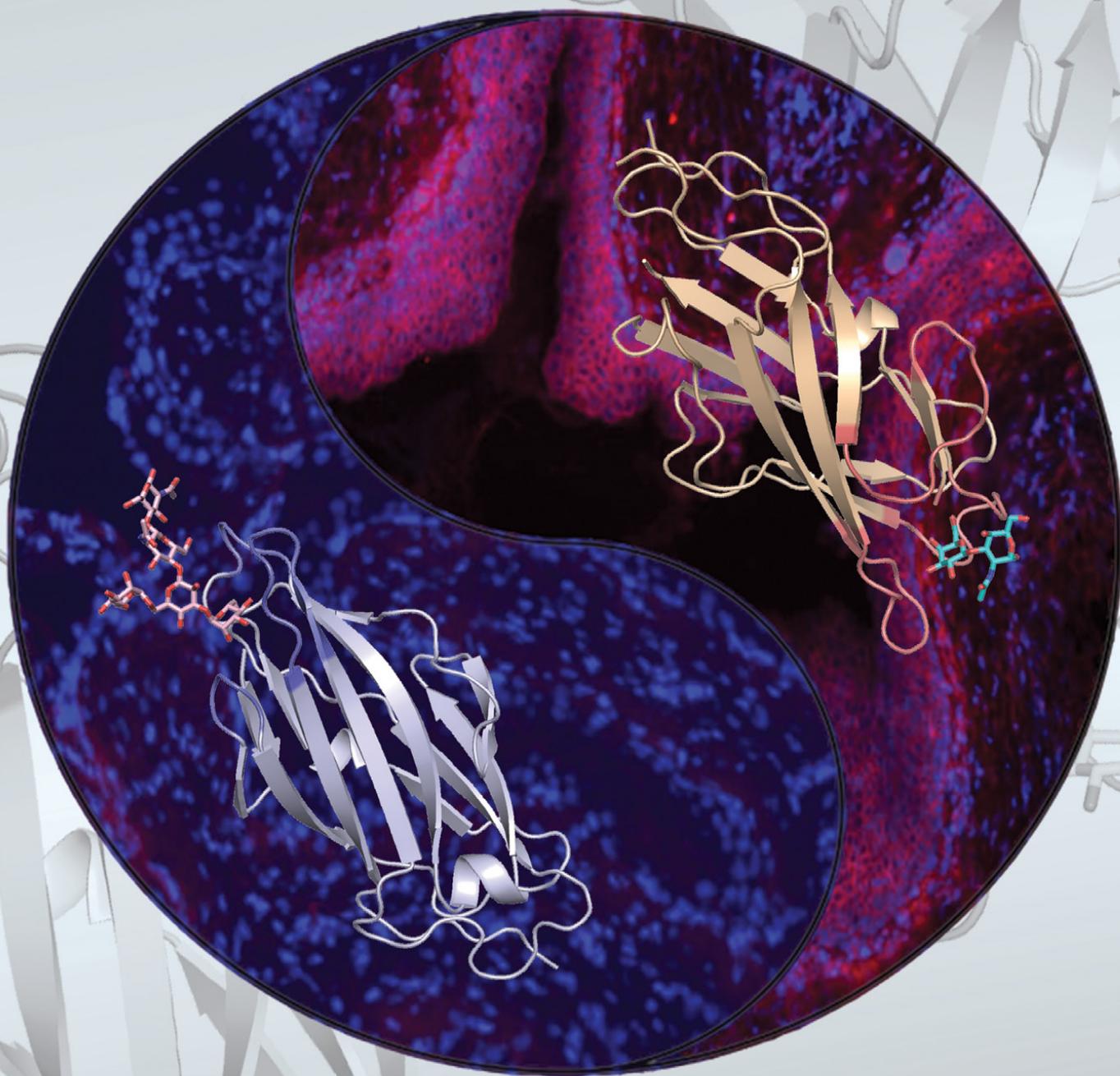


# NIDDK

## Recent Advances & Emerging Opportunities

January 2018



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

Cover caption: In the battles waged between the body and dangerous microbes that can lead to chronic or recurrent disease, each seeks to exploit potential vulnerabilities in the other to survive. As we learn more about the dynamic interactions between host cells and tissues and invading pathogens, this knowledge can be leveraged to improve human health. One such discovery is summarized in the illustration on the cover and described in more detail in this publication: Uropathogenic Escherichia coli bacteria, or UPEC, are the most common cause of human urinary tract infections (UTIs). These bacteria use pili (singular=pilus)—protein fibers that are tipped with adhesive domains—to bind to specific receptor molecules in the bladder lining and facilitate invasion of bladder cells. On the cover, bacterial pilus adhesive domains (“adhesins”) are depicted as folded ribbons, and bladder receptor molecules are depicted as multi-colored skeletal stick models; these are shown against a background of microscopy images of mouse bladder lining, in blue (left) or purple (right) as described further below. NIDDK-supported scientists have now found, through a study in mice, that UPEC employ two different pilus-adhesin combinations in a clever scheme to bolster bladder infection. They found that whereas one pilus adhesin (FimH, shown in silver) enables UPEC to adhere to receptors in a healthy bladder lining in mice (blue), the bacteria subsequently deploy a second type of pilus with a different adhesin (FmlH, shown in gold) that adheres to receptors in infected, inflamed bladder lining (purple), providing an advantage in establishing chronic infection. Antibiotic resistance is on the rise in UPEC, and these new insights into host and microbial interactions point to a potential new therapeutic target for human UTIs. The NIDDK continues vigorous support for basic, clinical, and translational research studies that can tip the balance into our favor and pave the way to healthier lives.

Images courtesy of Professor Han Remaut, PhD, VIB and Vrije Universiteit Brussel, Brussel, Belgium; and Scott Hultgren, Ph.D., Director of Center for Women’s Infectious Disease Research, Department of Molecular Microbiology, Washington University Medical School. Reprinted from Cell Host Microbe 20, Conover MS, Ruer S, Taganna J, Kalas V, De Greve H, Pinkner JS, Dodson KW, Remaut H, Hultgren SJ. Inflammation-Induced Adhesin-Receptor Interaction Provides a Fitness Advantage to Uropathogenic E. coli during Chronic Infection, Page 482, Copyright 2016, with permission from Elsevier.

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National Institutes of Health  
National Institute of Diabetes & Digestive & Kidney Diseases

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# 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 and obesity; kidney diseases, such as polycystic kidney disease; urologic diseases and conditions, such as interstitial cystitis/bladder pain syndrome and prostatitis; and hematologic diseases.

The 18th edition of this report illustrates recent NIDDK-supported scientific advances, such as:

- The discovery that youth with type 2 diabetes develop complications more often than peers with type 1 diabetes
- Identification of a critical role for an enzyme in the complex genetic disorder Prader-Willi Syndrome
- The development of a personalized medicine treatment plan following the discovery of a rare genetic mutation that causes a blood disease
- The finding that infection with a common virus, called a reovirus, may trigger celiac disease in people who are genetically susceptible to developing the disorder
- Optimizing screening schedules for an eye disease, retinopathy, in people with type 1 diabetes
- Insights into the kidney's role in salt and water balance and blood pressure regulation
- Multiple revelations from the first U.S.-based, long-term, observational study of gastric bypass in adults, including durability of weight loss and a reduction, by more than 90 percent, of new-onset type 2 diabetes
- The discovery that accumulation of a type of immune cell in the brain causes an inflammatory response that can lead to excess food intake and obesity in mice
- New insights into prediction and patterns of pain that could help pave the way to personalized care for people with urologic chronic pelvic pain syndromes

- Improved understanding of the potentially life-threatening outcomes from drug-induced liver injury and which racial/ethnic groups are at risk of suffering worse outcomes
- The development of a new method for calculating the average rate at which a single kidney nephron can filter blood—an important measure of kidney health
- Innovations in testing for a rare genetic disorder, Pompe Disease

In addition to reporting on recent advances, this publication traces the multi-step path to research achievements through three “Stories of Discovery.” These essays illustrate how major new discoveries that have greatly advanced biomedical science and are benefitting human health often emerge from many 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 teenager at high risk of developing type 1 diabetes describes her passion for participating in a clinical trial to test a strategy for stopping the disease progression at its earliest stage. An educator with type 2 diabetes gives an account of her longstanding participation in the largest, most diverse study of diabetes among U.S. youth ever conducted. A man shares his decade-long struggle with urinary urgency and describes his subsequent participation in a clinical trial to help researchers advance understanding of lower urinary tract dysfunction. A woman tells her story of living with debilitating gastroparesis, and how participating in a national gastroparesis registry gives her hope for the future.

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)

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
- Preserve a stable 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 Core Values” section at the end of this report and on our website at [www.niddk.nih.gov](http://www.niddk.nih.gov)

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.

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



**The NIDDK Information Network—dkNET—was launched in 2014 to promote sharing of information within and among NIDDK’s research communities. dkNET’s goal is to ensure that data and resources generated from NIDDK-supported research can easily be found, used, and re-used to answer future scientific questions. dkNET accomplishes this by being a search engine for many data repositories and for sources of cells, mouse models, and other research tools. For more information about dkNET, see the feature in this chapter.**

# 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.*

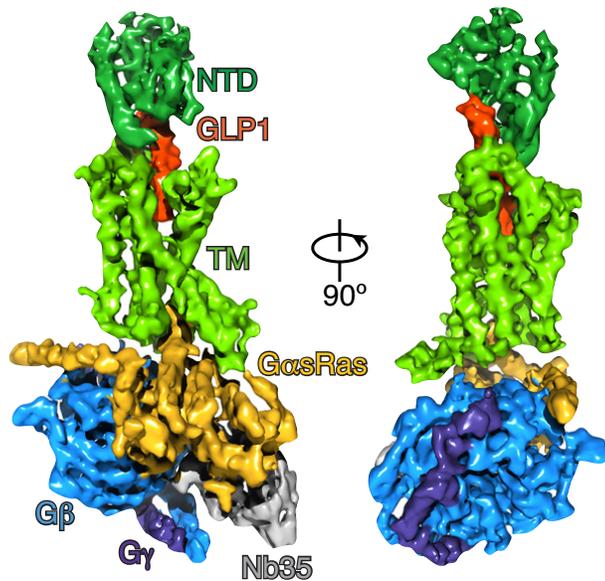
*With the development of innovative scientific technologies and the emergence of new scientific disciplines as talented and creative research teams join together to tackle ever more complex challenges, new opportunities to make exciting discoveries arise each day. The insights gained through this research can be expected to further scientific progress in many research areas, for today's discoveries may hold the seeds of tomorrow's cures.*

This chapter provides a few examples of the Institute's commitment to basic and applied research relevant across a broad spectrum of scientific disciplines. For example, features in this chapter highlight: the research of NIDDK-funded investigators who have won the distinguished Presidential Early Career Award for Scientists and Engineers; a workshop on the emerging role of branched chain amino acids in human diseases; and an exciting set of research resources available to the scientific community through the NIDDK Information Network (dkNET).

## FIRST STRUCTURE OF AN IMPORTANT CLASS OF MEMBRANE PROTEINS

**Painting a Portrait of a Trio of Proteins Important in Health: A G-protein-coupled Receptor and Its Molecular Partners:** Using a cutting-edge technique, researchers have determined the structure of a protein that helps control blood glucose (sugar) levels, the GLP-1 receptor (GLP1R). When GLP-1 hormone binds to it, the GLP1R interacts with another protein partner that helps carry the hormone's message within the cell. This receptor is part of a family of proteins, called class B G-protein coupled receptors (BGPCRs), that have key roles in many critical physiologic processes, including regulating levels of calcium in the blood and maintaining the balance of

water and electrolytes in the body. Each member of this protein family spans cellular membranes in the tissues where they function. In general, it is known that when the part of a BGPCR that lies outside the cell binds to its particular hormone, changes occur in the receptor's structure within the cell membrane that promote binding to and activation of a second protein, called a G protein. The G protein then carries the signal into the cell and triggers the appropriate response to the hormone. Given their important roles in human health, understanding the details of BGPCR interactions with hormones and G proteins might aid in development of therapeutics that stimulate or inhibit individual BGPCRs, potentially improving treatment for multiple human diseases. Indeed, some medicines for type 2 diabetes act by stimulating GLP1R, which helps promote production of insulin in response to elevations of blood glucose (sugar) that occur after a meal, while slowing stomach emptying. Unfortunately, determining the structure of BGPCRs in complex with their hormones and G proteins has proved difficult using traditional techniques. In the hope of one day improving such medications and providing insights that could promote development of medicines that act on other GPCRs, scientists turned to a cutting-edge method called cryo-electron microscopy to determine the structure of GLP1R in complex both with the hormone GLP-1 and with a partner G protein.



**Figure legend:** In this image, the portion of GLP1R located outside the cell is shown in dark green, and the membrane-spanning portion is light green. The GLP-1 hormone is shown in red, and various parts of the G protein are shown in gold, blue, and purple.

Image courtesy of Georgios Skiniotis. Reprinted by permission from Macmillan Publishers Ltd: *Nature* 546: 248-253, copyright 2017.

In this approach, the researchers flash-froze membranes containing large numbers of GLP-1 receptors in complex both with the GLP-1 hormone and the receptor's G protein partner. The very rapid drop to an extremely low temperature maintains the three-dimensional structure of the proteins and their relationship to one another and to the membrane, while avoiding the accumulation of ice crystals that might otherwise distort this arrangement. Next, they took transmission electron micrographs of the resulting samples. In this technique, a beam of electrons is passed through the sample. The frozen proteins interact with some of the electrons in the beam, and a receiver reconstructs an image of the sample based on these interactions. The resulting images of each individual GLP1R protein complex in the sample were somewhat fuzzy and indistinct; but by "averaging"

the results from the many GLP1Rs in the sample using sophisticated software, the researchers were able to determine the structure of the receptor with remarkable precision. The adjacent figure shows two views of the GLP1R structure, where colors have been added to highlight specific parts of the protein, as well as GLP-1 and the G protein partner. (See the figure legend for details.) The researchers were able to use the resulting images to glean new information about how the proteins in the complex fit together, how the protein complex is predicted to move and bend as it carries out its function, and what these findings could teach about the structure and function of other GPCRs. This "portrait" is a remarkable technical achievement, and may one day advance health through improved understanding of the biology of GPCRs.

Zhang Y, Sun B, Feng D,...Skiniotis G. *Cryo-EM structure of the activated GLP-1 receptor in complex with a G protein.*

*Nature* 546: 248-253, doi: 10.1038/nature22394, 2017.

## EFFECTS OF THE BRAIN ON AGING

### Cellular Understanding of How the Brain Controls Aging:

Researchers have discovered cellular mechanisms by which the brain regulates aging in mice, providing novel therapeutic targets to combat health consequences associated with aging. The research focused on cells, called adult neural stem/progenitor cells (NSCs), which through a complex signaling cascade called neurogenesis turn into mature nerve cells. NSCs are found in the hypothalamus, a part of the brain governing many physiologic functions, and in other brain regions, most notably, the hippocampus, involved in the formation of new memories and the site of early age-related degeneration in Alzheimer's disease and related dementia (ADRD). The study examined whether NSCs in the hypothalamus were involved in regulating aging in male mice. First, the scientists observed that the number of NSCs declined with aging in mice. NSCs were abundant in the hypothalamus of young mice, diminished in middle-age mice, and were nearly entirely lost in aged animals. To determine if the loss of NSCs was causing the mice to age or was a consequence of aging, the scientists experimentally depleted most of the NSCs in the animals' hypothalami. Compared to control animals, mice lacking NSCs showed accelerating signs of aging, such as a shortened lifespan. These findings suggest that NSCs control the speed of aging.

Confirming this role, aging was slowed when NSCs were implanted into middle-age mice, with the animals living longer than their control counterparts. Because NSC loss or implantation produced effects on aging in a relatively short timeframe, the scientists hypothesized that the outcomes might be mediated by factors produced by the NSCs rather than by their maturation. Indeed, additional experiments showed that NSCs exert some of their anti-aging effects by secreting small particles called exosomes that contain microRNAs (miRNAs) into the cerebrospinal fluid of mice. miRNAs are involved in regulating gene activity and can be taken up by other cells. Thus, it appears that it is a specialized function of NSCs related to secreting these particles, rather than their function of turning into new mature nerve cells, that plays a key role in how NSCs govern aging in mice.

If NSCs play a similar role in women and men, they represent novel therapeutic targets to combat the health consequences associated with aging, such as diabetes and ADRD. Further research that delves into the detailed mechanisms by which these cells exert their effects—such as identifying what miRNAs are released by NSCs—may illuminate other targets and potential therapies.

*Zhang Y, Kim MS, Jia B,...Cai D. Hypothalamic stem cells control ageing speed partly through exosomal miRNAs. Nature 548: 52-57, doi: 10.1038/nature23282, 2017.*

# dkNET: The NIDDK Information Network



Developing innovative strategies to enhance the sharing of data and resources remains an important focus for NIDDK. One of these strategies to enhance the ability to find, search, and reuse the science NIDDK supports is dkNET, the NIDDK Information Network.

Data science advances also continue to provide increasingly powerful and sophisticated technologies for integration and mining of data, offering new opportunities to use existing data to answer new questions. As biomedical tools and technologies improve, researchers are producing a rapidly increasing amount of data, tools, and resources. Organizing this information so that it can be easily found, searched, and reused is a continuing challenge. Resources and data from different studies can be stored in different public or private repositories, on different computer platforms, and in different formats. As a result, it can be extremely challenging for a researcher to locate resources relevant to their work, or to integrate data from different repositories in a useful way.

dkNET started in 2014 to promote sharing of information within and among NIDDK's research communities. Its goal was to ensure that NIDDK-supported data and resources can easily be found, used, and reused to answer future scientific questions. dkNET accomplishes this by being a search engine for multiple data repositories

along with sources of cells, mouse models, and other research tools.

dkNET has captured and indexed a wide variety of resources, including protocols and assays, antibodies, cell lines, animal models, data sets, and funding opportunities. dkNET's resource catalogs are accessible via a user-friendly web portal, allowing users to search through millions of database records stored in hundreds of databases. dkNET also provides tools to enhance search efficiency; to create individualized workflows; and to analyze, visualize, and reuse data.

Currently, dkNET includes resources from sites such as AddGene, the Antibody Registry, the Beta Cell Biology Consortium, the Diabetic Complications Consortium, Grants.gov, the Nuclear Receptor Signaling Atlas, and various research animal repositories. dkNET continues to extend its reach by adding additional NIH programs and resource centers to its search function.

dkNET also supports the NIH's Policy on Rigor and Reproducibility, which was released in 2015. dkNET was instrumental in supporting the development of Research Resource Identifiers, or RRDs, which are unique authentication tags for biological resources such as mouse models and antibodies. Using RRDs in their research allows scientists to specifically identify and track the reagents and resources used in a way that is not possible when using brand names, catalog numbers, or other identifiers that can change over time. dkNET provides services to help the scientific community easily find, use, and obtain RRDs, which helps enhance transparency and reproducibility.

Resources like dkNET help ensure that scientists can build upon the work of the past to address "Big Science" questions now and in the future. As scientists continue to profile human biology in extraordinary depth, the ability to integrate, analyze, and search existing data sets will provide new opportunities to accelerate and transform biomedical research.

# NIDDK-supported Scientists Receive Presidential Award



**Anna Greka, M.D., Ph.D.**



**Benjamin F. Voight, Ph.D.**

Two scientists supported by the NIDDK were among the recipients of the Presidential Early Career Award for Scientists and Engineers (PECASE) in 2017. The PECASE is awarded annually to scientists and engineers who, while early in their research careers, have pursued innovative research and shown outstanding scientific leadership. The two NIDDK extramural grantees who received the PECASE are Anna Greka, M.D., Ph.D., and Benjamin F. Voight, Ph.D.

***“These innovators are working to help keep the United States on the cutting edge, showing that Federal investments in science lead to advancements that expand our knowledge of the world around us and contribute to our economy.” - Barack Obama***

Dr. Greka, an Assistant Professor at Harvard Medical School, received a PECASE award in recognition of her research investigating the roles of calcium ion channels in inherited kidney diseases. Her studies on the ion channel Transient Receptor Potential Channel 5 (TRPC5) revealed that blocking its activity with an inhibitor can protect mice from damage to the kidney’s filtration system, a finding that could lead to a new strategy to treat kidney disease.

Dr. Voight, an Associate Professor of Systems Pharmacology and Translational Therapeutics and

Associate Professor of Genetics at the University of Pennsylvania, received a PECASE award for his work using statistical and computational informatics to identify genetic variants that contribute to the risk of type 2 diabetes. Determining these variants could lead to improved understanding of the biology that contributes to type 2 diabetes, creating opportunities to develop novel therapeutics and prevention strategies and to personalize an individual’s risk.

In addition to the NIDDK-supported recipients, other scientists supported by 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.

# The Porphyria Rare Disease Clinical Research Consortium



## What Are the Porphyrias?

The porphyrias are a group of metabolic disorders. The symptoms associated with porphyrias can be diverse and can be severe including chest and abdominal pain, emotional and mental disorders, seizures, and muscle weakness. These debilitating symptoms often appear quickly and can last for long periods of time. Environmental factors can trigger the symptoms of porphyria and include alcohol, smoking, certain drugs, hormones, stress, dieting and fasting, and exposure to sunlight. Avoiding sunlight has a major negative impact on quality of life, and the use of sunscreen is ineffective for porphyria symptoms.

The porphyrias mostly arise from an inherited mutation in any of eight genes that code for essential proteins in the heme production pathway—resulting in the accumulation of heme precursors and a blockage of heme production. Heme is a vital chemical compound that contains iron and gives blood its red color. It enables red blood cells to carry oxygen from the lungs to all parts of the body, and it also plays a role in the liver where it assists in breaking down chemicals including some drugs and hormones.

## Diagnosis and Treatment

Diagnosis is difficult because the range of symptoms is common to many disorders, and interpretation of the tests may be complex. Although a large number of tests are available, the results among clinical laboratories are not always reliable. Treatments need to be improved and specific therapies developed.

## The Porphyria Rare Disease Clinical Research Consortium

To advance understanding of how these diseases progress and what other factors influence them, the NIDDK along with the NIH's National Center for Advancing Translational Sciences (NCATS) support the Porphyria Rare Disease Clinical Research Consortium. The Consortium brings together porphyria experts at eight academic institutions with the American Porphyria

Foundation for the purposes of improving diagnosis; studying the causes, characteristics, and genetics of the porphyrias; as well as developing and testing new treatments; and training the next generation of porphyria clinicians and researchers. The eight participating academic institutions are: the Icahn School of Medicine at Mount Sinai, New York, NY; the University of Alabama at Birmingham, AL; the University of California at San Francisco, CA; the University of Miami, Miami, FL; the University of Texas Medical Branch, Galveston, TX; the University of Utah School of Medicine, Salt Lake City, UT; the University of Washington, Seattle, WA; and Wake Forest University Health Sciences, Winston-Salem, NC. In addition to the Consortium, NCATS supports 20 other rare disease clinical research consortia and a data coordinating center that enables uniform high-quality data collection and analysis and facilitates information sharing across the network. This robust data source helps scientists better understand the common elements of rare diseases, so they may apply that knowledge to improving diagnosis and treatment for these conditions.

## Recent Findings from the Consortium

Inherent challenges to the development of rare disease treatments include difficulties in diagnosis, geographically dispersed patients and scientific experts, and lack of data on health of a group of people with a specific medical condition over time. The Consortium has begun to make inroads to overcome these challenges. For example, researchers have reported that different clinical laboratories have variability in measurement of erythrocyte (red blood cell) protoporphyrin, a biological molecule that is made during the production of heme. Because some of the measurement tests may lead to missed diagnoses, this finding identifies an area for improving diagnostic tests. Another study by the Consortium showed that higher levels of erythrocyte protoporphyrin were associated with increased disease severity and risk of liver dysfunction in people with forms of porphyria called erythropoietic protoporphyria or X-linked protoporphyria. The Consortium has also issued recommendations for the diagnosis, treatment, and counseling of patients with acute hepatic porphyrias.

# Scientific Conference: Emerging Role of Branched-chain Amino Acids in Human Diseases

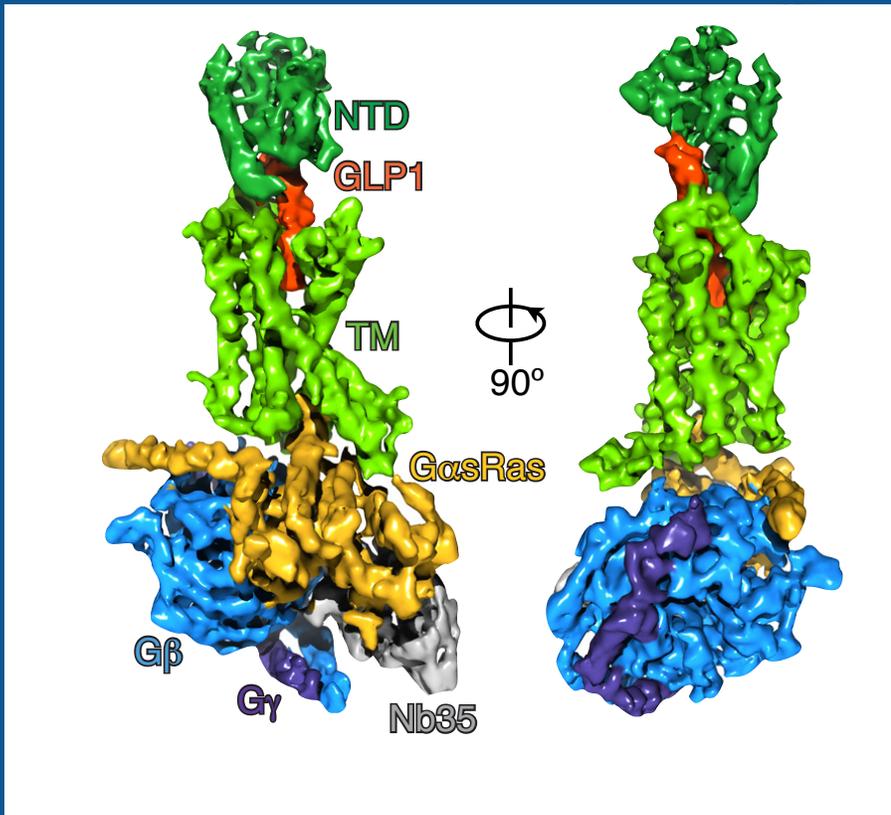
The branched-chain amino acids (BCAAs), including leucine, isoleucine, and valine, are among the most highly represented amino acids in dietary protein. Based on a wealth of studies, they have emerged as more than simply essential components of our diet. Leucine in particular appears to be a key nutrient signal of a protein-containing meal and to play an important role in regulating metabolism and satiety signals. For example, leucine promotes release of the hormones GLP-1, insulin, and leptin, but inhibits the release of the hormone ghrelin. BCAAs and insulin are signals that promote energy storage and alter the growth of energy-consuming tissues. Intravenous infusion of BCAAs or consumption of dietary protein increase diet-induced thermogenesis (production of body heat) and energy wasting, more so than is associated with other nutrients, and may therefore help promote weight loss.

In May of 2017, the NIDDK held a conference, in cooperation with the NIH Office of Dietary Supplements and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, to clarify whether these various actions of BCAAs are beneficial or detrimental to human health. This question is not straightforward, unfortunately, because scientific research exists in support of either conclusion.

For example, BCAA supplementation or BCAA-rich diets are often associated with improved body weight regulation, satiety, lean body mass, diet-induced thermogenesis, and muscle protein synthesis. Supplementation with BCAAs seems to result in health benefits in certain liver diseases, and has been investigated as a possible means to combat age-related loss of muscle mass.

On the other hand, there are reports of potentially adverse effects associated with BCAAs, particularly when present at high levels. For example, circulating levels of BCAAs are consistently increased in people with type 2 diabetes, insulin resistance, or obesity, where they positively correlate with insulin resistance and high levels of hemoglobin A1c, a marker of consistently elevated blood glucose (sugar). Several longitudinal studies have also reported that increased blood levels of BCAAs are predictive of future insulin resistance or type 2 diabetes, leading to speculation about a potential causative role for BCAAs. Abnormalities in BCAA metabolism leading to elevated levels are associated with various health problems. Some can lead to heart failure, while others underlie the pathology of Maple Syrup Urine Disease and a number of other rare genetic diseases. Additionally, a signaling system that has been implicated in cancer is activated by BCAAs. Another issue is that when circulating levels of BCAAs increase, they compete with the uptake of amino acid precursors of neurotransmitters in the brain, which can contribute to an increased risk of depression in individuals with obesity.

The NIDDK is the lead Institute at the NIH supporting studies of metabolism, nutrient signaling, obesity, type 2 diabetes, liver disease, and nutrition—all areas in which BCAAs appear to play an important role. Through this symposium, the NIDDK encouraged further research in this area, brought together the community studying BCAA signaling and metabolism with those interested in studying their role in health and disease, and sought to identify new research opportunities for understanding disorders affected by BCAAs.



The hormone GLP-1, produced by the gut in response to food intake, serves as a signal to the pancreas to prepare to release insulin (a hormone that lowers blood glucose levels) and to cease production of glucagon (a hormone that raises blood glucose levels). In addition, GLP-1 acts on other tissues, briefly slowing the digestive process and reducing appetite. This combination of effects makes GLP-1 an important contributor to maintaining healthy glucose levels, though its effects are brief as it is degraded rapidly in the blood. Past research has led to the development of both long-lived analogs of GLP-1 as well as inhibitors of GLP-1 degradation, which have become important classes of medication for people with type 2 diabetes. GLP-1 stimulates its effects by binding to a receptor, designated GLP1R, that spans cell membranes and transmits the hormone's signal to the cell's interior. Understanding how GLP1R interacts with GLP-1 on the outside of the cell, and then acts on protein partners called G proteins that carry the GLP-1 signal within the cell, might lead to improvements in therapy that take advantage of this pathway. In research described in the Cross-Cutting chapter of this book, a cutting-edge technique called cryo-electron microscopy was used to determine the structure of GLP1R in complex with GLP-1 and a G protein. In this image, the portion of GLP1R located outside the cell is shown in dark green, and the membrane-spanning portion is light green. The GLP-1 hormone is shown in red, and various parts of the G protein are shown in gold, blue, and purple. GLP1R is similar to other receptors that are important for a variety of functions in the body. A better understanding of these receptors, from this and other studies, may help inform the development of new treatments for multiple diseases.

*Image courtesy of Georgios Skiniotis. Reprinted by permission from Macmillan Publishers Ltd: Nature 546: 248-253, copyright 2017.*

# 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 rates of diabetes-related complications have declined substantially in the past two decades, and the rate of new cases of diagnosed diabetes has begun to fall, disease burden remains significant as the number of people with diabetes is still very high.<sup>3,4</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 2012—including costs of medical care, disability, and premature death—was \$245 billion.<sup>5</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 is similar to type 2 diabetes but unique to pregnancy. 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  $\beta$  cells of the pancreas. If left

- <sup>1</sup> *Diabetes in America*, 3rd ed. Cowie CC, et al., Eds. Bethesda, MD, National Institutes of Health, NIH Pub No. 17-1468, 2017.
- <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> Gregg EW, et al. *N Engl J Med* 370: 1514-1523, 2014.
- <sup>4</sup> Centers for Disease Control and Prevention. *Diabetes—working to reverse the US epidemic, at a glance 2016*. Atlanta, GA: U.S. Department of Health and Human Services, 2016.
- <sup>5</sup> American Diabetes Association. *Diabetes Care* 36: 1033-1046, 2013.

untreated, type 1 diabetes results in death from starvation: without insulin, glucose is not transported from the bloodstream into the body's cells, where it is needed. 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, with current technologies to test blood glucose levels and administer insulin, it is still not possible for people with type 1 diabetes to control 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, a milestone was achieved in 2016 when the U.S. Food and Drug Administration approved the first commercial "hybrid artificial pancreas" device that automatically links glucose monitoring and insulin delivery. The NIDDK supported early research that contributed to the development of the approved device and continues to support research to test and improve artificial pancreas technologies. 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>4</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>6</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 controlling 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 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 is an alarming trend for many reasons. For example, the NIDDK-supported Treatment Options for type 2 Diabetes in Adolescents and Youth (TODAY) clinical trial 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 both the duration of diabetes and control of blood glucose levels; thus, those with early disease onset are at greater risk with respect to complications than those who develop the disease later in life. 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 women could contribute to a cycle of ever-growing rates of diabetes. Therefore, the advent of type 2 diabetes

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<sup>6</sup> Kim C, et al. *Diabetes Care* 25: 1862-1868, 2002.

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.

## NEW INSIGHTS ON THE COMPLICATIONS OF DIABETES

**Personalizing Eye Exam Schedule for People with Type 1 Diabetes:** Researchers have developed an evidence-based screening schedule for an eye disease (retinopathy) in people with type 1 diabetes, with the frequency of screening tailored to an individual's current level of eye disease and hemoglobin (Hb) A1c level, a measure of average blood glucose (sugar) control. Diabetes is the leading cause of new cases of blindness in adults. Vision loss, however, can be prevented if the damage is detected and treated in a timely manner. Currently, for people with type 1 diabetes, annual retinal examinations are recommended to screen for signs of retinopathy, starting 3 to 5 years after diagnosis. Previous results from NIDDK's landmark Diabetes Control and Complications Trial (DCCT) and its follow-up study, Epidemiology of Diabetes Interventions and Complications (EDIC), demonstrated that a period of intensive blood glucose control lowered the risk of complications, including those involving the eyes. This led researchers to ask whether an annual eye screening is necessary for people with type 1 diabetes who intensively control their blood glucose levels.

In the over 30 years that DCCT/EDIC participants have been followed, data from approximately 24,000 eye examinations were collected along with information about each participant's eye health. From these data, DCCT/EDIC researchers modeled the likelihood that a person would progress from a lower level of retinopathy to very severe retinopathy in specific

periods of time. They found that, for people with no or mild retinopathy, annual examinations might not be necessary. For people with moderate to severe retinopathy, however, more frequent examinations might be needed to detect retinopathy severe enough that timely treatment is needed to prevent vision loss. They also found that the risk of eye disease progression was closely related to the participant's HbA1c level.

With the goal to limit the likelihood of progression to very severe retinopathy between examinations to approximately 5 percent, the scientists developed an eye examination schedule based on a person's current state of retinopathy and additionally on HbA1c level. Averaged over all levels of HbA1c, they estimated that a person could go 4 years between examinations if they had no initial retinopathy, 3 years if they had mild retinopathy, and 6 months if they had moderate retinopathy. For people with severe retinopathy, monitoring more frequently than every 3 months would be necessary to reduce the probability to approximately 15 percent of developing very severe retinopathy before their next examination. People at all levels of retinopathy with higher HbA1c levels were predicted to need more frequent eye exams, as they are at higher risk to develop eye disease. For example, the eye examination schedule for people at a current HbA1c level of 6 percent would be 5 years if they had no initial retinopathy, 5 years if they had mild retinopathy, 6 months if they had moderate retinopathy, and 3 months if they had severe retinopathy; whereas for people at a current HbA1c level of 10 percent, the corresponding schedule would be 3 years, 2 years, 3 months, and 1 month, respectively.

Taking into consideration both the reduced number of screenings the scientists propose for people with no or mild retinopathy and the more frequent examinations needed for people with moderate or severe retinopathy, they also calculated how their schedule would affect the number of screenings overall for people with type 1 diabetes. They found that, over 20 years, screening according to their tailored examination schedule would, on average, result in a 58 percent reduction in number of exams overall, compared to annual screening for everyone. Combining this overall reduction with an approximate \$200 cost for screening and approximate 1 million people with type 1 diabetes, the scientists estimate

that this screening schedule could result in a savings of approximately \$1 billion over 20 years. Although the additional screening for those with worse retinopathy would increase their screening burden, it could also increase the likelihood of detecting further progression of retinopathy, so that therapy proven to preserve vision can be delivered in the timeframe before irreparable vision loss occurs. Importantly, this schedule has yet to be tested in real-life situations in people with type 1 diabetes. In addition, it is not known if it will be appropriate for people with type 2 diabetes, as it remains to be determined whether retinopathy progresses similarly in people with type 2 diabetes and people with type 1 diabetes. Regardless, this risk-based screening schedule has the potential to personalize treatment to reduce both undetected diabetic eye disease and the burden of annual retinal exams for some people, which may result in cost savings overall and better health for people with type 1 diabetes.

*Nathan DM, Bebu I, Hainsworth D,...Lachin JM; for the DCCT/EDIC Research Group. Frequency of evidence-based screening for retinopathy in type 1 diabetes. N Engl J Med 376: 1507-1516, doi: 10.1056/NEJMoa1612836, 2017.*

**Identification of Signaling Pathway Involved in Diabetic Nerve Damage Leads to Discovery of Promising New Therapy:** Researchers have discovered that blocking a signaling pathway involved in diabetic nerve damage with a drug already being given to people for other conditions could prevent or reverse that damage in mice. People with diabetes can, over time, develop nerve damage throughout the body. The most common type of diabetic nerve damage, called peripheral diabetic neuropathy, causes pain or loss of feeling in the feet, legs, hands, and arms and is a major risk factor for amputation. Currently, people with diabetic nerve damage may be treated with medications to manage pain, but there is great need to find therapies to prevent and reverse this serious complication to reduce its significant health burden.

Toward this goal, scientists screened a library of molecules for those that could enhance repair of male rat nerve cells in laboratory culture by stimulating the outgrowth of neurites, which project from nerve cell bodies and connect the cells to other nerve cells. The more neurite outgrowth the better, in terms of repairing nerve damage. They found that molecules that blocked

“muscarinic acetylcholine type 1 receptors” ( $M_1R$ ) stimulated neurite outgrowth. Further experiments confirmed a key role for  $M_1R$  in regulating neurite outgrowth: neurite outgrowth was inhibited when  $M_1R$  protein levels were experimentally increased in cultured rat nerve cells, whereas neurite outgrowth was higher in nerve cells from an experimental mouse model lacking  $M_1R$  compared to control mice. To take these observations a step further, the researchers studied an  $M_1R$ -blocking drug in mouse models of diabetes, focusing on a drug called pirenzepine because it is already used clinically for other conditions. They found that pirenzepine treatment prevented and reversed diabetic nerve damage in male and female mouse models of type 1 and type 2 diabetes. The drug also prevented nerve damage associated with other conditions, such as chemotherapy, suggesting that blocking  $M_1R$  with pirenzepine stimulates nerve repair/regeneration even when the underlying cause of the damage varies in animals.

This research suggests that  $M_1R$  plays a key role in the nerve damage associated with diabetes and other conditions in mice, and that blocking its activity with pirenzepine could prevent or reverse that damage. Interestingly, the scientists found no evidence that the normal  $M_1R$  signaling pathway is altered in diabetes, so the mechanism by which pirenzepine is exerting its effects is not clear. Nonetheless, the promising results of the study are already being translated to people: the NIDDK is funding a pilot clinical trial to test whether another drug (topical oxybutynin) that blocks  $M_1R$  can reverse diabetic neuropathy.

*Calcutt NA, Smith DR, Frizzi K,...Femyhough P. Selective antagonism of muscarinic receptors is neuroprotective in peripheral neuropathy. J Clin Invest 127: 608-622, doi: 10.1172/JCI88321, 2017.*

### **Study Suggests Possible Approach for Early Detection of Diabetic Kidney Disease Risk:**

Researchers have identified a set of molecules that appear to predict loss of kidney function and correlate with kidney damage in people with type 2 diabetes. Diabetes is the leading cause of kidney disease in America and is one of the leading causes of kidney failure. However, early in the course of disease available methods for assessing kidney function are not sufficient to predict who is likely to progress to advanced loss of kidney function; knowing this information could help inform clinical care.

Persistent hyperglycemia, or elevated blood glucose (sugar) levels, is the chief culprit in most diabetes health complications and is thought to wield this effect in part through the production of tissue-damaging chemical byproducts. These molecules are called advanced glycation end products (AGEs) and oxidative end products, and are detectable in the blood. Leveraging biological samples and data from 168 women and men with type 2 diabetes who participated in a kidney-disease-focused clinical trial and follow-up period, researchers tested the hypothesis that increasing levels of one or more of these molecules at an early stage might predict progression of diabetic kidney disease. They found through their analyses that increases in certain AGEs during the 6-year clinical trial predicted a threshold loss of kidney function during the trial or follow-up period. Higher levels of these AGEs correlated with the appearance of kidney lesions typical of diabetic kidney disease that were detected in kidney biopsies from a subset of participants at the end of the clinical trial. Notably, there is evidence that increasing levels of AGEs in the blood are partly due to a vicious cycle in which kidney impairment leads to reduced clearance of these toxic molecules from the body—reinforcing the message of other clinical trials that good control of blood glucose is important to staving off diabetes complications. The participants in this study were Pima Indians of the Gila River Indian Community in Arizona. Future studies may both help determine the clinical utility of AGEs as noninvasive predictive biomarkers of progression of diabetic kidney disease and whether the findings extend to other populations.

*Saulnier P-J, Wheelock KM, Howell S,...Beisswenger PJ. Advanced glycation end products predict loss of renal function and correlate with lesions of diabetic kidney disease in American Indians with type 2 diabetes.*

*Diabetes* 65: 3744-3753, doi: 10.2337/db16-0310, 2016.

## UNCOVERING CLUES TO METABOLIC CONTROL AND THE DEVELOPMENT OF TYPE 2 DIABETES

### Identification of a Key Metabolic Regulator:

Scientists have determined that the protein  $\beta$ -arrestin-2 plays critical roles in the pancreas and liver to regulate metabolism.  $\beta$ -arrestins are a family of proteins that regulate a wide variety of functions in different tissues in the body. Previous studies, in which mice were genetically engineered to lack  $\beta$ -arrestin-2, suggested

that this protein might be involved in metabolic functions, but it remained unclear where  $\beta$ -arrestin-2 was needed to affect metabolism.

To explore a potential role for  $\beta$ -arrestin-2 in  $\beta$  cells (the pancreatic cells that produce insulin) and in hepatocytes (a cell type in the liver), the researchers genetically engineered mice to lack  $\beta$ -arrestin-2 specifically in  $\beta$  cells or in hepatocytes. The scientists found that male mice lacking  $\beta$ -arrestin-2 only in their  $\beta$  cells showed mild metabolic changes (compared to mice with  $\beta$ -arrestin-2 in their  $\beta$  cells) when fed a normal diet. These mice, however, showed significant metabolic changes when fed a diet that induces obesity and type 2 diabetes in mice. Compared to mice with  $\beta$ -arrestin-2 in their  $\beta$  cells consuming the same diet, the mice without  $\beta$ -arrestin-2 showed an increase in blood glucose (sugar) levels and almost no glucose-stimulated insulin secretion.

To complement these studies, the researchers also genetically engineered mice to have higher levels of  $\beta$ -arrestin-2 than usual. They found that, when fed the diabetes-inducing diet, these mice showed an increase in glucose-stimulated insulin secretion, greatly improved glucose tolerance, and lower blood glucose levels, compared to normal mice on the same diet. This suggested that  $\beta$ -arrestin-2 can counter the metabolic effects of a diet that induces obesity and type 2 diabetes. Importantly, the researchers found that glucose-stimulated insulin secretion was significantly reduced in a human pancreatic  $\beta$  cell line lacking  $\beta$ -arrestin-2, suggesting that  $\beta$ -arrestin-2 may be similarly important to human metabolism.

The researchers found that  $\beta$ -arrestin-2 has a similarly important role in hepatocytes: eliminating the protein in these liver cells in mice fed the diabetes-inducing diet worsened metabolic control, while increasing the protein's levels in the cells helped ameliorate the effects of the diet.

These two studies implicate  $\beta$ -arrestin-2 as a key regulator of  $\beta$  cell and hepatic function in maintaining normal metabolism. Additional research will be necessary to determine the extent to which  $\beta$ -arrestin-2 acts similarly in humans, but this research suggests that strategies to promote  $\beta$ -arrestin-2 activity in  $\beta$  cells and/or hepatocytes

could be clinically useful in the treatment of type 2 diabetes.

Zhu L, Rossi M, Cui Y, ...Wess J. Hepatic  $\beta$ -arrestin 2 is essential for maintaining euglycemia. *J Clin Invest* 127: 2941-2945, doi: 10.1172/JCI92913, 2017.

Zhu L, AlMaça J, Dadi PK, ...Wess J.  $\beta$ -arrestin-2 is an essential regulator of pancreatic  $\beta$ -cell function under physiological and pathophysiological conditions. *Nat Commun* 8: 14295, doi: 10.1038/ncomms14295, 2017.

### **Uncovering Cellular Factors Linking the Immune System, Obesity, and Insulin Resistance:**

New research suggests a protein secreted by certain immune system cells not only promotes obesity-associated inflammation, but also drives insulin resistance in key tissues, and identifies a key player in activation of the cells in question. Obesity has long been known to cause chronic inflammation, a state in which macrophages—immune-system cells that help defend the body against infection—are inappropriately and continuously activated in metabolically important tissues such as adipose (fat), muscle, and liver. This obesity-associated chronic inflammation is thought to contribute to the development of insulin resistance—a condition that often leads to type 2 diabetes—possibly through the effects of some of the many proteins macrophages secrete. Although several of these proteins have been investigated, none has clearly been shown to be a major driver of insulin resistance. A new study focuses on the macrophage-secreted galectin-3 (Gal3) protein. Gal3 had previously been shown to be elevated in a mouse model of diet-induced obesity. Gal3 levels in these mice correlated significantly with insulin resistance, so researchers sought to determine whether Gal3 has a role in causing this condition, or whether the correlation was coincidental.

Indeed, through a variety of experiments they found compelling evidence that Gal3 plays a significant role in promoting insulin resistance. For example, mice that lack the gene for making Gal3 remained sensitive to insulin, even when they had diet-induced obesity. Moreover, treating obese mice that have the normal gene for Gal3 with a compound that inhibits the action of the protein improved their insulin sensitivity. In other experiments, exposing laboratory-grown mouse adipose, muscle, or liver cells to Gal3 protein reduced the cells' ability to respond properly to insulin, suggesting that Gal3 could drive insulin

resistance in the absence of other macrophage-secreted substances. Exploring the mechanism by which it may promote insulin resistance, scientists found that Gal3 binds to the insulin receptor, a protein on the surface of cells to which insulin binds to exert its effects. Although it does not seem to prevent insulin from binding to its receptor at the same time, Gal3 seems to inhibit the receptor's capacity to trigger the normal cellular response to insulin. Complementing these findings, the team found evidence that Gal3 promotes the inflammatory state by attracting additional macrophages to tissues where it is being secreted. The experiments in living mice were performed in male animals, so it is possible that there are differences in the effects of Gal3 in female mice.

The researchers also examined Gal3 levels in 52 human volunteers, and found the protein to be elevated in those with obesity, and to correlate significantly with insulin-resistance, suggesting the protein might have a similar effect in human and mouse physiology. It remains unknown whether inhibition of Gal3 could be a safe or effective approach to treating human insulin resistance and type 2 diabetes, but taken together, these experiments suggest it is well worth exploring that possibility.

Understanding how macrophages become activated to cause inflammation and insulin resistance could provide another potential target for battling type 2 diabetes. In a study using male mice and laboratory grown cells, a different group of researchers focused on a cellular protein called fatty acid synthase, or FAS. FAS enables cells to make simple fat molecules, called fatty acids, from precursor molecules. These fatty acids can then become part of the cell membrane, which is the cell's structural boundary and site of interaction with its environment. Mice lacking FAS in their macrophages gained weight similarly to control mice when fed a diet that causes obesity and diabetes, but had fewer macrophages in their fat tissue, less inflammation, and better insulin sensitivity. At the same time, laboratory-grown macrophages with artificially reduced FAS activity also had lower than normal inflammatory responses to a pathogen-derived molecule that should elicit a potent immune response. These and other results suggested that FAS may play a key role in macrophage activation, both in obesity-associated inflammation and in the response to infection. Because macrophage activation involves changes in molecules present in the cell membrane, the scientists compared the composition of FAS-deficient and normal macrophage cell membranes under a variety of conditions. These

experiments indicated that while FAS deficiency does not disturb overall membrane integrity, it disrupts the normal assembly and molecular content of specialized membrane structures that help macrophages migrate into tissues and incite inflammation. Should FAS prove to have a similar role in human macrophage activation as it does in mice, these findings may one day lead to new approaches to preventing inflammation and insulin resistance in people with obesity.

Li P, Liu S, Lu M,...Olefsky JM. Hematopoietic-derived Galectin-3 causes cellular and systemic insulin resistance. *Cell* 167: 973-984. e12, doi: 10.1016/j.cell.2016.10.025, 2016.

Wei X, Song H, Yin L,...Semenkovich CF. Fatty acid synthesis configures the plasma membrane for inflammation in diabetes. *Nature* 539: 294-298, doi: 10.1038/nature20117, 2016.

**In Obesity, an Immune System Signal Can Lead to Loss of Beneficial Bacteria and Loss of Blood Glucose Control:** New research suggests that a key molecular signal produced by immune cells as a result of obesity may worsen blood glucose control through a system that limits the abundance of a beneficial species of bacteria in the gut. The molecular signal in question is interferon gamma (IFN $\gamma$ ), a protein released as part of the inflammatory response to obesity in both mice and humans. Diet-induced obesity can promote type 2 diabetes in mice, as it often does in humans, and previous research had shown that glucose levels remain healthy in obese mice that lack the gene for IFN $\gamma$ .

Because previous research has shown that bacteria in the gut can have a significant impact on glucose levels in their hosts, and because IFN $\gamma$  can modulate the body's immune response to bacteria, it is plausible that the protein might be promoting type 2 diabetes indirectly by causing the immune system to act against beneficial microbes. To test this concept, researchers treated obese male mice with a combination of antibiotics that wipes out nearly all intestinal bacteria. Doing so eliminated differences in glucose levels between mice that had the gene for IFN $\gamma$  and those that did not. Then they assessed the population of gut bacteria in obese male and female mice to find bacterial species that differed in abundance depending on whether the mice have the gene for IFN $\gamma$ . From among the species that were significantly more, or less, abundant, the researchers asked which of these species correlated positively or negatively with glucose levels. This process led them to focus on a type of bacteria called

*Akkermansia muciniphila*, which is commonly found in both the mouse and human gut. Higher levels of *A. muciniphila* in the mouse gut correlated with better blood glucose control. *A. muciniphila* levels were sharply reduced in obese mice that had the IFN $\gamma$  gene, but obese mice lacking IFN $\gamma$  had levels of these bacteria similar to those found in lean mice. Conversely, in obese male mice that lacked *A. muciniphila*, IFN $\gamma$  no longer seemed to affect glucose control: those without IFN $\gamma$  were no healthier than those that could make the protein, until and unless *A. muciniphila* was added back.

Overall, these results suggested that *A. muciniphila* helps mice maintain proper blood glucose control, and that IFN $\gamma$  production, elevated in obesity, worsens blood glucose control through its adverse effects on these beneficial bacteria. The scientists also found evidence that *A. muciniphila* may have a similar beneficial effect on metabolism in people: higher levels of these bacteria correlated modestly but significantly with better blood glucose control in a group of people participating in other metabolic studies. In any case, these results show that inflammation due to obesity can cause changes in the gut microbiome that have a deleterious effect on blood glucose control, and suggest that interventions to support a healthful gut microbiome may one day be a viable approach to helping treat or prevent type 2 diabetes.

Greer RL, Dong X, Moraes AC,...Shulzhenko N. Akkermansia muciniphila mediates negative effects of IFN $\gamma$  on glucose metabolism. *Nat Commun* 7: 13329, doi: 10.1038/ncomms13329, 2016.

## RESEARCH ON CYSTIC FIBROSIS AND OTHER RARE DISEASES

### Protein-folding Chaperones Have Both Positive and Negative Roles To Play in Cystic Fibrosis:

New research has found that proteins involved in ensuring that other proteins are in their proper shapes may have a role to play in mitigating the effects of cystic fibrosis (CF). People with CF lack a functional copy of the *CFTR* gene, and the most common variant of the gene found in people with CF is designated  $\Delta F508$ -*CFTR*. In  $\Delta F508$ -*CFTR*, the absence of a single amino acid interferes with proper folding of the CFTR protein, not only interfering directly with its function as a channel that allows chloride ions to pass through the cell membrane, but also destabilizing the protein and causing it to be a target for degradation. The destruction of the  $\Delta F508$ -CFTR protein is due in

part to the activities of a group of different proteins collectively known as “chaperones,” which help ensure that other proteins are properly folded through a variety of mechanisms. For example, some chaperones assist in the initial folding of a protein, as it is being synthesized by the cell; others identify proteins that have adopted improper shapes, such as  $\Delta F508$ -CFTR, and target them for degradation; and, in some cases, chaperones may be able to refold misfolded proteins into a functional state.

In new research, using cells from both mice and humans, scientists have found that the chaperones Hsc70 and Hsp90 have a limited capacity to nudge  $\Delta F508$ -CFTR closer to the properly folded shape of normal CFTR protein, allowing a modest amount of chloride to flow through the channel. The researchers found that selective, chemical inhibition of Hsc70 caused the amount of  $\Delta F508$ -CFTR in the cell membrane to increase, indicating that this chaperone contributes to the destruction of the misfolded channel. However, this approach further showed that despite there being more of the protein in the membrane, the ability of chloride to move through the membrane either did not rise or diminished slightly depending on the type of cell used for the experiment: this indicates that Hsc70 also has the capacity to help  $\Delta F508$ -CFTR adopt a conformation closer to normal CFTR protein. In other words, the higher levels of protein in the membrane are offset by the fact that the proteins do not work as well, which is why net chloride transmission stays the same or diminishes. Selective inhibition of Hsp90 had little impact on the amount of  $\Delta F508$ -CFTR in the membrane, but significantly reduced the ability of the channels present to transmit chloride, indicating that Hsp90 can help  $\Delta F508$ -CFTR adopt a more functional conformation—presumably closer to that of normal CFTR protein—without promoting its degradation. Inhibiting both chaperones had an even more profound effect, reducing chloride transmission by more than half. Importantly, addition of Hsc70 and Hsp90—along with specific protein partners called co-chaperones—increased the ability of  $\Delta F508$ -CFTR to permit passage of chloride through the membrane. Experiments further suggested that when working with one co-chaperone, Hsc70 may promote  $\Delta F508$ -CFTR destruction, while with another co-chaperone Hsc70 might promote improved channel function.

These discoveries suggest that manipulating the activity of chaperones is worth exploring as a potential means of treating CF and perhaps other diseases caused by destabilized cell surface proteins.

*Bagdany M, Veit G, Fukuda R,...Lukacs GL. Chaperones rescue the energetic landscape of mutant CFTR at single molecule and in cell. Nat Commun 8: 398, doi: 10.1038/s41467-017-00444-4, 2017.*

### **Finding the Genetic Cause of a Form of**

**Hyperparathyroidism:** Researchers have identified genetic differences that can cause familial isolated hyperparathyroidism (FIHP), a discovery that could lead to improved testing for the disorder and may one day lead to improved treatment for people with either too much or too little parathyroid hormone (PTH). PTH stimulates release of calcium from bones when blood calcium levels are too low. FIHP is an inherited disease, and is one of several conditions characterized by high basal levels of PTH that lead to dangerous elevations of blood calcium levels. This calcium dysregulation in FIHP results in symptoms that may include kidney stones, osteoporosis, and neuromuscular problems like weakness, drowsiness, and depression. Other causes of excess PTH that may yield similar symptoms include various distinct syndromes, and most commonly benign or malignant tumors of the parathyroid gland—conditions that may be best treated in different ways. Understanding the genetics of FIHP would potentially allow it to be differentiated more easily and accurately from other causes of excess PTH, allowing clinicians to tailor treatment to patient needs. Toward this goal, researchers studied families that have FIHP to pinpoint specific genetic differences unique to people with this disease.

Focusing initially on a subset of gene regions in DNA from eight unrelated families in which some family members have FIHP, the scientists looked for rare genetic variations that were present in people with FIHP, but not in their family members without the disease. This approach helped them narrow the investigation to 30 genes of interest, which they examined in detail in 32 other families where two or more individuals had symptoms of FIHP. In this way, they discovered that FIHP-affected members of several of the families had one or more rare mutations in the gene *GCM2*. This gene encodes a protein known to be involved in development and function of the parathyroid gland by turning on other genes needed for parathyroid function. Interestingly, other *GCM2* variations had previously been shown to reduce expression of those genes, leaving people without enough PTH; and mice genetically engineered to lack *GCM2* do not develop functional parathyroid glands. Thus, it was surprising that some *GCM2* variations have the opposite effect, leading to too much PTH in people with FIHP. The research team showed that at least some of the *GCM2* mutations found

in families with FIHP increased the activation of other genes by the *GCM2*-encoded protein, resulting in excess PTH. Through additional experiments, they showed that some of the FIHP-associated *GCM2* mutations alter an important regulatory region that is needed to prevent over-activation of the other parathyroid genes.

Importantly, people with FIHP in most of the families that participated in the study were found not to have mutations in *GCM2*, suggesting that other genetic causes of FIHP remain to be discovered. But the discoveries that *GCM2*-activating mutations can cause excess PTH, while inactivating mutations can result in insufficient PTH, suggest this gene or the protein it encodes could be valuable drug targets for treating some people whose PTH levels are either too high or too low.

Guan B, Welch JM, Sapp JC, ...Agarwal SK. *GCM2-activating mutations in familial isolated hyperparathyroidism. Am J Hum Genet* 99: 1034-1044, doi: 10.1016/j.ajhg.2016.08.018, 2016.

### Innovations in Testing for a Rare Genetic

**Disease—Pompe Disease:** Researchers have developed methods that could significantly improve accuracy of testing for a rare genetic disorder called Pompe disease. Pompe disease results from lack of a protein called acid  $\alpha$ -glucosidase (typically abbreviated GAA), and causes progressive muscle weakness, enlarged heart, and other problems—and in severe cases can lead to death in early childhood. Fortunately, the disease is treatable, particularly if GAA deficiency is identified early. Because the disease can cause significant, irreversible damage before symptoms are apparent, it is of great importance to be able to screen newborn children to identify those who would benefit from treatment.

A currently available screening method uses a chemical that becomes fluorescent when acted on by GAA. This screen can clearly distinguish individuals with normal GAA from individuals who have severe Pompe. However, some people are born with intermediate levels of GAA activity. These individuals may have a milder form of Pompe in which symptoms manifest later in development, but they also may not have the disease at all. Because the fluorescence test cannot clearly differentiate these groups from one another, some babies may be treated for Pompe unnecessarily. To get around this problem, researchers developed methods that can distinguish much more subtle differences in GAA activity. In one new study, they used a test method they estimated to be 15

times more sensitive to differences in GAA activity than is the fluorescent method. Using dried blood samples from males and females, including 11 people with early onset Pompe, 12 with more mild, later onset Pompe, and 230 with GAA levels that are lower than normal, but who have no disease, the new screening method correctly identified all of the people who have the disease, while incorrectly identifying just 4 percent of the healthy individuals who have intermediate GAA levels as having possible Pompe disease. That is, there were no false negatives, while there was a very low rate of false positives. In contrast, the fluorescence method had a much higher rate of false positives, incorrectly identifying about 90 percent of samples with intermediate levels of GAA as being from individuals with possible Pompe disease. The new method could be scaled up to allow rapid screening of dried blood spots from newborns, suggesting it could be practical as a standard infant screen for the disease.

Infants with the most severe, early-onset form of Pompe disease require more aggressive treatment than do babies with the milder, late-onset form. To enhance the ability to distinguish small differences in GAA activity, scientists developed a similar test, adding an additional step, and using fresh samples of leukocytes (white blood cells), rather than dried blood spots. This approach yields about a 700-fold improvement in sensitivity relative to the fluorescence method. Because this method requires a fresh blood sample, it is less suitable for initial screening of a large population of newborns than the version that utilizes dried blood spots. But with the more sensitive assay the researchers could clearly distinguish individuals with early-onset from those with late-onset Pompe. Even this highly sensitive approach identified a few individuals with low GAA levels but no symptoms as having possible late-onset Pompe. This suggests that some people may need more GAA than others to maintain health. Further research may be warranted to determine what factors affect susceptibility to late-onset Pompe in people with intermediate levels of GAA.

Liao HC, Chan MJ, Yang CF, ...Gelb MH. *Mass spectrometry but not fluorimetry distinguishes affected and pseudodeficiency patients in newborn screening for Pompe disease. Clin Chem* 63: 1271-1277, doi: 10.1373/clinchem.2016.269027, 2017.

Lin N, Huang J, Violante S, ...Yu C. *Liquid chromatography-tandem mass spectrometry assay of leukocyte acid  $\alpha$ -glucosidase for post-newborn screening evaluation of Pompe disease. Clin Chem* 63: 842-851, doi: 10.1373/clinchem.2016.259036, 2017.

# Medicare To Provide Benefits for Diabetes Prevention and Treatment Stemming from NIDDK-supported Research

In April of 2018, the Centers for Medicare & Medicaid Services (CMS) is beginning coverage of a group-based adaptation of the lifestyle intervention for beneficiaries with prediabetes; this prevention approach was first pioneered in NIDDK's Diabetes Prevention Program (DPP) clinical trial. The original DPP showed that an intensive lifestyle intervention could reduce the likelihood of developing type 2 diabetes by 58 percent over 3 years among people at high-risk for the disease. For DPP participants who were at least 60 years old at the beginning of the clinical trial the intervention was even more effective, reducing the likelihood of developing type 2 diabetes by 71 percent over the same time period. On the strength of these findings, the NIDDK funded research to develop cost-effective adaptations of the intervention that could be delivered efficiently to millions of Americans who would stand to benefit. The Centers for Disease Control and Prevention scaled up one such adaptation, calling it the National Diabetes Prevention Program. CMS tested this adaptation in 2014 and 2015, concluding in a report from the CMS Office of the Actuary on March 14, 2016, that if offered as a benefit nationally, the program would, on average, be expected to save money and lead to better health for beneficiaries. Accordingly, the Secretary of Health and Human Services determined that the benefit would begin in 2018.

Similarly, NIDDK-supported research contributed to the development of continuous glucose monitors (CGMs)—technology that is now being covered

by CMS under Medicare Part B for beneficiaries who take insulin for either type 1 or type 2 diabetes. CGMs automatically test a wearer's blood glucose (sugar) levels every few minutes, helping them ensure that their dose of insulin is keeping them as near as possible to optimal blood glucose levels. The NIDDK has long supported the development of new tools and technologies for managing diabetes, including CGMs. For example, NIDDK-supported research contributed to the seminal finding that glucose levels in "interstitial" fluid in tissues under the skin reflected glucose levels in the blood, enabling the development of CGMs, which rely upon this route for glucose sensing. Today, all current CGM technology on the market benefitted from NIDDK support early in development, such as through grants to small businesses. At first, the U.S. Food and Drug Administration (FDA) only approved CGMs as a supplement to, but not replacement for, conventional monitoring, such as with blood glucose test strips. With the recent FDA approval of at least one CGM as a primary method of blood glucose monitoring for making diabetes treatment decisions, as of January 2017 (CMS-1682-R), CMS is covering certain CGMs.

These examples show ways in which past NIDDK research has led to improvements in care and prevention for people with or at risk for diabetes, and demonstrate how other recent and ongoing discoveries may lead to further improvements in the future.

# The SEARCH for Diabetes in Youth

## Expanding Understanding of Diabetes in Children and Young Adults

Diabetes is one of the most serious health challenges facing the United States, with rising numbers of both adults and children affected. Type 1 and type 2 diabetes can affect many parts of the body and are associated with serious complications, such as heart disease and stroke, blindness, kidney failure, and lower limb amputation. To combat diabetes and its associated complications, the NIDDK and the Centers for Disease Control and Prevention began the SEARCH for Diabetes in Youth study in 2000. Today, this unique study continues to provide critical information about diabetes among children and young adults in the United States.

Effective diabetes research requires accurate data on the scope and trends of the disease: how many children and adults have diabetes? Does diabetes affect some populations differently than others? What complications do people with diabetes encounter, and how does this disease affect their quality of life? The SEARCH study has been investigating these questions.

Currently, SEARCH is conducted at five centers, with research sites located across seven states. More than 27,000 SEARCH study participants represent a geographically and racially/ethnically diverse group of people who were under 20 years of age when they were diagnosed with type 1 or type 2 diabetes. These volunteers' participation has been critical in determining the extent of diabetes in the United States and its impact on different populations.

In the over 17 years since its inception, SEARCH has provided a wealth of information about the effects of diabetes on children and young adults. SEARCH investigators have assessed trends in diabetes diagnosis, prevalence, and health care, and have gathered data on complication rates and quality of life. SEARCH was instrumental in discovering that diabetes is frequently found among U.S. youth and that rates of both type 1 and type 2 diabetes diagnoses are increasing in both male and female youths of various racial/ethnic backgrounds and ages. SEARCH also demonstrated that many youths with diabetes are at risk for complications such as eye, nerve, heart, and kidney problems, and that this risk is particularly prominent

among those of racial/ethnic minority groups. For example, a recent SEARCH report showed that from 2002 to 2012, the annual increase in type 1 diabetes diagnosis among U.S. youth—when adjusted for age, sex, and racial/ethnic group—was 1.8 percent, and the annual increase in type 2 diabetes diagnosis was 4.8 percent. However, SEARCH investigators found that the increase varied across racial/ethnic groups. A significant annual increase was found in type 2 diabetes diagnoses among all racial and ethnic groups except non-Hispanic Whites. Type 1 diabetes incidence also increased significantly more in Hispanic youths than in non-Hispanic White youths.

SEARCH investigators also recently published several reports on diabetes complications in youth. They estimated that by about age 21, approximately 32 percent of SEARCH study participants with type 1 diabetes and 72 percent of participants with type 2 diabetes would have (or be at high risk for) at least one diabetic complication. SEARCH researchers also described high rates of diabetic peripheral neuropathy in study participants (seven percent and twenty-two percent in youth with type 1 and type 2 diabetes, respectively) and found that poor blood glucose (sugar) control and greater waist-to-height ratio were associated with elevated cholesterol and lipid levels in the blood of youth with type 1 diabetes.

Increases in type 1 and type 2 diabetes diagnoses in youth are worrying because these populations face unique challenges in managing their diabetes and may be at greater risk of complications later in life due to their long disease duration. These findings suggest that early monitoring of youth with diabetes could lead to earlier diagnosis and treatment of complications, and ultimately to better health. Additionally, the variation in diabetes incidence along racial/ethnic lines suggests that the future burden of these diseases and their complications may fall disproportionately upon certain groups.

SEARCH is planned to continue at least through 2020, with SEARCH investigators building upon past progress to investigate important questions about diabetes trends and outcomes in youth.

# NIDDK Director Testifies on Type 1 Diabetes Research

On July 26, 2017, 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 “Progress Toward a Cure for Type 1 Diabetes: Research and the Artificial Pancreas,” 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.

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 the research include:

- progress toward the development of artificial pancreas technology—devices that automate blood glucose sensing and insulin administration—and four new clinical trials testing these technologies (see Story of Discovery in this chapter);
- results of a clinical trial testing islet transplantation as a treatment approach for people with difficult-to-control type 1 diabetes;
- progress toward engineering a biological cure from NIDDK’s Human Islet Research Network; and
- results of a comparative effectiveness clinical trial testing different treatments for diabetic eye disease.

Testifying with Dr. Rodgers were Mr. Paul Sparks, an actor and patient advocate; JDRF Children’s Congress Chairwoman Ms. Angie Platt; and JDRF Children’s Congress Delegates Charlie Albair, age 10, Lorynn Watt, age 17, and Jonathan Platt, age 14.



Shown at the dais (left to right): Sen. Elizabeth Warren (D-MA), Sen. Bob Casey (D-PA), and Sen. Susan Collins (R-ME, Committee Chair). Photo credit: Larry Lettera



NIDDK Director Dr. Griffin P. Rodgers testifying before the Senate Special Committee on Aging. Also shown at the table (left to right): Actor Paul Sparks, Children’s Congress Delegates Charlie Albair and Lorynn Watt, and 2017 Children’s Congress Family Chair Angie Platt. Photo credit: Larry Lettera

The *Special Diabetes Program*’s current authorization expired September 30, 2017. Should the *Program* be renewed, an extension would provide NIDDK with an opportunity to support new and emerging research in type 1 diabetes and its complications. To solicit input on future directions that could be supported with the new funds, the NIDDK convened a planning meeting in April 2017, which was held under the auspices of the statutory Diabetes Mellitus Interagency Coordinating Committee. At the meeting, a panel of external scientific experts and a lay representative provided input on concepts for potential new research initiatives developed by the NIDDK, other institutes at NIH, and the Centers for Disease Control and Prevention; the panel also provided input on the continuations of programs that are already supported by the *Special Diabetes Program*. 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. If the *Special Diabetes Program* is renewed, it would be poised to continue its exceptional track record of supporting cutting-edge type 1 diabetes research.

## Artificial Pancreas for Managing Type 1 Diabetes: Cutting-edge Technology 50 Years in the Making

Aristotle once said: “the whole is greater than the sum of its parts.” This could be said for a revolutionary new management tool for type 1 diabetes: artificial pancreas technology. This technology builds on decades of advances in type 1 diabetes management—such as the advent of insulin pumps and continuous glucose monitors (CGMs). Now, with long-standing support from NIDDK, private funding entities, and industry, researchers have combined these individual “parts” to develop a “whole” device that can benefit people more than each component alone.

A major milestone occurred in 2016 with the first U.S. Food and Drug Administration (FDA) approval of a commercial “hybrid” artificial pancreas device developed by industry; NIDDK-supported research contributed to the early development of the approved device. This milestone promises to be just the beginning of a new era in the development of cutting-edge artificial pancreas technologies that could benefit people with type 1 diabetes.

### What Is an Artificial Pancreas?

Artificial pancreas technology strives to mimic the exquisite control of blood glucose (sugar) maintained by a healthy pancreas. The pancreas produces a number of hormones, including insulin and glucagon, to enable the body to use glucose for fuel and store glucose when it is in excess, or release it from stores when glucose levels get too low. In people with type 1 diabetes, the immune system destroys insulin-producing pancreatic  $\beta$  cells. Thus, people with the disease need to administer

insulin for survival and measure blood glucose levels to determine how much insulin to take.

An artificial pancreas, or “closed-loop system,” is technology that would replace the function of the pancreas that does not work in people with type 1 diabetes: delivering insulin in response to blood glucose levels. It links three technologies: (1) a sensor, such as a CGM, that measures blood glucose levels and sends data to a computer; (2) an insulin delivery device, such as an insulin pump; and (3) a computer that calculates the amount of insulin needed and instructs the pump to deliver it. The goal is that the technology would require minimal human input, thus acting much like a healthy pancreas.

The need for improved type 1 diabetes management tools is underscored by the 1993 results of the NIDDK-supported landmark Diabetes Control and Complications Trial (DCCT). This trial and the ongoing follow-up study showed that intensive blood glucose control greatly reduced the development of eye, kidney, and nerve complications in people with type 1 diabetes, and even suggested that the increased mortality historically seen in those with type 1 diabetes can be reduced or eliminated through careful management of blood glucose. The ongoing follow-up study has continued to demonstrate long-term, improved health benefits of early and intensive glucose control. The intensive control regimen is difficult, however, because it requires that people frequently and carefully monitor blood glucose levels throughout the day with finger sticks or a CGM and determine how much insulin to

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administer. The calculation is a “best guess” that requires monitoring and modification, particularly when everyday experiences like eating, exercising, and illness can affect blood glucose levels in unexpected ways. Additionally, the DCCT showed that intensive control increased people’s immediate risk for hypoglycemia, or dangerously low blood glucose levels. With the difficulties of intensive glucose control and the threat of hypoglycemia, people with type 1 diabetes still rarely achieve recommended glucose levels to reduce the risk of complications. Thus, there is a critical need for better management tools, such as artificial pancreas technology that could help people achieve recommended blood glucose control while preventing hypoglycemia and reducing management burden.

## Early Artificial Pancreas Development

The idea of replacing the pancreas with an artificial one is not new. In fact, the first experimental artificial pancreas was developed in 1964. The device—about the size of a large backpack—measured blood glucose levels and delivered insulin intravenously and thus was not intended for everyday use in free-living conditions. Despite this early effort, the technologies it used to measure blood glucose levels and deliver insulin would take decades to develop for daily, home use. Additional progress was made in the mid- to late-1970s with several research groups publishing results of their closed-loop studies, as well as with the introduction of the first commercial artificial pancreas device intended to be used for inpatient hospital use—the Biostator. These big and bulky devices also used intravenous glucose sensing and insulin administration. While these advances were important for showing that this technology was feasible, much more research was needed to develop artificial pancreas technology that could be used in everyday life.

## Advances in the “Parts” of Artificial Pancreas Technology

Before the artificial pancreas as a whole could be improved, it was necessary to improve the individual parts. One key component of artificial pancreas technology is the insulin pump, which was developed with NIH and industry support. Insulin pumps are small devices that deliver insulin continuously in a small basal amount and provide larger boluses when needed, for example at mealtime. They provide an alternative to multiple injections of insulin and deliver insulin subcutaneously (*i.e.*, under the skin). The first commercially available insulin pump was introduced in 1978, and was bulky and not very user-friendly, relative to more recent devices. However, clinical studies of insulin pump therapy beginning in the late 1970s, and supported by the NIDDK and others, demonstrated that pumps were an effective alternative to multiple daily insulin injections, which helped promote their adoption into clinical practice. The development of insulin pumps made the DCCT possible, and the results of the DCCT demonstrating the importance of intensive blood glucose control stimulated future development of improved pumps.

Insulin pumps are widely used today and have improved over the years. For example, in 2013, the FDA approved a device, consisting of a pump linked to a sensor, that suspends the delivery of insulin when glucose levels reach a preset low threshold. This approach helps reduce the risk of hypoglycemia from excess insulin. Further research has led to newer devices that *predict*, based on trends in the sensor data, when this level will be reached and preemptively suspend insulin delivery. Such devices have been tested in NIDDK-supported studies and are being used in today’s artificial pancreas systems. Advances in insulin engineering, which has produced faster acting and longer duration forms to use in insulin pumps, have also been important to artificial pancreas development.

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Another critical component of artificial pancreas technology is a CGM, which provides a way to measure glucose levels every few minutes. For years, people with diabetes monitored their glucose levels with urine tests, which recognized high but not dangerously low glucose levels and reflected past, not current, glucose levels. By the 1980s, blood glucose meters had been developed and were widely used, enabling people to self-monitor blood glucose levels at home with finger sticks.

By the late 1990s, measurement of glucose in the blood had proven useful for several checks per day, but it was not readily amenable to continuous monitoring by a device that people could use in their daily lives. Researchers supported by the NIDDK and others found another route that would be safe and practical for continuous monitoring: glucose levels in “interstitial” fluid in tissues under the skin reflected glucose levels in the blood. One aspect of this advance was the development of miniaturized electrodes to measure the reaction of glucose with an enzyme called glucose oxidase to assess glucose levels in the small fluid volumes present under the skin. A continuous monitor was first approved by the FDA in 1999. However, the glucose values obtained from this device could only be assessed retrospectively, not in real time. Further research led to FDA approval, in 2006, of next-generation continuous monitors that allowed real-time glucose sensing. Since then, CGMs have continued to improve, are used by some people with diabetes, and are part of today’s artificial pancreas systems.

With the advances in the two major parts of artificial pancreas systems, also needed was a way to link them together. Thus, at the same time, researchers were working on the mathematical part of artificial pancreas technology by developing algorithms so that a computer can calculate how much insulin is needed based on CGM readings and tell the insulin pump to deliver it. Many algorithms being tested

in some of today’s artificial pancreas devices, called “model predictive control algorithms,” have their roots in an NIDDK-supported 1979 study that evaluated the feasibility of using a mathematical model of glucose disappearance to estimate insulin sensitivity. Additionally, computers themselves have significantly advanced over the last few decades, becoming smaller, more portable, faster, and capable of advanced processing. These technological advancements laid the foundation for research to link together these individual technologies to develop next-generation artificial pancreas devices.

The NIDDK supported development of these components—and continues to support the next generation of these devices—through grant awards to small businesses, as well to academic institutions. Many of the businesses were created by academic investigators to work toward commercializing the new technologies. Often these NIDDK-supported small businesses became larger companies, or the technologies were sold to larger businesses toward bringing the technologies to market. These important investments in small businesses played a significant role in ensuring that these advances moved from the laboratory to the people who could benefit from them.

## **A New Era of Artificial Pancreas Research—Making the Whole Greater Than the Sum of Its Parts**

A new era of research began in the 2000s as these technologies were becoming available. A 2006 NIDDK-supported study conducted by researchers in industry and academia was the first to show the feasibility of simultaneously using a continuous glucose monitor for subcutaneous glucose sensing and a subcutaneous pump for insulin delivery in an artificial pancreas system in people with type 1 diabetes. This study propelled the field toward the advanced trials being conducted today.

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Since that time, there has been tremendous progress in developing artificial pancreas technologies and testing them in people, which has been supported, for example, by the NIDDK, JDRF, the Leona M. and Harry B. Helmsley Charitable Trust, and industry. The first small clinical trials, in the early 2010s, were conducted in hospital or clinical settings using laptops to control the artificial pancreas device and with constant monitoring by researchers and study staff, restricting participants' activity. The trials showed very promising results. For example, in 2010, results from the first randomized controlled trial showed that use of an artificial pancreas device reduced the risk of nighttime hypoglycemia in children and adolescents with type 1 diabetes compared to standard therapy. Two years later, NIDDK-supported studies showed that artificial pancreas technology could improve blood glucose levels without increasing hypoglycemia in adolescents and adults with type 1 diabetes.

The promising results from hospital-based trials, as well as the advent of portable smartphone technology to replace laptops, enabled the next step—moving to “transitional” outpatient settings, such as hotels, that more closely mimicked free-living conditions but still allowed the participants to be monitored closely by study staff. For example, in 2013, NIDDK-supported researchers working with adult participants who had type 1 diabetes showed that smartphone technology could be used to run a closed loop system. Another group of NIDDK-supported researchers tested a “bihormonal” artificial pancreas system that delivers two different pancreatic hormones—insulin to lower blood glucose levels and glucagon to increase them—with the hope of more closely mimicking the function of a healthy pancreas. They tested their wearable, automated device in adults staying at a hotel, and in adolescents at a diabetes camp. Results reported in 2014 showed that compared to usual care, participants had lower mean glucose levels and reduced episodes of hypoglycemia. In fact, the device allowed nearly all participants to achieve

recommended levels of blood glucose control. Armed with positive results from transitional settings, scientists took the next step: moving to at-home studies to replicate real-life conditions. The first studies tested overnight artificial pancreas use, as it is easier for the artificial pancreas to control blood glucose levels when it is not being challenged with daytime activities, like meals and exercise, that cause unpredictable blood glucose swings. In 2014, NIDDK-supported researchers studied adolescents with type 1 diabetes who participated in school and other activities during the day using standard diabetes management tools, and wore an artificial pancreas at home at night. Results showed that unsupervised, *i.e.*, not monitored continually by study staff, closed-loop control at night improved glucose control during the day and night, and reduced the number of episodes of nighttime hypoglycemia.

More recently, researchers have begun to test day and night artificial pancreas use under free-living, home-use conditions, for a short duration in small numbers of people who went about their daily lives (*e.g.*, going to school or work) and had no restrictions on their diet and exercise. For example, in 2016, NIDDK-supported researchers showed that unsupervised day-and-night artificial pancreas use was feasible and safe in adolescents with type 1 diabetes. In 2017, NIDDK-supported researchers found that day-and-night use of a bihormonal artificial pancreas improved blood glucose control compared to conventional insulin pump therapy in adults. The positive results of these and other studies testing 24/7 closed-loop control at home under free-living conditions underscore the promise of this therapy for everyday use. Encouraged by this progress in moving from carefully controlled studies in hospitals to hotels to free-living conditions, researchers are now testing artificial pancreas technologies in even more challenging environments, like the extended vigorous outdoor exercise of snowboarding camp.

# STORY OF DISCOVERY

## First FDA Approval of a Hybrid Artificial Pancreas Device

A key milestone in artificial pancreas technology development was achieved in 2016 with the publication of results of an industry-supported trial. It was the largest outpatient artificial pancreas study done to that date, including 124 people with type 1 diabetes ages 14 and older who used the device for 3 months. The study tested a “hybrid” system: although the device automatically adjusts insulin levels, it requires users to count and enter mealtime carbohydrates. Thus, it is not completely automated and still requires human input. The study showed that the hybrid artificial pancreas system was safe for use. Based on the results of the trial, in September 2016 the FDA announced that it approved the device for use in people 14 years of age and older with type 1 diabetes—the first commercial hybrid artificial pancreas to be FDA approved. The NIDDK supported early research that contributed to the development of the approved device, such as testing some initial versions of the different components and algorithms that preceded the currently approved device. To improve upon this approved device, the NIDDK is supporting research comparing the current computer algorithm used in the device to another algorithm that might provide better control.

## Collaborations Stimulate Progress

Critically important to the success of this effort have been partnerships that the NIDDK has forged with FDA, and voluntaries such as JDRF and the Leona M. and Harry B. Helmsley Charitable Trust, all of whom are working toward the same goal of making the technology available to people with type 1 diabetes. For example, the NIDDK, FDA, and JDRF have sponsored a series of scientific workshops that have brought together experts in the field to stimulate discussion of the current state of the art, technical challenges and possible solutions, safety issues, and next steps. Since 2007, the NIDDK

and FDA have also worked together on a federal Interagency Artificial Pancreas Working Group, which has been instrumental in promoting the field. NIDDK collaboration with JDRF and the Leona M. and Harry B. Helmsley Charitable Trust has also resulted in coordinated efforts with more efficient investment of resources and has greatly expedited progress. Many of the studies described above have also had support from these funding entities.

## Future Research Directions

The FDA approval of the hybrid artificial pancreas device is a critical milestone, moving the technology from a research setting to the marketplace. However, there are still limitations with the approved device. For example, it requires users to prick their fingers to determine blood glucose levels to calibrate the device’s glucose sensors. Other approved sensors, however, do not require this type of calibration and may be incorporated into future devices. Also, the device requires users to count carbohydrates and put that information into the system at mealtimes. Thus, the device is not completely automated and still places management burden on patients. Still needed are improved components of artificial pancreas systems, as well as research testing different systems so that people could have choices about which system may best suit their needs and lifestyle.

Toward these goals, the NIDDK continues its vigorous support of artificial pancreas research through grants to small businesses and academia. For example, the Institute is supporting four new clinical trials to test technologies in larger groups, with wider age ranges, over longer periods of time, and in largely unrestricted conditions. These trials are generating data to determine whether the technologies meet the safety and efficacy requirements for FDA approval of these systems. Research is also ongoing toward improving the “parts” of artificial pancreas technology, such as developing implantable glucose

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sensors and novel hormone formulations, which could help overcome some of the limitations of current systems toward making fully automated devices. Additionally, developing implantable components is important toward achieving the goal of giving people with diabetes freedom from having devices attached to their bodies and having to manage the devices. Because new technology will only benefit people if they can use it, research optimizing incorporation of artificial pancreas technologies into clinical care and enhancing their “usability” is essential, so that people are not overwhelmed with excessive data volume or complexity. This includes research on special populations—such as young children, people with more difficult-to-control diabetes or vision complications, pregnant women, and older adults—to ensure that all people with type 1 diabetes could use and benefit from this new technology.

This technology could also be beneficial beyond type 1 diabetes. Many people with type 2 diabetes manage their diabetes with insulin. Hypoglycemia in this population is a major cause of morbidity, and artificial pancreas technology may help people using insulin to manage their type 2 diabetes to avoid this dangerous complication while improving their blood glucose control. A recent study found that adults who used continuous glucose monitoring on a daily or near-daily basis for 6 months showed improved blood glucose control. Another study tested artificial pancreas technology in people with type 2 diabetes

who had not previously used insulin to treat their diabetes. This study found that participants spent more time in the target glucose range and less time in hyperglycemia. This small, short trial suggested that artificial pancreas use in people with type 2 diabetes could improve glucose levels, especially overnight, without increasing the risk of hypoglycemia. There is also potential for these devices to be used in hospital settings to control glycemia; lack of glycemic control is a major problem, even in people who do not have diabetes, but who have hyperglycemia due to trauma or a critical illness.

While the artificial pancreas represents near-term technology that could improve the health of people with type 1 diabetes, it is not a cure. Therefore, the NIDDK also supports research toward a biological cure for type 1 diabetes, such as developing therapies to replace pancreatic  $\beta$  cells that have been destroyed by the immune system.

With the significant progress to date and with additional research, it is expected that the FDA approval of the first hybrid artificial pancreas device is just the beginning of a new era of technologies that can improve the short- and long-term health of people with type 1 diabetes, while also reducing management burden. Artificial pancreases truly represent a life-changing advance for people with this chronic disease.

# PATIENT PROFILE

## Jadah: Paving the Path to Preventing Type 1 Diabetes



Jadah (right) and Travis

“All that matters to me is that I’m making a difference,” explains Jadah matter-of-factly when asked why she is part of type 1 diabetes research. Although she hasn’t been diagnosed with type 1 diabetes, 14-year-old Jadah is at high risk of developing clinical symptoms of the disease, such as high blood sugar (glucose) levels. With the goal of making a difference, she is participating in a clinical trial in the NIDDK’s Type 1 Diabetes TrialNet (TrialNet) to test a strategy for stopping the disease process at its earliest stage. People at this early stage have biological factors (autoantibodies) that characterize the beginning of the disease process and are at very high risk of developing clinical symptoms, but they still have normal blood sugar levels. Researchers are also developing therapies for individuals with diagnosed diabetes, which is marked by high blood sugar levels. Type 1 diabetes is an autoimmune disease in which a

**“All that matters to me is that I’m making a difference,” says Jadah, of her participation in type 1 diabetes research.**

person’s immune system destroys the cells that make insulin. People with the disease (or parents of children with the disease) must carefully monitor blood sugar levels and administer insulin. It is very difficult for people with type 1 diabetes to achieve a balance between too much sugar in the blood and too little. Too much sugar could lead to serious eye, nerve, and kidney complications; too little sugar could lead to confusion, loss of consciousness, and death. While a goal of type 1 diabetes research is a cure for people who have the disease, a way to prevent the disease could spare future generations from type 1 diabetes.

### The Benefits of Learning Type 1 Diabetes Risk

Type 1 diabetes has been part of Jadah’s family life since 2009, when her brother, Travis, was diagnosed. Because people with a first-degree relative with type 1 diabetes have a 15 times greater likelihood of developing the disease, scientists believe that Jadah and others like her could hold the clues for

uncovering how the disease develops and identifying a prevention strategy.

Research has revealed that before type 1 diabetes symptoms appear, the immune system produces specific autoantibodies—proteins that can be detected in the blood. TrialNet’s Pathway to Prevention study screens first- and second-degree relatives of people with type 1 diabetes for these autoantibodies. From the number of different autoantibodies in a person’s blood, they can determine that person’s risk of developing type 1 diabetes. As of January 2018, TrialNet researchers had screened over 160,000

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individuals. TrialNet offers yearly rescreening for children under 18 years of age, so a total of over 250,000 screenings have been conducted to date.

Jadah's mom, Susie, had first seen TrialNet information booths at fundraising walks held by the type 1 diabetes advocacy organization JDRF. Susie was amazed that researchers could determine if there's further risk of type 1 diabetes in her family. But she was also concerned: would she want to know if Jadah were at high risk?

Susie learned that there were several reasons to find out Jadah's risk status. People screened by TrialNet and found to be at high risk are eligible to be monitored for the appearance of more autoantibodies

and/or for an increase in their blood sugar levels, which

would indicate development of type 1 diabetes. TrialNet's tests are likely to detect progression to type 1 diabetes before the appearance of potentially serious clinical symptoms. For example, early detection decreases the likelihood of a life-threatening complication, called diabetic ketoacidosis (DKA), at diagnosis.

Travis had DKA when he was diagnosed, and Susie didn't want the same thing to happen to Jadah. Additionally, if Jadah were found to be at high risk, she could be eligible to participate in a TrialNet prevention trial. Susie realized she did want to know, feeling that "[it] is incredible that you could find out ahead of time if there's a [high] risk [of type 1 diabetes]." But she and Terry, Jadah's dad, felt strongly that it was Jadah's decision.

## From Reluctant Screener to Trial Participant

It wasn't learning about her risk for type 1 diabetes that concerned Jadah most—it was the blood draw needed for the test. "I just hated needles when I was

a kid. I mean, I was so scared of them," she recalls. While visiting Travis at a diabetes camp in 2011, Jadah reluctantly agreed to be screened. Although she did the blood draw, the experience was too much for her. "Right after that I just wanted to leave that camp without even saying goodbye to my brother," she remembers.

Not long after, Susie and Terry received a call from TrialNet telling them that Jadah was at high risk, but that another blood draw was needed to confirm the result. This time Jadah said no. Susie and Terry tried everything they could think of to change Jadah's mind, but she held her ground for 4 years. In the meantime, Susie was constantly worried, wondering if every time Jadah seemed sick she was really showing early signs of type 1 diabetes. Eventually, it was Jadah's

grandmother who changed her mind about the rescreening. "I just thought that

***"It's not like you are in a study. It's like you are hanging out with your friends," says Jadah of her experience in a TrialNet clinical trial.***

I should take her advice," says Jadah, "...I thought this was something that I could do for her, that she would want me to do."

In April 2015, Jadah went to her local University of Minnesota TrialNet site for her second screening and shortly after learned the result, which confirmed the earlier screening: she was at high risk for developing type 1 diabetes. Because she was at high risk for the disease, but had not yet developed it, that September Jadah was offered a unique opportunity to join a clinical trial testing a drug for prevention of type 1 diabetes.

Whether or not to enroll in the trial wasn't a difficult decision for Jadah. She had made friends with the staff at the University of Minnesota TrialNet site and enjoyed spending time with them. "I loved these nurses so much that I wanted to be in the study right away, just so I could be with these nurses," says Jadah. Susie shared her daughter's enthusiasm for the trial, and they enrolled her.

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## Taking Part in the Abatacept Prevention Trial

Jadah is participating in a trial testing the agent CTLA-4lg, also known as abatacept, for its ability to delay or prevent type 1 diabetes in people at high risk for the disease. In 2011, TrialNet investigators found that over a 2-year period, abatacept slowed disease progression for 6 to 9 months, compared to placebo, in people newly diagnosed with type 1 diabetes. This resulted in higher insulin production in the people who had been treated with abatacept. Moreover, this improvement persisted for 1 year after the drug was discontinued. These results suggested that abatacept could be used in combination with other agents for a possibly more robust and prolonged effect in people newly diagnosed with type 1 diabetes,

***“Being able to have the opportunity to be part of this [research] is amazing,” Susie remarks. “We want to stop type 1 diabetes in this generation, and we are excited to be a part of this important research.”***

and that it should be tested for its ability to prevent the disease in those at high risk. This led to the trial in which Jadah is participating.

Jadah's experience in the trial required her to make three visits to the University of Minnesota TrialNet site in the first 4 weeks, then one visit a month for 11 months. Each visit took about 2 hours, including a 30-minute infusion of the drug or a placebo, followed by 1 hour of observation to make sure there were no side effects or complications, like an allergic reaction. Jadah and Susie turned the visits into a bonding experience, going out to breakfast or lunch afterwards before returning Jadah to school.

## A Bond Inspires the Future

Now that Jadah has finished her treatment course, she only returns to TrialNet for blood tests every 3 months and has her blood sugar levels tested every 6 months. She could even do the blood tests at her local clinic, but Jadah quickly said no to that. “Even if you're in there for 5 minutes,” she explains, “it's worth it because they [the nurses at the University of Minnesota TrialNet site] take their

time and they make you feel comfortable... It's not like you are in a study. It's like you are hanging out with your friends.” The TrialNet staff have even inspired her to work toward becoming a pediatrician in the future, as long as she can still have time to visit the lake, camp, and ski.

As for the possibility that she might develop type 1 diabetes, Jadah says, “I know that, even if I do get diagnosed, I'll still have my family, and they're going to know what to do. I'm going to know what to do because I see Travis doing it.”

Susie is so grateful that Jadah has had this wonderful experience and what she views as the chance to

be part of changing history. “Being able to have the opportunity to be part of

this [research] is amazing,” Susie remarks. “We want to stop type 1 diabetes in this generation, and we are excited to be a part of this important research.”

For now, Jadah and her family will continue to raise funds for type 1 diabetes research—with JDRF walks and, for Susie this past summer, through JDRF's Ride to Cure Diabetes—while they eagerly await the results of the abatacept prevention trial. Does Jadah wonder whether she received the drug or placebo? “Nope, I don't wonder at all,” she answers. “I just want to find a cure for all the other people out there who have type 1 diabetes.” Jadah is ready to participate again, if needed, in another prevention trial or in a trial for people who are newly diagnosed, if she develops type 1 diabetes.

Jadah and her family encourage anyone who can to get screened by TrialNet and to consider participating in research. “The sooner people get involved, the better we are going to be in the future,” says Susie. “We can't wait... All we need right now is participants [in clinical trials]. If we get participants, we'll get a prevention and a cure.”

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## Kinyatta: Dedicated to the Search for Better Health for People with Diabetes



**Kinyatta**

Kinyatta is, in her own words, “a helper.” That helping spirit shines through in her work as an instructional assistant co-teaching the first grade. It also shows in her long-standing participation—for 15 years and counting—in the SEARCH for Diabetes in Youth study, a unique research study committed to improving the quality of life of children and young adults with diabetes.

### Learning by Example

Kinyatta's participation in SEARCH is a gift to medical research and all those with diabetes, but it is just one way she works to help children. Kinyatta has always wanted to be an educator. To find the reason, you need look no further than Kinyatta's own first-grade teacher. “She was awesome,” Kinyatta says. One particularly memorable example involved a classroom hamster named Fluffy. In first grade,

Kinyatta loved Fluffy so much that she would cry when she had to go home and leave Fluffy behind. Then, one day, her teacher arrived at her home, with a hamster of her very own for Kinyatta to keep. Referring to that generous gift, Kinyatta says, “Those are the kind of things that I do for my kids.” Whether it is listening to and supporting the children, buying them a snack if they are hungry, or making sure they have the right clothes for a school performance, Kinyatta tries to be there for her students. “I just want to be that teacher that kids can go to with their problems. If they're having a bad day, they can talk to me. That's just the kind of person I am.”

### Living with Type 2 Diabetes

Kinyatta was diagnosed with type 2 diabetes when she was 12 years old. She had gained weight between third and sixth grades, and her mother, a nurse, was concerned. She brought up the issue to Kinyatta's doctor, and a blood test showed that Kinyatta's blood sugar (glucose) level was higher than normal. In type 2 diabetes, the body becomes resistant to the action of insulin, a hormone that controls blood sugar levels, and the pancreatic cells that produce insulin also don't function normally, causing blood sugar levels to rise.

Although her family had a history of type 1 diabetes, Kinyatta didn't know much about type 2 diabetes when she was diagnosed. Her doctors gave her basic information about the disease, showed her how to monitor her blood sugar level with a blood glucose meter, and taught her how to recognize the signs of high and low blood sugar. They also recommended that she manage her weight through diet and exercise. Kinyatta took nutrition classes

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that helped her make changes to her diet, such as controlling her portions and making healthy choices about what to eat.

Kinyatta had already picked up a lot of unhealthy eating habits, so changing what and how much she ate wasn't easy. Her family's support helped. They changed their diet as well, and joined Kinyatta in her efforts to get more exercise. She and her mother would walk every day. A few of her teachers knew what she was going through and supported her, as well.

Still, having type 2 diabetes added an extra complication to the already challenging transition to middle school. Other kids noticed that Kinyatta ate differently than they did, and some asked why she didn't eat candy, for example. Kinyatta didn't want to tell them she had

type 2 diabetes, so she would sometimes try to deflect the questions about her

choice of foods, saying it was just what she liked to eat. "It was little things like that, that I would have to fight off," Kinyatta says. "I've never been a self-conscious person, but...you know, as a child that stuff starts to get to you."

But Kinyatta's efforts paid off: she lost a lot of excess weight over the next few years. She also stayed active throughout high school by participating in color guard and winter guard, marching with the school band, and taking dance classes. "I always tried to just stay healthy," Kinyatta remembers.

## SEARCHing for Better Health

About a year after she was diagnosed, Kinyatta was asked to join a diabetes research study called the SEARCH for Diabetes in Youth.

SEARCH is currently the largest, most diverse study of diabetes among U.S. youth ever conducted. The

study is funded by the Centers for Disease Control and Prevention and the NIDDK, and SEARCH investigators seek to answer questions such as: how many children have type 1 or type 2 diabetes? How does that number change over time? What complications are these children experiencing as they age? How does having diabetes affect their lives? More than 27,000 volunteers with either type 1 or type 2 diabetes have participated in SEARCH, providing the information to help answer these questions.

Kinyatta agreed to become one of them.

Kinyatta says that she always feels well informed during her SEARCH visits. First, SEARCH coordinators contact her to begin the process. She is then sent

paperwork or surveys to fill out beforehand to help expedite her visit. Once she arrives at the

appointment, the SEARCH staff go over the visit with her in detail, describing what questions they are investigating, what tests they will run, and how they will use the results. The visit can take most of the day and can involve a variety of questionnaires and tests depending on the particular information the study is collecting at that time. For Kinyatta, some of those tests have included having her heart monitored, having her eyes examined, screening for depression, and taking part in a sleep study.

Being in SEARCH has also been educational in several ways. The SEARCH staff share Kinyatta's test results with her, giving her information about her own health. She's also learned about type 2 diabetes and strategies to manage the disease. "I'm really thankful for [the study staff], because I wouldn't know what I know now about diabetes if it wasn't for SEARCH."

***When Kinyatta made diet and lifestyle changes after her diagnosis, her family's support helped. They changed their diet as well and joined Kinyatta in her efforts to get more exercise.***

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Since the study began in 2000, SEARCH investigators have used data provided by volunteers like Kinyatta to assess not only how widespread diabetes is in the population, but also clinical and public health implications of the disease. As a result, SEARCH has provided a wealth of information about the effects of diabetes on children and young adults, including data on the total number of youth with diabetes in the United States, the number of new cases per year, and trends in diabetes diagnosis.

Findings from SEARCH are also providing data on the complications youth with diabetes are experiencing and how diabetes

affects their quality of life. For instance, before SEARCH it was unclear how soon signs of various diabetic complications

appear in children and young adults. All SEARCH participants were under 20 years of age when they were diagnosed, and the tests that they have participated in at SEARCH study visits have provided sobering news about diabetes complications in those who develop type 1 or type 2 diabetes early in life. A recent SEARCH analysis estimated that by about age 21, approximately 32 percent of SEARCH participants with type 1 diabetes and 72 percent of participants with type 2 diabetes would have at least one complication from diabetes or would be at high risk for a complication such as kidney, heart, nerve, or eye disease. These findings suggest that screening for risk factors and early monitoring of youth with diabetes could result in prevention or earlier diagnosis and treatment of complications. This could ultimately contribute to the goal of better health over the lifespan.

SEARCH is planned to continue at least through 2020. Such long-term studies offer valuable insights

into health trends over time, and they depend upon volunteers willing to participate for many years, as Kinyatta has. Kinyatta has no plans to quit, either. She says, “to see all the progress that I’ve helped them make is amazing to me.” She’s made strong connections with the SEARCH study staff, and they are often the first to send her a birthday card every year. “It’s just been a good experience,” Kinyatta says.

## Following Her Dreams

Kinyatta says that her diabetes status has not changed much since she was diagnosed. Her

HbA1c (a measure of her blood sugar level over time) has remained steady, and her doctors’ recommendations have been to continue

exercising and eating a healthy diet. Kinyatta takes this advice to heart. She feels that making healthy food choices and staying active helps keep her diabetes under control, which lets her avoid the need for diabetes medications and reduces her chances of having diabetic complications later in life.

Maintaining a healthy lifestyle can still be challenging, and Kinyatta admits that she doesn’t always do as well at it as she’d like. She’ll go through “health kicks” and cravings for unhealthy food the same as anyone else,

but through it all she tries to focus on the long-term goal: staying active and being mindful of what she eats. She goes to the gym and

takes walks, aiming to maintain her weight, and she continues to put those childhood nutrition classes to good use. When it comes to making food choices, she says, “I’m really good at saying, ‘Well, if I had this, I don’t need this.’” Such trade-offs—a not-so-healthy breakfast in the morning, for instance, and then a salad for lunch—help her maintain balance in her diet.

***“I love the connections that I’ve made with the people that work for SEARCH,” Kinyatta says of the research study staff. “It’s just been a good experience.”***

***“That’s the one thing that I’ve gotten out of my life: you can’t let people deter you from your dreams.”***

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Kinyatta still doesn't share the knowledge that she has diabetes widely, and some people she's known her whole life still haven't guessed. Kinyatta thinks it's because many people mistakenly believe that all people who have type 2 diabetes use insulin. But now Kinyatta is more likely to be forthcoming about her own experiences, pointing out that even those not on insulin therapy can have type 2 diabetes.

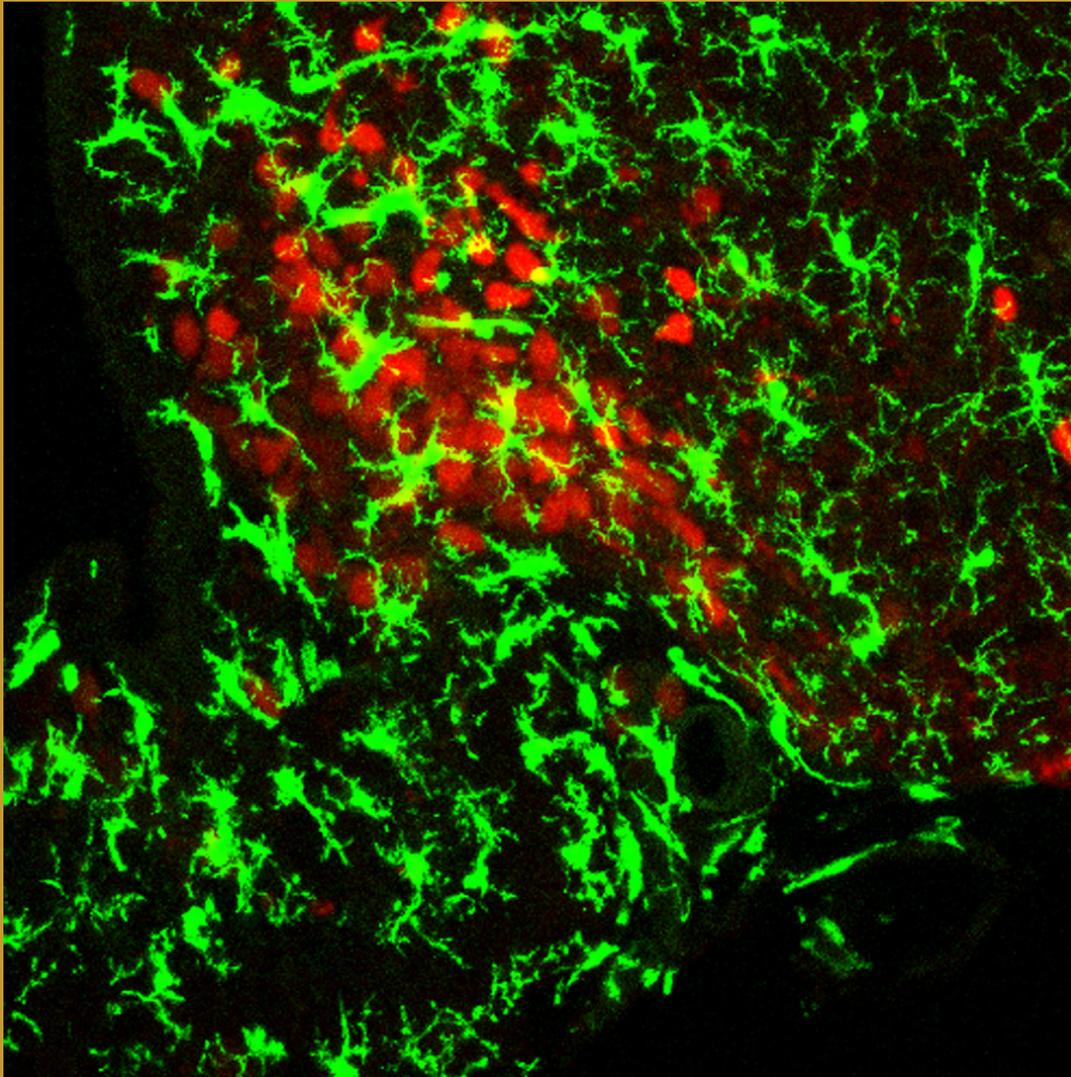
However, Kinyatta does not let having type 2 diabetes define her. She considers herself to have a normal life. She loves to travel and particularly loves spending time on the water. "I'm very adventurous. I just don't let anything hold me back from trying anything new."

Kinyatta carries that sense of adventure into her career, as well. In addition to her classroom teaching, she is studying for her master's degree in counselor education, so that she can become a school counselor. Her ultimate career goal is to own her own business or run her own school, where she can pursue

her passion for helping inner-city kids. She'd like to focus on teaching writing, reading comprehension, and literature, "because those were my biggest struggles as a child."

She also encourages others to do what they can for their health and to make the most of their lives. No matter what, Kinyatta says, don't give up on yourself. "I never saw myself graduating college: I did it. I never saw myself graduating with honors, and I did. I never thought about getting a master's degree—let alone a Ph.D.—and here I am working on my master's, and I have professors encouraging me to get a Ph.D."

The student has become the teacher, and if there's one thing that Kinyatta wants to pass on, to her students or to others with type 2 diabetes, it's this: do what you're capable of doing, no matter what others say. "That's the one thing that I've gotten out of my life: you can't let people deter you from your dreams."



**The brain plays a crucial role in feeding behavior and the regulation of body weight, and brain cells have long been the target of drugs to treat obesity. Recent research in mice, however, has shown that immune cells that reside in the brain can trigger inflammation in response to a high-fat diet, leading to weight gain. This figure shows immune cells (green) interacting with specific brain cells (red) that are critical in the regulation of food intake and body weight. As described in this chapter, this recent discovery could lead to new targets for obesity treatment that could avoid side effects of current drugs in use.**

*Image courtesy of Martin Valdearcos, Ph.D., laboratory of Suneil Koliwad, M.D., Ph.D.*

# 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, Institute-supported investigations into the biologic processes associated with body weight have continued to spark new ideas for intervention approaches. To help bring research results to those affected by obesity and their families, health professionals, and the general public, the Institute sponsors health information programs.<sup>3</sup>

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.

## AN ENZYME'S ROLE IN A COMPLEX GENETIC OBESITY SYNDROME

**An Enzyme Deficiency Contributes to Disease Symptoms in Prader-Willi Syndrome:** Researchers have discovered a critical role for the enzyme prohormone convertase 1 (PC1) in the complex genetic disorder, Prader-Willi Syndrome (PWS). PWS is caused when a part of the genome is missing, resulting in several genes not passing down from a father to a child, leading to many detrimental effects on the infant's body that persist throughout adulthood. Beginning in childhood, affected individuals are often short in stature and develop insatiable appetites, which leads to chronic overeating, obesity, and an increased risk for diabetes and other disorders. Physical symptoms arise from poor regulation of various hormones, including insulin; growth hormone (GH); possibly the appetite-regulating hormone, ghrelin; and others.

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

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

<sup>3</sup> NIDDK Weight-control Information Network, [www.win.niddk.nih.gov](http://www.win.niddk.nih.gov)

Most PWS instances are due to a large genetic deletion on chromosome 15. However, researchers identified five PWS patients with a smaller deletion, defining a critical region sufficient to cause the major PWS-associated traits. This region contains three genes, including one known as *SNORD116*. While none of the existing PWS mouse models develop obesity, mice lacking a paternal copy of *Snord116* (referred to as *Snord116*<sup>p-/m+</sup> mice) develop many of the other clinical features exhibited in their human counterparts, including overeating, decreased body length, and hormone impairments.

It is well-established that a part of the brain called the hypothalamus plays a crucial role in regulating appetite through production of and interactions with various hormones. To study how the PWS genetic deletions affect the brain, investigators generated brain cells (neurons) in the laboratory from another type of cell that, unlike neurons, can be obtained from patient volunteers. Using a technique developed by other researchers, they first “reprogrammed” samples of patients’ skin cells to an early stage, stem cell-like (or pluripotent) state in the laboratory and then had them differentiate into (become) neurons. Because the induced pluripotent stem cell-derived (iPSC-derived) neurons contain the PWS patients’ genetic material, scientists could study the effects of the gene deletion encompassing *SNORD116*. In addition, they studied male *Snord116*<sup>p-/m+</sup> mice. Analysis of human iPSC-derived neurons revealed that the gene *PCSK1*, which codes for the PC1 enzyme, had reduced activity, suggesting the possibility of PC1’s involvement in the development of PWS. Furthermore, mice lacking paternal *Snord116* had decreased PC1 levels compared to normal mice.

PC1 processes prohormones (precursors to hormones) including proinsulin, pro-GH-releasing hormone, and proghrelin, into their bioactive forms—insulin, GH, and ghrelin, respectively. To determine if the hormonal impairments observed in PWS are a consequence of impaired prohormone processing by deficient PC1, researchers measured hormone levels *in vivo* in *Snord116*<sup>p-/m+</sup> mice and human PWS patients. Compared to normal mice, *Snord116*<sup>p-/m+</sup> mice exhibited increased levels of proinsulin, pro-GH-releasing hormone, and proghrelin, indicating an inability of PC1 to properly

process the prohormones. The ratio of proinsulin to insulin in the blood of PWS patients was elevated, but not to the extent as that of a patient completely lacking PC1. These data suggest impaired PC1 activity due to paternal deletion of *SNORD116* drives the hormonal features of PWS.

This research highlights the effectiveness of a combined approach using human cells and blood samples along with mouse models to study a complex genetic disorder. While the findings contribute to a growing body of knowledge investigating how the loss of a gene alters hormone levels and function in PWS, more research is necessary to determine if other mechanisms are involved.

*Burnett LC, LeDuc CA, Sulsona CR,...Leibel RL. Deficiency in prohormone convertase PC1 impairs prohormone processing in Prader-Willi syndrome. J Clin Invest 127: 293-305, doi:10.1172/JCI88648, 2017.*

## NEW INSIGHTS INTO FAT CELL DEVELOPMENT

**A Strategy for Synthesizing Potential New Drugs and Research Tools Yields Insights into Fat Cell Development:** With a creative strategy for generating new knowledge that may lead to future drug development, researchers developed a set of small chemicals that bind many different proteins in cells and can be used to learn the proteins’ functions. Using this approach, they discovered a protein important in fat cell development.

Different types of proteins perform key tasks throughout the body, and many drugs are chemicals that target specific proteins, either blocking or increasing their functions for therapeutic effects. Chemicals that bind to proteins can also be used to gain new understanding of what the respective proteins do. However, only a fraction of human proteins are targets of current drugs. Thus, scientists devised a way to target many previously untargeted proteins with small chemicals. To get a sense of how many proteins could be targeted this way, they developed a collection of small chemicals of varying structure (which they also called “fragments”), each with a special tag, and mixed these with human cells grown in the lab. Using the tags, they retrieved the chemical-protein

pairs that formed in the cells. In their initial screen, they identified thousands of interactions between the chemicals and different types of proteins; each chemical could bind multiple proteins. A search of a large database revealed that most of these proteins were not targets of existing drugs. These results showed that a large variety of proteins—more than previously thought possible—could be bound by small chemicals. Further experiments with a few of the chemicals showed that these inhibited the functions of the bound proteins. The researchers also found that, by modifying a chemical's structure, they could strengthen its interaction with a particular protein of interest, an approach similar to the methods scientists use to fine-tune the structure of a potential new drug.

The researchers then explored whether their small, tagged chemicals could be used to discover proteins important for biological processes. As a test case, they chose the process of fat cell development. They screened for chemicals that could prompt precursor (immature) cells from mice to develop into fully mature fat cells. They found one chemical that could do this; identified the protein it bound, PGRMC2; and showed that the chemical stimulated the protein's activity. Through these and additional experiments, they discovered that PGRMC2 plays a role in fat cell development.

This research demonstrates that small, tagged chemicals can be used to target a wide variety of proteins in human cells. Future studies may advance understanding of the proteins they bind, and these chemicals could potentially serve as starting points for new drug development.

*Parker CG, Galmozzi A, Wang Y, ... Cravatt BF. Ligand and target discovery by fragment-based screening in human cells. Cell 168: 527-541, doi: 10.1016/j.cell.2016.12.029, 2017.*

**Large-scale Human Genetics Study of Fat Depots Identifies Gene Regions Associated with Depot- and Gender-specific Fat Cell Development:** In the largest-scale human genetics study of its kind, researchers have identified seven new gene regions associated with individual variability in body fat traits. In addition, genetic analysis in a mouse model suggests an important physiological role for two of these genes in fat cell development.

Differences in fat tissue (adipose tissue) distribution affect disease risk, including diabetes and heart disease. Mammals store different kinds of adipose tissue in various repositories, or depots, in the body. Subcutaneous adipose tissue (SAT) is found just beneath the skin, while visceral adipose tissue (VAT) surrounds abdominal organs, and pericardial adipose tissue (PAT) surrounds the heart. Research has shown that various traits, such as the volume, density, and relative distribution of adipose tissue have an associated genetic component and are important predictors of disease risk. To gain new insights into the genetic contribution to adipose tissue traits, investigators searched the genomes of 18,332 ethnically diverse men and women to identify small, common variants in their DNA. Then, using non-invasive imaging techniques, they analyzed adipose tissue traits of study participants; each trait was examined in all or a large number (several thousand) of the participants. They found that these traits were associated with common genetic variants in seven gene regions. Of these, three gene regions were associated with SAT and VAT volume, two were associated with PAT volume, one with SAT density, and one with the relative distribution between VAT and SAT depots. In some cases, associations were gender specific. Further evaluation found that genetic variations associated with PAT volume and a higher amount of VAT relative to SAT were also significantly associated with different metabolic conditions, including type 2 diabetes and levels of cholesterol and circulating fat in both men and women.

To examine the functional significance of these findings, they turned to an animal model. They measured activity of four analogous mouse genes in different fat depots in male mice and found variable activity for two genes: the *Ube2e2* gene was more highly activated in VAT than in SAT or PAT during fat cell development. The *Atxn1* gene was more active in the SAT of mice with diet-induced obesity than in lean mice. These data suggested a potential regulatory role for the genes in adipose tissue development. To explore this possibility, the team isolated early-stage cells from SAT and VAT depots of mice and allowed them to differentiate into (become) fat cells in the laboratory. Both *Atxn1* and *Ube2e2* showed evidence of dynamic regulation of activity at different timepoints. When they used a molecular technique to silence the activity of either of these genes in the cells, they observed impaired fat cell formation in SAT, whereas only *Ube2e2* disruption impaired adipose tissue development in VAT.

Through a combined approach of a large-scale human genetics study coupled with experiments in a mouse model, this research provides new insight into the genetics of body fat distribution and supports physiological roles for specific genes in fat cell development. Future studies are necessary to determine the mechanism by which *ATXN1* and *UBE2E2* affect adipose tissue formation in humans and if they influence the development of cardiometabolic disease.

*Chu AY, Deng X, Fisher VA,...Fox CS. Multiethnic genome-wide meta-analysis of ectopic fat depots identifies loci associated with adipocyte development and differentiation. Nat Genet 49: 125-130, doi: 10.1038/ng.3738, 2017.*

## REGULATING METABOLISM VIA SIGNALS FROM FAT, LIVER, AND BONE

### **An Appetite-suppressing Hormone in Bones:**

Researchers discovered, in a study in mice, that bones secrete an appetite-suppressing hormone called lipocalin 2 (LCN2). This hormone also regulates blood glucose (sugar) levels.

Building on previous findings that hinted at a possible role for bones in regulating appetite, the scientists searched for hormones produced in bone cells of mice and identified LCN2. This hormone had earlier been found to be made in fat cells and was associated with body weight. However, as shown in this study, compared to fat cells, bones produce much larger quantities of LCN2. To gain insight into the function of LCN2, the researchers explored what happens in its absence. They genetically engineered a group of male mice to be deficient specifically in bone-derived LCN2; these mice could still produce LCN2 in their fat cells. Without LCN2 from bones, these mice ate more than normal mice, gained more weight, and accumulated more body fat. The absence of bone-derived LCN2 also impaired blood glucose regulation. Thus, they concluded that LCN2 from bones normally dampens appetite and regulates blood glucose.

To confirm these results, the research team next studied the effects of excess LCN2. When they injected LCN2 into normal mice, the extra hormone reduced food intake, body fat, and weight. The researchers then found that levels of LCN2 naturally fluctuate with fasting and feeding. Delving deeper into how LCN2 affects

appetite, the researchers discovered, in experiments in male and female mice, that LCN2 can travel to the brain, where it partners with another molecule, MC4R, to suppress appetite. An additional study revealed clues that LCN2 might work similarly in humans. Examining men with type 2 diabetes, the researchers found that higher levels of LCN2 in the blood correlated with lower body weights and better blood glucose levels.

This study highlights the importance of a bone-produced hormone in regulating appetite and blood glucose. Other research teams had previously identified LCN2 as playing a role in blood glucose control and other biologic processes, but had not detected its production in bones, and, curiously, had not observed any effects on appetite in mice. Further investigation may yield additional insights into the complexity of this hormone's activities. If LCN2 suppresses appetite and improves blood glucose levels in people, it could potentially be targeted in the development of new therapies for type 2 diabetes and obesity.

*Mosialou I, Shikhel S, Liu JM...Kousteni S. MC4R-dependent suppression of appetite by bone-derived lipocalin 2. Nature 543: 385-390, doi: 10.1038/nature21697, 2017.*

### **Signals from Body Fat to Other Organs—Small Packages with Big Metabolic Impact:**

Researchers discovered a way that fat tissue regulates metabolism—it produces a variety of tiny but powerful molecules, called miRNAs (or microRNAs), and sends these out through the bloodstream to control biological processes elsewhere in the body. Previous studies had shown that fat tissue also releases hormones with far-ranging effects. The new discoveries thus add miRNAs to the metabolic toolbox of fat.

The researchers investigated miRNAs from fat tissue because past studies had illuminated their importance in cells throughout the body; and altered levels of miRNAs had been observed in obesity, diabetes, and another condition, lipodystrophy, associated with abnormalities in body fat. To gain new insights, the researchers began by comparing normal male mice to male mice that were deficient in miRNA production in fat tissue (but not elsewhere), to see whether this deficiency affected miRNAs in the blood. In the bloodstream, miRNAs often travel in small packages called exosomes, and the researchers found that levels of hundreds of different types of miRNAs were reduced in exosomes from the mice deficient in

fat-derived miRNAs. These mice also had metabolic problems, including impaired blood glucose (sugar) regulation. When the researchers transplanted fat tissue, particularly the type known as brown fat tissue, from normal mice into the miRNA-deficient mice, they found partial restoration of miRNAs in the blood and improvement in glucose levels. These results demonstrated that normal fat tissue is a key source of circulating miRNAs, with effects on health. Other experiments also showed that fat deploys miRNAs to control metabolism from afar. For these experiments, the researchers drew upon knowledge that miRNAs block the flow of information encoded by various genes, so that the information cannot be used to make proteins. They found that miRNA-containing exosomes from mouse fat tissue travel to the liver and regulate a gene there, reducing levels of the protein it encodes.

The researchers also studied miRNAs in blood samples from human volunteers, including healthy individuals and people with lipodystrophy, a condition marked by loss of body fat where it should be, accumulation of fat where it can be toxic, and increased risk for diabetes. The levels of many miRNAs were lower in people with lipodystrophy than in healthy people, a sign that normal fat tissue is a source of circulating miRNAs in humans.

This study highlights a role for miRNAs in the regulation of metabolism by body fat. With further research, scientists may be able to develop interventions that target fat-derived miRNAs to improve health.

*Thomou T, Mori MA, Dreyfuss JM,...Kahn CR. Adipose-derived circulating miRNAs regulate gene expression in other tissues. Nature 542: 450-455, doi :10.1038/nature21365, 2017.*

## THE BRAIN'S REGULATION OF APPETITE AND OBESITY

### Brain Immune Cells Control Food Intake and Obesity:

Researchers have discovered that a type of brain immune cell, called microglia, controls food intake and obesity in mice, providing a novel therapeutic target to combat obesity and its associated health consequences. The research focused on the hypothalamus, a part of the brain governing hunger and other physiologic functions, and was conducted primarily in male mice. Scientists built on

previous research showing the association between obesity and the accumulation of microglia in the hypothalamus of people and mice. It was not known, however, whether microglia-induced inflammation played a role in regulating food intake or rather was a consequence of excess food intake. To examine this question, researchers used a drug called PLX5622 to deplete microglia. They found that the drug had no effect on mice eating a standard, low-fat diet. They then compared two groups of mice that were on a high-fat diet. Although both groups gained more weight than mice fed the standard diet, drug-treated mice eating a high-fat diet ate less, gained less weight, and had lower body fat compared to untreated mice also eating a high-fat diet. These and other results suggested that microglia play a role in regulating food intake. To confirm this finding, the researchers performed the opposite experiment: using genetic techniques, they generated mice in which they could activate microglia and induce microglial inflammation. They found that mice with activated microglia were vulnerable to weight gain even on the standard diet, experiencing a rapid, 4-fold increase in the amount of weight gained compared to control mice on the same diet. Contributing to this weight gain, the experimental mice ate more food and had lower energy expenditure (calorie burning) compared to control mice, further suggesting that microglia directly regulate metabolism. Other experiments showed that, when exposed to a high-fat diet, microglia signal to immune cells found in the bone marrow and recruit them to the hypothalamus to participate in the inflammatory response. These results suggest that microglia are key regulators of food intake, calorie burning, and obesity in mice.

If found to play a similar role in women and men, microglia represent novel therapeutic targets for obesity and its associated health consequences. Further research into the detailed mechanisms by which these cells exert their effects—such as understanding how a high-fat diet promotes microglial inflammation in mice—could shed light on other targets and potential therapies.

*Valdearcos M, Douglass JD, Robblee MM,...Koliwad SK. Microglial inflammatory signaling orchestrates the hypothalamic immune response to dietary excess and mediates obesity susceptibility. Cell Metab 26: 185-197, doi: 10.1016/j.cmet.2017.05.015, 2017.*

**Brain Control of Obesity-related Behaviors:** Two studies in mice have demonstrated new links between nerve cell activity in specific brain regions and both physical activity and the drive to eat, further defining the critical roles of hormonal signaling in obesity-related behaviors.

The first study focused on understanding the association between obesity and physical inactivity. Although it is well known that physical activity benefits overall health, it is not known why people with obesity have reduced physical activity levels. In other words, does obesity cause physical inactivity, or does physical inactivity cause obesity? To begin to address this question, researchers fed male mice either a standard, low-fat diet or a high-fat diet for 18 weeks. As expected, mice on the high-fat diet gained more weight and were less physically active than the animals eating the standard diet. Next, the researchers sought to identify the mechanisms underlying the observed physical inactivity in obese mice and focused on studying dopamine signaling. The chemical dopamine is produced by neurons (nerve cells) and binds to protein “receptors” found on the surface of nerve and other cells to exert its effect of transmitting signals. Experiments showed that obese mice had deficits in the function of dopamine D2-type receptors (D2R) in the brain’s basal ganglia, meaning that the animals had less active dopamine signaling. Experimentally restoring signaling in obese mice increased their physical activity, whereas lean mice genetically altered to lack D2R in a subset of nerve cells in the basal ganglia were not as physically active as control animals. When the researchers further studied those genetically altered mice, they uncovered a surprising finding: the animals did not gain more weight on a high-fat diet compared to control mice eating the same diet. That is, even though the experimental mice were less active, they did not gain more weight than control mice with intact dopamine signaling. This result suggests that inactivity is more a consequence than a cause of obesity in mice.

A second study examined hunger and the drive to eat in relation to other competing behaviors in mice. Previous research has primarily studied feeding behavior in isolation. However, in nature, mice must navigate feeding while engaging in other behaviors, such as avoiding predators. The scientists hypothesized that hunger could suppress other behaviors in favor of eating. To test this, they used three different groups

of male mice: (1) fed (sated) mice, (2) hungry (fasted) mice, and (3) fed mice with experimentally-activated AgRP neurons found in the arcuate nucleus (ARC) of the brain’s hypothalamus (or fed-ARC<sup>AgRP</sup>-activated mice). It is known that ARC<sup>AgRP</sup> neurons play an important role in feeding behavior, as experimentally activating them promotes feeding even when mice are not hungry. Confirming prior results, the researchers first demonstrated that activating ARC<sup>AgRP</sup> neurons in fed mice promoted feeding, and thus their fed ARC<sup>AgRP</sup>-activated mice represented a model of hunger. The researchers then conducted several behavioral tests using the three different groups of mice to see where hunger/feeding would rank related to engaging in other behaviors. It turns out that, when food is present, hunger wins. For example, the scientists found that when housed in a large open-field apparatus, hungry mice and fed ARC<sup>AgRP</sup>-activated mice were more likely to spend time in the center—a location that is anxiety-provoking in mice—compared to fed mice when food was placed there. Similarly, when placed in a two-chamber apparatus with a chemical produced by a predator, foxes, in one chamber, the hungry mice and fed ARC<sup>AgRP</sup>-activated mice were more likely to venture over to the “unsafe” side if that side also had food. By contrast, the fed mice tended to stay on the safe side, which had no food but also lacked the fear-inducing chemical. Other experiments showed that hunger won over other behaviors, such as drinking water and promoting social interactions. The scientists suggest that it is possible that ARC<sup>AgRP</sup> neurons produce signals to suppress these other behaviors in favor of feeding.

These studies shed important new light on the complex neurological signaling pathways that regulate obesity-related behaviors such as physical inactivity and the drive to eat. Future research could help determine whether the same results are observed in women and men, as well as delve deeper into the underlying molecular mechanisms toward finding safe strategies to modify behavior to prevent or treat obesity.

*Friend DM, Devarakonda K, O’Neal TJ,...Kravitz AV. Basal ganglia dysfunction contributes to physical inactivity in obesity. Cell Metab 25: 312-321, doi: 10.1016/j.cmet.2016.12.001, 2017.*

*Burnett CJ, Li C, Webber E,...Krashes MJ. Hunger-driven motivational state competition. Neuron 92: 187-201, doi: 10.1016/j.neuron.2016.08.032, 2016.*

### **Identification of Brain Cells That Drive Binge Eating and Weight Gain:**

Researchers have identified a group of brain cells (referred to here as ZI-GABA cells) that, upon activation, induces rapid binge eating and weight gain in mice. Previous studies have shown that humans who receive brain stimulation of the region including these cells, as a form of therapy to treat neurological disorders, can develop compulsive eating habits. However, it was not clear why this treatment would elicit such a response.

To determine the role ZI-GABA cells play in eating behavior and body weight regulation, investigators used a technique that allowed them to use a light source to selectively control brain cells in mice that had been genetically modified to have light-responsive proteins on the surface of these cells. When they activated the ZI-GABA cells in these mice with laser light, the mice rapidly consumed large quantities of food compared to mice without light-activated proteins. Because previous observations have shown that ZI-GABA cells project (extend long fibers) into an area of the brain that may help regulate feeding, they investigated whether this area is a critical target for ZI-GABA cell control of food intake. Upon light stimulation, they found that ZI-GABA cells sent signals to cells in this target area that reduced their activity, subsequently inducing food foraging behavior, and dramatically increasing food intake. To assess whether activation of the ZI-GABA pathway leads to weight gain, they selectively light-stimulated the cells several times per day over the course of two weeks. Repeated activation led to increased feeding and weight gain in mice, both of which were significantly reduced when the light stimulus was removed. Consistent with the notion that mice resume normal feeding behavior and body weight in the absence of ZI-GABA activation, the researchers then selectively deleted ZI-GABA cells from a group of mice and found that these mice reduced their food intake and gained less weight.

Finally, the researchers explored the idea that, because ZI-GABA cells project an inhibitory signal to their target cells, in turn increasing feeding behavior, the target cells have an opposite effect on food intake. When they selectively activated the target cells, bypassing ZI-GABA, mice significantly reduced their food intake. Moreover, when they selectively eliminated the target cells, mice substantially increased feeding and body weight.

Taken together, these data suggest that activation of a robust inhibitory pathway involving ZI-GABA brain cells is capable of evoking rapid binge eating episodes. With further research, knowledge gleaned from these and future results could lead to the development of novel treatment strategies for binge eating disorders in humans.

*Zhang X and van den Pol AN. Rapid binge-like eating and body weight gain driven by zona incerta GABA neuron activation. [Science](#) 356: 853-859, doi: 10.1126/science.aam7100, 2017.*

### **Explaining the Link Between an Antipsychotic Medication and Excessive Weight Gain:**

Researchers have identified a receptor (cell-surface protein) in the mouse brain responsible for the metabolic syndrome caused by an antipsychotic medication, as well as a therapy that may prevent this side effect. Olanzapine is part of a drug family called the “atypical antipsychotics” (AATPs). AATPs can be effective treatments for disorders such as schizophrenia, bipolar disorder, and depression. However, AATPs can also cause side effects like food cravings and binge eating that can lead to obesity and type 2 diabetes within months of starting treatment. AATPs have been known to interact with multiple receptors in the brain, including HTR2C, which helps regulate food intake, body weight, and glucose metabolism. Blocking HTR2C signaling in mice was known to cause overeating and obesity, which resembled side effects induced by AATPs. Moreover, many AATPs, including olanzapine, interfere with HTR2C function, which led researchers to suggest that it was olanzapine’s block of HTR2C function that was causing the metabolic syndrome.

To test this hypothesis, researchers fed mice the drug to reproduce the olanzapine blood concentrations seen during human olanzapine therapy. This mouse model was then used to investigate how olanzapine causes weight gain. Female mice fed olanzapine ate more and moved less, leading to excessive weight gain and other indications of metabolic syndrome similar to that seen in humans. Overeating was less prominent in male mice fed olanzapine than in female mice, and the male mice gained less weight, though the reason for the difference between the males’ and females’ responses was unclear. This result, compared with that of other experiments, suggested that, at least in mice, the main contributor to olanzapine-induced weight gain was overeating.

The researchers performed further experiments with genetically modified mice to identify factors responsible for olanzapine-induced metabolic syndrome. Mice who were fed olanzapine but also lacked the HTR2C protein did not overeat, develop altered glucose metabolism or insulin levels, or gain weight, indicating that HTR2C is important for these side effects. The researchers then asked: if olanzapine's metabolic side effects are mediated by its blockage of HTR2C function, then could the side effects be alleviated by activating HTR2C? To test this, they fed mice olanzapine and treated the mice with an FDA-approved weight-loss drug called lorcaserin, which activates HTR2C. Lorcaserin blocked olanzapine's overeating, weight gain, and metabolic syndrome side effects.

Since other existing anti-obesity and anti-diabetic medications have limited effectiveness against AATP-induced metabolic syndrome, this work offers hope that HTR2C activators like lorcaserin may be useful tools to prevent these side effects.

*Lord CC, Wyler SC, Wan R,...Elmqvist JK. The atypical antipsychotic olanzapine causes weight gain by targeting serotonin receptor 2C. J Clin Invest 127: 3402-3406, doi: 10.1172/JCI93362, 2017.*

## HOW APPETITE COUNTERS WEIGHT LOSS

### **Weight Loss Leads to Strong Increase in Appetite—The Body's Internal Feedback Control of Calories:**

Studying people in a clinical trial of a type 2 diabetes drug that causes weight loss, researchers discovered that as people lost weight, their appetite increased proportionately, leading to increased calorie consumption and a leveling off of weight loss. The findings provide the first measurement in people of how strongly appetite counters weight loss, as part of the body's feedback control system regulating weight.

For this study, the researchers sought further insight into why it is so difficult to maintain a lower body weight after loss of excess weight. To investigate whether the body may have internal, biological controls that compensate for weight loss by boosting appetite, they developed a strategy for measuring real-world calorie consumption over the long term in response to weight loss. They examined body weight data from a year-long clinical trial of canagliflozin, a type 2 diabetes drug that substantially increases

the amount of glucose (sugar) that is excreted in the urine. This drug causes a gradual decrease in weight, averaging about eight pounds. In the clinical trial, participants were randomly assigned to either the drug or a placebo, without knowing which they received. The participants were not directly aware of the loss of calories in the excreted glucose, and they were not on a restricted diet or in an exercise program. Thus, because the participants were not making intentional behavior changes, the researchers could study what the body is inherently wired to do.

Analyzing data from 153 men and women who received the drug and 89 who received a placebo, the research team calculated calorie intake using a previously validated mathematical model. They compared the measured body weight changes with the weight changes expected based on the loss of glucose calories, and realized that calorie intake increased. For every pound of lost weight, the people treated with canagliflozin consumed about 50 calories per day more than they had been eating before the trial. The increased appetite and calorie intake led to a slowing of weight loss after about 6 months. These changes were not seen in people who got a placebo.

The researchers then analyzed data from a separate trial of a lifestyle weight-loss program for overweight and obese individuals. Based on the weight-loss and regain data, the researchers calculated the compensatory changes in appetite. Although the participants did not maintain their initial diet through the trial, they still consumed fewer calories than expected based on appetite changes—a sign that they persisted in their efforts despite an increased appetite.

These findings underscore the challenges faced by those seeking obesity treatment. Past research had shown that, after weight loss, the body also slows its rate of burning of calories, yet the change in appetite shown in this study is even stronger. Weight loss strategies thus need to overcome the body's multiple systems designed to regain that weight.

*Polidori D, Sanghvi A, Seeley RJ, and Hall KD. How strongly does appetite counter weight loss? Quantification of the feedback control of human energy intake. Obesity 24: 2289-2295, doi: 10.1002/oby.21653, 2016.*

## HEAT PRODUCTION, BROWN FAT, AND OBESITY

### Finding Factors Important to Stoking Brown Fat:

An international research team including NIDDK-supported scientists has newly identified a molecular factor key to the activation of energy-burning brown fat, furthering possibilities for targeting brown fat in treatment of obesity.

Unlike white adipose tissue (fat), which stores energy derived from the food we eat and expands in volume when calorie consumption exceeds bodily energy expenditure (calorie-burning), brown fat “burns” energy, releasing it as heat. Because active brown fat has been found in adult humans, its characteristics and development are being studied intensively to determine if it might be or harbor a treatment target for obesity and metabolic diseases. Through experiments in laboratory-grown mouse cell lines, male mice, and human tissue samples, researchers sought in this study to identify key molecular factors, called transcription regulators, controlling activation and maintenance of patterns of gene expression—turning genes “off” or “on”—specific to brown fat. Using a technique that enabled them to assess thousands of active chromosomal regions in cells simultaneously, they detected in mouse brown fat a DNA sequence motif associated with binding by a family of transcription regulators called nuclear factor I. Further experiments revealed that one member of this family, nuclear factor I A (NFIA), was highly enriched in mouse brown fat compared to other tissues, such as white fat and muscle. Brown fat cells share a common progenitor with skeletal muscle cells. Thus, to confirm a role for NFIA in defining brown fat, the scientists introduced NFIA into laboratory-grown mouse muscle precursor cells. These cells developed characteristics of fat cells and their gene expression was modified, with brown fat genes “turned on” and muscle genes “turned off.” Conversely, when NFIA was inhibited in laboratory-grown mouse brown fat cells, expression of brown fat-specific genes decreased significantly, indicating that NFIA is needed for both activation and maintenance of genes determining brown fat identity. Examining the underlying mechanism involved, the researchers found evidence in cell-based experiments that NFIA facilitates binding of PPAR $\gamma$ —the master transcriptional regulator governing fat cell development—to regions of the genome associated with brown fat-specific genes.

This co-localization resulted in significantly increased expression of the genes examined.

Moving from cells to whole organisms, experiments in mouse models appeared to confirm that NFIA is important to brown fat development and suppresses muscle cell development. The researchers then analyzed samples of human brown fat from volunteers. They found that samples from patients with a tumor causing activation of brown fat surrounding the kidneys showed higher expression of the genes encoding NFIA, as well as other brown fat-specific genes, than similar samples obtained from patients with other tumors. Analyses of brown fat cells and white fat cells cultured from tissue samples from shoulder and belly areas, respectively, of other volunteers, also showed higher NFIA expression in the brown fat cells. Taken together, the study results suggest that NFIA is a key factor in brown fat activation and development in mice and may act similarly in humans. Future studies may reveal ways to manipulate NFIA and its ability to “reprogram” cells to become brown fat as part of therapeutic strategies for obesity.

*Hiraike Y, Waki H, Yu J, ...Kadowaki T. NFIA co-localizes with PPAR $\gamma$  and transcriptionally controls the brown fat gene program. *Nat Cell Biol* 19: 1081-1092, doi: 10.1038/ncb3590, 2017.*

### Insights into How the Body Adjusts Its Thermostat To Regulate the Balance Between Food Intake and Calorie Burning:

Researchers identified two factors that contribute to the critical balance of food intake and energy expenditure (calorie burning), in studies in mice. Mammals have precise and linked regulatory mechanisms to maintain body temperature and stabilize body weight. Food is converted to chemical energy, which the body uses, stores as fat, or converts to and dissipates as heat to adapt to various conditions (a process referred to as “adaptive thermogenesis”). The body can adjust its calorie burning, to some extent, based on the amount of food eaten. Decreased food intake leads to reduced energy expenditure, which limits weight loss. Conversely, increased food intake stimulates calorie-burning brown and beige fat cells to increase energy expenditure through the generation of heat (a form of adaptive thermogenesis referred to as “diet-induced thermogenesis”), limiting weight gain. When this regulation is disrupted, excess food intake and/or reduced energy expenditure can eventually

lead to an increase in storage of the excess calories as fat, resulting in obesity. Scientists hope that, by understanding the body's regulatory mechanisms, they can develop strategies to stimulate increased energy expenditure and to help combat the growing prevalence of obesity. Toward that goal, two studies from the same research group provided important insights into these regulatory mechanisms.

Previously the investigators demonstrated that a small molecule known as creatine enhanced energy expenditure. To explore the role of creatine specifically in adipose (fat) tissue, the researchers genetically engineered mice to lack a gene critical to the synthesis of creatine in fat cells and characterized the male mice. When these mice were transferred to a cold environment, the researchers observed that the mice lacking creatine in fat had reduced body temperatures, indicating that creatine plays a role in the production of heat to maintain body temperature. When the mice were fed a diet that can induce obesity, the researchers found that the mice lacking creatine in fat more rapidly developed obesity, compared to mice with creatine, and exhibited mild metabolic dysfunction, such as impaired glucose tolerance.

Importantly, both types of mice were eating the same amount of food and exhibiting similar levels of physical activity, so the scientists measured the resting energy expenditure in the mice to see if that would explain the acceleration of obesity and metabolic dysfunction. They found that, on the obesity-inducing diet, mice with creatine in their fat increased their energy expenditure and metabolic rate concomitantly to balance the extra calories. Mice lacking creatine in their fat, however, did not increase their energy expenditure and metabolic rate to the same extent; their diet-induced thermogenesis was suppressed. To determine whether this was specifically due to the loss of creatine, the scientists fed a creatine-supplemented diet to mice lacking creatine in their fat and observed that these mice showed an increase in metabolic rate, "rescuing" the loss of diet-induced thermogenesis. These results indicated that creatine in fat promotes diet-induced thermogenesis and combats obesity.

Equally important as the processes that promote thermogenesis are the processes that put a "brake" on thermogenesis, protecting animals from wasting energy by counterbalancing thermogenic processes. In a second study, the researchers identified a protein

in fat, named KCNK3, that appears to act as this brake. Male and female mice genetically engineered to lack KCNK3 specifically in fat cells had increased body temperatures when placed in a cold environment compared to mice with KCNK3 in fat, indicating increased adaptive thermogenesis. When fed a diet that induces obesity, the genetically engineered mice gained significantly less weight than mice with KCNK3 in their fat cells and demonstrated increased energy expenditure. These mice also showed metabolic benefits, including improved glucose tolerance. Furthermore, the researchers demonstrated how KCNK3 activity leads to these outcomes: it increases the amount of potassium flowing out of a brown or beige fat cell, which limits the amount of calcium that can enter the cell; this process subsequently suppresses thermogenesis.

These two studies provide important details toward understanding the balance of energy intake and expenditure in mammals, and identify two new potential therapeutic targets in brown and beige fat for treatment of obesity and type 2 diabetes. As both these studies were conducted in mice, additional research will be necessary to determine the roles that creatine and KCNK3 play in human energy metabolism, and if creatine supplementation and/or KCNK3 antagonists can promote energy expenditure in humans.

*Kazak L, Chouchani ET, Lu GZ, ... Spiegelman BM. Genetic depletion of adipocyte creatine metabolism inhibits diet-induced thermogenesis and drives obesity. Cell Metab 26: 660-671, doi: 10.1016/j.cmet.2017.08.009, 2017.*

*Chen Y, Zeng X, Huang X, Serag S, Woolf CJ, Spiegelman BM. Crosstalk between KCNK3-mediated ion current and adrenergic signaling regulates adipose thermogenesis and obesity. Cell 171: 836-848, doi: 10.1016/j.cell.2017.09.015, 2017.*

## CLINICAL RESEARCH ON WEIGHT-LOSS INTERVENTIONS

### **Gastric Bypass Surgery Provides Long-term Health**

**Benefits:** In one of the first of its kind, a long-term, observational study of outcomes from gastric bypass, a form of bariatric surgery, has shown durability of weight loss and effective remission and prevention of type 2 diabetes in U.S. adults for more than a decade. More than 800 participants with severe obesity

who sought to undergo “Roux-en-Y” gastric bypass at a bariatric surgical center were studied. Of the participants who sought bariatric surgery, half of them proceeded with the operation, and half initially did not, although some of those individuals had surgery at a later time. The researchers also recruited a group of more than 300 people with severe obesity who were not seeking surgery, as another control group for the study. The study included women and men; a majority of the participants were women. The investigators conducted clinical examinations of participants upon enrollment, and 2, 6, and 12 years later to assess weight and to determine the presence of type 2 diabetes, high blood pressure, and a disorder marked by abnormal amounts of fat in the bloodstream called “dyslipidemia.” Remarkably, most of the participants took part in follow-up exams at the 12-year mark. Of these, 388 people had sought and undergone bariatric surgery. Their health outcomes were compared with data from participants who did not have surgery, including 217 individuals who originally sought but did not undergo surgery, and 262 who had not sought surgery.

Previously, the investigators reported that participants who underwent bariatric surgery lost significantly more weight than people who did not at the 2-year mark and at 6 years after surgery. Although the participants who had surgery, on average, gained back some of the weight, at 12 years after the surgery, more than 70 percent of these participants maintained greater than 20 percent of the weight loss, and 40 percent maintained greater than 30 percent weight loss. Additionally, on average, the surgery participants’ weight remained stable between the 6- and 12-year follow-up exams. Those who did not have bariatric surgery did not lose weight. Moreover, among participants who had type 2 diabetes at the study’s onset, those who had surgery were significantly more likely to experience remission than those who did not have surgery, especially if the diabetes had not progressed to the point of needing medication prior to the surgery. The investigators also observed a reduction—by more than 90 percent—of new-onset type 2 diabetes at 12 years among people who had the surgery.

Finally, when the researchers assessed other obesity-related conditions in the participants, they found a significant reduction in the occurrence of both high blood pressure and dyslipidemia in people who underwent bariatric surgery compared to those who sought out the surgery but did not receive it. Although

the overall effects of the surgery were of strong benefit, there were 7 suicide deaths, all in people who had surgery; this finding highlights the need for greater attention to patients’ psychological health before and after surgery.

The results from this first U.S.-based, 12-year, observational study of bariatric surgery in adults indicate long-term durability of weight loss after “Roux-en-Y” gastric bypass. Moreover, the weight loss was associated with improvement and prevention of type 2 diabetes and obesity-related cardiovascular conditions.

*Adams TD, Davidson LE, Litwin SE,...Hunt SC. Weight and metabolic outcomes 12 years after gastric bypass. N Engl J Med 377: 1143-1155, doi: 10.1056/NEJMoa1700459, 2017.*

### **A Weight-loss Intervention Shows Promise for Low-income Mothers Post-childbirth:**

A recent study has shown that an Internet-based weight loss intervention produces greater weight loss in low-income women who have recently given birth than a standard care program alone.

Weight retention post-childbirth can increase a woman’s risk of developing obesity and associated complications later in life. In addition, maintaining excess weight can compromise future pregnancies, affecting the health of both mother and child. However, few effective interventions exist for new mothers in low-income populations, who are at higher than average risk for weight retention. Researchers enrolled more than 370 new mothers, most of them Hispanic, from a federally funded nutrition assistance program in a 12-month study to assess the effects on weight of adding a primarily Internet-based weight-loss program to the standard care of the nutrition assistance program versus the standard care alone. While women in the standard care group received supplemental foods and newsletters with information about nutrition, exercise, and weight loss, women in the intervention group additionally received interactive and motivational Internet-based weight loss guidance, activity trackers, and dietary assessment tools, as well as multiple weekly text messages providing information and feedback, and monthly face-to-face group meetings with study staff; the entire program was available in English and Spanish. At the end of the trial, the researchers compared the weights of participants

in both groups and found that participants in the intervention group achieved a greater weight loss (7 pounds) than those who received standard care alone (2 pounds), indicating that the addition of the Internet-based program was effective in promoting weight loss in low-income women post-childbirth who were enrolled in the nutrition assistance program. In addition, more women in the intervention group returned to their self-reported pre-pregnancy weight, providing more evidence that the intervention is an effective weight-loss tool.

Even a modest reduction in weight can improve blood glucose (sugar) and lower the risk of developing obesity later in life. This study highlights a practical

and successful method of losing weight after giving birth among women at high risk of weight retention, potentially improving their long-term health. Future studies could help determine which components of this multi-faceted intervention were most critical to promoting weight loss, what might be added to enhance weight loss, and whether a primarily Internet-based weight loss intervention such as the one tested is cost effective.

*Phelan S, Hagobian T, Brannen A,...Tate DF. Effect of an Internet-based program on weight loss for low-income postpartum women: A randomized clinical trial. [JAMA](#) 317: 2381-2391, doi: 10.1001/jama.2017.7119, 2017.*

# Shining a Light on Obesity Genetics



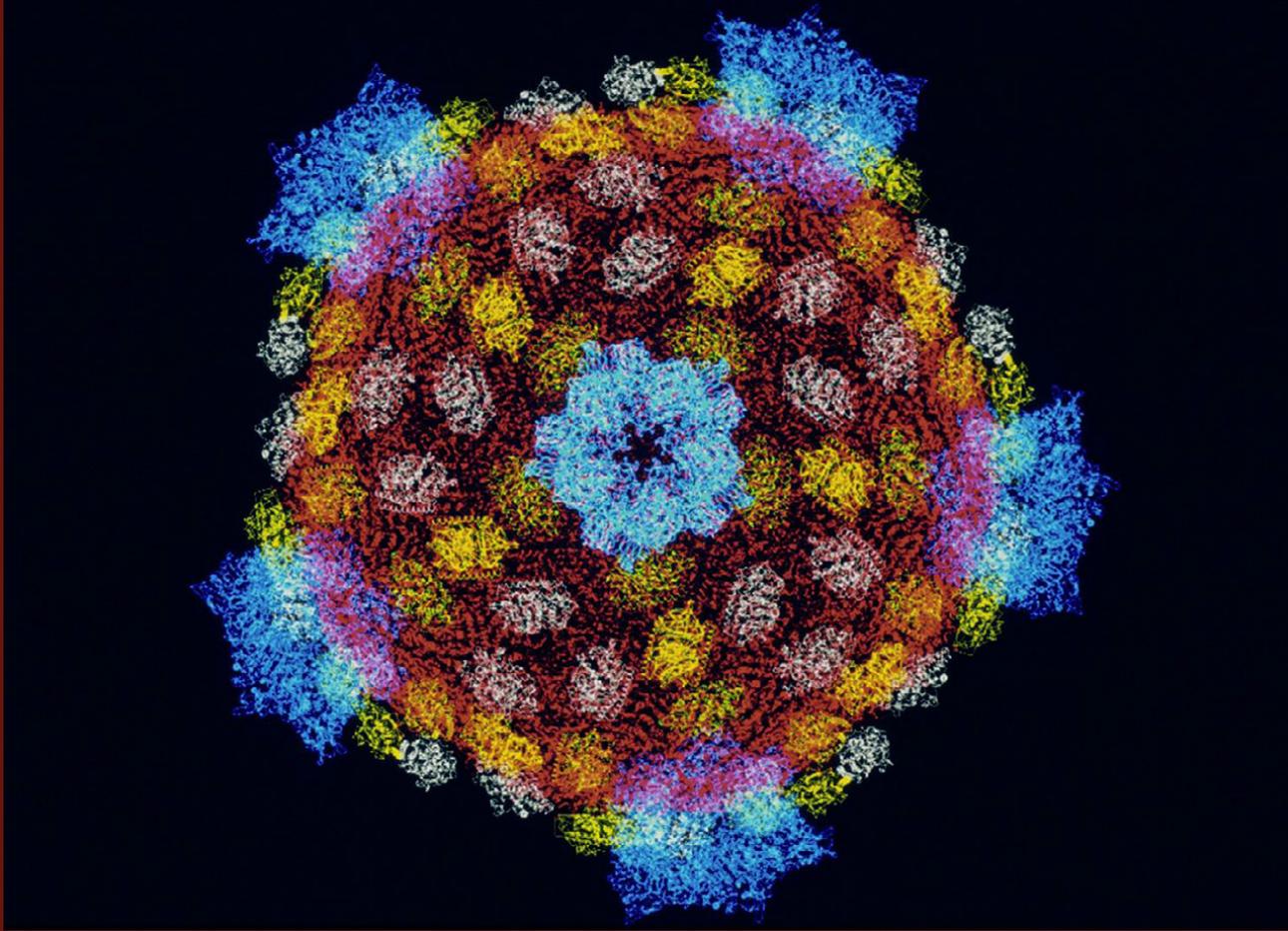
It takes a lot of detective work to understand what is written in our DNA—and how changes in the genome’s DNA sequence affect health. Yet, deciphering the nature of genetic variants can enhance understanding of diseases and disorders and identify molecular targets for developing novel interventions. Three leading scientists, Drs. Ruth Loos, Rudolph Leibel, and Manolis Kellis, highlighted their research on the genetics of obesity at a 2017 seminar on DNA sequence variants in a gene called *FTO* (fat mass and obesity-associated). The research was supported by several NIH Institutes, including the NIDDK, and other sources. The seminar was organized by the NIDDK as part of the NIH Obesity Research Task Force seminar series.

Dr. Loos and others discovered the obesity-associated genetic variants in *FTO*. These variants are common, and they contribute to increased body mass index (a measure of weight relative to height) in people

of European, African, and Asian ancestries. The association between *FTO* variants and obesity is also stronger than that of other genetic variants. But, genetics is not always destiny. Dr. Loos and her team found that people with *FTO* variants do not necessarily develop obesity, and physical activity can attenuate the risk. How do these variants increase body weight? They may, in part, affect the *FTO* gene, which encodes a protein. Intriguingly, however, the genetic variants are not in protein-coding regions of *FTO*; rather, they reside in intervening segments of DNA.

Drs. Leibel and Kellis discovered that the variant-containing segments of *FTO* have regulatory effects: they modulate the activity of *FTO* and several other genes, or the extent to which these genes are turned on or off, in different cells. Dr. Leibel and his team found that *FTO* variants modify the activity of *FTO* and an adjacent gene, called *RPGRIP1L*, in brain cells. They also showed that lower *RPGRIP1L* activity in the brain leads to overeating and excess body fat, likely by blunting response to an appetite-reducing hormone. Dr. Kellis and his team focused on a different part of the body, early-stage fat cells, and found that an *FTO* variant boosts the activity of other genes, *IRX3* and *IRX5*, which are further from *FTO* in the genome. As a result, these cells, which could have matured into cells that burn calories (“beige” fat cells), instead turned into the more common type of fat cells, which store extra calories as body fat.

The multiple effects of *FTO* genetic variants—increasing appetite through brain cell pathways, and decreasing calorie burning in fat tissue—may help explain their strong association with obesity. These research findings not only advance our understanding of body weight, but also provide insights for genetic research in general. Shining light on different areas of the genome, and on different cells and tissues, can reveal different clues as to how genetic variants affect biological processes and lead to new ideas for prevention and treatment strategies.



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

*Image courtesy of Professor Stephen C. Harrison, HHMI and Harvard University School of Medicine; and Professor Karin M. Reinisch, Yale University School of Medicine. Reprinted by permission from Macmillan Publishers Ltd: [Nature](#) 404: 960-967, copyright 2000.*

# Digestive Diseases and Nutrition

***Digestive diseases are among the leading causes of doctor visits, hospitalizations, and disability in the United States each year. These conditions span a wide spectrum of disorders that affect the gastrointestinal (GI) tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. The latest concerted effort to address the burden of all digestive diseases combining multiple big data sources estimated that digestive disease is the primary diagnosis in a total of 72 million ambulatory care visits to physicians' offices and hospital emergency and outpatient departments in the United States each year.<sup>1</sup> In addition, 4.6 million hospitalizations with a primary diagnosis of digestive diseases and 13.5 million hospitalizations with a primary or secondary diagnosis of digestive diseases were reported.<sup>1</sup> More recently, a study focusing specifically on the clinical and economic burden of emergency department visits reported 15.1 million emergency department visits with a primary diagnosis of digestive diseases and costs totaling \$27.9 billion in 2007.<sup>2</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 determine how widespread these diseases are across the United States and in specific population groups, to identify their causes and how they progress, and to test new interventions for prevention and treatment, including drugs, surgery, and behavior modification.

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

damaged intestinal tissue will help catalyze the design of novel therapeutic strategies. Research on controlling intestinal inflammation has potential benefits not only for patients with IBD, but also for those at risk of developing colorectal cancer.

Diseases of the stomach and intestines include some of the most common digestive diseases, such as peptic ulcer disease, which is typically caused by an infection with the bacterium *Helicobacter pylori* or use of non-steroidal anti-inflammatory drugs. Stomach and intestinal disorders also include functional bowel disorders, which result in symptoms of abdominal pain and altered bowel habits. For example, irritable bowel syndrome (IBS) causes pain and constipation or diarrhea. IBS more frequently affects women, who may display a different range of symptoms and respond differently from men to pharmacologic treatments for the disease. While diet and stress contribute to this disorder, its underlying causes are unknown. Gastroesophageal reflux disease, in which stomach acids rise up into the esophagus, is a common functional bowel disorder that can lead to a condition known as Barrett's esophagus. This condition, in which cells lining the esophagus turn into an intestinal type of cell, is associated with a heightened risk of esophageal cancer—one of the cancer types still on the rise in the United States. Scientists are working to understand the

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

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

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

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

Fecal incontinence, or impaired bowel control, is a bowel disorder that poses a major public health burden. Although fecal incontinence is more common in older adults, it can affect people of any age. Because it is difficult to talk about, many people suffer without seeking professional treatment for this surprisingly prevalent condition. Researchers thus aim both to examine barriers in addressing fecal incontinence and to develop improved treatment strategies.

Some digestive diseases can be triggered by the body's reaction to certain foods. For example, in individuals with celiac disease, the immune system reacts to the protein gluten—a component of wheat, barley, and rye—and damages the small intestine. This damage interferes with the ability of the intestine to absorb nutrients from foods and can result in chronic diarrhea, bloating, anemia, and, in children, slower growth and short stature. The only current treatment for celiac disease is maintenance of a strict gluten-free diet, which is difficult for many people. Recent and continued research advances in the understanding of genes and environmental triggers that are involved in the development of celiac disease may contribute to improved diagnosis and new ways to treat this condition in the future.

The microbes that inhabit the GI tract are important factors in maintaining or tipping the balance between digestive health and disease. These bacteria and viruses can affect long-term health and nutritional status in some surprising ways, depending on their interactions with each other, with intestinal cells, and with nutrients ingested by their human host. Scientists are gaining insights into the ways these GI microbes influence the development and function of the digestive tract and other systems

throughout the body, such as those with immune and metabolic functions, as well as how the composition of the GI microbial community changes with factors such as age, geography, diet, and antibiotic usage.

The exocrine pancreas, which secretes enzymes required for digestion, is vulnerable to disorders such as acute and chronic pancreatitis and their complications. Common causes of pancreatitis include gallstones, heavy alcohol use, inherited genetic factors, and drugs. In all forms of pancreatitis, digestive enzymes attack the pancreas from within, causing inflammation, loss of function, and severe pain. Advanced pancreatitis can be debilitating and may lead to cancer or diabetes, but because pancreatitis is difficult to detect in its early stages, many cases are advanced by the time they are diagnosed. Research has elucidated genetic and other factors contributing to pancreatitis that may lead to ways to treat or prevent this disorder.

The liver is an organ within the digestive system that performs many critical metabolic functions, including processing and distribution of nutrients such as fats. When the liver is functionally compromised by disease, serious adverse effects on health can occur, which sometimes leads to complete liver failure. Some liver diseases primarily affect children, such as biliary atresia (a progressive inflammatory liver disease), while others generally affect adults, such as a form of nonalcoholic fatty liver disease (NAFLD) or its more severe form, nonalcoholic steatohepatitis. In recent years, however, NAFLD has been increasingly diagnosed in children in the United States as well, concurrent with rising overweight and obesity. Some forms of liver disease are caused by viral infection, as in most cases of hepatitis, or by genetic mutations such as alpha-1-antitrypsin deficiency; others arise from diverse factors such as autoimmune reactions, drug toxicity, bile duct obstruction, and other triggers, some of which are unknown. Many liver diseases, such as chronic hepatitis B and C, place individuals at elevated risk for developing liver cancer. A healthy liver is necessary for life, and the only treatment for end-stage liver disease is a liver transplant. Because the number of livers available from deceased donors is limited, sometimes a healthy living person will donate part of his or her liver, most often to a family member who is recommended for a liver transplant. The living donor's liver eventually regenerates and grows back to normal size, as does the part of the liver that is donated.

Research is critical to identify liver disease early, find methods to preserve liver function in people with liver disease, and develop and further study new treatment options, including experimental, cell-based approaches to liver regeneration.

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

Other nutrition-related disorders under investigation involve specific, inherited alterations in nutrient metabolism. NIDDK-supported research has enhanced knowledge of how these nutritional disorders develop and how they can best be treated. The NIDDK's Office of Nutrition Research is overseeing a process to develop the first NIH-wide strategic plan for nutrition research, including providing support for activities of the NIH Nutrition Research Task Force, which is chaired by the NIDDK Director and co-chaired by the directors of the National Heart, Lung, and Blood Institute, the National Cancer Institute, and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development. Staff from these and a number of other NIH components participate on the Task Force.

## GUT MICROBIOME AND HEALTH

### **Molecules Produced by Gut Bacteria Extend**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Griffin NW, Ahern PP, Cheng J,...Gordon JI. Prior dietary practices and connections to a human gut microbial metacommunity alter responses to diet interventions. *Cell Host Microbe* 21: 84-96, doi: 10.1016/j.chom.2016.12.006, 2017.

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

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

### **Immune Molecule Defends Against Bacterial**

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

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

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

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

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

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

### **AUTOIMMUNE DISORDERS OF THE GUT AND LIVER**

#### **Uncovering the Genetic Basis for Primary Sclerosing**

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

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

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

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

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

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

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

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

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

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

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

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

### **Uncovering Factors Linked to Celiac Disease:**

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

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

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

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

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

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

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*Bouziat R, Hinterleitner R, Brown JJ,....Jabri B. Reovirus infection triggers inflammatory responses to dietary antigens and development of celiac disease. Science 356: 44-50, doi: 10.1126/science.aah5298, 2017.*

## UNDERSTANDING LIVER DISEASE

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

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

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

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

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

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*Li X, Liu R, Yang J,...Zhou H. The role of long noncoding RNA H19 in gender disparity of cholestatic liver injury in multidrug resistance 2 gene knockout mice. Hepatology 66: 869-884, doi: 10.1002/hep.29145, 2017.*

### **Studies Document Some Severe Outcomes After**

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

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

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

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

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

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

*Hayashi PH, Rockett DC, Fontana RJ,...Hoofnagle JH, for the Drug-Induced Liver Injury Network (DILIN) Investigators. Death and liver transplantation within 2 years of onset of drug-induced liver injury. Hepatology 66: 1275-1285, doi: 10.1002/hep.29283, 2017.*

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## LIVER TRANSPLANT OUTCOMES

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

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

*Shemesh E, Bucuvalas JC, Anand R,...Shneider BL. The medication level variability index (MLVI) predicts poor liver transplant outcomes: a prospective multi-site study. Am J Transplant 17: 2668-2678, doi: 10.1111/ajt.14276, 2017.*

### **Donor Outcomes in the Years After Living Donor Liver Transplantation:**

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

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

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

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

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

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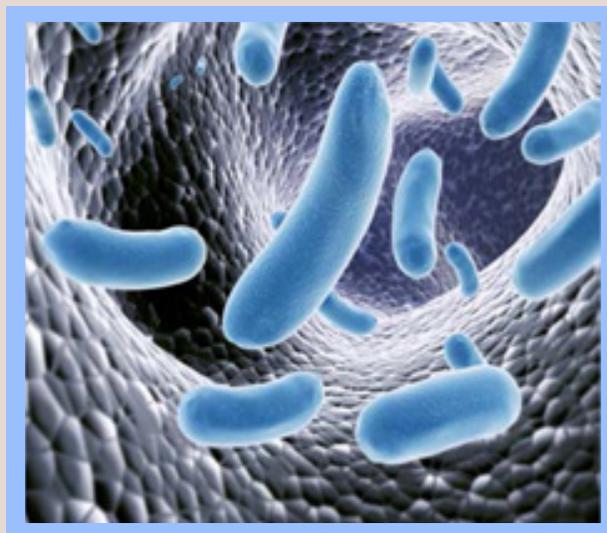
# Workshop Highlights Best Practices for Studies of Diet and Intestinal Microbiome

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

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

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

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



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

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

# New NIH Nutrition Research Task Force Guiding Nutrition Research Planning

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

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

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

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

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

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

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

# Biliary Atresia Workshop Focuses on Clinical and Translational Advances

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

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

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

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

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

## Intestinal Stem Cells

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

### The Cellular Structure of the Intestine

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

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

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

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

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

# STORY OF DISCOVERY

## Intestinal Stem Cells

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

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

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

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

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

## The Intestinal Stem Cell Consortium

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

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

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

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

## **Making Mini-intestines**

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

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

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

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

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

## **Studies on Stem Cell Renewal and Plasticity**

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

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

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

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

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

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

## The Future of Intestinal Stem-cell Research

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

## PATIENT PROFILE

# Ronetta: Finding Strength Within the Turmoil of Gastroparesis



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

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

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

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

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

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

### **Living with Gastroparesis: “It’s Complicated”**

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

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

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

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

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

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

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

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

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

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

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

***Through the turmoil of constant nausea, vomiting, and pain, Ronetta was resolute: "I'm a fighter," she says. "And I refuse to let anything beat me."***

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

## Learning To Cope with Gastroparesis

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

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

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

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

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

***"Sometimes it doesn't matter what I eat at all," Ronetta says. "It could just be one of those days.... And for whatever reason, I will be just doubled over in pain from my stomach."***

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

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

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

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

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

## Hope in Research

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

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

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

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

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

## A Network of Support

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

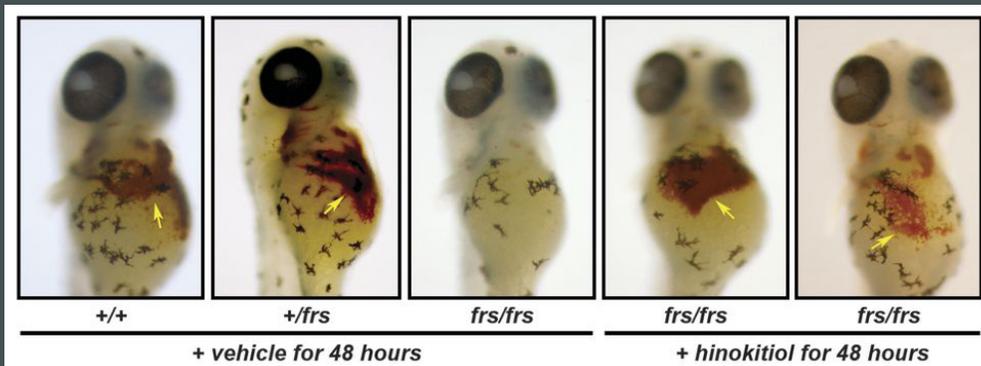
**Ronetta's motivation for joining the Gastroparesis Registry was altruistic: "Anything I can do to help someone else," she says. "It helps bring meaning to all of this."**

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

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

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

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



Humans need iron to make hemoglobin, the oxygen-carrying molecule in red blood cells. The body uses an array of iron transport proteins to properly maintain iron levels in cells and tissues. Defects in iron transport proteins have been identified in humans, and result in too little iron or too much iron in the body. Strategies that restore normal iron transport in these conditions would be very beneficial to these people. Research described in this chapter shows that a compound called hinokitiol is able to correct an iron transport defect in zebrafish. In this figure, brown staining indicates hemoglobin production in (from left to right): normal zebrafish (+/+); zebrafish partially missing an iron transport protein (+/frs); zebrafish completely missing an iron transport protein (frs/frs); and (final two panels) zebrafish completely missing an iron transport protein (frs/frs) that were treated with hinokitiol.

Image courtesy of Dr. Marianne Wessling-Resnick, Harvard T.H. Chan School of Public Health. From *Science* 356: 608-616, doi: 10.1126/science.aah3862, 2017. Reprinted with permission from AAAS.

# 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 afflict 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 treatments for them, 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 increase our understanding of kidney, urologic, and hematologic diseases in order to enhance approaches to prevent and treat these serious conditions.*

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, represents a life-threatening condition.

It has been estimated that more than 30 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 2015, over 701,000 patients received treatment for ESRD: over 493,000 received either hemodialysis or peritoneal dialysis, and over 207,000 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 and Asians, ESRD prevalence in 2015 was about 3 times greater in African Americans, 1.3 times greater in Hispanics, 1.2 times greater in American Indians and Alaska Natives.<sup>2</sup> All these represent reductions in relative risk of ESRD for these minority populations compared to non-Hispanic Whites over the past 15 years. 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. The NIDDK also supports studies

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<sup>1</sup> Centers for Disease Control and Prevention. *National Chronic Kidney Disease Fact Sheet, 2017*. Atlanta, Georgia: U.S. Department of Health and Human Services, 2017.

<sup>2</sup> U.S. Renal Data System, *USRDS 2017 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States*, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland, 2017.

of inherited diseases, such as polycystic kidney disease, congenital kidney disorders, and focal segmental glomerulosclerosis; and immune-related kidney diseases, such as IgA nephropathy and hemolytic uremic syndrome. The CKD Biomarkers Consortium 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.

The NIDDK's National Kidney Disease Education Program (NKDEP) is designed to raise awareness about the problem of kidney disease and steps that should be taken to treat CKD and prevent kidney failure. NKDEP represents a major educational outreach effort to patients, physicians, and the public. NKDEP also promotes the inclusion of estimates of kidney function as a part of routine blood testing and seeks to standardize measurements of protein in the urine, often a sign of underlying kidney disease.

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 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—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 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 1 in 10 U.S. adults (18 years of age and older) suffer from daily urinary incontinence; most of those affected are women.<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.

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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> *Urological Diseases in America*. NIDDK, NIH Publication Number 12-7865, 2012.

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). LURN will develop new questionnaires that will improve diagnosis and more comprehensively assess outcomes of interventions. 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 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, which is currently focusing on regulatory determinants of hematopoietic stem cell fate; role of non-coding RNA in hematopoiesis; role of macrophages in blood cell development; effects of aging on hematopoiesis; metabolic modulators of hematopoiesis; and remodeling the hematopoietic stem-cell niche. 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 stem cells, including adult hematopoietic (blood) stem cells, which are needed for bone marrow transplants and may have broader application in gene therapy research.

## THE KIDNEYS AT WORK

### Measuring How Well the Kidney Works—One

**“Nephron” at a Time:** Scientists developed a new method for calculating the average rate that a single kidney nephron filters blood—an important measure of kidney health. The basic functional unit of the kidney is the nephron, which consists of various cells and structures that work together to filter waste products, remove excess fluid from the blood, and balance various body chemicals. Of these structures, the glomerulus is the fundamental filtering apparatus. A common kidney function measurement called the glomerular filtration rate (GFR) is an estimate of blood filtered per minute by all the nephrons within the kidneys. However, calculating single nephron GFR is complicated for a number of reasons: there is substantial individual variation in the number of nephrons per kidney; there is variation in nephron size and in the amount of blood filtered per nephron; and availability of kidney biopsy samples is limited. Researchers have now developed a method for determining single-nephron GFR using biopsies from almost 1,400 people, with ages ranging from 20s to 70s, 58 percent of whom were women. These tiny biopsy samples were collected at the time of transplantation. People who donate their kidneys typically do not have chronic kidney disease or its major risk factors (e.g., diabetes, hypertension).

To calculate single-nephron GFR, the scientists first measured the total GFR and used a three-dimensional imaging technique that can determine the kidney's volume. Then they obtained a biopsy sample at the time of donation to determine the kidney's nephron density, as well as the average nephron size. With these measurements in hand, simple calculations revealed the number of nephrons per kidney (average of 860,000) and the single-nephron GFR for each individual. Several critical findings emerged from their analyses of the kidney donor cohort. The single-nephron GFR did not vary significantly by, sex, age (when under 70) or height (when under 6 feet, 2 inches). However, larger nephron size was associated with higher single-nephron GFR, as were hardening of glomeruli or blood vessels beyond what would be normally expected over time. In addition, some participant characteristics were linked to elevated single-nephron GFRs, including obesity, family history of end-stage renal disease, and height (over 6 feet, 2 inches).

Some of the characteristics found to be associated with elevated single-nephron GFR are known risk factors for kidney disease—seemingly contradictory findings, considering the clear association between lower total GFR and kidney disease. However, the scientists explain that some of these risk factors are often also associated with low numbers of nephrons in the kidney. This lower total number of nephrons could in turn cause individual nephrons to compensate by filtering blood at a higher rate, raising the total GFR to a normal level. However, in some people, over time the increased single nephron work load and other risk factors could lead to further nephron losses and kidney function deterioration, leading to eventual declining total GFR and kidney function. Further research would be needed to understand better how these characteristics affect the relationships between single-nephron GFR, total GFR, and kidney health.

The scientists recognize limitations in the study, such as the lack of diversity in the cohort. For example, only 2 percent of participants were Black. Also, the difficult and intensive process of kidney biopsy collection limited the study to donated kidneys. Due to the relatively healthy status of the kidneys selected through the donor screening process, additional research is needed to determine the generalizability of the results to those with reduced kidney function. Despite these limitations, this report establishes the first method for calculating single-nephron GFR from human kidneys, potentially leading to a greater understanding of the link between nephron GFR and overall kidney function and health.

Denic A, Mathew J, Lerman LO...and Rule AD. Single-nephron glomerular filtration rate in healthy adults. *N Eng J Med* 376: 2349-2357, doi: 10.1056/NEJMoa1614329, 2017.

**Insights into Salt Handling, Water Balance, and Blood Pressure Regulation by the Kidneys:** Two studies in mice have shed light on the complex relationships between kidney physiology, salt intake, water balance, and hypertension. One of the kidney's critical functions is to achieve electrolyte balance in the body by controlling urine salt concentration and water retention. Impairment of this essential function can lead to hypertension (high blood pressure). Two recent reports explored the links between salt, hypertension, and kidney function using rodent model systems.

Scientists have long believed that the body removes excess dietary salt through urination, leading to water loss that must be replenished—in other words, eating salty foods makes people thirsty. Recently, however, research has cast doubt on this simple relationship between salt and water consumption. In one previous study in 10 men, researchers found, surprisingly, that over time, increased salt consumption was associated with reduced water intake. In the present study, the team of researchers tested their previous observation experimentally using male mice that were fed either a low-salt diet with water or a high-salt diet with saline (salted water). Mice consuming a high-salt diet excreted more concentrated sodium in their urine than did mice on the lower-salt diet. Interestingly, over time the mice on a high-salt diet drank less fluid, retained more water, and consumed more food than did mice on a low-salt diet.

These results raised an important question: how does the body remove excess salt without simultaneously expelling too much water? The scientists considered that urea, a biological chemical abundantly found in urine, could be a key factor because urea in the kidney is known to drive reabsorption of water from developing urine. They found that the kidneys of mice on a high-salt diet contained higher levels of urea compared with those on a low-salt diet, helping to explain the observed water retention. Further examination of the mice revealed that additional urea was produced by muscle and liver tissue in response to increased salt. The muscle tissue appeared to be breaking down some of its molecular components as fuel to generate energy, likely to compensate for the energy-intensive process of urea production. This need for additional energy could also help explain the increased appetite observed in mice fed a high-salt diet. Together, these results uncover a novel coordinated, energy-intensive response to dietary salt by the liver, muscles, and kidneys to elevate urea levels, thereby conserving water.

In a separate study, scientists sought to gain a better understanding of the molecular basis of water maintenance and blood pressure regulation by the kidney. A segment of the nephron (the basic functional unit of the kidney) called the collecting duct fine-tunes the amounts of various essential substances, such as sodium, that can be retained in the body or excreted into the developing urine. The protein angiotensin II was

previously shown to control water reabsorption in the collecting duct. To better understand angiotensin II's role in the kidney, the researchers genetically engineered mice to lack the gene encoding the type 1 angiotensin (AT<sub>1</sub>) receptor, its essential protein partner, specifically in the collecting ducts. AT<sub>1</sub> receptor-deficient mice had the same blood pressure as normal mice, and both groups experienced hypertension similarly when they were fed high-salt diets. Mice were then administered angiotensin II, which is also known to induce hypertension. Blood pressure in normal mice predictably increased, but surprisingly, blood pressure in AT<sub>1</sub> receptor-deficient mice rose even higher; this finding was unexpected because elimination of AT<sub>1</sub> receptors was expected to prevent angiotensin II's ability to raise blood pressure. These AT<sub>1</sub> receptor-deficient mice excreted less sodium than did normal mice when given angiotensin II, suggesting that the higher salt levels may have been responsible for the elevated blood pressure. The researchers then asked whether cyclooxygenase-2 (COX-2), a known regulator of angiotensin II function, was affected by AT<sub>1</sub> receptor deficiency. Drugs that inhibit COX-2 function have been shown to influence blood pressure, leading the scientists to ask whether there could be a link between COX-2 and AT<sub>1</sub> receptor activity in this segment of the kidney. They examined collecting ducts, and found that those of normal mice given angiotensin II contained higher levels of COX-2 than did their untreated counterparts, but the absence of AT<sub>1</sub> receptors attenuated this response. Finally, the scientists again treated mice with angiotensin II to induce hypertension, but also administered a chemical inhibitor of COX-2 function. The COX-2 inhibitor eliminated the difference between AT<sub>1</sub> receptor-deficient mice and normal mice, allowing the blood pressures of both groups to rise to similar levels, further implicating COX-2 as a mediator of angiotensin II-induced hypertension. Taken together, these results define a surprising, novel role in the collecting duct for the angiotensin II-AT<sub>1</sub> receptor-COX-2 molecular pathway as a regulator of blood pressure.

These studies in mice challenge long-standing views and reveal the complexity of the kidney's role in salt and water balance, and in blood pressure regulation. If the molecular pathways described are found to work similarly in people, these two studies could pave the way for a more detailed understanding of how the human body maintains water balance in response to salt intake, and could generate novel therapeutic approaches for reducing the risk of hypertension.

Kitada K, Daub S, Zhang Y,...and Titze J. High salt intake reprioritizes osmolyte and energy metabolism for body fluid conservation. *J Clin Invest* 127: 1944-1959, doi: 10.1172/JCI88532, 2017.

Stegbauer J, Chen D, Herrera M,...and Coffman TM. Resistance to hypertension mediated by intercalated cells of the collecting duct. *JCI Insight* 2: e92720, doi: 10.1172/jci.insight.92720, 2017.

**The Sweet Smell of Success—A Protein in the Kidney Helps the Body Retain Sugar:** In a new study, scientists identified a protein in the mouse kidney that helps prevent the body from excreting glucose (sugar), potentially revealing a molecular target for novel treatment strategies for type 2 diabetes. This protein is one of a family of proteins called olfactory receptors. Decades ago, these receptors were discovered in mammals as the molecular sensors of odorants. Olfactory receptors were originally found in the nose, detecting chemicals to give us our sense of smell. Members of this protein family have since also been found in numerous other tissues throughout the body, recognizing a variety of substances and serving an array of physiological functions. Scientists previously discovered one particular olfactory receptor, called Olfr1393, in the mouse kidney, but its physiological function was unknown.

To extend their previous research, the scientists investigated the role of Olfr1393 in the kidneys of male and female mice. They determined that the *Olfr1393* gene was turned on specifically in the kidney's proximal tubules, basic functional units of the kidney. Tubules reabsorb various substances filtered from the blood to retain nutrients, such as glucose, in the body, so they are not excreted in the urine. The scientists then genetically engineered mice to lack the *Olfr1393* gene. In many ways, *Olfr1393*-deficient mice were similar to normal mice: their kidneys were of similar size and filtered blood at the same rate, and the mice had similar blood pressures and body weights. However, the researchers observed that the urine of *Olfr1393*-deficient mice contained significantly higher levels of glucose, suggesting that their kidneys did not reabsorb the sugar as well as their normal counterparts. Mice lacking *Olfr1393* also tolerated higher levels of glucose than the normal mice could.

Glucose reabsorption is a critical function of the kidney. Two related proteins in the kidney proximal tubule are responsible for glucose reabsorption: SglT1 and SglT2.

Sgt12 returns 90 percent of the filtered glucose to the blood, and Sgt1 returns the remaining 10 percent.\* The scientists found that overall levels of Sgt1 and Sgt2 were normal in *Olfr1393*-deficient mice. They also examined some of the traits of the mice for potential differences between the sexes, and, interestingly, found that the kidneys of male mice that lacked *Olfr1393* contained higher levels of Sgt2 protein than did kidneys from female mice lacking *Olfr1393*; this finding is consistent with reduced levels of urine glucose observed in these male mice. Next, the researchers more closely examined the kidney tissue and found that while overall Sgt1 levels were similar in all mice, the protein was not found in its normal location, the cell membranes of proximal tubule cells, in mice deficient in *Olfr1393*. These data suggest that Sgt1 function was impaired due to its inability to reach its intended location within the cell.

These results demonstrate the critical role of *Olfr1393* in regulating glucose reabsorption by the kidney. Improved tolerance of glucose in *Olfr1393*-deficient mice suggests that the olfactory receptor could influence the body's ability to regulate glucose levels. If *Olfr1393* functions similarly in people, it could represent a potential new target for the development of therapeutic strategies for type 2 diabetes.

*\*Sgt2 inhibitors are a new class of diabetic medications indicated only for the treatment of type 2 diabetes. In conjunction with exercise and a healthy diet, they can improve glycemic control.*

Shepard BD, Cheval L, Peterlin Z,...and Pluznick JL. A renal olfactory receptor aids in kidney glucose handling.

*Sci Rep* 6: 35215, doi: 10.1038/srep35215, 2016.

## INSIGHTS INTO LOST KIDNEY FUNCTION

**Kidney Damage from High Blood Pressure Leads to New Lymphatic Vessel Formation:** Scientists have discovered that in rodents, hypertension- or aging-induced kidney injury and inflammation leads to the development of new lymphatic vessels in the kidneys. Hypertension (high blood pressure) is a leading cause of chronic kidney disease and kidney failure. Previous research has shown that a type of immune cell, called the macrophage, accumulates in the kidney and contributes to inflammation—a process that can lead to hypertension, which in turn

increases risk for kidney disease. In a new study, researchers examined the effects of hypertension on lymphatic vessels, which drain fluid, immune cells (such as macrophages), and waste products away from tissues. Hypertension can lead to the formation of new lymphatic vessels, a process known as lymphangiogenesis, in various organs. However, lymphangiogenesis can either be harmful or beneficial depending on various factors, such as the timing of the inflammatory response. Because lymphatic vessels have been implicated in kidney disease, the scientists hypothesized that hypertension could affect the dynamics of their formation in the kidneys.

The researchers used well-studied rat models, including two types of rats that spontaneously develop hypertension, referred to as SHR (for spontaneously hypertensive rat). One of these types of hypertensive rats also develops kidney damage (a strain called SHR-A3), while the other does not (called SHR-B2). These two strains were compared with a third that does not develop high blood pressure under similar conditions (called WKY). By examining molecular signals of lymphangiogenesis from kidney biopsies, the researchers found that kidneys of male SHR-A3 rats (prone to kidney damage) had a higher density of lymphatic vessels and increased lymphangiogenesis than did the kidneys of male rats without hypertension (WKY). By contrast, male SHR-B2 rats' kidneys had fewer lymphatic vessels and reduced lymphangiogenesis compared to kidneys from WKY rats. Molecular signals indicating the presence of macrophages and inflammation were stronger in SHR-A3 rats' kidneys than in kidneys from SHR-B2 or WKY rats, suggesting that immune cells had invaded the injured kidneys.

The researchers then examined the effect of aging on lymphangiogenesis in the kidneys. They used Fischer 344 rats, which spontaneously develop kidney disease over time, but not hypertension. When 4-month old male rats were compared to 20- and 24-month old rats, the scientists found that kidney injury from aging led to lymphangiogenesis and increased immune cell invasion, similar to the SHR-A3 strain.

These findings suggest that kidney damage, due to either a hypertensive state or the course of aging, can lead to increased macrophage-induced inflammation and density of lymphatic vessels in the kidney.

However, the rodent models in the study were used because they exhibit particular traits (e.g., strong resistance to hypertension or kidney damage) that may not represent a real-world situation in people. A more likely explanation in patients, the scientists suggest, is that hypertension induces subtler kidney inflammation, injury, and lymphangiogenesis than is seen in these animal models, consistent with the slower progression of the disease that is often observed in chronic kidney disease. Additional research would help determine whether lymphatic vessels could be useful targets for the development of therapeutic strategies to reduce risk for hypertension and kidney disease.

*Kneeder SC, Phillips LE, Hudson KR,...and Mitchell BM. Renal inflammation and injury are associated with lymphangiogenesis in hypertension. Am J Physiol Renal Physiol 312: F861-F869, doi: 10.1152/ajprenal.00679.2016, 2017.*

**Predicting Kidney Function Decline in People Who Are at High Risk for Kidney Disease:** A new study has found that levels of the protein suPAR in the blood can help predict whether kidney function will deteriorate in people with high-risk genetic variants of the gene *APOL1*. Genetic variants of *APOL1*, which are found primarily in individuals of African ancestry, are arguably the most important discovery about the pathogenesis of chronic kidney disease over the past several decades, and among the only established genetic factors contributing to the well-appreciated health disparities in kidney diseases in Blacks compared to Whites. Individuals with one or two copies of either the G1 or G2 variants of the *APOL1* gene are protected from a potentially deadly infectious disease (African sleeping sickness) compared to people with only the G0 variant. However, those with any combination of two G1 and/or G2 variants of the gene are at increased risk of developing kidney disease.

Previous research showed that high levels of a protein called suPAR in the blood is associated with decline in kidney function and progression to chronic kidney disease. Because many people with the high-risk G1 and G2 *APOL1* variants do not develop kidney disease, researchers explored whether blood suPAR levels can help predict whether African Americans with these genetic variants will experience declining kidney function. The scientists analyzed data that had been collected from participants of two other studies, which together included almost

1,100 African-American participants. Kidney function was determined by evaluating estimated glomerular filtration rate, which is a commonly used measurement of how well the kidneys are filtering wastes and extra fluid from the blood. The scientists found that in people with high-risk *APOL1* variants, kidney function was likely to decline more rapidly over time in those with elevated plasma suPAR levels than those with lower suPAR levels.

Using another series of tests, the researchers asked whether suPAR could physically associate with the *APOL1* protein, which is encoded by the *APOL1* gene, and found that the G0, G1, and G2 proteins all interacted directly and tightly with suPAR. They also examined whether *APOL1* and suPAR could bind to  $\alpha_v\beta_3$  integrin, a protein complex known to mediate the action of suPAR in kidney cells. When in an activated state,  $\alpha_v\beta_3$  integrin formed strong protein complexes with suPAR and the high-risk G1 and G2 *APOL1* complexes; by contrast, it formed very weak complexes with low-risk, G0 variant *APOL1* proteins.

The scientists then examined the functional effects of these protein interactions by measuring the stimulation of  $\beta_3$  integrin, a component of the  $\alpha_v\beta_3$  integrin protein complex, in cultures of a type of human kidney cells called podocytes. The G1 and G2 variants of *APOL1* could activate  $\beta_3$  integrin, but only when suPAR was added to the culture; the *APOL1* G0 variant could not activate  $\beta_3$  integrin under any condition. They then injected female mice with DNA encoding either human G0, G1, or G2 *APOL1* variants and found that mice with G1 or G2 excreted high levels of protein in their urine, and their podocytes appeared to be physically injured—two indicators of kidney damage. The G0 variant had no effect on these mice. However, when the *APOL1* G2 variant was produced in mice that were genetically engineered to lack suPAR, urine protein levels were the same as in normal mice.

Taken together, these findings reveal that high levels of suPAR may play an important role in the kidney function decline observed in some people with *APOL1* G1 and G2 variants, and thus may serve as a useful predictor of kidney disease. The direct interactions observed between *APOL1*, suPAR, and  $\alpha_v\beta_3$  integrin proteins help provide a mechanistic explanation of how kidney damage develops in those with high-risk

APOL1 genetic variants, and thus may pave the way for new therapeutic strategies to prevent or treat kidney disease in these populations.

Hayek SS, Koh KH, Grams ME,...and Reiser J. A tripartite complex of suPAR, APOL1 risk variants and  $\alpha_v\beta_3$  integrin on podocytes mediates chronic kidney disease. *Nat Med* 23: 945-953, doi: 10.1038/nm.4362, 2017.

## KIDNEY STONE PREVENTION

**New Treatment Prevents Formation of Cystine Stones, a Type of Kidney Stone, in Mice:** In a recent study, the antioxidant  $\alpha$ -lipoic acid was shown to prevent the formation of cystine stones in mice. Kidney stone disease, also referred to as urinary stone disease, is a painful and increasingly common problem, with some people experiencing recurrent episodes. Despite the high prevalence and health and economic burden of the disease, little is known about how stones form or which are easily passed through the urinary tract. Advances in treatments in the past 30 years have evolved from open surgery to remove large stones, to new technologies. Unfortunately, these new advances have not benefited people who form cystine stones, and thus research efforts continue, toward preventing or slowing the formation of this type of stone.

Researchers recently used a mouse model of cystine stone disease to identify compounds that slowed the growth of this stone type. This genetically engineered model readily forms cystine stones in the bladder of the male mouse. Two compounds believed to have anti-cystine stone formation properties, tiopronin and L-CDME, were initially evaluated in this model system but were ineffective. In contrast to these two drugs,  $\alpha$ -lipoic acid significantly slowed the growth rate of cystine stone formation compared with untreated animals in the model system. Prior to the time when these mice would begin to form stones, they were treated with or without  $\alpha$ -lipoic acid to assess its ability to alter cystine stone formation. This experimental design demonstrated that  $\alpha$ -lipoic acid significantly prevented stone formation, delayed the time needed for stone formation, and/or reduced the overall size of stones that did form. Notably, when  $\alpha$ -lipoic acid treatment was discontinued, the mice subsequently resumed producing cystine stones, indicating that  $\alpha$ -lipoic acid treatment is reversible.

Additional results of the study indicated similar amounts of cystine were present in the urine from both untreated and  $\alpha$ -lipoic acid-treated animals in the model system. Given this finding, the investigators hypothesized that the urine obtained from  $\alpha$ -lipoic acid-treated animals provided an environment in which cystine is less likely to crystalize and begin to form stones compared to untreated animals. The investigators confirmed this is a likely mechanism by demonstrating that the urine from  $\alpha$ -lipoic acid-treated animals formed significantly less cystine precipitate (solid deposits that form from the compound in solution) during a 3-day period in the laboratory. Thus, it appears that the urine obtained from  $\alpha$ -lipoic acid-treated animals can maintain larger amounts of cystine “in solution”—thereby shutting down the pathway that ultimately leads to stone formation.

Research efforts continue that ultimately seek to prevent cystine stone formation in people. One effort is assessing the efficacy of cystine mimetics (compounds with similar structure) to block the cystine crystallization process in human urine and stone formation in the model system used above. A second effort will evaluate how daily  $\alpha$ -lipoic acid supplementation over 3 years affects cystine stone recurrence in 50 people.

Zee T, Bose N, Zee J,...Kapahi P.  $\alpha$ -lipoic acid treatment prevents cystine urolithiasis in a mouse model of cystinuria. *Nat Med* 23: 288-290, doi: 10.1038/nm.4280, 2017.

## ADVANCING UNDERSTANDING, TREATMENT, AND PREVENTION OF URINARY TRACT INFECTIONS

Urinary tract infections (UTIs) are common and occur more frequently in women, many of whom experience recurrent infections. UTIs are currently treatable with antibiotics. However, infections recur even after treatment in many women; and the emergence of antibiotic-resistant bacterial strains, as well as the personal burden and medical costs of care, make finding improved prevention and curative strategies a high priority.

The bacterium *Escherichia coli* (*E. coli*) is the primary culprit in UTIs. Normally an integral part of a healthy digestive tract, some *E. coli* acquire the ability to invade and wreak havoc in the urinary tract. These UTI-causing

*E. coli*, also referred to as uropathogenic *E. coli*, or UPEC, bind to and invade cells lining the inside of the bladder to initiate an infection. Researchers have been tackling the problem of UTIs from two main angles: seeking a better understanding of the factors produced by bacteria that enable them to thrive in the urinary tract; and infection-associated factors and defenses produced by the “hosts” (humans or animals). These factors could be targeted for therapeutic development. Researchers have also sought greater understanding of dynamic interactions between the bacterial and host factors. Several recent discoveries in animal models and humans about the biology of UTIs, including potential vaccine targets, summarized below, are taking scientists steps closer to new approaches to reduce the burden of this urologic disease in people.

### **Seeking a Genetic Signature for Urinary Tract Infections:**

In a recent study, scientists sought to determine whether there is a universal “genetic signature(s)” defining the ability of UPEC to cause disease. Potential candidates for such a signature include so-called putative urovirulence factor (PUF) genes that have been found to be enriched in UTI-associated *E. coli* versus “regular” *E. coli*. To pursue this question, the researchers studied 21 representative *E. coli* strains obtained from the urine of 14 women who suffered from recurrent UTIs. They compared and “scored” these strains based upon how many PUF genes each one possessed. By comparing this score with how well each strain infected bladders in a standard female mouse model, the researchers determined that having a higher PUF gene score did not correlate with either strength (robust, variable, or deficient) of an acute infection, or with the ability to cause chronic infection in one mouse model. However, the investigators found that mice that differed genetically reacted differently to infection—for example, two bacterial strains that caused robust bladder infection in one mouse model were much less effective in a second mouse model. Rather than ignore the differences between the mouse models, the scientists investigated whether that could provide a clue to differences in infectivity. Using the first mouse model, they found that variation in the activity of certain genes regulating core *E. coli* functions correlated with how effectively the strains infected the animals. Bacterial strains that more robustly infected the mice correlated with markers of greater UTI severity in women, such as higher white blood cell counts in urine. Together, the

study findings suggest that rather than a signature set of genes consistently determining virulence, a more complex and dynamic interplay between *E. coli* strains and their host environment determines infection and subsequent disease.

Schreiber HL 4th, Conover MS, Chou WC,...Hultgren SJ. Bacterial virulence phenotypes of *Escherichia coli* and host susceptibility determine risk for urinary tract infections. *Sci Transl Med* 9 pii: eaaf1283, doi: 10.1126/scitranslmed.aaf1283, 2017.

### **Dynamic Bladder and Bacterial Changes Hold Clues to Therapies:**

In two recent studies, researchers delved into changes that occur in the bladder and invading bacteria that affect both acute and recurrent infection. Repeated infections in women with a history of UTI are not always due to the same strain of UPEC or even to *E. coli*, and the majority of UPEC studies have been performed in “infection-naïve” mice—those that had no prior infection. Thus, there is a need to better understand how and what changes occur in the bladder during acute infection affect susceptibility to future infection, and how microbes have adapted to use these changes to persist in the bladder.

In one study, investigators examined more closely the impact of a history of UTI on risk for recurrent UTI. Similar to what is seen in human UTIs, mice infected with UPEC, in the absence of antibiotic treatment, have different outcomes—some spontaneously resolve the infection within a couple of weeks, while others go on to develop a chronic infection. To study the interaction of these outcomes with future infection, the researchers developed an experimental mouse model. They infected female mice with a standard UPEC strain commonly used in the laboratory, and separated mice in which the infection spontaneously resolved from mice that became chronically infected after 4 weeks. They then provided both groups with a 4-week convalescent period of antibiotic treatment. (A third, control group of mice was mock-infected but otherwise treated the same way.) Subsequently, the researchers exposed the three groups of mice to a fresh dose of UPEC. They found that the group of mice that previously developed chronic infections was predisposed to develop severe, recurrent infection, whereas mice from the spontaneously resolving group developed mild, acute infections that were quickly over, earning these groups the titles of “sensitized” and “resolved,” respectively. The researchers then compared how

sensitized, resolved, and naïve mice reacted to infection with several different strains of *E. coli* associated with urinary tract infections in people as well as a non-*E. coli* bacteria that can also cause UTIs. They found that the sensitized mice were highly susceptible to developing chronic infections from these strains, whereas virtually all of the resolved mice did not develop any chronic infection at all from these strains. These observations in mice suggest that a history of UTI and whether it resolved or became chronic could have an important impact on susceptibility to future infection.

Microscopic examination of the experimental model mouse bladders revealed dramatic structural changes. Cells at the surface of the bladder lining were smaller in both sensitized and resolved compared to naïve mice, whereas sensitized mice showed additional changes in deeper tissue layers that were suggestive of incomplete repair and regeneration of the bladder lining during convalescence. Intriguingly, results of other experiments suggested that this bladder remodeling also altered the dynamics of UPEC infection. Two further experiments shed light on possible targets for therapy. One experiment indicated that heightened susceptibility to chronic infection in sensitized mice is likely driven by a pro-inflammatory factor called cyclooxygenase-2 (COX-2). Treating sensitized mice with a COX-2 inhibitor prior to second infection with UPEC ameliorated the burden of infection. The other experiment demonstrated that vaccination against a UPEC protein essential to infection also protected sensitized mice from developing either acute or chronic infections. Important to potential translatability to humans, defects in the bladder lining have been observed in human chronic and recurrent UTI. Thus, such therapeutic targets could be useful if UTI-induced bladder changes and their impact as revealed in this study in mice turn out to occur similarly in some people.

In a second study, scientists focused on a specific UPEC factor and learned critical new information about its role in persistent UTIs. UPEC carry on their surface a number of protein fibers, called pili (singular: pilus), that enable them to attach to different surfaces. It has long been known that specific pili, called type 1 pili, are essential to UPEC's ability to initiate infection. The pili are tipped with adhesive domains that enable binding to specific receptor molecules in the bladder lining and facilitate bacterial invasion of bladder cells. The adhesive domains are referred to as "adhesins."

Through experiments in female mice, the researchers discovered that whereas the type 1 pilus adhesin, called FimH, enables UPEC to adhere to the cells at the surface of a healthy ("naïve") bladder lining at the outset of infection, UPEC then deploy a second type of pilus with a different adhesin, called FmlH, that adheres specifically to infected, inflamed bladder lining. This appears to occur as the superficial cells bound by FimH are shed during the bladder response to acute infection, exposing inflamed tissue bearing the target for FmlH binding. Notably, additional experiments indicated that the expression of the target is likely increasing as inflammation-induced remodeling of the bladder tissue occurs. The deployment of the second type of pilus provides an advantage in establishing chronic infection in a mouse model: UPEC lacking FmlH were much less effective at causing or maintaining chronic infection in mice. When the researchers tested a vaccine against FmlH in mice, they found that vaccinated mice initially had the same bacterial burden as mock-vaccinated mice, but within 2 to 3 days of infection the burden dropped significantly; the vaccine thus protected the mice against progression of infection. This study reveals a mechanism by which UPEC have adapted to and leveraged the host response to acute infection to their advantage, and a new therapeutic target that could help halt chronic or recurrent infection in its tracks.

Conover MS, Ruer S, Taganna J,...Hultgren SJ. Inflammation-induced adhesin-receptor interaction provides a fitness advantage to uropathogenic *E. coli* during chronic infection. *Cell Host Microbe* 20: 482-492, doi: 10.1016/j.chom.2016.08.013, 2016.

O'Brien VP, Hannan TJ, Yu L,...Hultgren SJ. A mucosal imprint left by prior *Escherichia coli* bladder infection sensitizes to recurrent disease. *Nat Microbiol* 2: 16196, doi: 10.1038/nmicrobiol.2016.196, 2016.

**Possible New Trigger for Recurrent Urinary Tract Infections Identified:** Researchers have identified a naturally occurring component of the vaginal microbiota as a possible trigger of recurrent UPEC UTI and a subsequent kidney infection.

A current model of the "infection life cycle" for UPEC-caused UTIs starts with acute infection, in which UPEC attach to and invade cells lining the bladder. There, they are protected from immune responses and form intracellular bacterial communities (IBCs), expand in number, and then burst forth from

the bladder cells. As the bladder tries to clear the bacteria by sending out immune cells and sloughing off the superficial layer of infected bladder cells, remaining UPEC go deeper within the bladder tissue and form additional IBCs, contributing to the acute infection. In some cases, these IBCs, which are also protected from antibiotics, go on to exist in a quiescent state (latency) until a signal in the environment causes them to emerge and start a new infection. Together with other evidence, such as the detection of IBCs in human urine during infection, this model provides a possible explanation for some of the cases in which recurring UTIs in humans are caused by the same bacterial strain—complementing other likely sources of same-strain reinfection, such as the gastrointestinal tract and vagina. However, factors that can trigger the emergence of quiescent UPEC from intracellular reservoirs in humans remain unknown.

Building on evidence implicating vaginal bacteria in susceptibility to bladder and kidney infections, researchers sought to determine if certain bacteria might trigger recurrent UPEC infection. Using a female mouse model carrying latent UPEC reservoirs, they exposed bladders of these mice to one or the other of two different types of bacteria that can predominate in the vagina, providing two doses one week apart. They found that in the case of one of the bacteria, *Gardnerella vaginalis* (*G. vaginalis*), this transient exposure was sufficient to induce the appearance of UPEC in the urine of over 50 percent of the tested mice. Notably, mice that responded to *G. vaginalis* as a trigger for UPEC reemergence were more likely to have had a more severe UTI prior to its resolution. *G. vaginalis* is associated with bacterial vaginosis, a form of inflammation caused by bacterial overgrowth. Many risk factors for bacterial vaginosis in humans overlap with risk factors for kidney infection. Upon examination, the researchers found that *G. vaginalis* was detectable in about one-third of mouse kidneys within 3 hours of exposure, and was able to cause kidney injury in the absence of *E. coli*. Exposure of UPEC reservoir-containing mice to *G. vaginalis* also increased the risk for these animals to develop severe *E. coli* kidney infections. This result suggests that *G. vaginalis* is a trigger for recurrent UTIs in mice, and future studies could help delineate whether *G. vaginalis* exposure is also a trigger for recurrent UTIs in humans, and the potential relevance to clinical care and prevention of UTIs and possibly associated kidney infections.

Gilbert NM, O'Brien VP, and Lewis AL. Transient microbiota exposures activate dormant *Escherichia coli* infection in the bladder and drive severe outcomes of recurrent disease. *PLoS Pathog* 13: e1006238, doi: 10.1371/journal.ppat.1006238, 2017

### Urinary Tract Infection Vaccines—Putting Metal

**to the Pedal:** Metals such as iron and copper are necessary co-factors in many microbial molecular pathways. Thus, during an infection, host organisms will try to limit access to these essential nutrients to help quash microbial growth, and microbes will try to acquire them to thrive and survive. Microbial acquisition of metals is usually accomplished by small metal-binding molecules, such as siderophores, which are small, high-affinity compounds that bind iron. In one recent report, investigators describe new evidence suggesting that UPEC use a siderophore called yersiniabactin (Ybt) more broadly to also modulate uptake of copper, preserving its availability while minimizing its toxicity to the bacteria. This multi-metal tasking aspect of Ybt adds to its importance to UPEC.

In a second study, researchers focused on using Ybt and another UPEC siderophore, aerobactin (Aer) as vaccination agents in mice, with the goal of raising host immune responses to UTI-causing bacteria. The mice were then challenged with exposure to a UPEC strain known to encode Ybt and Aer, which are not normally detected by host immune responses, and enterobactin (Ent), a siderophore that is targeted by innate immune host defenses, thus making Ybt and Aer essential to iron acquisition by this strain. The results showed that vaccination with Ybt or Aer reduced acute bacterial burden in the bladder by 12- and 19-fold, respectively. The mouse model they used is one that experiences “ascending” UTIs—i.e., infections that move into the kidneys—and the researchers observed that the siderophore vaccines also significantly reduced bacterial burden in the kidneys. Unlike innate immune responses, which act quickly, inducing adaptive immune responses—such as the formation of antibodies in response to a vaccine agent—takes time. Thus, although the researchers did not have the means to confirm that anti-siderophore antibodies were being generated, an additional experiment showed that their vaccination approach was not effective if the mice were exposed to bacteria only 1 week after administration, providing indirect evidence that the siderophore vaccines are inducing an adaptive immune response. These

encouraging results point toward an additional pool of candidate targets for vaccines to prevent UTIs.

Koh EI, Robinson AE, Bandara N, Rogers BE, and Henderson JP. Copper import in *Escherichia coli* by the yersiniabactin metallophore system. *Nat Chem Biol* 13: 1016-1021, doi: 10.1038/nchembio.2441, 2017.

Mike LA, Smith SN, Sumner CA, Eaton KA, and Mobley HL. Siderophore vaccine conjugates protect against uropathogenic *Escherichia coli* urinary tract infection. *Proc Natl Acad Sci USA* 113: 13468-13473, doi: 10.1073/pnas.1606324113, 2016.

**Reducing Catheter-associated Urinary Tract Infections in Nursing-home Residents:** A recent report highlights a successful effort to prevent catheter-associated urinary tract infection (CAUTI) in people residing in nursing homes. CAUTI is a chronic, costly, and potentially dangerous problem for people living in managed care facilities. Residents of nursing homes may use a urinary catheter for several reasons including urinary incontinence, urinary retention, urinary obstruction, accurate measurement of urinary output, required immobilization following trauma or surgery, and hospice or palliative care. Unfortunately, CAUTI can lead to hospitalization, cause a life-threatening condition called sepsis, and result in the spread of bacteria that are resistant to antibiotic treatment in this vulnerable population. While efforts to reduce such infections have included approaches to reducing the frequency of catheter usage, strategies need to be developed and tested to prevent infections in people who continue to require the use of urinary catheters.

Researchers now have reported the success of a multicomponent strategy to reduce CAUTI, as tested in 404 community-based nursing homes in 38 states. The strategy included, for example, training in proper aseptic (germ-free) insertion of catheters, catheter care and incontinence care planning, and catheter removal. Other components of the strategy addressed empowering facility teams, offering solutions to overcome barriers, resident and family engagement, and effective communications. This multi-pronged strategy did not reduce the frequency of catheter use, yet it succeeded in decreasing urinary tract infection rates by 54 percent—as compared to the infection rate at the same facilities before the study began.

The compelling results of this Agency for Healthcare Research and Quality (AHRQ)-led study, to which NIDDK and other organizations provided additional support, suggest this approach would be highly beneficial for people in nursing homes nationwide. To facilitate adoption of these methods, AHRQ has developed a toolkit to reduce CAUTI and other healthcare-associated infections in long term care facilities.

Mody L, Greene MT, Meddings J, ...Saint S. A national implementation project to prevent catheter-associated urinary tract infection in nursing home residents. *JAMA Intern Med* 177: 1154-1162, doi: 10.1001/jamainternmed.2017.1689, 2017.

## UROLOGIC CHRONIC PELVIC PAIN SYNDROMES

**Novel Insights into Present and Future Pain in People with Urologic Pain Syndromes:** Two recent reports have revealed important new information about pain patterns and other symptoms in people with the urologic chronic pelvic pain syndromes (UCPPS) interstitial cystitis/bladder pain syndrome (IC/BPS) and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), and the detection of future pain trends through noninvasive brain imaging. This research was conducted by investigative teams from the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network. The Network is using approaches that “look beyond” the bladder and prostate—the traditional focal points for study of these syndromes—to uncover meaningful information about the not-well-understood UCPPS and their relationships to other chronic pain conditions that could set the stage for future interventions.

In the first report, the research team set out to capture information about pain location, severity, and associated health and quality-of-life factors in women and men with UCPPS enrolled in the Network. To do this, 233 women with IC/BPS and 191 men with either IC/BPS or CP/CPPS were surveyed using multiple questionnaires assessing urologic, nonurologic, and psychosocial symptoms, and quality of life. For example, questionnaire items included measures or ratings of urinary frequency and urgency, pelvic pain, sleep, commonly co-occurring chronic pain conditions such as irritable bowel

syndrome, and stress. They were also asked to look at a “body map”—two drawings representing the front and back of the body partitioned into 45 numbered sites, comprising eight body regions—and to check off any site in which they had experienced pain in the past week. From these data, the investigators found that, whereas a quarter of participants reported pain only in the pelvic region, 75 percent reported pain in both pelvic and nonpelvic regions. They then subdivided the latter group into two groups: “intermediate pain” for people who checked sites in one to two nonpelvic regions, and “widespread pain” for people who checked sites in three to seven nonpelvic regions. Intriguingly, there were no differences in pelvic pain severity or urinary symptoms across the three groups. In contrast, nonpelvic pain severity, prevalence of chronic overlapping pain conditions, worsening psychosocial health, and poor quality of life increased as the number of pain regions increased. Notably, more women than men experienced widespread pain, and women were more likely to report a greater burden of nonpelvic and nonurinary symptoms and conditions as their pain locations increased.

The investigators then compared just the intermediate and widespread pain groups to see if there were any significant differences among study participants with UCPPS who reported nonpelvic pain. This comparison yielded results similar to the three-group comparison for pelvic pain and urinary symptoms, nonpelvic pain, and several other health measures, but also revealed more gender-specific differences. For example, compared to individuals in their respective intermediate pain groups, men with widespread pain were more likely to have migraines and anxiety, while women were more likely to have irritable bowel syndrome and sleep disturbance. The differences found in this study among people diagnosed with UCPPS have significant implications for better understanding the cause(s) and/or development of these syndromes, for research studies on potential treatment approaches, and for clinical diagnosis and personalized care.

Numerous studies have uncovered brain changes in people with UCPPS compared to people without these syndromes, and a second Network research team investigated whether there was a brain “signature” in people with UCPPS of several years duration that could predict future changes in pain. Such knowledge could potentially identify brain

factors involved in worsening or relief of pain and reveal a therapeutic target(s) for study. Using a brain imaging technique that measures the strength of functional interactions among different brain regions while at rest, researchers conducted voluntary brain scans on a subset of participants shortly after their enrollment in the Network. These scans and subsequent analyses yielded 13,530 brain connectivity measures per person. The team then compared analyses of the brain imaging data from 34 women and 18 men to their pelvic pain symptom status—categorized as “improvement” or “nonimprovement”—at 3, 6, and 12 months, to see if the brain scans could predict symptom trends. They found that their imaging analyses correctly predicted symptom trends in 73 percent of participants for the 3-month period following the scan. Further analyses revealed that the top 100 brain region connections that were more robust in “improvers” versus “nonimprovers” fell predominantly within a part of the brain associated with attention to sensory information.

Additional experiments will need to be performed in larger groups of people and under varying conditions to confirm, optimize, and extend these findings. However, this study is the first to demonstrate the feasibility of predicting pain symptom changes in women and men with UCPPS and has provided preliminary insights important to understanding the biology of pain and improving symptom management in these people.

*Kutch JJ, Labus JS, Harris RE,...Mayer EA; for the MAPP Research Network. Resting-state functional connectivity predicts longitudinal pain symptom change in urologic chronic pelvic pain syndrome: a MAPP network study. Pain 158: 1069-1082, doi: 10.1097/j.pain.0000000000000886, 2017.*

*Lai HH, Jemielita T, Sutcliffe S,...Landis JR; for the MAPP Research Network. Characterization of whole body pain in urological chronic pelvic pain syndrome at baseline: a MAPP Research Network study. J Urol 198: 622-631, doi: 10.1016/j.juro.2017.03.132, 2017.*

## UNDERSTANDING AND TREATMENT OF BLOOD DISEASES

**A Personalized Medicine Treatment Plan Developed After Identification of a Rare Pathogenic Mutation:** After discovering a rare genetic mutation responsible for a previously unknown severe blood disorder in a 6-year old boy, researchers developed a personalized treatment plan for his newborn sibling, also born with

the same blood disorder. Doctors at first diagnosed the boy when he was 1 year of age with Diamond-Blackfan anemia (DBA). DBA is a serious medical condition characterized by insufficient level of red blood cells, also known as anemia. Red cells carry oxygen from the lungs to the body's organs and tissues. The boy was given standard therapy for DBA. He was treated initially with blood transfusions, which provide a source of needed red cells, and then underwent a bone marrow transplant with a fully matched donor at 6 years of age. Although the doctors hoped the bone marrow transplant would be curative, as is typically the case for people with DBA, early signs showed that the transplant was not working as expected, and unfortunately the boy subsequently did not survive due to complications of the procedure.

Clinical scientists became aware of this 6-year old boy while they were conducting research to discover yet unknown genetic causes of DBA. Given the unanticipated transplant outcome, the researchers were interested to learn the cause of the boy's severe anemia, thinking it might be different from typical DBA, and that new insights might help in developing a better treatment approach for others. An analysis of the boy's genes did not reveal mutations known to cause DBA but did identify, for the first time, a mutation in the gene encoding the small protein hormone erythropoietin (EPO). The genetic mutation alters one of the 160 amino acid building blocks of the EPO protein; it changes an arginine amino acid to a glutamine amino acid. EPO normally stimulates red cell production by interacting with other proteins, called EPO receptors, on the surface of early stage (progenitor) red cells in the bone marrow.

The investigators determined that although mutant EPO had slightly less attraction for the EPO receptor than normal EPO had, the EPO mutation decreased its ability to remain attached to the EPO receptor, apparently resulting in significantly diminished ability to stimulate red cell proliferation (*i.e.*, an increase in number of cells) in culture, as compared to normal EPO. These laboratory findings are consistent with the inability of mutant EPO to produce adequate levels of mature red cells in the young boy.

While these research findings were undergoing review for publication, the researchers learned that the parents of the 6-year old boy had a newborn child, who also had anemia. Further testing confirmed that the newborn carried the same arginine to glutamine mutation in EPO. Equipped with new knowledge gained from research, the clinical scientists

developed a treatment strategy. They obtained appropriate permissions to initiate a personalized medicine treatment plan consisting of injections of normal EPO produced in the laboratory, also called recombinant EPO. Recombinant EPO is used often to increase levels of red cells in patients whose red cells have been depleted by a different condition, for example as a result of kidney disease or chemotherapy for cancer. The researchers reasoned that recombinant normal EPO might also work for this child, by latching onto EPO receptors more productively than the child's own EPO, and thus restore red cell production. After 11 weeks of treatment, the child's red cell production had increased—eliminating the need for blood transfusions.

This study underscores the benefit of research to greatly improve the life of a patient. Additionally, this personalized medicine treatment plan potentially could help others with the same disorder.

*Kim AR, Ullirsch JC, Wilmes S,...Sankaran VG. Functional selectivity in cytokine signaling revealed through a pathogenic EPO mutation. Cell 168: 1053-1064, doi:10.1016/j.cell.2017.02.026, 2017.*

#### **Correcting Iron-delivery Defects in Animals:**

Scientists recently identified a small molecule compound capable of correcting iron-delivery defects in animal models. Iron is essential to the body's oxygen-delivery system. Humans need iron to make hemoglobin, the oxygen-carrying molecule in red blood cells. Iron is shuttled to various tissues of the body by an exquisitely well-controlled series of proteins called iron transporters. Defects in iron delivery can lead to too little iron (also called iron deficiency anemia) or a buildup of too much iron (also called hemochromatosis) and are associated with more than 25 human diseases. Thus, approaches that restore normal iron delivery would be beneficial to those with these conditions.

The researchers began by studying iron transport in baker's yeast, an organism readily amenable to experimentation. While evaluating a series of candidate compounds for their ability to restore growth capacity to yeast missing a specific iron transport protein, researchers discovered that the natural product hinokitiol—originally isolated from the essential oil of a tree—restored normal growth rates. After showing that hinokitiol binds tightly to two forms of iron (ferrous and ferric) found in cells, the investigators demonstrated that this small molecule had the inherent ability to transport both ferrous and ferric iron across a model cell membrane. Further studies suggested that hinokitiol dissipates iron buildup by

transporting iron from areas of high iron concentration to areas of low concentration. In a set of experiments in mice and rats genetically engineered to lack specific iron transport proteins in the gut, the researchers showed that orally administered hinokitiol restored iron uptake in the animals. The researchers also demonstrated that hinokitiol restored normal hemoglobin production in zebrafish lacking a specific iron transport protein. This suggests that hinokitiol can work with the body's existing regulatory systems to supply iron where it is needed without interfering with other cell functions.

The findings from this study suggest that small molecules like hinokitiol may one day be used to substitute for missing or defective human iron transport proteins. More research into hinokitiol's function and safety will be needed to explore this fascinating possibility.

Grillo AS, SantaMaria AM, Kafina MD,...Burke MD. Restored iron transport by a small molecule promotes absorption and hemoglobinization in animals. *Science* 356: 608-616, doi: 10.1126/science.aah3862, 2017.

#### **Small, Yet Powerful Mitochondria and Blood Stem Cells:**

Two recent studies of blood stem cells highlight the importance of cellular components called mitochondria in determining how these cells function. Mitochondria are referred to as the “powerhouses” of the cell because these small organelles take energy that is ingested in the form of sugars or fats and convert it to fuel for the cell in a process called respiration. Blood stem cells (also called hematopoietic stem cells) have the potential to self-renew into two identical daughter stem cells or give rise (mature) to specialized cell types: red blood cells, white blood cells, or platelets. Scientists have sought to discover the cellular players that tip the balance in favor of self-renewal versus maturation.

In one report, scientists discovered that blood stem cells that retain the capacity to self-renew have a protein on their cell surface called Tie2, a feature distinguishing them from blood stem cells that may be on a path to become mature blood cells. When examining human and mouse blood stem cells with and without Tie2, they found that those with Tie2 have superior ability to grow and repeatedly give rise to new daughter stem cells over longer periods of time in the laboratory, creating many generations of blood stem cells. In

experiments in mice, blood stem cells with surface Tie2 were also more effective at migrating to the bone marrow, where they normally reside and self-renew. Further analysis of mouse Tie2-containing blood stem cells showed that these cells “turn on” several genes involved in the degradation of mitochondria, which helps cells remain healthy by selectively removing damaged mitochondria. Based on additional experiments, the researchers proposed that the process of mitochondrial clearance may be a key determinant that commits these cells to the self-renewal pathway.

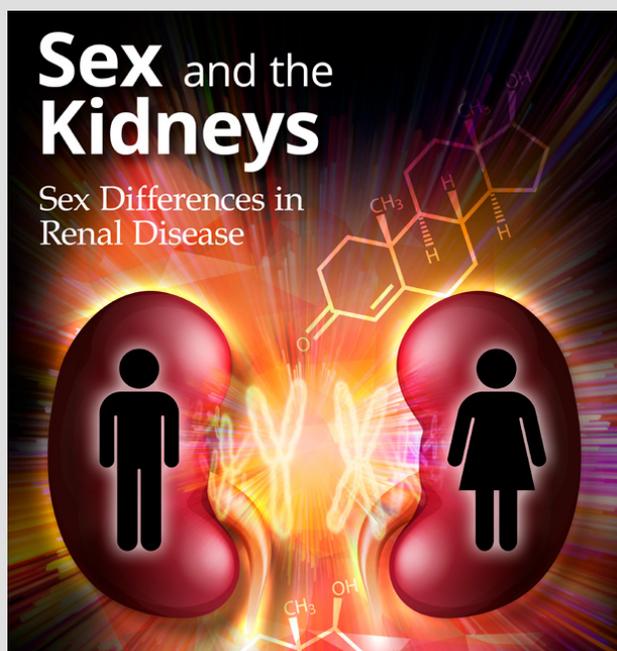
A second advance assessed whether intact, functional mitochondrial respiration is required for the function of mouse fetal and adult blood stem cells. The investigators found that deficiency in a protein component of the mitochondrial respiration process, called RISP, resulted in a mouse fetus that developed fewer red blood cells than normal and subsequently died. In addition to the lack of red blood cells, the late-stage mouse fetus contained fewer white blood cells and platelets—an indication that the blood stem cells were unable to mature into other critical blood cells. Another set of experiments confirmed that intact mitochondrial respiration is also essential for adult mouse blood stem cell functions. In contrast to normal adult blood stem cells, those lacking RISP initially generated more cells, but soon these cells died—resulting in an inadequate number of blood stem cells to self-renew or mature into specialized blood cells. These findings, in mice, suggest that mitochondrial respiration is required for normal blood stem cell function.

These two studies serve to underscore the significance of mitochondrial activity in normal blood stem cell function. Approaches to engineer these pathways may enable investigators to direct these cells to undergo self-renewal or maturation, which could be useful for future study of these important cells and for potential therapeutic strategies.

Ito K, Turcotte R, Cui J,...Ito K. Self-renewal of a purified Tie2+ hematopoietic stem cell population relies on mitochondrial clearance. *Science* 354: 1156-1160, doi: 10.1126/science.aaf5530, 2016.

Ansó E, Weinberg SE, Diebold LP,...Chandel NS. The mitochondrial respiratory chain is essential for haematopoietic stem cell function. *Nat Cell Biol* 19: 614-625, doi: 10.1038/ncb3529, 2017.

# Sex Differences in Kidney Disease



Women now account for roughly half of all participants in NIH-supported clinical research. However, basic and preclinical biomedical research has often focused on male animals and cells. An over-reliance on male animals and cells may obscure understanding of key sex influences on health processes and outcomes. Accounting for sex as a biological variable begins with the development of research questions and study design. It also includes data collection and analysis of results, as well as reporting of findings. Consideration of sex may be critical to the interpretation, validation, and generalizability of research findings. Adequate consideration of both sexes in experiments and separation of data by sex allows for sex-based comparisons and may inform clinical interventions. Appropriate analysis and transparent reporting of data by sex may therefore enhance the rigor and applicability of preclinical biomedical research.

The NIH has recently re-focused the research community on the role of sex as a biological variable—requiring all research grant submissions to deliberately consider sex as a modifier of biological response. As part of its efforts to address this important issue, the NIDDK sponsored a workshop on “Sex

and the Kidneys: Sex Differences in Renal Disease,” held on July 13-14, 2017, on the NIH campus in Bethesda, Maryland. This workshop follows a previous conference held in 1999, “Women in Renal Disease,” which focused on identifying the unique risks of chronic kidney disease (CKD) and progression to end-stage renal disease present in women across their lifespans. At the conclusion of that conference, the organizers proposed several avenues of research to advance our understanding of the pathophysiology of sex differences in CKD and improve clinical care of women with CKD. While some advances have been made since then in both clinical and basic research, much remains poorly understood, both at the molecular and clinical level.

The purpose of the 2017 workshop was to afford the kidney research community an opportunity to re-visit the role of sex in disease risk and etiology, in light of the advances made in our understanding of sex hormone action in the body’s tissues. Basic and clinical scientists, clinicians, and industry and regulatory representatives gathered to assess the state of the science, identify needed research, and set priorities for future efforts. A wide range of presentation topics were discussed, including the molecular mechanisms of sex hormone activity; lessons of sex difference epidemiology learned from other disease systems; basic mechanisms and clinical manifestations of kidney disease; and approaches and tools to identify, understand, and treat sex differences. In-depth discussion among experts in four breakout sessions led to a series of recommendations to address needs in basic, translational, and clinical science, and to develop necessary tools and methodologies. These recommendations are expected to inform experimental design and interpretation, ultimately leading to a better understanding of sex differences in kidney disease and improved clinical care.

# The Kidney Precision Medicine Project: A Bold Vision for the Future of Nephrology

Acute kidney injury (AKI) and chronic kidney disease (CKD) impose a significant global health burden; however, only a few drug therapies are available for CKD, and none currently exist for AKI. Development of pharmacologic agents for AKI and 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 AKI and CKD. A growing consensus suggests that CKD and AKI are not homogeneous diseases; rather, they are heterogeneous disorders that contain specific subgroups that are driven by different disease pathways. Thus, a better understanding of disease heterogeneity will likely inspire the development of more effective individualized treatment options. In 2017, the NIDDK began the Kidney Precision Medicine Project (KPMP)—a bold initiative that will begin to chart a course toward a more personalized approach to clinical care for people with kidney disease.

One might envision a more individualized future for clinical practice, where each person with kidney disease can find answers to important, patient-centered questions: “What do I have?” “What will happen to me?” “What can I do about it?” A nephrologist (kidney disease specialist) in this vision of the future might evaluate the person’s disease profile using blood and urine tests, image the kidney in real-time to identify and biopsy areas of kidney damage, then analyze the biopsy tissue using a kidney tissue atlas (a tool designed to classify the location and health of kidney tissue components), and select the appropriate drug to start individualized treatment.

How will this individualized approach to patient care be achieved? The KPMP outlines several goals with the overall objective of bringing

precision medicine to AKI and CKD: to ethically and safely obtain and evaluate human kidney biopsies from research participants with AKI or CKD; create a kidney tissue atlas; define disease subgroups; and identify critical cells, extracellular components, and pathways that can be targeted for novel therapies.

The kidney biopsy is essential to this vision of the future, as it will identify the specific subtype of AKI or CKD to provide the information needed to answer the patient-centered questions. However, advancing science to a point where subtypes are well defined will require technological leaps that can only be made by analyzing biopsy tissue from people with kidney disease. Today, kidney biopsies are of limited benefit to an individual, and the biopsy procedure carries some risk for well-defined complications (e.g., bleeding, pain). A central component of the KPMP will be to build a strong case to explain to patients and clinicians how biopsies could have long-term benefit because of their critical role in advancing research progress toward precision medicine.

Human kidney biopsies obtained through the KPMP will be analyzed using sophisticated technologies that have matured over the past few years, identifying new molecular markers that will reveal cellular and tissue heterogeneity in exquisite detail to define specific kidney structures. Markers characterized by the KPMP will help establish a complex kidney atlas that can classify and locate different cell types, cell states (healthy, injured, dying, recovering, undergoing repair, etc.), and important molecules. These new markers will then be linked to important patient clinical outcomes. The emerging kidney tissue atlas will be used as a foundation to better understand kidney disease heterogeneity and will be able to inform decision-making by pathologists, nephrologists, and patients with AKI and CKD.

Because the current kidney biopsy procedure is risky, with well-defined complications, ethical and participant safety considerations must be a primary concern. The NIDDK is also supporting research through its small business research programs to develop safer biopsy methods and novel techniques to analyze human kidney tissue. All resulting resources from the KPMP will be public, open, and transparent, and will be made available to everyone (e.g., patients, academic researchers, industry scientists). These findings and resources will help nephrologists better understand human kidney disease, and will invigorate kidney research, attract top talent from inside and outside nephrology, and seed new investigator-initiated research. To achieve maximal success, the KPMP will foster

partnerships among patients, academic researchers, private industry, advocacy organizations, and the NIDDK.

This patient-centered, individualized vision for the future of nephrology aims to keep the patient voice front and center in the design and implementation of the KPMP. Over time, results and resources from the KPMP are expected to drive the evolution of nephrology toward this future. An increased understanding of human kidney diseases is likely to catalyze the development of new therapies. Biopsy results will likely become more informative to clinical care as pathologists and nephrologists can better predict a drug's effectiveness based on an individual's specific kidney profile.

# STORY OF DISCOVERY

## *APOL1* Gene Variants: Unraveling the Genetic Basis of Elevated Risk for Kidney Disease in African Americans

African Americans have higher rates of end-stage renal disease (ESRD) than European Americans, but for many years the reasons for this health disparity were largely unknown. In 2008, researchers reported that genetic variations on chromosome 22, later revealed to be in the *APOL1* gene, were linked to a greater incidence of non-diabetic kidney disease among African Americans. The identification of *APOL1* kidney disease risk variants, which are found primarily in African Americans, is arguably the most important discovery about the pathogenesis of chronic kidney disease over the past several decades, and these variants are among the only known genetic factors contributing to the well-appreciated health disparities in kidney diseases. In the years since the initial groundbreaking discovery of *APOL1* risk variants, numerous NIDDK-supported studies have shed light on their important roles in disease risk and the underlying mechanisms of *APOL1* protein function, creating new research avenues to improve health in populations at risk.

### **The Heavy Burden of Kidney Disease in Some Populations**

Early-stage kidney disease often has no symptoms. Left unchecked, however, it can silently progress to kidney failure, a condition in which the kidneys are no longer able to filter waste and excess fluids from the blood. Millions of U.S. adults are estimated to have chronic kidney disease (CKD), and despite

recent advances in preserving kidney function in individuals with early-stage kidney disease, serious health complications are common.

The two most common causes of kidney failure are diabetes and hypertension (high blood pressure), which account for a majority of new cases. Both conditions are more frequent in minority populations, and African Americans bear an especially heavy burden of kidney disease. African Americans are more likely to develop diabetic kidney disease and kidney failure than Whites. One cause is a form of kidney disease called focal segmental glomerulosclerosis (FSGS), in which the glomeruli—the tiny filtering units of the kidneys—are damaged and scarred. Most FSGS arises from unknown causes and is termed “idiopathic” FSGS. African Americans are significantly more likely to develop idiopathic FSGS compared to individuals of other racial backgrounds. The health disparity increases dramatically with HIV infection: African Americans are far more likely than Whites to develop FSGS related to infection with HIV, the virus that causes AIDS. These rather striking disparities represent a serious public health problem, not only because of the kidney disease itself, but also because people who have even mild- to moderately-severe kidney disease typically have high blood pressure and other risk factors for serious complications such as cardiovascular disease.

What accounts for this dramatically increased risk of severe kidney disease in African Americans? Scientists and physicians have long known

# STORY OF DISCOVERY

that kidney disease tends to run in families and to cluster in racial/ethnic groups. These observations indicate that kidney disease is likely to have a genetic component. It is also almost certain that environmental and other factors also play a role in disease susceptibility and outcomes. However, studies that have attempted to identify genes that confer susceptibility to kidney disease and kidney failure had not generally been successful.

Moreover, it is not clear that all forms of kidney disease originate from a common starting point or progress through a shared pathway. For example, while patients with diabetes or those with hypertension are at increased risk of developing kidney disease and kidney failure, not all patients at risk go on to develop kidney disease. In addition, it is not clear that the underlying disease mechanisms which initiate injury and facilitate progression in diabetic and hypertensive kidney disease are the same. If, in fact, these two conditions cause kidney disease through different pathways, then treatment strategies for people whose kidney disease is a consequence of diabetes could be very different from those for people whose kidney disease is attributed to hypertension. Because of these considerations, it has been especially important to identify the genetic contribution to disease development and progression and characterize the biological pathways that lead to diminished kidney function.

## **A Foundational Discovery**

In 2008, members of the NIDDK-supported Family Investigation of Nephropathy and Diabetes (FIND) Consortium, along with scientists in the NIDDK's Intramural Research Program, reported that genetic variations on chromosome 22 were linked to greater incidence of non-diabetic kidney disease

among African Americans. Initially, attention focused on the region surrounding the *MYH9* gene. Further analyses revealed that much of the increased risk of kidney disease is actually due to two variations in the adjacent *APOL1* gene, which encodes the protein apolipoprotein L1 (also designated as APOL1 protein,) a component of so-called "good" cholesterol that is found circulating in the blood and in kidney cells. Two specific variants of this gene, termed G1 and G2, have been shown to account for nearly all of the excess risk of kidney failure in African Americans arising from causes other than diabetes—a major breakthrough in understanding the increased burden of the disease. The low-risk *APOL1* genetic variant is termed G0.

## **NIH Scientists Identify Critical Associations Between *APOL1* Variants and Kidney Diseases**

Soon after this association was reported, scientists found that African Americans with two copies of *APOL1* high-risk variants are at increased risk of developing kidney disease, particularly FSGS and kidney disease related to infection with HIV. This finding comes from collaborative research led by scientists in NIDDK and NCI's intramural research programs. Investigators in the United States and Europe were also part of the research team. The scientists studied patients with kidney disease who came to the NIH Clinical Center or other collaborating medical centers and provided blood samples for genetic studies.

Human cells typically have two copies of each gene—one inherited from each parent. African Americans with no normal copies of the *APOL1* gene, but instead two kidney disease risk variant copies, have about a 4 percent lifetime risk of developing FSGS.

# STORY OF DISCOVERY

Those who develop this disease tend to do so at younger ages than other FSGS patients, with 70 percent diagnosed between ages 15 and 39, compared to 42 percent in that age group for people with one or no *APOL1* high-risk variants. FSGS patients with two *APOL1* high-risk variants respond as well to steroid treatments, the therapy with the best chance of inducing a partial or complete remission of the disease, as people without the variants. However, the scientists found that the disease progresses more rapidly to kidney failure in patients with two *APOL1* variants. Among African Americans who are HIV-positive, but not receiving anti-viral therapy, possessing two *APOL1* variants raises the risk of developing HIV-associated kidney disease to 50 percent. (Anti-viral therapy appears fairly effective at preventing HIV-associated kidney disease.)

The persistence of *APOL1* variants in people of African descent may be partly explained by the ability of the *APOL1* protein to destroy certain parasites. Although the normal *APOL1* protein can destroy the parasite *Trypanosoma brucei brucei* (*T. b. brucei*), it is unable to destroy two related parasites, *T. b. rhodensiense* and *T. b. gambiense*. These parasites cause African sleeping sickness, a hematologic and neurological disease, spread by the tsetse fly, that kills thousands of people in sub-Saharan Africa each year. However, people with at least one copy of the G1 or G2 variants are protected against infection because they are able to destroy *T. b. rhodensiense* and *T. b. gambiense*. These two *APOL1* variants appear to have evolved relatively recently—in the past 10,000 years or so. Their relatively recent appearance and high frequency in chromosomes in individuals of African descent suggest that the variants may support protection against parasitic infection.

It should be noted that most people with two *APOL1* variants do not develop kidney disease.

Indeed, the much higher risk of kidney disease in patients with HIV suggested that a second triggering event, or “hit,” either with a virus or another factor, contributes to kidney injury in people who have two high-risk *APOL1* variants. Nevertheless, the observed increased risks of FSGS and HIV-associated kidney disease were the strongest effects yet discovered for common variants in a complex disease.

## A New Understanding of Kidney Disease Progression and Treatment

The link between *APOL1* gene variants and kidney disease risk led scientists in different studies to seek associations between these variants and measures of kidney disease severity. One NIDDK-supported study examined biosamples from participants in the African American Study of Kidney Disease and Hypertension (AASK). The AASK study enrolled African American patients with mild kidney disease due to hypertension and found that an angiotensin-converting enzyme inhibitor was better than two other drug options at slowing kidney disease progression. The investigators asked whether *APOL1* and other gene variants were associated with an increased risk of worsening kidney disease in 700 AASK participants. They analyzed archived DNA samples and found that the presence of the G1 variant was associated with a faster decline of kidney function compared to study participants without this variant. Another NIDDK-supported study examined over 400 African Americans with kidney failure and asked whether the presence of one or two copies of either *APOL1* variant was associated with a younger age at which the participants began hemodialysis, a therapy used to cleanse the blood of waste products and excess fluids and salts when the kidneys no longer function. The researchers found that African Americans with two copies

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of the G1 variant began hemodialysis at a significantly younger age (approximately 49 years old), than those with one copy of the variant (about 56 years old). People with two normal copies of the *APOL1* gene began hemodialysis at around 62 years of age.

## ***APOL1* Gene Variants and Cardiovascular Disease**

Because hypertension is a leading cause of kidney failure, researchers have also sought to determine the relationship between the *APOL1* variants and cardiovascular disease. Scientists analyzed data from people enrolled in AASK and the Chronic Renal Insufficiency Cohort (CRIC) Study. The CRIC study, also supported by the NIDDK, is one of the largest and longest ongoing studies of CKD epidemiology in the United States; it is examining the health of both White and African American people with CKD, about half of whom also have diabetes. In their analysis, the researchers found a correlation between the presence of high-risk variants of the *APOL1* gene and an increased risk of CKD progression among African Americans. This effect was seen regardless of whether patients maintained good blood pressure control or had diabetes. In a separate study, over 2,500 African American volunteers in the Systolic Blood Pressure Intervention Trial (SPRINT) clinical study agreed to undergo genetic testing to allow researchers to examine their *APOL1* status as it related to their kidney function and risk of developing cardiovascular disease. SPRINT was led by the National Heart, Lung, and Blood Institute (NHLBI), and co-sponsored by the NIDDK and other NIH institutes. In the genetics study, which also was supported by the NIDDK and other NIH Institutes, researchers found that SPRINT study participants with two risk variants of the *APOL1* gene were more likely to have mild kidney disease than people with a single risk

variant or none. However, they were not more likely to have cardiovascular disease.

More recently, scientists supported by the NIDDK analyzed data from multiple independent studies, overall including over 5,000 African American study participants, to explore whether *APOL1* variants affect the age of hypertension onset. They found that in young African Americans (20-29 years old), high-risk *APOL1* variants were linked to higher blood pressure levels and younger age of hypertension diagnosis than low-risk variants. Another NIDDK-supported research team asked whether *APOL1* gene variants were linked to differences in blood pressure trajectories over time. The scientists analyzed data from NHLBI's Coronary Artery Risk Development in Young Adults (CARDIA) Study, which began in 1986 and has been examining the development and determinants of cardiovascular disease and its risk factors by following study participants for 25 years. The scientists found that while blood pressure levels over the years rose to higher levels in African Americans than in Whites, blood pressure trajectories in those with high-risk *APOL1* variants did not appear to be different than in people with the low-risk variants.

## **Cellular and Molecular Mechanisms of *APOL1* Protein Function**

Great strides have been made over the past few years in defining various aspects of the genetic risk of kidney disease associated with *APOL1* variants in some populations. In addition, understanding the mechanisms of *APOL1* protein action in the kidney that underlie disease risk could inform clinical decisions and the development of new therapeutic strategies for people with high-risk variants. To begin exploring these mechanisms, one important experimental challenge had to be overcome by scientists—the *APOL1* gene is only found in humans and some primates. Thus,

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researchers needed to develop innovative strategies for investigating APOL1 protein function in animal models and other laboratory systems. Recent reports have shed light on the proteins and pathways that mediate APOL1 action in the kidney.

In one study, NIDDK-supported researchers genetically engineered mice to produce the human APOL1 G0, G1, or G2 variants in specialized cells in the kidney, called podocytes, that wrap around the glomerulus and are key components of the filtration apparatus. Mice that produced the G0 variant appeared normal, but those with a high-risk G1 or G2 variant exhibited hallmarks of human kidney disease (e.g., high levels of protein in the urine, physical injury to podocytes). These results in mice lend strong support to the hypothesis that these human APOL1 variants cause kidney disease. Another team of scientists supported by the NIDDK used the *Drosophila melanogaster* fruit fly system to investigate cellular and molecular mechanisms of APOL1 activity. The researchers engineered flies to produce human APOL1 G0 or G1 variants in their nephrocytes, which are cells in *Drosophila* that have a similar function as human podocytes. Both variants over time led to increased protein uptake by nephrocytes and impaired acidification of critical cellular compartments. These cellular changes over time were accompanied by decreased function, increased size, and premature death of nephrocytes. In each experiment, the results were much more severe in flies with the G1 variant than in those with the G0 variant. Together, these findings suggest that APOL1 variants are causative agents of kidney disease, and point to cellular toxicity as a potential mechanism for decline in kidney function.

High levels in the blood of a protein called suPAR are associated with decline in kidney function and progression to CKD. Because

many people with the high-risk G1 and G2 APOL1 variants do not develop kidney disease, an NIDDK-supported research team explored whether blood suPAR levels can help predict whether African Americans with these genetic variants will experience declining kidney function. The scientists analyzed data from the Emory Cardiovascular Biobank (EmCAB) and the AASK trial, which together included almost 1,100 African American participants. The scientists found that in people with high-risk APOL1 variants, kidney function was likely to decline more rapidly over time in those with elevated plasma suPAR levels than those with lower suPAR. To gain additional insights, the researchers studied APOL1 variants and suPAR in mice. Female mice engineered to express either the G1 or G2 variant showed evidence of kidney damage, but mice with the G0 variant appeared normal. However, when suPAR was genetically deleted from mice, the kidney damage caused by the G2 variant was gone. Taken together, these findings suggest that high levels of suPAR may be necessary for the kidney function decline observed in some people with APOL1 G1 and G2 variants, and thus may serve as a useful predictor of kidney disease. (For more details about this study, please see the advance earlier in this chapter).

## Looking Forward

A clear picture is emerging that links APOL1 gene variants to kidney diseases under a range of conditions that need further study, such as HIV-associated nephropathy, pediatric kidney disease, sickle cell nephropathy, and kidney transplantation. The NIDDK continues to support research at multiple levels to understand these relationships in African Americans and other populations. For example, the NIDDK held a conference in June 2015 on APOL1 and kidney disease to assess gaps in knowledge, including

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the function of the *APOL1* protein and its role in kidney transplantation. The conference developed new ideas regarding how *APOL1* gene variants lead to disease susceptibility, what kidney and cardiovascular outcomes are associated with these variants, which additional genetic variants or environmental factors play a role in differences in disease symptoms, and the possible role of determining whether patients have *APOL1* gene variants in guiding treatment as well as preventive strategies for patients.

Additionally, in 2016 the NIDDK, the National Institute on Minority Health and Health Disparities, and the National Institute of Allergy and Infectious Diseases began the *APOL1* Long-term Kidney Transplantation Outcomes Network (APOLLO) initiative. This initiative aims to determine the impact of *APOL1* genetic variants as risk factors in U.S. kidney transplant recipients who received kidneys from African American donors. The consortium will examine the rate of change of kidney function in recipients, and rates of acute rejection of

the kidney transplant, graft failure, and return to maintenance dialysis in the recipients, who received kidneys from patients of African descent, in the presence and absence of the *APOL1* genetic variants. Outcomes in kidney donors, including vital and renal functional status, will also be assessed.

These seminal studies, as well as many others over the past several years, have revealed the importance of *APOL1* in understanding some key differences in kidney disease risk across populations. Unraveling the molecular mechanisms by which *APOL1* variants contribute to kidney injury could provide key insights into the causes and possible treatments for kidney disease in African Americans. Moving forward, clinicians may be able to make more informed choices about when to start screening for kidney disease and how to choose an appropriate therapy by identifying which patients have these gene variants and are therefore at increased risk of developing kidney disease and progressing to kidney failure.

## PATIENT PROFILE

# Olivier: Participating in Clinical Research To Help Others with Benign Prostatic Hyperplasia Down the Road

Symptoms began gradually for 57-year old Olivier, so it is difficult to pinpoint exactly when he began dealing with urinary urgency, but he estimates he first noticed the problem 10 years ago. Urinary urgency is the sudden, strong need to urinate immediately. Although he has been dealing with urgency for the better part of a decade, he only learned the source of his symptoms—a condition called benign prostatic hyperplasia (BPH)—relatively recently. He admits that he is not good at going to the doctor right away when anything happens. “You kind of ignore what you have, and then you don’t want to think of the worst. There’s always the question of prostate cancer... Initially, you try to cope with it.” Although Olivier put off going to the doctor to find out what the problem was, he explains that he could not control or influence his symptoms very much. As he says, a natural way “was to drink less water, which is really not what you want to do ... because you’re thirsty all the time. Definitely not good for your metabolism to restrict your water intake. You realize that’s not the solution.” To better understand the nature of the symptoms that characterize his urinary problems, Olivier recently began participating in an NIDDK-supported research study—turning his own experiences with this difficult condition into a way to help others.

### **It’s Progressive**

After Olivier’s urinary urgency started, it got progressively worse over time, and began to

seriously interfere with his life. It was hard to predict when the urinary urge symptom would start, complicating the family’s social life and his everyday activities. “It’s not that you can choose when you go—you have to go right now.” Describing these challenges further, he says that with urinary urgency, “basically you cannot be in a room for more than an hour. Sometimes less. You have a diminished ability to interact with people and [need to] leave at specific moments.” Olivier, a husband and father of two boys, comments that it became very apparent to the people he interacted with that he had this pretty serious issue. “Everyone knew in my family that when I have to run out—it was mostly a joke. But,” he adds with a laugh, “I guess a good way to take it.”

Urinary urgency affected his life in other ways, too. He notes that air travel is especially difficult, “because once your seatbelt is fastened and you’re told you cannot move, that becomes a serious issue. You know ... sometimes ... it takes a long time before the plane taxis out from the gate and you actually take off [and finally] you are in the air and able to go use a restroom.” An owner of a business that imports specialty foods from Europe, he noticed that when he’s in a food warehouse, which is kept at about 40 degrees Fahrenheit, that really triggers the need to urinate. “I couldn’t stay in the cold environment more than 40 minutes. I would need to run to use the restroom.” Olivier also has struggled to get a good night’s sleep because of his night-time urinary symptoms.

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"Start waking up at night once. And then it becomes two. And then it becomes three.... You don't get any rest." As the nightly urinary symptoms became worse, exhaustion became an additional challenge during the day.

To complicate things further, he also developed another urinary problem. "Going to the bathroom to urinate became a real struggle, too. It's not just going more often—suddenly you're having a harder and harder time to try to urinate. It's increasingly hard to handle. Hard on your body too, just to go through that process."

## Benign Prostatic Hyperplasia Diagnosis and Treatment Plan

The combination of these and other symptoms significantly contributed to Olivier's diminished quality of life and work experiences, and finally brought him to a tipping point. He says, "That's what started to tell me that I need to do something about it, because it was

***"That's what started to tell me that I need to do something about it, because it was getting out of control" says Olivier of his decision to finally seek medical help for his severe urinary symptoms.***

getting out of control." After about 5 years of urinary urge and urinary stream problems, he made an appointment with his general practitioner, who referred him to a urologist, who diagnosed Olivier with BPH. BPH is a condition in which a man's prostate gland is enlarged but not cancerous. The prostate surrounds the urethra, which is the tube that carries urine from the bladder to the outside of the body. In BPH, the enlarged prostate presses against and pinches the urethra, causing urinary urgency, trouble starting a urine stream, and/or other symptoms. BPH is a fairly common condition in men around Olivier's age and older, and men with a family history of BPH are more likely to develop the condition. Olivier eventually

realized that his father probably developed this condition, although with his father it didn't occur until much later in life.

"I was put on tamsulosin (a medicine that relaxes the muscles in the prostate and those that connect the bladder to the urethra in the bladder neck) for several years" to treat the BPH, Olivier says. Initially, there was a slight improvement with tamsulosin, but, he adds, "there were a lot of side effects [from the medicine], which were not really emphasized. These side effects were marked enough to lead to some deterioration of quality of life" which led him to completely stop taking the medicine.

The approach to Olivier's treatment changed in the summer of 2016, when he received a transurethral resection of the prostate (TURP), a surgical procedure that removes some of the

prostate gland; the procedure is performed by a urologist. Olivier says "the surgery was a tremendous improvement. I

am thankful to Dr. John L. Gore and his team [at the University of Washington Medical Center] for the excellent care and surgery procedure that I received."

## Participating in the LURN Clinical Study

Olivier learned of the Symptoms of the Lower Urinary Tract Dysfunction Research Network (LURN) observational study from his urologist at the time of his surgery, and agreed at that time to participate in the study. To hasten advances in assessing and treating lower urinary tract dysfunction, whether caused by prostate-related or other conditions, the NIDDK started LURN in 2012. Expanded in 2013, LURN

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includes six clinical research sites and one data coordinating center. LURN was designed for people like Olivier, to better understand lower urinary tract conditions. Knowledge gained from LURN could then be used in other research efforts to develop and test new treatments to improve the health and quality of life of people with lower urinary tract symptoms (LUTS).

LUTS are highly prevalent in both males and females, but many people who seek help from healthcare providers for LUTS experience neither total nor permanent resolution of their symptoms with current management approaches. One of the barriers to improving diagnosis and management of LUTS is incomplete knowledge and imprecise classification of subtypes of LUTS and their associated causes. There are a wide variety of lower urinary tract symptoms that people can experience, which may be caused by problems in the urinary tract or may originate elsewhere in the body. Even people with similar symptoms may have different underlying urinary tract conditions. The researchers aim to identify and understand the different subgroups of people with LUTS based on their urinary symptoms, other health conditions they may have, and other characteristics; improve measurements of people's experiences with urologic symptoms; share novel findings with other researchers, clinicians, and patients; and generate data, samples, and research tools for future studies. Ultimately, the researchers hope that knowledge gained from this study will help researchers and healthcare providers improve prevention, management, and treatment strategies.

During his participation in LURN, Olivier underwent a standardized clinical examination and medical

history, and completed questionnaires designed to gather information about urinary and other symptoms and health-related quality of life. He also provided blood, urine, and other samples for storage in the NIDDK Sample Repository for future study by the LURN investigators and the broader research community.

Olivier speaks well of his participation in the LURN study. "I wouldn't say there was a hard part at all. I just have to go to the hospital a few times, and they were very flexible. The appointments were no more than a half hour

at a time." And he describes the LURN study staff as "very good. Very fine. They are

***"I wouldn't say there was a hard part at all," Olivier says of his participation in the LURN study.***

understanding. Good interactions." Olivier also mentions that his wife, a medicinal chemist by training, also thinks his participation in LURN is a good thing because the more people participate, the more data is collected and analyzed to improve understanding of these conditions. Asked whether other men who are experiencing urinary tract symptoms similar to his should consider enrolling in a study like LURN, Olivier says "I would encourage them to do so. That way, there would be more information [collected to help improve] counseling in how to manage this."

## **Additional Efforts To Help People with LUTS**

As part of the overall LURN research efforts, there are several sub-studies designed to examine certain aspects of urinary tract problems in smaller groups of participants. For example, one of these is a neuroimaging and sensory testing study to provide additional data to help identify subtypes of LUTS. In this effort, researchers are using functional magnetic resonance imaging (fMRI) to examine brain

# PATIENT PROFILE

structure and function as it relates to an empty versus full bladder. This approach may identify differences in brain areas involved in bladder control in participants with LUTS compared with those who do not have this condition.

With other procedures, the researchers plan to explore whether people with LUTS have

different central nervous system responses to auditory (hearing) and pain stimuli.

LURN also plans to compare how participants report LUTS experiences over different time periods (e.g., 3-day, 7-day, and 30-day recall). These “recall” data will help LURN investigators determine the most appropriate reporting period for specific symptoms.

A LURN pilot study effort will test the feasibility of using urodynamics—procedures that look at how well the bladder, sphincters, and urethra are storing and releasing urine—in healthy, asymptomatic women to inform the design of a larger study to identify subgroups of people with LUTS. In the long term, LURN seeks to develop diagnostic organ testing protocols for both women and men with LUTS to identify different mechanistic causes that may give rise to similar LUTS profiles.

A second LURN pilot study seeks to discover potential biomarkers in women and men that could ultimately be used in clinical practice as a tool to measure the presence, severity and/or subtype of LUTS. In addition to providing

***Asked whether other men who are experiencing urinary tract symptoms similar to his should consider enrolling in a study like LURN, Olivier says “I would encourage them to do so. That way, there would be more information [collected to help improve] counseling in how to manage this.”***

potential insights into the biological mechanisms underlying LUTS, these biomarkers

could one day be used as tools by clinicians to initiate more effective treatments and monitor the response.

## For People with LUTS

The NIDDK is committed to acquiring new knowledge that will help lead to future strategies to better manage and, when possible, prevent the development of LUTS. Olivier's struggle to attain an effective treatment plan is a reminder that more research needs to be conducted to help people with this burdensome condition. And Olivier's participation in research, along with the many other study volunteers, is key to progress toward improving people's lives.





# Extramural Funding Trends and Support of Core Values

*The NIDDK's core values emphasize maintaining a vigorous investigator-initiated R01 research portfolio, supporting pivotal clinical studies and trials, preserving a stable pool of talented new investigators, and continuing to foster exceptional research training and mentoring opportunities, consistent with the vision of NIDDK Director, Dr. Griffin P. Rodgers (see Director's Message).*

At the NIDDK's May 2012 Advisory Council meeting, NIDDK Deputy Director Dr. Gregory Germino highlighted these values and reviewed the NIDDK's resource focus on areas supporting them.

Following that presentation, the NIDDK generated additional data on application and funding trends to help our research community understand application and funding dynamics over recent years and demonstrate the NIDDK's commitment to research and programs associated with the NIDDK's core values. The Institute posted these data on the NIDDK website and has since updated them annually. The data shown here were recently updated to include Fiscal Year (FY) 2017.

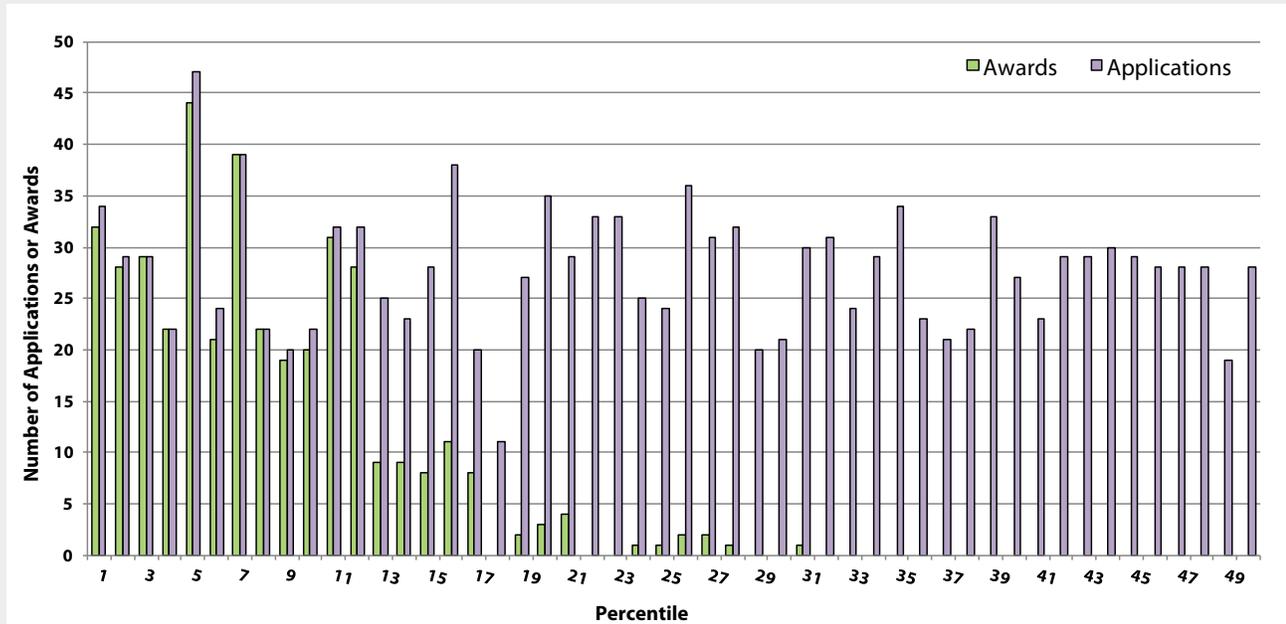
## **NIDDK Funding Outcomes for Fiscal Year 2017 and Historical Application and Funding Trends**

With the exception of Figure 8 (which includes initiative data), the data in all charts exclude initiatives (*i.e.*, Requests for Applications, or RFAs), grants funded through the *Special Statutory Funding Program for Type 1 Diabetes Research*, and funds appropriated through the American Recovery and Reinvestment Act (ARRA).

Most charts and tables in this report show data for the past 10 Fiscal Years. In Figures 4-7, the time horizon is expanded to include Fiscal Years starting in 1997, the year before the start of the doubling of the NIH budget from Fiscal Years 1998 through 2003. This expansion provides some perspective on application and funding trends occurring through the doubling period and then in the post-doubling era. Figures 11-12 are focused on Early Stage Investigators and build upon an initial set of charts that include data starting in FY 2010.

# FIGURE 1

**Number of NIDDK Competing R01 Applications Scoring Within the Top 50<sup>th</sup> Percentile and Number of NIDDK Percentiled R01 Applications Funded in FY 2017**

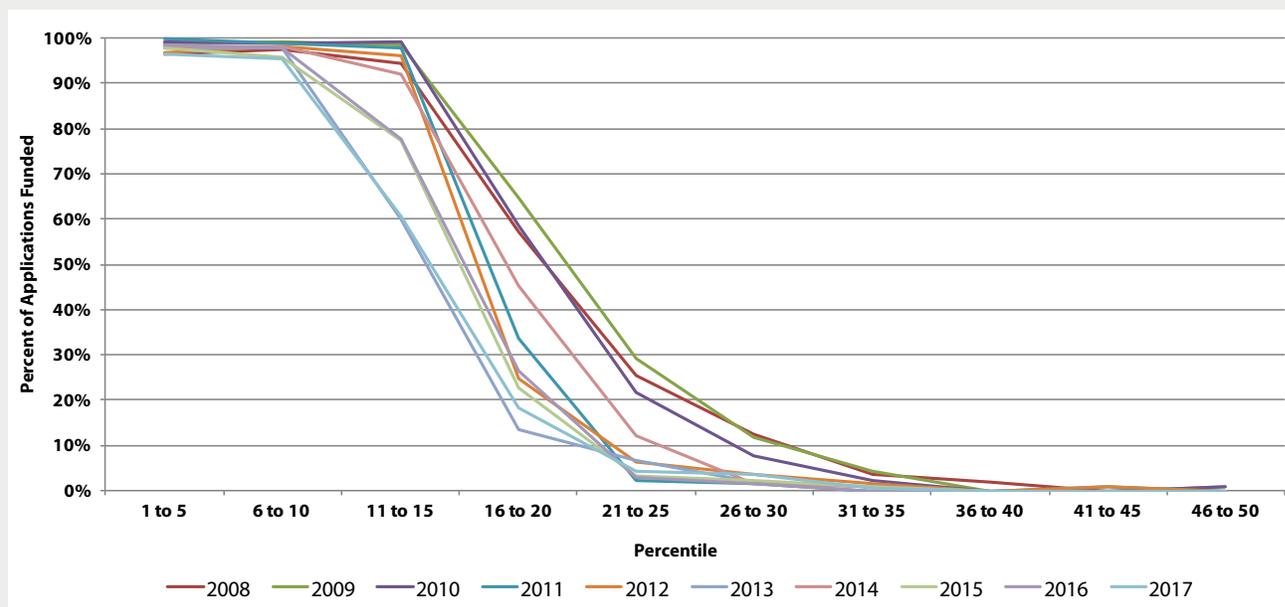


Note: “Applications” shown in the chart above include all R01 applications that scored 50<sup>th</sup> percentile or better. Unscored applications, scored applications with no percentiles, and applications scoring above the 50<sup>th</sup> percentile are not shown. (Fifty percent [n=1,397] of the applications received were unscored, scored but did not receive a percentile, or scored above the 50<sup>th</sup> percentile.) No unscored applications were funded in FY 2017.

The NIDDK nominal payline in FY 2017 was the 12<sup>th</sup> percentile for established investigators and the 17<sup>th</sup> percentile for Early Stage Investigators (ESIs). The payline and additional programmatic scrutiny for R01 applications requesting more than \$500,000 in direct costs are substantially more stringent. These data show that the NIDDK adheres closely to its payline, but does exercise programmatic discretion to include a limited number of programatically important applications.

# FIGURE 2

## NIDDK Competing R01 Application Funding Curves for FYs 2008-2017



To generate the data for Figure 2, R01 applications were placed into “percentile bins” as follows: bins 1 to 5 include all applications with percentile scores from 0.1 to 5.0, bins 6 to 10 include applications with percentile scores from 5.1 to 10.0, etc. Only R01 applications that scored 50th percentile or better were included in the analysis.

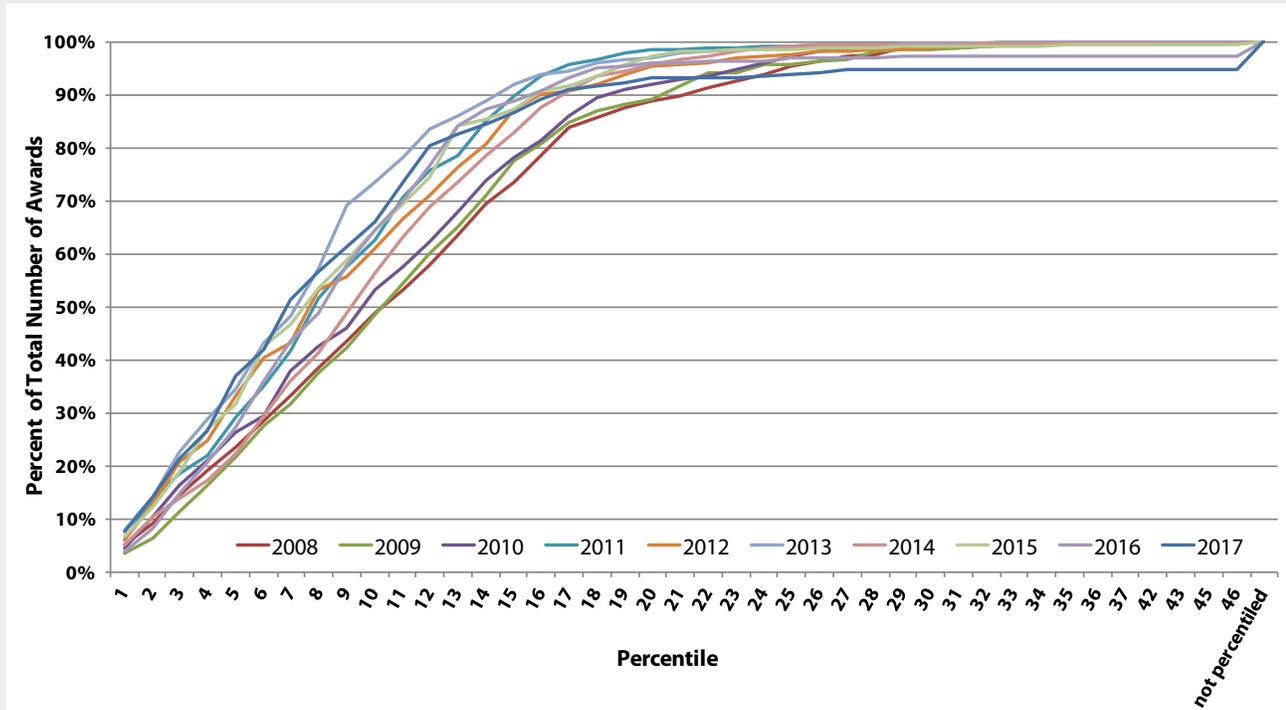
The data demonstrate steep deflections in the percentage of applications funded at or above the nominal payline for each year. The R01 paylines for the years included in Figure 2 are shown in the table to the right.

Fiscal Year	General Payline	\$>500K Payline	New Investigator Payline	Early Stage Investigator (ESI) Payline	ESI First Competitive Renewal Payline
2008	17	11	19	-	-
2009	17	11	19	-	-
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

**Note:** In FY 2012, the NIDDK began focusing on Early Stage Investigators (ESIs; see definition on the NIH “New and Early Stage Investigator Policies” webpage at [http://grants.nih.gov/grants/new\\_investigators/index.htm](http://grants.nih.gov/grants/new_investigators/index.htm)), a subset of New Investigators. For more information on the benefits that the NIDDK conveys to ESIs, see the NIDDK New and Early Stage Investigators page at <https://www.niddk.nih.gov/research-funding/process/apply/new-early-stage-investigators> (See also Figures 11 and 12.)

# FIGURE 3

Cumulative Percentage of R01 Awards Across Percentiles (FYs 2008-2017)



Only funded R01 applications are considered in the data set used to generate Figure 3. Percentile bin size equals one percentile and there is no overlap between bins. Percentiles with decimal places were summed into the next highest integral percentile as follows: 0.1-0.9 was summed into 1, 1.1-1.9 was summed into 2, etc. These cumulative funding data again demonstrate that the vast majority of R01 applications funded by the NIDDK fall within the payline, but that the NIDDK does exercise programmatic discretion to include a limited number of programatically important applications. Note that in FY 2016 and FY 2017 a limited number of R01 applications in response to specific Funding Opportunity Announcements received a priority score, but not a percentile score. Some of these applications were funded and hence included in this chart. No unscored/streamlined applications were funded in FY 2017.

# FIGURE 4

Number of Competing NIDDK R01 Applications (Including Resubmissions) Received for Funding Consideration in FYs 1997-2017

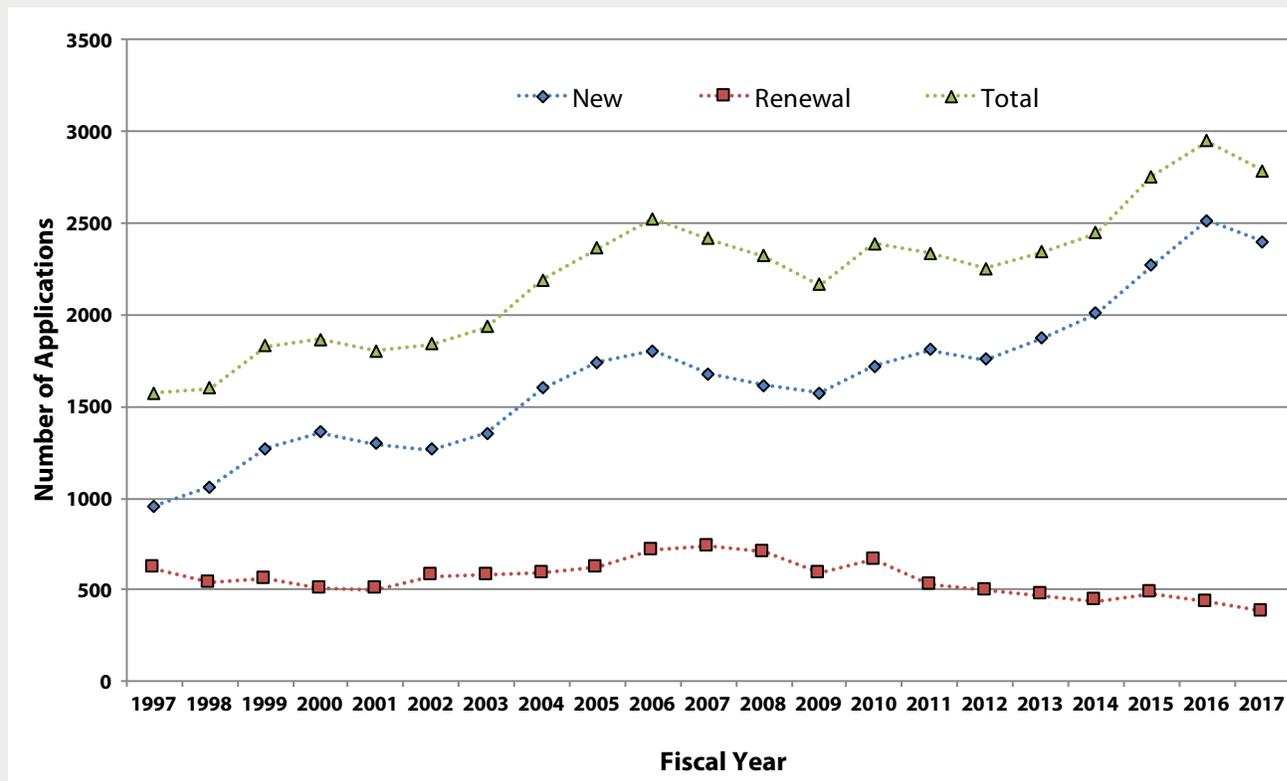
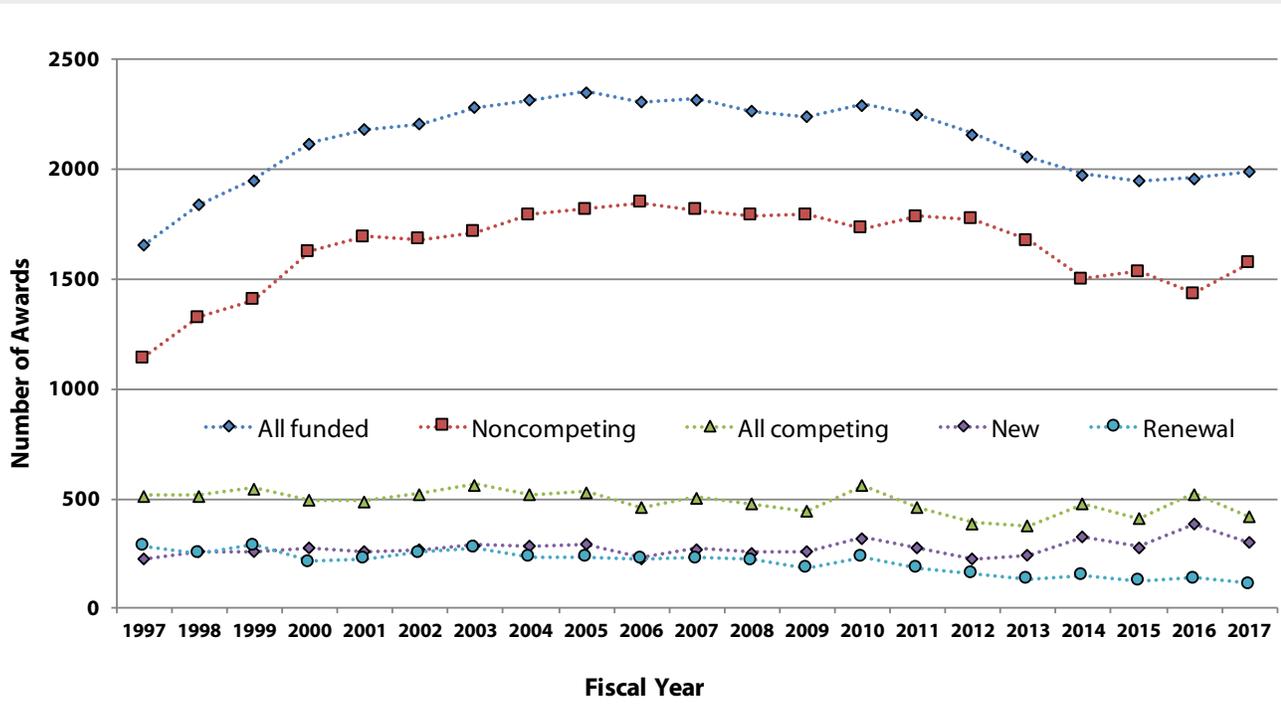


Figure 4 shows a substantial increase in the number of competing R01 applications received by the NIDDK between FYs 1997 and 2017. After some years of relatively flat growth (FYs 2006-2012), the number of competing R01 applications received by the NIDDK increased in FYs 2013-2016. This pattern of growth in R01 application numbers in successive years was not sustained in FY 2017. The observed increases between FYs 1997 and 2006 and between FYs 2013 and 2016 were primarily due to increases in the number of new (Type 1) applications. The number of competing renewal applications showed some fluctuation between FYs 1997 and 2017, but overall the number of renewal applications has slightly decreased.

# FIGURE 5

Number of NIDDK R01 and R37 Grants (Competing and Non-competing) Funded in FYs 1997-2017



During the doubling of the NIH budget (FYs 1998-2003), the total number of R01 and R37 grants funded by the NIDDK increased significantly. After leveling off following the doubling, the number of R01 and R37 grants funded by the NIDDK has declined since FY 2007. Prior to FY 2009, slightly fewer than half of the competing grants funded by the NIDDK were new (Type 1) awards in most years. However, since FY 2009 that proportion has risen to 72 percent (in FY 2017).

# FIGURE 6

Overall NIDDK Expenditures (Includes Direct and Facilities and Administrative Costs) on R01 and R37 Awards (Competing and Non-competing) in FYs 1997-2017

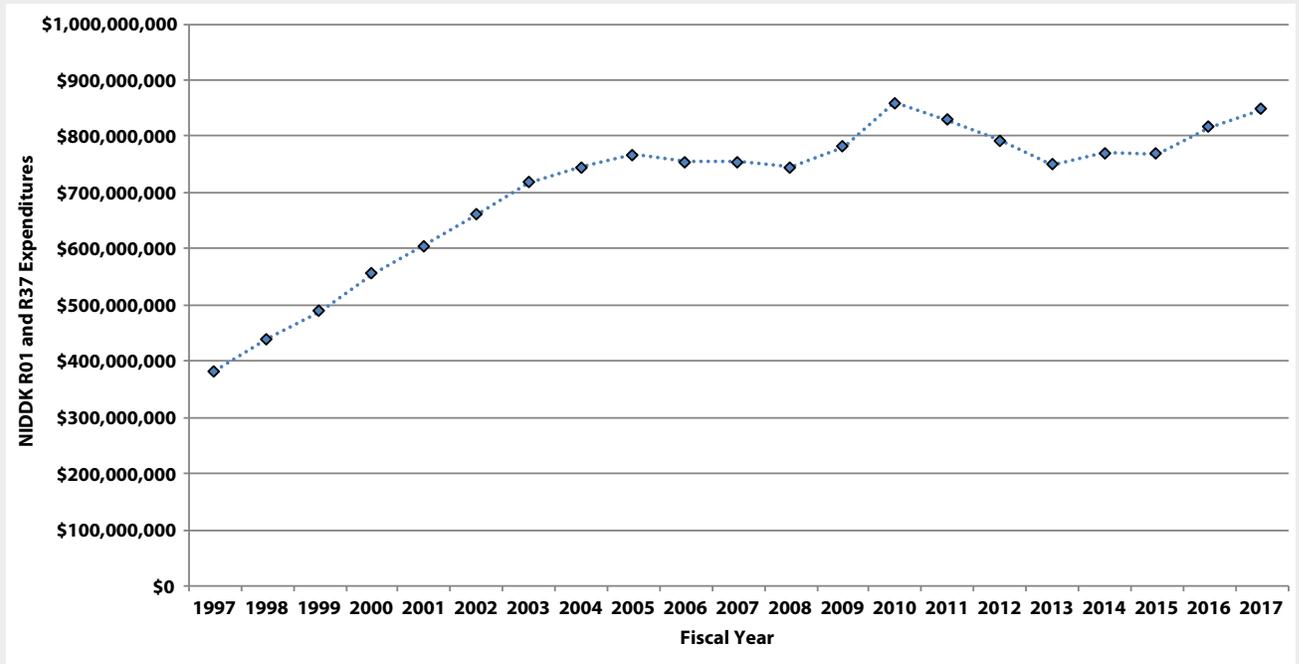


Figure 6 shows that NIDDK expenditures on R01 and R37 grants have more than doubled (121 percent increase) since FY 1997. This is because the NIDDK is funding a larger number of these awards (Figure 5) and because the median cost of an R01 has increased substantially (Figure 7).

# FIGURE 7

Median Total Costs (Includes Direct and Facilities and Administrative Costs) of NIDDK R01 and R37 Grants (Competing and Non-competing) in FYs 1997-2017

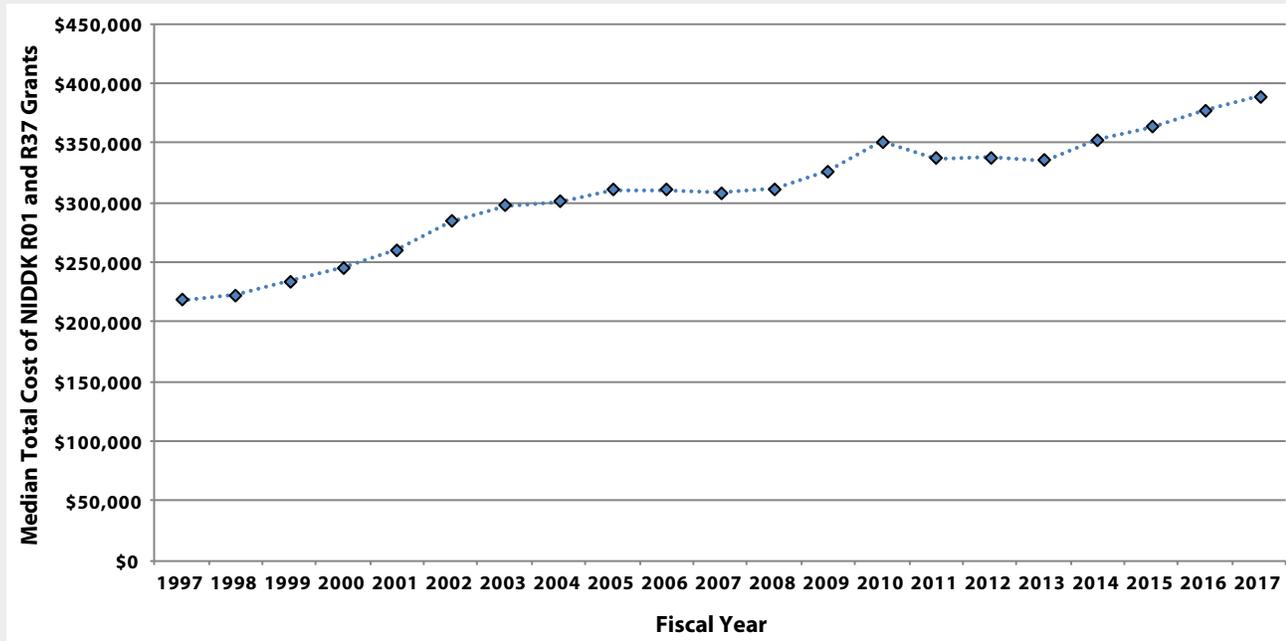


Figure 7 illustrates that the median cost of R01 and R37 awards has increased approximately 78 percent since FY 1997.

# FIGURE 8

## NIDDK Extramural Research Funding by Category (Competing and Non-competing) in FYs 2008-2017

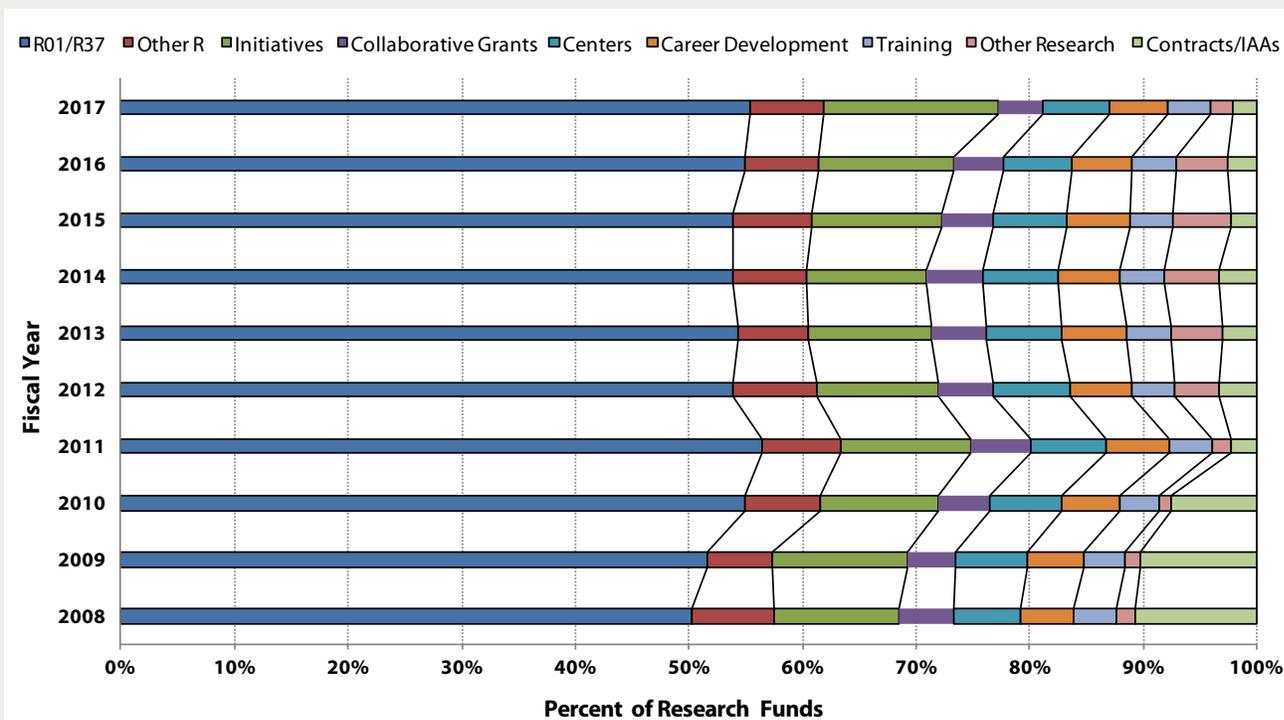


Figure 8 shows that relative funding levels of most NIDDK extramural research categories have remained fairly stable since FY 2008.

### NIDDK Portfolio Categories:

- **R01/R37** – Investigator-initiated (excludes R01s responding to NIDDK RFAs)
- **Other R** – Includes other R activities (*i.e.*, R03, R13, R15, R18, R21, R34, SBIR/STTR, etc.) but excludes R24s and applications submitted to NIDDK RFAs
- **Initiatives** – Awards made in response to NIDDK RFAs; includes most NIDDK large clinical trials and consortia
- **Collaborative Grants** – P01s and R24s that are not “mini-Centers”
- **Centers** – Includes all non-P01 P awards and R24 “mini-Centers”
- **Career Development** – Includes all Ks (including K99/R00)
- **Training** – Includes all F and T activities
- **Other Research** – Everything not captured in the other categories
- **Contracts and Interagency Agreements (IAAs)** – Includes some large clinical studies

# FIGURE 9

## Maintaining a Stable Pool of NIDDK Investigators—Number of Investigators Supported by at Least One R01 or R37

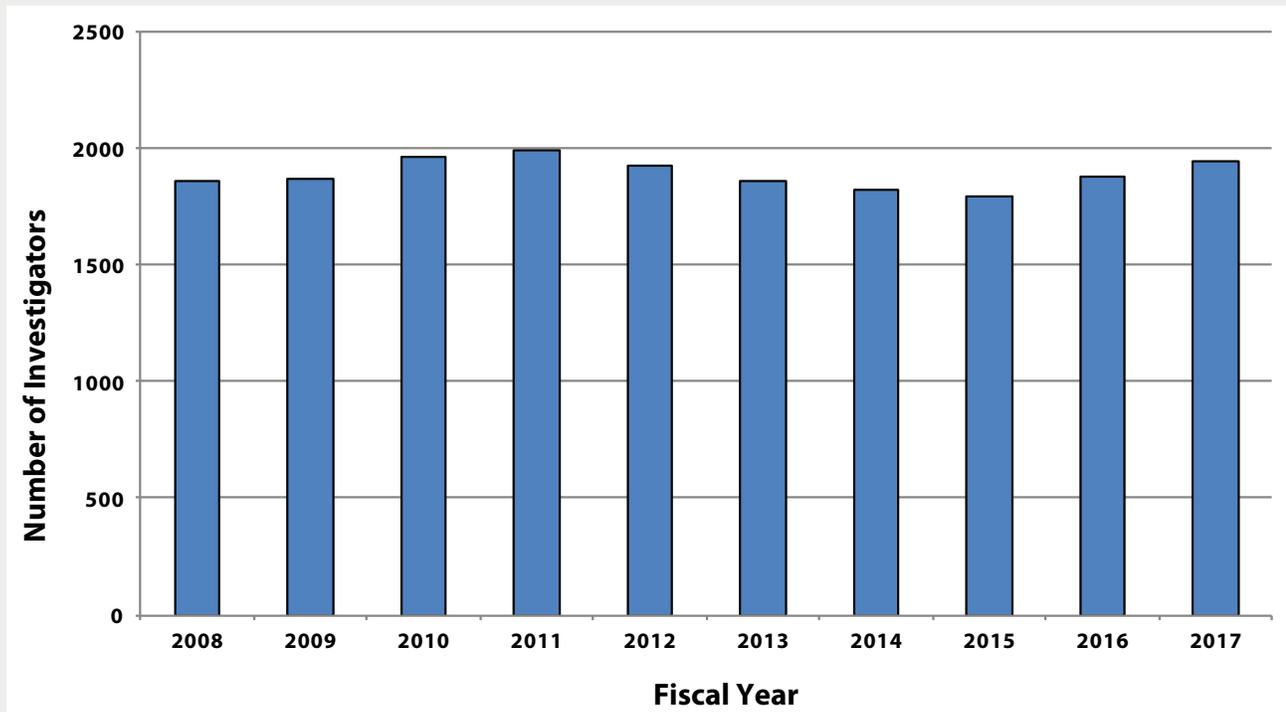


Figure 9 shows that the number of principal investigators (PIs) supported by at least one R01 or R37 remained relatively stable between FYs 2008 and 2017, with slight increases from FYs 2010-2012 and FYs 2016-2017. It should be noted that in FY 2008, NIH, for the first time, began making multiple principal investigator R01 awards to support team science projects. The observed increases in numbers of PIs supported by the NIDDK immediately following FY 2008 are largely attributable to multiple PI R01 awards. The reduction in the number of PIs supported by the NIDDK from FYs 2012-2015 may be the result of more stringent paylines, as well as other factors, during that period.

# FIGURE 10

Preserving a Stable Pool of New Investigators—Number of NIDDK New Investigator R01 Applications and Awards

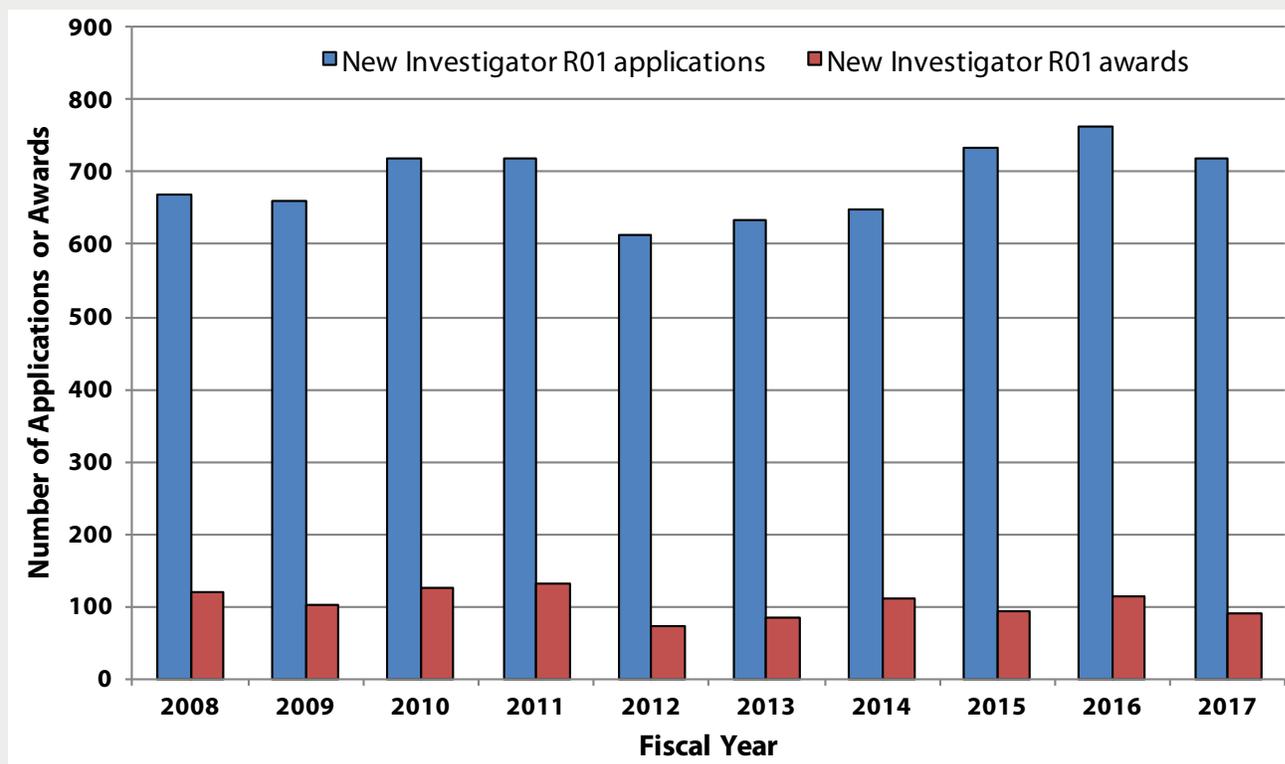
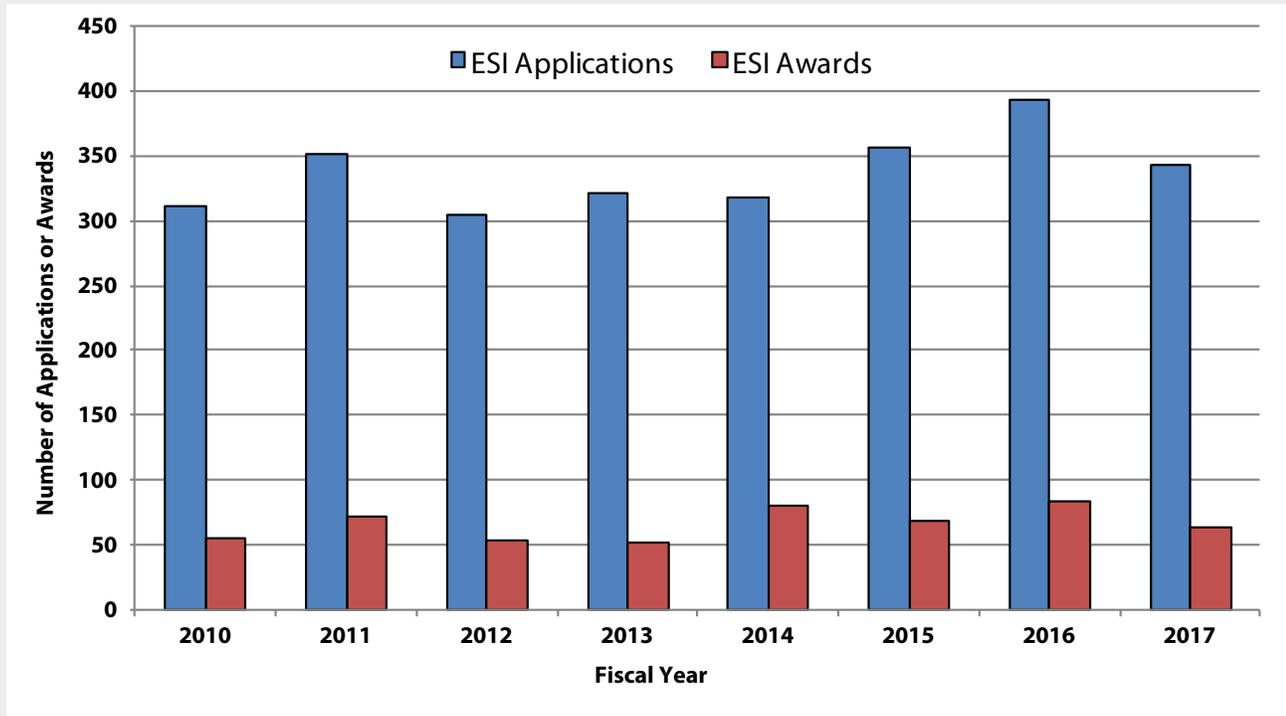


Figure 10 shows that over the last decade, the numbers of New Investigator (NI) applications and awards have remained fairly stable, fluctuating around about 100 per year. It should be noted that these data count applications and awards, not persons. The NIH issued a policy in 2008 to encourage early transition to funding independence for New Investigators within 10 years of completing their terminal research degree or their medical residency (<https://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-121.html>). This policy was modified or superseded by subsequent notices. The current policy, the Next Generation Researchers Initiative (<https://grants.nih.gov/grants/guide/notice-files/NOT-OD-17-101.html>), began in 2017. The NI definition includes all investigators who are new to NIH, both those who qualify as Early Stage Investigators (ESIs) and those who do not. ESIs are the focus of Figures 11 and 12.

# FIGURE 11

## Preserving a Stable Pool of New Investigators—Number of NIDDK Early Stage Investigator R01 Applications and Awards



Comparison of Figures 10 and 11 shows that while Early Stage Investigator (ESI) applications fell in FY 2012 essentially in proportion to the total drop in New Investigator applications, the proportional drop in number of awards to ESIs was not as great. This is attributable in part to the NIDDK’s differential payline for ESI applications (see table accompanying Figure 2 and the NIDDK New and Early Stage Investigators page at <https://www.niddk.nih.gov/research-funding/process/apply/new-early-stage-investigators>). Although there is moderate fluctuation from year to year in the numbers of ESI applications and awards, the differential payline is contributing to a healthy success rate for these applications.

# FIGURE 12

## Preserving a Stable Pool of New Investigators (NIs)—Percent of NIDDK NI Applications Submitted and Awards Received by Early Stage Investigators (ESIs)

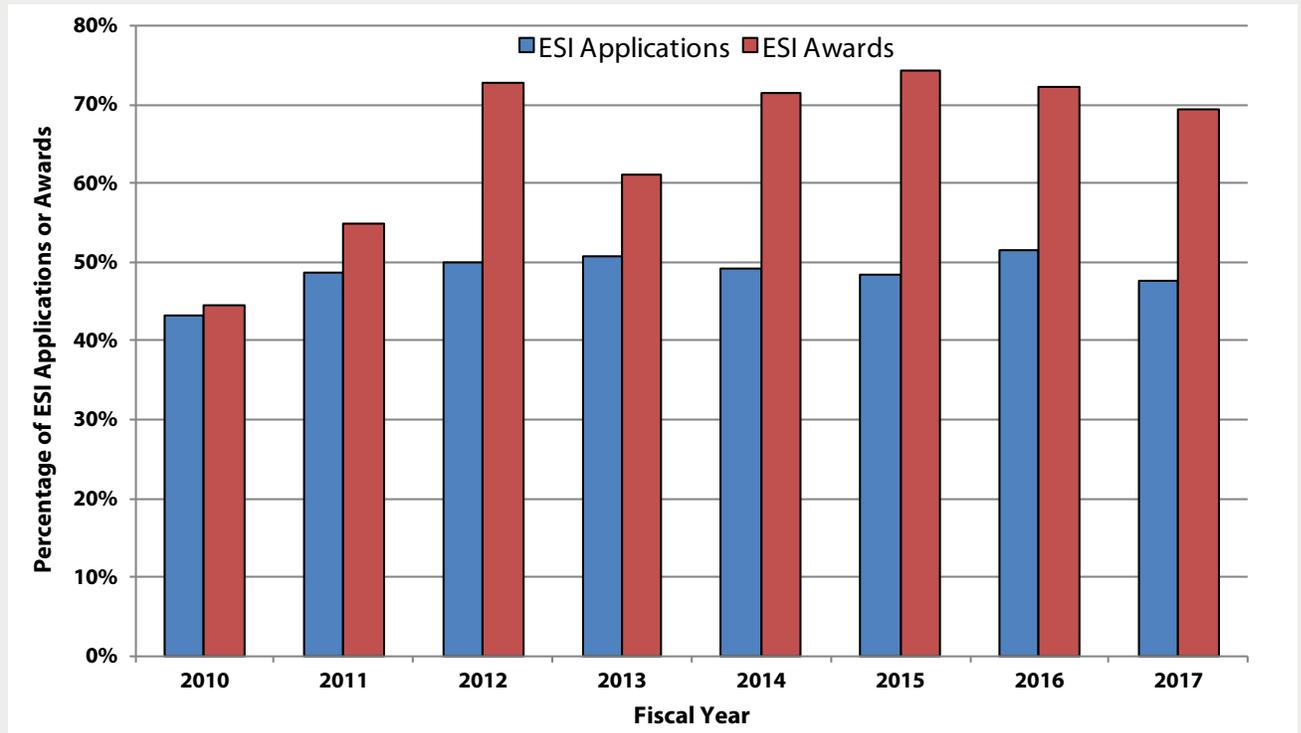
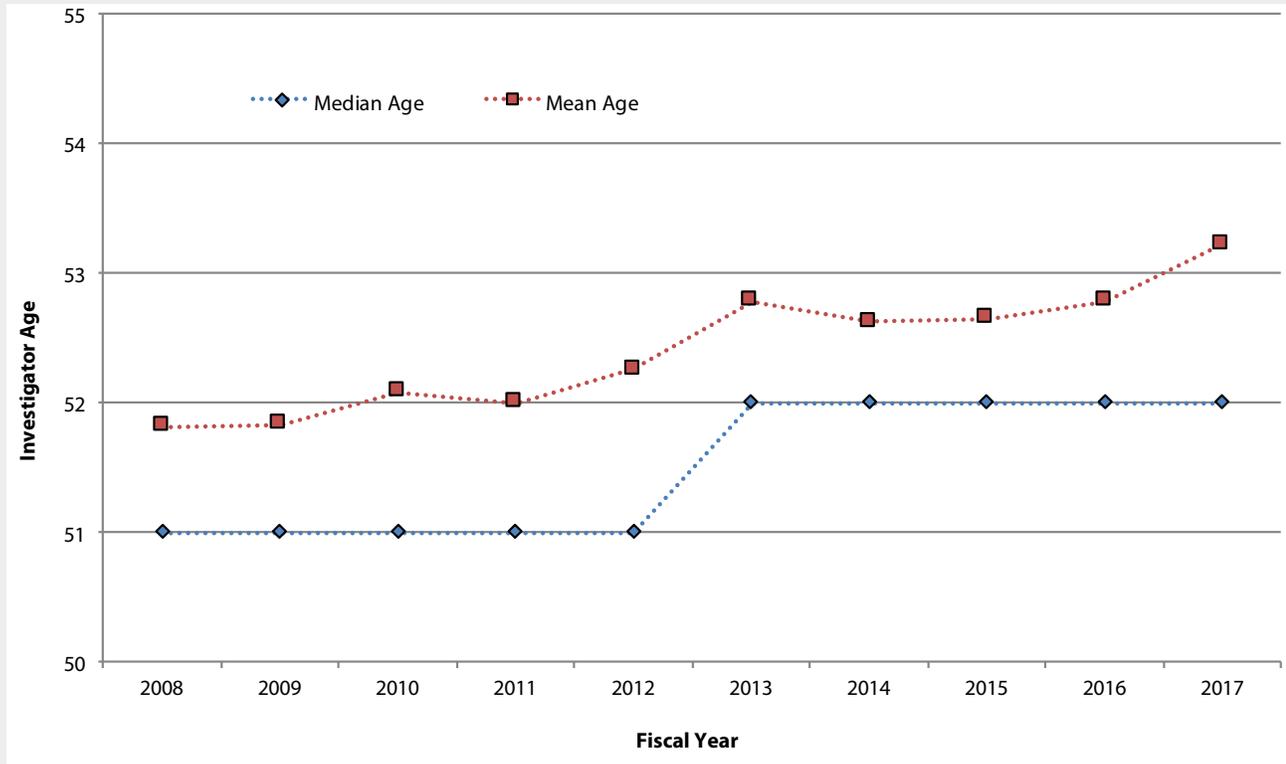


Figure 12 shows that the NIDDK’s differential payline for ESIs from FYs 2012-2017 (see table accompanying Figure 2 and the NIDDK New and Early Stage Investigators page at <https://www.niddk.nih.gov/research-funding/process/apply/new-early-stage-investigators>) has been effective in enhancing ESI representation among NI awards. ESI applications comprise about 49 percent of all NI applications received since FY 2011. The ESI differential payline instituted in 2012 has increased the number of awards to ESIs, so that about 68 percent of awards to NIs are awarded to ESIs. NIs who do not qualify as ESIs are paid according to the nominal payline each fiscal year.

# FIGURE 13

## Median and Mean Ages of NIDDK R01 and R37 Investigators



Over the past 10 years, the median ages of investigators holding R01 or R37 awards (competing and non-competing) increased by 1 year, and mean age of these investigators has increased by 1.4 years. Mean age increased gradually from FYs 2008 through 2013, then held relatively constant from FYs 2013 through 2016. In FY 2017, mean age increased to approximately 53.2 years, continuing the overall upward trend over time. Median age increased from 51 to 52 in FY 2013 and has remained constant since then.

# FIGURE 14

## Support Pivotal Clinical Studies and Trials—NIDDK Human Subjects Research Funding as a Proportion of All Extramural Research Funding

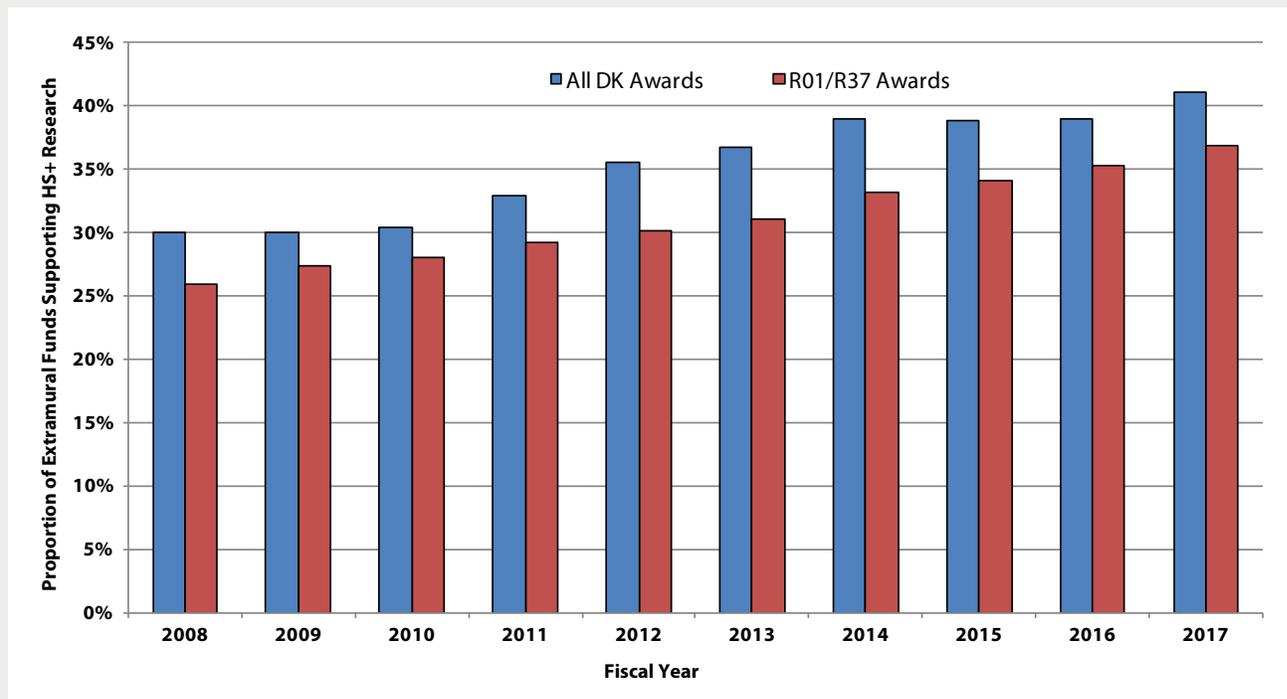


Figure 14 demonstrates that the NIDDK continues to commit a substantial proportion of its research funding to the support of clinical research involving human subjects. The proportion of NIDDK funds supporting Human Subjects research has increased steadily over the last 10 years, from 30 percent of all NIDDK-funded research in FYs 2008-2010 to about 40 percent in FYs 2014-2017. This same steady increase was seen in the fraction of R01 and R37 funding for human subjects research. For the purpose of this analysis, we used the definition described in Kotchen *et al.*, 2004 (*JAMA* 291: 836-843, doi:10.1001/jama.291.7.836, 2004) and included all studies coded as using human subjects (HS+). We are monitoring this trend to be sure that we maintain an appropriate balance between clinical and basic research.

# FIGURES 15A TO 15E

## Figures 15A To 15E: The NIDDK Is Committed to Training the Next Generation of Scientists

Figures 15A through E demonstrate that the NIDDK's commitment to training and developing the careers of the next generation of scientists remains strong.

**Figure 15A: NIDDK Fellowship (F), Career Development (K), and Training (T) Awards as a Percent of Total Extramural Research Funding**

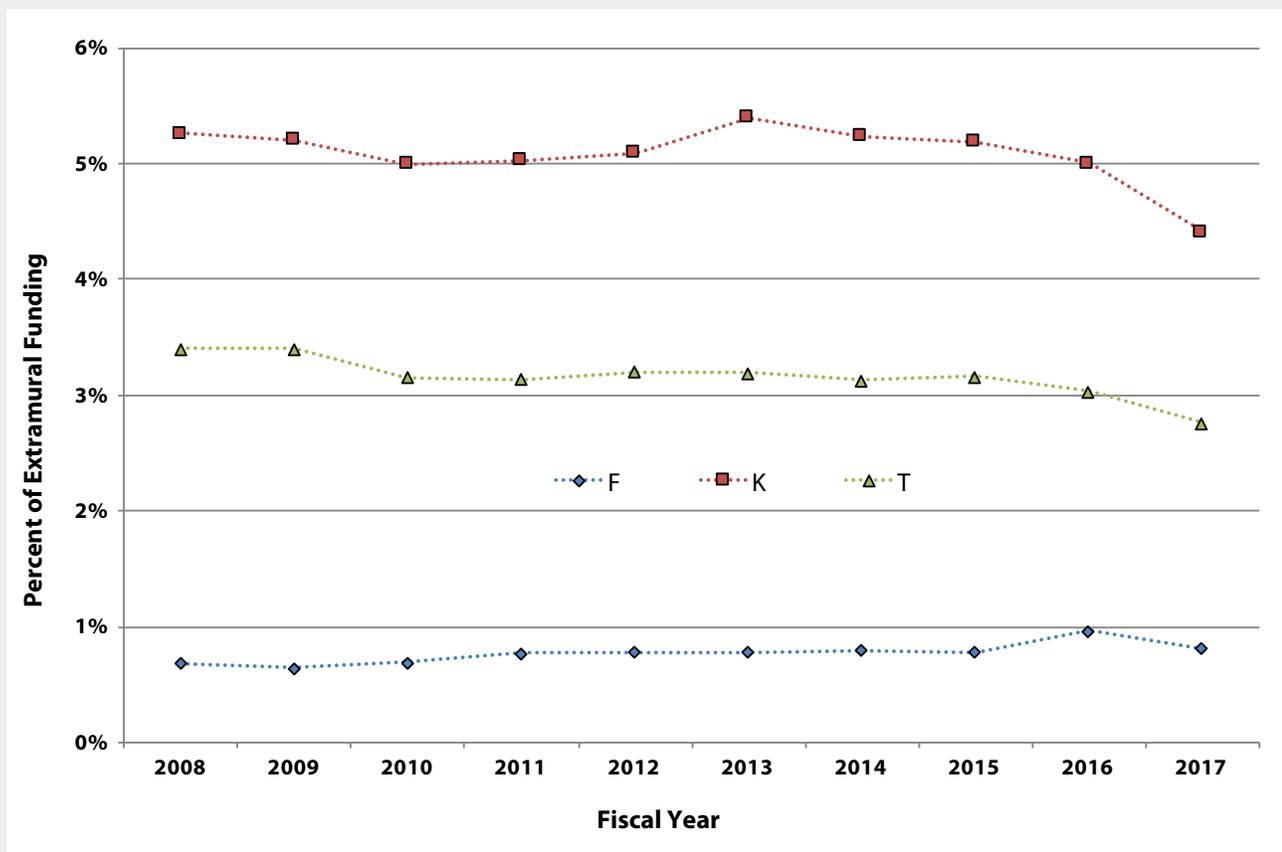


Figure 15A shows that overall support of training and career development programs has remained fairly stable since FY 2008. Funding for K awards has remained stable at about \$72 million a year since FY 2010, though in FY 2017 this represented a slightly lower proportion of the overall extramural research budget.

# FIGURE 15B

## Numbers of NIDDK Fellowship (F), Career Development (K), and Training (T) Awards by Fiscal Year

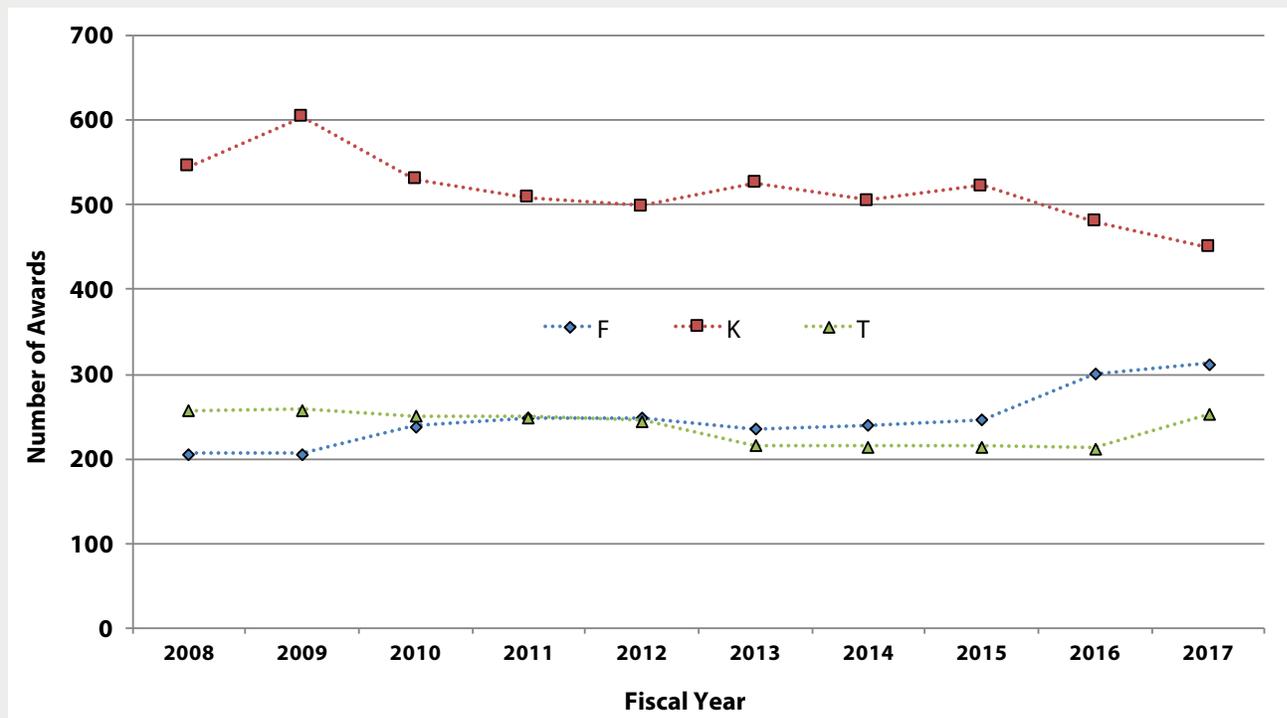


Figure 15B shows that the numbers of NIDDK F and T awards increased slightly in FY 2017, while the number of K awards dipped somewhat in FY 2017 for a second year in a row. Trends in specific K mechanism awards that contributed to this effect are shown in detail in Figure 15C. In addition, salary cap increases implemented in FY 2017 for competing and non-competing K08 and K23 awards may have contributed to this slight decrease in K award numbers.

# FIGURE 15C

## Numbers of NIDDK Career Development (K) Awards by Activity and Fiscal Year

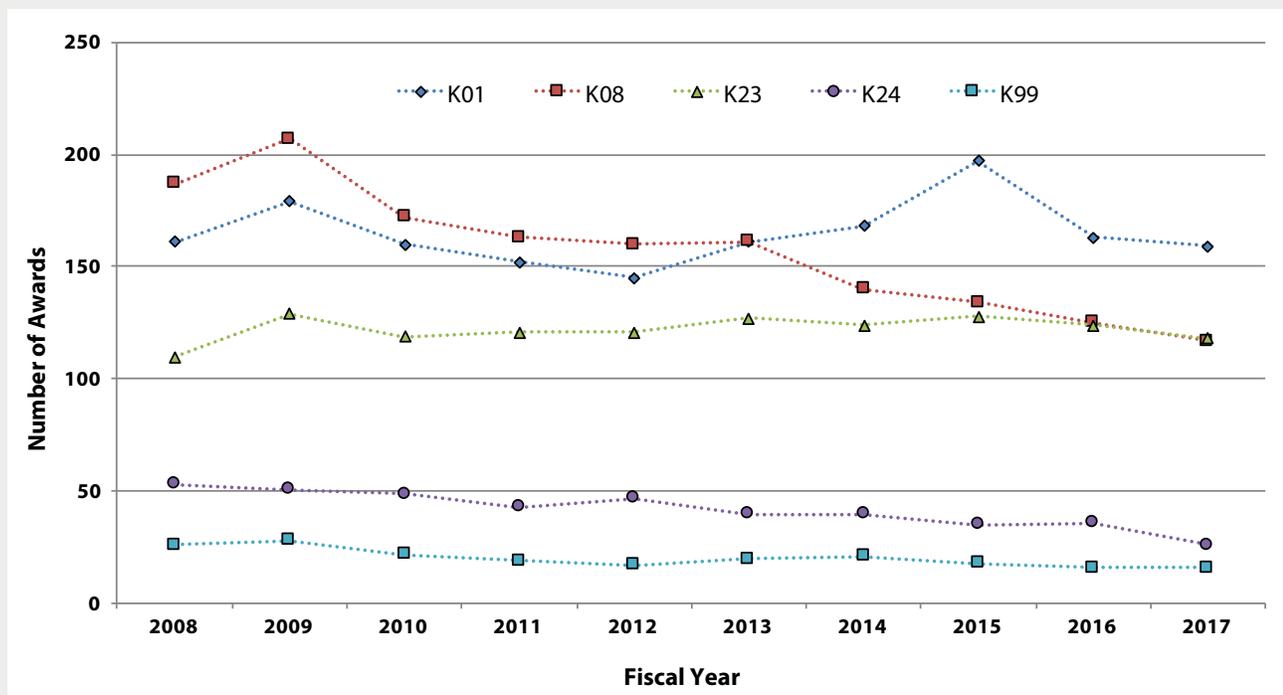


Figure 15C shows that the numbers of NIDDK K08 (Mentored Clinical Scientist Development Awards) and K24 (Midcareer Investigator Awards in Patient-Oriented Research) have decreased since FY 2008; the numbers of other K mechanism awards have shown no such overall trend. (FY 2017 was the last year that NIDDK accepted K24 applications, and no new NIDDK K24 awards are expected starting FY 2018.)

# FIGURE 15D

## Numbers of NIDDK Career Development (K) Applications by Activity and Fiscal Year

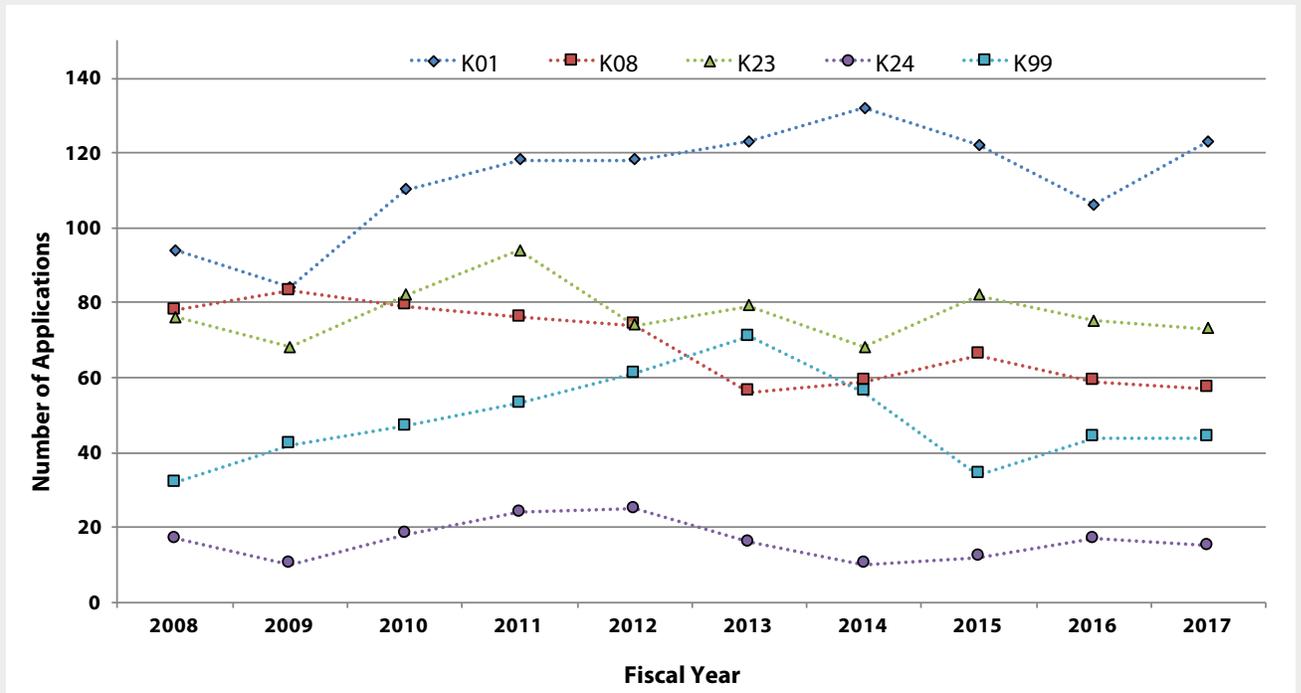


Figure 15D shows that K application numbers have fluctuated over time with some overall indication of declining numbers of K08 applications and increasing numbers of K01 applications. Other K application types show some year-to-year fluctuations or short-term trends but relatively comparable numbers of applications overall between FY 2008 and FY 2017.

# FIGURE 15E

## Numbers of NIDDK Training (T32) Award Slots by Fiscal Year

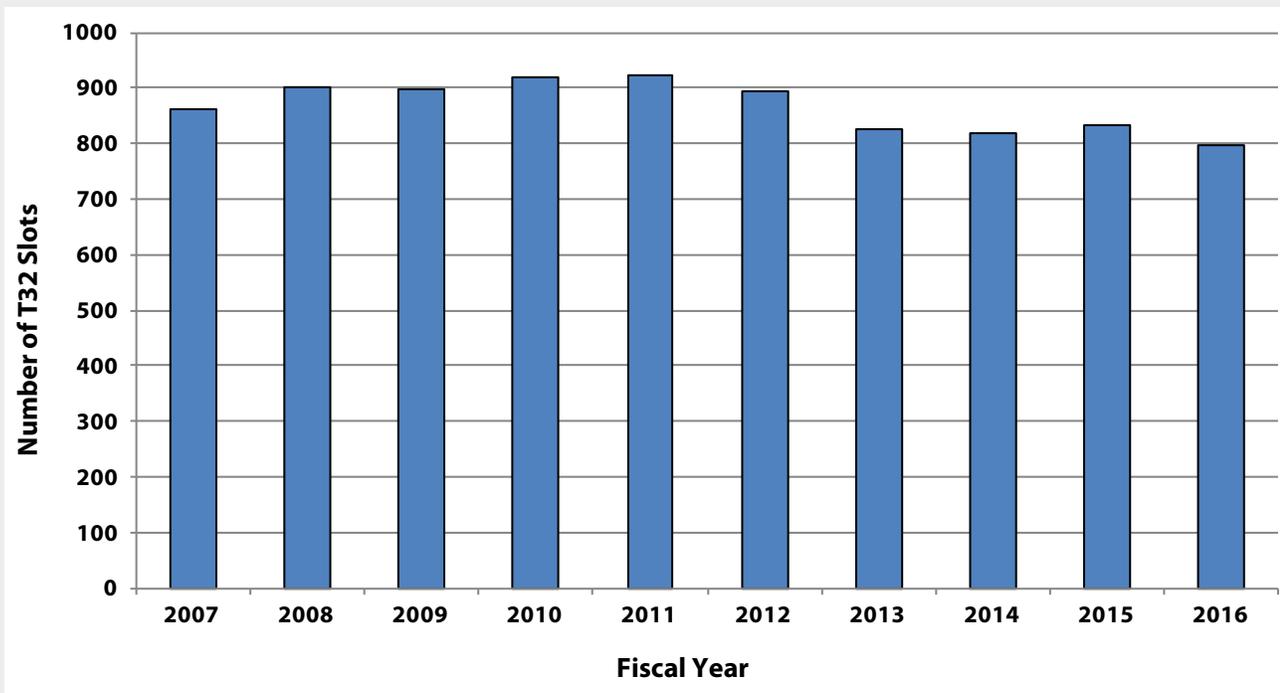


Figure 15E illustrates that the number of NIDDK T award training slots has remained relatively stable. The NIDDK will continue to monitor carefully its training and career development programs to identify factors behind trends and to ensure appropriate balance.

Note: T32 awards made in FY 2017 continue into FY 2018. The total number of T32 slots are reported at the end of the award period. Therefore, the FY 2017 information on T32 slots will not be available until later in FY 2018; thus, unlike the other charts in this section, FY 2017 data are not included here.



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