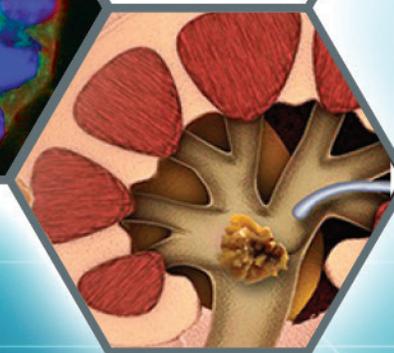
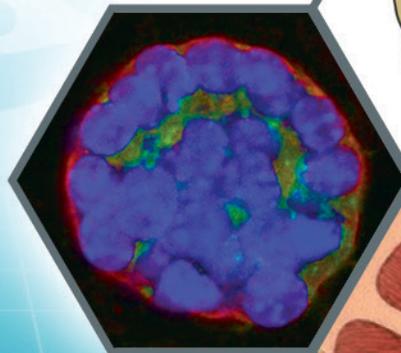
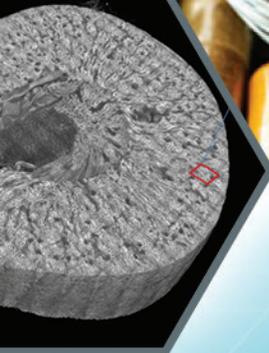
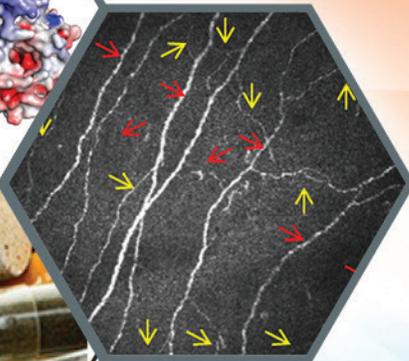
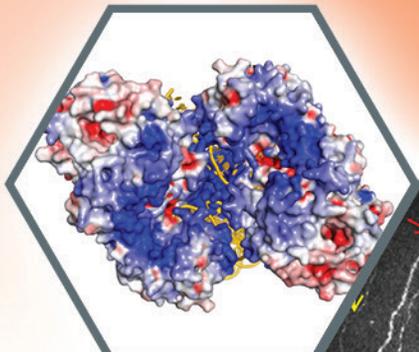


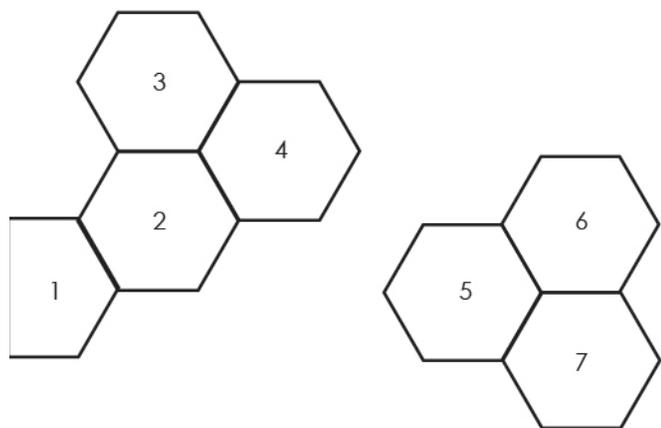
# NIDDK

## Recent Advances & Emerging Opportunities

January 2016



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



Cover Legend: The NIDDK has a long history of supporting research that employs cutting-edge tools and technologies to improve diagnoses, prevention strategies, and treatments for a broad array of human diseases and conditions. At the same time, the limits of technology are continually expanding, allowing an ever-deepening understanding of the biological processes underpinning health and disease. Images on the cover represent these interactions among technology, biology, and medicine in the quest to improve human health, exemplified by the NIDDK-supported research highlighted in this annual publication.

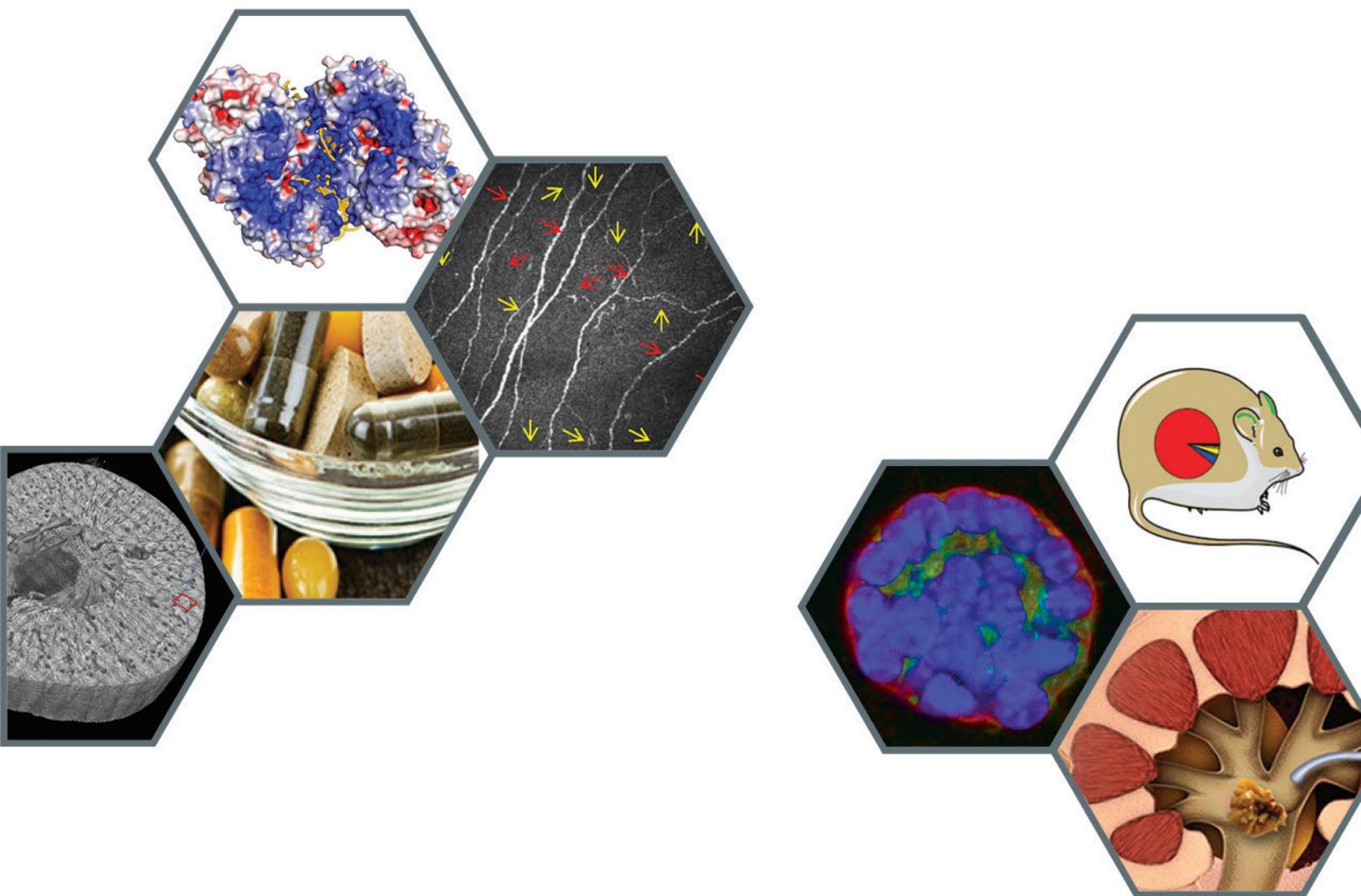
*Inset image credits:*

1. Image provided by Dr. Richard Torres and Dr. Robert Safirstein. Republished with permission of the *Journal of the American Society of Nephrology*, from Torres R, Velazquez H, Chang JJ, et al. Copyright 2015; permission conveyed through Copyright Clearance Center, Inc.
2. From graphics for Liver Injury from Herbal and Dietary Supplements Workshop.
3. Image provided by Dr. Wei Yang. Reprinted by permission from Macmillan Publishers Ltd: *Nature*, Kim M-S, Lapkouski M, Yang W, and Gellert M. Copyright 2015.
4. Image provided by Dr. Xin Chen and Dr. Rayaz A. Malik. From: Chen X, Graham J, Dabbah MA, et al. American Diabetes Association *Diabetes Care*, American Diabetes Association, 2015. Copyright and all rights reserved. Material from this publication has been used with the permission of American Diabetes Association.
5. Image provided by Dr. Rebecca G. Wells. From: Lorent K, Gong W, Koo KA, et al, *Sci Transl Med*, 2015. Reprinted with permission from AAAS.
6. Image provided by Dr. C. Ronald Kahn. Reprinted from *Cell Metab*, Ussar S, Griffin NW, Bezy O, et al, copyright 2015, with permission from Elsevier.
7. From graphics for Scientific Workshop: Urinary Stone Disease—Research Challenges and Opportunities.

# NIDDK

## Recent Advances & Emerging Opportunities

January 2016



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

## TABLE OF CONTENTS

### MESSAGE FROM THE DIRECTOR

### CROSS-CUTTING SCIENCE..... 1

Insights into Protein Structure and Function..... 1

*Solving Three-dimensional Structure of  
Immune System Proteins..... 1*

*Protein Folding Machinery—  
GroEL Gives the Assist..... 2*

Friends of NIDDK Coalition Holds  
Congressional Reception ..... 3

NIDDK Workshop: How Microbes Residing in the  
Gut Influence the Health of Their Hosts ..... 4

SPARC and NIDDK Organs of Interest ..... 6

Scientific Presentation: Dr. Wei Yang—  
Molecular Machines in DNA Biology ..... 8

### DIABETES, ENDOCRINOLOGY, AND METABOLIC DISEASES..... 13

Diabetes Prevalence ..... 15

*First National Data on Diabetes  
Rates in Asian Americans..... 15*

Type 1 Diabetes—Health Benefits of  
Good Glucose Management ..... 16

*Early and Intensive Glucose Control Can  
Result in Better Eye Health and Longer  
Life for Those with Type 1 Diabetes ..... 16*

Combating Hypoglycemia Associated with  
Insulin Therapy ..... 17

*Discovery of Brain Pathway That  
Responds to Low Blood Glucose Levels..... 17*

Replacing Beta Cells ..... 18

*Molecule Shows Promise in Promoting  
Beta Cell Replication..... 18*

Advancing Type 2 Diabetes Treatments ..... 19

*A Clearer Picture of the Molecular  
Underpinnings of Insulin Resistance and  
Type 2 Diabetes..... 19*

*Genetic Differences Throughout the  
Genome Affect the Function of a Key  
Metabolic Regulator..... 20*

*Discovery of Naturally Occurring  
Fats That May Alleviate Diabetes and  
Inflammation ..... 21*

Understanding and Diagnosing  
Diabetes Complications ..... 22

*Insights into Development of  
Diabetes Complications..... 22*

*For Diagnosing Diabetic Nerve Damage, the  
Eyes May Have It ..... 23*

Effects of Insulin Resistance in the Brain ..... 24

*Linking Insulin Resistance in the Brain to  
Behavioral Disorders ..... 24*

Metabolic Regulators of Health and Disease ..... 25

|                                                                                                                                             |           |                                                                                                                                              |    |
|---------------------------------------------------------------------------------------------------------------------------------------------|-----------|----------------------------------------------------------------------------------------------------------------------------------------------|----|
| <i>Regulating the Cell's Internal Recycling Program During Feast and Famine</i> .....                                                       | 25        | <i>Ease of Weight Loss Influenced by Individual Biology</i> .....                                                                            | 57 |
| <i>Attention to Starving Has Been Starving for Attention</i> .....                                                                          | 26        | Healthier Weight in Impoverished Children.....                                                                                               | 57 |
| Cystic Fibrosis Research .....                                                                                                              | 27        | <i>Head Start Participation Associated with Healthy Changes in Body Weight Among Impoverished Overweight and Obese Children</i> .....        | 57 |
| <i>Modest Benefit Seen from Treatment for Most Common Cystic Fibrosis Mutation</i> .....                                                    | 27        | Multiple Brain Cell Pathways That Regulate Appetite .....                                                                                    | 58 |
| NIDDK Director Testifies on Type 1 Diabetes Research.....                                                                                   | 29        | <i>Brain Cells That Forge a Path from Hunger to a Quest for Food</i> .....                                                                   | 58 |
| Exploring the Fourth Dimension on a Cellular Level: The 4D Nucleome.....                                                                    | 31        | <i>Hungry When You're Full? Cannabinoids Trigger a Surprising Switch in Brain Cells Known for Their Role in Suppressing Appetite</i> .....   | 59 |
| Story of Discovery: SGLT2 Inhibitors: Harnessing the Kidneys To Help Treat Diabetes .....                                                   | 33        | Circadian Rhythm and Calorie Intake.....                                                                                                     | 60 |
| Scientific Presentation: Dr. Jean E. Schaffer—An Unexpected Role for Certain Small RNAs in Diabetic Complications: sno in the Forecast..... | 39        | <i>Time To Eat?</i> .....                                                                                                                    | 60 |
| Patient Profile: Bariatric Surgery Offers Hope as a Treatment for Some People with Type 2 Diabetes .....                                    | 42        | Gut Microbiome, Genetics, and Obesity.....                                                                                                   | 61 |
| Patient Profile: Improved Diagnosis Leads to an Easier Treatment for Monogenic Diabetes .....                                               | 48        | <i>Nature, Nurture, and Nutrition—Gut Bacteria Native to Different Environments, Genetics, and Diet Influence Metabolism</i> .....           | 61 |
| <b>OBESITY</b> .....                                                                                                                        | <b>55</b> | Bariatric Surgery Research .....                                                                                                             | 62 |
| Biological Factors in Human Body Weight.....                                                                                                | 56        | <i>Bariatric Surgery May Help Reduce Urinary Incontinence</i> .....                                                                          | 62 |
| <i>Belly Fat, or Hip Fat? And How Much? Mining the Genome for Answers</i> .....                                                             | 56        | <i>Redirecting the Flow of Bile to the Distal Small Intestine Could Provide Metabolic Health Benefits Similar to Bariatric Surgery</i> ..... | 63 |

Burning Calories: Brown Fat, Beige Fat, and Body Temperature ..... 64

*Mimicking a Meal To Reduce Weight Gain* ..... 64

*A New Role for a Class of Immune Cells in Regulating Beige Fat Development*..... 65

*Improved Utility of Mice as Models of Human Obesity*..... 67

A Technological Partnership Between the NIH and the USDA Aims To Help People Achieve and Maintain a Healthy Weight..... 68

**DIGESTIVE DISEASES AND NUTRITION ..... 71**

Gut Microbes in Health and Disease..... 73

*Gut Microbial Communities Shaped by Human Genetic Factors*..... 73

*Harmful Gut Bacterial Effects Behind Persistent Childhood Undernutrition* ..... 74

*Viruses Can Cover for Helpful Bacteria in the Gut’s Microbial Community* ..... 75

Early Nutrition and Gastrointestinal Disease ..... 76

*Understanding Breast Milk’s Protective Effects Against Deadly Gastrointestinal Disease in Newborns* ..... 76

Workshop on Biomarkers of Pancreatic Disease ..... 78

Risk Factors for Inflammatory Bowel Disease ..... 80

*Digging Deeper for the Genetic Roots of Inflammatory Bowel Disease*..... 80

*Viruses in Gut Linked to Inflammatory Bowel Disease*..... 80

Insights into Gut Development and Activities ..... 81

*Gut Sensory Cells Can Signal Directly to the Nervous System* ..... 81

*Animal Models Provide Important Clues to Gut Vessel Development*..... 82

*Human Intestinal Model from Stem Cells Grown in Culture and Transplanted into Mice* ..... 83

Intestinal Cancer Genetics..... 84

*Identification of Genetic Mutation May Lead to Better Screening for Carcinoid Tumors, a Type of Intestinal Cancer*..... 84

Symptom Reporting in Irritable Bowel Syndrome..... 85

*“Total Recall”? Variations in Memory of Pain and Other Irritable Bowel Syndrome Symptoms Over Time* ..... 85

Understanding and Treating Liver Disease ..... 86

*Plant Toxins Linked to Biliary Atresia in Newborn Animals*..... 86

*Promise and Caution Mark Results from New Drug Trial for Fatty Liver Disease*..... 87

|                                                                                                                           |     |
|---------------------------------------------------------------------------------------------------------------------------|-----|
| Liver Injury from Herbal and Dietary Supplements Workshop.....                                                            | 89  |
| New Directions in Hepatitis Treatment.....                                                                                | 91  |
| <i>Over-the-Counter Antihistamine May Be Effective in Treating Hepatitis C</i> .....                                      | 91  |
| <i>Clinical Trial Shows Promising Results of New Chronic Hepatitis D Treatment</i> .....                                  | 91  |
| Story of Discovery: Hepatitis C: From Non-A, Non-B Hepatitis to a Cure .....                                              | 93  |
| Scientific Presentation: Dr. David Brenner— Mapping the Origins and Fates of Cells Underlying Liver Fibrosis .....        | 98  |
| Patient Profile: Surgical Procedure for Chronic Pancreatitis Transforms Young Person's World from Pain into Promise ..... | 102 |

## **KIDNEY, UROLOGIC, AND HEMATOLOGIC DISEASES ..... 111**

|                                                                                                   |     |
|---------------------------------------------------------------------------------------------------|-----|
| Insights into Kidney Disease and Damage.....                                                      | 114 |
| <i>Link Found Between Kidney and Bowel Diseases</i> .....                                         | 114 |
| <i>Genetic Variations in Young Patients with Chronic Kidney Disease May Be Risk Factors</i> ..... | 114 |
| <i>Delving Deep into the Kidney with a Novel Imaging Technique</i> .....                          | 115 |
| Kidney Disease Treatment.....                                                                     | 115 |

|                                                                                                                                                                  |     |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| <i>Single Drug as Effective as Pair in Treating Polycystic Kidney Disease</i> .....                                                                              | 115 |
| <i>Living Kidney Donors Fare Well Over Time</i> .....                                                                                                            | 116 |
| <i>Targeting Cells Involved in Fibrosis May Reduce Chronic Kidney Damage</i> .....                                                                               | 117 |
| NIDDK ApoL1 and Kidney Disease Conference .....                                                                                                                  | 118 |
| Scientific Workshop: Urinary Stone Disease— Research Challenges and Opportunities .....                                                                          | 119 |
| Advancing Understanding and Treatment of Urinary Tract Infections .....                                                                                          | 121 |
| <i>Identifying Players in Urinary Tract Infections</i> .....                                                                                                     | 121 |
| <i>Infected Bladder Cells Send Bacteria on a Trip via TRP</i> .....                                                                                              | 122 |
| <i>Too Much of a Good Thing— Host Defense of the Bladder</i> .....                                                                                               | 122 |
| <i>Fighting Fire with Fire To Treat Urinary Tract Infections</i> .....                                                                                           | 123 |
| Evaluating Pain in Women and Men.....                                                                                                                            | 124 |
| <i>What's the Difference? New Insights into Pain in Women and Men</i> .....                                                                                      | 124 |
| The Multi-Disciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network: Yielding New Insights into Challenging Urologic Pain Syndromes..... | 126 |

Prostate Disease Research ..... 133

*Benign Prostatic Hyperplasia—  
Gaining Insight and Potential New  
Treatment Identified in Mice..... 133*

Blood Cell Development and Survival ..... 134

*Elegant Study Documents How  
Hematopoietic (Blood) Stem Cells  
Find Their Niche ..... 134*

How Cells with a Choice  
Choose Their Fate..... 135

*Learning To Leverage the Potential of  
Pluripotent Stem Cells ..... 135*

Story of Discovery: Erythroferrone—  
New Regulator of Iron Balance ..... 136

Patient Profile: A Trip to the Emergency Room  
Prompts One Man To Join a Clinical Trial for the  
Treatment of Kidney Stones..... 140

**NIDDK FUNDING TRENDS AND  
SUPPORT OF CORE VALUES ..... 145**

**ACKNOWLEDGEMENTS**

# 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, such as cystic fibrosis; 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 16th edition of this report illustrates recent NIDDK-supported scientific advances, such as:

- Finding that early and intensive blood glucose control can result in better eye health and longer life for people with type 1 diabetes
- Identification of a group of fatty acids that improves blood glucose control and reduces inflammation in mice, with implications for treating or preventing type 2 diabetes
- Demonstration that a noninvasive technique for imaging small nerves in the eye appears to be just as effective as a skin biopsy at detecting evidence of diabetic nerve damage, a result that could have clinical implications
- Results showing modest improvements in breathing and resistance to infection using a two-drug approach for treating the underlying cause of cystic fibrosis, providing a new therapy for people with the most common disease-causing genetic mutation
- Finding that overweight or obese preschoolers participating in Head Start programs were more likely to reach healthier weights by kindergarten age than other groups of overweight and obese children
- New research in mice showing that restricting eating to a shorter period of the day might confer metabolic benefits or allow weight loss without reducing caloric intake, eating a rigid diet, or taking weight-loss medications
- Discovery that human genetic factors shape the composition of the gut microbial community and that some gut microbes may in turn affect the metabolism of their human “hosts,” knowledge that could inform future health-promoting interventions

- Identification of viruses in the gut that are part of the “microbiome” and linked to inflammatory bowel disease, which could pave the way to new prevention or treatment approaches
- Discovery that an over-the-counter drug indicated for treating allergy symptoms limited hepatitis C virus activity in infected mice, opening up possibilities for a low-cost therapy
- Results showing that using two drugs to treat high blood pressure in people with autosomal dominant polycystic kidney disease is no more effective than using a single drug, and that decreasing blood pressure below usual targets slowed growth of kidney cysts but had little effect on kidney function
- Insights into how bacteria such as *E. coli* use different yet critical processes to survive and promote urinary tract infections in people, which could have therapeutic implications
- New understanding of how the hematopoietic (blood) stem cell microenvironment promotes the survival and function of these cells, with implications for bone marrow or other transplant procedures

In addition to reporting on recent advances, this publication traces the multi-step path to research achievements through several “Stories of Discovery” and “Scientific Presentations.” These essays illustrate how major new discoveries that have greatly advanced biomedical science and are benefitting human health often emerge from 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 woman enthusiastically shares her experience of participating in a clinical trial focused on the risks and benefits of bariatric surgery for persons with mild obesity and type 2 diabetes. A teenager and her family share their story of how research advances and a correct diagnosis dramatically reduced the burden of treatment for her rare—and often misdiagnosed—form of diabetes. Another girl and her family share the story of her journey from incapacitating abdominal pain to an active teenage life following a surgical procedure to treat chronic inflammation of the pancreas. A man with a kidney stone describes his participation in a clinical trial testing whether a drug could help stones pass more quickly and/or with less pain.

The NIDDK is continuing efforts to ensure that knowledge gained from its research

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)

advances is disseminated to health care providers, patients, and the general public. Such efforts include the Institute's education programs: the National Diabetes Education Program and the National Kidney Disease Education Program. Additionally, the Weight-control Information Network, the National Diabetes Information Clearinghouse, the National Digestive Diseases Information Clearinghouse, and the National Kidney and Urologic Diseases Information Clearinghouse develop and distribute science-based information on diseases and disorders within the NIDDK mission. Several hundred brochures, fact sheets, and publications are available to patients, health care providers, and the public both in printed format and on the NIDDK 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 new Twitter feed: [@NIDDKgov](https://twitter.com/NIDDKgov)

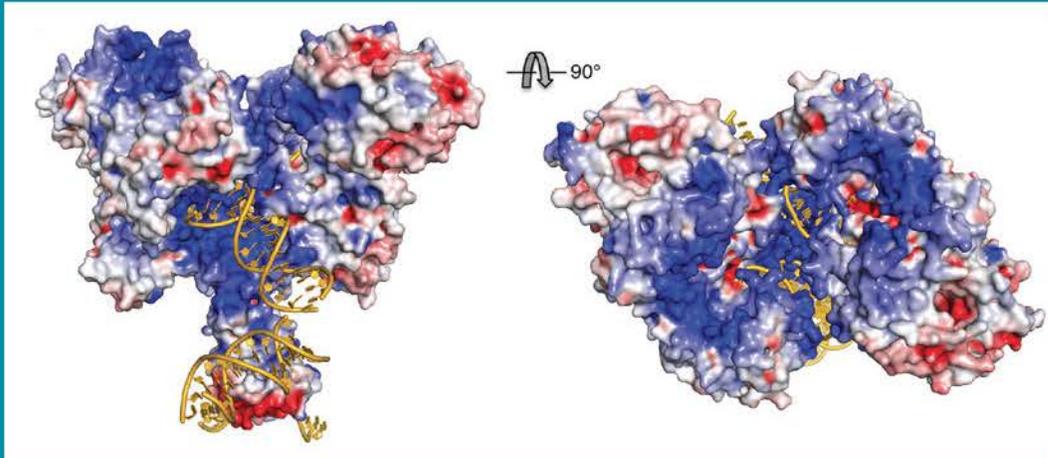
This report reflects only a fraction of the immense body of NIDDK-funded research performed by basic scientists, clinical investigators, and patient volunteers. Moving forward, we remain committed to supporting these important areas of research and translating scientific discoveries into improvements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



Scan the QR code  
with your mobile  
device to link to the  
NIDDK website at  
[www.niddk.nih.gov](http://www.niddk.nih.gov)



**Determining the three-dimensional structure of a protein allows researchers to better understand its role in health and disease and identify the regions that are important for function. As described in this chapter, the RAG1 and RAG2 protein complex is critical to generating a diverse population of antibodies that defend us against pathogens but could also be culprits in autoimmune diseases. The images above show two views of the computer modeling of the RAG1-RAG2 complex associated with its DNA targets. The blue color represents regions of the protein that are positively charged, red represents negatively charged regions, and the bound DNA is in yellow. Modeling the complex revealed that its surface is highly positively charged, promoting its interaction with the negatively charged backbone of DNA. Understanding the three-dimensional structure of RAG1-RAG2 provides insight not only into how antibodies are made, but into human disease as well. Antibodies are essential for recognizing pathogens, while misguided antibodies can recognize the body's own proteins and lead to diseases that affect the pancreas, gastrointestinal tract, kidneys, and immune system.**

*Image courtesy of Drs. Wei Yang and Martin Gellert, NIDDK. Reprinted by permission from Macmillan Publishers Ltd: [Nature](#), Kim M-S, Lapkouski M, Yang W, and Gellert M. Crystal structure of the V(D)J recombinase RAG1-RAG2. 518: 507-511, copyright 2015.*

*For an animated view of this image, please see [http://www.nature.com/nature/journal/v518/n7540/fig\\_tab/nature14174\\_SV1.html](http://www.nature.com/nature/journal/v518/n7540/fig_tab/nature14174_SV1.html)*

# Cross-Cutting Science

*Medical advances are not always achieved in great, intuitive leaps. More often, new prevention strategies, treatments, and cures result from a long, gradual accumulation of new 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.*

Described in this chapter are several recent studies that illustrate the Institute's commitment to basic and applied research relevant across a broad spectrum of scientific disciplines. Also featured are the NIDDK's transdisciplinary research efforts to understand how microbial communities in the intestine affect health and diseases/conditions, especially obesity, digestive, and liver diseases. Another feature describes a new NIH program, co-led by the NIDDK, that aims to advance neuromodulation therapies toward precise neural control of organ function to treat diseases and conditions of interest to the NIDDK. This chapter also highlights research in the NIDDK's Intramural Research Program that is determining the structure of molecular machines and thus providing insights into human health and disease.

## INSIGHTS INTO PROTEIN STRUCTURE AND FUNCTION

### **Solving Three-dimensional Structure of Immune System Proteins:**

Scientists in the NIDDK Intramural Research Program have determined the three-dimensional structure of a protein complex known as RAG1-RAG2 that is critical to the immune system. Because animals encounter a wide variety of potential infectious agents, the immune system must be able to generate a large and diverse population of antibodies to recognize these various agents. This diversity is generated by a process that joins different gene segments together in different combinations, a process known as "V(D)J recombination."

The proteins RAG1 and RAG2 are critical to this process; over 60 mutations that lead to severe combined immunodeficiency (SCID) in humans or a milder type of immunodeficiency called Omenn syndrome have been mapped to changes in the genes coding for RAG1 and RAG2. Understanding the three-dimensional structure, therefore, could provide insight not only into the critical process of V(D)J recombination, but into human disease as well. Solving the structure of mouse RAG1-RAG2 revealed that the disease-involved components of the proteins (amino acids), which are identical between mouse and human RAG proteins, fell into four classes based upon how the changes would disrupt the structure and/or affect RAG1-RAG2 activity. These data highlighted the amino acids critical to RAG1-RAG2, and may help explain how RAG1-RAG2 carries out its role in V(D)J recombination. (See also the *Scientific Presentation later in this chapter.*)

*Kim M-S, Lapkouski M, Yang W, and Gellert M. Crystal structure of the V(D)J recombinase RAG1-RAG2. Nature 518: 507-511, 2015.*

### **Protein Folding Machinery—GroEL Gives the Assist:**

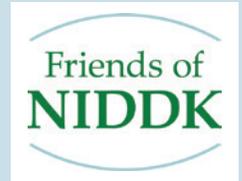
Scientists from the NIDDK's Intramural Research Program have designed a new experimental approach to visualize better how a bacterial protein called GroEL helps other proteins to fold properly. Proteins carry out many of the biological functions underlying normal health and disease, and these molecules need to be "folded" into the right shape to properly function. In many instances, disease-causing mutations alter a protein's function by disrupting its three-dimensional structure, resulting in a misfolded protein that may accumulate in the cell and/or function poorly or not at all. Thus, an important part of biomedical research is to understand the relationship between the structure and biological function of proteins and how cells promote proper protein folding. GroEL, a member of the chaperonin family of proteins, assists other proteins in the folding process and is also suspected of having the ability to recognize misfolded proteins and unfold

them to allow proper refolding. However, getting a clear picture of how GroEL interacts with the proteins it is folding has been difficult, because GroEL essentially engulfs the protein. Conventional techniques for determining molecular structures are unable to "see" the engulfed protein or how it is being manipulated in GroEL's folding chamber. As a result, very little is known about precisely how GroEL folds or unfolds proteins.

To obtain more information about how GroEL works, researchers took advantage of several different nuclear magnetic resonance (NMR) techniques. These techniques, instead of looking at the outside of GroEL as it folds a protein, examine the activity of GroEL and of the folding protein itself to collect detailed information about molecules' structures and how they are moving. Researchers used these techniques to investigate how GroEL interacts with a modified form of the protein Fyn SH3. Fyn SH3 exists largely in the folded state but also naturally unfolds a small amount of the time. Scientists monitored the transition of Fyn SH3 from unfolded to folded and back again, demonstrating that GroEL accelerates this transition by about 20-fold. Notably, these events took place in the absence of any cofactors or accessory proteins, giving conclusive evidence that GroEL possesses an innate or intrinsic ability to both fold and unfold proteins. Furthermore, the researchers were able to reconstruct how Fyn SH3 interacts with the inside of the GroEL folding chamber, identifying for the first time what parts of the two structures interact as Fyn SH3 folds and unfolds. These results provide new information about how GroEL (and possibly other protein-folding proteins) functions and how it helps protect cells from the detrimental effects of misfolded proteins.

*Libich DS, Tugarinov V, and Clore GM. Intrinsic unfoldase/foldase activity of the chaperonin GroEL directly demonstrated using multinuclear relaxation-based NMR. Proc Natl Acad Sci USA 112: 8817-8823, 2015.*

# Friends of NIDDK Coalition Holds Congressional Reception



On June 23, 2015, the Friends of NIDDK held their inaugural event, a Congressional Reception. The Friends of NIDDK is a coalition of approximately 40 organizations who share the Institute's commitment to research to improve the lives of people living with, or at risk for, diseases and conditions within the NIDDK's mission. Through the coalition, these organizations have come together to speak with one voice, with the goal of promoting and sustaining the vital research activities of the NIDDK. Their collective interests span the Institute's research portfolio, including diabetes and other endocrine and metabolic diseases, liver and other digestive diseases, obesity, nutrition, kidney diseases, urologic diseases, and hematologic diseases.

At the June event, NIDDK Dr. Griffin P. Rodgers and other NIDDK staff had the opportunity to speak with leaders from many of the Friends organizations. Welcoming remarks were provided by Ms. Shereen Arent, Chief Advocacy Officer at the American Diabetes Association, who expressed the shared interests of the Friends organizations in research supported by the NIDDK. She also commented that nearly everyone's family is affected by a disease within the NIDDK's mission. Dr. Rodgers

then addressed the group, highlighting the NIDDK's research areas and expressing appreciation to the attendees for their efforts in support of biomedical research.

Three Congress Members then provided remarks: Senator Dick Durbin (D-Illinois), Senator Jeanne Shaheen (D-New Hampshire), and Representative Robin Kelly (D-Illinois). Senator Durbin spoke of his legislative work to promote biomedical research, and of the new bipartisan Senate NIH Caucus, which he co-launched with Senator Lindsey Graham (R-South Carolina) earlier in 2015. Senator Shaheen, who had extended the invitation to Dr. Rodgers for this event, also spoke of the many benefits of research. Representative Kelly expressed her support and spoke in particular about health disparities, a topic relevant to many of the diseases in the NIDDK's mission. Congressional staff also attended the event, representing both Republican and Democratic members.

The NIDDK greatly appreciates the support and passion for biomedical research that was clearly evident at this event, and looks forward to the continued efforts of the Friends of NIDDK.



*Left to right: Dr. Rodgers addressing the Friends of NIDDK; Dr. Rodgers with Sen. Durbin (D-Illinois), with Sen. Shaheen (D-New Hampshire), and with Ms. Arent (American Diabetes Association) and Rep. Kelly (D-Illinois). Photo credit: Hilary Schwab Photography*

# NIDDK Workshop: How Microbes Residing in the Gut Influence the Health of Their Hosts



Humans host trillions of microbes in the gut, including bacteria and viruses and other microorganisms, referred to collectively as the “gut microbiota” or “gut microbiome.” These diverse intestinal inhabitants process dietary components and contribute to nutrient absorption and metabolism, interact with the immune system, and produce molecular signals that affect the intestines and other organs throughout the body. The types and proportions of different microbes in the microbiome can vary, and these differences may confer different effects on health. Although distinct from the bacteria and viruses that typically cause infectious disease, various communities of resident gut microbes have been associated with other diseases and conditions, such as obesity, diabetes, and inflammatory bowel disease.

In September 2014, the NIDDK convened a workshop to advance understanding of how disease pathways and normal biological processes

are affected by gut microbes and to define research needs and opportunities for further investigating host-microbiome interactions. These include both exploration of how gut microbes influence the health of their hosts, and how differences among hosts might affect the composition of the gut microbial community, for example, through diet, inflammation, or genetics. As researchers have been cataloguing the numerous types of bacteria in the gut, it will be valuable to identify the many molecules these microbes produce and what functions they may have. While scientists have observed correlations between the gut microbiome and multiple diseases, future research would help determine whether certain microbial communities actually cause these diseases, and if so, how. These research avenues would benefit from collaborations among researchers from diverse disciplines, including basic science and clinical investigation, the study of microbes and animal

models, and the analysis of large amounts of complex data (bioinformatics). Finally, there is considerable interest in manipulating the microbiome for health benefits. For example, there is a clinical application of fecal microbiome transplantation to restore a normal microbiome for treatment of recurrent infections with a type of bacteria called *Clostridium difficile*, which can be severe. However, it is not clear whether manipulation of the normal gut microbiome may affect other diseases processes, or whether any of the molecules these microbes produce might be useful as therapeutics.

Following this workshop, the NIDDK released Funding Opportunity Announcements to encourage further research: “A Community Research Resource of Microbiome-Derived Factors Modulating Host Physiology in Obesity, Digestive and Liver Diseases, and Nutrition” ([RFA-DK-15-012](#)) and “Exploratory Studies for Delineating Microbiome: Host Interactions in Obesity, Digestive and Liver Diseases and Nutrition” ([RFA-DK-15-013](#)).

# SPARC and NIDDK Organs of Interest



NIDDK leadership provides direction to the NIH Common Fund Program “Stimulating Peripheral Activity to Relieve Conditions” (SPARC).

Peripheral nerves, the nerves outside of the brain and spinal cord, make connections with and influence the function of every organ in the body. Modulation of peripheral nerve signals (neuromodulation) to control the functions of the organs they supply has been recognized as a potentially powerful way to treat many diseases and conditions, such as hypertension, heart failure, gastrointestinal disorders, diabetes and its complications, inflammatory disorders, and more. However, the underlying physiology and mechanisms of action for neuromodulation therapies are poorly understood. The design

of more effective and minimally invasive neuromodulation therapies requires knowing exactly how to stimulate nerves to achieve the desired effect on organ function. It also requires knowing exactly what nerves must be avoided to prevent unwanted side effects.

SPARC is uniquely positioned to serve as a community resource that provides the broader public and private research communities with the scientific foundation necessary to advance neuromodulation therapies towards precise neural control of end-organ system function to treat diseases and conditions. This high-risk, goal-driven program is structured as a consortium of four distinct research areas that will function in an integrated and iterative way, fostering discovery and broad dissemination of the fundamental physiology and biological mechanisms underlying peripheral control of internal organ function (autonomic nervous system) and sensory control, along with changes attributable to disease states and conditions. In turn, these discoveries will enable development of next generation closed-loop neuromodulation therapies, investigation of approved devices for new indications, and adoption of improved computational tools and modeling methods.

To spur applications to develop new and/or enhance existing tools and technologies tailored to elucidate the neurobiology and neurophysiology underlying autonomic control of internal organs in health or disease, which will ultimately inform next-generation neuromodulation therapies, the SPARC program released a research solicitation (Funding

Opportunity Announcement), “Exploratory Technologies to Understand the Control of Organ Function by the Peripheral Nervous System for SPARC,” in January 2015. Of 12 projects funded in September 2015, eight related to NIDDK organs of interest:

#### Gastric and Intestinal Motility Disorders

- An implantable wireless system to study gastric (stomach) neurophysiology
- Defining gastric vagal mechanisms underlying nausea and vomiting using novel electrophysiological and optical mapping technology
- Establishment of *in vitro* and *in vivo* models of human gastrointestinal organoids with a functional enteric nervous system

#### Bladder Dysfunction and Pain

- Generate novel viral tools that target sensory neurons for control of bladder function and pain

- Develop a novel multi-modal spinal root interface to better understand nervous system control of the bladder
- Integrate optogenetic technologies with fully implantable wireless systems for inhibition of neurons innervating the bladder

#### Colon and Bladder Dysfunction

- Selective peripheral neuromodulation through transfer of genes designed for this purpose into organ-specific nerve cells

#### Acute Kidney Injury

- Tailoring ultrasound technology and imaging to explore mechanisms of neuromodulation of inflammation to protect kidneys from acute kidney injury

For more information on SPARC, please see:  
<http://commonfund.nih.gov/sparc/index>

## Dr. Wei Yang— *Molecular Machines in DNA Biology*

*Dr. Wei Yang is the Chief of the NIDDK Intramural Research Program's Mechanism of DNA Repair, Replication, and Recombination Section in the Laboratory of Molecular Biology. Dr. Yang received her Ph.D. in Biochemistry and Molecular Biophysics from Columbia University and conducted her postdoctoral work at Columbia and Yale Universities. She joined the NIDDK's Intramural Research Program in 1995 and was tenured in 2000. A fellow of the American Association for the Advancement of Science since 2012, Dr. Yang was also elected to the National Academy of Sciences in 2013 and the American Academy of Arts and Sciences in 2015. Her research focuses on DNA recombination, repair, and replication using X-ray crystallography, molecular biology, and other biochemical and biophysical approaches to understand the mechanisms underpinning these biological processes. At the September 2015 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, Dr. Yang presented a lecture highlighting two recent advances to understand how DNA processes are carried out at the molecular level.*

### **Critical Molecular Processes Protect DNA**

Cancers are initiated by changes (mutations) or aberrations in DNA—changes that could be inherited or could result from exposure to

a cancer-causing agent. To prevent these changes, cells have protective mechanisms, the three “Rs” of DNA as Dr. Yang calls them: replication, repair, and recombination. Cells replicate their own DNA to create new, identical, healthy cells. Repair pathways correct mistakes or damage to DNA. Recombination is a process that can repair damaged DNA as well, and enables genetic diversity by allowing pieces of DNA to move in the genome; as discussed below, this process is critical for the immune system. When these protective pathways become inactive or malfunction, disease can result. For example, a genetic cause of hereditary colon cancer is mutations that affect the mismatch pathway by which cells reduce DNA mutation rates. Dr. Yang's research investigates these pathways that maintain genomic stability. Although it may seem as though she is studying many different pathways, all of these processes converge on DNA. The processes are linked and share commonalities, as Dr. Yang and her colleagues have discovered.

Dr. Yang utilizes a variety of biochemical and biophysical strategies to study these pathways. In particular, she and her colleagues utilize techniques in structural biology—a field that focuses on elucidating the three-dimensional configurations of molecules, and how the

# SCIENTIFIC PRESENTATION

details of molecular structures influence their functions. Determining how a molecule is built at the atomic level provides insight into how it works mechanically and reveals how mutations disrupt its function. Structural biology provides detailed portraits of molecules, identifies their dynamic states, and reveals the molecular properties that allow them to function. With recent improvements in techniques and methodology, Dr. Yang's research has been able to answer more challenging questions in DNA biology and to probe the mechanics of DNA processes more deeply.

## Key Insights into DNA Recombination in the Immune System

In her first example, Dr. Yang discussed her research that determined the structure of the RAG1-RAG2 protein complex, an important enzyme in the immune system. The RAG1-RAG2 complex helps create diversity in antibodies, proteins that are produced by the immune system to identify and target pathogens. Because the body can encounter a large number of different pathogens, this ability to create antibodies that recognize diverse pathogens is critical to survival. The RAG1-RAG2 complex helps create this diversity by recombining—cutting and rejoining—segments of DNA that encode the pathogen-recognizing sites of antibodies. This process is called “V(D)J recombination” for the different V, D, and J segments of DNA that are shuffled to form a large variety of combinations.

Twenty years after the discovery of V(D)J recombination, Dr. Martin Gellert's group in

the NIDDK's Intramural Research Program reconstituted a critical step *in vitro* (in a test tube) and showed that the RAG1-RAG2 complex cleaves the DNA at specific DNA sites, initiating the recombination process. When Dr. Yang joined the NIDDK in 1995, she and Dr. Gellert launched a collaboration to study how the RAG1-RAG2 complex functions by understanding its three-dimensional properties. It took many different innovations and another 20 years of effort to determine the structure of RAG1-RAG2; the breakthrough research paper describing their findings was published in 2015 (see also “Solving Three-dimensional Structure of Immune System Proteins” in the Cross-Cutting Science chapter). Dr. Yang explained that one of the challenges they faced was that only 1 percent of the purified protein was functional from the insect cells they were using to make the protein. Although insect cells are often used for protein production, the researchers eventually found a mammalian cell type that was more permissive—generating over 50 percent functional purified protein—which was the quantum leap they needed.

Dr. Yang and her colleagues utilized a method called X-ray crystallography to determine the structure of the RAG1-RAG2 complex. In this technique, the arrangement of atoms in a crystallized protein or protein complex can be determined by the pattern in which x-ray light is diffracted from the crystal. The structure, determined by Drs. Yang and Gellert's group, provided important insights into how RAG1 and RAG2 combine to form the active complex. In addition, the structure explained over

# SCIENTIFIC PRESENTATION

60 mutations in human immune disease. These changes in the DNA sequences of the *RAG1* and *RAG2* genes (which encode the RAG1 and RAG2 proteins) cause severe combined immunodeficiency, a disease in which people are highly susceptible to severe, recurrent infections, or a milder form known as Omenn syndrome. Dr. Yang and her colleagues were able to predict how the disease-causing mutations affect the RAG1-RAG2 complex's structure and function. This important discovery not only provides answers to one of the biggest and most important questions in biology about how V(D)J recombination proceeds, it also reveals critical information about human disease and could lead to improved diagnostics and treatments.

## Watching DNA Repair in Action

In a second example, Dr. Yang described her research visualizing the process of DNA synthesis, by studying a protein called "DNA polymerase  $\eta$ ." DNA polymerase  $\eta$  replicates DNA that has been damaged by ultraviolet (UV) light. This process is important; people who have defects in this protein have a severe sensitivity to sunlight—a heritable disease called xeroderma pigmentosum. Sunlight causes some of the building blocks of DNA to become linked aberrantly, making a "UV lesion." This linkage blocks DNA and RNA synthesis at that site; DNA polymerase  $\eta$  allows bypass of the blockage. Dr. Yang and her colleagues were able to determine the structure of DNA polymerase  $\eta$ , revealing that the UV lesion fits snugly into DNA polymerase  $\eta$ , whereas a normal DNA building block does not fit well; therefore DNA

polymerase  $\eta$  is prevented from inappropriately working on undamaged DNA.

This discovery answered questions about DNA repair and synthesis and, not surprisingly, generated many more. DNA polymerase  $\eta$  is just one of the many polymerases found in human cells. Although Dr. Yang and her colleagues had determined how DNA polymerase  $\eta$  functions in bypassing UV lesions, they were interested in probing more deeply into the chemical mechanism for DNA synthesis, which is similar among all DNA polymerases. They also wanted to explore fundamental questions, such as how the many polymerases of human cells slightly differ from those found in bacteria and viruses, in order to design inhibitors to eliminate pathogens without preventing our own enzymes from working. To answer these questions, Dr. Yang needed to see DNA polymerase  $\eta$  in action. Solving a single crystal structure is like taking a still photograph, she noted. While looking at a photograph can give an idea of how something might work, a series of photographs of a dynamic process is needed to reveal the sequence of steps and transiently associated key ingredients. As an analogy, Dr. Yang told a famous story: in 1878, the English photographer Eadweard Muybridge was hired to help solve a popular question of the day—when a horse runs, are all four feet off of the ground? Muybridge took a series of photographs of a trotting horse. In many of the photographs, the horse had at least one foot on the ground. However, in one photograph all four feet were off the ground. If Muybridge had only looked at one photograph in the series, he would have likely answered "no" to the question. The series of

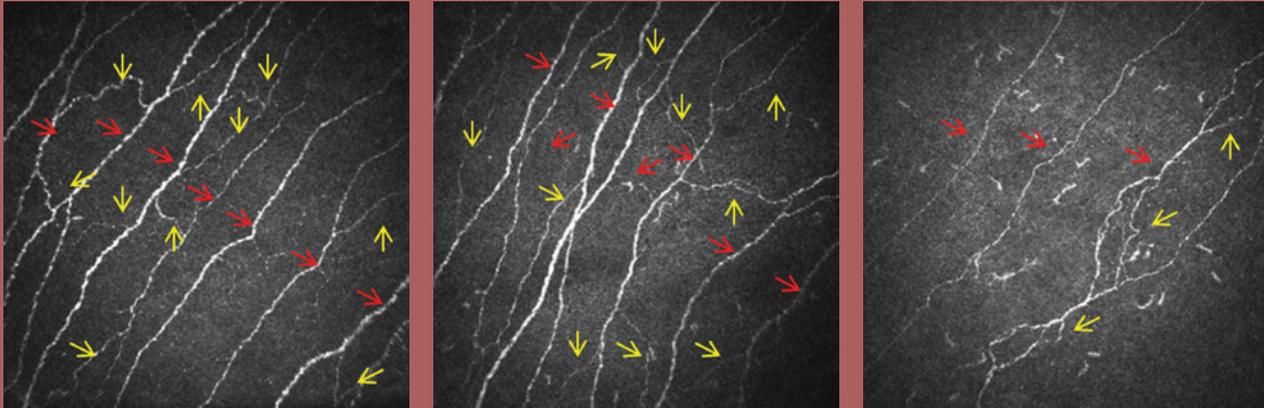
# SCIENTIFIC PRESENTATION

photographs, however, revealed the true answer of the dynamic process.

Dr. Yang and her colleagues took the same approach to watching DNA polymerase  $\eta$  in action. In an amazing feat, they were able to control the activity of DNA polymerase  $\eta$  and obtain sequential crystals of the DNA polymerase  $\eta$  in successive stages in action, and determine their structures. One of the observations they made was that a previously unknown third magnesium ion was essential to the reaction, and that this third magnesium ion is essential for every DNA polymerase. However, each polymerase has a different environment surrounding the third magnesium ion, providing a handle to design unique inhibitors for individual proteins. The identification of the critical role of this metal ion could lead to new drug targets and provides a new understanding of how this important protein functions.

## Conclusion

Structural biology, as Dr. Yang discussed, provides key information to determining the causes and treatments for disease beyond identification of the genetic mutation. Understanding the structure of RAG1-RAG2 revealed how specific mutations in this complex lead to severe combined immunodeficiency and Omenn syndrome. Being able to watch DNA polymerase  $\eta$  in action exposed a critical metal ion that had not been previously appreciated and opened new avenues for developing potential new treatments for cancers and infections. A protein's structure tells the story of its function, pinpoints the key regions, and explains how mutations lead to disruption of its activity. These details are critical to designing new, efficacious drugs and revealing new targets. As Dr. Yang demonstrated, structural biology is an essential tool and continues to play a central role in biomedical research.



Up to half of people with diabetes suffer damage to nerves throughout the body, damage that can lead to decreased sensation, difficulty moving, and pain and tingling in arms, legs, hands, and feet—a condition called diabetic sensorimotor polyneuropathy, or DSPN. Neuropathy is a major risk factor for amputation. Because the earliest signs of nerve damage occur in small nerves, tools to assess small nerve damage are critical for detecting and developing new therapies for DSPN. Currently, the most reliable tool for assessing small nerve damage is an invasive skin biopsy. A noninvasive technique to measure small nerve damage has the potential to improve the ability to detect and monitor DSPN early and to speed research to develop new approaches to prevent and treat it. Research described in this chapter indicates that a noninvasive laser-based imaging technique called corneal confocal microscopy (CCM), which allows users to image small nerves in the front of a person's eye, could be a new tool for detecting DSPN. The three panels above show CCM images of nerve fibers (white) in the eyes of three study participants. Compared to a person without diabetes (left) and a person with type 1 diabetes but no DSPN (middle), a person with type 1 diabetes and DSPN (right) has lower nerve fiber density, as illustrated by fewer arrows pointing to main (red arrow) and branch (yellow arrow) nerve fibers. As described in the chapter, both CCM and the invasive skin biopsy technique were similarly effective for diagnosing DSPN. These new findings suggest that the noninvasive nature of CCM, as well as its potential for automation, could make it preferable to skin biopsy for detecting DSPN.

*Images courtesy of Dr. Xin Chen, University of Manchester and Dr. Rayaz A. Malik, University of Manchester and Weill Cornell Medicine Qatar. From: Chen X, Graham J, Dabbah MA, Petropoulos IN, Ponirakis G, Asghar O, Alam U, Marshall A, Fadavi H, Ferdousi M, Azmi S, Tavakoli M, Efron N, Jeziorska M, Malik RA. Small nerve fiber quantification in the diagnosis of diabetic sensorimotor polyneuropathy: comparing corneal confocal microscopy with intraepidermal nerve fiber density. American Diabetes Association [Diabetes Care](#), American Diabetes Association, 2015. Copyright and all rights reserved. Material from this publication has been used with the permission of American Diabetes Association.*

# 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.*

Diabetes is a debilitating disease that affects an estimated 29.1 million people in the United States—or 9.3 percent of the total population—and is the seventh leading cause of death.<sup>1</sup> Compared with people of similar age without the disease, overall rates of death are about 1.5 times higher in people with diabetes, and rates of death from cardiovascular disease are 1.7 times higher.<sup>1</sup> Although rates of diabetes-related complications have declined substantially in the past two decades, disease burden remains significant as the number of people with diabetes continues to increase.<sup>2</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 diabetes in the United States in 2012—including costs of medical care, disability, and premature death—was \$245 billion.<sup>3</sup> Effective therapy can prevent or delay diabetic complications, but approximately one-quarter of Americans with diabetes are undiagnosed and therefore not receiving therapy.

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

---

<sup>1</sup> Centers for Disease Control and Prevention. *National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States, 2014*. Atlanta, GA: U.S. Department of Health and Human Services, 2014.

<sup>2</sup> Gregg EW, et al. *N Engl J Med* 370: 1514-1523, 2014.

<sup>3</sup> American Diabetes Association. *Diabetes Care* 36: 1033-1046, 2013.

diabetes cases in adults, and the majority of diagnosed cases in children and youth.<sup>1</sup> It most often develops during childhood but may appear at any age. Type 1 diabetes is an autoimmune disease in which the immune system launches a misguided attack and destroys the insulin-producing  $\beta$  (beta) cells of the pancreas. If left untreated, type 1 diabetes results in death 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  $\beta$  cells do. Thus, researchers are actively seeking new methods to improve blood glucose monitoring and insulin delivery. This includes continued development and testing of “artificial pancreas” technologies in real-world settings, as well as 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>1</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.

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, and Native Hawaiians and Pacific Islanders.<sup>1</sup> Gestational diabetes is also a risk factor: shortly after pregnancy, 5 to 10 percent of women with gestational diabetes continue to have high blood glucose levels and are diagnosed with diabetes, usually type 2.<sup>1</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 86 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>1</sup> This population is at elevated risk of developing 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 10 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 lead to a cycle of ever-growing rates of diabetes. Therefore, the advent of type 2 diabetes in youth has the potential to worsen the enormous health burden that diabetes already places on the United States.

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

## DIABETES PREVALENCE

**First National Data on Diabetes Rates in Asian Americans:** New research has found that more than half of Asian Americans with diabetes are undiagnosed, and that the prevalence of diabetes for all American adults increased between 1988 and 2012. In the new study, researchers analyzed data from 26,415 adults in the National Health and Nutrition Examination Survey (NHANES), which is a study to monitor nutritional and health status in the U.S. population. From this ongoing survey, they were able to generate national data on diabetes rates from 1988 to 2012. Understanding the prevalence (proportion of the population with the disease) of diabetes in the United States, how it changes over time, and which populations are disproportionately affected by the disease, could inform future research, public health, and educational awareness efforts to combat it.

The analyses showed that, between 1988 and 2012, diabetes prevalence for U.S. adults increased from nearly 10 percent to over 12 percent when measured by the tests commonly used to diagnose diabetes in clinical practice; more sensitive research tests yielded somewhat higher rates. Diabetes prevalence in U.S. adults also went up in both sexes and every age, level of education, income, and racial/ethnic subgroup. On positive notes, the proportion of people with diabetes that was undiagnosed decreased by 23 percent during the same time period, and diabetes prevalence has remained largely unchanged in more recent years (between 2007 and 2012).

For the 2011-2012 survey, NHANES surveyed a disproportionately large number of Asian Americans, which allowed researchers to quantify diabetes prevalence in this population for the first

time. Using the more sensitive research tests to define diabetes rates, they found that nearly 21 percent of Asian Americans had diabetes, with 51 percent of those individuals undiagnosed—the highest proportion of undiagnosed diabetes among all ethnic and racial subgroups studied. However, one difference between Asian Americans and the other groups studied is that Asian Americans often develop type 2 diabetes at a lower body mass index (BMI, a measure of weight relative to height). The NHANES data showed that the average BMI for all Asian Americans surveyed was just under 25; for the U.S. population overall, the average BMI was just below 29. (A BMI of 25 to under 30 is considered overweight, and a BMI of 30 or greater is considered obese.) These findings underscore the American Diabetes Association’s recommendation that Asian Americans get tested for type 2 diabetes at a BMI of 23 or higher, which is lower than the BMI threshold of 25 or higher that is recommended for the general population.

The study also provided data on other ethnic and racial subgroups in the United States. Again using the more sensitive research test to define diabetes rates, the scientists found that Hispanic Americans had the highest prevalence of diabetes at nearly 23 percent, with 49 percent of those individuals undiagnosed. Nearly 22 percent of non-Hispanic black adults had diabetes; however, they had a lower proportion of diabetes that was undiagnosed than the Asian or Hispanic subgroups, at about 37 percent. Non-Hispanic whites had the lowest prevalence of diabetes at 11 percent, and the lowest proportion of diabetes that was undiagnosed, at just over 32 percent.

These findings give important information about how diabetes affects the U.S. population and provide the first national data on total diabetes (diagnosed and undiagnosed) prevalence in Asian Americans.

The high prevalence of undiagnosed diabetes in Asian Americans suggests the need for increased awareness about type 2 diabetes screening at a lower BMI threshold in this population.

*Menke A, Casagrande S, Geiss L, and Cowie CC. Prevalence of and trends in diabetes among adults in the United States, 1988-2012. JAMA 314: 1021-1029, 2015.*

## **TYPE 1 DIABETES—HEALTH BENEFITS OF GOOD GLUCOSE MANAGEMENT**

### **Early and Intensive Glucose Control Can Result in Better Eye Health and Longer Life for Those**

**with Type 1 Diabetes:** People with type 1 diabetes who intensively control their blood glucose (sugar) early in their disease are likely to live longer and require fewer eye surgeries than those who do not. These findings are the latest results of the Diabetes Control and Complications Trial (DCCT) and its follow-up, the Epidemiology of Diabetes Interventions and Complications (EDIC) study. In type 1 diabetes, the body does not make insulin, and people with the disease need to take daily insulin to live. Beginning in 1983, the DCCT enrolled 1,441 males and females between ages 13 and 39 with type 1 diabetes. The goal of the trial was to determine whether intensive blood glucose control would result in fewer complications later in life. In the DCCT, half of the participants were assigned at random to intensive blood glucose control designed to keep blood glucose levels as close to normal as safely possible, and half were assigned to what was conventional treatment at the time. The DCCT ended in 1993 when the intensive control group was found to have substantially less eye, nerve, and kidney disease. Because of this result, intensive blood glucose control is now the standard of care for people with type 1 diabetes, though achieving such intensive control can be difficult. After the DCCT

ended, all participants were taught intensive blood glucose control and the researchers have continued monitoring their health during the ongoing EDIC study, which showed that blood glucose control has since been similar in both groups.

To further study the lasting effects of the different treatments tested by the DCCT, the researchers examined differences in overall lifespan between the intensive and conventional control groups. They found that after an average of 27 years of follow-up, the former intensive control group had a 33 percent reduction in deaths from all causes (43 versus 64) compared to the former conventional treatment group. The most common causes of death were cardiovascular disease (22 percent), cancer (20 percent), dangerously high or low blood glucose (18 percent), and accidents/suicide (17 percent). Additionally, fewer people in the former intensive treatment group than the former conventional treatment group died from diabetic kidney disease (one versus six). Higher average blood glucose levels and increased protein in the urine—a marker of diabetic kidney disease—were the major risk factors for death. A related study involving the DCCT/EDIC cohort showed that, after an average of 23 years of follow-up, participating in the intensive glucose control treatment group during the DCCT was associated with a 48 percent reduction in the risk of diabetes-related eye surgery, such as surgery to treat retinopathy, cataracts, or glaucoma. This reduction in eye surgeries led to a 32 percent reduction in the costs of surgery for those who had been in the DCCT's intensive control group, demonstrating how prevention of diabetes complications can have significant cost implications over the long term. Analysis of the rates of eye surgeries in conjunction with markers for blood glucose control indicated that improved glucose control accounted for virtually all the benefit seen from the intensive treatment.

These results from DCCT/EDIC further emphasize the importance of good glucose control in maintaining health and underscore the need for new tools and technologies to help people with type 1 diabetes achieve recommended blood glucose levels. The findings also add to the growing evidence that even a finite window of intensive glucose control early in the course of type 1 diabetes can have lasting benefits, resulting in reduced rates of diabetes complications and contributing to longer, healthier lives.

*Writing Group for the DCCT/EDIC Research Group: Orchard TJ, Nathan DM,...Lachin JM. Association between 7 years of intensive treatment of type 1 diabetes and long-term mortality. JAMA 313: 45-53, 2015.*

*Writing Group for the DCCT/EDIC Research Group: Aiello LP, Sun W,...Nathan DM. Intensive diabetes therapy and ocular surgery in type 1 diabetes. N Engl J Med 372: 1722-1733, 2015.*

## **COMBATING HYPOGLYCEMIA ASSOCIATED WITH INSULIN THERAPY**

**Discovery of Brain Pathway That Responds to Low Blood Glucose Levels:** Researchers have discovered a novel role for the hormone leptin in combating low blood glucose (sugar) and found that leptin acts through a previously unknown brain pathway to exert this effect, with potential implications for diabetes management. People with type 1 diabetes require insulin for survival; research has shown that intensive insulin therapy to control blood glucose levels is critical for improving their long-term health. However, too much insulin increases the risk of hypoglycemia (low blood glucose), which could result in life-threatening consequences. When glucose levels fall too low, the body reacts with a counter-regulatory response (CRR) to raise them; the brain is known to play an

important role in glucose sensing and in regulating the CRR. However, in people with type 1 diabetes, the CRR is impaired and worsens with each episode of low blood glucose. Thus, it is important to identify ways to preserve a robust CRR to protect people from adverse consequences of hypoglycemia so that they can achieve intensive blood glucose control.

Hypoglycemia can also occur in a fasted or starved state. Researchers hypothesized that, under such conditions, the body may respond by mounting a more robust CRR to overcome the nutrient deprivation, and that leptin may play a role. Leptin is a hormone secreted by fat cells; low leptin levels signal to the body that energy stores are low and promote hunger. Researchers speculated that low leptin levels may also enhance the CRR as a means to protect against hypoglycemia.

To test this hypothesis, the scientists conducted a series of experiments in mice. Previous research showed that leptin targets nerve cells (neurons) in several regions of the brain by binding to a cell surface protein called the leptin receptor. In a new study, researchers focused on a brain region, called the parabrachial nucleus (PBN), where they identified many neurons with the leptin receptor. They found that PBN neurons targeted a different brain region known to be involved in the CRR; that low glucose levels activated PBN neurons; and that leptin decreased their activity. These observations suggest that when glucose and leptin levels are low, PBN neurons are activated, which may enhance the CRR to protect against hypoglycemia. To examine this further, the scientists genetically engineered mice to lack the leptin receptor in a subset of PBN neurons (so that leptin cannot bind to the cells) and found evidence of an enhanced CRR when insulin was used to induce hypoglycemia. Because leptin has other functions, the researchers further examined the mice. They saw no effect on the

animals' body weights or on normal regulation of glucose levels in the absence of insulin-induced hypoglycemia. Other experiments showed that a hormone called cholecystokinin (CCK), which is involved in regulating food intake, is also expressed in PBN neurons with the leptin receptor; CCK was found to play an important role in this newly identified pathway. These findings suggest that low levels of leptin enhance the CRR through PBN neurons that express both CCK and the leptin receptor, and this effect is separate from leptin's well-known roles in regulating appetite and calorie burning (energy balance).

This research not only led to the discovery of a previously unknown brain pathway that plays a key role in regulating blood glucose levels, but also identified a novel role for leptin. Because PBN neurons were not involved in regulating energy balance or glucose levels under normal glucose conditions, the research suggests that this new pathway provides a potential specific therapeutic target to protect against hypoglycemia associated with insulin therapy. Further research could help determine the specific cells targeted by the PBN neurons that are involved in the CRR and if the findings are applicable in humans.

*Flak JN, Patterson CM, Garfield AS,...Myers MG Jr. Leptin-inhibited PBN neurons enhance responses to hypoglycemia in negative energy balance. Nat Neurosci 17: 1744-1750, 2014.*

## REPLACING BETA CELLS

### **Molecule Shows Promise in Promoting Beta Cell**

**Replication:** Researchers have found that a small molecule called harmine may promote  $\beta$  (beta) cell replication, potentially creating new approaches for expanding  $\beta$  cell mass. In both type 1 and type 2 diabetes, critical insulin-producing  $\beta$  cells

are lost. Because  $\beta$  cell proliferation is greatly diminished after infancy, older children and adults cannot replace lost  $\beta$  cells through regeneration. Research to identify strategies for expanding the number of  $\beta$  cells, throughout the lifespan, is therefore important to the treatment of both types of diabetes. Although an attractive strategy, inducing human adult  $\beta$  cells to regenerate has proven to be a difficult goal. One strategy involves stimulating  $\beta$  cell replication—inducing existing mature  $\beta$  cells to divide and produce an exact copy of themselves.

To identify new agents that promote  $\beta$  cell replication, researchers in this study screened over 100,000 small molecules with a high-throughput assay measuring cell growth signals that drive the replication of human  $\beta$  cells. They found one promising molecule, called harmine, that represents a newly discovered class of molecules. Harmine and other closely related molecules were found to increase human  $\beta$  cell replication both in  $\beta$  cells cultured in the laboratory and in intact animals. Importantly, researchers were able to probe the mechanism of harmine's action: first, they identified a specific target of its action as DYRK1A (Dual-specificity tyrosine-regulated kinase-1a); and second, they determined that a group of proteins that regulate gene activity (transcription factors) that belong to the NFAT (nuclear factors of activated T cells) family serve as mediators of human  $\beta$  cell proliferation and differentiation (the process by which cells become specialized).

To explore whether harmine induced  $\beta$  cell replication in live animals, the scientists tested its effects in three different mouse models. In the first, part of the pancreas of male mice was removed to prompt  $\beta$  cell growth. When the mice were treated with harmine, the scientists observed a rapid and robust replication of  $\beta$  cells and an increase in  $\beta$  cell mass. In the second model, mice were transplanted with both

male and female human  $\beta$  cells. The transplanted human  $\beta$  cells increased their replication in response to harmine treatment. Finally, harmine treatment improved glucose (sugar) control in non-obese diabetic mice transplanted with human  $\beta$  cells. Based on these findings, researchers have demonstrated harmine can induce  $\beta$  cell replication of both rodent and human  $\beta$  cells, leading to increased  $\beta$  cell mass and improvements in blood glucose levels.

This study provides promising evidence that harmine may promote human  $\beta$  cell replication, opening potential new therapeutic approaches for expanding  $\beta$  cell mass. There are several challenges before harmine, or an optimized version of it, could be ready for market. Further research will be needed to determine if the results in rodents hold true in humans. Additionally, because the mechanism through which harmine induces cell replication is not unique to  $\beta$  cells, and because harmine is known to have effects elsewhere in the body, harmine delivery will need to be tailored specifically to  $\beta$  cells to avoid off-target effects. Additional studies are also needed to determine the proper dose of harmine and duration of treatment because too much  $\beta$  cell replication could be harmful.

*Wang P, Alvarez-Perez JC, Felsenfeld DP, ... Stewart AF. A high-throughput chemical screen reveals that harmine-mediated inhibition of DYRK1A increases human pancreatic beta cell replication. *Nat Med* 21: 383-388, 2015.*

## **ADVANCING TYPE 2 DIABETES TREATMENTS**

### **A Clearer Picture of the Molecular Underpinnings of Insulin Resistance and Type 2 Diabetes:**

Recent experiments in mice have shed greater light on the regulation of insulin resistance and type 2 diabetes, suggesting a possible new approach to medical treatment for these

conditions. The thiazolidinediones, or glitazones, are insulin-sensitizing medications that act by targeting PPAR $\gamma$ , a protein known to be one of the key molecular arbiters of insulin resistance and type 2 diabetes. While glitazones are effective at improving glycemic control in people with type 2 diabetes, they have also been associated with a variety of side effects. In previous studies, researchers showed that one effect of glitazone treatment is to block the addition of a phosphate group to a particular site on PPAR $\gamma$  (a process called phosphorylation). Disruption of this phosphorylation event experimentally promotes the expression of genes that increase insulin sensitivity, leading to improved glycemic control, but without the side effects often seen with glitazones.

In a new study, the researchers sought to better understand the regulation of PPAR $\gamma$  by creating mice whose adipose (fat) tissue lacked the protein they thought to be responsible for the key PPAR $\gamma$  phosphorylation. Surprisingly, however, this caused an increase (rather than the expected decrease) in phosphorylation of PPAR $\gamma$  and the mice became more (rather than less) susceptible to the diabetes-inducing effects of a high-fat diet. Through further experimentation, they were able to identify the enzyme responsible for the increased phosphorylation, a protein called ERK. Working with male mice that had obesity and diabetes either as a result of diet or due to a genetic mutation, the researchers tested two different compounds known to block activation of ERK. Both were well-tolerated by the mice and proved effective at treating their diabetes without affecting their body weight, suggesting one or both might be useful therapeutically for treatment of type 2 diabetes in people. One of these compounds is approved by the U.S. Food and Drug Administration for the treatment of melanoma, a type of skin cancer. Further testing will be needed, however, to determine whether either compound is safe and effective for long-term use in the treatment of people with diabetes.

*Banks AS, McAllister FE, Camporez JP,...Spiegelman BM. An ERK/Cdk5 axis controls the diabetogenic actions of PPAR $\gamma$ . Nature 517: 391-395, 2015.*

### **Genetic Differences Throughout the Genome Affect the Function of a Key Metabolic Regulator:**

New research has led to the discovery of an important way that genomic variation can affect metabolic health and response to one class of medication for type 2 diabetes; these findings could lead to development of personalized treatments for the disease. Our genes contain the encoded recipes for making thousands of different proteins. When genetic differences alter these recipes in ways that inactivate or change the activities of the proteins they encode, there can be significant consequences for human health. But a surprisingly large portion of the genome is made up of stretches of DNA that do not code for proteins, and it has been something of a mystery as to why many genetic differences that are known to influence risk for various diseases have been found in these non-coding regions. One part of the explanation likely stems from the fact that proteins are not produced at the same rate throughout the body: their respective genes are “expressed” (activated so that the protein they encode can be produced) to differing extents in different tissues, at various stages of development and disease, and in response to nutritional and environmental cues. This pattern of gene utilization is enforced by expression-regulating proteins that bind to DNA sequences typically located near to (but not usually within) the genes they regulate. Thus, changes in non-coding DNA could affect health by altering the binding sites of these regulatory proteins, thereby influencing the expression of nearby genes.

In the new study, researchers investigated binding sites (in both mice and humans) for one such regulatory protein, called PPAR $\gamma$ , which plays a key role in controlling metabolic gene expression. They

began by comparing male mice from two genetically different strains, one of which was more susceptible to type 2 diabetes and other metabolic diseases than the other. PPAR $\gamma$  can bind approximately 35,000 sites in the mouse genome, and the investigators found that in male mice genetic differences between the two strains affected PPAR $\gamma$ 's ability to bind about 2,000 of these sites. In some cases, variations in the DNA sequence within PPAR $\gamma$  binding sites (or within the binding sites of other regulatory proteins PPAR $\gamma$  sometimes works with) had large effects on PPAR $\gamma$  binding. The researchers were able to document that, in general, stronger PPAR $\gamma$  binding correlated with stronger expression of nearby genes, indicating that sequence variation at these sites had a significant impact on gene expression in the two strains of mice. These differences in gene expression were accentuated when the mice were treated with rosiglitazone, a medication for type 2 diabetes that works by activating PPAR $\gamma$ . What makes this particular finding so significant is that the response to rosiglitazone is quite variable: it dramatically lowers blood glucose (sugar) in most (but not all) people with type 2 diabetes, and the drug is linked to significant side effects in some who take it. These findings suggest that the positive and negative variations in rosiglitazone response may be linked to differences in PPAR $\gamma$  binding sites near certain key genes.

To test whether people have significant, physiologically relevant differences in the binding sites of PPAR $\gamma$  and the proteins it works with, the investigators compared samples from five obese female volunteers (one Hispanic, one African American, and three White). As in mice, numerous differences were found that affect how well PPAR $\gamma$  binds various regulatory sites. The researchers looked at expression of genes near these sites using data from a previous study of samples from a group of 1,381 Finnish men with diabetes or at risk for the disease. They found that stronger

binding of PPAR $\gamma$  or its partners correlated well with higher expression of nearby genes, suggesting that natural differences in PPAR $\gamma$  binding sites correlate with markedly different gene expression patterns in people as well as mice. In fact, the analysis uncovered a previously undescribed PPAR $\gamma$  binding site difference that appears to have a significant impact on levels of good cholesterol and other metabolic factors and thus, presumably, on human health. Further analyses of variation in human PPAR $\gamma$  binding sites may lead to identification of genetic variations associated with beneficial and/or harmful responses to rosiglitazone, and may one day facilitate personalized treatment for type 2 diabetes by identifying those most likely to benefit from and least likely to be harmed by the drug. Indeed, these findings chart a course toward better understanding the effects of any drug that acts on proteins that regulate gene expression.

*Soccio RE, Chen ER, Rajapurkar SR,...Lazar MA. Genetic variation determines PPAR $\gamma$  function and anti-diabetic drug response in vivo. Cell 162: 33-44, 2015.*

**Discovery of Naturally Occurring Fats That May Alleviate Diabetes and Inflammation:** Working with a mouse model system, investigators have identified a group of fatty acids (a subgroup of lipids, or fat molecules) that appears to improve blood glucose (sugar) control and reduce inflammation. Previously, they noticed that mice from a particular genetically engineered strain were obese, with high levels of fatty acids, but—unlike many other obese mice—these had very good blood glucose control. The investigators wondered whether one or more of the elevated fatty acids might be playing a role in maintaining glucose control in these mice, so they compared lipids recovered from the adipose (fat) tissue from the engineered strain to lipids from normal control mice. This analysis led to the discovery of a family of fat molecules called PAHSAs

(palmitic acid-hydroxy stearic acids), which were present in both groups of animals but found at much lower levels in the control mice. In a test of normal mice with diet-induced obesity and type 2 diabetes, they found several of the PAHSAs were significantly reduced in some tissues compared to levels seen in normal mice on a healthier diet. The researchers also found similar relationships between PAHSA levels and insulin resistance in a small group of human volunteers, suggesting that low PAHSA levels may be associated with increased risk for type 2 diabetes.

To test whether PAHSAs may have therapeutic potential, the researchers orally administered two forms of these fatty acids (designated 5-PAHSA and 9-PAHSA) to mice with diet-induced type 2 diabetes. They found that each of the compounds lowered fasting glucose levels in the mice, and greatly improved their ability to manage glucose levels after feeding. The results suggest that these PAHSAs improve blood glucose control in at least two ways: by increasing the animals' insulin sensitivity, and—when they are fed—by increasing their insulin-production response. When investigators examined insulin-producing  $\beta$  (beta) cells from non-diabetic human donors (one man and one woman), they found that exposure to 5-PAHSA resulted in a modest increase in glucose-stimulated insulin production. They observed a more pronounced effect in mouse intestinal cells: both 5- and 9-PAHSA stimulated these cells to produce the hormone GLP-1, which itself can stimulate  $\beta$  cells to produce more insulin in response to glucose. Because obesity-induced inflammation in adipose tissue has been linked to type 2 diabetes, the researchers also studied whether these compounds might have an effect on inflammation. They found that oral administration of 9-PAHSA to mice for 3 days significantly blunted the subsequent response of immune cells within adipose tissue to a potent inflammatory trigger

compared to corresponding cells from control mice that did not receive 9-PAHSA treatment. Because some of these results were from experiments in male mice, and others from experiments with females, further research could help determine whether the findings apply equally to both males and females, and whether PAHSAs may be safe and effective for treating people with or at risk for type 2 diabetes.

*Yore MM, Syed I, Moraes-Vieira PM,...Kahn BB. Discovery of a class of endogenous mammalian lipids with anti-diabetic and anti-inflammatory effects. Cell 159: 318-332, 2014.*

## UNDERSTANDING AND DIAGNOSING DIABETES COMPLICATIONS

### Insights into Development of Diabetes

**Complications:** By studying cells derived from people with type 1 diabetes, researchers have revealed an important role in prevention of diabetes complications for a pathway that detects DNA damage and gives the cell a chance to repair it. Diabetes-related complications of the eyes, kidneys, nerves, heart, and other organs greatly affect the personal health of people with diabetes and contribute significantly to the costs of health care in the United States. Despite this toll, an understanding of the mechanisms underlying diabetes complications has remained elusive, in part due to the lack of cellular and animal models that mimic the human disease; and there is a need for novel, effective therapies. Though many people with type 1 diabetes will develop complications, valuable insights can be made from those who do not. In this research, the scientists studied “Medalists”—a group of people who have lived with type 1 diabetes for at least 50 years post-diagnosis—and compared Medalists who have experienced significant complications with Medalists who have not. The scientists reasoned that these people must have factors that protect them from

complications and that identification of these factors could help others who do develop complications.

To find these protective factors, the scientists needed to study the Medalists at a cellular and molecular level. They took advantage of a recent technological development and generated induced pluripotent stem (iPS) cells from skin cells of female and male Medalists with and without complications. These iPS cells have the potential to develop into different tissue types, allowing the scientists to study multiple tissues that are involved in diabetes complications.

Using genomic and proteomic tools, the scientists “profiled” cells from the Medalists to look for biomarkers of complications. Interestingly, genes involved in the DNA damage checkpoint pathway were suppressed in cells from Medalists with complications, but not in cells from Medalists without complications. The DNA damage checkpoint pathway monitors DNA damage and, when detected, pauses cell division to allow the cell to repair the damage. Without this checkpoint, DNA damage accumulates in a cell and can lead to cell death. The scientists found that DNA damage checkpoint pathway proteins were greatly reduced in cells from Medalists with complications, and that these cells were more likely to show signs of DNA damage.

Further investigation revealed that many of the DNA damage checkpoint pathway genes are downregulated by a small, circulating RNA molecule known as “miR200”; elevated levels of miR200 were found in cells and blood from Medalists with complications. The scientists found that increasing levels of miR200 in cells led to loss of DNA damage checkpoint control, whereas decreasing levels of miR200 in cells restored levels of the checkpoint proteins in cells from Medalists with complications. This finding is key, as it indicates that altering levels of miR200 could have value as a therapeutic approach.

The study also revealed differences between iPS cells from Medalists with complications and from Medalists without complications that may reflect other molecular pathways involved in diabetes complications. The researchers observed that the iPS cells from Medalists with complications showed significant impairment in their ability to grow and to generate different tissue types. Further research into this impairment in cell renewal may yield additional insights.

Collectively, these results revealed a role for the DNA damage checkpoint pathway in development of complications, suggested miR200 could be a therapeutic target, and demonstrated the utility of iPS cells as a cellular model to study complications and test future therapies. Additional research is necessary to determine whether targeting miR200 can protect people with type 1 diabetes from the onset and progression of diabetes complications; whether people with other forms of diabetes also get complications due to loss of the DNA damage checkpoint; and the mechanism by which type 1 diabetes can lead to elevation of miR200 in some people but not others, which could also lead to therapies tailored for individuals.

*Bhatt S, Gupta MK, Khamaisi M,...Kulkarni RN. Preserved DNA damage checkpoint pathway protects against complications in long-standing type 1 diabetes. Cell Metab 22: 239-252, 2015.*

### **For Diagnosing Diabetic Nerve Damage, the Eyes**

**May Have It:** A noninvasive technique for imaging small nerves in the front of the eye appears to be just as effective as a skin biopsy at detecting evidence of diabetic nerve damage. Up to half of people with diabetes suffer damage to nerves throughout the body. When this damage affects both the nerves that allow feeling and those that cause movement, it can lead to decreased sensation, difficulty moving, and pain and tingling

in arms, legs, hands, and feet—a condition called diabetic sensorimotor polyneuropathy, or DSPN. Detecting and evaluating nerve damage, especially damage to the smaller nerves more often involved in early disease, is key to early diagnosis of DSPN and assessment of its progression or regression (e.g., response to clinical treatment). One test that has been used to detect small nerve fiber damage quantifies the density of these fibers in a small skin sample, usually taken from the foot or leg. While robust, however, the reliability of this technique for diagnosing DSPN remains to be thoroughly validated in large cohorts of people with diabetes, and its invasiveness makes it less useful for repeated testing.

The cornea of the eye is rich in small nerve fibers, which can be visualized and quantified using images obtained with a noninvasive laser-based imaging technique called corneal confocal microscopy (CCM). To determine whether CCM could be an alternative to skin biopsy for diagnosis of DSPN, researchers directly compared the two methods in people. Study participants included 63 men and women with type 1 diabetes who had been tested for DSPN using independent criteria, 17 of whom were diagnosed with the condition; the study also included 26 people without diabetes. Each participant underwent both the skin biopsy and CCM. Both techniques detected lower nerve fiber density in the DSPN group compared with the group without DSPN and the group without diabetes. Notably, CCM images evaluated either manually or by computer yielded similar results. While neither skin biopsy nor CCM detected 100 percent of DSPN cases, both techniques had comparable diagnostic performance. More research needs to be done in larger and more diverse groups of people with diabetes; however, these results suggest that, while both techniques are less than perfect for diagnosing DSPN, the noninvasive nature of CCM and its potential for

automation could make it preferable for use in the clinic and in clinical trials.

*Chen X, Graham J, Dabbah MA, ...Malik RA. Small nerve fiber quantification in the diagnosis of diabetic sensorimotor polyneuropathy: comparing corneal confocal microscopy with intraepidermal nerve fiber density. Diabetes Care 38: 1138-1144, 2015.*

## EFFECTS OF INSULIN RESISTANCE IN THE BRAIN

### Linking Insulin Resistance in the Brain to

**Behavioral Disorders:** New studies in mice have demonstrated that insulin resistance in the brain can lead to anxiety and depression-like behaviors, suggesting a possible link between behavioral disorders and diabetes. Insulin is a hormone that plays a major role in metabolism by helping cells to absorb and use glucose (sugar) obtained from foods. Insulin resistance, a condition that occurs when cells do not respond properly to insulin, causing glucose to accumulate in the bloodstream, is a condition that often leads to type 2 diabetes. Previous research has suggested an association between diabetes and a variety of brain-related diseases and conditions, such as depression, age-related cognitive decline, and Alzheimer's disease. Insulin signaling in the brain has also been shown to be important for brain function and regulation of metabolism, but how insulin might regulate complex behavioral disorders is not well understood.

One proposed role for insulin in brain function involves the function of mitochondria, the structures in cells that unlock the energy stored in molecules like sugars and fats to make it useful to the cell. Mitochondria are also the home of two enzymes (called monoamine oxidase A and B or MAO A and B) that degrade many of the molecules nerve cells

use to signal one another. Dysregulation of these enzymes has been linked to depressive behaviors in people. To investigate how insulin activity in the brain affects mitochondria and behavior, scientists used certain genetically engineered mice (called NIRKO mice) that almost completely lack the insulin receptor in their brains. This insulin receptor deficiency disrupts signaling in the brain of NIRKO mice. At 10 months of age (“middle age” for mice), NIRKO mice are much like other mice based on body weight, food intake, blood glucose levels, and anxiety- and depressive-like behaviors. However, NIRKO mice as young as 4 months old had reduced mitochondrial function in their brains, and older NIRKO mice of both sexes displayed behavioral differences compared to controls. By 17 months of age (“older age”), NIRKO mice showed increased anxiety- and depressive-like behaviors on multiple behavioral tests, compared to their non-NIRKO counterparts. Analysis of the brains of female NIRKO mice revealed decreased signaling of the nerve cell signaling molecule, dopamine, as is often observed in human depression. Further investigation also found increased levels of MAO A and MAO B proteins in parts of the NIRKO brain, suggesting that insulin activity plays a role in signaling in both neuronal and some non-neuronal cells in the brain. Treatment of older female NIRKO mice with antidepressants that target MAO A and B virtually eliminated depressive-like behavior in one behavioral test. The scientists also found other alterations in mitochondrial structure and function. This research proposes a new explanation for how insulin resistance could affect brain function related to anxiety- and depressive-like behaviors in mice—by increasing MAO A and B activity, altering mitochondria, and decreasing dopamine activity. These findings suggest that improving brain insulin signaling may be a potential therapeutic target for the treatment of insulin resistance and diabetes-associated mood disorders. Further

research will be needed to learn if insulin plays the same role in humans and to determine if that role is similar in both women and men.

*Kleinridders A, Cai W, Cappellucci L,...Kahn CR. Insulin resistance in brain alters dopamine turnover and causes behavioral disorders. Proc Natl Acad Sci USA 112: 3463-3468, 2015.*

## **METABOLIC REGULATORS OF HEALTH AND DISEASE**

### **Regulating the Cell's Internal Recycling Program**

**During Feast and Famine:** New research in mice has described interlocking regulatory pathways in the liver that are involved in important cellular responses to nutrient availability or fasting. Autophagy, a process by which cellular components are routed to the cell's “recycling centers” and broken down for use, is a key survival mechanism that can provide nutrients to maintain metabolism. Autophagy is controlled by many factors, including whether the organism has eaten lately and thus whether abundant nutrients are available to cells. When an organism fasts and nutrient levels are low, autophagy can provide raw materials and fuel to maintain the cell. Many questions remain about how this occurs, including the details of how autophagy is triggered during fasting but repressed when an organism is well-fed.

Two research groups investigating how nutrient availability regulates autophagy independently discovered that several transcriptional regulators (proteins that control whether or not genes are expressed or “turned on”) play a crucial part in this process. Using male mice as a model, both groups found that FXR, a protein in the cell nucleus that regulates various aspects of metabolism, repressed autophagy in the liver when nutrients were abundantly available. The two groups' experiments then revealed two distinct, but complementary, FXR functions. One

group determined that FXR worked in opposition to another protein involved in metabolism, PPAR $\alpha$ , to control the activity of genes involved in autophagy. The scientists found that FXR and PPAR $\alpha$  competed to bind to DNA sites that specifically regulate genes involved in autophagy. When nutrient levels were high, FXR was activated and bound to these autophagy regulatory sites, reducing the expression of autophagy-related genes. However, when nutrient levels fell during fasting, PPAR $\alpha$  was activated and competed with FXR for binding to the DNA regulatory sites. PPAR $\alpha$  binding caused autophagy genes to be expressed and autophagy to increase to help support the cell.

The other group of researchers discovered that FXR interferes with another protein that regulates gene expression. This protein, called CREB, attaches to a crucial partner protein and then binds to regulatory sites of autophagy genes to turn on the genes. When mice were well-fed, FXR disrupted the interaction between CREB and its partner, reducing autophagy. When the mice fasted, however, FXR was inactivated, and CREB regained its ability to bind to and promote expression of autophagy-related genes, triggering autophagy. The complementary findings of these two groups shed new light on the complex interactions governing the important process of autophagy. Further research is needed to clarify how FXR, PPAR $\alpha$ , CREB, and other proteins interact to regulate this process. If similar pathways are active in humans, future studies may reveal important therapeutic targets for metabolic disease—in which dysregulation of autophagy may alter fat storage, insulin sensitivity, and other metabolic processes—and other diseases, such as cancer and neurodegenerative diseases.

Seok S, Fu T, Choi SE,...Kemper JK. *Transcriptional regulation of autophagy by an FXR-CREB axis.* *Nature* 516: 108-111, 2014.

Lee JM, Wagner M, Xiao R,...Moore DD. *Nutrient-sensing nuclear receptors coordinate autophagy.* *Nature* 516: 112-115, 2014.

### **Attention to Starving Has Been Starving for**

**Attention:** The discovery of a hormone and receptor that suppress insulin secretion during periods of fasting broadens our understanding of the intricate ways in which the body controls blood glucose (sugar) levels. During digestion, glucose from food enters the bloodstream, and insulin is secreted by the pancreas to signal cells to absorb that glucose and use it for metabolism. Hormones that signal the body to increase insulin secretion after eating—called incretins—have been the topic of considerable research, which has led to a new class of therapeutics for type 2 diabetes, called incretin mimetics. But observations in humans and other animals suggest that an opposing mechanism also exists: after a prolonged fast, the insulin-producing  $\beta$  (beta) cells of the pancreas are much slower to respond to an influx of nutrients by excreting insulin than they are when an individual eats more regularly. The result can be a transient elevation of blood glucose levels sometimes called “starvation diabetes,” which resolves as the  $\beta$  cells return to normal function. To account for this, it has been suggested that a different group of hormones—termed decretins—exists to suppress insulin release, preventing blood glucose from going too low during periods of fasting. The identities of these theorized decretin hormones and their receptors have remained a mystery. Surprisingly, the solution to the puzzle came from studying animals that do not have  $\beta$  cells, or even a pancreas: fruit flies.

As is typical in insects, flies respond to rising glucose levels by releasing insulin-like proteins from a group of cells in the brain. But, as in mammals, starvation results in a delayed insulin response upon re-feeding. To look for a fly decretin, investigators focused on a set of proteins that flies produce in greater abundance during starvation. They created a set of fly strains, each of which had increased levels of one of these starvation proteins, even when the flies were well-fed.

They found that the strain producing one such protein had elevated blood glucose and reduced insulin levels, suggesting the protein might be a incretin. They named it “limostatin” after Limos, the Greek goddess of starvation.

They next created flies that lack the gene encoding limostatin, and found them to have the opposite characteristics: low blood glucose and high insulin levels, as might be expected for an animal with reduced capacity to suppress insulin release. These flies also had atypically large amounts of adipose (fat) tissue, compared to normal flies, presumably because they release excessive amounts of insulin, causing too much sugar to be absorbed and stored as fat. Limostatin, the researchers went on to discover, was produced and excreted (during fasting) by certain cells of the digestive tract—and its excretion was specifically inhibited by the presence of sugars in the fly gut (rather than by fats or dietary proteins). They identified a limostatin receptor produced by insulin-producing cells, and found that flies with reduced levels of this receptor look much the same as flies lacking limostatin: they have low blood glucose and high insulin levels.

Although limostatin itself is not strikingly similar to any human proteins, the limostatin receptor is quite similar to a human protein—NMUR1—that is found on the surface of  $\beta$  cells, but not on other pancreatic cells. NMUR1 is the receptor for a hormone known as NMU, which is produced, they noted, in cells of the human upper intestinal tract. The researchers found that treating human  $\beta$  cells with purified NMU strongly inhibited insulin production, suggesting it functions in humans as limostatin does in flies—as a incretin. Interestingly, people with a very rare mutation in NMU have low blood glucose, high insulin, and early-onset obesity. These observations suggest that NMU plays an important role in inhibiting inappropriate insulin secretion in

normal, day-to-day life, not just in cases of extreme starvation. Further research may help determine whether modulating the activity of NMU or its receptors might be of clinical benefit to people with obesity or type 2 diabetes.

*Alfa RW, Park S, Skelly KR,...Kim SK. Suppression of insulin production and secretion by a incretin hormone. Cell Metab 21: 323-333, 2015.*

## CYSTIC FIBROSIS RESEARCH

**Modest Benefit Seen from Treatment for Most Common Cystic Fibrosis Mutation:** New research shows that modest but significant improvements in breathing and resistance to infection can be achieved through a two-drug approach to treating the root cause of cystic fibrosis (CF) in people with the most common CF-causing mutation. CF is caused by mutations in the *CFTR* gene, which encodes a protein normally found on the membrane covering cells. This membrane controls the passage of substances into and out of the cells, and the CFTR protein contributes by serving as a channel for chloride ions. CF results when mutations inactivate both of a person's copies of the *CFTR* gene. The most common CF-causing mutation is *cfr-ΔF508*: about 90 percent of people with CF have at least one copy of this mutation, and nearly half have two copies. This mutation disrupts the chloride channel in multiple ways: it creates a channel that is unstable, that rarely gets inserted properly into the cell membrane, and that is not functional even when it is inserted. Researchers previously identified a compound, called lumacaftor, that increases the amount of  $\Delta F508$ -CFTR protein that gets to the right place in the cell membrane, although its capacity to serve as a channel for chloride remains limited. A few years ago, a breakthrough medication became available for people with a different, rarer CF-causing

mutation. That mutation encodes a channel that makes it to the cell membrane but is non-functional. The new drug, called ivacaftor, works by making the non-functional CFTR channel permeable to chloride. For people with the rarer form of CF, ivacaftor greatly improved lung function and overall health.

In the new research, investigators tried a combination of lumacaftor (to increase the amount of the channel in the membrane) and ivacaftor (to allow chloride to pass through it) to treat people—both male and female—who have two copies of *cftr-ΔF508*. After 24 weeks, participants taking the drug combination had slightly better lung function than a control group taking placebo—roughly a 5 percent improvement in the amount of air they were able to exhale in 1 second. This improvement is modest, but was statistically significant. More strikingly, the participants receiving the drug combination were about one-third less likely to need to start or change antibiotics to treat symptoms of upper airway infection (a common and serious complication of CF). On the basis of these results, and because the drug combination is the first and only existing therapeutic approach that treats the underlying molecular problem in people with two copies of *cftr-ΔF508*, the

U.S. Food and Drug Administration granted expedited review and approval for its use in people 12 years of age and older with this genetic profile. It should be noted that the modest improvements conferred by lumacaftor/ivacaftor treatment do not appear to be without risk. For example, some participants receiving the drug combination experienced spikes in levels of enzymes indicative of liver injury or of damage to the heart or other muscles, and overall 4.2 percent of those taking the combination experienced adverse events that caused them to stop taking the study drugs, compared to 1.6 percent of those in the placebo group. For these reasons, it will be very important to monitor carefully the health of people taking these drugs, to limit adverse events, and to assess the long-term effects of the treatment. Research to find a drug or drug combination that is more effective and/or less prone to adverse events is ongoing; but in the meantime, there is now a therapy available that treats the underlying molecular cause of CF in about half of people with the disease.

*Wainwright CE, Elborn JS, Ramsey BW, ...Boyle MP; for the TRAFFIC and TRANSPORT Study Groups. Lumacaftor-ivacaftor in patients with cystic fibrosis homozygous for phe508del CFTR. N Engl J Med 373: 220-231, 2015.*

# NIDDK Director Testifies on Type 1 Diabetes Research

On July 15, 2015, 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 Claire McCaskill (D-Missouri). The hearing, entitled “Diabetes Research: Improving Lives on the Path to a Cure,” was held in conjunction with the Children’s Congress, an event sponsored every 2 years by JDRF (formerly the Juvenile Diabetes Research Foundation) 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)*, including progress from clinical trials testing approaches to delay or prevent type 1 diabetes; recent advances toward the development of an artificial pancreas—technology to automate blood glucose sensing and insulin administration; progress on islet transplantation as a treatment approach for people with difficult-to-control type 1 diabetes; progress toward producing large quantities of insulin-producing cells in the laboratory for cell replacement therapies; and results of a comparative effectiveness clinical trial testing



NIDDK Director Dr. Griffin P. Rodgers. Photo copyright: Larry Lettera



Shown at table (left to right): Amelia Cooper, Kate Hall, Bob Amato, Dr. Griffin P. Rodgers, and Dr. Habib Zaghouni. JDRF Children's Congress delegates sit in the foreground. Photo copyright: Larry Lettera



Dr. Griffin P. Rodgers delivering testimony before the Senate Special Committee on Aging. Also shown at table (left to right): Amelia Cooper, Kate Hall, Bob Amato, and Dr. Habib Zaghouni. Photo copyright: Larry Lettera

different treatments for diabetic eye disease. The NIDDK administers the *Special Diabetes Program* on behalf of the HHS Secretary.

Testifying with Dr. Rodgers were Ms. Kate Hall, a recent high school graduate and track and field star with type 1 diabetes; Mr. Bob Amato, a former collegiate runner and coach who has had type 1 diabetes for 67 years; JDRF Children's Congress delegates Amelia Cooper, age 15, and Isabelle Levesque, age 10; and Dr. Habib Zaghouani, the J. Lavenia Edwards Chair in Pediatrics at the University of Missouri School of Medicine.

Dr. Rodgers noted in his testimony that the *Special Diabetes Program* had recently been extended through Fiscal Year 2017. The extension provides the NIDDK with an opportunity to support new and emerging research in type 1 diabetes and its complications. To solicit input on future research directions that could be supported with the new funds, the NIDDK convened a planning meeting in April 2015, 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 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



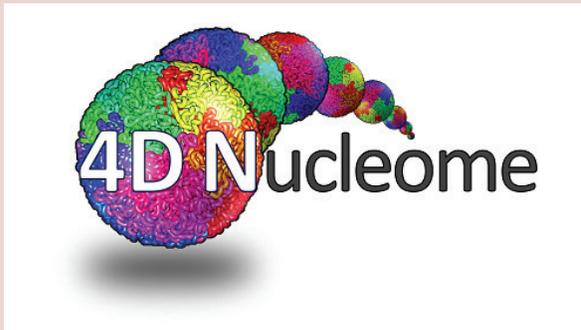
Shown at dais (left to right): Sen. Joe Donnelly (D-Indiana), Sen. Bob Casey (D-Pennsylvania), Sen. Elizabeth Warren (D-Massachusetts), Sen. Claire McCaskill (D-Missouri), Sen. Susan Collins (R-Maine), Sen. Jeanne Shaheen (D-New Hampshire), and Sen. Thom Tillis (R-North Carolina). Photo copyright: Larry Lettera



Dr. Griffin P. Rodgers and Sen. Susan Collins (R-Maine). Photo copyright: Larry Lettera

workshops, the Institute is identifying the most compelling areas of current research opportunity to pursue with the new funds, ensuring that the funds are used in the most efficient and scientifically productive manner possible. With the new funding, the *Special Diabetes Program* is poised to continue its exceptional track record of supporting cutting-edge type 1 diabetes research.

# Exploring the Fourth Dimension on a Cellular Level: The 4D Nucleome



Imagine a stage scene in a play, with people and props bounded by the stage wings left and right, the floor front, back, and below, curtain, lights, and supports above. Now imagine that same scene, but on the tiniest of scales, with the actors now the millions of DNA base pairs in a human cell. They fill the space in three dimensions as if on winding staircases, tightly holding props of proteins for support, clustered into small groups that are organized into still larger groups we would call chromosomes. Distinct characters and activities are indicated by chemical markers costuming the DNA and proteins. All around they are bounded not by wood and curtains and lights, but by a porous yet highly structured membrane that delimits the nucleus of human and other mammalian cells. Every actor has a mark in the intricate stage directions, every costume has a meaning, every prop a purpose. But although we can see it, what principles direct staging and other choices, the architecture of this molecular level scene? And what of when the scene is no longer still, and we enter time—the fourth dimension—and the play moves forward? The actors move, in smaller and larger groups; they interact, change

costumes, change props—but what if they stumble and fall, ignore cues, lose props, drop or misdirect lines, miss marks? How does the play change—and thus, the life of a cell, a tissue, a whole organism? Scientists involved in an ambitious new NIH program called the 4D Nucleome are seeking answers to these questions and more.

The 4D Nucleome Program is supported by the NIH Common Fund. Established in 2006 and administered through the NIH Division of Program Coordination, Planning, and Strategic Initiatives, the purpose of the Common Fund is to develop and invest in scientific programs that are transformative and have clear goals and deliverables within a specific time frame. These programs are also trans-NIH in nature and scope, transcending the missions of individual Institutes as they benefit the scientific enterprise. Focused on the cell's nucleus, in which chromosomes—the individual structures containing the genetic blueprint for a cell—reside, the 4D Nucleome Program aims to understand the principles underlying nuclear organization in space and time, the role nuclear organization plays in gene expression—how genes are “turned on” or “turned off”—and cellular function, and how changes in nuclear organization affect normal development as well as various diseases.

To establish the 4D Nucleome Program, in 2014 the NIH issued six related Funding Opportunity Announcements, each representing a core research or research support initiative important

to the overall goal of the program. In October 2015, the NIH announced that the 4D Nucleome Program is supporting its first set of 29 awards under these six core initiatives, totaling approximately \$25 million. The program includes support for an interdisciplinary consortium to explore nuclear organization and function; development of new chemical, biochemical, and imaging tools; studies of structural and functional subregions within the nucleus; an organizational hub to facilitate collaboration and resource sharing; and a data center to coordinate and integrate data generated by the 4D Nucleome investigators.

One of the core initiatives, the Nuclear Organization and Function Interdisciplinary Consortium, is being administered by the NIDDK. The NIDDK has taken a leadership role in the 4D Nucleome Program because complex diseases and conditions, such as diabetes and metabolic syndrome, are suspected to involve disruptions in normal nuclear architecture. For example, many genome-wide association studies have been performed in recent years to better understand the genetic basis for individual predisposition to

diabetes. When analyzed as an aggregate, these studies reveal the existence of over a hundred genetic variations associated with diabetes, with approximately 90 percent of them located in non-coding regions of the genome. These genetic variants likely contribute to disease by disrupting genome architecture and/or the ability of regulatory elements within the nucleus to properly control the spatial and temporal expression of genes involved in metabolic functions. It is also known that the activity of genes involved in metabolism is affected by circadian clocks—central mechanisms that allow most organisms to coordinate biological functions and behavior with environmental changes in light and dark cycles. Evidence suggests that there are regular spatial as well as functional changes in circadian clock genes within the nucleus during specific time periods, and hence a dynamic point of vulnerability to mishaps that could, in turn, affect metabolic gene expression. Thus, information from the 4D Nucleome efforts could be highly beneficial to a better understanding of metabolic and other diseases within the NIDDK mission, and the discovery of novel drug targets.

## SGLT2 Inhibitors: Harnessing the Kidneys To Help Treat Diabetes

Existing treatments are helping many people with diabetes live healthier lives, but there is still an urgent demand for new diabetes medications. Many years of research have enhanced understanding of diabetes and its effects on the body, including its role in kidney damage. Likewise, research into how the kidneys function has led to a better understanding of how the kidney manages glucose (sugar) and fluid in the body. Collectively, this research has led to the discovery of a new class of drugs that targets the kidneys to help control blood glucose in people with diabetes.

Glucose is a sugar that serves as the body's chief energy source. For those with diabetes, their cells have difficulty using glucose properly, leading to hyperglycemia (high blood glucose). Some people with type 2 diabetes can control their condition with physical activity and diet, while others require diabetes medications. As the disease progresses, many require injections of insulin, a hormone which helps the body utilize glucose. Existing diabetes drugs can help people with diabetes maintain their blood glucose levels in a healthy range, reducing their chances of complications later in life. However, these existing treatments sometimes carry side effects (such as hypoglycemia, or low blood glucose) and/or restrictions that can limit their usefulness. Moreover,

even with the expanded choice of treatments now available, meeting recommended blood glucose level targets can be challenging.

From many years of dedicated research, a new approach to reducing blood glucose levels has emerged: a new class of diabetes drugs, called SGLT2 inhibitors, that allows the kidneys to dispose of excess blood glucose in the urine. Clinical studies in people with type 2 diabetes have shown that these medications can safely and effectively lower blood glucose levels and improve glycemic control.

The path of discovery from basic research to effective, U.S. Food and Drug Administration (FDA)-approved diabetes drugs was paved with decades of work by many scientists, including NIDDK-supported researchers. The SGLT2 inhibitors are a prime example of how discovery research into how the body works can result in new disease treatments.

### **The Kidneys and Diabetes**

Every day, a healthy adult's two kidneys, each about the size of a fist, together filter 120 to 150 quarts of blood. Blood carrying wastes enters the kidneys, and the kidneys' millions of filtering units, called nephrons, filter that blood in a two-step process. First, blood passes through

# STORY OF DISCOVERY

the glomerulus, a structure which keeps blood cells and larger molecules, such as proteins, in the blood, while allowing wastes and excess fluid to pass through. The filtered fluid then passes through the tubule, which reclaims needed minerals and glucose, sending them back to the bloodstream. Wastes and extra fluid continue on to the bladder as urine. In this way, the kidneys maintain blood's healthy composition, keep levels of electrolytes such as sodium and potassium stable, and (through fluid management) contribute to healthy blood pressure.

The kidneys play an important role in managing glucose levels in the body. Because glucose is small enough that it can pass through the glomerulus, it will end up in the urine if it is not reclaimed or "reabsorbed." Because the body uses glucose as fuel, losing significant amounts of glucose in the urine would be wasteful for a healthy person. To prevent this loss, healthy kidneys in people without diabetes recapture virtually all the filtered glucose and return it to the bloodstream.

After the blood is filtered through the glomerulus, the filtered fluid (or "filtrate") moves on to the tubule, where the business of glucose reabsorption takes place. In the tubule wall, one side of each cell faces the filtrate and the other faces the circulation. In this way, tubule cells can act as both sensors monitoring the components of the filtrate, and conduits that can move materials from the filtrate back into the blood. The filtrate flows over the tubule cells, and transport proteins on the tubule cell surface recapture the glucose, much like workers plucking items from a conveyor belt.

Glucose is transported into the tubule cells and then pumped out the other side, back into the blood.

However, the kidneys' glucose reabsorption system is optimized to work best when blood glucose concentrations are in a normal range. In people with poorly controlled diabetes, who have increased blood glucose levels, this system begins to break down. The amount of glucose in the blood exceeds the kidneys' ability to recapture it, and some glucose continues through the tubules and is lost in the urine, a condition called glucosuria.

## **The Identification of SGLTs—Novel Proteins That Transport Glucose in the Kidney and Other Tissues**

For many years, the exact details of how kidney cells reabsorb glucose were unknown. The first clue as to how the kidneys accomplish this task was discovered in the early 1980s by NIH-supported researchers who noticed differences in glucose transport capacity throughout the rat kidney tubule: the early part of the tubule could absorb more glucose more quickly than the downstream part of the tubule. Understanding of how this worked on the molecular level emerged from studies of how glucose from food is absorbed by the cells lining the intestine. NIDDK-supported researchers studying the cells lining the intestine discovered the gene for the intestinal glucose transport protein. The protein belonged to a new class of glucose transporters called sodium-glucose cotransporters, or SGLTs. The intestinal transport protein was named SGLT1. Scientists then found a second, closely related

# STORY OF DISCOVERY

protein, SGLT2. Both SGLT1 and SGLT2 are responsible for glucose transport in the kidney.

SGLT1 and SGLT2 are proteins on the cell surface of the tubule cells. Both reclaim glucose from the kidney filtrate, moving glucose together with sodium into the tubule cells, where they can then be returned to the blood. SGLT2 is found earlier in the tubule and is a very high-capacity glucose transporter, while SGLT1 is found later in the tubule and is a lower-capacity transporter. Thus, filtered glucose will first encounter SGLT2 before encountering SGLT1. SGLT2 is responsible for 90 percent of the total glucose absorption as urine is made, while SGLT1 is responsible for the remaining 10 percent. In addition to the kidney and intestine, SGLT1 is also found in many other tissues of the body.

Because of their key role in glucose reabsorption, the SGLTs, particularly SGLT2, were promising drug targets to alter blood glucose levels. Healthy kidneys can reabsorb up to 180 grams (roughly 0.40 pounds) of glucose per day. If a medication could safely block SGLT2 activity and encourage the kidneys to pass that glucose out with the urine rather than reclaim it back into the blood, that might be an elegant solution to persistently high blood glucose levels. In fact, a condition called familial renal glucosuria (FRG) already demonstrated this approach in nature. This condition is caused by changes in the gene coding for SGLT2, resulting in reduction in SGLT2 activity. This reduced activity prevents most glucose in the filtrate from being reclaimed, and people with FRG lose significant amounts of glucose in the urine. Interestingly, for reasons that

are not entirely understood, this condition does not seem to cause hypoglycemia or any serious side effects. Therefore, researchers asked, could SGLT2 inhibitors be safe and effective for use in people with diabetes?

## Treating Diabetes with the Help of the Kidneys

By the time the SGLT proteins were discovered, an SGLT inhibitor called phlorizin had been studied for over 150 years, although only in recent decades have scientists discovered its mechanism of action. Phlorizin came from the root bark of the apple tree. As early as 1933, it was briefly tested in a very small number of people, and scientists found that it could increase glucose in the urine, lower blood glucose levels, and prevent reabsorption of glucose. However, its effects were not limited to the kidney. Because it inhibited glucose absorption in the intestine, was poorly absorbed when taken orally, and interfered with glucose transport in other parts of the body, it was not suitable for use in people. Nonetheless, studies of phlorizin were important to understanding how sodium-glucose transporters worked, and scientists suspected that it might inhibit the SGLTs. Indeed, in 1995, NIDDK-funded researchers found that phlorizin inhibited both SGLT1 and SGLT2. Because SGLT1 is found in many tissues and plays a key role in absorbing glucose in the intestine, this explains some of phlorizin's side effects.

As more became known about phlorizin and SGLTs, scientists became interested in using phlorizin as a starting point to develop a treatment

# STORY OF DISCOVERY

for diabetes. Work over the next few decades focused on developing phlorizin derivatives that were more potent, more specific to SGLT2, and that lasted longer in the bloodstream. This research resulted in the discovery and testing of improved SGLT2 inhibitors.

The SGLT2 inhibitors have been extensively studied in industry-supported clinical trials and were found to be safe and effective at improving glucose control in adults with type 2 diabetes. This research has culminated in FDA approval of several drugs for treatment of type 2 diabetes. The first SGLT2 inhibitor to be FDA-approved was canagliflozin (marketed as Invokana®) in March 2013, followed by the approval of dapagliflozin (marketed as Farxiga®) in January 2014 and empagliflozin (marketed as Jardiance®) in August 2014. These medications provide new tools to help control blood glucose levels in adults with type 2 diabetes.

The approved SGLT2 inhibitors are effective in reducing hemoglobin A1c (HbA1c), a measure of blood glucose levels. SGLT2 inhibitors can be used with other oral or injectable diabetes medications, including insulin. This is important because diabetes is a progressive disease which often requires additional medicines over time as insulin production decreases. Another advantage of SGLT2 inhibitors is that they do not cause hypoglycemia (low blood sugar) in the absence of other drugs with this side effect. Because sodium, like glucose, is reabsorbed by the SGLTs, SGLT inhibition also increases loss of sodium in the urine. SGLT2 inhibitors can cause a modest reduction in systolic blood pressure, but because this effect is so small it may not be clinically

meaningful, and these drugs are not indicated for the control of blood pressure.

One recent industry-supported clinical trial found that people with type 2 diabetes and cardiovascular disease had a lower rate of death from cardiovascular causes when they added the SGLT2 inhibitor empagliflozin to their standard care. Cardiovascular death was reduced by 38 percent, although there was no significant effect on nonfatal heart attacks or strokes. Of note, the study was limited to participants with established cardiovascular disease and a previous cardiovascular event such as a heart attack or stroke. Also, most participants were older (average age 63) and had long-standing diabetes (57 percent had diabetes for more than 10 years). Studies are ongoing to determine the effects of other SGLT2 inhibitors on cardiovascular disease. More research is also needed to determine the effect of empagliflozin on cardiovascular disease in the broader population with diabetes—those who are younger, have shorter durations of diabetes, and do not have pre-existing cardiovascular disease. Research is also needed to understand whether the reduction in cardiovascular death was due to reduced blood pressure, lower fluid volume, or other mechanisms.

SGLT2 inhibitor use does have some restrictions and side effects, however. The glucose-lowering action of these drugs is dependent on adequately functioning kidneys. People should not take SGLT2 inhibitors if they have severely impaired kidney function. Some of the SGLT2 inhibitors are not effective and may have more side effects in

# STORY OF DISCOVERY

people with moderate kidney dysfunction. SGLT2 inhibitors are also not approved to treat patients with type 1 diabetes. Because SGLT2 inhibitors are diuretics, common side effects include increased thirst and urination. People taking SGLT2 inhibitors may develop low blood pressure when going from lying down or sitting to standing. Older people are particularly susceptible to this drop in blood pressure, called orthostatic hypotension, which can cause dizziness and falls. People taking SGLT2 inhibitors also have an increased risk for genital fungal infections (such as yeast infections in women) and urinary tract infections. At least one SGLT2 inhibitor (canagliflozin) increases both the risk of loss of bone density in the hip and lower spine and the risk of bone fractures. The FDA has warned that fractures can occur as early as 12 weeks after starting this drug and with only minor trauma. Because these drugs impact mineral metabolism and may increase the risk of falls, health care providers should be vigilant regarding bone health. There have also been reports that these medications may cause ketoacidosis, a potentially dangerous metabolic condition, and the FDA has warned patients and caregivers to be alert for the signs and symptoms of this condition. Diabetic ketoacidosis is usually seen only with very high blood glucose levels, but ketoacidosis has been reported to occur with only mild or moderately increased glucose in people taking SGLT2 inhibitors. This side effect may be more common in people taking insulin who reduce their insulin dose, and in those with acute illness, infection, alcohol use, or reduced food and fluid intake.

## The Future of SGLT Inhibitors

Building on the successful use of existing SGLT2 inhibitors in people with type 2 diabetes, more research is being done on this class of medications. SGLT2 inhibitors provide a significant reduction in blood glucose levels, but they do not reduce blood glucose to healthy levels in all people who have been given these medications. Thus, some compounds that inhibit both SGLT2 and SGLT1 are under investigation to increase glucosuria even more than can be achieved with SGLT2 inhibitors alone. Additionally, several new SGLT2-specific inhibitors are in pre-clinical development, and some have been approved for type 2 diabetes treatment in other countries. Ongoing studies will provide information about whether SGLT2 inhibitors are safe and effective in people with type 1 diabetes.

After drugs receive FDA approval, new information about risks and benefits often emerges as more people receive the drug. As described above, the SGLT2 inhibitors' risks and benefits will require further study. More research will determine the magnitude of the risks (such as bone fractures) and benefits (such as reductions in cardiovascular-related deaths), in which patients they occur, and whether these effects are specific to certain drugs or are common to all SGLT2 inhibitors. The FDA continues to work closely with manufacturers to monitor emerging information about the safety of the three drugs in the SGLT2 inhibitor class that have now been approved and to alert caregivers and patients to the latest information.

# STORY OF DISCOVERY

## Better Treatments Through Research

Diabetes is a costly, chronic disease that can be difficult to manage effectively. People with diabetes can find it difficult to keep their blood glucose levels within a healthy range, and new medications that help them achieve these goals are needed. SGLT2 inhibitors, a new class of medications for the treatment of diabetes, are the result of decades of dedicated research by many scientists across the globe. The development of the SGLT2 inhibitors built upon years of research into how the kidneys function, and their story is a wonderful example of how research in one area can lead to advances in

another. The NIDDK and the NIH have supported many stages of this research: basic inquiries into how the kidneys function, discovery of the SGLT proteins, pre-clinical development and testing of SGLT inhibitory compounds, and elucidation of how SGLT2 inhibitors work to help those with diabetes meet their health goals. Until recently, people with type 2 diabetes had only a few classes of drugs to choose from when diet and exercise were not sufficient to control their blood glucose. Basic research expanding our knowledge of how the body works has paid off by laying a firm foundation for the discovery of new medicines that are helping people with diabetes build a healthy future.

## Dr. Jean E. Schaffer— An Unexpected Role for Certain Small RNAs in Diabetic Complications: sno in the Forecast

*Dr. Jean Schaffer is the Virginia Minnich Distinguished Professor of Medicine, and Director of the Diabetic Cardiovascular Disease Center and Diabetes Research Center at Washington University School of Medicine, St. Louis, Missouri. She earned her M.D. from Harvard Medical School, where she also served as a resident and intern at the Brigham & Women's Hospital. She was a Clinical and Research Fellow in Cardiology at Beth Israel Hospital, and a Post-doctoral Fellow at the Whitehead Institute for Biomedical Research at the Massachusetts Institute of Technology. Her laboratory seeks to understand the physiological links between dyslipidemia and heart disease in people with diabetes and obesity. Dr. Schaffer described some of her laboratory's research studies at the January 2015 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, of which she is a member.*

Many of the heaviest burdens of diabetes—death, disability, and financial cost—stem from its complications, rather than from the disease itself. In particular, the cardiovascular complications of diabetes, which include heart attack, stroke, and heart failure (a dangerous reduction in the heart's

ability to pump blood through the circulatory system), are the leading causes of death among people with diabetes. What are the biological links between diabetes and cardiovascular disease? And, other than treating or preventing diabetes in the first place, how might we intervene to prevent the cardiac complications of diabetes? Through the use of genetic screens in cultured cells and the study of mouse models, Dr. Schaffer and her colleagues have discovered one such potential intervention point: a group of molecules, designated small nucleolar RNAs, or snoRNAs, that appear to play an important role in tissue damage from metabolic stress.

### **Fatty Acids as Drivers of Cardiac Complications of Diabetes**

"Dyslipidemia"—abnormally high levels of harmful lipids (fats) and/or low levels of beneficial lipids—is a common problem in Americans, especially those who are overweight or obese. The insulin resistance that often leads to type 2 diabetes has also been shown to promote dyslipidemia by driving the liver to increase production of certain lipids and by causing the failure of fat

# SCIENTIFIC PRESENTATION

tissue to appropriately regulate the release of lipids. Abnormalities in lipid metabolism are also seen in type 1 diabetes. Dr. Schaffer described how elevated blood levels of free fatty acids can induce damage to the heart muscle and lead to heart failure in people with diabetes, even in the absence of underlying blockages in the blood vessels of the heart.

A healthy heart muscle has limited capacity to store the energy it needs for its ceaseless, life-long task of pumping blood through the circulatory system. It has therefore evolved a tremendous capacity to scavenge calorie-containing molecules from the blood supply, including lipids. In healthy individuals—those without dyslipidemia—about two-thirds of the heart's caloric needs are fulfilled by its uptake of free fatty acids from the blood. In the setting of dyslipidemia, the diabetic heart takes up fatty acids to an even greater extent, but not all of this lipid can be metabolized by the heart muscle cells. Dr. Schaffer noted that work from others has shown that people with diabetic heart failure tend to have abnormal deposits of fat within the muscle cells of the heart. She speculated that in people with diabetes, increased absorption of free fatty acids due to dyslipidemia might lead not only to these deposits, but also to the functional defects seen in diabetic heart failure.

To test this hypothesis, she and her colleagues generated and analyzed a strain of mice whose hearts absorb these fats even more efficiently than normal. This has the effect of promoting accumulation of fat deposits in the heart muscle cells of these animals. They found that the hearts

of these animals also rapidly develop features of diabetic heart failure. Further, as is sometimes observed in other tissues where excessive amounts of various metabolites occur in disease, they saw evidence that these accumulating lipids were triggering formation of “reactive oxygen species,” which are types of molecules that can cause cell damage or even cell death if they become abundant enough. Thus, these results support a model in which increased deposition of lipid in the heart muscle can have damaging effects.

## A Large Role for Small Nucleolar RNAs

To identify novel ways to treat or prevent heart failure and other diabetic complications, Dr. Schaffer and colleagues used a rodent cell line to look for mutations that would allow the cells to withstand what are normally toxic levels of free fatty acids and glucose (sugar). In this way, they identified a mutation that inactivated a gene called *Rpl13a*. Like other genes in mice and humans, the *Rpl13a* sequence is interrupted by sections of DNA called introns that do not encode portions of the Rpl13a protein. To utilize the gene to make the Rpl13a protein, the cell produces an RNA version of the gene, called the gene's transcript, from which the introns are then removed. However, while the introns of most genes are rapidly destroyed following removal from the initial transcript, four short sections of the *Rpl13a* introns, called small nucleolar RNAs (snRNAs), have a function of their own, and are retained.

Because the mutation disrupted production of both the Rpl13a protein and the snRNAs,

# SCIENTIFIC PRESENTATION

an important question became whether it was elimination of the protein that conferred resistance to toxic lipid levels (lipotoxicity), or was it the elimination of one or more of the snoRNAs? To answer this question, Dr. Schaffer's group first used genetic complementation to identify the critical regions of *Rpl13a* that could restore sensitivity to metabolic stress in the mutant cells. The genomic region was sufficient to complement the mutant, but only if the snoRNAs were intact. In a second approach they showed that knocking down the snoRNAs, but not the Rpl13a protein, rendered wild type cells resistant to lipotoxic stress. These findings point to a key role for the snoRNAs in the deleterious effects of high lipid levels.

## Nucleolar RNAs That Can Leave the Nucleolus

snoRNAs are generally found within the nucleolus, a large structure within the cell's nucleus that produces ribosomes, molecular machines that perform the critical role of translating RNA from genes into proteins. As expected, Dr. Schaffer and colleagues found that *Rpl13a* snoRNAs are found in the nucleolus under normal conditions. Interestingly, however, when cells are subjected to unhealthy levels of fatty acids, the *Rpl13a* snoRNAs exit both the nucleolus and the nucleus and accumulate in the cytoplasm (*i.e.*, outside the nucleus but still inside the cell).

Dr. Schaffer's group observed this unusual location for the *Rpl13a* snoRNAs not only when cellular fatty acid levels rise, but also following other triggers of

reactive oxygen species. It is unknown what these snoRNAs might be doing outside of the nucleolus, but the data suggest they may be involved in destroying cells damaged by reactive oxygen species, leading to tissue damage. Indeed, using a technique that selectively reduced levels of the *Rpl13a* snoRNAs in the cell, they found they could increase the cell's resistance to these toxic molecules. Similarly, they found that reducing the levels of snoRNAs in the liver of a mouse helped protect the animal from oxidative stress. This suggests that the snoRNAs may play an important role in response to many environmental stimuli that damage tissues through reactive oxygen species.

To better understand the physiological function of the *Rpl13a* snoRNAs, they recently created mice that lacked these non-coding RNAs entirely. (That is, with a version of the *Rpl13a* gene that encodes the Rpl13a protein, but not the snoRNAs.) Dr. Schaffer's group is testing how these animals respond to different types of metabolic stress. These "sno-less" mice will provide a powerful new tool for understanding the damaging effects of ectopic lipid accumulation in tissues like the heart. They may also provide new insights into mechanisms that underlie other diabetic complications that result from excess metabolites.

Further testing will be needed to determine whether the corresponding human snoRNAs promote diabetic heart disease. If they do, it may one day be possible to improve treatment of diabetes by blunting their impact through the development of therapies that lower snoRNA levels.

# Bariatric Surgery Offers Hope as a Treatment for Some People with Type 2 Diabetes

Several years ago, when Karen Voll learned that she had been accepted as a participant in the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-funded Triabetes research study and would receive bariatric surgery, she was ecstatic. “That was the happiest day,” she laughs, “I can still remember that day!” The Triabetes clinical trial aims to understand the health benefits and risks of bariatric surgery in people who have mild or moderate obesity along with type 2 diabetes that has been particularly difficult to control by other means. Soon after an evaluation showed that it could be an appropriate treatment option for Karen, she underwent Roux-en-Y gastric bypass surgery, and the health improvements that she experienced were immediate and dramatic. Her previously uncontrolled type 2 diabetes was completely reversed, even without any medications.

### A Difficult Diagnosis

The relief and hope that Karen felt when she first joined the Triabetes study were so strong because she was no stranger to type 2 diabetes and its health consequences. Several members of her family are either living with the disease, or have succumbed to its complications. That’s why, in

2005, when she was diagnosed with the disease in her late forties, she had a clear idea of what this difficult news meant. Following her diagnosis, she began taking the type 2 diabetes medication metformin and tried unsuccessfully to lose weight, as she was mildly obese. Despite her best efforts to control her blood (glucose) sugar levels, her health deteriorated. “I just felt sick all the time,” she remembers, “some days I didn’t even feel like crawling out of bed.” This was a challenging time, considering Karen worked a full-time job in addition to helping with her husband’s electrical contractor business. As she describes it, she “constantly just didn’t feel right at all. Just felt off.”

Over the years following her diagnosis, Karen’s health continued to decline. During that time, she remembered witnessing the progressive deterioration in health of her mother-in-law and her own father, both of whom died as a result of type 2 diabetes. It “just scared me to death,” Karen recalls. These memories filled her with a determination to find a way to manage her diabetes.

Karen knew about bariatric surgery as a weight-loss treatment; her husband, who had more severe obesity and also suffered from type 2 diabetes, had bariatric surgery 10 years earlier. However,

# PATIENT PROFILE

her BMI (body mass index, a measure of weight relative to height) was in a range that is considered mildly obese. By contrast, guidelines generally recommend bariatric surgery only for patients with higher levels of obesity, and particularly for patients with severe obesity. Through her own research on the Internet, she learned of a study, called “The Triabetes Study: A Trial to Compare Surgical and Medical Treatments for Type 2 Diabetes,”

that was being conducted in nearby Pittsburgh, Pennsylvania, by a group led by Dr. Anita Courcoulas, a bariatric surgeon. The Triabetes study was enrolling participants

with BMIs in the range of mild obesity whose type 2 diabetes was particularly difficult to control. Karen felt she might be a good candidate, so she contacted Dr. Courcoulas' office to see if she was eligible to participate in the clinical trial. After consultations with staff associated with the research study (including a dietician, psychologist, and physician), she learned the great news that she was a good fit.

## Roux-en-Y Gastric Bypass Surgery and the Triabetes Study

Previous research had shown that, in people with severe obesity (a BMI of 40 or higher), bariatric

surgical procedures can have dramatic benefits, such as significant and sustained weight loss, improved control of blood sugar levels, and even reversal (remission) of type 2 diabetes. However, there has been little scientific evidence to define the risks and benefits of bariatric surgery for people with lower levels of obesity, and particularly for people with mild obesity, who suffer from uncontrolled type 2 diabetes. (For a

woman of average height, about 5 feet 4 inches tall, a BMI of 30, or mild obesity, would correspond to a body weight of 175 pounds; and a BMI of 40, severe obesity, would correspond

to a weight of 233 pounds.) Among individuals with severe obesity who experience remission of their type 2 diabetes after bariatric surgery, some find that their diabetes subsequently recurs. However, the longer-term health effects of bariatric surgery have not been well studied, and for people with milder levels of obesity, there was limited data even on shorter-term outcomes.

To begin addressing the important question of the effects of bariatric surgery in people with type 2 diabetes and lower levels of obesity, like Karen, the Triabetes clinical trial compared two different bariatric surgery procedures—Roux-en-Y gastric bypass (RYGB) and laparoscopic

***When Karen learned that she had been accepted as a participant in the NIDDK-funded Triabetes research study and would receive bariatric surgery, she was ecstatic. “That was the happiest day,” she laughs, “I can still remember that day!” The Triabetes clinical trial aims to understand the health benefits and risks of bariatric surgery in people who have mild or moderate obesity along with type 2 diabetes that has been particularly difficult to control by other means.***

# PATIENT PROFILE

adjustable gastric band (LAGB)—with an intensive lifestyle weight loss intervention. The goal was to determine each intervention's relative effectiveness at reducing weight and improving blood sugar levels. Karen and the other volunteers were randomly assigned to receive one of these three treatments, and the researchers then evaluated their health outcomes.

In 2011, Karen was randomly assigned to receive RYGB surgery. The most commonly performed procedure at this time, RYGB reduces the size of the stomach and connects the upper part of the stomach to the lower part of the small intestine, so that food bypasses a large portion of the gastrointestinal tract in which digestion and nutrient absorption normally take place.

## Maintaining Health Benefits Through Lifestyle Changes

Karen began feeling health benefits soon after the RYGB procedure. Remarkably, her diabetes was completely reversed within weeks after surgery. She no longer required insulin or metformin to manage her blood sugar levels. "Three weeks after I had the surgery," she remembers, "I was no longer on the insulin. My numbers were perfect. I no longer had high cholesterol. I no longer had high blood pressure. I was off all that medication.

Within 3 weeks!" Within 6 months, her weight fell dramatically, and continued to drop for the following 6 months. Her weight then increased slightly and stabilized at a level well within the healthy range for her height.

The first weeks and months following surgery were challenging nonetheless. "The first few weeks, you're on pure liquids," she recalls about her initial diet, "then you start slowly introducing solids."

Dieticians associated with the Triabetes trial

provided clear recommendations for her diet moving forward (e.g., lean proteins, vegetables, fiber), which she

has been following quite closely over the years. Unexpectedly, Karen's taste preferences seemed to change after the surgery. She no longer craves certain foods that were a regular part of her diet. "I'm Italian, so pasta was a big thing for us, that we grew up on," Karen reminisces. But now, she doesn't have the desire for pasta that she once had. "My taste buds have completely changed. I don't even think about it now." A few staple dishes now constitute the bulk of her daily dietary routine, such as salads, homemade chicken soup (without noodles), and tuna fish, and she eats only small quantities at a time.

In addition to diet, Triabetes staff recommended at least 200 minutes of exercise per week, working up to 300 minutes. Due to a back problem,

***"Three weeks after I had the surgery," Karen remembers, "I was no longer on the insulin. My numbers were perfect. I no longer had high cholesterol. I no longer had high blood pressure. I was off all that medication. Within 3 weeks!"***

# PATIENT PROFILE

Karen has not been able to engage in strenuous physical activity, but she walks “at a pretty good pace” for 30 to 45 minutes, 5 days a week. In addition to walking, she is very active in various other aspects of her life, including mowing her lawn and gardening. “I love doing my gardening...babying my tomatoes right now!”

The health benefits and weight loss associated with bariatric surgery have given Karen a level of energy that she simply did not have before. “I know I’m a lot happier now, I’m a lot healthier. I feel better. I have more energy to do things. Before, I could hardly walk up and down steps; now I can run up...to my second floor. I can run up those steps with no problem at all.”

Like Karen, many other participants in the Triabetes study also experienced health benefits. Evaluating outcomes 12 months post-surgery, the researchers found that RYGB was more effective than LAGB for weight loss and improved control of type 2 diabetes. Both of these surgical treatments were significantly more effective than lifestyle interventions alone for this group of individuals. Importantly, however, only a few of the participants who received RYGB surgery experienced complete remission of type 2 diabetes, as Karen did. Those with complete remission of type 2 diabetes had normal

blood sugar levels without need for diabetes medications. Half of the participants who received RYGB experienced partial remission: their blood sugar levels, although above normal, were no longer in the range of diabetes, and they were able to discontinue their diabetes medications. None of the participants in the original lifestyle treatment group experienced complete or partial diabetes remission.

Karen and others continued participating in the study, so that the researchers could gather

data on their health outcomes several years after surgery. All of the participants, including those who originally

received surgery, were given lifestyle instruction on weight-control behaviors, with the hope that it would help them maintain their weight loss. Among those who had received RYGB, the overall rate of diabetes remission (partial or complete) was 60 percent 1 year after surgery, but by 3 years after surgery, fewer people (40 percent of the participants) had the benefit of diabetes remission. In the LAGB group, the rate of complete or partial diabetes remission remained stable at 29 percent at the two time periods. A few individuals in the surgical groups experienced complications (such as needing another surgery, ulcers, kidney stones, or hospitalization for dehydration), but Karen did not.

***“I know I’m a lot happier now, I’m a lot healthier. I feel better. I have more energy to do things. Before, I could hardly walk up and down steps; now I can run up...to my second floor. I can run up those steps with no problem at all.”***

# PATIENT PROFILE

The Triabetes study was relatively small, with only about 20 participants in each group. Nonetheless, the study yielded important knowledge about the health outcomes from bariatric surgery, and useful information to help researchers plan future studies on longer-term risks and benefits. For example, based on the study's findings of

differences among the participants in diabetes remission and surgical complications, future research could begin to

provide important insights about which people are likely to benefit from bariatric surgery, like Karen did, and which potential patients might not achieve remission of their diabetes, or might experience complications. This research could help inform treatment decisions.

Longer-term studies are also necessary because type 2 diabetes can recur even after surgery. Type 2 diabetes is a disease that progresses over decades, and it remains unclear how long the effects of bariatric surgery will last for different individuals. Additionally, people who have undergone gastric bypass surgery need lifetime health monitoring to help avoid nutritional deficiencies. Because only a few of the participants, like Karen, experienced complete diabetes remission, future research could also yield insights that might further increase benefits for people who choose surgery.

***Longer-term studies are also necessary because type 2 diabetes can recur even after surgery. Type 2 diabetes is a disease that progresses over decades, and it remains unclear how long the effects of bariatric surgery will last for different individuals.***

## Life After Bariatric Surgery—Continuing Health Benefits Four Years Later

Now, 4 years after her bariatric surgery, Karen remains at a healthy weight and free from diabetes and high blood pressure. She is grateful for the support of her family, which has been

helpful in her adherence to a healthful lifestyle. Because her husband previously had RYGB surgery, she was well aware of the associated

challenges and lifestyle changes. Unlike Karen, he did experience some complications, but overall he has been able to maintain his healthier weight. "My husband...was my number one cheerleader," she says.

Karen continues to have monthly follow-up phone calls with Triabetes study staff, and annual visits to Dr. Courcoulas' office. In addition to providing valuable longer-term data for the study, these communications and interactions ensure that she can receive regular guidance should any problems arise. But thankfully, the lifestyle changes that she adopted following her surgery have successfully led to sustained health.

Despite the fact that Karen was only mildly obese prior to her surgery, her uncontrolled type 2 diabetes forced her to consider all

# PATIENT PROFILE

treatment options. Based on the Triabetes and other studies, bariatric surgery appears promising as a treatment for some people with type 2 diabetes and milder levels of obesity, along with those who have severe obesity. Dr. Courcoulas and other scientists caution that it is important to build on these results and evaluate

longer-term outcomes in more people. Bariatric surgery is more invasive than other intervention strategies and thus comes with some inherent risk—but in Karen's case, it has been very effective while other strategies were unsuccessful. She says, "I've been asked, 'Would I do it again?' And I've said, 'in a heartbeat I would.'"

## Improved Diagnosis Leads to an Easier Treatment for Monogenic Diabetes



Lilly (center) with father, Mike (left), and mother, Laurie (right)

Sixteen-year-old Lilly remembers the pressure of being a young child on insulin therapy for what was thought to be type 1 diabetes—the monitoring of her blood sugar (glucose), the pain of needle sticks, the lack of independence and freedom. “It was very stressful,” Lilly says, “and it was really hard.”

But now, instead of shots and constant monitoring, Lilly only needs to take several pills twice a day.

This new treatment is possible because Lilly actually has a rare—and often misdiagnosed—

form of diabetes known as monogenic diabetes, and some types of monogenic diabetes can be treated with a class of drugs called sulfonylureas. This medication helps her pancreas release the

insulin it makes, allowing her to live without the need for insulin injections.

Lilly’s mother, Laurie, describes Lilly’s transition from insulin to sulfonylurea as a miracle. “We prayed for a long time after Lilly was diagnosed that there would be a cure,...but we in our wildest dreams didn’t think that it would happen so quickly, and certainly not that it would come in the form of a pill!”

### Growing Up with Diabetes

Lilly was diagnosed with type 1 diabetes when she was 1 month old. Type 1 diabetes is an autoimmune disease in which the body launches a mistaken attack that destroys the insulin-producing beta cells in the pancreas. As a result, people with this disease must carefully monitor their blood sugar levels and must receive insulin either by injection or through an insulin pump. Lilly’s pancreas was not releasing insulin, though her case was atypical because tests indicated that her immune system wasn’t

attacking her beta cells. Additionally, though type 1 diabetes is most often

diagnosed in young people, it is not often found in month-old babies. However, as Laurie says, the doctors diagnosed Lilly with type 1 diabetes because “there was nothing else to call it.”

**“You can never let your guard down when you have a child with diabetes, let alone a baby with diabetes,” Laurie says.**

# PATIENT PROFILE

Lilly's parents provided the rigorous monitoring and treatment Lilly needed. Laurie would prick baby Lilly's heels—sometimes 10 to 15 times a day—to check her blood sugar levels and then give her food if her blood sugar levels were too low or give her insulin if her blood sugar levels were too high. “It made me very sad, as a mother, to have to withhold food from my baby when she was hungry because her blood sugar was [too]

high,” Laurie says.

Likewise, if Lilly's blood sugar was too low, Laurie would have to feed her, even

if Lilly wasn't hungry. Laurie kept tubes of cake frosting around for such times, because it was a ready source of sugar she could rub on Lilly's gums to help bring her out of her “lows.”

Maintaining Lilly's health those first few years meant constant vigilance. Laurie says that diabetes was like having an extra child in the family, in addition to Lilly and her brother, Nathan, and sister, Charlotte. “You can never let your guard down when you have a child with diabetes, let alone a baby with diabetes,” Laurie says.

When Lilly was 4 years old, she was put on an insulin pump after she had two seizures in 3 months due to low blood sugar. The pump made insulin administration easier by providing insulin through a tube that stays inserted under the skin. The pump helped stabilize Lilly's blood sugar

levels and also freed her from having to endure individual insulin shots throughout the day.

However, changing the pump's infusion set still required being stuck with a long needle, which Lilly hated. “When my mom was trying to stick the needle in me, I would run away from her,” Lilly remembers. “And it was very frustrating, obviously, for my mom, because that was

something I needed.” The pump made life easier, but Lilly was still too young to monitor her blood sugar on her own.

***Living with diabetes as a young child can be confusing. When Lilly was put on the insulin pump, she says, “A lot of my friends would ask, ‘Oh, why do you have to wear that?’ And I really didn't know.”***

She needed an adult to calculate how much insulin she required and operate the pump.

Living with diabetes as a young child can be confusing, Lilly says. She didn't always understand why she couldn't do the same things that her siblings did, such as go on sleepovers, or why her mother had to come along and monitor how much she ate at birthday parties. And sometimes other children didn't understand, either. When Lilly was put on the insulin pump, she says, “A lot of my friends would ask, ‘Oh, why do you have to wear that?’ And I really didn't know.”

## **A New Diagnosis and Hope for Easier Treatment**

After Lilly's diagnosis, her parents became involved with the type 1 diabetes advocacy organization, JDRF. In June 2006, her father, Mike,

# PATIENT PROFILE

attended a meeting sponsored by JDRF where a speaker mentioned new research on a rare form of diabetes called “monogenic” diabetes. Mike was immediately intrigued, as the description of monogenic diabetes fit Lilly perfectly.

Monogenic forms of diabetes result from changes in a single gene (as opposed to other forms of diabetes, which result from the activity of multiple genes). Monogenic diabetes accounts for about 1 to 5 percent of all cases of diabetes in young people. A subset of monogenic diabetes is called neonatal diabetes, which is diagnosed before 1 year of age and which is often misdiagnosed as type 1 diabetes. In most cases of monogenic diabetes, the person has an altered form of a gene involved in insulin production, and that change reduces the amount of insulin the person makes or secretes into the bloodstream. Most excitingly, as Mike heard at the meeting, a common and inexpensive class of oral medication used to treat type 2 diabetes, called sulfonylureas, had shown promise as a treatment in specific types of monogenic diabetes. These medications help the body’s beta cells release insulin. A “transition” therapy had been tested that involved slowly replacing insulin treatment with sulfonylurea pills.

Laurie was skeptical, but hopeful. After years of constant testing and insulin administration, the idea that Lilly could instead just take some pills seemed preposterous. Nonetheless, Mike and Laurie decided to have Lilly’s DNA tested. The test confirmed that Lilly had monogenic diabetes and that she was a candidate for the transition to sulfonylurea therapy. But this therapy had

not been widely used in the United States, and there were no guarantees that sulfonylurea would be able to replace Lilly’s insulin treatment. Finally, the transition attempt might also cause Lilly’s blood sugar levels to fluctuate wildly, a frightening proposition for a family that had spent so much time working to keep Lilly’s blood sugar in a healthy range.

Lilly, then 6 years old, also had reservations. When her parents explained what would be involved—a hospital stay, and then taking pills instead of needing her pump—she started crying. “I did *not* want to go to a hospital,” Lilly remembers. “I did *not* like them at that time, and I was not happy going.” Additionally, she had lived her whole life needing insulin and had grown very emotionally attached to her pump. “It was something I’d always had when I was younger...,” Lilly explains. “It was very important to me.”

Despite these reservations, the fact that Lilly’s health and quality of life could be greatly improved ultimately convinced Lilly and her family to take the risk and go ahead with the treatment. Lilly would be starting first grade that fall, and they hoped that she could start school no longer needing insulin.

## Trading One Therapy for Another

To begin the therapy transition, Lilly’s clinical team cut her insulin dose in half and gave her a small dose of the oral sulfonylurea medication. It was a balancing act to find the right dosage of the new medication while reducing her insulin use.

# PATIENT PROFILE

At first, the results were frightening: Lilly's blood sugars went, in Laurie's words, "sky high." Lilly was also scared, at first. Because she was a pioneering patient in this sort of procedure, there was a lot of attention focused on her, a lot of hospital staff coming and going, and a lot of tension all around. Lilly says that her friends were an incredible source of support while she was in the hospital. Their visits were a comfort and a welcome time of normalcy where Lilly could play with her friends.

"They helped me get through hard times," Lilly says.

***When a blood test showed that the treatment was working, Laurie says, "We all started crying and hugging, and it was just this incredible moment."***

On day 4 of the transition, Lilly reached a dramatic turning point. When she entered the hospital, a blood test had confirmed that her beta cells were not releasing insulin. By day 4, the medication had allowed her insulin dosages to be significantly reduced. Then, another test showed that her beta cells were now able to release insulin into her bloodstream. The new medication was working. When the doctor informed them of the good news, Laurie says, "We all started crying and hugging, and it was just this incredible moment. Even the doctor was blown away."

By the time Lilly left the hospital on day 5, she was still taking small doses of insulin, but her doctors were confident that the family could continue her treatment transition at home. On day 9—August 23, 2006—Lilly took off her insulin pump for the last time. Then, after taking her medication (but no insulin) for the night, Lilly celebrated with a big

bowl of ice cream while her parents looked on nervously. An hour later, her blood sugar levels were completely normal.

## A Life Without Insulin Therapy

When asked how it felt to no longer need insulin therapy, Lilly says, "It felt really good, because I got to be more independent at that time."

Finally, she could do many of the things that

other children her age could do, unconstrained by her diabetes. As of publication, the

sulfonylurea therapy has continued to keep Lilly's blood sugar levels in a normal range without the need for insulin for almost 10 years.

Unfortunately, not all people with diabetes can be helped by sulfonylureas. Lilly remembers hoping that her cousins and friends who have type 1 diabetes would be able to switch from insulin to pills. She was sad when her parents explained that the therapy wouldn't work for kids with type 1 diabetes, as they have a different form of diabetes than she has. Even for some people with monogenic diabetes, sulfonylurea treatment may work partially or not at all, depending on the genetic change that causes their disease and on their particular circumstances.

Lilly and her family have continued to share their story to raise public awareness about monogenic diabetes and the sulfonylurea therapy that can help some people with the disease. Most children

# PATIENT PROFILE

are not tested for monogenic diabetes, and many health providers are not aware that infants with diabetes may have monogenic diabetes rather than type 1 diabetes.

Lilly's family's advocacy led to the first neonatal diabetes registry in the United States. In 2009, Illinois passed "Lilly's Law," establishing a registry of Illinois children diagnosed with neonatal diabetes before their first birthday. This registry helps doctors connect children and their families with appropriate treatments and could also help scientists identify new genes that cause neonatal diabetes. Laurie and Mike have also produced a television documentary featuring their and other families' stories ("Journey to a Miracle: Freedom from Insulin"), which was released in early 2015.

Support for scientific research is critically important, Laurie says. "Research takes time. It takes decades. Research builds on research that builds on research." Thinking about the research that led to Lilly's diagnosis of monogenic diabetes from a DNA test, and her new treatment, Laurie explains, "This breakthrough seems sudden, but...it was decades in the making." Laurie is excited about the benefits that future research might bring.

## Looking to the Future, Reflecting on the Past

Now 16 years old, Lilly's future is bright. A high school sophomore, she enjoys history and vocal ensemble. She attends theater and acting programs and has enjoyed performing for years. She particularly loves singing and dancing, and she wants to pursue a career in musical theater.

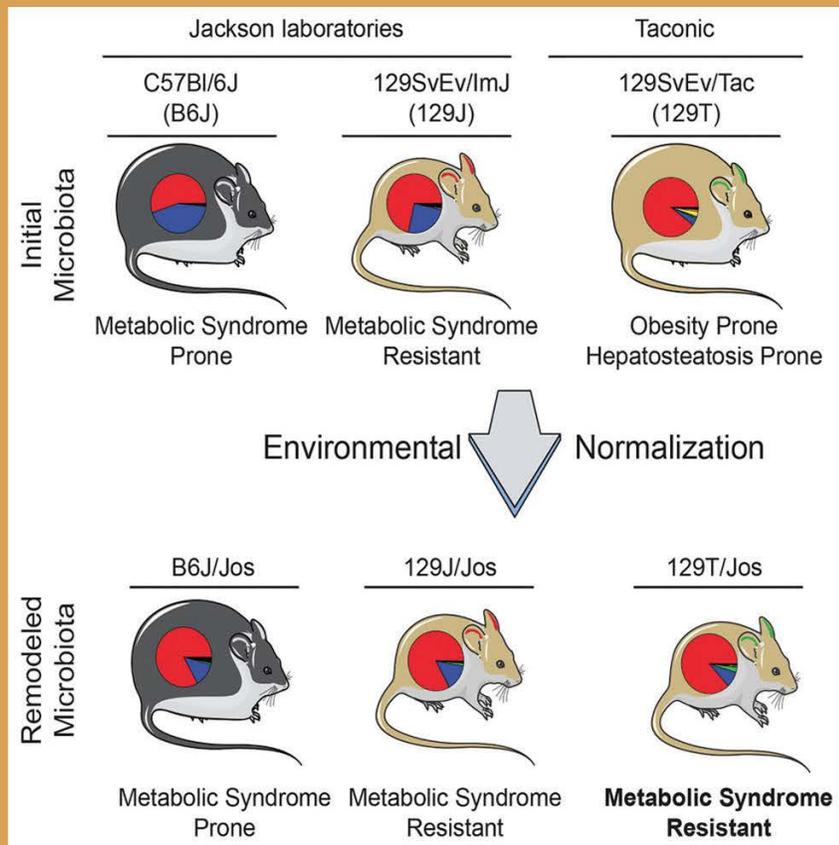
***"Research takes time. It takes decades. Research builds on research that builds on research. This breakthrough [in Lilly's treatment] seems sudden, but...it was decades in the making."***

And what of Lilly's old insulin pump, that symbol of her first 6 years on insulin? They still have it...in a closet. They sometimes bring out the pump at interviews, using it as a visual reminder of how their lives have changed. In the past, there was the pump and all the supplies that went with it...and now, there is only Lilly's pills.

"I feel like I am very lucky," Lilly says. When she thinks about being on insulin, and of all the other children and families dealing with diabetes, she is very thankful. "I just feel really grateful to have this amazing thing happen to me."

*For more information on monogenic diabetes, please see [www.niddk.nih.gov/health-information/health-topics/Diabetes/monogenic-forms-diabetes-neonatal-diabetes-mellitus-maturity-onset-diabetes-young/Pages/index.aspx](http://www.niddk.nih.gov/health-information/health-topics/Diabetes/monogenic-forms-diabetes-neonatal-diabetes-mellitus-maturity-onset-diabetes-young/Pages/index.aspx)*





Genetics, diet, and various types of bacteria that reside in the gut all modify susceptibility to obesity and metabolic conditions. In a recent study, researchers discovered that these factors are all interrelated, as illustrated in the figure and described further in this chapter. The top row depicts two genetically related strains of mice bred in different locations (129J and 129T) and an unrelated strain (B6J). Their gut bacteria (part of the “microbiota” or “microbiome”) are represented by multicolored pie charts. The bottom row shows these strains of mice after several generations of breeding in the same environment (“environmental normalization”) and the resulting similarities among their gut bacteria. From the B6J mice, the researchers found that a strong genetic susceptibility to obesity and metabolic syndrome can overshadow environmental factors. By contrast, environment prevailed over genetics for the 129 strains: bred apart, they differed in metabolic traits (top row), such as propensity for obesity and fatty liver (hepatosteatosi s). These differences disappeared after the 129 strains were bred in the same place and acquired similar gut bacteria (bottom row). Insights from this study may lead to new strategies to improve health by modulating gut bacteria.

*Image courtesy of Dr. C. Ronald Kahn, Harvard Medical School and Joslin Diabetes Center. Reprinted from Cell Metab, Vol 22, Ussar S, Griffin NW, Bezy O, Fujisaka S, Vienberg S, Softic S, Deng L, Bry L, Gordon JI, Kahn CR, Interactions between gut microbiota, host genetics and diet modulate the predisposition to obesity and metabolic syndrome, p. 516-530, copyright 2015, with permission from Elsevier.*

# Obesity

***Obesity has risen to epidemic levels in the United States. Individuals who are obese 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. More than one-third of U.S. adults are considered obese based on body mass index (BMI), a measure of weight relative to height.<sup>1</sup> Approximately 17 percent of children and teens ages 2 through 19 are also obese, and thus 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, schools, health care, and other settings using a variety of approaches and technologies; medical and surgical interventions; and combinations of these 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.

---

<sup>1</sup> Ogden CL, et al. NCHS Data Brief, No. 219. National Center for Health Statistics, Centers for Disease Control and Prevention. 2015.

<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, <http://win.niddk.nih.gov/index.htm>

These represent examples of the NIDDK's broad spectrum of research efforts toward reducing the burden of obesity so that people can look forward to healthier lives.

## BIOLOGICAL FACTORS IN HUMAN BODY WEIGHT

**Belly Fat, or Hip Fat? And How Much? Mining the Genome for Answers:** In recent international studies, researchers examining data from hundreds of thousands of people have discovered areas of the genome that are associated with obesity and other areas of the genome that help determine whether extra calories are more likely to be stored around the waist or hips. Researchers have long known that genetic and environmental factors, along with behaviors influenced by both, contribute to obesity. Genetic factors also direct where body fat accumulates. However, it has been difficult to identify genetic variants (genome sequence differences among individuals) associated with these conditions.

In one study, researchers scanned the genome for genetic variants associated with body mass index (BMI), a measure of weight relative to height, in more than 320,000 people, most of European ancestry. They identified variants in 97 different genomic regions, 56 of which were not previously known to be associated with BMI. Variants in two of the genomic regions differed between men and women, with stronger effects in women. Some were associated with other, obesity-related health conditions, but the effects were not always as expected. For example, although obesity is a strong risk factor for type 2 diabetes, one of the variants was associated with higher obesity risk and, surprisingly, lower diabetes risk; these findings may help explain why not everyone who is obese develops metabolic disease. The genomic region around any variant

could contain multiple genes; as a first step toward identifying those likely to cause the effects on BMI, the researchers examined the genes near each variant. They found that many of the genes were known from prior research to function in brain pathways relevant to obesity, and some are involved in processes such as transmitting signals between brain cells. Other genes are associated with insulin action, fat cell development, regulating whether genes are turned on or off, and other, disparate functions.

In the other study, researchers explored genetic contributors to body fat distribution, or why some people tend to accumulate belly fat, or fat in the abdominal area, while others store more fat in their hips—sometimes referred to as apple-shaped compared to pear-shaped, respectively. Abdominal fat, often estimated by waist circumference, tends to be more detrimental to health. For people of the same weight and height, an individual with a larger waist circumference relative to hip circumference, or waist-to-hip ratio, is at heightened risk for metabolic conditions, including diabetes. For this study, researchers analyzed data from over 220,000 individuals, most of European ancestry, and found genetic variants associated with waist-to-hip ratio in 49 regions across the genome. Of these, 33 were newly discovered to be related to body fat distribution. Variants in 19 of the genomic regions had larger effects on body fat distribution in women, while variants in one region showed a larger effect in men. The researchers then cataloged the genes and other DNA sequences in the genomic regions around each of the variants: some are known to play a role in insulin resistance (a condition related to diabetes); others are involved in blood vessel formation, fat tissue functions, and regulation of gene activity.

These new studies build on prior findings, more than doubling the number of regions of the genome now known to be associated with obesity and whether

body fat accumulates more at the waist or hips. Future research could determine which specific genes are responsible for the effects, and may also identify additional genetic variants that affect obesity and body fat distribution. Further study of individuals of diverse ancestry may also yield more insights. These lines of research will continue to enhance understanding and yield targets for potential new therapies.

*Locke AE, Kahali B, Berndt SI,...Speliotes EK. Genetic studies of body mass index yield new insights for obesity biology. Nature 518: 197-206, 2015.*

*Shungin D, Winkler TW, Croteau-Chonka DC,...Mohlke KL. New genetic loci link adipose and insulin biology to body fat distribution. Nature 518: 187-196, 2015.*

### **Ease of Weight Loss Influenced by Individual**

**Biology:** Researchers have found evidence supporting the commonly held belief that people's biology influences how much weight they lose when limiting calories. It is well known that, when people decrease their caloric intake, there is wide variability in the amount and rate of weight loss from person to person. However, the reasons behind this variability are not fully known. To examine the biology underlying these differences, NIDDK intramural researchers studied energy expenditure (calorie burning) and weight loss in 12 men and women with obesity. Using a whole-room indirect calorimeter—which allows energy expenditure to be calculated based on air samples—researchers took baseline measurements of participants' energy expenditure in response to a day of fasting and a day of eating substantially more than usual (overfeeding). Fasting typically causes the body to reduce energy expenditure (burn fewer calories), while excess food can cause the body to increase energy expenditure (although usually not by enough to burn off all the extra calories). The researchers found that participants differed in how much their energy expenditure

changed in response to fasting or overfeeding. They next sought to determine whether these differences in individual biology correlated with differences in weight loss on a low-calorie diet.

Following the baseline period, the participants consumed a 50 percent calorie-reduced diet for 6 weeks. After accounting for age, sex, race, and baseline weight, the scientists found that the people who lost the least amount of weight during the calorie-reduced period were those whose energy expenditure had decreased the most when fasting and increased the least when overfeeding. In other words, their metabolism slowed down more when they fasted and did not increase as much when they overfed. Those participants had what the researchers called a "thrifty" metabolism—i.e., they saved calories. The people who lost the most weight had a "spendthrift" metabolism, in which energy expenditure decreased the least during fasting and increased the most during overfeeding. It is unknown if these biological differences are innate or develop over time. These findings show that when people who are obese try to lose weight by limiting calories, their metabolism influences how much weight they lose. Future research may help determine whether these insights could inform personalized approaches to help people who are obese achieve a healthy weight.

*Reinhardt M, Thearle MS, Ibrahim M,...Votruba SB. A human thrifty phenotype associated with less weight loss during caloric restriction. Diabetes 64: 2859-2867, 2015.*

## **HEALTHIER WEIGHT IN IMPOVERISHED CHILDREN**

### **Head Start Participation Associated with Healthy Changes in Body Weight Among Impoverished**

**Overweight and Obese Children:** A team of researchers found that preschoolers in Head Start

programs who were overweight or obese were more likely to reach healthier weights by kindergarten age than other groups of overweight and obese children. Head Start is a federally funded preschool program for children living in poverty. The researchers sought to determine whether Head Start might be a valuable setting for reducing childhood obesity because these programs serve impoverished children across the country, and because socioeconomically disadvantaged individuals are at greater risk for obesity.

For the study, the research team gathered children's weight and height measures from 12 Head Start programs in Michigan, both rural and urban, who agreed to participate, along with measures from other children for comparison. More than 19,000 children were in the Head Start group, including similar numbers of boys and girls, with race/ethnicities of 65 percent white, 11 percent black, and 14 percent Hispanic. The comparison groups, who were from a health care system in the same state, included 5,400 children who were on Medicaid, and over 19,000 children not on Medicaid. To examine the children's weight changes over time, taking into account the fact that children also grow in height, the researchers calculated body mass index (BMI), a measure of weight relative to height. They then examined how far the children's BMIs varied from what is considered healthy for their ages, based on standard growth charts for boys and girls. Among children who were obese or overweight, those in Head Start attained a healthier BMI during the first year of the study than children in the comparison groups, and were still at a healthier weight by the end of the second year. Children in Head Start who were underweight also reached healthier weights than those in comparison groups, although the data were more limited. It is not clear which aspects of Head Start may have contributed to these weight

changes, but the researchers suggested several possibilities. For example, Head Start programs are required to meet certain nutritional guidelines, provide space for active play, prohibit television watching, and facilitate access to health care. The results of this study provide evidence that Head Start programs may have beneficial effects on children's weight early in life.

*Lumeng JC, Kaciroti N, Sturza J,...Reischl TM. Changes in body mass index associated with Head Start participation. Pediatrics 135: e449-456, 2015.*

## **MULTIPLE BRAIN CELL PATHWAYS THAT REGULATE APPETITE**

Although it may seem intuitive that one would feel hungry after not eating for a while, and full after eating, the body's regulation of the desire to eat is actually quite complex, involving multiple biological pathways in the brain. Two recent studies in mice, described below, give new insights into different pathways that increase appetite; these focused on different cells—AgRP and POMC neurons—that reside in the same region of the brain. In the study of AgRP neurons, scientists discovered other groups of cells along what is likely a major pathway toward increasing appetite when the body needs more energy from food. Unlike AgRP neurons, POMC neurons are known to suppress appetite. However, as revealed by the other study, in the presence of chemicals called cannabinoids, POMC neurons take on the opposite role, and, surprisingly, promote excessive eating.

**Brain Cells That Forge a Path from Hunger to a Quest for Food:** Researchers have mapped out a series of cells in the brain that relay signals to drive appetite in mice, illuminating potential targets for new obesity drug development. The research

team included scientists from universities and the NIDDK's Intramural Research Program.

To gain new insights into the circuits of the brain that control hunger and satiety (feeling full), the researchers began by engineering mouse brain cells called AgRP neurons to fire in response to blue light, and then, literally, shined light on these cells to activate them. Known to promote hunger, AgRP neurons work by blocking cells that would otherwise promote satiety—but the other cells' identity was unclear. Based on clues from past research, the scientists focused on brain cells containing MC4R, a molecule known to play a role in satiety. They stimulated the modified AgRP neurons with light, and observed cells elsewhere in the brain that responded by firing (emitting characteristic bursts of electrical activity). Some of these cells were MC4R-containing neurons in a part of the brain referred to as the PVH. The researchers then examined the roles of these PVH<sup>MC4R</sup> cells in satiety. Activating PVH<sup>MC4R</sup> neurons (with another technique) caused mice to eat less than normal after a fast, when they should have been hungry. Conversely, when the researchers inhibited PVH<sup>MC4R</sup> neurons, the mice began poking a food-dispensing machine in their cage repeatedly to obtain more food pellets, even after a full meal. With further experiments, the researchers confirmed that AgRP neurons increase appetite by blocking the satiety-promoting activity of PVH<sup>MC4R</sup> neurons. They then discovered the next brain region involved in this pathway. Using a fluorescent protein “tag,” they traced projections from PVH<sup>MC4R</sup> neurons to neurons in another part of the brain, called the LPBN. Additional experiments demonstrated the importance of the connection between these groups of neurons for promoting satiety. By blocking PVH<sup>MC4R</sup> cells and their LPBN cell partners, AgRP neurons are able to send the mice on a quest for food.

The researchers have thus mapped a series of cells along a pathway of appetite regulation that traverses different parts of the brain. Additional research, conducted by these scientists and others, shows that this is one of multiple brain pathways that affect eating behavior. Future research may show whether the results of this study apply to both females and males, as many of the experiments included only male mice. Future research could also determine whether AgRP, PVH<sup>MC4R</sup>, and LPBN neurons work similarly in humans; if so, researchers may one day be able to target this pathway to develop obesity drugs that help people feel full after eating less food.

Garfield AS, Li C, Madara JC, ...Lowell BB. A neural basis for melanocortin-4 receptor-regulated appetite. *Nat Neurosci* 18: 863-871, 2015.

**Hungry When You're Full? Cannabinoids Trigger a Surprising Switch in Brain Cells Known for Their Role in Suppressing Appetite:**

Exploring how cannabinoids generate a strong urge to eat—even after a meal—researchers discovered, in mice, that these substances cause certain brain cells to abandon their characteristic role in appetite suppression and instead drive feeding behavior. Although cannabinoids are known as chemical components of marijuana, versions of cannabinoids are also made by the body, where they promote hunger, particularly for sweet and fatty food. The mechanisms by which they work, however, have not been well understood.

Because cells in the brain have docking sites, or receptors, for cannabinoids, a team of researchers thought that cannabinoids might block the activity of brain cells called POMC neurons, which have long been known for their role in feeling satiated. To test the theory, the researchers gave well-fed male mice doses of synthetic cannabinoids to stimulate one type of cannabinoid receptor, called CB<sub>1</sub>R, and then observed how much the mice ate and the chemical's effects

on the brain. To their surprise, at doses that induced the mice to eat to excess, the synthetic cannabinoids activated—rather than dampened—POMC neurons. For example, these neurons ramped up their characteristic firing, the bursts of electrical activity that nerve cells use for signaling. Delving further into this paradox, the researchers examined levels of two molecules produced by POMC neurons that, intriguingly, have opposite effects. One is a hormone that inhibits eating; the other,  $\beta$ -endorphin, can increase feeding, although POMC neurons were not previously known to produce this hormone. In experiments with male mice, CB<sub>1</sub>R activation amplified  $\beta$ -endorphin release from POMC cells in the brain but did not affect levels of the appetite-suppressing hormone. The researchers also tested the effects of a CB<sub>1</sub>R-inhibiting drug, rimonabant. Once used for obesity treatment in some countries (although not in the United States), rimonabant is no longer marketed due to serious psychological side effects. When administered to mice, rimonabant blocked cannabinoid-induced food intake and diminished  $\beta$ -endorphin release in the brain.

This research in mice reveals an unexpected biologic switch in POMC neurons from suppressing appetite to promoting excessive hunger. With further study of CB<sub>1</sub>R and innate cannabinoids in humans, investigators may be able to develop new and potentially safer therapeutics for obesity that target cannabinoid pathways.

*Koch M, Varela L, Kim JG,...Horvath TL. Hypothalamic POMC neurons promote cannabinoid-induced feeding. [Nature](#) 519: 45-50, 2015.*

## CIRCADIAN RHYTHM AND CALORIE INTAKE

**Time To Eat?:** New research in mice suggests that restricting eating to a shorter period of the day

might be a valuable adjunct to traditional diet and exercise recommendations, and might potentially confer metabolic benefits or allow weight loss even without reducing caloric intake, eating a rigid diet, or taking weight-loss medications. Several studies have established that healthy metabolism is tied closely to circadian rhythms, and that the body handles food and digestion most effectively during daytime hours. These findings may help explain observational findings that people who work night shifts or eat at night are at an increased risk for obesity and metabolic diseases such as type 2 diabetes. The observations also lead to the question: can people improve their health by eating only during specific hours of the day? Clinical research to test the effects of varying people's mealtimes is currently lacking. However, previous research has shown that if mice are given food only during 8 hours of their active period (at night, because they are nocturnal animals), they are protected from obesity and diabetes, even if they are fed a high-fat diet that normally causes these conditions in mice with all-day access to the same food. In a new study, researchers sought to expand upon this result. For example, they sought to determine how severely feeding time had to be restricted to protect mice from the adverse metabolic effects of a high-fat diet. Working with male mice, they found through a number of experiments that mice with constant, continuous access to a high-fat diet gained significantly more weight than mice whose access was limited to 9 hours, to 12 hours, or even to 15 hours (*i.e.*, 9 hours fasting). The shorter the eating period, the better the protection, though protection was substantial even in the 15-hour-fed group. Animals with access to the high-fat diet for 9 hours weighed no more than mice given a normal healthy diet. Interestingly, for any given diet, time-restricted feeding did not change the number of calories the mice consumed: those with 9-hour access to high-fat food ate no less than those with

the same diet available for 24 hours. This suggests that circadian coordination of metabolism is responsible for the effect—not a reduction in calories consumed, but simply timing that consumption to correspond to the animals’ natural active period. That is, a dieting and exercise program was not necessary to limit weight, at least in male mice. Indeed, even on a healthy diet, time-restricted feeding came with a surprise benefit: although the mice weighed the same as mice with 24-hour access to the healthy diet, they had less body fat and more lean muscle mass. Taking the experiment further, the investigators examined whether it was necessary to restrict food intake every day. When male mice fed a high-fat diet were restricted to 9-hour access for 5 days per week and then given 24-hour access on weekends, they still fared considerably better than mice given 24-hour access to the diet every day, doing about as well as mice given food for 12 hours a day, 7 days a week. Importantly, the mice with 5 days of time-restricted feeding not only weighed less than the mice with 24-hour food access, but also experienced significant metabolic benefits: better insulin sensitivity, lower blood glucose (sugar), fewer signs of inflammation, more healthy brown fat, less unhealthy white fat, and healthier blood and liver levels of fat and cholesterol.

In another series of experiments, mice that had grown obese through 24-hour access to the high-fat food were switched to time-restricted feeding. These animals lost weight, and then plateaued, while those still receiving food 24/7 continued to gain. The metabolic benefits seen after switching the obese mice to time-restricted feeding were significant. For example, their insulin levels fell and glycemic control improved, although they remained somewhat less metabolically healthy than mice that had been fed continuously in a time-restricted fashion. In contrast, when time-restricted feeders were switched to 24-hour food access, they gained substantial

amounts of weight, although they retained some of the metabolic benefits they had previously earned in their period of time-restricted feeding. Further, the researchers found that the time-restricted feeding approach not only benefited animals on a high-fat diet. The benefit of time-restricted feeding was also quite substantial in mice given diets that may be more representative of those contributing to obesity in humans: not quite as high in fat, but including sucrose (table sugar), for example, or a diet elevated in fructose (often found in processed foods). It is unclear to what extent this approach would be beneficial to people, whether there might be differences between women and men in metabolic benefits derived, and whether people will be more or less able to adapt to changing their mealtimes as opposed to changing their diets. However, if the effects are similar to those in mice, the discovery may be of profound importance: people who have difficulty restricting calories or curtailing fattening foods may find it more manageable to simply restrict the time when they can consume them.

*Chaix A, Zarrinpar A, Miu P, and Panda S. Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges. Cell Metab 20: 991–1005, 2014.*

## **GUT MICROBIOME, GENETICS, AND OBESITY**

### **Nature, Nurture, and Nutrition—Gut Bacteria Native to Different Environments, Genetics, and**

**Diet Influence Metabolism:** In a study in mice, researchers discovered that genetics, diet, and gut microbes acquired in different environments all interact to modify susceptibility to obesity and metabolic conditions. To examine genetic risk, the researchers compared mice known to be genetically related, strains 129J and 129T, and an unrelated strain, referred to as B6J. To investigate dietary

effects, they fed the mice regular mouse chow or a high-fat diet. Exploring potential environmental effects, and heeding the mantra “location, location, location,” they obtained mice from different vendors (the “J” and “T” in the strain names), and bred some of these mice for several generations in the same environment—their own research institution. They also catalogued the many different types of gut bacteria (the gut microbiome) in the mice.

Building on previous studies, the researchers found that all of these factors play a role in obesity and metabolism—and are interrelated. For example, the B6J mice had a strong genetic susceptibility to obesity and metabolic conditions. Regardless of birthplace, they gained more weight than both the 129T and 129J strains on either diet. The B6J mice also had worse metabolic health, including insulin resistance, impaired control of glucose (sugar) levels, inflammation, and liver damage. The genetically related 129T and 129J strains, when bred in separate locales, differed in weight gain and liver damage on a high-fat diet; thus, environment prevailed over genetics for some of their metabolic conditions. After the 129T and 129J strains were bred for several generations in the same place, these differences disappeared. The breeding environment, mouse genetic background, and diet all influenced the composition of gut bacteria, which in turn affected metabolism. From bacterial transplant experiments in the mice, the researchers found that some collections of gut bacteria confer better metabolic health than others to male recipient mice. They then discovered that specific types of gut bacteria were more abundant (and others less) in mice with higher body weight. Body fat, insulin levels, and markers of inflammation also correlated with the relative abundance of various types of gut bacteria.

This study provides new insights into obesity and metabolism. It also has implications for researchers

who are trying to confirm scientific results, in that genetically similar mice may exhibit different traits if bred in environments with different gut bacteria. Finally, this research may lead to strategies to improve health by modulating the composition of gut bacteria.

*Ussar S, Griffin NW, Bezy O, ... Kahn CR. Interactions between gut microbiota, host genetics and diet modulate the predisposition to obesity and metabolic syndrome. Cell Metab 22: 516-530, 2015.*

## BARIATRIC SURGERY RESEARCH

### **Bariatric Surgery May Help Reduce Urinary**

**Incontinence:** A new study has shown that men and women with severe obesity experienced fewer urinary incontinence episodes after bariatric surgery, up to 3 years following the procedure. One of the many negative health consequences associated with obesity is urinary incontinence, which is the accidental loss of urine, caused by the loss of bladder control. People with severe obesity often do not gain sufficient health benefits from lifestyle interventions alone, and thus may turn to bariatric surgery to help them lose weight and reduce their risk for obesity-associated health conditions. Although bariatric surgery can rapidly lead to weight loss, and weight loss from diet and exercise has been associated with reduced incontinence in overweight and obese individuals, it has not been known if bariatric surgical procedures can reduce urinary incontinence episodes in people with severe obesity. To address this question, scientists with the multi-center Longitudinal Assessment of Bariatric Surgery (LABS) Consortium asked study participants to complete questionnaires designed to assess frequency of urinary incontinence. More than 90 percent of study participants underwent either Roux-en-Y gastric bypass or laparoscopic adjustable gastric banding—two different commonly performed bariatric surgery procedures. Nearly

2,000 participants with severe obesity, 79 percent of whom were women, completed questionnaires within 30 days before bariatric surgery, and annually for 3 years after surgery for most participants. Prior to their surgery, 49 percent of women and 22 percent of men had reported prevalent (at least weekly) urinary incontinence. One year after surgery, the rates of prevalent urinary incontinence significantly dropped, to 18 percent of women and 10 percent of men. At 3 years after surgery, although rates rose to 25 percent of women and 12 percent of men, these were still both significantly lower than pre-surgery levels of prevalent incontinence. The researchers suggest that the improved continence could be a direct result of the dramatic weight loss often experienced by bariatric surgery patients. Supporting this idea, analysis of the results revealed that greater weight loss was associated with better continence, and that the amount of regained weight was associated with increased incontinence. Some study participants regained weight between the first and third years after surgery, which could explain the rise in reported urinary incontinence over that period of time. In addition, the scientists examined other independent factors among the participants, finding that older age, severe walking limitation, and recent pregnancy—known risk factors for urinary incontinence—reduced the likelihood for improvements after surgery. While the results are significant, researchers acknowledge that this observational study did not have a control group for direct comparison, and that self-reported assessments are not always completely accurate. The researchers do note, however, that the questionnaires used in this study have been previously validated for assessing urinary incontinence. The finding that urinary incontinence may be a health benefit of bariatric surgery could help inform people with severe obesity and their health care providers, as they weigh the risks and benefits of surgery as a treatment option.

Subak LL, King WC, Belle SH,....Huang AJ. Urinary incontinence before and after bariatric surgery. *JAMA Intern Med* 175: 1378-1387, 2015.

### **Redirecting the Flow of Bile to the Distal Small Intestine Could Provide Metabolic Health Benefits Similar to Bariatric Surgery:**

A new study in mice suggests that diversion of bile acids directly to the distal segment of the small intestine could lead to weight loss and metabolic benefits that are similar to gastric bypass bariatric surgery. For some people with severe obesity who are unable to lose sufficient weight through lifestyle changes alone, bariatric surgery could be an effective therapeutic option. Roux-en-Y gastric bypass (RYGB) surgery, currently the most common bariatric surgery procedure performed in the United States, connects the upper stomach to the middle part of the small intestine (called the jejunum), so that food bypasses a large portion of the gastrointestinal tract in which digestion and nutrient absorption normally take place. RYGB can have dramatic health benefits, including significant weight loss, improved control of blood glucose (sugar) levels, or even reversal of type 2 diabetes. Recent evidence suggests that bile acids help mediate some of the metabolic effects of RYGB. Bile acids, released from the gall bladder into the upper portion of the small intestine (called the duodenum), normally aid in the digestion and absorption of nutrients, and also act as hormones in the gut, influencing metabolism and other physiological processes.

To determine whether bile acids can directly lead to weight loss and metabolic benefit in the absence of RYGB, researchers tested in male mice different surgical procedures connecting the gall bladder to the three portions of the small intestine: the duodenum (GB-D); the jejunum (GB-J); or the ileum (GB-IL), which is farthest from the stomach. The GB-J and GB-IL procedures diverted bile flow,

while the GB-D procedure did not alter bile flow and served as a surgical control. They compared mice that underwent these procedures with mice that had RYGB, as well as with mice that did not have any surgical procedures. All mice were fed a high-fat diet to induce obesity. At 2 weeks following surgery, the GB-IL mice and RYGB mice consumed significantly less food than mice in the other groups. After 8 weeks, GB-IL mice exhibited sustained weight loss equal to or even greater than RYGB; GB-D and GB-J mice lost weight initially, but regained weight over time and eventually reached the weight of the normal control mice. Total circulating bile acid levels were elevated several-fold in the GB-IL mice over each other group. The researchers then examined various indicators of metabolic health in the different groups of mice. The GB-IL and RYGB procedures led to several health improvements over the other groups, including sustained weight loss, reduced overall body fat, improved blood glucose control and insulin sensitivity, lower levels of circulating fats, and protection from fat accumulation in the liver. Circulating cholesterol levels were only lower in GB-IL mice. By contrast, triglycerides in the blood were at about the same level among all groups. Because the gut microbiome—the community of bacterial species that symbiotically inhabit the gastrointestinal tract in mice and humans—is known to affect bile acid function, the scientists compared the microbiomes of the groups of mice that received bile diversion procedures. They found that 8 weeks after surgery, the pattern of GB-IL gut bacterial species more closely resembled that of lean mice than of the other surgical groups or control obese mice. These results suggest that surgical diversion of bile to the ileum yields many of the health benefits of RYGB, without the risks associated with dramatic alterations to the gastrointestinal tract. However, the researchers point out that there is still risk associated with

surgically diverting bile flow. While promising as a potential treatment for severe obesity, additional research will be necessary to determine if bile diversion surgical procedures could provide similar benefit to humans, or whether these findings could lead to therapeutic approaches that can harness the metabolic potential of bile acids without the need for invasive surgery.

*Flynn CR, Albaugh VL, Cai S,...Abumrad NN. Bile diversion to the distal small intestine has comparable metabolic benefits to bariatric surgery. [Nat Commun](#) 6: 7715, 2015.*

## **BURNING CALORIES: BROWN FAT, BEIGE FAT, AND BODY TEMPERATURE**

### **Mimicking a Meal To Reduce Weight Gain:**

Researchers have determined that tissue-selective delivery of a molecule called fexaramine (Fex) reduced weight gain and improved the metabolism of mice with diet-induced obesity without changes in appetite. Fex is a specific and potent activator of FXR, a factor that, when activated, “turns on” genes in diverse tissues including the kidney, stomach, intestines, and liver. It is known that FXR plays a complex, but not fully understood, role in metabolism. FXR is selectively activated in the intestine in response to a meal. It was unknown whether experimentally activating FXR only in the intestines could mimic eating a meal and lead to improvements in metabolism without consumption of calories. This selective activation was attractive to scientists because it could potentially avoid side effects that often result from drugs that systemically affect the body.

First, the scientists demonstrated that oral delivery of Fex to male mice resulted in intestinally restricted FXR activation due to its poor absorption into the bloodstream. They then gave the mice daily

treatment of Fex for 5 weeks and fed them a normal or a high-fat diet. Mice on a normal diet showed similar weight gain and metabolic characteristics whether or not they received Fex. However, mice on a high-fat diet who received Fex showed a reduction in weight gain and improved metabolic profiles, including reduced glucose (sugar) and cholesterol levels. The Fex treatment improved insulin sensitivity and glucose tolerance in the mice, as well as reduced levels of inflammatory factors that accompany obesity.

To further understand these effects, the scientists examined the adipose (fat) tissue of the mice. Mammals have different types of fat tissue: calorie-storing white adipose tissue (WAT) is the most abundant; brown adipose tissue (BAT), which burns calories to generate heat; and beige fat tissue, which exhibits some characteristics of classic BAT cells but also has distinct properties, and can appear within WAT depots in response to various triggers. Treatment with Fex led to increased energy expenditure in BAT, increased core body temperature, and the “browning” of WAT; the WAT took on characteristics of energy-burning BAT. Combined with the reduction in diet-induced weight gain and inflammation and improvement in metabolic profiles, these results suggest that Fex activation of FXR in the intestines mimics eating a meal in male mice. The selective activation may mean that Fex is safer than agents that activate FXR throughout the body, thus making Fex an attractive candidate for further development and testing in human clinical trials as a new approach in the treatment of obesity and metabolic syndrome. Further research will also be needed to determine if the results of this study hold true in females.

*Fang S, Suh JM, Reilly SM,...Evans RM. Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance. Nat Med 21: 159-165, 2015.*

### **A New Role for a Class of Immune Cells in Regulating Beige Fat Development:**

Two studies have revealed a critical role for an immunological cell type, called ILC2 cells, in the development and activity of calorie-burning beige fat. Mammals harbor different kinds of adipose (fat) tissue in various regions of the body. White adipose tissue (WAT)—the most abundant type of fat—stores calories, while brown adipose tissue burns calories to generate heat. A third, calorie-burning type of fat, called beige fat, emerges within WAT depots—a process referred to as “browning” of WAT—in response to cold exposure, nervous system triggers, or muscle activity. The metabolic potential of beige fat has led many scientists to believe that it could serve as a target for treatment strategies for obesity and associated metabolic diseases in humans, but the mechanisms controlling beige fat induction are not well understood.

Previous research showed that molecular signals from certain immunological cells within WAT activate pathways that induce beige fat formation. One such signal, called IL-33, has been shown to be present in WAT, and to protect mice from insulin resistance and other metabolic conditions associated with obesity. In an effort to further understand the role of IL-33 in weight gain and metabolism, two research teams recently discovered that, in mice, a type of immune cell, called group 2 innate lymphoid cells (ILC2s), which are known to respond to IL-33, plays an essential role in beige fat induction and energy balance—the state of balance between energy consumption (eating food), energy storage (fat), and energy expenditure (burning fat to fuel activity and generate body heat).

In one study, scientists treated male and female mice with IL-33 and found a robust induction of beige fat cells within areas of WAT under the skin (subcutaneous WAT depots). When exposed to

cold temperature, which triggers the browning of WAT, IL-33-treated mice exhibited a greater increase in overall calorie burning (whole-body energy expenditure) than did untreated mice. The researchers then used mice that were genetically modified to produce a fluorescent “marker” protein exclusively in ILC2s, enabling the scientists to track the formation of these cells as they arise. IL-33 treatment in these mice led to dramatic induction of active ILC2s as compared to untreated mice. In addition, the scientists found that IL-33 treatment led to increased numbers of “adipocyte precursor” cells—cells that could either develop into white or beige fat cells, depending on what signals they receive—in WAT. These precursor cells were characterized and shown to exhibit molecular hallmarks of beige fat, indicating that IL-33 triggers the expansion of adipocyte precursor cells in WAT that are committed to becoming beige fat cells. Moreover, IL-33 treatment in mice genetically modified to lack ILC2s failed to stimulate production of the precursor cells and beige fat cells, indicating a crucial role for ILC2s in the browning of WAT. Together, these results show that IL-33 induces ILC2s in WAT, which promotes the browning of WAT through the expansion of beige fat-committed adipocyte precursor cells. Based on additional experiments in this study and research from other scientists, it is likely that this newly identified pathway works in parallel with other cells and molecules to induce the browning of WAT.

In a separate study, scientists independently demonstrated that IL-33 induces ILC2s in WAT, and further addressed the role of these cells in energy balance using male mice. The scientists fed mice a high-fat diet, which leads to obesity, and found that WAT from the resulting obese mice contained fewer ILC2s than did normal-weight mice. Mice genetically modified to lack IL-33 gained more weight and fat mass on a normal diet than did normal

mice. In addition, mice lacking IL-33 had far fewer beige fat cells within WAT than did normal mice. Mice genetically engineered to lack ILC2s, when treated with IL-33, failed to induce browning of WAT. However, when ILC2s from normal donor mice were transplanted into mice lacking ILC2s, the browning phenomenon in WAT was restored. To identify the possible “browning” signals sent from ILC2s, the researchers compared the genes turned on in mouse ILC2s to those turned on in similar cells (ILC3s) that are not involved in the induction of beige fat. The analysis revealed one gene specific to ILC2s, whose protein product can activate a potential signal called methionine-enkephalin (or MetEnk). IL-33 treatment led to increased production of MetEnk by ILC2s. Mice treated with MetEnk exhibited increased browning of WAT, as well as increased body energy expenditure. Isolated WAT cells treated with MetEnk turned on beige fat genes, suggesting that MetEnk itself can directly promote the browning of WAT. To begin to address whether ILC2s similarly function in people, the scientists compared WAT biopsies from a small number of donors. They found that WAT from people who were obese (six women and one man) contained significantly lower levels of ILC2s than did WAT from people of normal weight (three women and four men).

Together, these studies suggest that IL-33 induces production of ILC2s in WAT. ILC2s then generate MetEnk, which signals the formation and activity of beige fat cells. Based on previous research, it is clear that these cells and signals work together with other immune cell pathways to induce the browning of WAT. While preliminary experiments revealed a correlation between larger numbers of ILC2s and lower body weight in people, additional studies will be needed to continue elucidating the roles of these cells and proteins. If human beige fat development works in a similar manner, IL-33, ILC2s, MetEnk, and their partners and pathways could be useful

therapeutic targets to protect against obesity and metabolic diseases.

Brestoff JR, Kim BS, Saenz SA, ... Artis D. Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. *Nature* 519: 242-246, 2015.

Lee M-W, Odegaard JI, Mukundan L, ... Chawla A. Activated type 2 innate lymphoid cells regulate beige fat biogenesis. *Cell* 160: 74-87, 2015.

### Improved Utility of Mice as Models of Human

**Obesity:** NIDDK scientists have improved the predictive value of mice as experimental models of human obesity by integrating body temperature into the analysis of factors affecting energy use and regulation. For decades, researchers studying obesity and metabolism have used the mouse as a model experimental system for understanding the mechanisms regulating energy balance, which is governed by a variety of factors, such as energy intake (calories from food), energy expenditure (calorie burning), metabolism, and heat production. To better reflect the complex control of energy use and expenditure in humans and understand how it contributes to weight maintenance and obesity, scientists in the NIDDK's Intramural Research Program used sophisticated technologies in mice to measure and integrate several factors that contribute to energy balance. Included in their analyses was body temperature—a measure that had not been previously integrated in similar studies—as well as the energy effects of physical activity, food intake, and basal metabolic rate. The scientists housed individual mice at 22 degrees Celsius (72 degrees Fahrenheit) for 3 days, and then adjusted the ambient (environmental) temperature to a range from 4 degrees Celsius (40 degrees Fahrenheit) to

33 degrees Celsius (91 degrees Fahrenheit) for 1 day each. For each ambient temperature, they took continuous energy measurements at the light and dark phases, which were both 12 hours each day. (Because mice are nocturnal, they are more active in the dark phase than they are in the light.) They then analyzed the series of energy measurements across different times and environmental temperatures. The scientists found that in male mice, body temperature was constant from 18 to 28 degrees Celsius, but was lower at 4 and 12 degrees Celsius. It was elevated during the dark phase compared to the light phase at all of the ambient temperatures. The light/dark cycle and physical activity, and to a lesser extent ambient temperature, were the factors that most affected body temperature. The researchers found that physical activity actually raised the body temperature that the mice were trying to maintain, as compared with their body temperature during periods of inactivity; the elevation was not just due to an overproduction of body heat from exercise. The researchers then compared heat loss in different living mice (normal, shaved mice, or genetically furless) with that of male or female mice that had just died, and found that fur provides a relatively minor contribution to insulation as compared with physiological mechanisms active while mice are alive. These findings provide a more comprehensive view of how mice respond to different environmental temperatures and reveal the complex interactions between body temperature, environmental temperature, and energy expenditure, which could help scientists enhance the utility of mice as research models of human obesity.

Abreu-Vieira G, Xiao C, Gavrillova O, and Reitman ML. Integration of body temperature into the analysis of energy expenditure in the mouse. *Mol Metab* 4: 461-470, 2015.

# A Technological Partnership Between the NIH and the USDA Aims To Help People Achieve and Maintain a Healthy Weight



The National Institutes of Health (NIH) and the U.S. Department of Agriculture (USDA) have partnered to add the NIH Body Weight Planner, developed by NIDDK scientists, to the

USDA's SuperTracker online food and activity tool as a goal-setting resource to help people achieve and stay at a healthy weight. Created in 2011, the SuperTracker tool empowers people to build a healthier diet, manage weight, and reduce risk of chronic disease. Users can determine what and how much to eat; track foods, physical activities, and weight; and personalize with goal setting, virtual coaching, and journaling. With science-based technology drawing on years of research, the new addition of the Body Weight Planner in 2015 will now enable SuperTracker's more than 5.5 million registered users to tailor their plans to reach a goal weight during a specific timeframe, and maintain that weight afterward.

The mathematical model behind the Body Weight Planner, an online tool published by the NIH in 2011, was created to accurately forecast how body weight changes when people alter their diet and exercise habits. The Planner's calculations reflect the discovery that the widely accepted paradigm that reducing 3,500 calories will shed one pound of

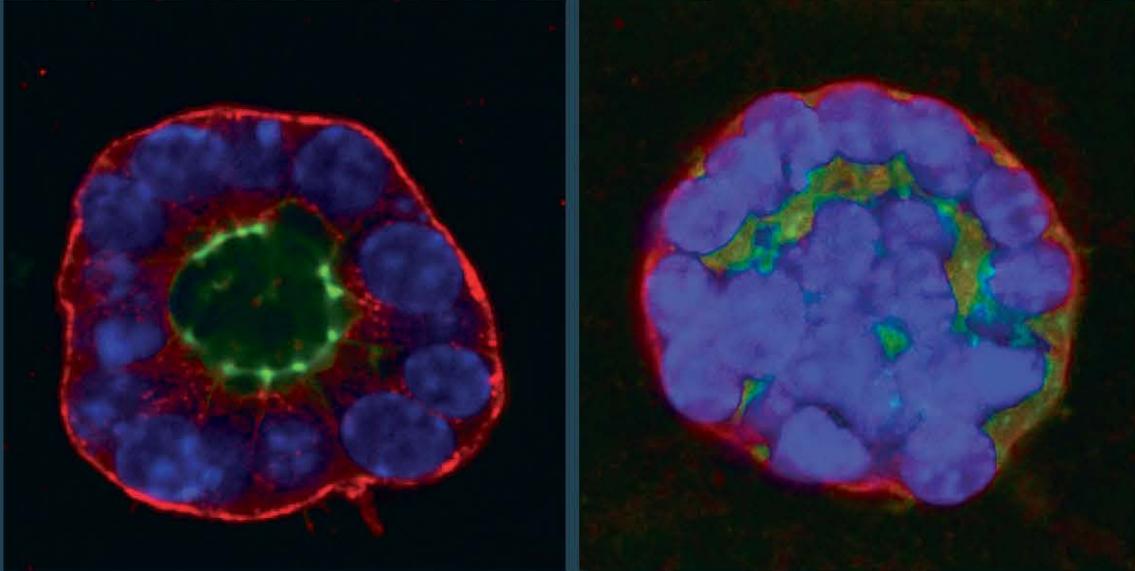
weight does not account for slowing of metabolism as people change their diet and physical activities. Computer-based simulations used in the online tool were developed in the laboratory of Dr. Kevin Hall, a scientist in the NIDDK's Intramural Research Program. The Planner's calculations more accurately model changes in a person's body by taking into consideration differences between people, such as age, height, weight, amount of body fat, whether they are male or female, and resting metabolic rate. The complex "dynamic" model incorporates these various parameters; the model also accounts for changes in metabolism during weight loss, and the variation in these changes among people. The mathematical model was validated using data from multiple controlled studies in people. Based on this research, the NIH and USDA worked together to incorporate the Body Weight Planner into the SuperTracker to enhance the web-based tool. This technological partnership could assist those striving for a healthier weight—the NIH Body Weight Planner helps users develop a realistic plan for reaching their goals, and the USDA SuperTracker helps them to achieve it.

**For more information, please visit the following websites:**

NIH Body Weight Planner: [www.niddk.nih.gov/health-information/health-topics/weight-control/body-weight-planner/Pages/bwp.aspx](http://www.niddk.nih.gov/health-information/health-topics/weight-control/body-weight-planner/Pages/bwp.aspx)

USDA SuperTracker: [www.supertracker.usda.gov](http://www.supertracker.usda.gov)





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

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

# Digestive Diseases and Nutrition

***Digestive diseases are among the leading causes of doctor visits, hospitalizations, and disability in the United States each year. These conditions span a wide spectrum of disorders that affect the gastrointestinal (GI) tract, liver, gallbladder, and pancreas, as well as obesity and other nutrition-related disorders. The latest concerted effort to address the burden of all digestive diseases combining multiple big data sources estimated a total of 72 million ambulatory care visits to physicians' offices and to hospital emergency and outpatient departments with a primary diagnosis of digestive diseases in the United States.<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 a total charge of \$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 better understand how widespread these diseases are across the United States and in specific population groups, to identify their causes and how they progress, and to test new interventions for prevention and treatment, including drugs, surgery, and behavior modification.

Inflammatory bowel diseases (IBD), which include Crohn's disease and ulcerative colitis, are marked by damaging inflammation in the intestinal tract leading to rectal bleeding, diarrhea, nutritional deficiencies, and other serious complications. These diseases

often strike early in life, with a peak age of onset in adolescence or young adulthood. Treatment frequently requires prolonged use of multiple drugs and may require surgery, including removal of the affected region of the intestine. Scientists are investigating the complex interactions among the genetic, environmental, immune, microbial, and cellular factors that contribute to, or protect against, the development of IBD. The continued discovery of predisposing genetic variations, potential autoimmune and microbial influences, and new methods to repair damaged intestinal tissue will help catalyze the design of novel therapeutic strategies. Research on controlling intestinal inflammation has potential benefits not only for patients with IBD, but also for those at risk of developing colorectal cancer.

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

---

<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.

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

Some digestive diseases can be triggered by the body's reaction to certain foods. For example, in individuals with celiac disease, the immune system reacts to the protein gluten—a component of wheat,

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

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

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

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

The number of Americans who are overweight or obese has risen dramatically in recent decades and is now at epidemic levels. Obesity is associated with numerous diseases, including type 2 diabetes, heart disease, and cancer. Multiple factors contribute to obesity. As scientists elucidate the molecular, genetic, microbial, and environmental factors that influence appetite, metabolism, and energy storage, they are identifying potential avenues for the development of new intervention strategies to

promote safe, long-term weight loss. In addition to new pharmacologic interventions for obesity that may arise from research, existing bariatric surgical techniques are being evaluated for their long-term impacts on weight loss, obesity-associated disease, and well-being. Investigators are also continuing research to help people achieve healthy lifestyles that include physical activity and improved diet. *(Additional information on NIDDK-supported research endeavors focusing on obesity is provided in the Obesity chapter.)*

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

## GUT MICROBES IN HEALTH AND DISEASE

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

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

gut microbial levels depending on an individual's genetic background.

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

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

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

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

the undernourished twins and single-born children, but the children's ability to launch an immune response with IgA offered some protection against the growth stunting caused by their heavy burden of pathogenic bacteria.

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

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

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

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

To learn more about the roles these viruses play in the gut, researchers studied the effect of a common type of mouse enteric virus called murine norovirus (MNV) in germ-free (GF) mice. Due to their lack of gut bacteria, GF mice have intestinal and immunological abnormalities that can be corrected by adding back some types of gut bacteria. To determine if a virus could also reduce these abnormalities, researchers used MNV to infect GF mice, who then passed the infection to their offspring. GF mice inoculated with MNV and their offspring both exhibited a partial reversal of the typical abnormalities seen in GF mice: their intestinal tissues more closely resembled those of conventional mice. MNV also reversed some of the irregularities in their immune systems, in some cases returning certain immune factors and immune cell types to near-normal levels. Thus, the mice's reactions to MNV were in some ways similar to their reactions to normal intestinal bacteria. To see if MNV could protect mice against some of the harmful effects of antibiotics, the researchers treated normal mice with antibiotics for 2 weeks, inoculated these antibiotic-treated mice with either MNV or gut bacteria, and observed the resulting intestinal and immunological changes. Both the viral and bacterial treatments reduced antibiotic-induced intestinal abnormalities, though the two treatments had slightly different effects on the immune system and on the genes activated. Antibiotic treatment can also leave the gut susceptible to damage, due

to its destruction of the normal gut microbiome. MNV, however, was able to protect the guts of antibiotic-treated mice from further damage or death caused by either a chemical agent used to mimic human inflammatory bowel disease or by pathogenic bacteria. These results demonstrated for the first time that a virus in the mouse intestinal tract could have beneficial effects on intestinal physiology, immune function, and disease protection similar to those granted by gut bacteria. Further research is needed to determine if human enteric viruses have similar functions and to define further the roles viruses play in the mammalian gut microbiome.

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

## EARLY NUTRITION AND GASTROINTESTINAL DISEASE

### Understanding Breast Milk's Protective Effects Against Deadly Gastrointestinal Disease in

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

Researchers studied both rodent intestinal cells in culture and newborn mice given breast milk from lactating mice under various conditions to get at the underlying reasons for the milk's NEC-protective properties. Pretreating intestinal cells in culture with breast milk reduced the activation of pro-inflammatory factors such as TLR4 within cells when in the presence of the bacterial toxin. Heating the breast milk at a high temperature, which inactivates proteins, abolished this effect. Based on this and previous studies, the scientists suspected a protein in breast milk called epidermal growth factor (EGF) might be involved. By treating the cells with breast milk depleted of its EGF, then adding the growth factor back in again, they confirmed their suspicions that EGF in breast milk plays a role in tamping down the intestinal inflammatory process by inhibiting TLR4. Studies in mice confirmed that these findings held true in a whole animal. Newborn mice whose cells were genetically engineered to light up when they expressed a key inflammatory molecule activated by TLR4 were injected with the bacterial toxin and then given breast milk or saline. The group given breast milk showed reduced inflammation and pro-inflammatory factors such as TLR4 in their intestines compared to those given

saline. The essential role of EGF in breast milk's protective effects was also replicated in the mice. In the mice fed breast milk but either treated with an EGF inhibitor, given breast milk depleted of its EGF, or genetically engineered to remove the receptor needed for EGF response in the intestine, the milk's protective effects were abolished. The milk was no longer able to protect the intestine by inhibiting inflammatory molecules produced in response to bacterial proteins, supporting intestinal cell replication, or protecting against cell death.

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

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

# Workshop on Biomarkers of Pancreatic Disease

## Advances in Biomedical Imaging, Bioengineering, and Related Technologies for the Development of Biomarkers of Pancreatic Disease

Gaps, Needs, and Opportunities

July 22, 2015 • University Club, University of Pittsburgh, Pittsburgh, PA



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

to build on recent research advances that are offering new approaches to diagnosing the presence and stage of pancreatic diseases at an early time point in their course, which would allow early treatment and possibly the ability to monitor response to therapy.

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

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

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

To propel research progress in this area, the NIDDK, with support from the National Cancer Institute (NCI), has recently funded a multi-center consortium to pursue clinical

research on pancreatic diseases, including chronic pancreatitis, acute recurring pancreatitis, pancreatic cancer, and the type 3c diabetes that may result from these diseases.

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

## RISK FACTORS FOR INFLAMMATORY BOWEL DISEASE

### Digging Deeper for the Genetic Roots of

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

To address this, an international group of researchers compared the genomes of people living in 15 countries across Europe, North America, and Australia, including 18,405 people with Crohn's disease, 14,308 people with ulcerative colitis, and 34,241 people without these diseases. The scientists employed a powerful technique, called high-density single nucleotide polymorphism typing, to identify variants of the HLA genes that were more common in people with IBD than in people without the disease. While the group found that most of these genetic variants were either associated with

Crohn's disease or ulcerative colitis, the researchers found that a certain variant, HLA-DRB1\*01:03, was highly associated with both diseases, suggesting that it may be involved in the development of both forms of IBD. The researchers also found that the HLA genes in people with ulcerative colitis, but not Crohn's disease, were less likely to be heterozygous—in other words, people with ulcerative colitis were more likely to have two copies of the same HLA variant. This suggests that having two different variants for each HLA gene might protect people from developing ulcerative colitis to some degree. These findings point to specific similarities and differences in immune-related molecules between the two most common forms of IBD, and they point to specific genetic traits that could be important in the development of these diseases.

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

### Viruses in Gut Linked to Inflammatory Bowel

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

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

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

These results introduce a new twist to the complicated understanding of IBD. The exact role gut viruses may play in this disease—or in any other diseases and conditions in which gut bacteria have been found to play a role, such as diabetes, obesity,

metabolic diseases, and cancer—remains to be determined. Nonetheless, the discovery of this link between gut viruses and IBD could open the door to designing better treatments or preventative measures in the future.

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

## INSIGHTS INTO GUT DEVELOPMENT AND ACTIVITIES

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

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

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

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

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

**Animal Models Provide Important Clues to Gut Vessel Development:** A recent study in animal models has uncovered details of how arteries and lymphatic vessels develop in the gut before birth. The gastrointestinal tract is enveloped by an intricate

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

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

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

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

#### **Human Intestinal Model from Stem Cells Grown in Culture and Transplanted into Mice:**

A research group has built a better model for studying the human small intestine by growing intestinal tissue from human stem cells and then successfully transplanting it into the mouse kidney capsule, where it performs digestive functions and responds to the physiological environment. To date, scientists have had limited options available for studying the human intestine under physiological conditions similar to the human body. Mouse models and human cells in laboratory culture fall short in terms of simulating what happens to the human intestine inside the body. By building on a previous advance that created three-dimensional mini-intestines, or “organoids,” from human stem cells, researchers have now produced a more accurate model for studying the human intestine. First, they grew human stem cells in laboratory cell culture with a special mix of growth factors for about 5 weeks to allow them time to form hollow, intestine-like organoids. They were able to use different types of human stem cells to form the organoids, including

embryonic stem cells approved for NIH research and “induced pluripotent stem cells” made from an adult human cell type called a fibroblast. The organoids were then transplanted into an immune-deficient breed of mice, which would not reject the human tissue, by placing them under the kidney capsule, a layer of connective tissue covering each kidney. In more than 130 transplant procedures performed, over 90 percent were successful. Transplants were allowed to grow and mature inside the mice for 6 weeks before they were collected. After collection, the transplants were seen to have grown 50- to 100-fold and exhibited signs of mature human intestinal tissue, particularly similar to the small intestine, including a diversity of differentiated intestinal cell types, appropriate intestinal structures and layers, and functions such as maintaining the intestinal barrier, producing digestive enzymes, and absorbing nutrients. The transplanted tissue’s maturation outstripped that of tissue grown for the same time period inside a culture dish. The researchers also tested the advantages of this model for studying human intestinal responses in a physiological (whole-body) system. Surgical removal of a portion of the intestine is known to stimulate some compensatory growth of the remaining intestine through factors present in the circulation. The researchers either surgically removed part of the intestine or performed a sham surgery in mice that had previously been transplanted with the human intestinal organoids for 6 weeks, then examined the effect on the transplant. The mice with the intestinal surgery had more robust growth in the transplanted intestinal organoids than the transplants in mice receiving the sham surgery, highlighting the transplanted intestinal organoids’ responsiveness to physiological signals. This study has created the first functional, *in vivo* model of human small intestine generated from stem cells. This unique model can be used to study more complex contributors to human intestinal function and disease than was possible before. In the future, the technology could be used for further research toward

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

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

## INTESTINAL CANCER GENETICS

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

A group of scientists working in the NIDDK's Intramural Research Program sought to identify genetic mutations that could increase the likelihood

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

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

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

## SYMPTOM REPORTING IN IRRITABLE BOWEL SYNDROME

### “Total Recall”? Variations in Memory of Pain and Other Irritable Bowel Syndrome Symptoms Over

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

To test the accuracy of self-reports, researchers assessed symptom recall in persons (mostly women) diagnosed with moderate to severe IBS who were enrolled in an IBS treatment clinical trial. They asked participants to rate and record every day for 7 days information about four discrete symptoms: abdominal pain (average and worst), typical stool consistency (on a standard seven point scale), number of bowel movements, and feelings of urgency to defecate (average and worst). Participants were to enter this information at home at the end of each day into an electronic diary, which was time-stamped to ensure that entries were made on the symptom day, or “real time.” Then, on day 8, participants returned to the clinic to fill out a clinical questionnaire assessing how well they recalled

symptoms that they had had for the past 7 days. Using data from 177 participants, the researchers found that, as a group, the average recall on day 8 reflected certain, although not all, symptoms as recorded in the daily diaries. Recall of stool frequency, worst intensity of pain and urgency, and days of urgency corresponded well with diary entries, whereas other symptoms tended to be over-reported (e.g., average intensities of pain and urgency and days of abdominal pain). In contrast, when responses were analyzed at the individual level—as they would be in a doctor’s office, for example—the researchers found that, for a significant number of participants, day 8 recall did not correspond well at all with the real-time entries, including those that corresponded well on the group level. The difference in accuracy observed for the group versus individual responses could apparently be explained by the balancing out of retrospective over- and under-reporting of symptoms—and also indicated how group level results could easily mask individual level responses.

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

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

## UNDERSTANDING AND TREATING LIVER DISEASE

### Plant Toxins Linked to Biliary Atresia in Newborn

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

An insight into a possible environmental factor came when Australian scientists identified a disease of newborn sheep that resembled biliary atresia. Strikingly, outbreaks of this condition occurred in Australian lambs during the immediate period following severe droughts. They subsequently found that pregnant sheep in searching for food would consume plant species that were growing on land that was usually under water. Analysis of the plants eaten by the Australian sheep herds during a recent drought pointed to a species of the genus *Dysphania*. Scientists in the United States imported samples of these plants from Australia and analyzed their components, isolating and examining 95 distinct

fractions. Each of these components was then tested for its effects on the larvae of a small translucent fish called the zebrafish, a “model system” that can be used to identify substances and genes that cause injury or disease states. In one of the plant extracts, they identified a toxin that caused a similar damage to the bile ducts of zebrafish larvae as was described in the newborn sheep. They named this toxin “biliatresone” and showed that in high doses it caused defects in the formation of both the gallbladder and bile ducts of zebrafish larvae. They also found that larvae with a certain genetic mutation were more sensitive to gallbladder and bile duct injury from biliatresone. The region of the zebrafish genome that contained this mutation was sequenced and was found to be similar to regions in the human genome that have been associated with increased susceptibility to biliary atresia in humans. Moving to a mammal model closer to humans, the researchers analyzed cells in culture taken from newborn mouse bile ducts that had been exposed to biliatresone. The exposure reduced the number of hair-like projections called cilia on the cell surface, which perform essential functions, including sensing fluid flow and detecting molecules such as bile acids. In a final experiment, the group used a mouse bile duct cell culture system in which the duct cells form spherical-shaped structures with hollow centers, similar to true bile ducts. Exposure to biliatresone disrupted their hollow centers and proper orientation of the cells, which could obstruct bile ducts in the whole animal and contribute to diseases such as biliary atresia.

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

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

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

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

The Farnesoid X Receptor Ligand Obeticholic Acid in NASH Treatment (FLINT) trial was conducted as part of the NIDDK's Nonalcoholic

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

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

This trial showed the promise of a once-a-day drug that targets a bile acid receptor, the farnesoid X nuclear receptor, for reducing harm from this common form of liver disease. The degree of improvement with this approach and its safety when

administered long-term remain issues. Larger, longer-term studies are now underway that will carefully evaluate the safety of this drug and its effects on reducing NAFLD.

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

# Liver Injury from Herbal and Dietary Supplements Workshop



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

Use of herbal and dietary supplements—including vitamins, minerals, herbs, and other bioactive compounds found in foods and plants—is high in

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

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

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

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

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

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

---

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

## NEW DIRECTIONS IN HEPATITIS TREATMENT

### Over-the-Counter Antihistamine May Be

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

Hepatitis C can lead to damaging liver disease, including cirrhosis, liver failure, and liver cancer. For years, standard therapy for hepatitis C was a combination of the antiviral drugs peginterferon and ribavirin, but in recent years, combination treatments with drugs specifically targeting the virus have made therapy more effective and tolerable, although at a high cost. Opportunities remain to find effective and affordable treatments for hepatitis C among existing drugs approved by the U.S. Food and Drug Administration (FDA) for other uses. A team of NIDDK scientists, in collaboration with staff of the NIH National Center for Advancing Translational Sciences, employed a system to rapidly screen large numbers of compounds against the hepatitis C virus (HCV). They grew human liver cells infected with HCV in multiple wells on small plates; in each, they added a dose of one of approximately 3,800 active FDA-approved drugs. The virus was tagged with a chemical that lights up when viral genes are activated. Among the many compounds tested, a common antihistamine drug first approved in 1940, called chlorcyclizine, was particularly potent at suppressing HCV activity. The effect of the antihistamine also held true in a more standard cell model of HCV infection using a liver cancer cell line. The drug was found to inhibit multiple subtypes or "genotypes" of HCV that infect

people and affect treatment response. Combining the antihistamine with drugs approved for treating hepatitis C led to additive effects inhibiting HCV. To evaluate the antihistamine's unique pharmacologic properties for treating HCV, including its metabolism and distribution throughout the body, the group tested it in mice injected with the drug. The drug showed long-lasting effects, and it was found in high amounts in the liver, where it would need to concentrate to fight off the HCV infection. The team also found large amounts of the drug in brain, a possible problem as antihistamines are known to cause drowsiness and sedation. To test how effective the drug was against HCV in an animal model designed to mimic human infection, they transplanted human liver cells into mice, infected them with two different genotypes of HCV, and treated them with the drug for several weeks. The drug was found to lower viral levels of both strains of HCV in a rapid and reversible manner. This research showed the potent antiviral activity of an affordable, over-the-counter drug in cell and mouse models of hepatitis C. Because this drug is already FDA-approved for long-term human use, it is known to be relatively safe. Studies are now underway in humans with hepatitis C with close monitoring for side effects and careful analysis of the effects on HCV levels and liver damage. Future research will explore ways to biochemically modify the drug to reduce levels in the brain and increase the activity against HCV, thus adding to its efficacy and reducing side effects.

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

### Clinical Trial Shows Promising Results of New

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

chronic hepatitis D. Chronic hepatitis D is found throughout the world and often causes severe liver disease. The hepatitis D virus (HDV) is unique in that it cannot cause infection on its own, but requires help infecting its host from the hepatitis B virus and, therefore, is found only in persons who also have hepatitis B. Peginterferon has been the standard therapy for chronic hepatitis D, but the response rate is quite low and side effects are frequent, dose-limiting, and sometimes severe. While many advances have been made in the therapy of chronic hepatitis B and C, there has been no progress in antiviral therapy of hepatitis D. Scientists in the NIDDK Intramural Research Program, in collaboration with an international group of investigators and the drug sponsor, performed a pilot, phase 2a clinical trial to test the safety, tolerability, and effectiveness of the drug lonafarnib in treating chronic hepatitis D. Lonafarnib inhibits an important biochemical process that modifies proteins and is essential for HDV replication in liver cells. In this first human trial, 14 men and women with chronic hepatitis D were given one of

two oral dose levels of lonafarnib or a placebo for 4 weeks and monitored during treatment and then for 6 months afterwards. In those who received lonafarnib, HDV levels decreased during treatment compared to the placebo group; the effect was more pronounced in those given the higher dose of the drug. The drug was well-tolerated, with no trial participant choosing to discontinue treatment due to an adverse event, though gastrointestinal side effects, such as diarrhea and nausea, were common. This clinical trial shows the promise of lonafarnib as a potentially groundbreaking new type of therapy for chronic hepatitis D. Studies of longer courses of treatment (6 months) using the higher dose of lonafarnib are now under way. Future studies will explore long-term therapy, dose adjustment, and combination with drugs to increase the antiviral activity and reduce side effects of treatment.

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

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

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

### **An Unknown Culprit**

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

a longer incubation period, and could lead to a chronic (long-lasting) infection. Because many cases of hepatitis seemed to be coming from blood transfusions, the identification of the viruses, particularly the blood-borne agent that causes hepatitis B, became imperative. If the virus was known, the blood supply could be screened to prevent spread of the disease.

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

# STORY OF DISCOVERY

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

## Interfering with Non-A, Non-B Hepatitis

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

In 1984, scientists in the NIDDK Intramural Research Program led a pilot study of interferon in 10 patients

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

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

# STORY OF DISCOVERY

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

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

## The Discovery of the Hepatitis C Virus

The non-A, non-B hepatitis virus was identified in 1989 by scientists at a California biotechnology company called Chiron who were collaborating with investigators at the Centers for Disease Control and Prevention (CDC). The research confirmed that this was a new virus—now officially called the hepatitis C virus, or HCV. This was a landmark advance in medicine that allowed for development of tests to detect HCV, which were rapidly applied to screen

blood donations. Over the next few years, as the testing improved, HCV was effectively eliminated from the blood transfusion supply. The identification of HCV also led to further studies, undertaken by NIAID- and NIDDK-funded researchers and others, to determine its molecular structure. This was crucial for the design of drugs that would specifically interact with components of the virus and inhibit its replication. The identification of the virus also allowed for a more accurate diagnosis and a better sense of its prevalence; in fact, it was eventually determined that HCV was the most common cause of chronic hepatitis, cirrhosis, and liver cancer in the Western world.

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

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

# STORY OF DISCOVERY

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

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

## Zeroing in on the Hepatitis C Virus

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

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

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

# STORY OF DISCOVERY

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

## The Future of Hepatitis C Therapy

With such high rates of success with current treatments, it may seem like the hepatitis C story is in its final chapters, but it is not over yet. A

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

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

*Dr. David Brenner is the Vice Chancellor for Health Sciences, Dean of the School of Medicine, and Distinguished Professor of Medicine at the University of California, San Diego (UCSD). Dr. Brenner earned his B.S. from Yale University, and his M.D. from the Yale University School of Medicine. Following his medical internship and residency, at the Yale-New Haven Medical Center, he completed fellowship training as a research associate in the Genetics and Biochemistry Branch of what is now the NIDDK and in gastroenterology at UCSD. He later joined the medical school faculty at UCSD and served as a physician in the Veterans Affairs San Diego Healthcare System. He was then appointed as Professor and Chief of the Division of Digestive Diseases and Nutrition at the University of North Carolina at Chapel Hill, before moving to the Columbia University College of Physicians and Surgeons, where he was Samuel Bard Professor, chair of the Department of Medicine, and physician-in-chief of New York Presbyterian Hospital at Columbia. Currently, Dr. Brenner leads the UCSD School of Medicine, Skaggs School of Pharmacy and Pharmaceutical Sciences, UCSD Medical Center, and UCSD Medical Group. Dr. Brenner is a member of several professional societies and has served on numerous editorial*

*boards, including his tenure as editor-in-chief of the journal Gastroenterology from 2001 to 2006. Dr. Brenner is a leader in the field of gastroenterological research, specializing in diseases of the liver. Dr. Brenner presented his laboratory's recent research findings at the May 2015 meeting of the National Diabetes and Digestive and Kidney Diseases Advisory Council, of which he is a member. The following are highlights from his presentation.*

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

# SCIENTIFIC PRESENTATION

## **Liver Fibrosis: One Process with Many Questions**

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

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

### **In Pursuit of the Cellular Source of Fibrosis**

As Dr. Brenner pointed out in his presentation, these questions surrounding the cell type(s) responsible

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

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

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

# SCIENTIFIC PRESENTATION

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

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

Again using the mouse model that glowed green where the fibrotic molecule collagen was produced, Dr. Brenner's group was able to find tell-tale signs of hepatic stellate cells due to their high amounts of vitamin A. This study showed that the hepatic stellate cells were activated into myofibroblasts and contributed to fibrosis characterized by liver cell death in response to chemical injury by carbon tetrachloride, which mimics hepatotoxic injury to cells in the liver called hepatocytes. In another study, they subjected the same mouse model to bile duct ligation, mimicking conditions of bile duct obstruction in humans and injury of the cells lining

the bile duct from a back-up of bile acids. They observed that fibroblasts surrounding the portal vein became activated into myofibroblasts, contributing to fibrosis in this scenario. Through profiling the genes activated in these cells in mice, the scientists discovered new markers unique to these activated cells that were once portal fibroblasts, which they also found in samples from patients with biliary cirrhosis.

## Charting a Path To Protect Against Future Liver Fibrosis

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

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

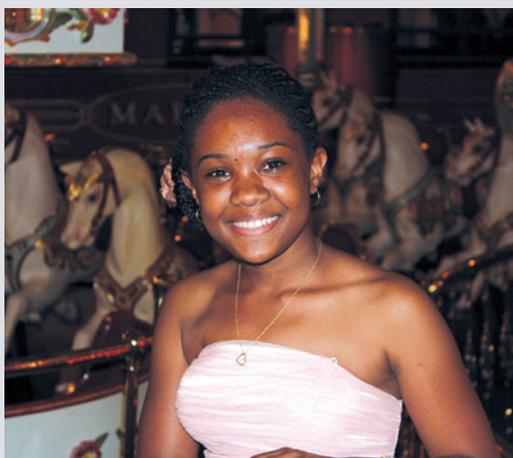
# SCIENTIFIC PRESENTATION

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

severe fibrosis. Dr. Brenner and his team have recently begun investigating the factors that help the once-activated liver cells escape death and persist in the liver after recovery from fibrosis.

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

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



**Sydney**

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

But just a few years ago, her life was dramatically different—marked by frequent attacks of abdominal pain so severe that they put her in the hospital for weeks at a time and kept her from going to school or engaging in any of the other typical activities of children her age. Her struggles with pancreatitis and her entire family's journey with managing the disease have not been easy. With the help of research on genetic factors

underlying this disease, however, much of which was performed by scientists with National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) support, and a surgical procedure called “total pancreatectomy-islet autotransplantation,” the future is bright for this young person who is eager to give back to the world of medicine and other children dealing with serious illnesses.

### **Living with Pancreatitis**

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

# PATIENT PROFILE

disease, often caused by gallstones as they pass through the duct connecting the pancreas and gallbladder to the intestine, resolves after a few days of treatment.

However, chronic pancreatitis is marked by frequent attacks of debilitating abdominal

pain that become worse over time, causing more long-term damage and preventing those affected from engaging in everyday activities such as attending school or work.

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

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

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

Typically, pancreatitis is diagnosed through a combination of medical history, physical examination, blood tests for elevated levels of

digestive enzymes, and imaging tests showing the pancreas, gallbladder, and surrounding ducts.

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

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

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

# PATIENT PROFILE

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

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

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

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

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

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

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

# PATIENT PROFILE

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

## Choosing a Life Without Pain

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

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

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

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

Sydney’s family weighed the pros and cons of having the procedure, including Sydney being free of the pancreatic attacks and less likely to develop

pancreatic cancer later, but also the serious risks of any surgical procedure and also the possibility

of developing diabetes if the transplanted islets did not produce enough insulin.

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

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

# PATIENT PROFILE

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

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

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

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

## The Long Road to Recovery

Sydney’s recovery in the following months after the TP-IAT surgery was slow and full of challenges.

Beginning immediately upon returning to her hospital room after her surgery, now without a pancreas, Sydney’s recovery would require many interventions in the weeks and months that followed, to help her maintain her blood sugar, manage pain, and help with some difficulty she experienced breathing.

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

Nutrition was also a major challenge during this time. Sydney remained on the IV TPN for a few months following the surgery. For weeks, Sydney

had to have her blood sugar monitored around the clock until the

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

transplanted islet cells showed signs that they were functioning normally in their new home inside the liver. Until then she was considered to have “brittle diabetes,” a condition in which blood sugar levels fluctuate unpredictably. Even after beginning to eat by mouth again, she was taking both short- and long-acting forms of insulin, in addition to pancreatic enzymes, with her meals, and closely counting her carbohydrates, to provide intensive control of her blood sugar while her islet cells recovered.

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

# PATIENT PROFILE

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

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

But a bright spot from that difficult recovery period came when, while in the hospital, Sydney was

able to meet another child who went through the procedure around the same time.

The two became "inseparable" afterwards and remain good friends.

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

## A New Quality of Life

Now, 4 years after the TP-IAT surgery, Sydney is back in school full time and studying diligently, even attending a math and science camp during her summer break. She no longer

experiences abdominal pain, takes minimal insulin medication and lower doses of the pancreatic enzymes with her meals, and only has to check her blood sugar after exercising. She also continues to take special care through her diet, including daily vitamin supplements.

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

"We believe this is what the surgery has given her, that quality of life," adds LaKindra. Though in Sydney's case, the decision to have the

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

surgery also meant a lifelong commitment to managing her blood sugar—a tradeoff they

willingly accepted to give Sydney a life free of chronic pain. Sydney, her family, and her health care team take care to preserve the function of the islet cells transplanted into her liver, which cannot replicate like islet cells within the pancreas.

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

# PATIENT PROFILE

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

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

## Hope Through Research

The NIDDK has supported research related to pancreatitis conducted by individual investigator-led teams, as well as larger, multi-center studies, such as the North American Pancreatic Study Group, which in 2012 performed the first genome-wide association study of pancreatitis, discovering additional genetic factors involved in the disease. The Institute has also sponsored

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

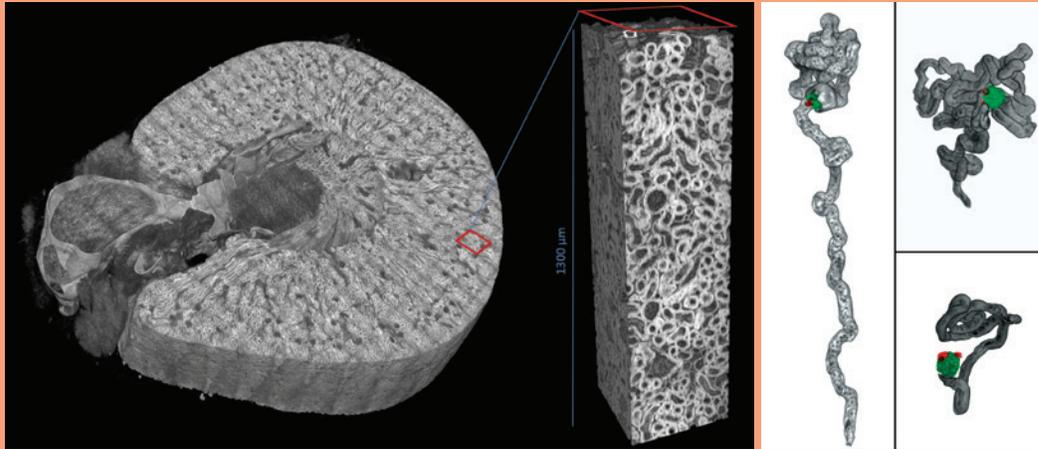
workshops in recent years to foster new ideas and collaborations that can further advance pancreatitis research. For example, a 2013 workshop co-sponsored by the NIDDK and the National Cancer Institute (NCI) focused on pancreatitis, diabetes, and pancreatic cancer; a 2014 workshop focused on opportunities for research on TP-IAT use as a treatment for chronic pancreatitis; and a 2015 workshop focused on the development of biomarkers to facilitate early diagnosis of pancreatic disease.

Also in 2015, a new Consortium for the Study of Chronic Pancreatitis, Diabetes and Pancreatic Cancer was established with support from the NIDDK and the NCI. The Consortium's work will include conducting studies of people with chronic pancreatitis to improve understanding

of disease processes and related outcomes such as diabetes and pancreatic cancer development. These efforts offer hope through

research to advance knowledge of pancreatic disease and improve its management.





Research described in this chapter uses a novel way to process mouse kidney tissue samples, in combination with a specialized imaging technique, called multiphoton microscopy, to get a deep view into the kidney's internal structures. Left panel: This novel technique was used to visually reconstruct a section of the kidney with excellent detail and depth. Right panel: Scientists also used this novel technique to visualize kidney damage caused by the chemotherapy drug cisplatin. *Clockwise from left:* Visual reconstruction of a normal mouse nephron (the basic functional unit of the kidney) and of two nephrons damaged by cisplatin. Within each nephron, the glomerulus—the fundamental filtering apparatus in the kidney—is indicated by gray (normal covering) or green (missing normal covering); small blood vessels are indicated in red. Cisplatin was found to damage the outermost layer of cells that encapsulate the glomerulus, thus disconnecting the glomerulus from another part of the nephron called the proximal tubule. This new technique could shed light on ways to make cisplatin safer for people, as well as be used to study other forms of kidney damage.

*Images courtesy of Dr. Richard Torres and Dr. Robert Safirstein, Yale University School of Medicine. Republished with permission of the [Journal of the American Society of Nephrology](#), from: Three dimensional morphology by multiphoton microscopy with clearing in a model of cisplatin induced CKD, Torres R, Velazquez H, Chang JJ, Levene MJ, Moeckel G, Desir GV, Safirstein R. Copyright 2015; permission conveyed through Copyright Clearance Center, Inc.*

# 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 two 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 20 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 2013, over 661,000 patients received treatment for ESRD: nearly 467,000 received either hemodialysis or peritoneal dialysis and over 193,000 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. African Americans are nearly four times more likely

---

<sup>1</sup> Centers for Disease Control and Prevention (CDC). *National Chronic Kidney Disease Fact Sheet: General Information and National Estimates on Chronic Kidney Disease in the United States, 2014*. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2014.

<sup>2</sup> U.S. Renal Data System, *USRDS 2015 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, MD, 2015.

to develop kidney failure than are non-Hispanic Whites.<sup>2</sup> American Indians and Alaska Natives and Hispanic and Latino Americans have twice the risk for kidney failure as do non-Hispanic Whites.<sup>2</sup> In recent years, scientists supported by the NIDDK have uncovered important genetic clues that may play a role in some of the health disparities related to kidney disease susceptibility and progression in minority populations.

The NIDDK supports a significant body of research aimed at understanding the biology underlying CKD. 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 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 (CKD BioCon) promotes the discovery and validation of novel biomarkers for CKD initiation, progression, and development of complications. A more complete understanding of biomarkers could allow physicians to detect kidney disease earlier and perhaps identify people at greater risk of progression, allowing them to tailor treatments to a specific individual. To encourage testing practical, sustainable, acceptable, and cost-efficient adaptations of efficacious strategies or approaches to prevent and treat kidney disease, the Institute issued in 2015 a research solicitation entitled "Translational Research to Improve Outcomes in Kidney Diseases."

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 urological diseases and disorders such as benign prostatic hyperplasia, urinary incontinence, urinary tract infections, and urinary stone disease. In 2015, the NIDDK held a meeting to discuss and prioritize the clinical and basic urinary stone disease research needs and identify strategies and recommendations on how best to address those needs. In follow-up to the meeting, the NIDDK issued a solicitation entitled "Urinary Stone Disease Research Network" in 2015 (see feature later in this chapter for more on the meeting and initiative). Other disorders of the genitourinary tract, such as interstitial cystitis/bladder pain syndrome (IC/BPS)—also known as IC/painful bladder syndrome (PBS)—in women and men and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) in men, are also important components 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 painful bladder syndrome.<sup>4</sup>

NIDDK-supported basic and clinical research on IC/BPS and on CP/CPPS is focused on elucidating the causes of these conditions, identifying important subsets of patients to aid diagnostic stratification, and improving treatment and interventions. One example of an ongoing study is the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network, which supports research designed to uncover the underlying causes of IC/BPS and CP/CPPS and to characterize the disease profiles in patients; this research network, described in a feature in this chapter, was recently renewed for an additional 5 years.

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 provide new insights into non-surgical alternatives. As researchers continue to investigate treatment options, an equally important challenge is to improve strategies for assessing both the impact of urinary incontinence and other lower urinary

tract symptoms in women and men and the effect of different diagnostic tools and interventions on patient outcomes. To address this challenge, the NIDDK launched and recently expanded the multi-site Symptoms of Lower Urinary Tract Dysfunction Research Network (LURN). The NIDDK is also leading new efforts to explore whether it may be possible to prevent symptom onset and/or progression, thereby improving health. In 2015, the NIDDK, in conjunction with the National Institute on Aging and the NIH Office of Research on Women's Health, 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 reissued the "New Directions in Hematology Research (SHINE II)" initiative in 2015. New principal areas of research outlined in the initiative include the identification and characterization of the production of the various types of blood cells in the bone marrow.

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.

---

<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.

## INSIGHTS INTO KIDNEY DISEASE AND DAMAGE

### Link Found Between Kidney and Bowel Diseases:

Researchers have found six new regions in the human genome that increase susceptibility to immunoglobulin A (IgA) nephropathy, a major cause of kidney failure worldwide. Using genome-wide screening, the scientists identified susceptibility genes in people of Asian and European ancestries that affect both the risk of developing IgA nephropathy and the age at which the disease develops. Along with nine previously reported genes, these genetic regions are also associated with the risk of developing inflammatory bowel disease. This observation suggests that IgA nephropathy may be part of a group of autoimmune and inflammatory disorders that share some risk genes.

IgA nephropathy is a kidney disorder that occurs when IgA—a protein that helps the body fight infections—forms aggregates in the kidneys, resulting in inflammation. After many years, the IgA deposits may damage the kidneys, causing them to leak blood and sometimes protein into the urine. Scientists do not know what causes IgA deposits to form in the kidneys; although, as with many common diseases, both genes and environmental factors are likely to play a role. In the current study, the researchers found that several of the genes that confer risk of developing IgA nephropathy may also provide protection against intestinal parasites, which may help explain why respiratory or digestive tract infections seem to be a trigger for IgA nephropathy in some individuals.

*Kirylyuk K, Li Y, Scolari F,...Gharavi AG. Discovery of new risk loci for IgA nephropathy implicates genes involved in immunity against intestinal pathogens. Nat Genet 46: 1187-1196, 2014.*

### Genetic Variations in Young Patients with Chronic Kidney Disease May Be Risk Factors:

A fraction of children and adolescents enrolled in the Chronic Kidney Disease in Children Study (CKiD) have been found to have changes in their genetic material that may be important in the development of kidney disease and its complications.

CKiD is a study of pediatric patients with mild to moderate kidney disease. For most children enrolled in CKiD, the cause of their kidney disease has been identified; however, in others it has not. In the current study, researchers used chromosomal microarrays, which detect duplications or deletions of chromosomal regions called copy number variants (CNVs), to analyze the genetic material in volunteers whose kidney disease was of unknown origin. They found that, of the 419 children in this group, 31 (7.4 percent) had CNVs. Further analysis revealed that these CNVs involved changes in 10 genes previously shown to be associated with kidney disease and 12 others that are thought to be related to kidney disease based on their function. The identification of patients with these genomic changes may warrant more personalized clinical care and evaluation, because the genes in some of these regions are associated with a higher risk of certain complications, such as diabetes, heart disease, eye disease, or neurological problems. These conditions may result from the kidney disease, or, potentially in some cases, may be independent of the kidney disease but caused by the same CNVs.

In the United States, the main causes of chronic kidney disease in adults are diabetes and high blood pressure. In children and adolescents, the causes of kidney disease are quite different and less well understood. A better understanding of potential CNV-related causes of kidney disease in children,

especially when there is no other identifiable cause, could pave the way to earlier interventions to preserve kidney function and better monitoring and treatments for associated conditions.

Verbitsky M, Sanna-Cherchi S, Fasel DA,...Gharavi AG. Genomic imbalances in pediatric patients with chronic kidney disease. *J Clin Invest* 125: 2171-2178, 2015.

**Delving Deep into the Kidney with a Novel Imaging Technique:** Scientists have developed a new imaging technique that allows them to see deep into the kidney's internal structures and gain novel insights about cisplatin-induced chronic kidney disease (CKD) in mice. Cisplatin is a chemotherapy drug that is used to treat various types of cancer. However, some people taking the drug develop CKD, which limits the drug's usefulness. To investigate how cisplatin treatment causes kidney damage, researchers first generated a new mouse model of cisplatin-induced CKD by administering two doses of the drug to mice 2 weeks apart. The animals developed CKD similarly to what is seen in people taking the drug. To examine the resulting kidney damage in the mice, the researchers used a specialized imaging technique, called multiphoton microscopy. However, a major limitation to using this technique is that it only gives a shallow view of the organ. To overcome this barrier, the scientists used a novel way to process samples of kidney tissue before looking at them under the microscope—they used a “clearing” solution that replaced the water in the tissue with other chemicals. Use of this clearing solution greatly increased the imaging depth so that the microscope could produce high-resolution three-dimensional images deep inside the kidneys of the mice; this allowed the scientists to observe the damage caused by cisplatin. For example, they found that cisplatin treatment reduced the number of a type of cell (cuboidal cells) in the capsule of the

glomerulus—the fundamental filtering apparatus in the kidney. The loss of cuboidal cells corresponded, almost exactly, to reduced kidney function. The observations made in this study, as well as future research using this new approach, could provide novel insights about kidney damage caused by cisplatin and identify prevention targets to make the drug safer for people. Additionally, the imaging technique could be used to study other forms of kidney damage, such as kidney disease caused by diabetes or high blood pressure.

Torres R, Velazquez H, Chang JJ,...Safirstein R. Three-dimensional morphology by multiphoton microscopy with clearing in a model of cisplatin-induced CKD. *J Am Soc Nephrol* 2015 Aug 24 pii: ASN.2015010079 [Epub ahead of print].

## KIDNEY DISEASE TREATMENT

**Single Drug As Effective As Pair in Treating Polycystic Kidney Disease:** Using two drugs is no more effective than a single drug in slowing disease progression in people with autosomal dominant polycystic kidney disease (ADPKD), according to two recent reports. One of the studies also showed that rigorous blood pressure treatment slowed growth of kidney cysts, a marker of ADPKD, but had little effect on kidney function compared to standard blood pressure treatment.

The HALT-PKD trials enrolled volunteers to test whether a combination of commonly used U.S. Food and Drug Administration (FDA)-approved drugs to treat high blood pressure, lisinopril and telmisartan, could shrink kidney cysts and therefore slow progression of ADPKD. One study examined 558 people with early-stage ADPKD and relatively healthy kidneys. Another study treated 486 people with more advanced disease and diminished kidney function. In each study, half of

the participants were randomly assigned to receive both drugs, while the other half received lisinopril plus a placebo. In both studies, adding the second drug did not change kidney function or rate of increase in kidney cyst size.

In the study of people with early ADPKD and healthier kidneys, researchers also tested whether decreasing blood pressure below usual targets would slow progression of ADPKD and preserve kidney function. One-half of the participants were assigned to a standard blood pressure group (between 120 to 130 over 70 to 80); the other was assigned to a lower blood pressure group (between 95 to 110 over 60 to 75), but still within the normal range. Participants in the lower blood pressure group, who took more medication to maintain the lower blood pressure, had a 14 percent decrease in kidney cyst size compared to those in the standard blood pressure group. However, kidney function—measured by estimated glomerular filtration rate (eGFR)—was about the same as the standard group at the end of the trial, yielding no clinical benefit.

ADPKD is a genetic disorder characterized by the growth of numerous fluid-filled cysts in the kidneys. Symptoms usually develop between the ages of 30 and 40, and many people with ADPKD experience a decline in their kidney function as the cysts grow. About one-half of them progress to kidney failure and require dialysis or a kidney transplant to live. High blood pressure and related cardiovascular complications, such as heart attacks and strokes, are common health problems for people with ADPKD. The results of the HALT-PKD studies demonstrate that more research is needed to better understand how ADPKD destroys kidney function over time, and to determine what combination of medications can most safely and effectively prevent or undo the damage caused by this serious condition.

Schrier RW, Abebe KZ, Perrone RD,...Chapman AB; for the HALT-PKD Trial Investigators. Blood pressure in early autosomal dominant polycystic kidney disease. *N Engl J Med* 371: 2255-2266, 2014.

Torres VE, Abebe KZ, Chapman AB,...Perrone RD; for the HALT-PKD Trial Investigators. Angiotensin blockade in late autosomal dominant polycystic kidney disease. *N Engl J Med* 371: 2267-2276, 2014.

**Living Kidney Donors Fare Well Over Time:** People who choose to donate one of their kidneys to someone with kidney failure remain relatively healthy 3 years after their donation. Relatively few studies have assessed the overall health, and specifically the kidney function, of living kidney donors over time after they donate one of their two kidneys compared to individuals who have not donated a kidney. In a recent study, researchers examined the health status of 182 kidney donors (who, after their donation, have only one kidney) and 173 non-donors (*i.e.*, people with two kidneys) over a 36-month period; 95 percent of the participants were white. Of those who did not donate a kidney, two clinical tests of kidney function—measured glomerular filtration rate (mGFR) and estimated glomerular filtration rate (eGFR)—declined over time, which is expected as people age. In the donor group, these measures were initially lower after donation but improved over time. Other health parameters were largely the same between the two groups. Blood pressure increased slightly over time but was similar in donors and non-donors. Another test of kidney function—protein excreted from the kidney into the urine—did not differ between donors and non-donors.

Once kidney disease progresses to kidney failure, the only treatment is dialysis or transplant. As donor organs are in short supply, there has been interest in the use of living donor organ transplants. The current study suggests that living donor kidney

donation may represent a potential source of organs that would allow recipients to live healthy lives while not compromising the health of the donor, at least within the first 3 years after donation. Future studies could determine longer-term health outcomes for kidney donors.

*Kasike BL, Anderson-Haag T, Israni AK,...Weir MR. A prospective controlled study of living kidney donors: three-year follow-up. Am J Kidney Dis 66: 114-124, 2015.*

**Targeting Cells Involved in Fibrosis May Reduce Chronic Kidney Damage:** Researchers have discovered that members of the GLI protein family can promote kidney fibrosis and that inhibiting these proteins can reduce kidney damage. Fibrosis—the deposition of large amounts of collagen-rich connective tissue that can lead to organ damage—is seen in many conditions related to inflammation and, unchecked, can diminish the ability of an organ to perform its normal functions. In the kidney, fibrosis is a common final pathway for many diseases. It may arise as the result of a brief, severe injury to the kidney or from a slowly progressing, chronic condition. Extensive kidney fibrosis can cause irreversible organ damage and, in severe cases, lead to kidney failure.

Cells called myofibroblasts are known to drive kidney fibrosis. Additionally, members of the GLI family of proteins have been found in precursor cells that become myofibroblasts, and these proteins become more active during kidney fibrosis. However, it was not known whether GLI protein activity, myofibroblast activity, and fibrosis are linked. Scientists have recently clarified this issue by asking whether GLI proteins regulate myofibroblast activity during kidney fibrosis.

To confirm that GLI proteins help control myofibroblast replication, the researchers first asked

what role GLI proteins play in cultured myofibroblast precursor cells in the laboratory. Reducing the levels of one of the GLI proteins, GLI2, caused the cells to stop replicating, suggesting that GLI2 was required for these cells to expand their numbers during organ injury. Male and female mice engineered to lack GLI2 also had less kidney fibrosis than their normal counterparts when kidney injury was induced, and this reduced fibrosis was due to myofibroblasts failing to replicate. These results suggested that GLI2 may be required for myofibroblasts to cause kidney fibrosis and that inhibiting GLI2 could be protective. To determine if GLI2 inhibition could protect against kidney fibrosis, researchers tested the effects of a GLI2 inhibitor drug called darinaparsin. In cultured myofibroblast precursor cells, darinaparsin reduced GLI2 protein levels and caused the cells to stop dividing. When male mice were exposed to kidney injury, darinaparsin again blocked the expected increase in GLI2 protein, significantly reducing kidney fibrosis compared to mice not given the drug. Importantly, darinaparsin reduced kidney fibrosis whether the mice were given the drug prior to kidney injury or after the fibrosis had already begun. Finally, the researchers found that the genes for GLI proteins were more active in kidneys from men and women who had severe kidney fibrosis than in people with less severe kidney fibrosis, though more research is needed to confirm that the GLI proteins work the same in people as they do in mice. Overall, these experiments demonstrate that GLI proteins contribute to kidney fibrosis through their control of myofibroblast replication. Furthermore, these findings suggest that GLI proteins such as GLI2 may be promising targets for developing kidney fibrosis treatments.

*Kramann R, Fleig SV, Schneider RK,...Humphreys BD. Pharmacological GLI2 inhibition prevents myofibroblast cell-cycle progression and reduces kidney fibrosis. J Clin Invest 125: 2935-2951, 2015.*

# NIDDK ApoL1 and Kidney Disease Conference



The NIDDK held a conference entitled “ApoL1 and Kidney Disease Conference” in June 2015 to assess gaps in knowledge including the function of the ApoL1 protein and its role in kidney transplantation. Variants of the *APOL1* gene, which are found primarily in African Americans, are arguably the most important discovery about the pathogenesis of chronic kidney disease over the past several decades, and among the only known genetic factors contributing to the well-appreciated health disparities in kidney diseases. These variants may explain 70 percent of the excess focal segmental glomerulosclerosis, HIV-associated nephropathy, and hypertensive kidney disease in African Americans.

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 roles of *APOL1* genotyping in guiding treatment as well as preventive strategies.

Experts were drawn from across multiple fields. They contributed a wealth of information from a variety of perspectives and identified a broad spectrum of areas in which additional information is needed to move the field forward. The input garnered included many suggestions for potential future research directions. Examples include: exploring the effects of *APOL1* variants on kidney transplantation outcomes in donors and recipients, identifying environmental and additional genetic factors that contribute to *APOL1*-related disease, and investigating how *APOL1* gene variants contribute to kidney disease.

# Scientific Workshop: Urinary Stone Disease— Research Challenges and Opportunities



To assess gaps in knowledge and evidence-based treatment, the NIDDK held a meeting titled “Urinary Stone Disease Research Challenges and Opportunities” in April 2015. Urinary stone disease (USD) is an important, increasingly common problem, with some people experiencing recurrent episodes. It is a costly condition and also carries the burdens of severe pain and loss of quality of life. Obesity and diabetes are associated with USD, and these conditions also are increasing in prevalence. USD is most common in Whites and in men, although USD prevalence is growing in all races and both sexes.

Despite the high prevalence and health and economic burden of the disease, little is known about how stones form or which are likely to be passed. Advances in treatments in the past 30 years have evolved from open surgery to remove large stones, to new technologies. Current treatments to prevent recurrence include increasing fluid intake, modifying diet, and

medications. These measures, however, have not decreased prevalence, suffering, recurrence rates, USD-related chronic kidney disease (CKD) incidence, or cost, representing limited progress in USD, especially compared to diseases such as cancer and cardiovascular disease. Few of the American Urological Association’s and American College of Physicians’ guidelines for USD treatment recommendations are based on level-one evidence; this level of evidence is obtained from randomized, well-controlled clinical trials.

The meeting’s objectives included presenting information on the epidemiology and pathophysiology of USD, as well as evidence for management strategies; discussing and prioritizing the areas in which additional USD research is needed, including studies to better understand the role of the microbiome, genetics, and exposome (defined as the measure of all the exposures—for example, diet, environmental conditions such as hot temperatures, constraints in many occupations

that limit urination frequency) of an individual in a lifetime and how those exposures relate to health; and providing input that could inform potential future NIDDK program initiatives, including input on the key question of the types of studies needed to inform management of USD.

Experts were drawn from across multiple fields—not just urology and nephrology, but also epidemiology, clinical trials, behavioral economics, and biology. Participants contributed a wealth of information from a variety of perspectives and identified a broad spectrum of areas in which additional information is needed to move the field forward. For example, the picture is incomplete regarding how genes and proteins cause USD. Disease gene identification could allow doctors to offer genetic diagnosis, provide novel insights into pathogenesis and physiology, and potentially permit personalized treatments. The lack of effective medications to prevent recurrence of USD was also highlighted, as well as side effects associated with the use of devices to pulverize stone(s) (e.g., bleeding and hospital re-admission).

Four break-out groups identified future research needs including: assessing how the diet and/or microbiome may affect USD, determining how genetic information can be used for personalized USD prevention, identifying strategies to prevent USD recurrences, and formulating the clinical studies needed to evaluate the impact of surgical stone removal therapies on stone clearance.

To encourage research that addresses some of the research needs identified during the meeting, the NIDDK released Funding Opportunity Announcements to establish a “Urinary Stone Disease Research Network” in 2016, which will include several Clinical Centers and a Scientific Data Research Center. The Network will seek to 1) design and conduct 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, 2) conduct clinical research to understand and mitigate ureteral stent-related pain and symptoms, and 3) provide data and collect biological samples from the studies to create a resource for future researchers.

## ADVANCING UNDERSTANDING AND TREATMENT OF URINARY TRACT INFECTIONS

Urinary tract infections (UTIs) are common and occur more frequently in women, many of whom suffer repeated bouts of infection. Although treatable with antibiotics, the emergence of antibiotic-resistant microbes in UTIs, combined with the personal and medical costs of care, makes finding better therapeutic strategies a priority. The primary culprit in UTIs is the bacterium *Escherichia coli* (*E.coli*); UTI-causing *E. coli* bind to and invade the cells lining the inside of the bladder to initiate an infection. A better understanding of how microbes such as *E. coli* respond to and even manipulate host defenses to promote their own survival—and how hosts counter these activities—could help uncover new approaches to UTI treatment. Several studies, summarized below, have provided new insights into aspects of both bacterial and host biology that are advancing efforts in this area.

### Identifying Players in Urinary Tract Infections:

Two recent studies shed light on how the bacterium *E. coli* causes infection in the human urinary tract.

An acute UTI caused by *E. coli* begins when bacteria attach to and invade the cells lining the inside of the bladder. The UTI-causing *E. coli* form intracellular bacterial communities within the bladder that help to promote sustained infection. This invasion and proliferation provokes a defense response in the infected individual, including activation of the immune system and sloughing off of bladder cells into the urine in an attempt to rid the body of offending bacteria. While this exfoliation of infected bladder cells can help clear infection, it also presents an opportunity for bacteria to infect deeper cell layers of the bladder.

One group of researchers sought to investigate further the bacterial and host factors modulating the exfoliation of infected bladder cells. Previous research has found that almost half of *E. coli* obtained from patients with a UTI produce a molecule called  $\alpha$ -hemolysin (HlyA). HlyA damages bladder cells by causing holes or pores in the bladder cell membrane. The investigators determined that HlyA also activates a host cell death signaling program in infected bladder cells. Further, they found that *E. coli* controls its production of HlyA by a sensor system called CpxRA, a system that senses environmental cues such as changes in the makeup of membranes. Taken together, the results of this study provide valuable information showing that some UTI-causing *E. coli* can control their ability to cause disease (virulence) through regulation of HlyA by CpxRA, possibly using this system to manipulate the timing of exfoliation to optimize infection.

Using a molecular biology approach, a second group of investigators identified “fitness” genes expressed (turned on) by *E. coli* during UTI in women. In this context, fitness genes include genes that contribute to *E. coli* survival in the host environment. Among the nine fitness genes identified was a gene called *cus* that helps *E. coli* survive the toxic effect of copper that the body uses to fight infection. Strategies that target the protein products of *cus* and other genes that support bacterial survival may be an effective approach to combat bacterial infection.

These studies provide new insights into how bacteria such as *E. coli* use different yet critical processes to survive and promote UTI in humans. Strategies that target these bacterial processes may yield new therapeutic approaches for UTIs that are synergistic with, or even more useful than, current antibiotics.

Nagamatsu K, Hannan TJ, Guest RL,...Hultgren SJ. Dysregulation of *Escherichia coli*  $\alpha$ -hemolysin expression alters the course of acute and persistent urinary tract infection. *Proc Natl Acad Sci USA* 112: E871-E880, 2015.

Subashchandrabose S, Hazen TH, Brumbaugh AR,...Mobley HL. Host-specific induction of *Escherichia coli* fitness genes during human urinary tract infection. *Proc Natl Acad Sci USA* 111: 18327-18332, 2014.

### Infected Bladder Cells Send Bacteria on a Trip via

**TRP:** A recent study reveals a mechanism used by infected bladder cells to rid themselves of invading bacteria. One common mechanism host cells use to purge themselves of invading pathogens is to round them up in cellular compartments called lysosomes, which are membrane-bound cell organelles containing digestive enzymes that break down excess or worn-out cell parts as well as invading viruses and bacteria. Researchers now have new insights into how bladder cells use lysosomes to combat *E. coli* infection. Previously, researchers found that bladder cells infected with *E. coli* expel the bacteria intact, rather than degrade them in lysosomes; now, they report the mechanism used by infected cells to accomplish this task. Through a series of experiments in mice and in human bladder cells, lysosomes filled with UTI-causing *E. coli* were found to have an altered pH—in this case a neutral pH rather than the acidic pH in which lysosomes exert their best degradative function. pH measures the acidity or alkalinity of a solution; for example, tomatoes are acidic with a pH of 4.6, ammonia has a pH of 11.6, and water is neutral with a pH of 7. It appears that the pathogenic *E. coli* cause the abnormal neutralization of lysosome pH. Lysosomes are unable to degrade the bacteria in neutral pH, so they activate a backup plan. The researchers found that a lysosomal membrane protein called mucolipin TRP channel 3 senses the altered pH and

sets in motion a series of events leading affected lysosomes to travel to and fuse with the bladder cell membrane, subsequently expelling the bacteria, wrapped up in small membrane-bound sacs, to the outside of the cell and into the urine.

These findings reveal a mechanism used by infected bladder cells to circumvent a malfunctioning lysosomal process and effectively rid the cell of bacterial pathogens. Molecules that simulate the activity of TRP and enhance bacterial expulsion from infected bladders may be an effective strategy to combat UTIs.

Miao Y, Li G, Zhang X, Xu H, and Abraham SN. A TRP channel senses lysosome neutralization by pathogens to trigger their expulsion. *Cell* 161: 1306-1319, 2015.

### Too Much of a Good Thing—Host Defense of the Bladder:

A recent study in humans and mice suggests that excessive activity by a cell of the immune system can predispose the bladder to recurrent UTI. Certain strains of *E. coli* can latch on to the epithelial cells lining the bladder wall and invade the bladder cells—establishing an intracellular reservoir leading to recurrent UTIs. Exactly how the host acts to rid itself of the infection and what factors may predict susceptibility to recurrence are poorly understood, although activation of the immune system—the “defense complex” of cells and molecules used by many organisms to ward off dangerous microbes and other threats—appears to play a role in both.

Researchers sought to determine whether a biomarker for susceptibility to recurrent UTI (e.g., an easily detectable and measurable molecule associated with this condition) could be identified in blood samples from women who sought treatment for a UTI less than 7 days in duration, and who were then followed for 3 months to

determine recurrence of UTI. Several molecules were found to be increased in women who developed recurrent UTI compared to those who did not experience a recurrence. These molecules are known to be produced in different types of immune system cells—neutrophils, monocytes, and macrophages—all of which can play a role in promoting inflammation. Acting on this finding, the researchers interrogated the role of these three immune system cells in a female mouse model of recurrent UTI through experiments that revealed an unknown contribution by the neutrophil. When the neutrophil population was depleted in the bladder, the mice developed severe infection and chronic UTI. However, when the neutrophil pro-inflammatory response was robust in the bladder, the mice were also susceptible to chronic UTI. In contrast, moderating this neutrophil response by partially reducing the abundance of neutrophils in the bladder led to a reduced incidence of chronic UTI. The investigators concluded that, while neutrophils are necessary to prevent severe bladder infection, an excessive neutrophil response damages the bladder lining and predisposes the bladder to chronic UTI.

Drilling down further, the researchers found that both neutrophils and cells lining the bladder produce the pro-inflammatory molecule COX-2 during infection. Notably, COX-2 is a target of non-steroidal anti-inflammatory drugs, such as ibuprofen, as well as other medications. While COX-2 is not detectable in the uninfected bladder, it is found in women with UTI. Similarly, the *Cox-2* gene is barely “turned on” in the uninfected mouse bladder but increases 50-fold in bladders infected by *E. coli*. Importantly, blocking COX-2 activity with a specific inhibitor reduced the severity of bladder inflammation and protected mice from chronic UTI—apparently, at least in part, by helping to prevent entry of neutrophils into the bladder.

These findings in humans and mice provide new information about the role of the immune system in promoting susceptibility to recurrent UTIs. Although more research is needed, they also suggest that inhibition of COX-2 may have beneficial effects in women by preventing recurrent UTI.

*Hannan TJ, Roberts PL, Riehl TE,...Hultgren SJ. Inhibition of cyclooxygenase-2 prevents chronic and recurrent cystitis. EBioMedicine 1: 46-57, 2014.*

### **Fighting Fire with Fire To Treat Urinary Tract**

**Infections:** A new study in mice suggests that bacteria that do not cause symptomatic UTIs may be an effective therapy against ones that do. Some *E. coli* strains can invade and multiply in the human urinary tract without causing the symptoms of pelvic pain and urinary urgency normally associated with UTIs. Recent evidence suggests that this may be due to differences between these asymptomatic bacteriuria (ASB) *E. coli* and uropathogenic *E. coli* (UPEC) in certain bacterial surface molecules, with consequent differences in host responses. A number of studies in humans and animal models have also suggested that ASB *E. coli* may prevent or disrupt infection by UPEC, and, furthermore, may have pain-relieving, or analgesic, properties.

Working in female mice, scientists recently tested whether delivering live ASB *E. coli* into the bladder could be used to both reduce the total number of UPEC and reduce UTI pain symptoms. They found that one time administration of a commonly used ASB *E. coli* strain both reduced bacterial numbers and reduced measures of pelvic pain in mice infected with UPEC. Additional tests showed that pain relief provided by ASB *E. coli* 24 hours after administration was similar to that seen after 1 hour treatment with a pain-relieving medication called lidocaine. They then tested other ASB *E. coli* strains isolated from humans for their analgesic

properties and found a range of efficacy; one strain in particular, termed isolate 2-12, was even more effective than the initial strain at reducing pelvic pain in mice infected with UPEC. But how well does the ASB *E. coli* treatment approach compare to antibiotic treatment? Initial tests with the original ASB *E. coli* strain suggested that its ability to reduce the total number of UPEC during infection is comparable to a 3-day course of the antibiotic ciprofloxacin—the clinical standard for treatment of human UTIs. More significantly, however, experiments using the highly analgesic 2-12 strain demonstrated that it provided dramatic and early relief of pain symptoms, whereas ciprofloxacin did not. Furthermore, isolate 2-12 was not only superior to ciprofloxacin in relieving pain induced by UPEC, but also pain induced by three other, non-*E. coli* UTI-causing bacterial species.

While further research is needed, a “probiotic” strategy using ASB *E. coli* to interfere with UTI-causing bacteria and the pain they cause may prove to be a safe and effective treatment strategy for women and men with UTIs, and may even prove useful in treating pelvic pain from other causes.

Rudick CN, Taylor AK, Yaggie RE, Schaeffer AJ, and Klump DJ. Asymptomatic bacteriuria *Escherichia coli* are live biotherapeutics for UTI. *PLoS One* 9: e109321, 2014.

## EVALUATING PAIN IN WOMEN AND MEN

**What's the Difference? New Insights into Pain in Women and Men:** Research has revealed significant differences in how women and men experience pain. Many chronic pain conditions, such as the pelvic pain conditions interstitial cystitis/bladder pain syndrome (IC/BPS) and irritable bowel syndrome (IBS), are much more

prevalent in women than in men, and women with these conditions tend to report more severe, frequent, and longer-lasting pain than their male counterparts. Evidence suggests that individual experiences of pain can be affected both by pain sensitivity—*i.e.*, the threshold at which a person detects a stimulus, such as heat or cold, as painful—and by complex, “top-down” neural pathways running from the brain to the body that can diminish (inhibit) a person’s response to such a stimulus. While additional studies have suggested that there are sex differences in these two factors affecting pain, results have been somewhat mixed. A research team set out to clarify whether being male or female can influence pain sensitivity and/or pain inhibition.

For the study, data were collected from 24 women and 24 men between the ages of 19 and 45 (average age of about 22) who were healthy and free of chronic pain conditions. Each study participant completed a series of tests to measure pain experience. The initial test measured pain sensitivity: Participants were asked to conduct exercises with one hand while blood flow to that hand was restricted by a blood pressure cuff, and to rate their pain on a numeric scale at multiple time points during the test. A second set of tests measured pain inhibition by seeing how well participants could be conditioned to pain: First, participants were asked to immerse one hand four times in a cold water bath that grew increasingly colder at each immersion, and to rate the pain midway and at the end of each immersion. Then, using the temperature that evoked moderate pain for each individual, participants were again asked to immerse one hand in cold water, but then a device that delivered increasing levels of pressure was applied to the other arm, and participants were asked to report when the pressure first

became painful. Importantly, the researchers sought to account for factors known to influence pain experience by having participants complete questionnaires about depressive symptoms and sleep quality prior to undergoing the pain tests.

When they analyzed the results, the researchers found that there were significant differences between healthy women and men both in their sensitivity to pain and in pain inhibition, even after controlling for differences in sleep quality and depressive symptoms. Men were more tolerant of pain and showed more efficient pain conditioning than women. These results suggest that there are underlying biological differences between women

and men in pathways affecting pain experience that could help explain observed differences in chronic pain condition prevalence and symptom severity. Future research could help clarify this and also help determine whether targeting pathways involved in pain sensitivity and pain inhibition in different ways in women and men—e.g., by using different therapies, or by administering the same intervention in different amounts—could help better alleviate pain burden in women.

*Bulls HW, Freeman EL, Anderson AJ, Robbins MT, Ness TJ, and Goodin BR. Sex differences in experimental measures of pain sensitivity and endogenous pain inhibition. J Pain Res 8: 311-320, 2015.*



# The Multi-Disciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network: Yielding New Insights into Challenging Urologic Pain Syndromes

Chronic, often debilitating pain in the pelvic or genital areas, frequently accompanied by urinary symptoms such as needing “to go” urgently or many times a day: These are hallmark symptoms of urologic chronic pelvic pain syndrome (UCPPS), a term that encompasses both interstitial cystitis/bladder pain syndrome (IC/BPS, also called IC/painful bladder syndrome (PBS)), which predominantly affects women, and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), which affects men. These pain conditions reduce quality of life and productivity and incur significant health care costs for millions of Americans.

Despite many years of committed basic and clinical research efforts supported by the NIDDK and other research funding agencies, the cause(s) of UCPPS has long remained elusive, as have widely or fully effective treatments. Moreover, diagnostic tests are not currently available. Instead, because many UCPPS symptoms can be suggestive of known diseases, those diseases need to be ruled out first—making IC/BPS or CP/CPPS a “diagnosis of exclusion.” While public and clinical awareness of UCPPS is increasing due to educational efforts by the NIDDK and major health advocacy organizations, such as the Interstitial Cystitis Association and the Prostatitis Foundation, many people suffer

for years with symptoms and no diagnosis. In the face of these challenges, discoveries emerging from the NIDDK-sponsored Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network are bringing scientists and clinicians closer to understanding the cause(s) of UCPPS, improved diagnosis, better characterization of patients to help identify more effective treatments, and ways to prevent onset.

## A New Approach

Historically, much of the NIDDK-supported research on causes and treatments for IC/BPS and CP/CPPS focused on the bladder and prostate, respectively. This was because these organs and tissues were thought to be the sites of origin for the pain and other symptoms suffered by persons diagnosed with UCPPS. Despite a broad array of studies exploring many hypotheses, however, researchers were unable to find satisfactory medical explanations for symptoms or to identify definitive risk factors or viable therapies, with the exception of a single study suggesting that myofascial physical therapy might be effective in some IC/BPS patients. At the same time, a growing body of evidence was revealing that many people with UCPPS frequently have other, overlapping chronic pain conditions, such as irritable bowel syndrome

(IBS) and fibromyalgia. Taken together, these outcomes and findings suggested that, at least in some people, UCPPS might not be a localized pain condition, but instead be part of a global pain process involving the central nervous system (*i.e.*, the brain and spinal cord) and potentially other body systems, such as the immune system. These insights also suggested that there might be a greater degree of diversity among persons grouped together under a UCPPS diagnosis than was initially thought.

In 2008, armed with these new perspectives and input from the scientific and health advocacy communities, the NIDDK launched Phase I of the MAPP Research Network. Established through a Request for Applications, this phase of the Network comprised six “Discovery Sites” at research institutions across the country, complemented by a Data Coordinating Center to manage and store clinical data and a Tissue and Technology Center to centrally process, store, and disburse clinical samples. This novel, multi-site research network embraced a unique, systemic (whole-body) approach to the study of IC/BPS and CP/CPSS to address clinically relevant questions. The Network gathered together scientists with diverse research expertise—including basic science, clinical urology, behavioral science, immunology, epidemiology, neurobiology, psychology, chronic pain, neurobiology/neuroimaging and infectious diseases—all working collaboratively to better understand UCPPS. In addition to moving beyond traditional bladder- and prostate-specific research directions to an innovative, multidisciplinary strategy for studying UCPPS, Network scientists sought to investigate potential relationships between these syndromes and the other chronic pain conditions that are often seen in people with UCPPS, such as IBS, fibromyalgia, and chronic fatigue syndrome. Thus, Network leaders included

not only urologists, but investigators specializing in these overlapping pain conditions.

## Foundation Studies and Novel Findings

In Phase I, the MAPP Research Network recruited 424 men and women with UCPPS in a central, Trans-MAPP Epidemiology and Phenotyping study to better understand how these conditions progress over time (the natural history) and to learn if patients might fall into different, distinguishable subgroups based on differing symptoms that may arise from different causes—and, thus, may require different treatments. To achieve its goals, the Network also recruited “control” participants—both healthy persons without any pain syndromes (415 individuals), and those who have one or more of the overlapping chronic pain conditions (200 individuals).

The Network also conducted a number of other collaborative studies across sites complementing the central Trans-MAPP study—for example, there is evidence that alterations in the brain and spinal cord play important roles in chronic pain, so brain-imaging studies looking at structure and function were conducted across the Network using standardized protocols. Other efforts included various studies to identify biomarkers of disease, to assess the possible role of infectious agents, and to provide a systemic view of disease. The development and assessment of clinically relevant animal models of UCPPS has also been part of the Network’s efforts. In addition, individual Network sites have conducted studies to test ideas complementary to the Trans-MAPP central clinical study. Importantly, the Network was structured so that investigators would draw upon a shared pool of participant data and samples from across the Network collected using common protocols.

To establish the central Trans-MAPP study, people with IC/BPS or CP/CPPS and control participants were initially asked to fill out a number of questionnaires covering a variety of topics, including urologic pain, emotional state, other types of pain, and other symptoms and quality of life issues. They were also asked to provide blood and urine samples for use in some of the research studies. Additionally, a subset of participants engaged in a simple pressure pain threshold procedure that is a way to directly assess pain sensitivity. For participants with UCPPS, this initial, in depth clinic-based visit was followed by two additional visits at 6 and 12 months after enrollment. During that year, these participants were also registered with an Internet-based system so that they could fill out assessments of their symptoms every 2 weeks. This regular symptom assessment, a key component of the central epidemiological study, was a particularly valuable tool, as it has enabled MAPP Research Network scientists to learn a great deal about UCPPS symptoms, symptom fluctuations, and their possible correlations with other factors. The data and samples collected for the central study have also served as a crucial resource for other collaborative studies and site-specific studies in the Network.

During Phase I, the Network made significant progress on a variety of fronts, and results of Network analyses continue to emerge in scientific meetings and in peer-reviewed publications. Recent scientific reports describing novel advances in four major areas—clinical research tools, symptom flares, brain changes, and biomarkers/potential mediators—are summarized below.

### **Clinical Research Tools**

As described previously, a large part of the central trans-MAPP study consisted of participants completing a variety of questionnaires to

describe the key symptoms of pain and bladder dysfunction, as well as issues such as depression, sleep quality, and general quality of life. While a few of these questionnaires were developed specifically for the MAPP Research Network, the majority have already been employed in health care settings and in clinical research to assess the impact of UCPPS on individuals and the outcomes of clinical trials, respectively. In the past, many of these questionnaires were used to generate a composite “score” for UCPPS that combined pain and bladder symptoms. However, a recent analysis of data from a subset of questionnaires administered in the trans-MAPP study allowed Network scientists to determine that such a composite score can “mask” independent responses in each of these areas—i.e., their analysis suggests that improvement or worsening can occur in pain independently of bladder symptoms, and vice versa, and that a composite score limits the ability of researchers and clinicians to detect such changes. In addition, they found a differential impact of these key symptoms on an important comorbidity, depression: only pain symptoms were associated with depression. These results indicate that, going forward, pain and urinary symptoms should be scored independently in UCPPS to enable more accurate research analyses and improved patient care.

### **Symptom Flares**

People with UCPPS have reported suffering from symptom “flares”—brief or extended periods of time when symptoms intensify. Understanding flares is important both for research studies—in which flares need to be taken into account when assessing the success of a therapeutic intervention for UCPPS, for example—and also in developing clinical tools to measure and improve patient quality of life. Network scientists conducted a collaborative study at four Discovery Sites to learn

more about the impact of flares on individuals' lives, as well as gain more insight into associated triggers and treatment. The study involved eight focus groups (two groups per site) of women with IC/BPS, for a total of 57 individuals.

As a result of the moderated focus group discussions and accompanying questionnaires, the researchers found that flares were common and varied widely in their nature (e.g., pain, diarrhea, nausea), intensity (moderate to severe), frequency (daily to once a year or less), and duration (minutes to years); there were also some distinctions in frequency and duration between mild or moderate flares and the more severe flares. The most disruptive flares seemed to be those that were painful in nature, were accompanied by bladder symptoms, and lasted for days. Most participants could identify at least some of their triggers, though not all. Examples included stress, diet, allergies, medications, brand of toilet paper, and emotional state. Also, many triggers were individual specific—e.g., exercise could be a trigger in one person and a management strategy in another. Participants described approaches they used for preventing and self-managing flares but, crucially, they also conveyed the immediate and longer-term impacts of flares, from having to cancel social engagements, to living in a state of constant vigilance and anxiety (which in turn affected family and other relationships), to losing jobs and educational opportunities due to the severity of flares. The very negative impact of flares suggests that future research focused on preventing and mitigating flares would have a positive impact on quality of life for these individuals. This study also revealed how a sense of control over some aspect of symptoms (through medication or other means) is an important coping mechanism for many persons experiencing flares, especially as the unpredictability of flares is part of

their negative impact; thus, empowering patients through, e.g., discussing treatment strategies for flares, could be integrated into clinical care.

### Brain Changes

Brain changes have been observed in individuals suffering from a variety of painful conditions, but identifying changes in brain structure and function in large numbers of well-characterized people with UCPPS in a standardized manner had not been attempted previously. Network scientists recently reported a variety of differences in brain structure and function between women and men with UCPPS and healthy counterparts. For example, one study focused on “white matter,” the structures that facilitate communication of information between and within brain regions. Using an imaging technique that detects a marker of white matter structural integrity, Network researchers found that, compared to healthy controls, women with IC/BPS exhibited white matter abnormalities in several different brain regions. Moreover, these alterations appear to be clinically relevant, as they correlated variously with pelvic pain severity, urologic symptoms, and quality of life as reported by participants. These results complement a prior Network study of women with IC/BPS that found that increases in pain and mood disturbance were associated with increases in the volume of “gray matter”—the brain tissues responsible for cognition, sensory perception, emotion, and muscle control, which can form connections via white matter.

Network scientists have also used a technique called functional magnetic resonance imaging (fMRI) to explore how brain regions work together to produce pain or how they may be modified in the context of chronic pain, and to possibly identify signature alterations in this “functional connectivity” germane to UCPPS. Comparing fMRI brain scanning data from 45

women with UCPPS but no comorbid conditions and 45 healthy controls, scientists found significant alterations in functional connectivity between several brain regions and networks in symptomatic women while at rest—i.e., not actively engaged in tasks. These alterations included decoupling of two brain regions from the brain's "default mode network," a pattern of brain activity that is engaged when people are involved in undisturbed, task-free, introspective thought—suggesting that persons with UCPPS may experience dysfunction in this default network. Moreover, the two decoupled regions exhibited altered functional connectivity—both increases and decreases—with other brain regions, including ones involved in pain; sensory, motor, and emotion regulation processes; reward; and higher executive functioning. These latter alterations were associated with clinical and behavioral measures reported by the participants within 48 hours of their brain scans, including pain, anxiety, and self-esteem, and may reflect a literal shift in brain focus in persons with UCPPS from introspective thought towards aspects of pain and emotion regulation.

In another study, Network scientists used fMRI to examine the relationship between chronic pain and brain involvement in pelvic floor muscle control in men with CP/CPSP. In addition to experiencing pain in this area, men with CP/CPSP are known to have abnormalities in pelvic floor muscle activity. In the study, researchers first identified in healthy men a brain region involved when actively contracting pelvic floor muscles, as well as a distinct region involved in muscle contraction in a non-painful area in men with CP/CPSP (the right hand). They then used brain scans from multiple Discovery Sites to look at the functional connectivity of these

regions in both men with CP/CPSP and healthy men while at rest to see if there were differences that could help explain the altered pelvic floor muscle activity in men with CP/CPSP. They found that, compared to functional connectivity of the hand control region, there was a significant alteration in functional connectivity of the brain region involved in pelvic muscle control in men with CP/CPSP versus healthy controls. The alteration affected functional connectivity to a brain region involved in processing and providing an emotional response to a broad spectrum of sensory inputs from the body (e.g., it is involved in experiences such as food cravings, nausea, pain, and disgust). This altered functional connectivity was significantly associated only with pain symptoms and not with other symptoms experienced by men with CP/CPSP, and the degree of alteration tracked with the severity of pain symptoms reported by participants. This study is the first to identify brain activity changes in men with CP/CPSP compared to healthy men, points to an important role for brain control of muscle activity in this disorder, and suggests a possible signature alteration that could be explored both as a biomarker of treatment success and a predictor of response to treatment.

These findings contribute significantly to the growing body of evidence for involvement of the central nervous system (CNS) in UCPPS. Importantly, as all of these studies are "snapshots" of the brain at one point in time, it remains unclear whether the structural and functional changes are causes or consequences of UCPPS. However, these findings can now be pursued to determine the potential role(s) of these differences in symptom manifestation, maintenance, and amelioration.

### **Biomarkers/Potential Disease Pathways**

Network scientists are pursuing a variety of hypotheses and efforts to identify molecules or biological changes, or “biomarkers,” that are easily detected and consistently associated with some aspect of UCPPS. Moreover, some biomarkers for UCPPS may differ among individuals and thus could potentially distinguish subgroups of people with this condition who may benefit from different therapies. (This approach would be similar to testing for *BRCA1* gene mutations to help in selecting a specific cancer therapy.) In a recent study, scientists at a Phase I Discovery Site investigated certain inflammatory responses as potential indicators of underlying biological processes in UCPPS, and whether they are also associated with differing pain profiles in some people with UCPPS. Inflammation is a bodily process that is normally used to help defend against infection; some typical signs of inflammation are redness, heat, and pain. However, if inflammation is activated inappropriately, people can suffer needlessly from its effects, including pain. In a prior study, the research team had found that there was an association between pelvic pain symptoms in women with IC/BPS and heightened inflammatory responses mediated by two cellular proteins called toll-like receptor (TLR)-4 and TLR-2. Building on these findings, the scientists investigated whether these inflammatory responses could further differentiate between women experiencing pelvic pain and women also reporting widespread pain outside the pelvic area. The magnitude of TLR-mediated inflammatory responses can be detected in a laboratory test using blood samples. Comparing the results of blood sample tests to pain symptom data from participants at their Discovery Site, the scientists found that women with IC/BPS who had a higher than average TLR-4-mediated inflammatory response were significantly more likely to be

reporting pain symptoms outside of, as well as in, the pelvic area. As might be expected from the first finding, TLR-4-mediated inflammatory responses were also higher among women with IC/BPS who had been diagnosed with one or more overlapping pain conditions. While additional studies will be needed in a larger and more diverse sample of women, these findings suggest that the magnitude of TLR-4-mediated inflammatory responses may be a biomarker that can differentiate between subgroups of women diagnosed with IC/BPS in a way that could advance both clinical research and clinical care.

Other findings emerging from Phase I Network studies include insights into the course of UCPPS in women and men; differences between people with UCPPS and healthy controls in microbes associated with the bladder; and identification of clinical characteristics that could help differentiate potentially relevant subgroups among participants with UCPPS.

### **Next Steps**

In light of the progress, novel findings, important new research resources, and resulting new hypotheses developed during Phase I of the MAPP Research Network, the NIDDK decided to support continuation of its efforts. With co-funding from the NIH Office of Research on Women's Health (ORWH), the NIDDK issued a second set of Requests for Applications and in FY 2014 renewed the MAPP Research Network for a second 5 year phase. In Phase II, the Network has been enhanced by the integration of three additional Discovery Sites.

As Network studies move forward, investigators are building upon Phase I discoveries and continuing efforts to provide a foundation for

effective clinical interventions for IC/BPS and CP/CPSP. For example, Network researchers are engaged in a multi-faceted Trans-MAPP Symptom Patterns Study (SPS) designed to better understand symptom change profiles over time and associated biological changes and risk factors. The SPS includes studies to help clarify whether structure and functional changes in the brain are cause or consequence of UCPPS, by looking at participant brain images over time. Other SPS studies include evaluating promising biomarkers/potential mediators identified in Phase I, and pursuing identification of UCPPS patient subgroups defined by differences in clinical symptoms and underlying biological factors. The Network is also implementing the results of the questionnaire analysis described previously to score pain and urinary symptoms separately. To better understand findings from the clinical research, Network researchers will study animal models to examine possible biological mechanisms underlying UCPPS. They will also explore observations made initially in animal models of UCPPS to determine their relevance to humans. Finally, in Phase II the Network is expanding its collaborative efforts to include scientists outside of the Network itself—including other NIDDK-supported research networks—thereby increasing the number of and speed with which critical scientific questions can be pursued, to the ultimate benefit of persons living with or at risk of developing UCPPS.

More information can be found at the MAPP Research Network website:  
[www.mappnetwork.org](http://www.mappnetwork.org)

Farmer MA, Huang L, Martucci K,...Apkarian AV; MAPP Research Network. Brain white matter abnormalities in female interstitial cystitis/bladder pain syndrome: a MAPP Network neuroimaging study. *J Urol* 194: 118-126, 2015.

Fitzgerald MP, Anderson RU, Potts J,...Nyberg LM; Urological Pelvic Pain Collaborative Research Network. Randomized multicenter feasibility trial of myofascial physical therapy for the treatment of urological chronic pelvic pain syndromes. *J Urol* 189: S75-S85, 2013.

Griffith JW, Stephens-Shields AJ, Hou X,...Landis JR. Pain and urinary symptoms should not be combined into one score: Psychometric findings from the Multi-disciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network. *J Urol* Nov 13 pii: S0022-5347(15)05226-X, 2015. [Epub ahead of print]

Kairys AE, Schmidt-Wilcke T, Puiu T,...Harris RE. Increased brain gray matter in the primary somatosensory cortex is associated with increased pain and mood disturbance in patients with interstitial cystitis/painful bladder syndrome. *J Urol* 193: 131-137, 2015.

Kutch JJ, Yani MS, Asavasopon S,...Mayer EA. Altered resting state neuromotor connectivity in men with chronic prostatitis/chronic pelvic pain syndrome: A MAPP Research Network Neuroimaging Study. *Neuroimage Clin* 8: 493-502, 2015.

Martucci KT, Shirer WR, Bagarinao E,...Mackey SC. The posterior medial cortex in urologic chronic pelvic pain syndrome: detachment from default mode network—a resting-state study from the MAPP Research Network. *Pain* 156: 1755-1764, 2015.

Schrepf A, Bradley CS, O'Donnell M,...Lutgendorf S; Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) Research Network. Toll-like receptor 4 and comorbid pain in Interstitial Cystitis/Bladder Pain Syndrome: a Multidisciplinary Approach to the Study of Chronic Pelvic Pain Research Network Study. *Brain Behav Immun* 49: 66-74, 2015.

Sutcliffe S, Bradley CS, Clemens JQ,...Berry SH. Urological chronic pelvic pain syndrome flares and their impact: qualitative analysis in the MAPP network. *Int Urogynecol J* 26: 1047-1060, 2015.

## PROSTATE DISEASE RESEARCH

### Benign Prostatic Hyperplasia—Gaining Insight and Potential New Treatment Identified in

**Mice:** Two recent studies have explored the role of fibrosis in benign prostatic hyperplasia (BPH). The prostate is a male gland about the size and shape of a walnut. It surrounds the urethra just below the bladder, where it adds fluid to semen before ejaculation. The prostate gland commonly becomes enlarged as a man ages. This condition is called benign prostatic hyperplasia (BPH) and is caused by the non-cancerous (benign) growth (hyperplasia) of two different cell types—epithelial and smooth muscle—in regions of the prostate called nodules. As the prostate enlarges, it may squeeze the urethra and affect the flow of the urinary stream. The lower urinary tract symptoms (LUTS) associated with the development of BPH rarely occur before age 40, but more than half of men in their sixties, seventies, and eighties have some LUTS. The most common symptoms vary, but involve changes or problems with urination, such as a hesitant, interrupted, weak stream; urgency and leaking or dribbling; more frequent urination, especially at night; and urge incontinence. Previous studies have suggested that formation of fibrosis in the prostate gland contributes to the development of BPH and LUTS. During pathological fibrosis, normal structures are replaced by scar tissue, which is characterized by excess production and deposition of proteins such as collagen—thus, collagen is often used as a marker of fibrosis.

One group of researchers sought to evaluate the contribution of fibrosis, specifically collagen, to the development of BPH in men. Normal prostate tissue was obtained from patients undergoing prostatectomy for surgical management of prostate cancer. BPH

nodule tissue was obtained from patients during prostate resection surgery (a procedure to remove part of the prostate gland in order for urine to more easily flow through the urethra). Total collagen content was determined to be similar between normal and BPH tissue samples. However, a significant increase in a type of collagen pattern called “thicker collagen bundles” was noted in BPH tissue compared to normal tissue—suggesting that these bundles may play a role in BPH. Medical management of LUTS due to BPH includes the use of prescription medications called  $\alpha$ -blockers (e.g., tamsulosin) and 5 $\alpha$ -reductase inhibitors (e.g., finasteride). Collagen levels in BPH prostate samples obtained from patients treated with either  $\alpha$ -blockers or 5 $\alpha$ -reductase inhibitors were compared to BPH prostate samples from patients not treated with medications. Collagen levels in tissue samples were found to be similar in medication-treated men compared to men not taking either of the two medications, suggesting that these medications do not lead to a decrease in fibrosis within the prostate.

Using a mouse model of bacterial-induced prostate inflammation, a second group of investigators examined the reversibility of fibrosis after eliminating the infection and inflammation with an antibiotic treatment. In this model, uropathogenic *Escherichia coli* (*E. coli*) bacteria (a leading cause of urinary tract infections) are placed in the urethra in close proximity to the ejaculatory ducts of the prostate, whereas saline is placed in the urethra in close proximity to the ejaculatory ducts of mice that serve as controls. Mice whose prostates were infiltrated with *E. coli* produced more collagen in their prostates compared to control animals. The investigators further showed that antibiotic treatment completely eliminated the bacterial infection and partly reduced the accompanying collagen buildup in the prostate.

These studies provide new information on the potential role of fibrosis in BPH. A better understanding of fibrosis, in general, could yield insights into how this process unfolds in other tissues (e.g., kidney), potentially opening new avenues to therapy for a range of conditions and diseases.

Bauman TM, Nicholson TM, Abler LL, ...Ricke WA. Characterization of fibrillar collagens and extracellular matrix of glandular benign prostatic hyperplasia nodules. *PLoS One* 9: e109102, 2014.

Wong L, Hutson PR, and Bushman W. Resolution of chronic bacterial-induced prostatic inflammation reverses established fibrosis. *Prostate* 75: 23-32, 2014.

## BLOOD CELL DEVELOPMENT AND SURVIVAL

**Elegant Study Documents How Hematopoietic (Blood) Stem Cells Find Their Niche:** A new study extends our understanding of how the hematopoietic stem cell (HSC) microenvironment, or “niche,” promotes the survival and function of these cells. HSCs, a type of stem cell, are able both to self-renew and to develop into any kind of blood cell. Immature HSCs arise from cells in a vascular structure called the dorsal aorta and then travel to the intermediate site before arriving at their final location in the body. In the model organism zebrafish, these sites are the tail and the adult kidney; in mice and other mammals, they are the fetal liver and bone marrow, respectively. The sites in these tissues in which the HSCs reside are termed niches, and the cell types and factors that interact with the immature HSCs have been of interest to investigators. Strategies that promote the interaction between the HSC and its niche may lead to greater numbers of blood stem cells that could be acquired from a blood or tissue

donor, which would be therapeutically advantageous for bone marrow and other transplant procedures.

To further understand how HSCs occupy the niche, researchers studied the intermediate maturation sites of HSCs. Because zebrafish embryos are transparent, the investigators “tagged” HSCs by genetic manipulation to allow live imaging of individual cells taking up residence. The researchers discovered a multi-step, dynamic remodeling process between the HSC and its niche in the zebrafish tail. On arrival, the HSC adheres to the inside of the blood vessel wall. Next, the HSC moves from the inside of the blood vessel wall to the outside of the blood vessel wall. A small group of endothelial cells from the blood vessel wall then surround and form a pocket around the HSC, creating a space for it. A similar process of HSC binding and pocket formation was observed in mouse fetal liver. This suggests that this mechanism may be a common feature of HSC-niche interactions across multiple species.

Further studies in zebrafish showed that the HSC is also either in direct contact or very near to a different type of cell, a mesenchymal stromal cell, in this pocket. The stromal cell seems to help the HSC to divide to form two daughter HSCs. These observations were confirmed using electron microscopy, which offers much higher resolution images than light microscopy.

Current and future research efforts may include the study of the cellular dynamics in the mammalian adult marrow and the identification of small molecules that have the ability to promote critical interactions within the niche and foster an expansion of the HSC population.

Tamplin OJ, Durand EM, Carr LA, ...Zon LI. Hematopoietic stem cell arrival triggers dynamic remodeling of the perivascular niche. *Cell* 160: 241-252, 2015.

## HOW CELLS WITH A CHOICE CHOOSE THEIR FATE

**Learning To Leverage the Potential of Pluripotent Stem Cells:** Results from a new study of mouse cells provide insight into the factors that contribute to the behavior of pluripotent stem cells (PSCs). Scientists have developed ways of reprogramming cells, such as those derived from blood or skin, to revert back to an embryonic stem cell-like state. PSCs—including embryonic stem cells and cells experimentally induced to be pluripotent—have the potential to give rise to more stem cells (self-renewal) and to cells of many different types of tissues (pluripotency). Scientists have been grappling with how to determine what controls or drives PSCs to either self-renew or undergo a state of pluripotency, which could lead ultimately to becoming more specialized cells. The ability or knowledge to control the path a PSC takes will allow for more efficient use of this cell in disease treatments or regenerative medicine.

Using an approach called single-cell expression profiling, researchers have begun to understand what cell and tissue types will arise from an individual PSC. In single-cell expression profiling, the “transcriptome” is characterized to provide a measure of a single cell’s gene activity. Active genes produce transcripts, which may have independent functions or may serve as instructions for making proteins. A transcriptome is a collection of all the transcripts present in a given cell. Because of technical limitations, most gene activity studies are performed on cell populations rather than an

individual cell. As the individual cells within a cell population may be quite variable in terms of their respective gene activity, this could have profound implications for understanding biological responses under differing experimental conditions. Using the single-cell expression profiling, the researchers were able to learn that different classes of genes have differing variability in PSCs. For example, “housekeeping” genes, which are required for basic cellular function, showed consistent activity across individual cells. Genes involved in signaling pathways, however, showed more variability. The variability of signaling pathways may be characteristic of the different types of cells that a PSC could become. From the extensive data obtained, the researchers constructed a model that reflected why a PSC would toggle from self-renewal to pluripotency. They identified several regulatory molecules called miRNA-294, miRNA-148, and Let-7—some of which drive a cell toward pluripotency and potentially specialization, and others toward self-renewal as stem cells.

The ability to analyze the transcriptome at the single cell level has allowed investigators to begin to identify regulatory circuits governing transitions between pluripotent and self-renewal cell states. This foundational information is critical to developing strategies to “program” PSCs to address needs associated with disease and injury.

*Kumar RM, Cahan P, Shalek AK,...Collins JJ. Deconstructing transcriptional heterogeneity in pluripotent stem cells. [Nature](#) 516: 56-61, 2014.*

## Erythroferrone – New Regulator of Iron Balance

The proper maintenance of blood iron levels is complex, and multiple diseases can result when iron balance goes awry. Hepcidin, a small protein made in the liver, has been previously shown to play a key role in preserving proper iron balance. Critically important to the overall understanding of iron balance is the recent discovery of erythroferrone—with potential implications for treating multiple blood disorders and diseases.

Iron is essential to the body's oxygen-delivery system. Humans need iron to make hemoglobin, the oxygen-carrying molecule in red blood cells. Most of the 3 to 4 grams (0.1 to 0.14 ounces) of iron in adults is in hemoglobin. Much of the remaining iron is stored in the liver, spleen, and bone marrow. Because excess iron damages tissues, total body iron is carefully regulated, with most of it being constantly recycled. While small amounts are absorbed daily via the digestive tract, about 10 times more iron is simply retrieved from aged red blood cells and reused. A protein called transferrin picks up this iron, along with dietary iron that has been absorbed via the digestive tract. Transferrin then carries the iron to the bone marrow, where it is used to produce new red blood cells. Unfortunately, the human body does not seem to have an efficient or regulated way to rid itself of excess iron.

If insufficient iron flows to the bone marrow, normal red blood cell production drops and anemia can result. Thus, an important, and the most common, cause of anemia is iron deficiency, which can be corrected through administration of iron supplements. Another form of anemia, however, is associated with inflammation. Called the anemia of inflammation and chronic diseases, this condition affects people who have infections, chronic inflammatory disorders—such as rheumatoid arthritis—and many other chronic disorders, including cancers. Patients with this form of anemia typically have inadequate red blood cell production, low levels of iron in the blood, and low levels of transferrin; they may also be resistant to the effects of erythropoietin, the hormone that normally stimulates and regulates red blood cell production.

Patients with anemia of inflammation and chronic diseases are usually not iron-deficient. Instead, the iron balance in their bodies has been altered, such that more iron is sequestered in the cells involved in iron recycling and absorption, as well as in liver cells that store iron. The cellular sequestration of iron leaves less available for transport to the bone marrow. Attempts to treat this condition with oral iron supplements typically do not work (a condition referred to as iron-refractory anemia), even though this form of anemia mimics iron deficiency.

# STORY OF DISCOVERY

Anemia of inflammation generally improves if the underlying condition resolves.

An additional form of anemia can arise in people with a condition called thalassemia. Characterized by the under-production of normal hemoglobin, some people with thalassemia are treated with blood transfusions to provide much-needed red blood cells. While improving anemia, these transfusions can also lead to iron overload.

## Regulation of Systemic Iron Levels by Hepcidin

NIH-supported research showed that hepcidin, a small protein produced in the liver, is the master regulator of iron absorption and tissue distribution. Hepcidin was identified in 1998 in a search for small molecules active in “innate immunity,” the body’s first line of defense against invading bacteria, fungi, and other microorganisms.

In 2001, a research team in France found that when the levels of hepcidin were disrupted in mice, the animals developed iron overload, while mice that were genetically altered to “turn on” the *Hepcidin* gene to a higher level than normal were severely anemic and died within hours of birth. In 2002, while studying abnormally high iron storage levels in the liver, using a mouse model of the most common form of inherited iron overload (hereditary hemochromatosis), researchers found that the *Hepcidin* gene was turned off to

a greater extent in the mice with the excess liver iron compared to normal mice. When rats were fed an iron-abundant diet and then switched to an iron-deficient diet, investigators reported that the *Hepcidin* gene was significantly turned off in the liver while genes encoding iron transporters were significantly turned on in the digestive tract.

From this research, hepcidin emerged as a fundamental regulator of iron balance that inhibits iron absorption and iron release from tissue stores when iron levels in the blood are high, and eases off when blood iron levels decline. When the *Hepcidin* gene was always turned on, the iron accumulation normally seen in two different mouse models of iron overload was prevented. Mechanistically, hepcidin was shown to bind to the iron transport protein ferroportin and induce its destruction, thereby leading to both decreased iron absorption and release of iron into the blood. In a proof of principle of its systemic action, investigators observed a significant decrease in blood iron levels within an hour when mice were injected with hepcidin. Thus far shown to detect elevated or diminished human hepcidin protein levels in a spectrum of human diseases and conditions, a clinical assay to standardize measurement of human hepcidin is under development for commercial release to the clinical community. NIH investments in discovery research have provided a clear understanding of the role of hepcidin in normal physiology and its role in certain disease conditions.

# STORY OF DISCOVERY

## Discovery of Erythroferrone as New Regulator of Iron Balance

During times of acute blood loss, there is an immediate need for the bone marrow to produce new blood cells, including red blood cells (RBCs), to replenish lost cells. Newly made RBCs demand an ample supply of iron—a component of hemoglobin. Erythropoietin drives RBC production within a few hours following blood loss, and this process continues for several days. Just how the body suppresses the action of hepcidin to allow increased iron absorption and mobilization from stores has been unclear. Recent NIH-supported research has been instrumental in identifying a key factor responsible for controlling the supply of iron needed for RBC production.

Research conducted in the mid-2000s strongly suggested that the factor responsible for hepcidin suppression during RBC production arose from the bone marrow. This critical piece of the puzzle came from a study in which drugs were used to interfere with RBC production in mice, and then the mice were subjected to blood loss. These animals lost their ability to turn off the *Hepcidin* gene, in contrast to animals not treated with these drugs.

NIDDK-supported scientists have conducted seminal studies designed to identify and characterize the factor emanating from the bone marrow that regulates hepcidin. For these studies, they used an animal model (male mice). They found that the time needed for erythropoietin administration or blood loss to significantly turn off the *Hepcidin* gene in liver or decrease hepcidin

protein blood levels was between 4 and 9 hours. Thus, the unknown hepcidin suppressor must be produced in the bone marrow within the first 4 hours following erythropoietin administration or blood loss.

While evaluating the set of bone marrow genes turned on in mice subjected to blood loss, a previously uncharacterized gene was identified that was turned on within 4 hours of blood loss and was predicted to encode for a secreted protein. In this case, the protein must be able to exit the bone marrow cell and travel to the liver to exert its anti-hepcidin activity. This protein was named “erythroferrone” (*Erfe*), as it functions as a link between production of red blood cells (erythrocytes) and the regulation of iron (which has a Latin name of ferrum). Erythropoietin administration in mice was also shown to significantly turn on the *Erfe* gene in bone marrow. The bone marrow cell types responsible for turning on *Erfe* were shown to be the developing RBCs called erythroblasts. Mice genetically engineered to lack a functional *Erfe* gene were incapable of turning off the *Hepcidin* gene. To further confirm that *Erfe* suppressed hepcidin activity, they administered laboratory-made *Erfe* protein to the mice, and found that it turned off the *Hepcidin* gene in liver and reduced hepcidin protein levels in blood. In a mouse model of  $\beta$ -thalassemia that recapitulates features of the human disease, including low levels of hepcidin in blood and iron overload in the liver, the *Erfe* gene was turned on to a significantly higher level in bone marrow compared to normal mice. When the *Erfe* gene was inactivated in the mouse model, hepcidin expression was restored and iron overload in liver

# STORY OF DISCOVERY

reduced—providing an initial proof of concept that this pathway may be useful to prevent iron overload in patients with thalassemia.

This groundbreaking study, published in 2014, was the first to demonstrate that *Erfe* is a biologically important regulator of hepcidin, with implications for supplying iron on demand for RBC production during blood loss. In addition, further experiments conducted in this study suggest that *Erfe* may serve as a target for treatment or prevention of iron overload in patients with  $\beta$ -thalassemia.

Building upon the critical finding that *Erfe* plays an important role in hepcidin suppression, NIDDK-supported researchers designed experiments to evaluate whether *Erfe* contributes to recovery from the anemia of inflammation and chronic diseases. Examining male mice with bacterial-induced anemia of inflammation, the researchers found that the *Erfe* gene was significantly turned on in the bone marrow. To gain insight into *Erfe*'s role in responding to bacterial-induced anemia of inflammation, the researchers compared normal mice to genetically engineered mice that could not produce *Erfe* (*Erfe*-deficient). Compared to normal mice, the *Erfe*-deficient mice: 1) exhibited a more severe form of anemia of inflammation (greater loss of hemoglobin), 2) turned on the *Hepcidin* gene to a significantly higher level, 3) were less capable of properly regulating serum hepcidin levels, 4) had significantly lower blood iron levels, and 5) had prolonged production of serum erythropoietin, reflecting the body's attempt to compensate for low hemoglobin levels. In addition, the

*Erfe*-deficient mice produced greater numbers of immature RBCs, as a step toward replenishing mature RBCs. However, these immature RBCs were smaller than those produced by the normal mice, because less iron was available for blood cell use in the *Erfe*-deficient mice. Taken together, this set of experiments highlights the important contribution *Erfe* makes to the recovery of anemia of inflammation by turning down hepcidin and increasing blood iron levels.

## Looking to the Future

As described in this story, knowledge gained from studying hepcidin has led to the identification of *Erfe* as a new regulator of iron balance in mammals. Future studies will determine how *Erfe* exerts its mechanism of action in liver to suppress hepcidin. If *Erfe* functions similarly in humans, then future studies may lead to potential therapies targeting *Erfe* or hepcidin for disorders of iron balance—absorption, storage, and mobilization.

The NIDDK continues to support a robust portfolio of research projects designed to shed new light on iron homeostasis—the body's establishment and maintenance of iron balance. For example, investigators are exploring various strategies for increasing hepcidin blood levels and bolstering the effectiveness of chelation therapy for iron overload. Other investigators, funded via the Stimulating Hematology Investigation: New Endeavors (SHINE) program, are examining the role of transferrin in red blood cell production and iron balance, and mouse models of iron recycling.

# A Trip to the Emergency Room Prompts One Man To Join a Clinical Trial for the Treatment of Kidney Stones



**Bob Schwarz**

The weather in Washington, DC, was crisp the December afternoon in 2014 when Bob Schwarz walked back to his office after having lunch with a friend. Bob had a long career working with the Peter Pan bus lines, overseeing the company's legislative interests, customer service, marketing, and real estate for 26 years. In his time with the family-owned business, he'd met five U.S. Presidents and been invited to the White House.

Now 67, Bob continues his work in the area of transportation as a government affairs representative for Greyhound Bus Lines and the American Highway Users Alliance. Although he works in Washington, Bob considers

Wilbraham, MA, "home," as does his wife of 27 years, who lives there year round.

As Bob settled back into his office and surveyed the stacks of papers on his desk, he was struck with a sharp, shooting pain in his lower back. "It was unlike anything I had ever experienced before. It felt like someone was putting a hot poker right into my back," he says. Although intense, the pain was also fleeting, leaving just 5 minutes after it arrived.

Bob was working late that evening, and around 8 p.m. his back pain returned, though this time it was worse and accompanied by abdominal pain. "It felt like someone had kicked me and totally knocked the wind out of me," he says. The pain was so intense he walked over to the sofa in his office to lie down, hoping that this second round of pain would pass as quickly as the first. A colleague, concerned about Bob's obvious discomfort, suggested that he go to the emergency room, but Bob demurred, describing himself as "stubborn." Instead, he placed a call to his primary care physician's office in Massachusetts. After hearing his symptoms, Bob's doctor said, "I think what you're describing is a kidney stone." Bob decided that he would wait until the next morning to go to the emergency room. After all, he describes himself as having been "blessed with very good

# PATIENT PROFILE

health" throughout his life, only missing a single day of work in his 40-year career due to illness. Certainly, whatever he was experiencing could wait until the next day. How bad could a kidney stone possibly be?

Reflecting on whether it was a good idea to wait until the next morning before seeking medical treatment for his suspected kidney stone, Bob notes dryly, "Knowing now what I didn't know then, I certainly would not have done that."

## The Urinary Tract and Kidney Stones

If the circulatory system can be thought of as the body's plumbing, the urinary tract represents the body's waste management system. It consists of two kidneys, which filter the blood to remove waste, salts, and excess fluid; the ureters, the two tubes that connect the kidneys to the bladder that stores urine; and a urethra, through which the urine in the bladder is excreted from the body. A kidney stone is a crystal that forms when substances in the urine become highly concentrated and can no longer stay dissolved. Once formed, a stone may remain in the kidney or travel through the urinary tract and be passed out of the body in the urine.

Kidney stones vary in size, but the severity of symptoms is influenced by factors other than simply the size of the stone. Some people who

have small kidney stones may pass them relatively easily and have mild or no symptoms at all. Larger stones can sometimes also pass with little difficulty, if they are smooth and rounded. However, if stones are irregularly shaped or have sharp edges, even small ones can cause great pain, because they can irritate or lodge in the ureter. In severe cases, where the stone cannot be passed, approaches including shockwave therapy to break up the stone into smaller pieces or surgery to remove it may be required.

## The Next Morning

A neighbor accompanied Bob to the emergency room at George Washington University Hospital (GW) the next morning. Once there, the staff took his medical history and questioned him about his symptoms. They determined that he was dehydrated, and started an intravenous drip that included fluids and pain-relieving medications. An abdominal scan revealed a relatively small stone, approximately 3 millimeters, or about one-tenth of an inch, in diameter. As Bob confirms, even a stone of this size can cause "excruciating" pain.

At this point, he was approached by the patient coordinator for a National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-supported clinical study of treatment for kidney stones that was underway at GW. This trial was testing whether

***Reflecting on whether it was a good idea to wait until the next morning before seeking medical treatment for his suspected kidney stone, Bob notes dryly, "Knowing now what I didn't know then, I certainly would not have done that."***

# PATIENT PROFILE

a drug could help stones pass more quickly and/or with less pain; would he be willing to participate? Bob didn't need to think long about his reply. "If I can do something to help future people with kidney stones not go through what I went through, let's do it," he said. With that decision, Bob became a volunteer in the STONE clinical trial.

## The STONE Trial

Study Tamsulosin for urolithiasis in the Emergency department (STONE) is an ongoing, multi-center clinical trial of the drug tamsulosin to treat kidney stones. STONE expects to enroll 500 people through four participating hospitals—the George Washington University Hospital (in Washington, DC), the University of Pittsburgh, the Thomas Jefferson Hospital (in Philadelphia, PA) and the University of Alabama at Birmingham—who come to the emergency room with symptoms of a kidney stone. If the presence of a stone is confirmed via an abdominal scan, patients are asked whether they would like to enroll in the study.

Tamsulosin (which is marketed under the brand name Flomax® and also is available in generic form) is already approved for the treatment of difficult urination caused by an enlarged prostate in men. It is a member of a class of drugs known as "alpha blockers." This medication relaxes the muscles in the bladder neck, the prostate gland, and the ureter, making it easier for urine to pass. Given these effects, many researchers and physicians think that this drug might also increase the rate at which people with kidney stones might successfully pass their stones.

Over the past 10 years or so, several trials have examined the usefulness of this drug in the treatment of kidney stones, with varying results. Many of these studies, however, were small or had other limitations. The STONE clinical trial is larger and more robust than these earlier studies. It is a randomized and placebo-controlled study, meaning that patients are randomly assigned to receive either a placebo (sugar pill) or tamsulosin. Additionally, it is a double-blind trial, meaning that neither the researchers who are examining the patients nor the study participants themselves will know into which arm of the study the patients have been enrolled until the study ends. Furthermore, STONE is testing the effectiveness of tamsulosin in both men and women who have kidney stones. This is important, because tamsulosin has historically been used to treat difficult urination in men with enlarged prostates, and has not been widely studied in women.

## Enrollment, Follow-up, and Resolution

After Bob agreed to enroll in the STONE study, he was given medications and sent home. As with all volunteers, follow-up consisted of contact via telephone, email, or text message several times over the next 29 days, when the participants are asked whether or not they have passed their stone and whether they have experienced any complications or side effects, such as additional medical visits related to their stone, urinary tract infections, dizziness, or headaches. A subsequent abdominal scan is scheduled on or around day 29 to determine definitively whether the stone has passed. A final follow-up conversation takes place on day 90.

# PATIENT PROFILE

Bob was conscientious about his participation in the study. The day-29 scan at GW revealed no sign of a stone in his urinary tract, and he was declared stone-free. Bob reports that he has felt fine ever since his participation in the trial ended. Moving forward, he has been advised to drink more water and cut back on coffee, in order to stay more hydrated and decrease the risk of a subsequent stone.

Because STONE is not yet complete, as of November 2015, neither Bob nor his doctor know whether he was given the placebo or tamsulosin. If, at the end of the trial, tamsulosin is shown to be effective, more widespread use of the drug in treating kidney stones could result in a significant improvement in the quality of life for patients with stones by reducing pain, shortening the time taken to expel the stone, and potentially decreasing the number of patients who require surgery to remove their stones. It could also reduce the so-called "indirect" (i.e., non-medical) costs related to kidney stones, such as days lost from work waiting for the stone to pass. If tamsulosin is shown to be not effective, then patients could be saved the unwanted side effects of the medication, and health care dollars would be saved.

Because Bob splits his time between Washington and Massachusetts, he admits to feeling a bit like

"a stranger in a community" when his kidney stone developed. He credits the staff at GW for helping him navigate his diagnosis and treatment, saying that they were welcoming and efficient, even

working around his schedule when he had an early morning meeting on Capitol Hill the same day as his day-29 scan. "I

can't say enough about the follow-up of staff at the hospital," Bob says, noting that he especially appreciated the team "just being there when I needed them. They treated me like a platinum card carrier." Six months after his kidney stone, Bob happily reported that he had not suffered a recurrence of a stone...and that he had still not taken a sick day off from work.

*In addition to this trial, the NIDDK supports a broad range of studies in urology, including basic research urinary tract biology and clinical trials of novel therapies. The NIDDK supports a George M. O'Brien Urology Cooperative Research Center Program to improve stone disease treatment and a Rare Kidney Stone Consortium. To discuss and prioritize research needs and identify strategies to address those needs, the NIDDK held a workshop titled "Urinary Stone Disease Research Challenges and Opportunities" on April 1-2, 2015, and intends to fund a Urinary Stone Disease Research Network in 2016.*

***"If I can do something to help future people with kidney stones not go through what I went through, let's do it," he said. With that decision, Bob became a volunteer in the STONE clinical trial.***



# Funding Trends and Support of Core Values

*NIDDK's core values emphasize maintaining a strong investigator-initiated R01 program, preserving a stable pool of talented new investigators, supporting key clinical studies and trials, and continuing strong support of training and career development programs, 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 the core values.

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 and posted these data on the NIDDK website. The NIDDK updates the charts

on its website annually; the data shown here were recently updated to include Fiscal Year (FY) 2015.

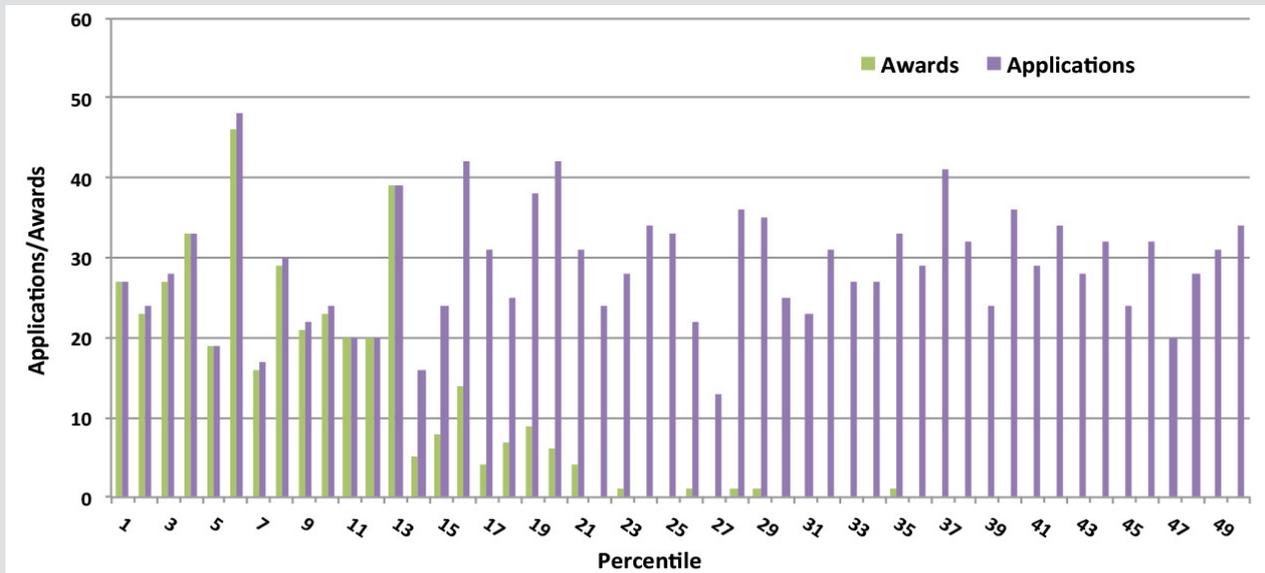
## NIDDK Funding Outcomes for Fiscal Year (FY) 2015 and Historical Application and Funding Trends

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

| Code | Title                                                        | Code      | Title                                                                               |
|------|--------------------------------------------------------------|-----------|-------------------------------------------------------------------------------------|
| K01  | Research Scientist Development Award – Research and Training | R13       | Conference                                                                          |
| K08  | Clinical Investigator Award (CIA)                            | R15       | Academic Research Enhancement Awards (AREA)                                         |
| K23  | Mentored Patient-Oriented Research Career Development Award  | R18       | Research Demonstration and Dissemination Project                                    |
| K24  | Midcareer Investigator Award in Patient-Oriented Research    | R21       | Exploratory/Developmental Grant                                                     |
| K25  | Mentored Quantitative Research Career Development Award      | R24       | Resource-Related Research Project                                                   |
| K99  | Career Transition Award                                      | R34       | Planning Grant                                                                      |
| P01  | Research Program Project                                     | R37       | Method to Extend Research in Time (MERIT) Award                                     |
| R00  | Research Transition Award                                    | SBIR/STTR | Small Business Innovation Research Grants/ Small Business Technology Transfer Grant |
| R01  | Research Project                                             | T32       | Institutional National Research Service Award                                       |
| R03  | Small Research Grant                                         |           |                                                                                     |

# FIGURE 1

**FIGURE 1: NUMBER OF NIDDK COMPETING R01 APPLICATIONS SCORING WITHIN THE TOP 50<sup>TH</sup> PERCENTILE AND NUMBER OF NIDDK COMPETING R01 APPLICATIONS FUNDED IN FY 2015**

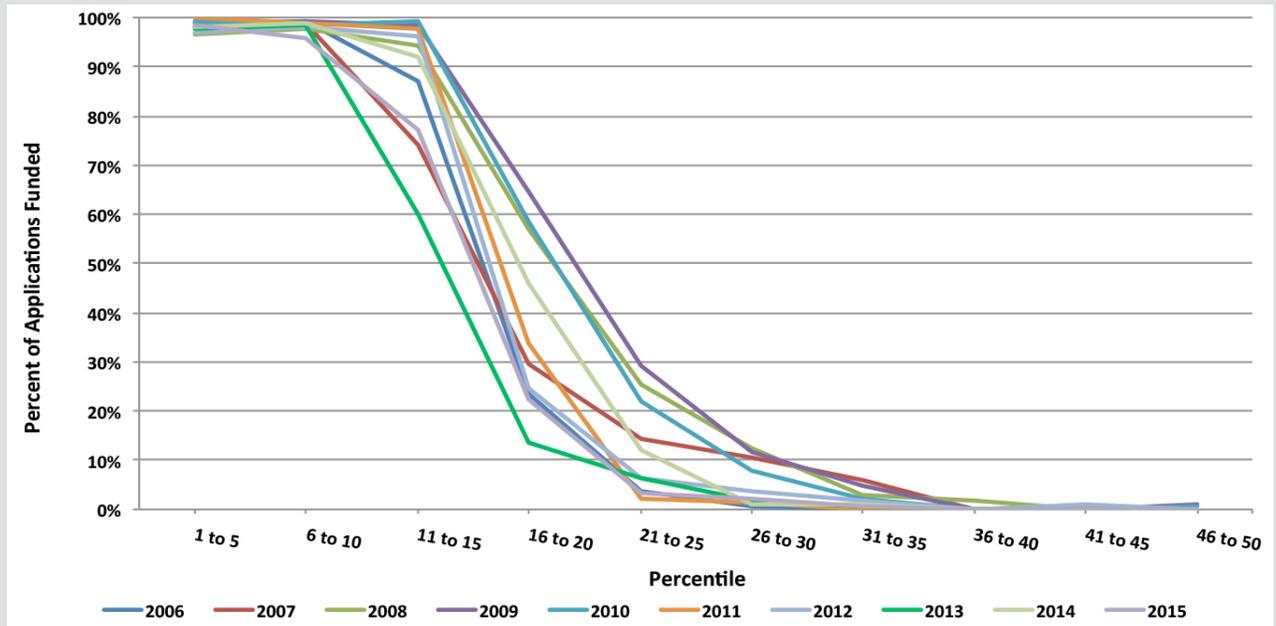


Note: “Applications” shown in the chart above include all applications that scored 50<sup>th</sup> percentile or better. Unscored applications, scored applications with no percentiles, and applications scoring above the 50<sup>th</sup> percentile are not shown (48 percent [n=1,332] of the applications received were unscored or scored above the 50<sup>th</sup> percentile). No unscored applications were funded in FY 2015.

The NIDDK nominal payline in FY 2015 was the 13<sup>th</sup> percentile for established investigators and the 18<sup>th</sup> percentile for Early Stage Investigators (ESIs). The payline and additional programmatic scrutiny for R01 applications requesting more than \$500,000 in direct costs are substantially more stringent. These data show that the NIDDK closely adheres to its payline, but does exercise some programmatic discretion to reach for a limited number of especially innovative or programmatically important applications.

# FIGURE 2

FIGURE 2: NIDDK COMPETING R01 APPLICATION FUNDING CURVES FOR FY 2006-2015



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

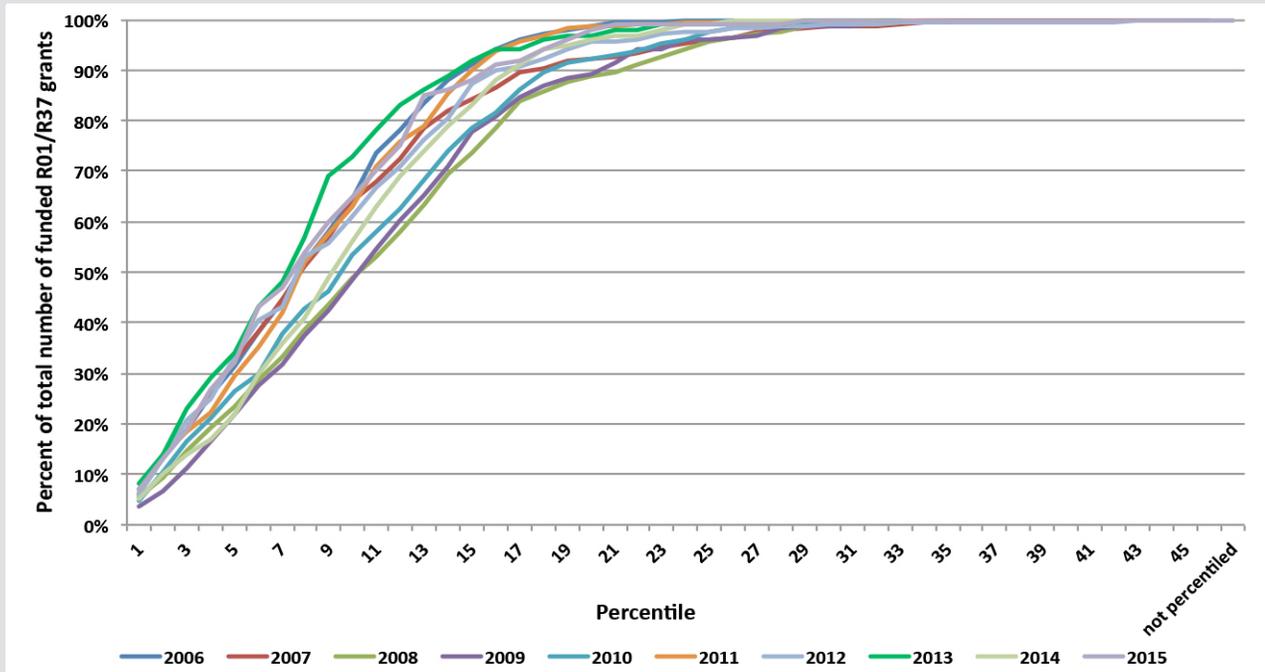
The data demonstrate steep deflections in the percentage of applications funded at the nominal payline for each year. The R01 paylines for the years included in Figure 2 are shown in the table to the right.

| Fiscal Year | General Payline | New Investigator Payline | Early Stage Investigator (ESI) Payline | ESI First Competitive Renewal Payline |
|-------------|-----------------|--------------------------|----------------------------------------|---------------------------------------|
| 2006        | 14              | 16                       | ####                                   | ####                                  |
| 2007        | 13              | 15                       | ####                                   | ####                                  |
| 2008        | 17              | 19                       | ####                                   | ####                                  |
| 2009        | 17              | 19                       | ####                                   | ####                                  |
| 2010        | 17              | 19                       | ####                                   | ####                                  |
| 2011        | 15              | 17                       | ####                                   | ####                                  |
| 2012        | 13              | 13                       | 18                                     | ####                                  |
| 2013        | 11              | 11                       | 16                                     | ####                                  |
| 2014        | 13              | 13                       | 18                                     | ####                                  |
| 2015        | 13              | 13                       | 18                                     | 15                                    |

Note: In FY 2012, the NIDDK began focusing on Early Stage Investigators (ESIs; see definition of and benefits conveyed to ESIs 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)), which is a subset of New Investigators (see also Figures 11 and 12).

# FIGURE 3

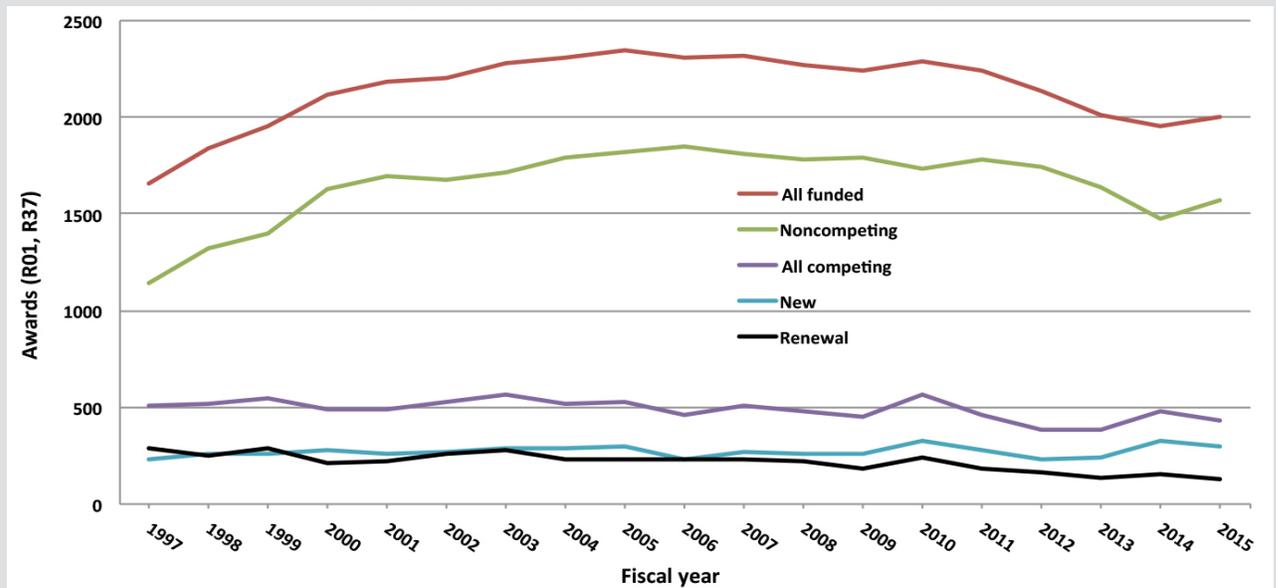
FIGURE 3: CUMULATIVE PERCENTAGE OF R01 AWARDS ACROSS PERCENTILES (FY 2006-2015)



Only funded applications are considered in the data set charted in 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 applications funded by the NIDDK fall within the payline, but that the NIDDK does exercise some programmatic discretion to reach for a limited number of especially innovative or programatically important applications.

# FIGURE 4

**FIGURE 4: NUMBER OF NIDDK R01 GRANTS (COMPETING AND NON-COMPETING) FUNDED IN FY 1997-2015**



Overall, the total number of R01 grants funded by the NIDDK has increased by slightly more than 20 percent since FY 1997. The major portion of this increase occurred during the years of the NIH budget doubling (FYs 1998-2003). Over the past 5 years, there has been a slight decline in the number of grants funded. In the past, approximately half of the competing grants funded by the NIDDK were new (Type 1) awards, but since FY 2009 that proportion has risen to what is now well over half.

# FIGURE 5

**FIGURE 5: NUMBER OF COMPETING NIDDK R01 APPLICATIONS (INCLUDING REVISIONS) RECEIVED FOR FUNDING CONSIDERATION IN FY 1998-2015**

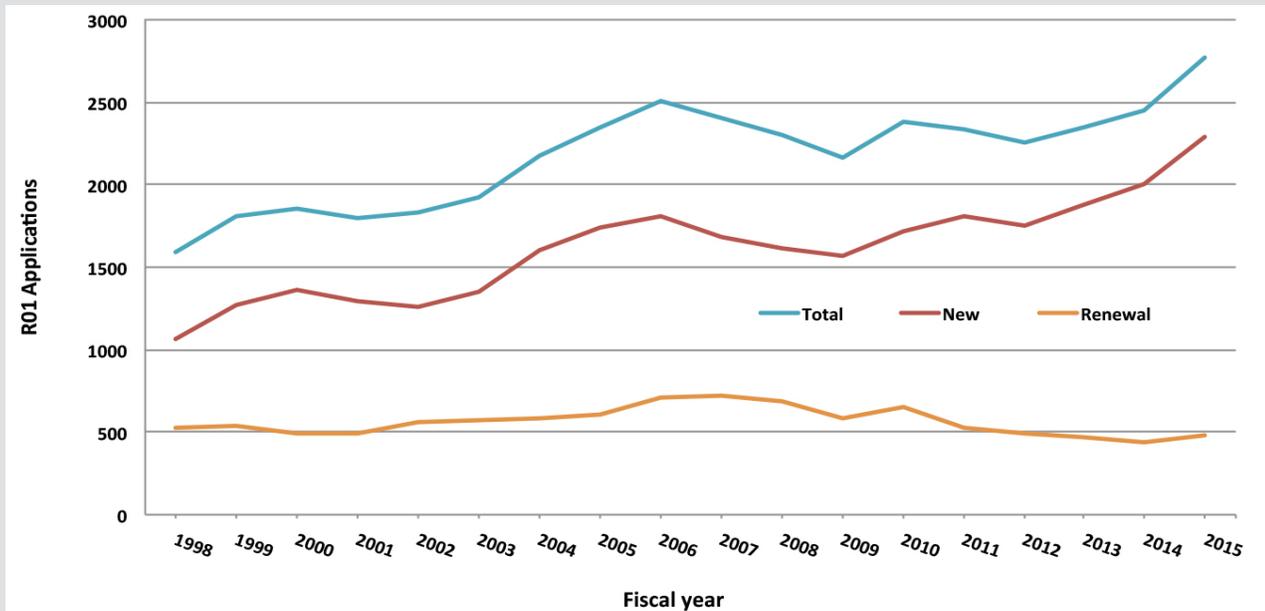


Figure 5 shows a substantial increase in the number of competing R01 applications received by the NIDDK between FYs 1998 and 2006. After approximately 5 years of relatively flat growth prior to FY 2013, the number of applications increased in FYs 2013-2015. Much of the observed increase between FY 1998 and FY 2006 and between FY 2013 and FY 2015 was largely due to new (Type 1) applications. Submission rates for competing renewal applications fluctuated somewhat between FY 1998 and FY 2014, but, overall, the numbers of renewal applications have remained relatively flat. It should be noted that only one amendment and resubmission of an application was allowed after January 25, 2009. The full implementation of the NIH policy eliminating a second amended application is coincident with a rise in “New” competing R01 applications in FY 2010.

# FIGURE 6

**FIGURE 6: OVERALL NIDDK EXPENDITURES (INCLUDES DIRECT AND FACILITIES AND ADMINISTRATIVE COSTS) ON R01 AWARDS (COMPETING AND NON-COMPETING) IN FY 1995-2015**

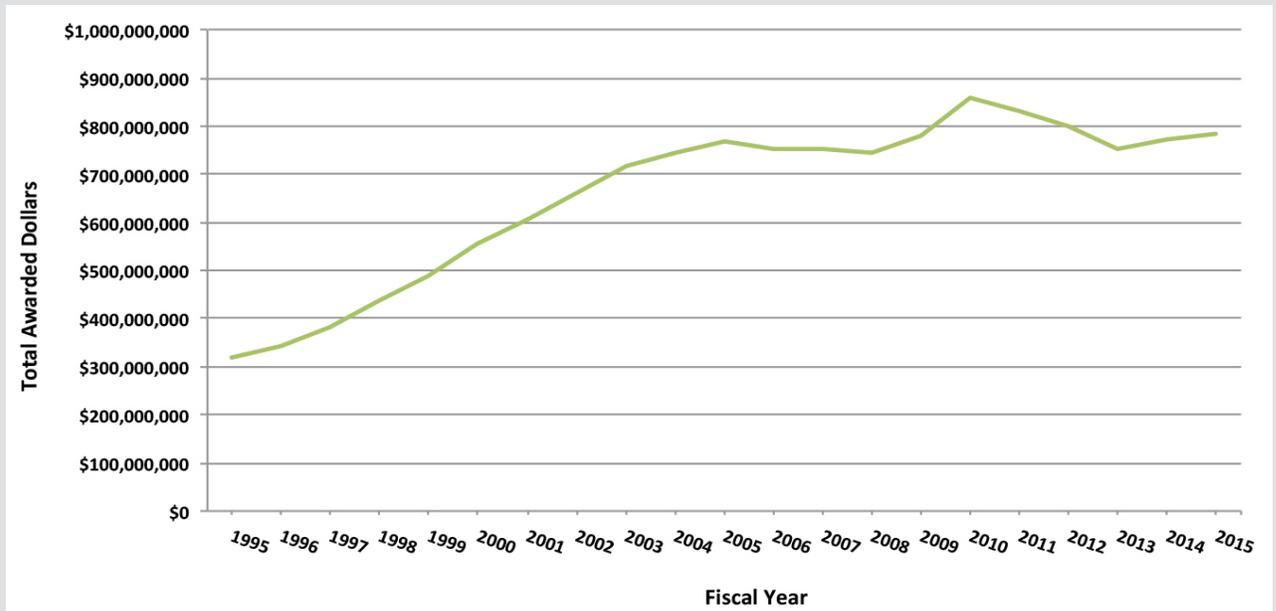


Figure 6 shows that NIDDK expenditures on R01 grants have increased markedly (147 percent) since FY 1995. This is because the NIDDK is funding a larger number of these awards (Figure 4) and also because the median cost of an R01 has increased substantially (Figure 7).

# FIGURE 7

**FIGURE 7: MEDIAN TOTAL COSTS (INCLUDES DIRECT AND FACILITIES AND ADMINISTRATIVE COSTS) OF NIDDK R01 GRANTS (COMPETING AND NON-COMPETING) IN FY 1995-2015**

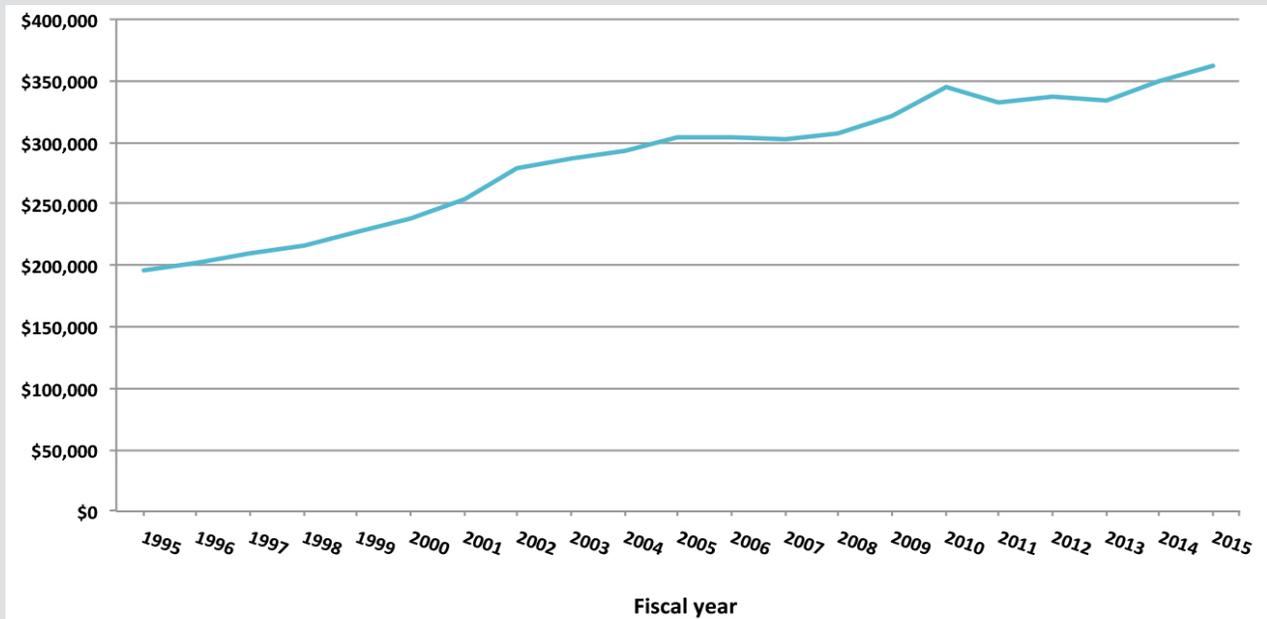


Figure 7 illustrates that the median cost of R01 awards has increased approximately 85 percent since FY 1995. Since FY 2004, the number of grants receiving \$500,000 or more in total costs has gone from 5 percent of the total number of awards to 19 percent of the total awarded R01s. The number of grants receiving \$250,000 or less in total costs has declined from 20 percent of the total awards to 8 percent.

# FIGURE 8

**FIGURE 8: NIDDK EXTRAMURAL RESEARCH FUNDING BY CATEGORY (COMPETING AND NON-COMPETING)**

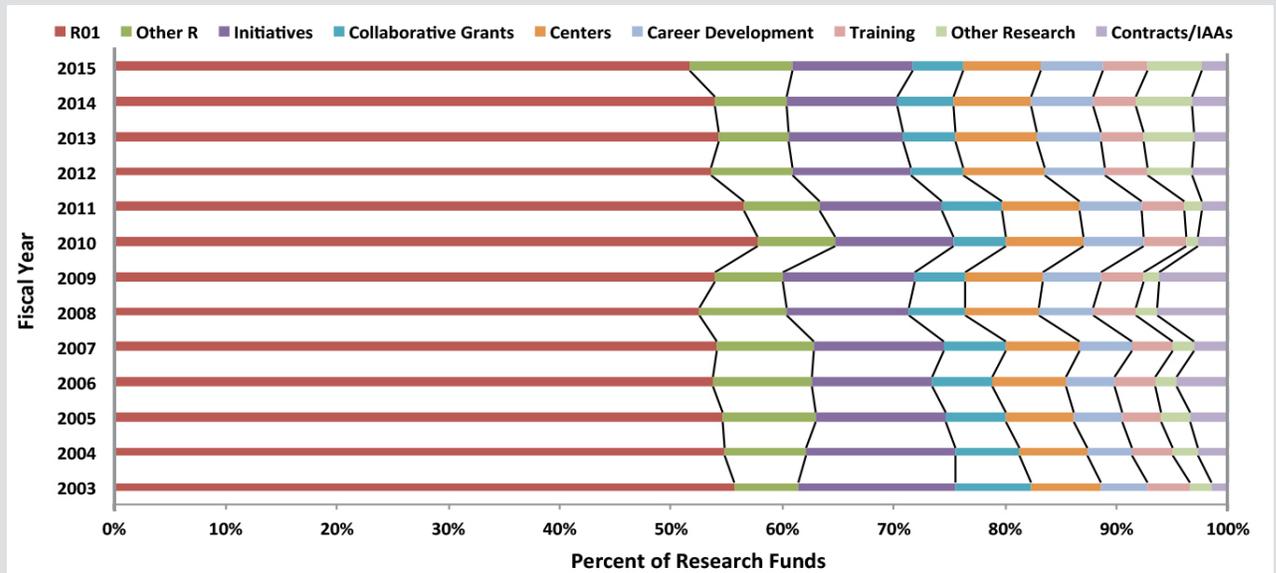


Figure 8 shows that relative funding levels of most NIDDK extramural research categories have remained fairly stable since FY 2003. These data were presented to the NIDDK’s Advisory Council in May 2012 in the context of the NIDDK’s core values. The NIDDK core values emphasize maintaining a strong investigator initiated R01 program, preserving a stable pool of talented new investigators, supporting key clinical studies and trials (support is generally represented in the Initiatives and Contracts categories), and continuing strong support of training and career development programs. Figures 9 to 12 illustrate other examples of how the NIDDK’s portfolio has reflected NIDDK core values over time.

**NIDDK Portfolio Categories:**

- **R01** – 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 categories above
- **Contracts and Interagency Agreements (IAAs)**– Includes some large clinical studies

# FIGURE 9

**FIGURE 9: MAINTAINING A STABLE POOL OF NIDDK INVESTIGATORS:  
NUMBER OF INVESTIGATORS SUPPORTED BY AT LEAST ONE R01**

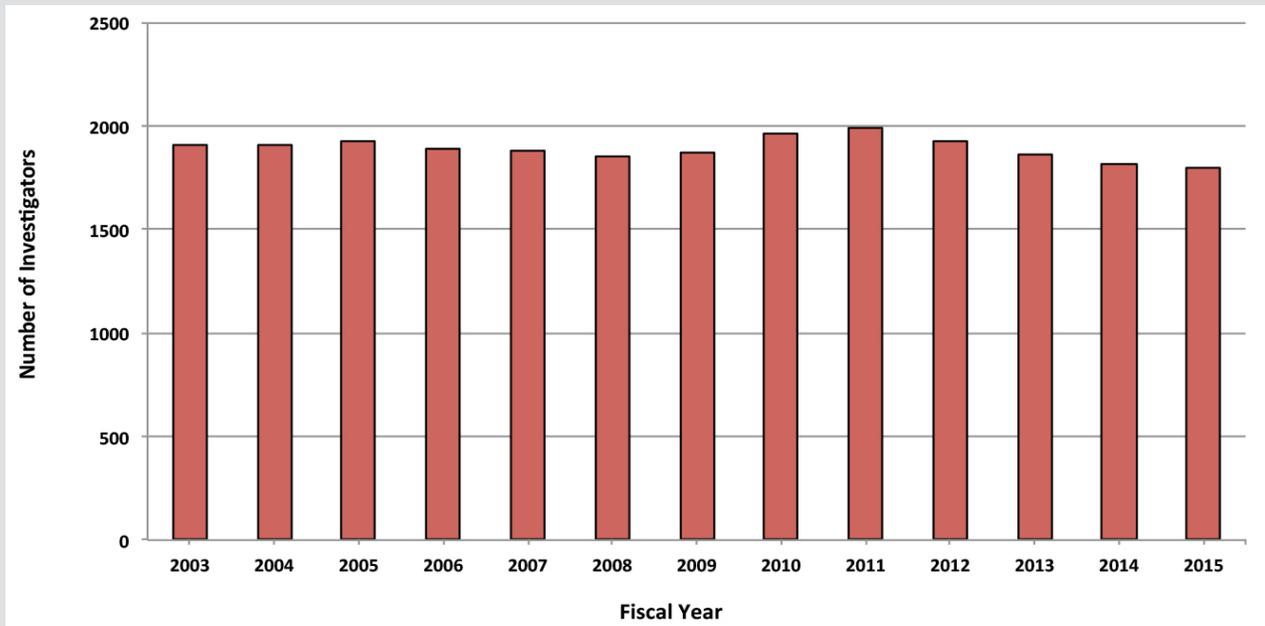


Figure 9 shows that the number of Principal Investigators (PIs) supported by at least one R01 remained relatively stable between FYs 2003 and 2009. In FYs 2010 and 2011, there were increases in the numbers of PIs supported with an NIDDK R01. It should be noted that in FY 2008 NIH, for the first time, began making multiple principal investigator R01 awards to support team science projects. The observed increases in numbers of PIs supported by the NIDDK in FYs 2010 and 2011 are largely attributable to multiple principal investigator R01 awards. The subsequent declines in FYs 2012-2015 are likely due in large part to paylines that became more stringent (*i.e.*, after FY 2011) and inflationary pressures in the context of flat or declining budgets.

# FIGURE 10

**FIGURE 10: PRESERVING A STABLE POOL OF NEW INVESTIGATORS: NUMBER OF NIDDK NEW INVESTIGATOR R01 APPLICATIONS AND AWARDS**

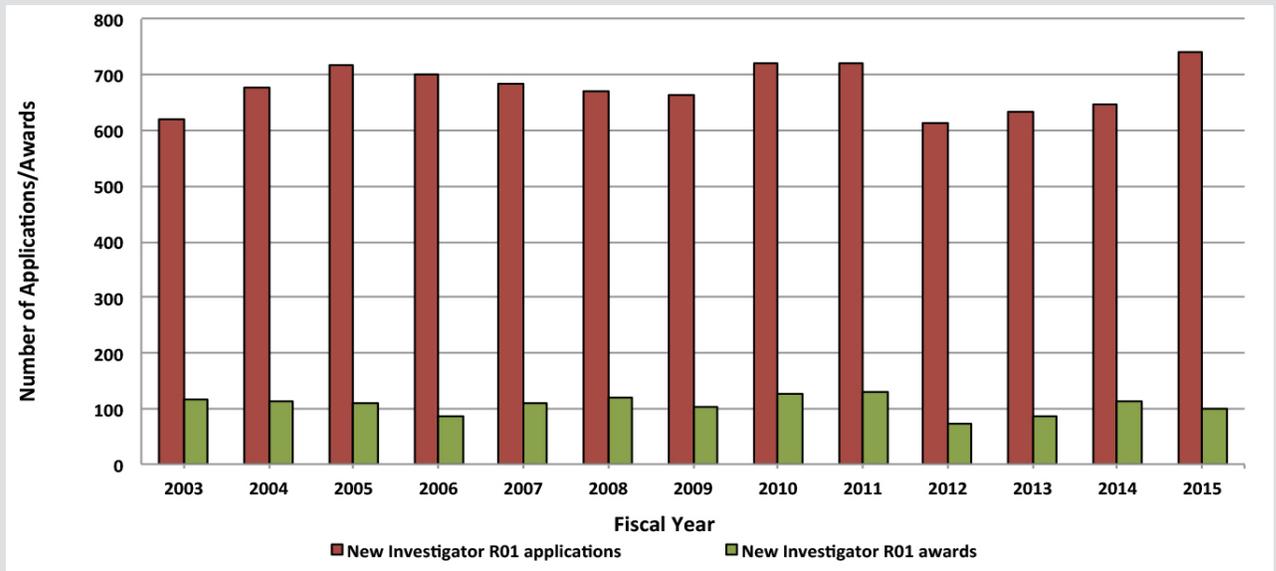
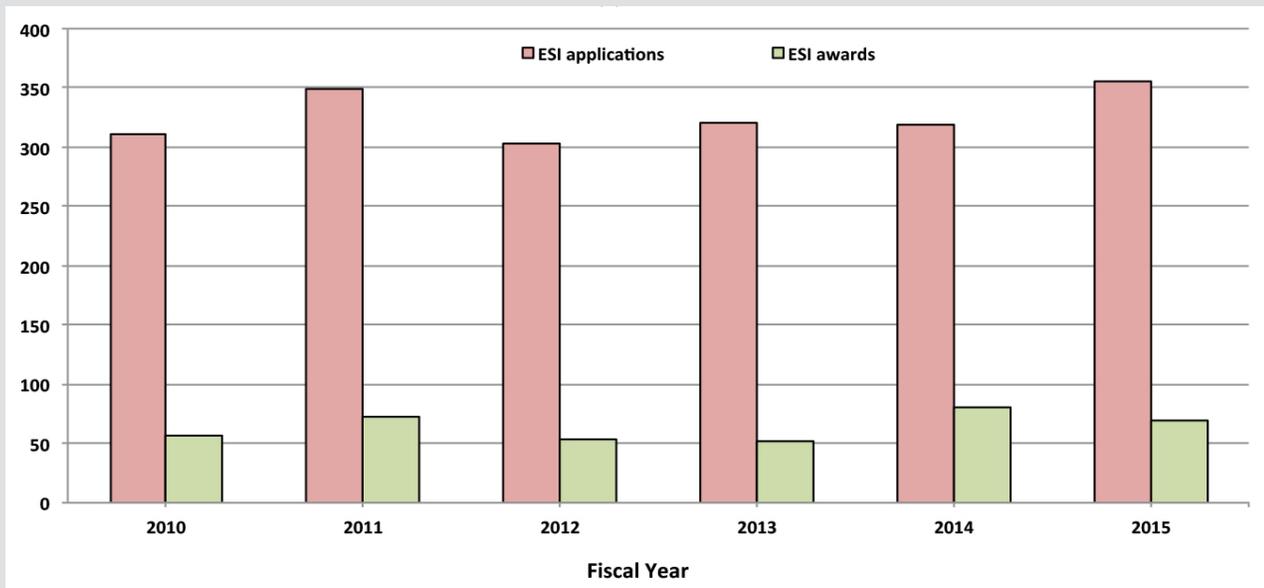


Figure 10 shows that while application rates for New Investigators have remained fairly high, there was a deceleration in the number of New Investigator awards between FYs 2003 and 2006. Starting in FY 2007, the NIH and the NIDDK established new policies focused on New Investigators and these policies appear effective in mitigating downward pressures on New Investigator awards. The decrease in number of New Investigator awards in FY 2012 reflects a decrease in the number of applications from New Investigators that year. Numbers of New Investigator applications and awards recovered somewhat in FY 2013 through FY 2015, although in FY 2015 the number of New Investigator awards did not keep pace with increased numbers of applications and in fact there was a slight decline in the number of New Investigator awards. In addition, in FY 2012 NIH and NIDDK began focusing on Early Stage Investigators (ESIs; see definition of and benefits conveyed to ESIs 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)), which is a subset of New Investigators (see table associated with Figure 2 and Figures 11 and 12). It should be noted that the data in this chart count applications and awards, not persons.

# FIGURE 11

**FIGURE 11: PRESERVING A STABLE POOL OF NEW INVESTIGATORS: NUMBER OF NIDDK EARLY STAGE INVESTIGATOR (ESI) R01 APPLICATIONS AND AWARDS**



Comparison of Figures 10 and 11 shows that while the subset of 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 Figure 2 and the “NIDDK Policies Related to First-Time Investigator Support” at [http://grants.nih.gov/grants/new\\_investigators/index.htm](http://grants.nih.gov/grants/new_investigators/index.htm)). The number of ESI awards in FY 2013 was essentially flat compared with the number of ESI awards in FY 2012, and the number of ESI awards in FY 2014 increased approximately 54 percent compared to FY 2013 ESI award numbers. In FY 2015, the number of ESI awards dipped slightly compared to FY 2014; however, the number of ESI awards in FY 2015 remains over 30 percent higher than the number in FY 2013.

# FIGURE 12

**FIGURE 12: PRESERVING A STABLE POOL OF NEW INVESTIGATORS: PERCENT OF NEW INVESTIGATOR APPLICATIONS AND AWARDS THAT ARE ESI**

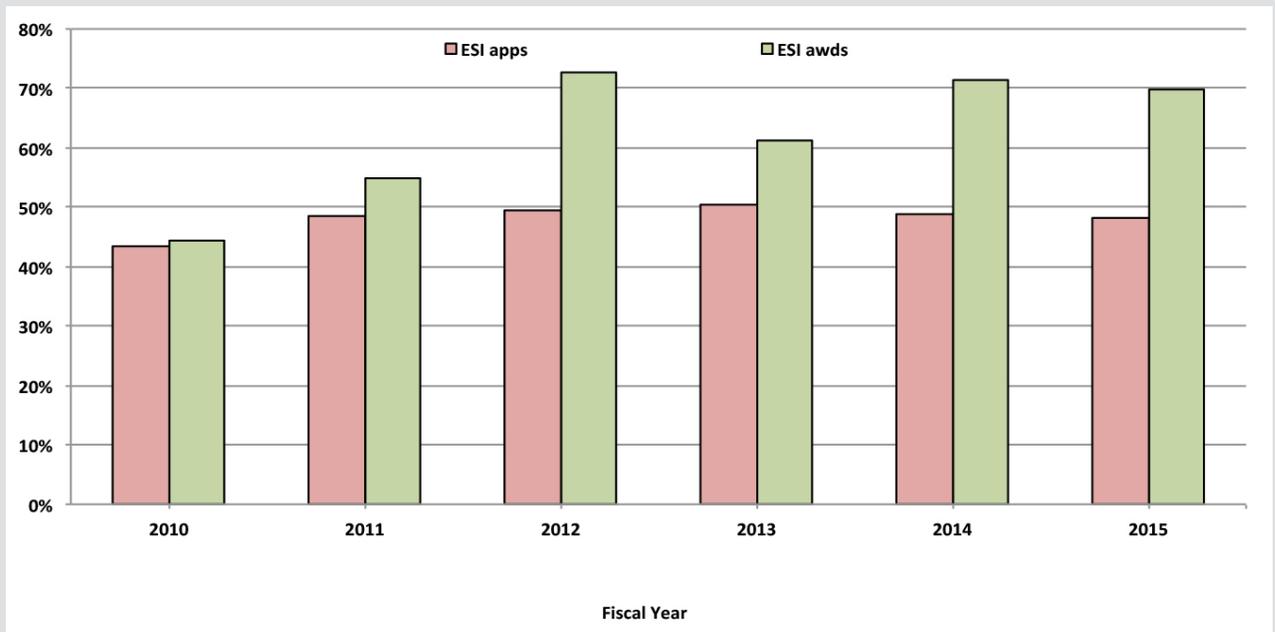


Figure 12 shows that the NIDDK’s differential payline for ESIs from FY 2012 to FY 2015 (see Figure 2 and the “NIDDK Policies Related to First-Time Investigator Support” at [http://grants.nih.gov/grants/new\\_investigators/index.htm](http://grants.nih.gov/grants/new_investigators/index.htm)) has been effective in continuing to enhance ESI representation among New Investigator awards.

# FIGURE 13

**FIGURE 13: SUPPORT PIVOTAL CLINICAL STUDIES AND TRIALS: NIDDK HUMAN SUBJECTS RESEARCH FUNDING AS A PROPORTION OF ALL EXTRAMURAL RESEARCH FUNDING**

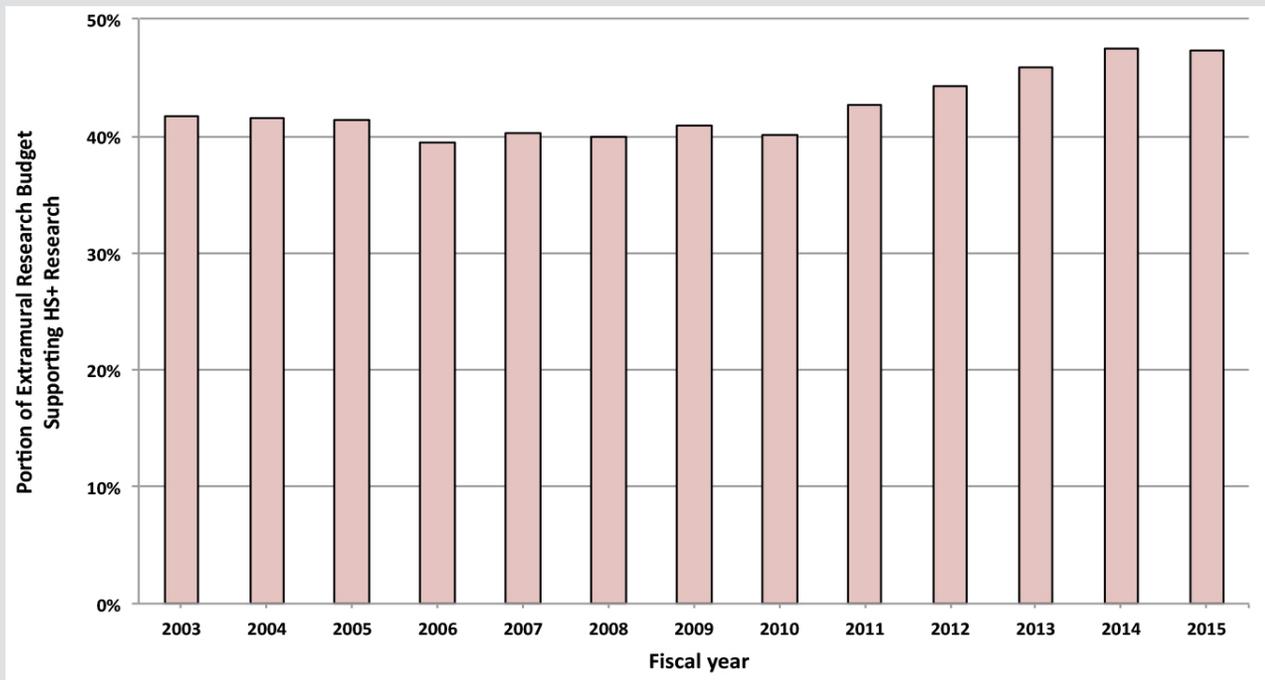


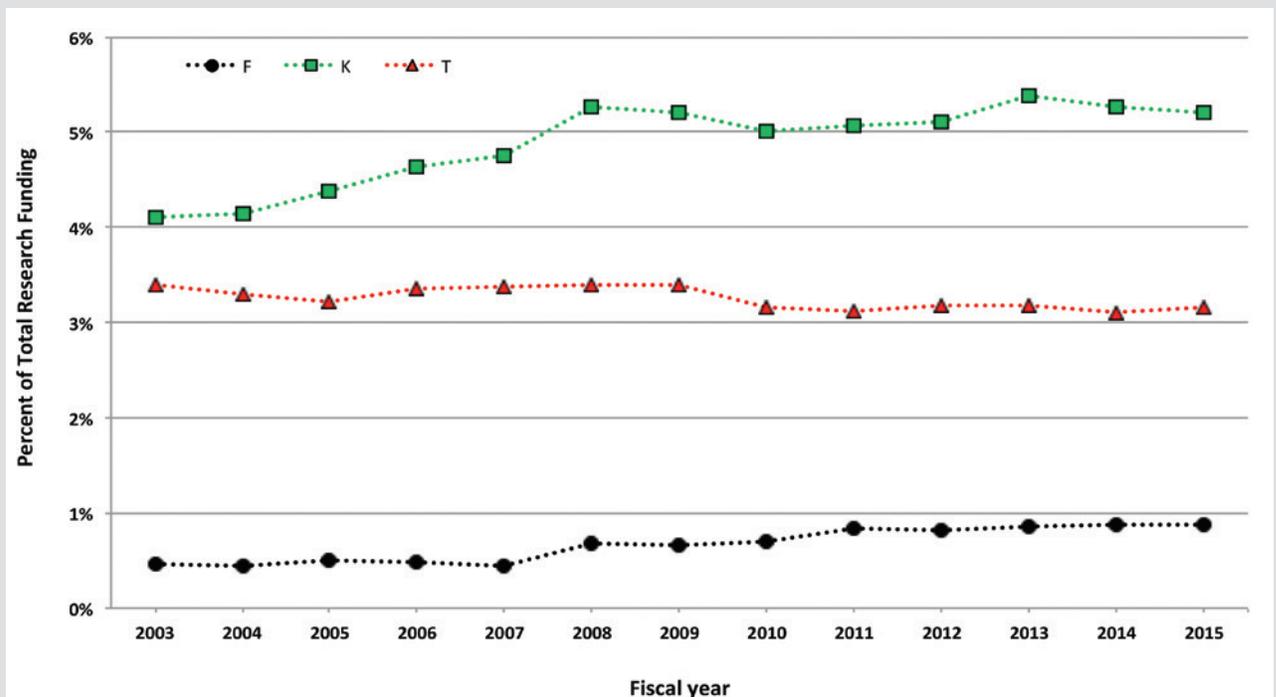
Figure 13 demonstrates that the NIDDK commits a substantial proportion of its research funding to the support of clinical research involving human subjects. For the purpose of this analysis, we used the definition described in Kotchen *et al*, *JAMA* 2004 Feb; 291(7):836-43 and included all studies coded as using Human Subjects (HS+).

# FIGURES 14A TO 14D

## FIGURES 14A TO 14D: THE NIDDK IS COMMITTED TO TRAINING THE NEXT GENERATION

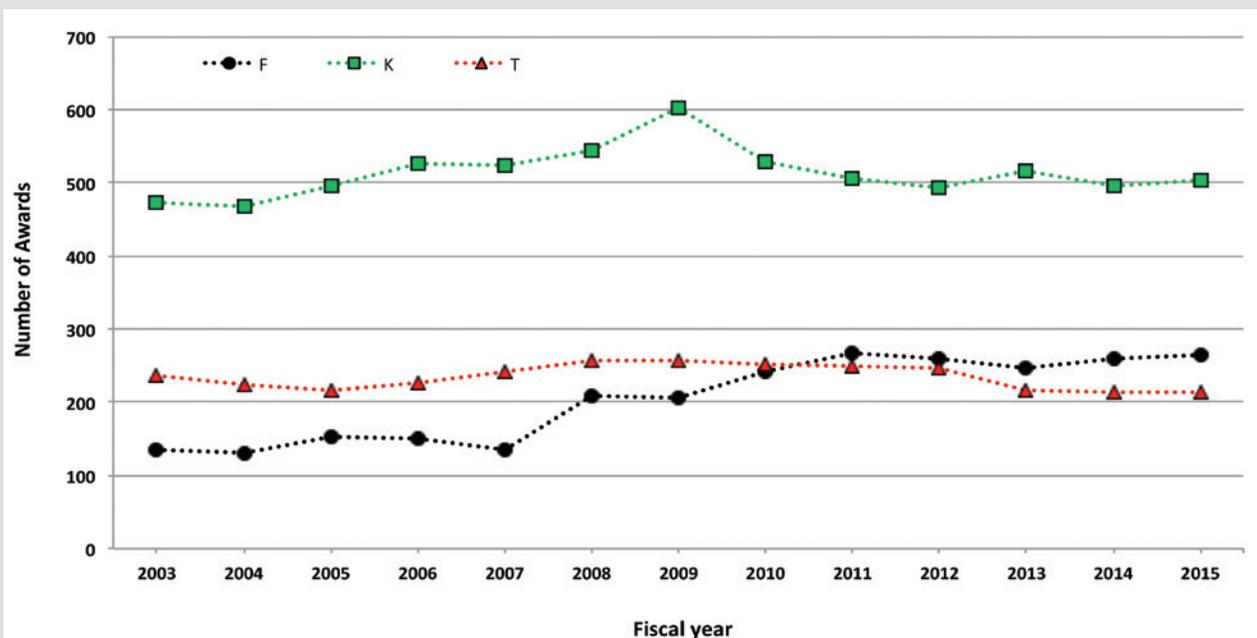
Figures 14A to 14D demonstrate that the NIDDK's commitment to training and developing the careers of the next generation of scientists remains strong. Figure 14A shows that overall support of training and career development programs has increased since FY 2003 and that the slight deceleration of T awards support was offset by an increase in support of F awards (by design). Figures 14B and D illustrate that the numbers of NIDDK T awards and associated training slots have decreased somewhat since FY 2012 as the NIDDK enhanced support of F awards. Figure 14C shows that while the numbers of NIDDK K08 (Mentored Clinical Scientist Development Award) awards decreased between FYs 2003 and 2013, the numbers of K01 (Mentored Research Scientist Development Award) and K23 (Mentored Patient-Oriented Research Career Development Award) increased. The NIDDK will continue to monitor carefully its training and career development programs to ensure appropriate balance.

**FIGURE 14A. NIDDK FELLOWSHIP (F), CAREER DEVELOPMENT (K), AND TRAINING (T) AWARDS AS A PERCENT OF TOTAL RESEARCH FUNDING**



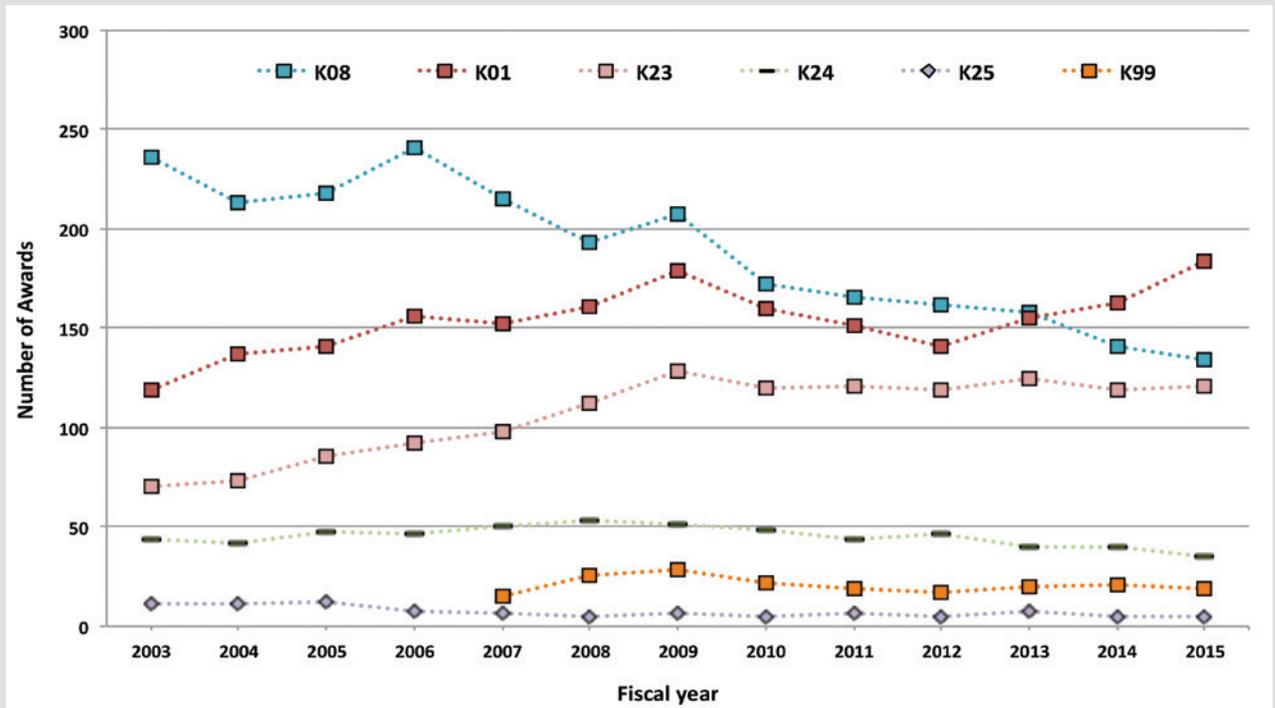
# FIGURES 14A TO 14D

FIGURE 14B. NUMBER OF NIDDK FELLOWSHIP (F), CAREER DEVELOPMENT (K), AND TRAINING (T) AWARDS BY FISCAL YEAR



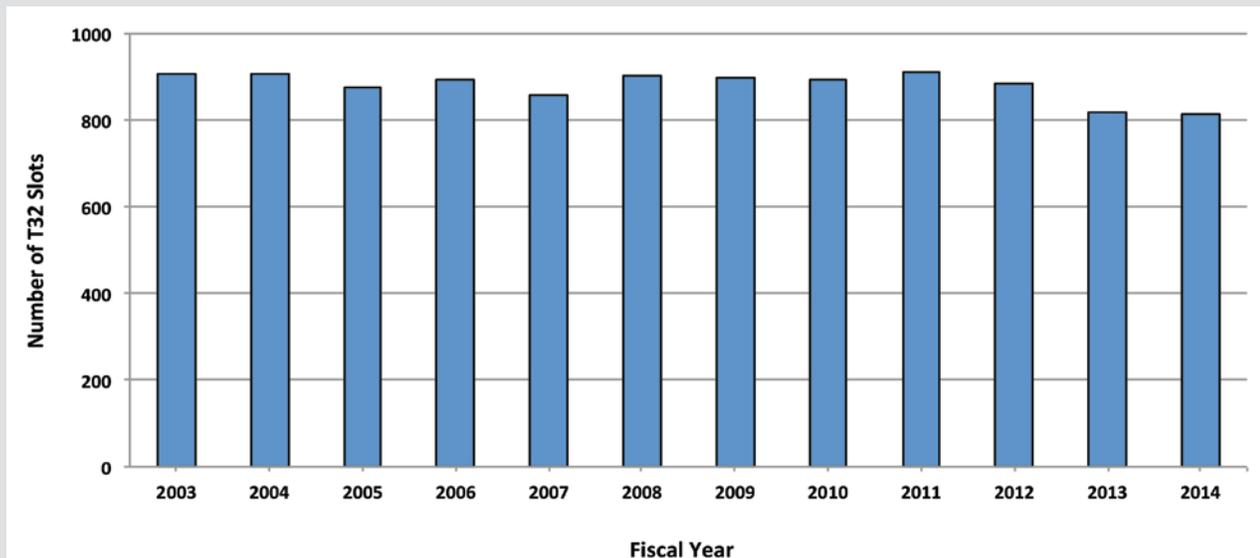
# FIGURES 14A TO 14D

FIGURE 14C. NUMBER OF NIDDK CAREER DEVELOPMENT (K) AWARDS BY ACTIVITY AND FISCAL YEAR



# FIGURES 14A TO 14D

FIGURE 14D. NUMBER OF NIDDK TRAINING (T32) AWARD SLOTS BY FISCAL YEAR



Note: T32 awards made in FY 2015 continue into FY 2016. The total numbers of T32 slots are reported at the end of the award period. Therefore, the FY 2015 information on T32 slots will not be available until later in FY 2016; thus, unlike the previous charts, FY 2015 data are not included here.





## ACKNOWLEDGEMENTS

Printed January 2016

### Research

The NIDDK gratefully acknowledges the contributions of the researchers whose studies are described in this report, and of the volunteers who participate in clinical research studies.

### Writing and Production

#### *Overall Production*

Eleanor Hoff, Ph.D., Office of Scientific Program and Policy Analysis

Robert Tilghman, Ph.D., Office of Scientific Program and Policy Analysis

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

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

Richard Farishian, Ph.D., Director

Lisa Gansheroff, Ph.D., Deputy Director

Rebecca Cerio, Ph.D.

Sandeep Dayal, Ph.D.

Patrick Donohue, Ph.D.

Anne Hanessian, B.S.

Mary Hanlon, Ph.D.

Eleanor Hoff, Ph.D.

David Miller, Ph.D.

Dung Pham

B. Tibor Roberts, Ph.D.

Megan Singh, Ph.D.

Robert Tilghman, Ph.D.

Julie Wallace, Ph.D.

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

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

Krysten Carrera, M.P.A.

Amy F. Reiter, M.S.

#### *NIDDK Funding Trends and Support of Core Values*

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

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

Teresa Lindquist, M.S.

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

Van Nguyen, B.Acc., Director

Michelle Shorter, B.Acc., Deputy Director

Shelby Andrews-Shigaki, M.Acc.

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







Scan the QR code with your mobile device to link to the NIDDK website at [www.niddk.nih.gov](http://www.niddk.nih.gov)



National Institute of Diabetes and Digestive and Kidney Diseases

NIH Publication Number: 16-7962