKD) in children and young adults receiving a kidney transplant, enabling clinicians to use precision medicine strategies to improve outcomes. When CKD worsens to kidney failure, transplantation is in many cases the preferable of the two main treatment options; the other option is dialysis. The underlying reasons that children and young adults develop CKD are different from older adults, and many of the gene variants that cause the different types of CKD have been determined over the past few years. Individual cases have demonstrated that knowing the genetic cause of CKD can help guide clinical decisions to improve outcomes in young patients who received a kidney transplant. Therefore, researchers in a recent study sought to systematically investigate whether sequencing portions of the genome previously linked to CKD could help diagnose the cause of kidney failure in kidney transplant recipients.

The scientists sequenced approximately 400 CKD-linked genes in 104 children and young adults who developed the disease under the age of 25, and who received a kidney transplant between 2007 and 2017. From their analyses, the scientists identified a genetic cause of CKD in 34 patients (32.7 percent). The likelihood of finding a genetic cause was highest in patients with certain types of CKD, such as conditions known as urinary stone disease and renal cystic ciliopathy. These results revealed that sequencing specific portions of the genome could help determine the type of CKD that led to kidney failure in about one-third of children and

young adults receiving a kidney transplant—findings that could be clinically useful because different types of CKD often require different patient care approaches (e.g., early screening for anticipated health problems; treatment strategies before and after transplantation). The researchers also point out that genetic analyses of potential living kidney donors may also help predict health outcomes and determine whether a kidney is suitable for donation; genetic testing of donors would be particularly important for close family members who are more likely to harbor the same genetic variants. Armed with this genetic knowledge, doctors, patients, and their families could make more informed clinical decisions and tailor treatment strategies to the patients' individual needs.

Mann N, Braun DA, Amann K,...Hildebrandt F. Whole-exome sequencing enables a precision medicine approach for kidney transplant recipients. *J Am Soc Nephrol* 30: 201-215, 2019.

## RESEARCH ON LOWER URINARY TRACT SYMPTOMS AND DISORDERS

### Achieving a Better Understanding of Symptom Flares in People with Urologic Pain Syndromes:

Scientists studying urologic chronic pelvic pain syndromes (UCPPS) have recently reported on risk factors and variation regarding symptom flares in people—information that could help improve care in the future. People with UCPPS—interstitial cystitis/bladder pain syndrome (IC/BPS) or chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS, in men only)—experience pelvic pain and urologic symptoms, such as urinary frequency and urgency. When one or both sets of symptoms becomes worse for a period of time, this is called a flare. Understanding frequency, duration, and risk factors for flares over time could help in finding ways to manage or prevent them. In a study lasting nearly a year, researchers with the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network collected information about flares from nearly 400 participants. About 75 percent of participants reported having at least one flare, and the duration of flares could be as short as one day or as long as 150 days. Further analyses revealed that both urologic and pelvic pain symptoms worsened during flares, although the degree of worsening in each flare event varied not only between people but also within persons who had multiple flares. Risk of worse and/or longer flares was greater in women, in individuals who had greater than average UCPPS

symptoms overall when not having a flare, and in those with bladder pain associated with filling and/or urgency to urinate (bladder hypersensitivity). Finally, people with UCPPS commonly have co-occurring chronic pain conditions, such as irritable bowel syndrome (IBS), and having IBS or any such pain condition was also a risk factor for worse flares. Based upon their findings, the researchers also generated some new ideas for investigation regarding mechanisms underlying risk and extent of flares, such as the potential involvement of pain pathways both in the central nervous system and in peripheral organs and tissues. This newly acquired knowledge of UCPPS symptom fluctuation over time, risk factors, and variability in flare characteristics both within and between individuals helps to identify subgroups of people with UCPPS, and provides a foundation on which to research further flare management with the ultimate goal of flare prevention.

*Sutcliffe S, Gallop R, Henry Lai HH,...Landis JR. A longitudinal analysis of urological chronic pelvic pain syndrome flares in the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network. BJU Int 124: 522-531, 2019.*

### **Fibrosis Underlies Male Lower Urinary Tract**

**Symptoms:** Two recent studies have highlighted the importance of fibrosis in lower urinary tract symptoms (LUTS) in men and in mouse models. Fibrosis is the deposition of large amounts of collagen-rich connective tissue that can lead to organ damage. The most common symptoms vary but LUTS can involve changes or problems with urination, such as a hesitant, interrupted, weak stream; urgency and leaking or dribbling; more frequent urination, especially at night; and urge incontinence. Previous studies have suggested that deposition of fibrosis in the prostate gland contributes to the development of LUTS/BPH (benign prostatic hyperplasia) in men. The extent to which fibrosis plays a role in the pathology of LUTS remains to be defined.

In 2003, NIDDK's Medical Therapy of Prostatic Symptoms (MTOPS) trial reported that combination therapy of two drugs (finasteride and doxazosin) is more effective than either drug singly in the treatment of BPH. Medical and surgical interventions, however, do not achieve symptom relief for all men with lower urinary tract dysfunction or may not provide a durable response. In one recent study, researchers examined tissue specimens

to test the hypothesis that fibrotic changes in the prostate may be associated with an increased risk for clinical progression to more severe symptoms among MTOPS participants. The researchers reported significant alterations in the architecture of collagen (i.e., a surrogate marker for fibrosis) in prostate specimens from men who exhibited clinical progression compared to those who did not. This finding suggests that anti-fibrotic medications might offer another potential treatment option to men with BPH. Furthermore, men who had worsening symptoms were also found to have a high body mass index (a measure of weight relative to height).

In a second recent study, investigators sought to better understand the inflammation and pain associated with LUTS/BPH using a mouse model. The model is created by infection of the male mouse urethra with a strain of *Escherichia coli* (*E. coli*) bacteria called CP1; the mice then display symptomatic changes that mimic those observed in human LUTS/BPH. The investigators showed that *E. coli* CP1 infection increases the percentage of collagen within the prostate. Furthermore, the researchers identified that the immune system of a certain mouse strain increased production of two proteins called IL-4 and IL-13 in response to CP1 infection. These two proteins were shown to be associated with fibrosis in previous studies. Strategies that target IL-4 and IL-13 could thus be tested to see if they reduce the fibrosis and pain associated with LUTS/BPH in this model system.

Taken together, these studies report that fibrosis underlies LUTS/BPH in both humans and the CP1-induced mouse model. Further research will be required to determine whether anti-fibrotic medications can be of clinical benefit in this burdensome condition.

*Macoska JA, Uchtmann KS, Levenson GE, McVary KT, and Ricke WA. Prostate transition zone fibrosis is associated with clinical progression in the MTOPS Study. J Urol 202: 1-8, 2019.*

*Bell-Cohn A, Mazur DJ, Hall C, Schaeffer AJ, and Thumbikat P. Uropathogenic *Escherichia coli*-induced fibrosis, leading to lower urinary tract symptoms, is associated with type 2 cytokine signaling. Am J Physiol Renal Physiol 316: F682-F692, 2019.*

### **Genetic Risk Factor Associated with Erectile**

**Dysfunction:** For the first time, researchers have identified a genetic variant that increases the risk of erectile dysfunction (ED); the genetic variant is

near a gene called *SIM1* and may affect this gene's activity. ED is a condition in which one is unable to get or keep an erection firm enough for satisfactory sexual intercourse. Symptoms of ED include being able to get an erection sometimes, but not every time; being able to get an erection but not having it last long enough for sexual intercourse; and being unable to get an erection at any time. Several different diseases and conditions can lead to ED including type 2 diabetes, heart and blood vessel disease, and chronic kidney disease, among others. Although men are more likely to develop ED as they age, aging does not cause ED. A twin study of middle-aged males reported that about one-third of ED risk is heritable—meaning that there is a genetic component(s), but specific genetic variants had not been identified.

Investigators recently undertook a genome-wide association study (GWAS) of erectile dysfunction in a racially diverse cohort of 36,649 men. GWAS can be used to search for rare or common susceptibility genes in large groups of individuals. Of the people studied, 14,215 men had reported ED symptoms, and these individuals were more likely to be older, have a slightly higher body mass index (a measure of weight relative to height), have diabetes, be smokers or former smokers, have a clinical diagnosis of ED, and have filled a prescription to treat ED, compared to the control population of 22,434 men. The researchers found that DNA sequence variations at a position in the genome (*i.e.*, genetic locus) near the *SIM1* gene are significantly associated with an approximate 26 percent increased risk of ED. The increased risk is independent of known risk factors such as higher body mass index. The DNA sequence variations were verified in a second cohort of 222,358 men. The researchers further showed a biological role for the implicated genetic locus. The locus resides within an “enhancer” element; enhancers are short DNA sequences that control the extent to which a gene is turned on or off. The researchers found that the variants they identified within the enhancer alter its ability to control the activity of the *SIM1* gene.

This study reveals a previously unknown mechanism associated with ED, and lays the foundation for efforts to develop approaches targeting *SIM1* to restore erectile function and, thus, help men achieve a healthy sex life.

Jorgenson E, Matharu N, Palmer MR,...Van Den Eeden SK. Genetic variation in the *SIM1* locus is associated with erectile dysfunction. *Proc Natl Acad Sci USA* 115: 11018-11023, 2018.

## TREATING BLOOD DISORDERS

**Expanding Numbers of Blood Stem Cells Prior to Therapeutic Transplantation:** Two recent studies highlight potential strategies to expand blood (hematopoietic) stem cells *in vitro* (in a laboratory dish) in order to generate more of these rare cells prior to therapeutic transplantation in people. Hematopoietic stem cell (HSC) transplants can be life-saving for people with a number of conditions. When introduced into a donor, HSCs migrate to the bone marrow where they normally reside and renew—and when needed mature into all types of blood cells (*e.g.*, red cells, white cells, and platelets). However, it can be challenging to find HSCs in needed quantities from a donor whose cells are similar enough to a patient's cells to be a sufficient “match” for transplantation. Researchers supported by NIDDK continue studies to discover key factors that promote HSC expansion *in vitro*, and with these insights increase the potential availability of transplantation to benefit many more people.

In the search for ways to promote expansion of HSCs, one team of scientists focused on a protein called DEK. Previous research has shown that DEK is an abundant protein found in most human tissues, and that it may regulate blood cell development. In a recent study, the researchers found that a synthetically produced secreted form of the DEK protein was shown to greatly enhance expansion of mouse (both male and female) and human HSCs within 4 days *in vitro*. This finding is important as the ability to transplant increased numbers of HSCs might improve transplantation outcomes in the recipient.

Another research team sought to optimize the components of the liquid culture medium in which HSCs are expanded *in vitro*, and the surface that they are grown on. For example, serum albumin has long been used as part of the culture medium for the expansion of HSCs, but it contains a complex mixture of proteins, often inadequately characterized. In a recent study in mice, the researchers described the development of a defined culture system that includes a component called “polyvinyl alcohol” (PVA) as a substitute for serum albumin, and optimized levels of two



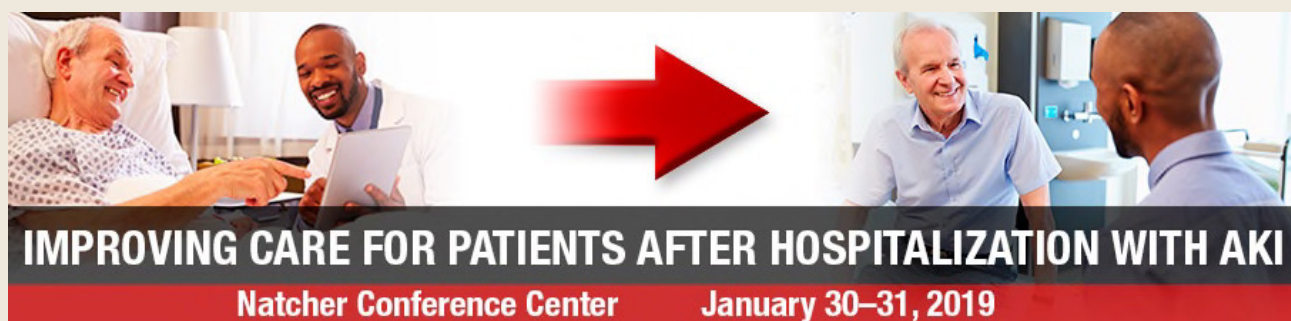
other components, thrombopoietin and stem cell factor. They also used another factor, fibronectin, to coat the dish on which the cells are expanded. The researchers found that this culture system supports long-term expansion of mouse HSCs. Both male and female mice were used in this study. This culture system facilitated expansion of HSCs between 236-fold and 899-fold during a 1-month timeframe. Furthermore, when transplanted into recipient mice, the expanded HSCs migrated to and engrafted into the bone marrow without the mice having to undergo standard, but toxic, pre-conditioning regimens.

Taken together, these research studies highlight potential new strategies to expand transplantable HSCs *ex vivo* prior to therapeutic transplantation. Future research could determine whether these strategies improve HSC transplantation in people.

Capitano ML, Mor-Vaknin N, Saha AK,...Broxmeyer HE. Secreted nuclear protein DEK regulates hematopoiesis through CXCR2 signaling. *J Clin Invest* 129: 2555-2570, 2019.

Wilkinson AC, Ishida R, Kikuchi M,...Yamazaki S. Long-term *ex vivo* haematopoietic-stem-cell expansion allows nonconditioned transplantation. *Nature* 571: 117-121, 2019.

# Workshop Explores New Approaches for Improving Care for Patients After Acute Kidney Injury



On January 30–31, 2019, the NIDDK sponsored a multidisciplinary workshop in Bethesda, Maryland, focusing on the development of strategies to improve clinical outcomes among patients surviving an episode of acute kidney injury (AKI).

AKI, also called acute renal failure, is characterized by a relatively rapid loss of kidney function, usually over a period of several hours or days. The resulting inability to excrete waste products and maintain fluid and salt balance poses urgent health problems for patients and their physicians. AKI may arise from a number of causes, such as sepsis (a serious, whole-body inflammatory reaction caused by infection), decreased blood pressure, or kidney damage from drugs or other toxins. AKI is an increasingly common condition affecting hospitalized patients that is associated with future chronic kidney disease (CKD), cardiovascular events, diminished health care-related quality of life, and death. Multiple studies highlight rapid growth in the incidence of AKI, and with it, parallel increases in the number of survivors.

A proportion of patients without preexisting CKD experience significant loss of kidney function over the long term after an episode of AKI, resulting in the development of new CKD. Approximately 20 percent of such patients with AKI, usually of the greatest severity, will develop CKD over the course of a few years. Risk factors for the development of CKD after an AKI episode include older age, race (e.g., African Americans are at higher risk), and disease severity.

Although several therapeutic interventions for AKI during hospitalization have been tested, none is effective in changing the course of the disease. How patients should be treated—including medications and level of blood pressure control—in the post-AKI outpatient setting is unknown. Current clinical practice guidelines lack high-quality evidence to inform care recommendations.

This workshop brought together experts with the goals of: 1) facilitating the development of strategies to improve clinical outcomes among patients surviving an episode of AKI; 2) reviewing the current state-of-the-science of interventions following AKI and mechanisms that drive susceptibility to future CKD and cardiovascular events, thus identifying knowledge gaps that need to be addressed to better inform clinical care; and 3) identifying possible interventions, focusing on parameters of key interest for evaluation and feasibility of studies.

Through fruitful discussions, there emerged a broad consensus that post-AKI care interventions warrant testing in clinical trials. Given the challenges expressed by AKI survivors with the post-hospitalization transition, such studies should focus on intermediate (90- to 180-day) outcomes. Furthermore, the primary concerns reported by AKI survivors were not necessarily hard clinical outcomes (e.g., re-hospitalization), but rather symptoms and quality of life (e.g., anxiety/depression) during the post-AKI transition, and such concerns need to be incorporated into clinical trial design.

# Catheter-associated Urinary Tract Infections Technology Workshop



On March 11-12, 2019, the NIDDK sponsored a multidisciplinary workshop in Bethesda, Maryland, focused on the development of strategies to improve clinical outcomes among patients having an indwelling urinary catheter.

An indwelling urinary catheter is a thin, hollow tube inserted through the urethra into the urinary bladder to collect and drain urine. Hospitalized patients receive urinary catheters during their hospital stay for indications including voiding management for patients with urethral obstruction, and before and after certain surgical procedures. However, prolonged use of a urinary catheter is a risk factor for developing a catheter-associated urinary tract infection (CAUTI).

CAUTIs are the most common health care-associated infection and are responsible for increased morbidity and mortality (e.g., due to bloodstream infections), excess length of stay, increased cost, and unnecessary antimicrobial use. In addition to implementing clinical best practices

to reduce CAUTIs, research toward development of new catheter technologies is expected to have a significant clinical impact.

The purpose of this workshop was to promote the development of new or improved technologies to reduce the incidence and severity of CAUTIs. As a baseline, the workshop attendees reviewed the state-of-the-science in this research area. The workshop afforded a venue for regulators to provide guidance to innovators on the catheter development and device approval path. Attendees also identified common hurdles, and discussed potential proactive solutions to these difficulties.

Challenges remain, however, including why some patients have bacteria in their urine without symptoms or other signs of an infection, while others progress to CAUTI. Additional research could address this and other issues, such as how to overcome obstacles to conducting clinical investigations in this area.

# STORY OF DISCOVERY

## Engineered Kidney Tissues and Organoids: Tools for Improved Disease Modeling and Development of Therapies

Chronic kidney disease (CKD) is a major public health problem in the United States. The impact of CKD is substantial and includes increased risk of death, diminished quality of life, numerous co-associated diseases and conditions, such as cardiovascular disease, and significantly increased risk of progression to kidney failure (end-stage renal disease). As symptoms are few or non-existent, most people are unaware that they have CKD until most kidney function has been lost. Development of drugs for CKD has been hampered by non-predictive animal models, the inability to identify and prioritize molecular factors in human kidneys that could be targeted with medication, and an underlying poor understanding of human CKD. Therefore, CKD research, as well as research on other kidney-related conditions (e.g., acute kidney injury, drug toxicity, cancer), would benefit greatly from the development of improved laboratory tools to model human kidney structure and function.

Kidneys are highly complex, bean-shaped organs that cleanse metabolic waste products from the blood and maintain proper salt and mineral balance and fluid volume in the body. Each human kidney contains about 1 million individual filtration units, called nephrons, in which blood vessels intertwine with other structures to achieve these remarkable functions. Kidney formation in the developing embryo is a dynamic, orchestrated process; clusters of cells move and interact, and various cells undergo tightly regulated molecular and physical changes that ultimately drive them to mature into functional nephrons. Due to this complexity, for many years, scientists struggled to develop models that accurately recapitulate human kidney structures and function, and the lack of such human kidney models has limited the ability to develop new drugs to treat or prevent CKD. However, due to rapid technological

advances made over the past few years, engineered kidney tissues and organoids—self-organizing, three-dimensional kidney assemblies often derived from adult human stem cells—have emerged as promising tools to accelerate CKD research.

### KIDNEY ORGANOID— A LEAP FORWARD

Over the course of many years, scientists painstakingly defined a number of laboratory conditions under which human pluripotent stem cells (hPSCs)—cells that are able to become any type of cell in the body—could be coaxed to develop into individual cell types found in the nephron. However, researchers faced the challenge of assembling the different kidney cell types together into three-dimensional structures that can recapitulate human kidney functions. Between 2013 and 2015, several research groups reported important technological breakthroughs that defined conditions under which hPSCs could develop into higher-order kidney-like structures. For example, in 2014, scientists in Japan used mouse models to carefully define a series of molecules that act sequentially to induce the stages of normal kidney development over time. The researchers then applied this knowledge from mice to a human system by adding these molecular signals step-wise to hPSCs growing in culture, coaxing the cells to grow in number, aggregate, and mature into organoids that recapitulate complex kidney structures. Other research groups also used a variety of methodologies to identify molecules and processes in normal kidney development that, when applied to cultured hPSCs, improve their ability to self-organize into organoids.

# STORY OF DISCOVERY

Kidneys filter the blood through interactions between very thin blood vessels (capillaries) and specialized kidney cells in the nephron. The incorporation of capillaries in developing kidney structures is a process called “vascularization,” and is essential for kidney function. Therefore, vascularization of kidney organoids in culture is critical, but has been a technological hurdle for researchers. In 2018, a team of scientists, supported in part by NIDDK, made a leap forward when they showed that hPSC-derived kidney organoids, when grown in certain culture conditions and transplanted into mice, could recruit the host mouse’s blood vessels into the organoids’ budding blood vessels. They observed over time that the organoids grew in size, formed critical structures such as filtration membranes between the mouse blood vessels and kidney cells, and developed vascular connections that were fully functional. These findings demonstrated that hPSC-derived organoids exposed to a physiological environment similar to that in the body (in this case, when transplanted into a mouse) are poised to vascularize and mature.

These experiments generated a relatively small number of organoids that could, under specific conditions, mimic kidney structures and functions. But another obstacle to translating this research to wider use in the laboratory, and potentially the clinic, is scalability—the amount of hPSC-derived kidney organoids will have to be increased. To address this need, researchers, supported in part by NIDDK, modified culture conditions to optimize the yield of kidney structures that could be produced in the laboratory. In 2019, they published their finding that certain conditions favored the generation of large quantities of kidney tissue in the form of much smaller organoids, which they term “micro-organoids,” than previous studies had reported. The cellular compositions and maturity levels of the micro-organoids were similar to those of standard organoids, but these laboratory conditions generated 3 to 4 times the total amount of kidney tissue from the same number of starting cells, with a 75 percent reduction in cost.

## MODELING BIOLOGICAL FUNCTIONS BY MOUNTING KIDNEY TISSUES TO PLATFORMS

While the laboratory protocols for growing organoids in culture have made impressive strides in producing tissue structures that resemble the kidney, kidney development and maturation has not been fully replicated under simple culture conditions. As an example, organoids in the previously described study required grafting into mice to achieve vascularization. To more closely replicate the conditions of normal kidney development, researchers have turned to “microfluidic” platforms, which are devices on which kidney cells or organoids can be mounted and exposed to tiny, controllable volumes of liquid. These modifiable platforms, or “chips,” can therefore be used to test various culture conditions with extraordinary precision to identify factors that promote maturation of kidney cells and organoids.

In recent studies in 2018 and 2019, two teams of NIDDK-funded scientists sought to use chips to improve functional models of tubules, which are specific portions of the nephron where various molecules (e.g., water, proteins, salts, sugars) are exchanged between the nephron and surrounding capillaries to achieve proper balance in the body. One research team developed a chip-mounted renal vascular-tubular unit (hRVTU), consisting of a permeable membrane with vascular (capillary) components on one side and tubule cells on the other. Over time, molecules produced by the cells “remodeled” the membrane between the two compartments to closely resemble the normal interface between tubules and capillaries in human kidneys. Moreover, proteins and sugars that normally pass between tubules and capillaries could flow between these two compartments, but others could not; this selectivity revealed the great extent to which the hRVTU could replicate kidney function.

The other group of scientists used “3-D bioprinting” technology to create chips on which three-dimensional tubules and capillaries were



# STORY OF DISCOVERY

“printed” directly adjacent to one another and are embedded in an engineered matrix that resembles the environmental conditions surrounding these structures within nephrons in the body. By measuring the contents within the two compartments, they observed the exchange of molecules between the two structures, demonstrating that the chip modeled normal tubule function. The scientists then exposed the chips to hyperglycemic (excess sugar in the fluid) conditions to model the effects of diabetes on nephron tubules and capillaries. Hyperglycemia led to cellular damage and dysfunction in the capillary and tubular cells, effects that were prevented by treating the chips with a drug that reduces the transport of sugar between the compartments. Thus, the chips proved useful for accurately modeling tubule-capillary dynamics in both normal conditions and immediately following high sugar exposure; future studies are needed to determine whether these chips could be useful for modeling the effects of long-term, chronic high sugar exposure characteristic of diabetes.

While these studies focused on creating chips modeling particular parts of the nephron, other researchers have sought to mount and develop entire organoids on microfluidic platforms. In a recent study published in 2019, NIDDK-supported scientists sought to overcome the barrier of organoid vascularization by reasoning that because developing kidneys normally are exposed to fluid flow, perhaps adding the stress of fluid flow to chips could mimic the natural environment. When the researchers grew chip-mounted kidney organoids in the presence of a high rate of fluid flow, they developed an array of blood vessels with varying diameters; by contrast, organoids exposed to low fluid rate or none at all had far fewer blood vessels. Under high flow conditions, the developing blood vessels successfully transported fluids and even assembled as networks connecting neighboring organoids, demonstrating that they were physiologically mature. (For more details, please see the advance summary earlier in this chapter.)

## ONGOING AND FUTURE RESEARCH EFFORTS

Tools developed through these advances are already proving their utility for accelerating CKD research. For example, one study used hPSC-derived kidney organoids to help overcome a long-standing technological roadblock. Previously, researchers had tried to treat damaged kidney cells with viral gene therapy, but research protocols to deliver genes to appropriate human cells were unsuccessful. NIDDK-supported scientists recently identified a specific subtype of virus that could successfully deliver gene therapy and prevent damage to kidney cells in mice. However, it is well known that many discoveries in mice cannot be translated to humans due to inter-species differences between kidneys. Therefore, the scientists tested the virus in hPSC-derived organoids, determining that the gene delivery vehicle could successfully work in these human cell-derived structures and providing a foundation for potential future therapeutic studies. This study exemplifies how using organoids that faithfully mimic human kidney structures and physiology can help predict drug or other treatment effectiveness and toxicity at a relatively low cost.

NIH and the International Space Station U.S. National Laboratory are currently collaborating on another fascinating use for kidney chips, as well as chips modeling other organs and diseases. Researchers will use the tissue chips in space to study aging and certain disease states that appear to be accelerated in microgravity and then later to test the potential effects of drugs on those tissues. The projects aim to provide insights that will speed the development of treatments for kidney stones, arthritis, and other conditions that affect us here on Earth.

Research to develop engineered kidney tissues and organoids have taken extraordinary leaps over the past few years. As technology continues to improve, these laboratory tools will undoubtedly play central roles in understanding human kidney development, modeling disease, accelerating drug discovery, and catalyzing innovation in renal replacement therapy.

# SCIENTIFIC PRESENTATION

## Dr. Lisa M. Guay-Woodford—Autosomal Recessive Polycystic Kidney Disease: New Insights Reveal Provocative Complexities

*Dr. Lisa M. Guay-Woodford is Professor of Pediatrics at George Washington University (GWU) School of Medicine and Health Science, and the McGehee Joyce Professor of Pediatrics at Children's National Research Institute (CNRI). She serves as the Associate Vice President for Clinical and Translational Research at GWU. Dr. Guay-Woodford leads the Clinical and Translational Science Institute, which is funded by the NIH Clinical and Translational Science Awards program and is a partnership between CNRI and GWU. A graduate of the College of the Holy Cross, she earned her M.D. degree from Harvard Medical School, and then completed pediatric training and a pediatric nephrology fellowship at Boston Children's Hospital.*

*Dr. Guay-Woodford is an internationally recognized pediatric nephrologist with a research program focused on identifying clinical and genetic factors involved in the pathogenesis of inherited kidney disorders, most notably autosomal recessive polycystic kidney disease (ARPKD). Her laboratory has identified disease-causing genes in several experimental models of ARPKD, and her research group participated in the identification of the human ARPKD gene as part of an international consortium. For her contributions to the field, Dr. Guay-Woodford was awarded the Lillian Jean Kaplan International Prize for Advancement in the Understanding of Polycystic Kidney Disease. Dr. Guay-Woodford has established and directed national and international collaborative research groups, as well as assumed numerous elected leadership positions, including: President of the Society for Pediatric Research, Councilor for the International Pediatric Nephrology Association, Chair of the NIH Cellular and Molecular Biology of the Kidney Study Section, Board of Trustee member for the Polycystic Kidney Disease Foundation, and Board member for the Association of Clinical and Translational Science. She currently serves*

*as a member of the National Diabetes and Digestive and Kidney Diseases Advisory Council, and at its May 2019 meeting, Dr. Guay-Woodford presented a lecture on the provocative complexities of ARPKD.*

Polycystic kidney disease (PKD) is a genetic disorder that results in the growth of multiple fluid-filled cysts in the kidneys. ARPKD is a rare and severe form of PKD that usually becomes apparent around the time of birth or in early childhood, and many affected patients do not survive this time period. Ninety-nine percent of people with ARPKD have been shown to have mutations in the *PKHD1* gene; researchers have found a wide variety of different mutations in this gene that are associated with the disease. As with most genes, people have two copies of *PKHD1*, and the disease results when there are mutations in both. ARPKD is characterized by fluid-filled kidney cysts and connective tissue build-up in the liver (i.e., hepatic fibrosis). The greatly enlarged kidneys have reduced kidney function. *PKHD1* mutations affect kidney development starting before birth, and can be detected first in the proximal tubule of the kidney, which reabsorbs water and salts, and then in the kidney's collecting ducts, which form a drainage system that opens into the tubes that carry urine from the kidney to the bladder. The kidney cysts in ARPKD involve dilatation or widening of virtually all of the collecting ducts. Dr. Guay-Woodford's research group has led efforts to understand the origin of ARPKD in order to establish a foundation on which to develop effective treatment strategies for this intractable disease.

The *PKHD1* gene encodes a protein called FPC that sits in the cell membrane. Working with NIDDK Deputy Director Dr. Gregory Germino's laboratory, Dr. Guay-Woodford and her team showed that FPC undergoes a processing event in which one end of the protein,

# SCIENTIFIC PRESENTATION

called the carboxyl-terminus, is cleaved off and travels to the nucleus of the cell, where the DNA is housed.

To better understand the function of FPC, researchers have genetically engineered mouse models for study. As there are hundreds of different strains of mice that differ in their genetic makeup, researchers must take into account that these differences could modify how the gene under study may function. Dr. Guay-Woodford showed results from several mouse models that were generated having different mutations in the mouse *Pkhd1* gene—including mixed-strain mice and inbred mice. Mice with a “mixed” genetic background and two mutated *Pkhd1* gene copies develop cystic kidney disease—primarily in the proximal tubule or collecting duct—as well as liver disease. By contrast, researchers found that an inbred strain of mice with these same mutations does not have kidney disease, but does still have liver disease. A research team led by Dr. Terry Watnick developed a mouse model with a specific mutation in the *Pkhd1* gene that results in the FPC protein missing most of the carboxyl-terminus. These mice did not have kidney or liver disease. Furthermore, when Dr. Germino’s research team completely knocked out the *Pkhd1* gene, resulting in no FPC protein being produced, they found that the mouse model did not have kidney disease but did have liver disease. From these investigations, it is clear that these mutations in *Pkhd1* in mice fail to consistently model human ARPKD.

To further study the disease, Dr. Guay-Woodford and her colleagues analyzed the *PKHD1* gene in 36 Afrikaner families with ARPKD, and found that a majority of them had the same *PKHD1* mutation. As a way to gain further insight into this disease, Dr. Guay-Woodford’s team has generated an analogous mutation in mice, which they are currently studying.

## ATYPICAL KIDNEYS IN PARENTS OF CHILDREN WITH ARPKD

Dr. Guay-Woodford presented findings from another research group that performed ultrasound evaluations on 62 parents from ARPKD families.

Ultrasound identified six parents—who have just one copy of the mutated gene—as having atypical kidney images called medullary echogenicity that resembled cysts—and intriguingly they were all female. Further research is needed to understand how the presence of one copy of the mutated gene may play a role in the development of the kidney cyst lesions in the six female parents.

## KIDNEY CYSTIC DISEASE AND THE C-MYC PROTEIN IN HUMANS AND MOUSE MODELS

While reflecting on the biological pathways that had been identified as important in kidney cyst formation, Dr. Guay-Woodford and her colleagues focused on a protein called c-Myc, which regulates gene activity. Dr. Marie Trudel and other investigators have shown that c-Myc protein levels are increased in patients with another form of PKD, autosomal dominant polycystic kidney disease (ADPKD). ADPKD is the most common form of the disease, and people are usually diagnosed between the ages of 30 and 50. Using different approaches to study c-Myc, research teams led by Dr. Trudel and Dr. Vincent Gattone had discovered that increased levels of this protein correlate with cystic kidney disease in both ARPKD and ADPKD mouse models, and that experimentally decreasing levels of this protein in mice could reduce kidney cysts and improve kidney function. More recently, Dr. Trudel and her colleagues showed that c-Myc regulates a gene called *Pkd*; mutations in this gene cause ADPKD. Dr. Guay-Woodford concluded her presentation by suggesting that c-Myc could be part of a molecular mechanism for PKD susceptibility, which could be targeted for the development of drugs to attenuate the disease in people.

# PERSONAL PERSPECTIVES

## SHAREing Their Experiences with Bladder and Urinary Tract Health: Five Women Talk About Their Participation in the PLUS Research Consortium

At some point in their lives, the majority of women will face at least one health issue having to do with the bladder and/or urination. Such issues range from acute infections to chronic, sometimes painful conditions. These urologic health challenges can have far-reaching negative effects on a woman's health and well-being. NIDDK-supported scientists with the Prevention of Lower Urinary Tract Symptoms (PLUS) Research Consortium have embarked upon a journey to improve both bladder health and overall health for women, with the help of hundreds of research volunteers across the country—including five women featured here who discussed their experiences participating in a foundational study called SHARE (see insets).

### BLADDER ISSUES: FROM HEALTH TO HEALTH BURDEN

Urinating is a way to rid waste products of daily metabolism that would otherwise build up in the bloodstream. These toxic substances, once filtered from the blood, are sent to the bladder for storage in the form of urine. Periodically, urine is voided from the bladder out of the body through a tube-like structure called the urethra.

However, many problems can affect the bladder and urethra, resulting in symptoms that disrupt normal voiding and overall health. These problems, such as urinary incontinence (UI), urinary tract infections (UTIs), overactive bladder (OAB), and interstitial cystitis/bladder pain syndrome (IC/BPS), occur much more frequently in women than in men. Bothersome lower urinary tract symptoms, or LUTS, can also be caused by behaviors such as drinking too much

fluid. Whatever the cause, LUTS can exacerbate or contribute to other chronic health problems in women,



### ADINA

With over 26 years of experience under her belt, 53-year-old Adina is in contact with people in her community every day as a workforce development specialist focused on helping

students prepare for employment. Outside of work, Adina spends time with her fiancée and young adult daughter and son, enjoying dancing, bike riding, and reading. Adina decided to join a SHARE focus group because she has a close relative with bladder health issues, and she also wanted to know more about these issues herself as she is getting older. In describing her experience, Adina observes that her group was very enthusiastic and supportive of women's health, and that she feels the organizers made the setting safe and comfortable to "ask uncomfortable questions" and "share openly, without being judged." Adina says she is glad to have participated in SHARE because when she thinks about bladder health she feels "people really don't talk about that" and are less aware of it compared to other medical conditions. "I don't come across a lot of advertisement or marketing for specifically bladder issues," she notes, adding that, especially in light of her relative's bladder problems, she is very eager to know "is there something I can do to be proactive and to prevent this?" Ultimately, Adina feels her SHARE group was both "wonderful" and "important."

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including obesity, diabetes, and depression, by creating barriers to engaging in physical and social activities (e.g., fear of embarrassment, risk of leaking urine, and need to maintain easy access to bathrooms).

Up to this point, almost all research on bladder conditions has been aimed at determining their causes and testing treatments. An understanding of healthy bladder function across the lifespan is still somewhat elusive. Such an understanding would help scientists and clinicians set goals for research to promote bladder health and develop prevention as well as improved treatment strategies for different lower urinary tract problems and symptoms.

## THE PREVENTION OF LOWER URINARY TRACT SYMPTOMS (PLUS) RESEARCH CONSORTIUM

In 2015, the NIDDK, in collaboration with other NIH components, established the PLUS Research Consortium. The overarching goal of this Consortium is to establish the scientific basis for future intervention studies that can promote bladder health and prevent LUTS and associated bladder conditions in girls and women.

PLUS is using several strategies to accomplish its mission: a transdisciplinary approach (i.e., one that harnesses multiple research disciplines to tackle important, complex questions); a research structure and research techniques to study not just biological factors, but also mental health, social and behavioral factors, and the impact of many other potentially important factors in a woman's "environment" (e.g., home life, school, and work) that may contribute to bladder health; and the inclusion of the experiences and perspectives of adolescents and women.

PLUS investigators decided early on that defining and measuring the state of bladder health was a necessary component of the evidence for future prevention efforts. In one step toward reaching this objective, PLUS recently developed and published a novel, multi-faceted research definition of bladder health

that can inform approaches for evaluation of bladder health promotion and prevention of LUTS both in research and in public health initiatives. Informed by the World Health Organization's definition of health, the PLUS Consortium defined bladder health as "a complete state of physical, mental, and social well-being related to bladder function, and not merely the absence of LUTS. Healthy bladder function permits daily activities, adapts to short-term physical or environmental stressors, and allows optimal well-



### ROXANNE

A self-described "Midwestern gal all around" whose attitude is to "keep moving and grooving" despite serious health challenges, 60-year-old Roxanne is a retired social worker who directed projects for

several different urban, community-based programs, including one that promoted career mentoring for high school girls. She participates in clinical studies regularly, especially those focused on women's health, as a way to "meet people ... and to give something back," as well as to benefit her health as she gets older. SHARE was of interest to Roxanne because of this drive and her own experience with a bladder condition. Describing her SHARE focus group experience, she recalls that a key thing she took away from it was validation—"it's not just me." Roxanne also participated in a second SHARE focus group, which brought together one participant from each of the focus groups at her research center. Recalling that group, she remembers sharing both information and personal stories, such as triggers that affect her bladder—"every time I go to the grocery store I have to pee ... it's like a cue in my brain!" she says. When asked if she would participate in a similar focus group and whether she would recommend it to other women, Roxanne's response is a simple one: a resounding "Yes!"



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being (e.g., travel, exercise, social, occupational, or other activities).” In a related effort, PLUS scientists analyzed comprehensive data on LUTS and LUTS-specific interference with physical and social activities from an existing community-based study. As a result, they estimate that only about one in five women ages 30 to 80 years old has optimal bladder health—underscoring the need for LUTS prevention and bladder health promotion.

Additionally, PLUS scientists have shed light on suspected associations between occupation, industry, and work environment and the risk of LUTS in women. They found that there was insufficient data from past studies to evaluate LUTS by occupation types. This represents a profound gap in knowledge and indicates that future studies should characterize factors such as voiding frequency and toilet access in a consistent manner by occupation and explore their relationships to LUTS development.

## PAVING THE WAY TO PREVENTION: PLUS CLINICAL STUDIES

To obtain the evidence base for intervention studies to prevent LUTS and promote bladder health, PLUS scientists identified the need for a nationally representative long-term study in women and adolescent girls—one that would determine the distribution of bladder health conditions and expand our understanding of factors that promote bladder health or contribute to bladder conditions. As a result of its novel and visionary approach, PLUS developed a multi-pronged research strategy to lay the foundation for such a study. This strategy included talking to adolescent and adult women; scientific literature reviews; analyses of existing databases; and identification, development, and validation of an array of items (questions) to obtain measurable information about a person’s bladder health and about novel factors that contribute to bladder conditions, such as how much adolescent and adult women know about their bladders. Key studies that depended upon human volunteers are known by the acronyms SHARE, CLEAR, and VIEW.

**SHARE:** Every journey begins with a step, and in the case of the PLUS, a key first step was the Study of Habits, Attitudes, Realities and Experiences, or SHARE.

From its inception, PLUS made inclusion of the voices of women who are not researchers or clinicians a priority. Using principles of community engagement, PLUS quickly developed a plan for gaining broader community input on each step of the process in developing a definition of bladder health and the elements of a research method (tool) to measure bladder health. This plan resulted in SHARE.

## MAXINE

A nurse for over 40 years, 74-year-old Maxine is retired now but still attends classes to keep up her license and stay current on what is happening in nursing, research, and patient care—particularly the impact of electronic health records, which she finds very exciting. She also spends a great deal of time focused on her family, especially her three great-grandchildren. Maxine’s reasons for joining a SHARE group included past bladder issues of her own and experience teaching students about bowel and bladder issues during one phase of her nursing career. In describing her SHARE experience, Maxine recalls the group being asked about the influence of TV and other media on thinking and decisions affecting bladder health, such as products they might buy, or treatments they might request from their physicians—the topic “sparked a pretty interesting conversation,” she says. Maxine also recalls the group discussing whether their mothers influenced their perception of bladder issues and how to talk about these conditions. This question resonated with Maxine as she recalled her own early life experiences and also brought up “responses [that] were interesting to me” from the other women, she says. The SHARE group was the first medically oriented focus group Maxine had participated in, and she says she would “definitely” recommend participation in such a group to others. “I’m looking forward to seeing the results of the entire study,” she adds.

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A large, multi-center, focus-group based, qualitative study, SHARE was conducted in 2017 to understand how women conceive of and talk about their bladders and bladder health. PLUS held 44 focus groups, with more than 300 women and girls 11 to 80 years of age from racially, ethnically, and sociodemographically diverse backgrounds. Many participants had bladder health issues, while others did not. This differentiated SHARE from earlier studies of this kind, which focused on people with particular bladder conditions. Participants met in groups of approximately 5 to 10 persons of similar ages, together with a discussion facilitator and a study coordinator from the local PLUS study site.

Although participants were provided some basic information about the bladder, the focus groups were not meant to be classes—rather, they were forums for women and girls to talk about the bladder and bladder problems, how they monitor their own bladder behavior, what bladder health “looks like” to them, their experiences with navigating toilet access in public spaces such as workplaces and schools, and a variety of other topics. For many participants, the focus groups represented a venue in which to discuss issues or problems that are often swept under the rug due to embarrassment or cultural norms. Five of the participating women from sites across the country discussed their experiences in their respective SHARE focus groups for this feature (see insets).

PLUS scientists are in the process of interpreting the findings and publishing the results from the SHARE study. The information garnered from SHARE participants has been instrumental to the development of a multi-component measure of bladder health that will ultimately be used in the long-term observational study, and is informing other PLUS activities as well. For example, the focus groups provided key insights into the terms and language used by a very diverse group of women and girls to discuss bladder health and issues, as well as the emotional and social aspects of such discussions. Also, because the participants were grouped by age, how women’s experiences and perspectives differed across the lifespan could be more easily explored, analyzed, and incorporated into

PLUS research efforts. Without SHARE, many of the other studies developed by PLUS would not have been possible.

**CLEAR and VIEW:** In preparation for its large-scale study, PLUS also needed to work methodically to identify the factors most likely to influence risk for or protection from bladder problems; develop ways to measure knowledge, attitudes, and beliefs about bladder function and health; and ultimately to draft research questions for the study. To put the draft questions through an initial test for usability, PLUS developed the Clarification of Language, Evaluation And Refinement, or CLEAR, study—a way to determine whether the questions are easily



## LAURA

Between working toward a Ph.D. in clinical psychology, running, and taking time to read and bake with her young daughter, 30-year-old Laura is super busy.

However, she was very excited to take time to participate in the SHARE focus group because of her general interest in women’s health and because of her own specific bladder health challenge—a diagnosis of interstitial cystitis when she was 19 years old, which she happily says is currently under control with medication. In talking about her SHARE experience, Laura notes that there was a point when the discussion turned to whether participants felt like bladder health was common knowledge, and that “I just remember being, like, yeah, we don’t think about women’s bladder health, and ... that struck me.” When asked what she thought was the best part of being in SHARE, Laura says that “considering that I’ve been struggling with this since I was a pre-teen, to have a group that focused on bladder health felt almost empowering.” Also, Laura notes she is aware that research is being done on bladder health, and as she puts it, “to be part of it felt encouraging to me.”

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understood or need to be further refined. Candidate questions for a “Bladder Health Instrument,” will undergo full validation in the Validation of Bladder Health Instrument for Evaluation in Women, or VIEW, study. Validated and age- and culturally-appropriate instruments will be developed for both English- and Spanish-speaking women. The validation process will reduce the number of items needed to determine the state of bladder health and the final instrument will be known as the Bladder Health Scale. PLUS researchers will then use the Bladder Health Scale to obtain the main results about bladder health and dysfunction in the planned long-term study in women and girls.

## GOING FORWARD IN PLUS

The PLUS Research Consortium is expected to enter its second 5-year phase later in 2020, during which recruitment of participants for the national, long-term study will begin. It is anticipated that this study will determine the state of bladder health in adolescent and adult women in the United States and monitor changes in bladder health over time. These assessments, together with additional medical

and other data gathered from a subset of study volunteers, will also expand the understanding of risk and protective factors to identify targets for future intervention studies.

Because of the suspected influence of access to toilets and the toilet environment on lower urinary tract symptoms, PLUS has also been developing a smartphone app called “Where I Go” to capture real time assessment of toilet access, safety, and cleanliness, and decision-making about when and where to go. PLUS researchers anticipate using this app in the long-term study. Finally, in anticipation of future efforts to promote bladder health and prevention strategies, PLUS will work on expanding its community engagement strategy to facilitate community-based participatory intervention and implementation research.

In all of its efforts, PLUS will continue to depend upon the individuals who contribute their time and experience to participate in clinical research that could benefit women and girls across the Nation and potentially even around the world.

*For more information, visit the PLUS website:*  
<https://plusconsortium.umn.edu/>.



## CINDI

A marketing professional for over 25 years, 49-year-old Cindi spends her time outside of work focused on the arts, music, volunteering, socializing with friends, and exercise—especially highly aerobic aqua exercise. She also has been managing bladder challenges since her youth, and thus was especially interested in the SHARE focus group. Cindi, who contends with other chronic health conditions as well, notes that her current bladder issues have led her to avoid some activities, such as long road trips where she might not be able to get to a bathroom easily. Cindi also says bluntly that she’s “happy that I’m not

having major accidents, but it is embarrassing—I feel like I can’t go very long without having to go to the bathroom.” When asked what part of SHARE stuck with her most, Cindi notes that the diversity of perspectives, norms, habits, and personal experiences the women described was “incredibly eye opening.” She adds that her SHARE group experience “sort of normalized” her situation for her—recalling the stories she heard in the group, Cindi observes that, “What I’m dealing with is hard, but what everybody is dealing with is hard ... it made me feel like at least I’m not the only one out there who’s struggling.” In addition to spurring her to follow up with a specialist for her own bladder health, Cindi says that she felt SHARE was “a safe place” and “a positive experience.”



# NIDDK Extramural Funding Trends and Support of Guiding Principles

*The NIDDK's guiding principles toward achieving its core mission include maintaining a vigorous investigator-initiated R01 research portfolio, supporting pivotal clinical studies and trials, promoting a steady and diverse pool of talented new investigators, and fostering exceptional research training and mentoring opportunities consistent with the vision of the NIDDK Director, Dr. Griffin P. Rodgers (see Message from the Director). To highlight its dedication to these principles, the NIDDK generates data and analyses of application and funding trends.*

## Maintaining Investigator-initiated Research

The NIDDK is committed to maintaining a strong investigator-initiated R01 research program, while also allowing appropriate flexibility for other priorities, such as training and career development of the next generation of scientists; infrastructure; key initiatives, including large clinical trials and consortia; and other efforts informed by input from various sources, including the scientific community. Historically, investigator-initiated research has been—and continues to be—the Institute's highest priority because it fosters ground-breaking and innovative research.

## Supporting Clinical Studies and Trials

The NIDDK commits a substantial proportion of its research funding to the support of clinical studies, from preliminary clinical feasibility studies to large multi-center studies. As part of this clinical research effort, the NIDDK seeks to ensure substantial representation and participation of women, minorities, and other historically underrepresented populations. The Institute also continues to expand investigator access to these valuable research resources through ancillary studies and a central repository of data and samples.

## Promoting a Steady and Diverse Pool of New Investigators

The NIDDK creates opportunities to help new investigators advance scientific discovery in their own laboratories. Each year, the NIDDK sets a more generous payline for Early Stage Investigators (ESIs) with the aim of enhancing ESI representation among new grant awardees. (ESIs are new investigators who completed their terminal research degree or medical residency within the past 10 years, and who have not yet competed successfully for a substantial, competing NIH research grant.)

## Fostering Research Training and Mentoring

The NIDDK also continues to foster the growth of a diverse biomedical research workforce by providing training and mentoring opportunities for talented young investigators, including the NIDDK's fellowship (F), career development (K), and training (T) awards. These and other NIDDK-supported opportunities aim to promote a diverse research pipeline.



In support of these guiding principles, the NIDDK funds a wide range of research awards across its extramural research portfolio, including investigator-initiated grants (R01s/R37s); other R grants (e.g., SBIR/STTR and many other types of grants); awards made in response to initiatives such as Requests For Applications (RFAs), including most of the Institute's large clinical trials and consortia; collaborative grants (e.g., P01s); centers; career development awards; training grants; contracts and interagency agreements (e.g., some large clinical studies); and other research.

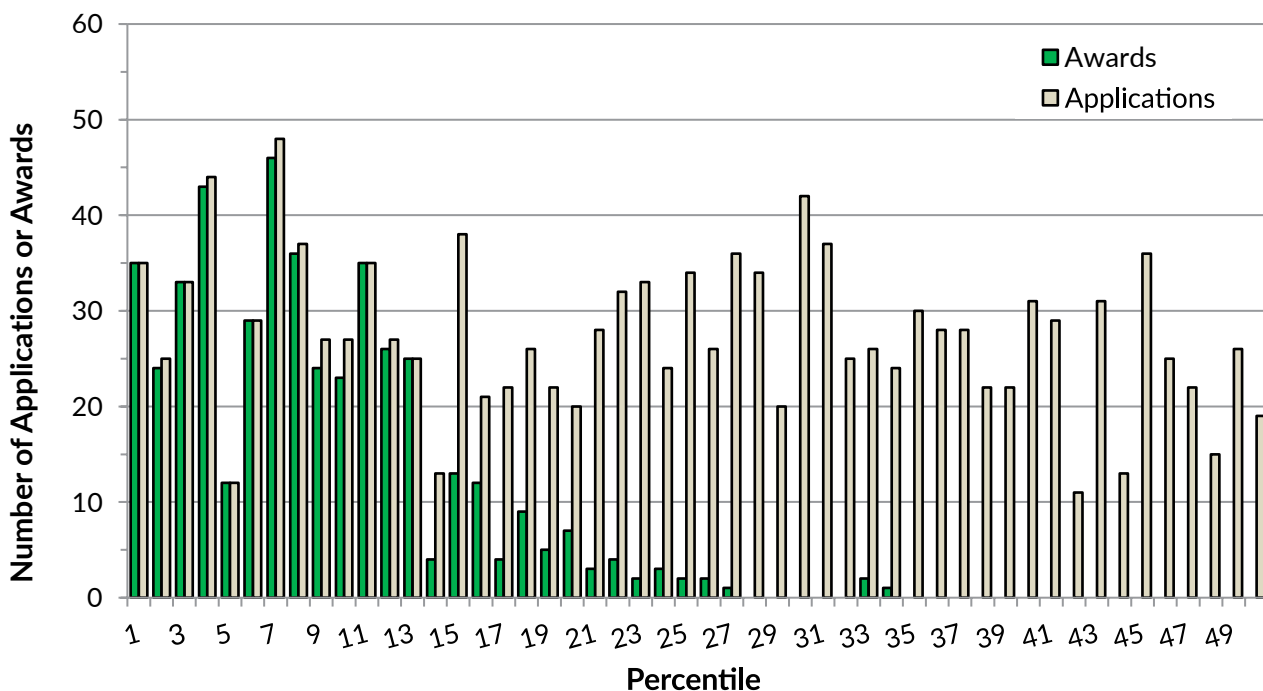
The data that the NIDDK generates on application and funding trends help the research community understand application and funding dynamics over recent years and demonstrate the NIDDK's commitment to research and programs associated with the guiding principles. The Institute posts these data on its public website and updates them

annually. The figures in this section were recently updated to include Fiscal Year (FY) 2019 and present some key examples from the posted data on funding trends that demonstrate the NIDDK's resource focus in each of these areas.<sup>1</sup> Please see the NIDDK website for additional funding trends analyses: <https://www.niddk.nih.gov/research-funding/funded-grants-grant-history/funding-trends-support-core-values>.

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<sup>1</sup> With the exception of the figures titled "NIDDK Extramural Research Funded in FYs 2010-2019, by Funding Mechanism" and "NIDDK Research Project Grants Funded in FYs 2010-2019, by Activity Code" (which both include initiative data), the data in all charts exclude initiatives (i.e., Requests for Applications, or RFAs) and funds appropriated through the American Recovery and Reinvestment Act (ARRA). Figures do not include grants funded through the Special Statutory Funding Program for Type 1 Diabetes Research.

## NUMBER OF NIDDK PAID AND UNPAID R01 APPLICATIONS IN FY 2019, BY PERCENTILE

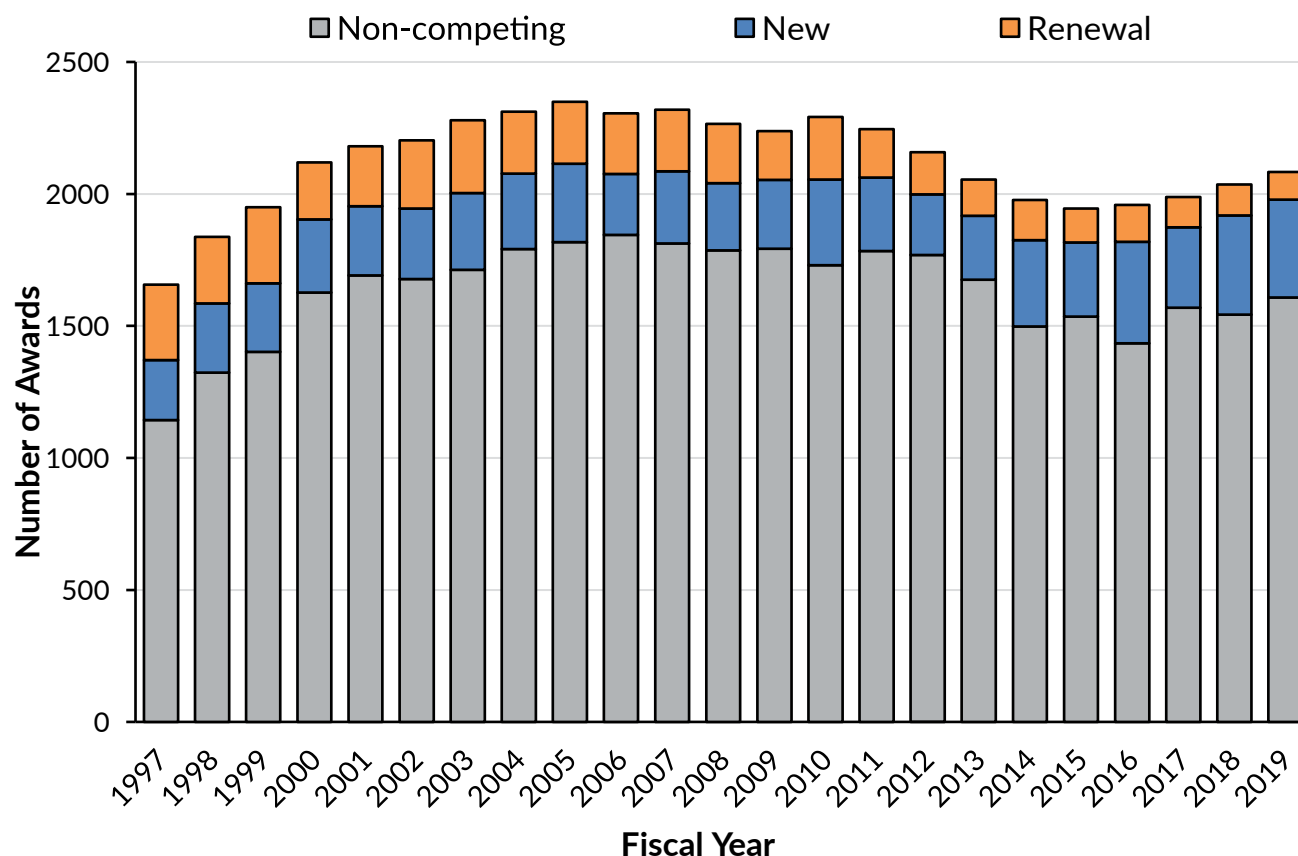


“Applications” shown in the chart above include all R01 applications that scored 50<sup>th</sup> percentile or better. Unsourced applications, scored applications with no percentiles, and applications scoring above the 50<sup>th</sup> percentile are not shown. No unsourced applications were funded in FY 2019.

The NIDDK general payline in FY 2019 for most R01 applications was the 13<sup>th</sup> percentile for established investigators and the 18<sup>th</sup> percentile for Early Stage Investigators (ESIs). The payline and additional programmatic scrutiny for R01 applications requesting \$500,000 or more in direct costs are substantially more stringent. These data show that the NIDDK adheres closely to its payline but does exercise discretion to include a limited number of programatically important applications beyond the payline. The R01 paylines for FY 2010 through FY 2019 are shown in the table.

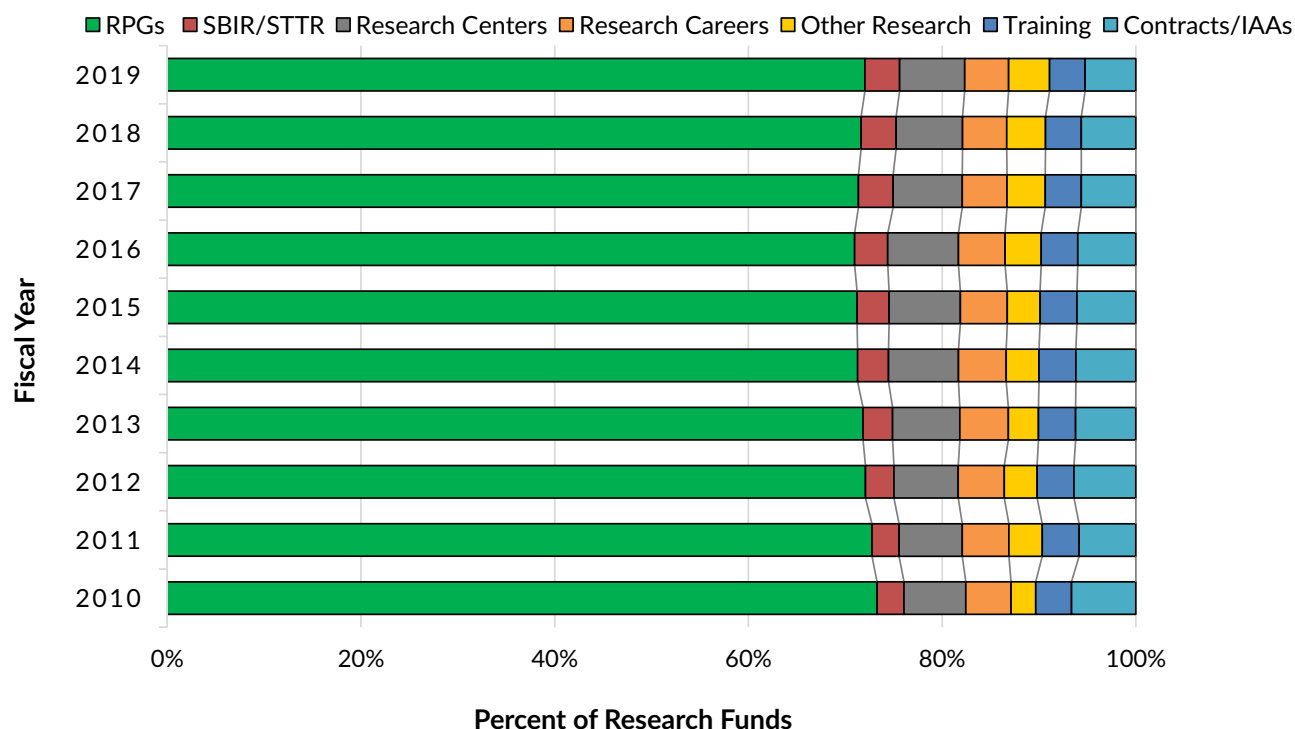
Fiscal Year	General Payline	≥\$500K Payline	New Investigator Payline	Early Stage Investigator (ESI) Payline	ESI First Competitive Renewal Payline
2010	17	11	19		
2011	15	10	17		
2012	13	9	13	18	
2013	11	7	11	16	
2014	13	8	13	18	
2015	13	8	13	18	15
2016	13	8	13	18	15
2017	12	7	12	17	15
2018	13	8	13	18	16
2019	13	8	13	18	16

## NUMBER OF NIDDK R01 AND R37 GRANTS (COMPETING AND NON-COMPETING) FUNDED IN FYs 1997-2019



During the doubling of the NIH budget (FYs 1998-2003), the total number of R01/R37 grants funded by the NIDDK increased significantly. After leveling off following the doubling, the number of R01/R37 grants funded by the Institute from FY 2010 to FY 2015 declined. From FY 2016 to FY 2019, there has been a slight but steady increase in the number of NIDDK-supported R01/R37 awards. Prior to FY 2009, approximately half of the competing grants funded by the NIDDK were new (Type 1) awards in most FYs. However, in the last 10 years, the majority of competing awards were new: in FY 2019, 78 percent of competing NIDDK R01/R37 awards were new.

## NIDDK EXTRAMURAL RESEARCH FUNDED IN FYs 2010-2019, BY FUNDING MECHANISM

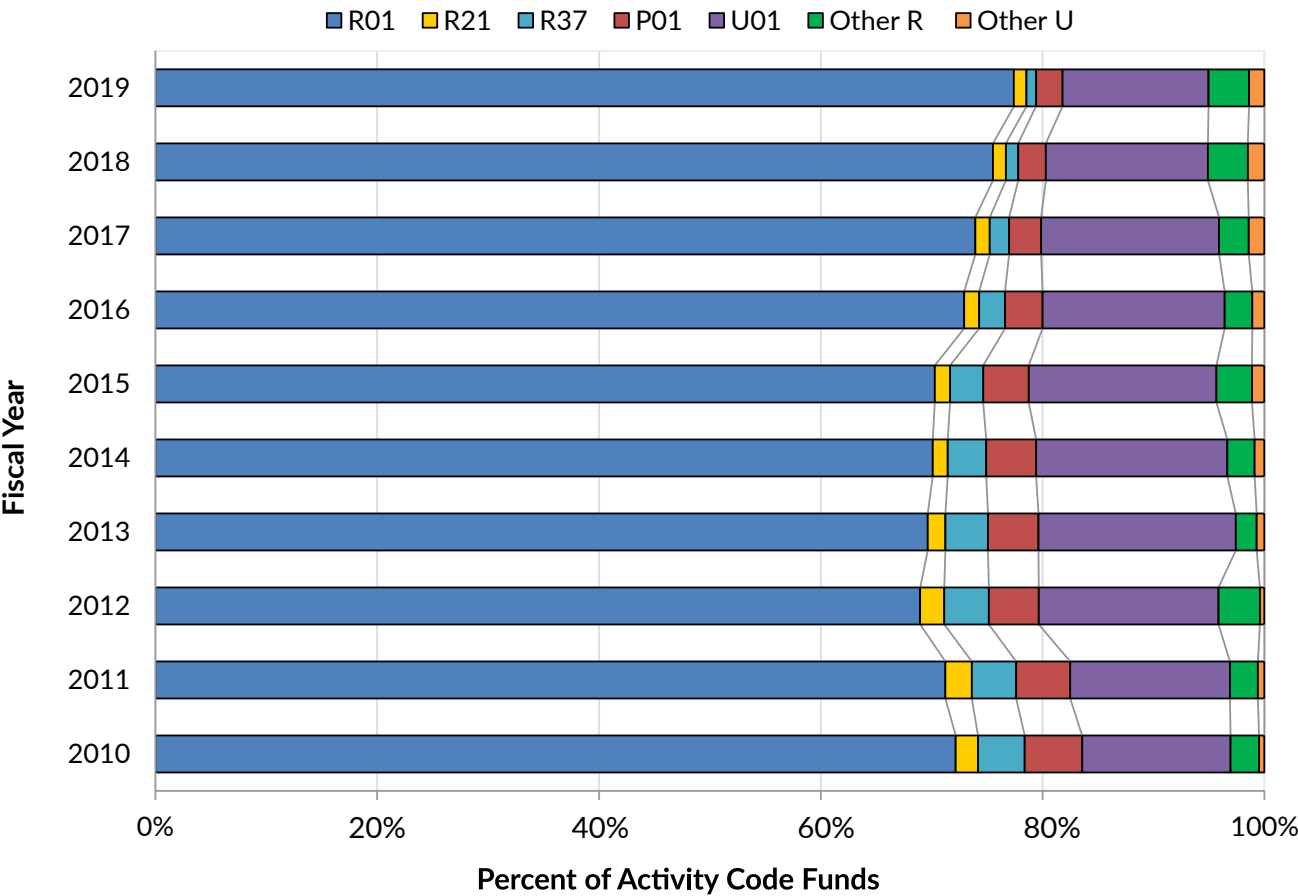


This figure shows that relative funding levels of most NIDDK extramural research funding mechanisms have remained fairly stable since FY 2010. The majority of the NIDDK's extramural research funding awards are Research Project Grants (RPGs), of which the largest portion goes to investigator-initiated research awards, particularly R01 grants.

### NIDDK Funding Mechanisms:

- **RPGs** – Research Project Grants, including investigator-initiated R01, R03, R15, R21, and R56, as well as U01 and P01 awards
- **SBIR/STTR** – Small Business Innovation Research/Small Business Technology Transfer, including R41, R42, R43, and R44 awards
- **Research Centers** – Includes P20, P30, P50, U42, and U54 awards
- **Research Careers** – Includes all K awards (including K99/R00 awards)
- **Other Research** – Everything not captured in other mechanisms, including R13, R18, R24, R25, U24, and U2C awards
- **Training** – Includes all F and T activities
- **Contract and Interagency Agreements (IAAs)** – Includes some large clinical studies

NIDDK RESEARCH PROJECT GRANTS FUNDED IN FYs 2010-2019, BY ACTIVITY CODE



This figure shows the NIDDK Research Project Grants (RPGs) category from the previous figure, broken down by activity code. The figure illustrates that the relative funding levels of most of these subcategories of awards have remained fairly stable since FY 2010.

**NIDDK Research Project Grant Activity Codes:**

- **R01** – The most common type of Research Project Grant funded by the NIH
- **R21** – Exploratory/Developmental Research Grant
- **R37** – Method to Extend Research in Time (MERIT) Grant
- **P01** – Research Program Project Grant
- **U01** – Research Project Cooperative Agreement
- **Other R in RPGs** – Includes R00, R03, R15, R34, R56, RC2, and DP grants
- **Other U in RPGs** – Includes U34, UG3, UH3, and UM1 grants







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### Writing and Production

#### *Overall Production*

Megan Singh, Ph.D., Office of Scientific Program and Policy Analysis

Mary Hanlon-Tilghman, Ph.D., Office of Scientific Program and Policy Analysis

#### *Highlights of Research Advances and Opportunities*

#### *Staff of the NIDDK Office of Scientific Program and Policy Analysis*

Heather Rieff, Ph.D., Director

Dung Pham

Lisa Gansheroff, Ph.D., Deputy Director

B. Tibor Roberts, Ph.D.

Rebecca Cerio, Ph.D.

Megan Singh, Ph.D.

Sandeep Dayal, Ph.D.

Jaime J. Smith, Ph.D.

Patrick Donohue, Ph.D.

Robert Tilghman, Ph.D.

Mary Hanlon-Tilghman, Ph.D.

Julie A. Wallace, Ph.D.

Eleanor Hoff, Ph.D.

*Input from staff within the NIDDK's scientific program and research divisions: the Division of Diabetes, Endocrinology, and Metabolic Diseases; the Division of Digestive Diseases and Nutrition; the Division of Kidney, Urologic, and Hematologic Diseases; and the Division of Intramural Research.*

#### *Contributions from the NIDDK Office of Communications and Public Liaison*

Alyssa M. Voss, M.P.H.

Elizabeth Waibel

#### *NIDDK Extramural Funding Trends and Support of Guiding Principles*

#### *NIDDK Division of Extramural Activities (DEA), Office of Research Evaluation and Operations (OREO)*

Karl Malik, Ph.D., Director (DEA)

Jennie Larkin, Ph.D., Director (OREO)

Teresa Lindquist, M.S. (OREO)

#### *NIDDK Office of Financial Management and Analysis*

Van Nguyen, B.Acc., Budget Officer

Michelle Shorter, B.Acc., Deputy Budget Officer

Mimi Bishop, B.S., Budget Analyst

For more information on the NIDDK and its mission, and links to other sites, visit: [www.niddk.nih.gov](http://www.niddk.nih.gov).



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